BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

NOTICE OF FILING

TO: See Service List

PLEASE TAKE NOTICE that I have today filed with the Illinois Pollution Control Board, Illinois EPA's MOTION FOR ACCEPTANCE; APPEARANCES; CERTFICIATE OF ORGINATION; STATEMENT OF REASONS; and PROPOSED AMENDMENTS TO 35 ILL. ADM. CODE 620, GROUNDWATER QUALITY STANDARDS, a copy of which is served upon you.

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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P.O. Box 19276
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Sara.Terranova@illinois.gov

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

MOTION FOR ACCEPTANCE

NOW COMES the Illinois Environmental Protection Agency (Illinois EPA), by and through its attorneys, and pursuant to 35 Ill. Adm. Code 102.106, 102.200, and 102.202, moves the Illinois Pollution Control Board to accept the Illinois EPA'S proposal for the adoption of a proposed amendments to 35 Ill. Adm. Code 620, Groundwater Quality.

This regulatory proposal includes:

1) Notice of Filing;
2) Appearances of Attorneys for the Illinois EPA;
3) Certification of Origination;
4) Statement of Reasons (including attachments);
5) Proposed Amendments; and
6) Certificate of Service

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

APPEARANCE

The undersigned hereby enters her appearance as an attorney on behalf of the Illinois Environmental Protection Agency.

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

APPEARANCE

The undersigned hereby enters his appearance as an attorney on behalf of the Illinois Environmental Protection Agency.

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Nicholas E. Kondelis
Nicholas E. Kondelis
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

CERTIFICATE OF ORIGINATION

NOW COMES the ILLINOIS ENVIRONMENTAL PROTECTION AGENCY (Illinois EPA) by its attorneys, and pursuant to 35 Ill. Adm. Code 102.202(i), the Illinois EPA certifies that the regulatory proposal in the above captioned matter amends the most recent version of Part 620 of the Illinois Pollution Control Board's regulation, as published on the Board's website.

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

CERTIFICATE OF SERVICE

I, the undersigned attorney at law, hereby certify that on , I served true and correct copies of the foregoing NOTICE OF FILING; MOTION FOR ACCEPTANCE; APPEARANCES; CERTFICATE OF ORIGINATION; STATEMENT OF REASONS; PROPOSED AMENDMENTS TO 35 ILL. ADM. CODE 620, GROUNDWATER QUALITY upon the persons and by the methods pursuant to the service list hereto attached.

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

DATED: 12/7/21

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	RXXX-XXX
PROPOSED AMENDMENTS TO	)	
GROUNDWATER QUALITY	)	(Rulemaking – Public Water
(35 Ill. Adm. Code 620)	)	Supplies)

STATEMENT OF REASONS

NOW COMES the Illinois Environmental Protection Agency (Illinois EPA or Agency) by and through its counsel and submits its Statement of Reasons to the Illinois Pollution Control Board (Board) pursuant to 415 ILCS 5/27 and 5/28, and 35 Ill. Adm. Code 102.200 and 102.202. Upholding the policy of the Illinois Groundwater Protection Act (IGPA), this proposal seeks to amend 35 Ill. Adm. Code 620, keeping groundwater quality standards current as scientific data and methods supporting the development of groundwater quality standards have evolved.

I. STATUTORY AUTHORITY

Part 620 of the Board's rules pertain to "various aspects of groundwater quality." *See* 35 Ill. Adm. Code 620.105. As such, Part 620 is subject to the IGPA.

Adopted in 1987, the IGPA set forth that:

> …it is the policy of the State of Illinois to restore, protect, and enhance the groundwaters of the State, as a natural and public resource. The State recognizes the essential and pervasive role of groundwater in the social and economic well-being of the people of Illinois, and its vital importance to the general health, safety, and welfare. It is further recognized as consistent with this policy that the groundwater resources of the State be utilized for beneficial and legitimate purposes; that waste and degradation of the resources be prevented; and that the underground water resource be

> managed to allow for maximum benefit of the people of the State of Illinois.

See 415 ILCS 55/2(b).

Implementing the policy above, Section 55/8(a) of the IGPA required the Illinois EPA to establish and propose comprehensive water quality standards specifically for the protection of the groundwater by July 1, 1989. *See* 415 ILCS 55/8(a). In preparing such regulations, the IGPA required the Agency to:

> …address, to the extent feasible, those contaminants which have been found in the groundwaters of the State and which are known to cause, or are suspected of causing, cancer, birth defects, or any other adverse effect on human health according to nationally accepted guidelines.

See 415 ILCS 55/8(a).

In addition, Section 55/8(b) of the IGPA required the Board to promulgate the water quality standards for groundwater within two years after the date upon which the Agency filed proposed regulations. *See* 415 ILCS 55/8(b).

II. BACKGROUND

In 1989, Illinois EPA established and proposed comprehensive water quality standards specifically for the protection of groundwater. In 1991, the Board promulgated water quality standards for groundwater. *See* Groundwater Quality Standards (35 Ill. Adm. Code 620, R89-14(B) (November 7,1991) (R89-14(B)). Although satisfying the mandate of the IGPA with the proposal and adoption of Part 620; Groundwater Quality, the Board acknowledged the need to continually revise the numeric groundwater quality standards as new information develops. *See* Id. at 19. Therefore, to ensure that the numerical groundwater quality standards continued to evolve with the supporting

scientific data and methods, the Board stated an expectation "of regular updates" to the groundwater quality standards from the Agency. *See* Id.

This point was reiterated in the most recent rulemaking, R08-18. There, the Board recognized the Agency's position, that to prevent the degradation of the groundwater resources it is periodically necessary to: 1) amend the groundwater quality standards to account for new scientific data, 2) update the groundwater standards that have been amended at the federal level, 3) update technical references that are in the Incorporations by Reference, and 4) address any additional groundwater parameters that have been discovered. *See* R08-18, at 4.

To meet the Board's stated expectations and to keep the groundwater quality standards current with the latest scientific data and methods, the Agency has proposed, and the Board has adopted amendments to the groundwater quality standards multiple times over the years. In 1994, the principal amendments included: 1) the addition of Class I and Class II groundwater quality standards for sixteen chemicals for which standards had not previously been promulgated, 2) the modification of certain preventive notification and response provisions, including listing of ten of the new sixteen chemicals, and 3) making various amendments of a conforming nature. *See* Groundwater Protection: Amendments to Groundwater Quality Standards 35 Ill. Adm Code. 620, R93-27 (Aug. 11, 1994) (R93-27).

In 1997, the Board adopted amendments to conform the groundwater quality standards to the Site Remediation Program. *See* Site Remediation Program (Brownfields) and Groundwater Quality Standards (35 Ill. Adm. Code 740 and 35 Ill. Adm. Code 620),

R97-11 (June 5, 1997) (R97-11).[1] In 2002, The Board adopted amendments which included a preventive response level in addition to Class I and Class II groundwater quality standards for methyl tertiary-butyl ether (MTBE). *See* Proposed MTBE and Compliance Determination Amendments to Groundwater Quality Standards: 35 Ill. Adm. Code 620, R01-14 (Jan. 24, 2002) (R01-14).

Finally, based upon new scientific data, federal amendments, and technical references, 35 Ill. Adm. Code 620 Groundwater Quality standards were last updated in 2012. *See* Proposed Amendments Groundwater Quality Standards (35 Ill. Adm. Code 620), R08-18 (October 4, 2012). The amendments added groundwater quality standards for chemical constituents detected in Illinois groundwater that had established toxicity values or that had groundwater remediation objectives under the Tiered Approach to Corrective Action Objectives (TACO) (35 Ill. Adm. Code 742). *See* Id. In all, 39 chemical constituents were added. In addition, revisions were made to the Class I groundwater quality standard for arsenic. *See* Id.

III. REGULATORY PROPOSAL

A. Regulatory Proposal: Purpose and Effect

The Illinois EPA now submits this proposal to amend 35 Ill. Adm. Code 620; Groundwater Quality. The purpose of this proposal is to uphold the stated policy of the IGPA. This is accomplished by keeping the groundwater quality standards current with the evolution of scientific data and methods supporting the development of the groundwater quality standards. Specifically, the proposal achieves this purpose with the inclusion of ten new chemicals, three new atrazine

[1] In 1997, the Agency proposed, and the Board adopted additional amendments to Part 620. However, these amendments were of a non-substantive, "housekeeping" nature. *See* Amendments to 35 Ill. Adm. Code Subtitle F (Parts 601-620), R96-18 (May 1, 1997).

metabolites, and updated procedures in Subpart F and Appendix A for selecting toxicity values consistent with current scientific data and methods. This includes methods for calculating groundwater quality standards when Maximum Contaminant Levels (MCLs) or Maximum Contaminant Level Goals (MCLG) are not available. In addition, definitions and references are updated. Site specific groundwater standards for designated Class III Special Resource Groundwater are also proposed. Finally, this proposal includes groundwater quality standards for six Per- and Polyfluoroalkyl Substances (PFAS) including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), perfluorobutanesulfonic acid (PFBS), and hexafluoropropylene oxide dimer acid (HFPO-DA).

B. Regulatory Proposal: Language

The following is a Section-by-Section summary of the Illinois EPA's proposed language. Illinois EPA is providing both Ms. Hawbaker's and Mr. Dunaway's pre-filed testimony for a more in-depth and technical review of the proposed language. *See* Carol Hawbaker Testimony (Attachment 1) and Lynn Dunaway Testimony (Attachment 2).

SUBPART A: GENERAL

Section 620.110 Definitions

The Agency proposes adding the following definitions to Section 620.110: "Chemical Abstract Service Registry Numbers or CASRN," "Lower Limit of Quantitation or LLOQ," "Lowest Concentration Minimum Reporting Level or LCMRL," and "Mutagen." In addition, the Agency proposes to update the

definition of "Carcinogen" to maintain consistency with the current U.S. EPA Integrated Risk Information System and the definition of "Detection" to language currently utilized in test methods. Finally, the Agency proposes to remove the definition of "Practical Quantitation Level or PQL" as it is replaced by the terms "Lower Limit of Quantitation or LLOQ" and "Lowest concentration minimum reporting level (LCMRL), as analytical methods have updated the terminology to describe levels of quantitation in analyses.

Section 620.125 Incorporations by Reference

The Agency proposes to add Illinois EPA's "Integrated Water Quality Report and Section 303(d) List" and the National Academy of Science "Water Quality Criteria" (1973) to this Section, while also adding and updating several test methods. The Agency proposes to add and update references from the U.S. EPA Office of Research and Development, National Center for Environmental Assessment, the U.S. EPA Risk Assessment Forum, and the U.S. EPA Office of Resource Conservation and Recovery. Finally, the Agency proposes updating the U.S. EPA groundwater guidance and the Code of Federal Regulation references to the most recent edition.

SUBPART B: GROUNDWATER CLASSIFICATION

Section 620.210 Class I: Potable Groundwater

The Agency proposes removing permeameter as an acceptable means to determine hydraulic conductivity. This removal is because the collection of samples for its use has a high potential to alter the sample's hydraulic conductivity and are therefore not sufficient representatives of in-situ hydraulic

conductivity. Further, the permeameter analysis accounts for vertical hydraulic conductivity, whereas in-situ methods measure hydraulic conductivity on a horizontal plane.

The Agency proposes adding the wellhead protection area of a community water supply well or well field as a specific area to which Class I groundwater quality standards are applicable. The wellhead protection area is added because site specific hydrogeologic parameters are used to estimate the area from which the groundwater will enter a community well or well field from within the aquifer material.

Section 620.250 Groundwater Management Zone

The Agency proposes adding a list of standard documentation that must be included with all groundwater management zone (GMZ) applications. Submission of a standardized list of documents makes review and approval of a GMZ more efficient. In addition, some of the information will provide documentation of the complete set of corrective actions required, the time required to complete them, and evidence supporting the efficacy of the corrective action(s).

SUBPART C: NONDEGRADATION PROVISIONS FOR APPROPRIATE GROUNDWATERS

Section 620.302 Applicability of Preventive Notification and Preventive Response Activities

Section 602.302(b)

Section 620.302(a) provides the applicability of preventive notification and preventive response activities.[2] For the purposes of subsection (a), Section

[2] 35 Ill. Adm. Code 620.302(a):
Preventive notification and preventive response as specified in Sections 620.305through 620.310

620.302(b) provides a list of persons that conduct groundwater monitoring. To make the list in this subsection more comprehensive, the Agency proposes adding examples of persons that conduct groundwater monitoring.

Section 620.310 Preventive Response Activities

The Agency proposes updating the table in Section 620.310(a)(3)(A)(i) to include the Chemical Abstracts Service Registry Numbers (CASRN) for each constituent.[3] The Agency also proposes adding a table at Section 620.310(a)(3)(A)(ii) depicting the constituents in the subsection. This table will provide a clear representation of the constituents subject to the preventive response activities. Along with this addition of the proposed constituents are carcinogens with health-based standards that are not set at the LLOQ or LCMRL, and carcinogens with MCLs that are no longer based on the LLOQ or LCMRL because these constituents can be reliably detected at lower concentrations. The Agency proposes not including chemicals in the table that now utilize LLOQs/LCMRLs as the Class I standard because they cannot be reliably detected at lower concentrations. Finally, the Agency proposes to remove the Board note, as many carcinogens with groundwater quality standards based on the MCL and set at PQLs, now have LLOQs or LCMRLs that are below the MCL, making these carcinogens subject to the non-degradation provisions at Section 620.310.

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applies to:

1) Class I groundwater under Section 620.210(a)(1), (a)(2), or (a)(3) that is monitored by the persons listed in subsection (b); or
2) Class III groundwater that is monitored by the persons listed in subsection (b).

[3] CASRNs are unique numerical identifiers that are directly tied to a specific chemical. Chemicals can be associated with several names or synonyms for identification. The CASRN allows the user to know the specific chemical in reference.

SUBPART D: GROUNDWATER QUALITY STANDARDS

Section 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater

The Agency proposes to add Class I groundwater quality standards for ten new chemicals as they have been identified in the groundwater in Illinois and may cause a hazard to human health. In addition, tables are updated to add a CASRN for each constituent. The Agency proposes to add footnotes detailing the basis of the groundwater quality standards. This allows users to easily identify the sources of the standards. In addition, the notations identify carcinogens (where previously an asterisk was used as an identifier) and include the identification of carcinogens that operate via mutagenic mode of action. The Agency also proposes to update the applicable Class I groundwater quality standards based on updated methodologies in 620 Subpart F and Appendix A. In addition, updates for the beneficial use of potable resource groundwater (the use of more stringent livestock and irrigation values, in lieu of action levels and MCLs for copper and selenium) are proposed. The Agency is proposing the use of livestock and irrigation criteria for these constituents because the livestock and irrigation criteria are more stringent than current standards. Potable resource groundwater is also used for the watering of livestock and the irrigation of the crops/produce. Finally, maintaining consistency with U.S. EPA, the Agency proposes to update the groundwater quality standards based on the MCLs for radium 226 and radium 228 to a groundwater quality standard based on an updated MCL for radium (combined 226+228).

The Agency proposes removing the explosive constituents table at Section 620.410(c) and more appropriately integrating those constituents into a table at Section 620.410(b). In addition, the Agency proposes moving atrazine from Section 620.410(b) to the complex chemical mixtures tables at Section 620.410(c) with the addition of atrazine metabolites. With the addition of the metabolites, it becomes a complex organic chemical mixture, and thus more appropriate for subsection (c).

Section 620.420 Groundwater Quality Standards for Class II: General Resource Groundwater

In Section 620.420, the Agency proposes adding Class II groundwater quality standards for ten new chemicals and two chemicals listed in Section 620.410 without prior Class II groundwater quality standards. In addition, the Agency proposes updating the constituent tables adding a CASRN for each constituent and updating applicable Class II groundwater quality standards. Footnotes detailing the sources of the standards and carcinogenicity are also proposed. The Agency proposes removing the explosive constituents table at Section 620.420(c) and more appropriately integrating the constituents into the table at Section 620.420(b). Finally, the Agency proposes moving atrazine from Section 620.420(b) to the complex chemical mixtures tables at Section 620.420(c) with the addition of atrazine metabolites. With the addition of the metabolites, it becomes a complex organic chemical mixture, and thus more appropriate for subsection (c).

Section 620.430 Groundwater Quality Standards for Class III: Special Resource Groundwater.

The proposed language in Section 620.430 establishes location specific Class III groundwater quality standards for six dedicated nature preserves (DNPs), as designated pursuant Section 620.230(b). Chloride and pH location specific Class III groundwater quality standards are established at four "cave" DNPs which will provide added protection to assist in the preservation and ideally the enhancement of the critical environment for the Federally and State endangered Illinois Cave Amphipod. In addition, a chloride location specific Class III groundwater quality standards is established for two "wetland" DNPs which will provide added protection for the preservation and ideally the enhancement of wetland ecosystems which provide habitat for numerous State threatened and endangered plant species. Please see Attachment 2 for the required justification in support of the location specific proposed groundwater quality standards. *See* R89-14(B) at 20.

Section 620.440 Groundwater Quality Standards for Class IV: Other Groundwater

The Agency proposes to update the names of explosive constituents.

Section 620.450 Alternative Groundwater Quality Standards

The Agency proposes to update the names of explosive constituents.

SUBPART E: GROUNDWATER MONITORING AND ANALYTICAL PROCEDURES

Section 620.510 Monitoring and Analytical Requirements

The proposed language in this Section requires the use of the 2009 Unified Guidance to determine background groundwater quality unless other methods are

specified by regulation. In addition, because "PQL" is an outdated term, the proposed language replaces the use of the "PQL" with either the "LLOQ" or "LCMRL" to the analytical method of the chemical.

SUBPART F: HEALTH ADVISORIES

Section 620.601 Purpose of a Health Advisory

Section 620.601(b)

The Agency proposes updating the Illinois Administrative Code references to more thoroughly and accurately reflect the factors that must be considered when establishing a new source of public water supply.

Section 620.605 Issuance of a Health Advisory

Section 620.605(b)(1)

Amendments in Section 620.605(b)(1) designate the more stringent toxicity value of the (Human Threshold Toxicant Advisory Concentration (HTTAC) or Human Nonthreshold Toxicant Advisory Concentration (HNTAC) as the guidance value in the absence of a Maximum Contaminant Level (MCL) or Maximum Contaminant Level Goal (MCLG).

Section 620.605(b)(2)

The Agency proposes removing the Human Nonthreshold Toxicant Advisory Concentration (HNTAC) language and equation and relocating it to Appendix A.

Section 620.APPENDIX A Procedures for Determining Human Toxicant Advisory Concentrations for Class I: Potable Resource Groundwater

Section 620.Appendix A(a)

The proposed language in Section Appendix A(a) includes updates clarifying language for the calculation of noncancer effects, and updates the per capita daily water ingestion rate from an average adult rate of 2 liters per day ("L/d") to an average child water ingestion rate 0.78 L/d. The Agency proposes updating the exposure population from an average adult to a child aged 0 – 6 years. The Agency proposes the use of a child population for evaluating noncarcinogenic effects as a more sensitive receptor than an adult when considering exposure via the oral, or ingestion, route. The use of a child exposure population is consistent with 35 Ill. Adm. Code 742 for calculating ingestion remediation objectives for noncancer effects. U.S. EPA methods found at U.S. EPA's Regional Screening Levels ("RSLs") calculate screening levels for both an adult and a child for exposure to residential tapwater and both the adult and child must be addressed.

Section 620.Appendix A(b)(1)

The proposed language to Section 620.Appendix A(b)(1) updates the body weight for calculating the Acceptable Daily Exposure from an average adult to 15 kg, representative of a child 0-6 years of age.

Section 620.Appendix A(b)(2)

The Agency proposes building upon the toxicity hierarchy used to derive oral reference doses in the 2012 amendments, based on updates in 2013 and 2021 to the original toxicity hierarchy referenced in PCB R08-18. See Ms. Hawbaker's

testimony for more detailed information regarding U.S. EPA's updates to its toxicity hierarchy. The updates to the toxicity hierarchy are incorporated into RSLs decision tree for selecting toxicity values for development of its screening levels. The Agency generally uses the toxicity values found in the RSL table when calculating TACO Tier 2 remediation objectives and intends to utilize the RSLs when updating its TACO amendments. The toxicity values for calculating groundwater quality standards in Part 620 and remediation objectives in Part 742 need to be consistent.

Section 620.Appendix A(b)(3)

The Agency proposes revising the methodology used to calculate oral references doses when an oral reference dose is not available from the toxicity hierarchy. This update will make the language consistent with the U.S. EPA Reference Dose Guidance and Species Extrapolation Guidance. The method currently described in (b)(3), (b)(4), (b)(5), and (b)(6) of Appendix A is outdated. Updated methods and models to derive oral reference doses have replaced the method described in these subsections.

Section 620.Appendix A(b)(4)

The Agency proposes removing the language as described in Section (b)(3) of Appendix A and clarifying the usage of uncertainty factor consistent with updated methods for calculating oral reference doses.

Section 620.Appendix A(b)(5)

The Agency proposes removing the outdated language as described in Section (b)(3) of Appendix A.

Section 620.Appendix A(b)(6)

The Agency proposes removing the outdated language as described in Section (b)(3) of Appendix A.

Section 620.Appendix A(d)

Proposed Section (d) in Appendix A adds methods describing the calculation of HNTAC guidance levels (removed from Section 620.605(b)(2)) and updates the methods in accordance with U.S. EPA guidance. The updated methods are based on processes prescribed in the Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility for Early-Life Exposure to Carcinogens, both added to the Incorporations by Reference. It also updates the exposure factors based on 2011 updates to the Exposure Factor Handbook. The methods are used by U.S. EPA to calculate RSLs for carcinogen risks of drinking contaminated residential tapwater.

Section 620.Appendix A(d)(1)

Proposed Section (d)(1) in Appendix A adds an equation for calculating HNTAC guidance level for chemicals designated as carcinogens that operate through a mutagenic mode of action. Mutagens are expected to cause irreversible changes to DNA, which would exhibit a greater risk in early-life versus later-life exposures. The equation for calculating a standard for mutagens uses potency adjustments to account for greater exposure risk during early life periods. The methods are based on Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens; both incorporated by reference in Section 620.125.

Section 620.Appendix A(d)(2)

Based on updated methods in the Guidelines for Carcinogen Risk Assessment and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, proposed Section (d)(2) of Appendix A updates the equation for calculating HNTAC guidance levels for chemicals designated as carcinogens that are not designated as mutagens.

Section 620.APPENDIX B Procedures for Determining Hazard Indices for Class I: Potable Resource Groundwater for Mixtures of Similar Acting Substances

Section 620.Appendix B(c)

The Agency proposes removing the language specific to mixtures of ortho-dichlorobenzene and para-dichlorobenzene, and 1,1-dichloroethane and 1,1,1- trichloroethane, and adding a reference to Appendix E. Many chemicals have similar-acting health effects. Adding Appendix E assists the user in determining if chemicals detected together in groundwater have similar-acting effects, making them subject to the mixture rule.

Section 620.Appendix B(f)(2)

The Agency proposes replacing the use of the "PQL" with the "LLOQ" or "LCMRL," as appropriate, to the analytical method of the chemical.

Section 620.APPENDIX C Guidelines for Determining When Dose Addition of Similar-Acting Substances in Class I: Potable Resource Groundwaters is Appropriate

The Agency proposes replacing the use of the "PQL" with the "LLOQ" or "LCMRL," as appropriate, to the analytical method of the chemical.

Section 620.APPENDIX E Similar Acting Substances

The Agency proposes adding tables of similar acting non-carcinogenic and carcinogenic constituents for convenient reference.

IV. OUTREACH

A. Community Outreach

On September 14, 2018, the Agency presented to stakeholders proposed amendments to the groundwater quality standard. This proposal included groundwater quality standards for PFOA and PFOS based on the Health Advisory Level of 70 nanograms per liter finalized by the U.S. EPA in May 2016. The Agency held a 21-day comment period.

On December 24, 2019, the Agency again presented to stakeholders updated proposed amendments to the groundwater quality standards. This came after an update in scientific data which resulted in a significant change to the Agency's proposed groundwater quality standards. Specifically, the ATSDR published the Peer Reviewed Toxicological Profile establishing toxicity criteria for PFOA and PFOS using updated studies resulting in lowered dose minimum risk levels and providing toxicity values for PFHxS and PFNA in units of milligrams per kilogram per day. The Agency held a 38- day comment period.

On January 28, 2020, due to stakeholder response, the Agency redistributed to stakeholders the proposed amendments to the groundwater quality standards and extended the comment period for an additional 30 days.[4] On

[4] Comments were received from 3M; the City of Springfield, Office of Public Utilities (CWLP); the Illinois Attorney General's Office, Environmental Bureau; Susan Smith; the Illinois Department of Transportation, Bureau of Design & Environment; PDC Laboratories, Inc.; Eurofins; PDC Technical Service, Inc., Andrews Engineering (on behalf of Republic Services), Millennium Waste Incorporated, and Waste

February 13, 2020, the Agency held a question-and-answer session regarding the proposed amendments. During this session, the Agency outlined the methodology used in developing the PFAS groundwater quality standards. *See* Illinois EPA's Development of Proposed PFAS Groundwater Quality Standards for 35 Ill. Adm. Code Part 620; Hawbaker, Carol Proposed Updates to 35 Ill. Adm. Code 410 and 420, Power Point (February 13, 2020); and 620 Update Tables for Outreach (Attachment 3). In addition, the Agency summarized the proposed amendments and key provisions, and answered questions during the session.

On May 12, 2021, the Agency presented to stakeholders updated proposed amendments to the groundwater quality standard amendments. After a review of potential exposure receptors for calculating noncarcinogen standards, the Illinois EPA utilized child exposure factors for children 0-6 years of age to protect more sensitive populations from the harmful effects of drinking contaminated groundwater. Illinois EPA updated all groundwater quality standards calculated using the HTTAC, including groundwater standards for the newly proposed constituents, for a child population, which resulted in more protective groundwater quality standards. In addition, the Agency utilized the California EPA cancer toxicity value for PFOA. Calculating PFOA as a carcinogen with this value and the proposed updated methodology to account for childhood exposure to carcinogens resulted in more protective groundwater quality standards.

Management - each as members of the Illinois Chapter of the National Waste & Recycling Association; the PFAS Regulatory Coalition; the Illinois Environmental Council; Dynegy Midwest Generation, LLC, Kincaid Generation, LLC, Illinois Power Resources Generating Company, and Electric Energy Inc.; Illinois American Water; the American Chemistry Council; the Illinois Environmental Regulatory Group; the Department of the Army, United States Army Corps of Engineers; and the Illinois Association of Wastewater Agencies.

The Agency accepted and considered all public comments regarding the proposed groundwater quality standards for six weeks, until June 25, 2021.[5] During this time, the Agency held a public meeting on May 26, 2021 to outline the updated methodology used in developing the PFAS groundwater quality standards. *See* Hawbaker, Carol Illinois Environmental Protection Agency Proposed Updates to 35 Ill. Adm. Code 620 Power Point, (May 26, 2021) (Attachment 4). In addition, the Agency summarized the proposed amendments and key provisions, and answered questions. *See* 35 Ill. Adm. Code 620; Groundwater Quality Pre-Filing Public Comment Period Factsheet and Overview of Proposed Changes; and Table – Part 620 Proposed Groundwater Quality Standards Excel Spreadsheet (Attachment 4). Industry, environmental groups, State agencies, and other potentially interested parties were invited.

During both the 2020 and the 2021 public comment periods, a significant number of commenters asked about the process that Illinois EPA used to develop the groundwater quality standards. In response, the Agency presented on and outlined the methodology used in developing the groundwater quality standards at both the question-and-answer session on February 13, 2020, and the public meeting on May 26, 2021. *See* Attachments 3 and 4.

In addition, several commenters asked if Illinois EPA considered the impact of the groundwater standards on cleanup and permit programs (e.g., solid waste landfills). The Agency acknowledges that certain regulations administered by the Bureau of Land

[5] The Agency received comments from the Illinois Environmental Regulatory Group; the PFAS Regulatory Coalition; PDC Technical Services; the Natural Resources Defense Council in collaboration with the Illinois Environmental Council Sierra Club, Illinois Chapter; GSI Environmental, Inc. on behalf of Midwest Generation LLC.; PDC Laboratories, Inc.; Eurofins; The International Molybdenum Association; the National Waste and Recycling Association; the American Chemistry Council; St. John-Mittelhauser & Associates, Inc.; Illinois American Water; and the Illinois Groundwater Advisory Council.

are tied to the Board's groundwater quality standards, and as such the adoption or amendment of any groundwater quality standard could impact land cleanup and permit programs. Separate and apart from this proceeding, the Agency has reached out to commenters and the regulated community involved in the land cleanup and permit programs to begin discussions regarding potential changes to those programs following the completion of this rulemaking. Specifically, on June 25, 2021, the Agency met with members of the Site Remediation Advisory Committee of the Illinois Chamber of Commerce's Illinois Environmental Regulatory Group. This group represents parties that are interested in impacts to the Agency's remedial programs. On August 19, 2021 and November 15, 2021, the Agency met with representatives of the landfill industry.

B. Groundwater Advisory Council and Interagency Coordinating Committee on Groundwater

In addition to engaging with the public and stakeholders during the drafting process of Part 620, the Agency coordinated with both the Groundwater Advisory Council (GAC) and the Interagency Coordinating Committee on Groundwater (ICCG). On September 19, 2021, following three meetings and a request from the ICCG Chairman to provide comments, the GAC provided a Recommendation to the ICCG Chairman and Agency representatives regarding the proposed groundwater quality standards amendments.[6] The Recommendation contained a list of items the GAC believes the Agency has not provided along with a statement indicating that

> …if the Agency continues to move forward with the proposal, it is the GAC's position that the Illinois EPA sufficiently address these questions in the Proposed rulemaking and/or Statement of Reasons to provide the most robust and transparent proposal to the Illinois Pollution Control Board for a more effective and workable standard.

[6] 415 ILCS 55/5 requires the GAC to, among other things, review, evaluate, and make recommendations on State groundwater protection laws, regulations, and procedures.

See GAC Recommendation (Attachment 5).

On October 14, 2021, following a review of the GAC Recommendation, the ICCG provided to both the Agency and GAC a written Response stating the following:

> The ICCG as a whole entity does not have the expertise to answer or comment on the GAC questions/comments on the proposed changes to the 35 Ill. Adm. Code 620 Groundwater Quality standards. These changes to the Groundwater Quality standards are being proposed by the Illinois EPA, who has the expertise and knowledge to address this (GAC) Recommendation. Therefore, it is the Committee's stated opinion that the GAC Recommendation should be addressed by the Illinois EPA in the Statement of Reason or before the Illinois Pollution Control Board. Further, this Response by ICCG does not specifically endorse or disapprove of the proposed rule changes and individual ICCG member reserves the right to provide additional comment, questions, or concerns during the rule making process.

See ICCG Response (Attachment 6). The ICCG concluded that "by providing this written memorandum and the Response within, the ICCG has met its Statutory obligations under the Illinois Groundwater Protection Act." [7] *See* Id.

On November 18, 2021, the Illinois EPA responded in writing to the GAC Recommendation, indicating that while the Agency believes each applicable point of concern raised by the Council has been sufficiently addressed in the Statement of Reasons and Testimony, each point may be further addressed during the proposed rulemaking proceeding before the Board. *See* Agency Reply (Attachment 7).

V. AFFECTED SOURCES AND FACILITES

While Part 620 establishes groundwater quality standards, it is the various permitting and cleanup programs that implement the standards. Therefore, it is those

[7] 415 ILCS 55/4 requires the ICCG to, among other things, review and evaluate State groundwater protection laws, regulations, and procedures, to consider findings and recommendations that are provided by the GAC, and to respond to the GAC findings and recommendation in writing.

sources and facilities subject to the permitting requirements and cleanup programs that will be impacted by the proposed groundwater quality standard amendments.

VI. TECHNICAL FEASIBILITY AND ECONOMIC REASONABLENESS

When promulgating regulations under the Act, Section 27 requires the Board to consider "the technical feasibility and economic reasonableness of measuring or reducing the particular type of pollution." *See* 415 ILCS 5/27(a). In addition, the Board must also determine "whether the proposed rule has any adverse economic impact on the people of the State of Illinois." *See* 415 ILCS 5/27(b). Consistent with prior Board determinations, the groundwater quality standards proposed today are technically feasible and economically reasonable and will not adversely impact the people of the State of Illinois.

A. Prior Evaluations and Determinations

The Board has evaluated the technical feasibility and economic reasonableness of groundwater standards on multiple occasions. Each evaluation concluding the amendments to be technical feasible and economically reasonable.

First, in 1991, with the initial adoption of the groundwater quality standards. Those regulations included numerical standards for 60 constituents. The mandated[8] (*See* 415 ILCS 55/8(d)) economic impact study (EcIS) of those 60 constituents "determined that the most significant costs of the regulations can be expected to be groundwater remediation costs, i.e., those costs associated with returning contaminated groundwater to compliance with standards." *See* R89-14(B) at 22. Following the EcIS determination, the Board noted the importance in the distinction

[8] The Board determined in R08-18, that from a plain reading of the statutory language in 415 ILCS 55/8(d), the mandate for IDNR to conduct an economic impact study on the Agency's proposal only applied to the initial promulgation of Part 620 and not to subsequent amendments. *See* R08-18 at 25.

between groundwater quality standards and cleanup standards or requirements. *See* Id. at 24. As such, the Board pointed out that "site specific considerations would most likely determine the nature of the required remediation and what actual cost is to be borne by any particular entity, industry, or government." *See* Id. at 24-25.

The Board continued in its discussion stating:

> Another factor in consideration of the EcIS' cost estimates is that the instant regulations do not create or require any new corrective action program; all such programs are part of other regulations already in place or proposed (e.g., RCRA, CERCLA, LUST, waterwell setback regulations, etc.). The EcIS investigators recognized that the remedial costs properly associated with the instant rules should be "incremental costs over and above the costs associated with the currently applicable regulations for water quality standards and cleanup criteria."

See Id. at 25, emphasis in original.

The Board stated further:

> The fact that the EcIS investigators attributed to today's groundwater quality standards all the costs of any potential future remedial action is a serious flaw in the EcIS analysis. Cleanup of contaminants to the levels stated in these rules as required by an appropriate agency during remediation does not mean that all the costs of cleanup should be attributed to adoption of today's rules.

See Id.

What is more, the Board indicated the following economic benefits would result from the adoption of groundwater standards: reduction of carcinogenic health risks; reduced expenses for treatment of water at wellheads; and reduced expenses for obtaining water supplies. *See* Id at 23-26.

Next[9], the Board adopted new groundwater standards for Methyl Tertiary-Butyl Ether (MTBE) on January 24, 2002. *See* R01-14. There, the Board recognized the

[9] The Board amended the ground water quality standards in 1994 with the addition of 16 Class I and II standards, however the Agency could not find any discussion regarding technical feasibility and economic reasonableness in the Board Orders. *See* R93-27.

Agency's position that the addition of MTBE as a groundwater quality parameter would not change the Board's economic reasonableness and technical feasibility conclusions from the regulatory proceeding in which the Board's original groundwater regulations became effective – docket R89-14(B). *See* R01-14 at 7. Similarly, just as was concluded in R89-14(B), the Board found any significant economic impacts to be a result of remediation efforts. *See* Id. However, the Board balanced this consideration with the safety of drinking water supplies and the costs of changing water supplies if a water supply became contaminated. The Board stated that it "places a very high value on the safety of drinking water supplies and finds that safety must be paramount in this matter." *See* Id at 10.

Finally, the Board last updated the groundwater standards in its Order dated October 4, 2012. *See* R08-18. There, the Board again recognized the Agency's position that the addition of the proposed numeric standards would not change the Board's economic reasonableness and technical feasibility conclusions from the regulatory proceeding in which the Board's original groundwater regulations became effective – docket R89-14(B). *See* Id. at 26. In addition, the Board reiterated the Agency's statement that the proposed revisions would not create new corrective or monitoring programs, and TACO remediation objectives had already been established for most chemicals at issue. *See* Id. at 27. Further, the Board agreed with the Agency's statement that the revised standards would be phased into existing programs over time and the economic impact of applying the revised standards in other programs would be incremental. *See* Id.

The Board concluded its discussion on technical feasibility and economic

reasonableness adding "that in the IGPA, the General Assembly found 'contamination of Illinois groundwater will . . . adversely impact the economic viability of the State' and 'protection of groundwater is a necessity for future economic development in this State.' 415 ILCS 5/2(a) (2010)." *See* Id. After noting economic benefits that would result from adopting groundwater standards, the Board found the proposed amendments "to be designed for furthering the General Assembly's intent of protecting groundwater not only for the health of Illinois citizens, but also for their economic well-being. *See* Id. Ultimately, the Board reiterated its first notice determination that the proposed amendments would not have an adverse economic impact on the people of Illinois. *See* Id.

B. Current Proposal

Consistent with the Board's numerous prior evaluations and determinations, the proposed groundwater quality standard amendments are technically feasible and economically reasonable. As the Board stated in R89-14(B), there is an important distinction between the Part 620 groundwater quality standards and cleanup standards or requirements, and it is through the cleanup programs implementing the groundwater quality standards where the economic impacts can be expected. *See* R89-14(B) at 22. Maintaining this difference, the proposed amendments simply establish the groundwater quality standards. They do not establish clean-up standards or requirements. In addition, the proposed groundwater quality standards do not require new corrective action or monitoring programs. It is through these existing programs cleanup standards and programs in which the proposed groundwater quality standards will be implemented.

Consistent with the Board's determination after reviewing the IGPA required EcIS and with the numerous prior Board Orders updating the groundwater quality standards, any significant economic impacts will be a result of remediation efforts (*See* R89-14 (B) at 22, R01-14 at 7) and would be incremental (*See* 89-14(B) at 25 and R08-18 at 27). The proposed groundwater quality standards will be phased into existing programs, as appropriate, over time. For example, the adoption of new groundwater quality standards will trigger changes to the cleanup standards used in Bureau of Land cleanup programs. These changes will be implemented through amendments to the cleanup standards set forth in the Board's TACO rules. The economic impact resulting from those amendments will be addressed in the appropriate rulemakings as they occur over time.

As the Board stated in R08-18, there is no information suggesting that the proposed amendments would impose an economic or technical burden significantly different from that resulting from prior Part 620 rulemakings. *See* R08-18 at 27. Therefore, as previously determined by the Board, the groundwater quality standards proposed today are technically feasible and economically reasonable and will not adversely impact on the people of the State of Illinois.

VII. AGENCY WITNESSES AND TESTIMONY

The Illinois EPA will present two witnesses during the Board's hearing on this proposal, Ms. Carol Hawbaker and Mr. Lynn Dunaway. Pre-filed written testimony for each witness is attached hereafter. *See* Attachments 1 and 2.

VIII. SUPPORTING DOCUMENTS

A. Relied Upon Documents

The Illinois Administrative Procedure Act provides that all proposed rulemakings must include:

> a descriptive title or other description of any published study or research report used in developing the rule, the identity of the person who performed such study, and a description of where the public may obtain a copy of any such study or research report. If the study was performed by an agency or by a person or entity that contracted with the agency for the performance of the study, the agency shall also make copies of the underlying data available to members of the public upon request if the data are not protected from disclosure under the Freedom of Information Act.

See 5 ILCS I00/5-40(b)(3.5). The Board's procedural rules require the same information to be included with any rulemaking proposal filed with the Board. *See* 35 Ill. Adm. Code 102.202(e). The following list constitutes the studies and reports that the Illinois EPA used in developing the proposed amendments:

Title 35: Environmental Protection, Subtitle G: Waste Disposal, Chapter I: Pollution Control Board, Subchapter f: Risk Based Cleanup Objectives, Part 742 Tiered Approach to Corrective Action Objectives, effective July 15, 2013.

In the Matter of: Proposed Amendments to Groundwater Quality Standards, R08-18 Prefiled Testimony of Richard P. Cobb and Thomas Hornshaw, Illinois EPA, File Date May 29, 2008.

In the Matter of: Groundwater Quality Standards, 35 Ill. Adm. Code 620, R89-14(B), Final Order, Illinois Pollution Control Board, November 7, 1991.

Office of Solid Waste and Emergency Response (OSWER) Directive 9200.1-120, Subject: Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors U.S. EPA, February 6, 2014.

Regional Screening Levels (RSLs), available at https://www.epa.gov/risk/regional-screening-levels-rsls , U.S. EPA, last accessed October 15, 2021.

Office of Solid Waste and Emergency Response (OSWER) Directive 9285.7-53, U.S. EPA, December 5, 2003.

Office of Solid Waste and Emergency Response (OSWER) Directive 9285.7-86: Tier 3 Toxicity Value White Paper, U.S. EPA, May 16, 2013.

Office of Land and Emergency Management (OLEM) Memorandum: Recommendations on the Use of Chronic or Subchronic Noncancer Values for Superfund Human Health Risk Assessments, U.S. EPA, May 26, 2021.

In the Matter of Proposed MTBE and Compliance Determination to Groundwater Quality Standards: 35 Ill. Adm. Code 620. Final Order and Opinion, R01-14, Rulemaking – Public Water Supply. Illinois Pollution Control Board. January 24, 2002.

Integrated Risk Information System (IRIS) Chemical Assessment Summary Vanadium pentoxide; CASRN 1314-62-1, U.S. EPA, June 30, 1988.

Provisional Peer-Reviewed Toxicity Values for Vanadium and Its Soluble Inorganics Compounds Other Than Vanadium Pentoxide (CASRN 7440-62-62 and Others), Derivations of Subchronic and Chronic Oral RfDs, EPA/690/R-09/070F, U.S. EPA, September 30, 2009.

Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA822-R-16-003, U.S. EPA Office of Water, May 2016.

Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). EPA822-R-16-002, U.S. EPA Office of Water, May 2016.

Toxicological Profile for Perfluoroalkyls, U.S. Department of Health and Human services, Agency for Toxic Substances and Disease Registry, May 2021

IARC Monographs on the Identification of Carcinogenic Hazard to Humans, International Agency for Research on Cancer, available at: https://monographs.iarc.who.int/agents-classified-by-the-iarc/ , last accessed October 15, 2021.

Notification Level Recommendations Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Drinking Water, California EPA Office of Environmental Health Hazard Assessment, August 2019.

Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). U.S. EPA, Office of Water. October 2021.

A Review of the Reference Dose and Reference Concentration Processes EPA/630/P-02/002F, U.S. EPA, December 2002.

Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation, EPA/R-14/002F, U.S. EPA, September 2014.

Guidelines for Carcinogen Risk Assessment, EPA/630/P-03/001F, U.S. EPA, March 2005.

Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, EPA/630/R-03/003F, U.S. EPA, March 2005.

Non-Community Public Water Systems, Illinois Department of Public Health, available at: http://dph.illinois.gov/topics-services/environmental-health-protection/non-community-public-water-systems , last accessed October 15, 2021.

Atrazine and Its Metabolites in Drinking-water, Background document for development of WHO Guidelines for Drinking-water Quality, World Health Organization, WHO/HSE/WSH/10.01/11, 2010.

Herbicides and Their Transformation Products in Source-Water Aquifer Tapped by Public-Supply Wells in Illinois, 2001-02, Water-Resources Investigations Report 03-4226, U.S. Geological Survey, in cooperation with the Illinois EPA, 2004.

Provisional Peer-Reviewed Toxicity Values (PPRTV) Assessments, U.S. EPA, available at: https://www.epa.gov/pprtv/provisional-peer-reviewed-toxicity-values-pprtvs-assessments , last accessed October 15, 2021.

Water Quality Criteria, National Academy of Sciences, 1972.

Integrated Risk Information System (IRIS), U.S. EPA, available at: https://www.epa.gov/iris , last accessed October 15, 2021.

California Office of Environmental Health Hazard Assessment (CalEPA), California Environmental Protection Agency, available at: https://oehha.ca.gov/chemicals , last accessed October 15, 2021.

Agency for Toxic Substance and Disease Registry (ATSDR), U.S. Department of Health and Human Services, available at: https://www.atsdr.cdc.gov/toxprofiledocs/index.html, last accessed October 15, 2021.

Illinois Integrated Water Quality Report and Section 303(d) List, 2018, Illinois EPA, February 2021.

Curriculum Vitae of Lynn Dunaway

List of Dedicated Nature Preserves for which Class III Groundwater has been final listed in the Environmental Register

Map of the Class III Groundwater areas for Spring Grove Fen and Cotton Creek Marsh Dedicated Nature Preserves

Map of the Class III Groundwater areas for Pautler Cave, Stemler Cave, Armin Kreuger Speleological and Fogelpole Cave Dedicated Nature Preserves

Impact of Urban Development on the Chemical Composition of Ground Water in a Fen - Wetland Complex (Panno et al 1999)

Spatial and Temporal Analyses of the Bacterial Fauna and Water, Sediment and Amphipod Tissue Chemistry Within the Range of Gammarus Acherondytes (Taylor, Webb and Panno, 2000)

Subspecies, Morphs and Clines in the Amphipod Gammarus Duebeni from Fresh and Saline Waters (Sutcliffe 2000)

Status Survey for a Cave Amphipod, Gammarus Acherondytes, in Southern Illinois (Webb 1993)

United States Geological Survey, Water Resources Investigations Report 91-4011 Effects of Septic Tank Effluent on Ground Water Quality in Northern Williamson County and Southern Davidson County, Tennessee (USGS 1991)

Washington State Department of Health, Rule Development Committee Issue Report Draft, Septic Tank Effluent Values, Wastewater Management Program (2004)

B. Incorporations by Reference

Section 102.202(d) of the Board's procedural rules requires the Agency to submit "[a]ny material to be incorporated by reference within the proposed rule pursuant to section 5-75 of the IAPA [5 ILCS 100/5-75)." The Agency incorporates by the reference the following documents and each is attached hereafter:

"Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)" ASTM D7979-20.

"Illinois Integrated Water Quality Report and Section 303(d) List, 2018," Agency, February 2021. https://www2.illinois.gov/epa/topics/water-quality/watershed-management/tmdls/Pages/303d-list.aspx

"Water Quality Criteria 1972", EPA.R3.73-033, 1973. https://nepis.epa.gov

"Selected Analytical Methods for Environmental Remediation and Recovery (SAM), 2017. Record last revision date February 10, 2020. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=339252

"Method 533: Determination of Per-and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry," November 2019. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf).

Shoemaker, J. and Dan Tettenhorst. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, l Center for Environmental Assessment, Washington, DC. Version 2.0, March 2020.

"A Review of the Reference Dose and References Concentration Process", EPA/630/P-02/002F, December 2002".

"Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation", EPA/R-14/002F, September 2014.

"Guidelines for Carcinogen Risk Assessment", EPA/630/P-03/001F, March 2005".

"Supplemental Guidance for Assessing Susceptibility for Early-Life Exposure to Carcinogens", EPA/630/R-03/003F, March 2005.

IX. CONCLUSION

WHEREFORE, for the reasons stated above, the Illinois EPA asks the Board to accept this Statement of Reasons and proceed to hearings on the above-captioned rulemaking proposal.

Respectfully submitted,

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

By: /s/ Sara Terranova
Sara Terranova
Assistant Counsel
Division of Legal Counsel

ATTACHMENT 1

TESTIMONY OF CAROL L. HAWBAKER ON PROPOSED

UPDATED GROUNDWATER QUALITY STANDARDS

My name is Carol L. Hawbaker. I am the Lead Worker in the Office of Toxicity Assessment ("OTA") of the Illinois Environmental Protection Agency's ("Illinois EPA") Associate Director's Office ("ADO"). I have been employed at Illinois EPA since September of 2000, first as a Project Manager in Bureau of Land's Leaking Underground Storage Program; then, as an Environmental Risk Assessor in OTA. As an Environmental Protection Specialist IV, or Lead Worker, for OTA, my primary responsibilities include the development and use of procedures for toxicity and environmental risk assessments, review of toxicity and risk data in support of Illinois EPA programs, review of human health and ecological risk assessment for projects enrolled in state and federal programs, and review of exposure, risk assessment, and fate and transport models.

I am a member of U.S. Environmental Protection Agency's ("U.S. EPA") Environmental Council of the States and Association of State and Territorial Health Officials PFAS Science Group and a participant in State Risk Assessors Teleconference Group. I also participate in workgroups within the Illinois EPA focusing on updates to cleanup objectives and procedures utilized in developing cleanup objectives, including taking a lead role in drafting updates to 35 Ill. Adm. Code 742.

I received a Bachelor of Science in History from Illinois State University in 1995 and subsequently received 48 hours of educational credit towards a Master in Science degree in

Environmental Studies with a focus on environmental risk assessment and toxicology; after which I received certification in Environmental Risk Assessment.

Testimonial Statement

Background and Basis

My testimony will focus on the following topics:

- The addition of new chemical constituents proposed to be added to Part 620;
- Updates to Part 620.310(a)(3)(A);
- Updates to Part 620.410 Class I: Groundwater Quality Standards for Potable Resource Groundwater ("standards");
- Updates to Part 620.420 Class II: Groundwater Quality Standards for General Resource Groundwater ("standards");
- Updates to methodologies used to calculate when a Maximum Contaminant Level ("MCL") or Maximum Contaminant Level Goal ("MCLG") is not available for a constituent, located at Part 620, Subpart F and Appendix A; and
- Updates to Appendix B for similar-acting substances, including a new Appendix E, providing tables listing similar-acting constituents and affected target organs or health effects.

For clarity, I will begin with updates to Subpart F and Appendix A. These updates form the basis for updates to the Class I standards at Part 620.410 for several constituents.

I. Updates to Subpart F: Health Advisories and Appendix A

Part 620, Subpart F and Appendix A, provide the basis for developing Illinois Pollution Control Board ("Board") rulemaking proposals for new or revised numerical standards (35 Ill.

Adm. Code 620.601(c)). The Board adopted Subpart F: Health Advisories and Appendix A procedures for determining a Human Threshold Toxicant Advisory Concentration ("HTTAC") for developing noncancer health-based standards for Class I: Potable Resource Groundwater on November 7, 1991. The Board adopted Subpart F: Health Advisories procedures for determining a Human Nonthreshold Toxicant Advisory Concentration ("HNTAC") for developing cancer risk-based standards for Class I: Potable Resource Groundwater on October 4, 2012. Since those times, Illinois EPA developed Class I standards using this procedure for contaminants found in groundwater that do not have MCLs or MCLGs. Of the 115 constituents currently listed in Part 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater, 39 constituents currently have Class I standards based on the procedures specified in Subpart F and Appendix A.

Subpart F: Health Advisories

Part 620.601(b): Updates the applicable regulation for siting and source water requirements.

Part 620.605(b)(1): Updates the language to allow for a Class I standard to be set at the lower value calculated from either the HTTAC noncancer equation or the HNTAC cancer equation. By choosing the lower of the two concentrations, a person ingesting groundwater is protected from both cancer effects and other noncancer adverse health effects. The method of choosing the lower of the concentrations is consistent with the methods prescribed in 35 Ill. Adm. Code 742: Tiered Approach to Corrective Action Objectives ("TACO"). Thomas C. Hornshaw, in prefiled testimony dated May 29, 2008 for the Board's rulemaking docket PCB R08-18 (Attachment 1A), discussed Illinois EPA's desire to make Part 620 consistent with TACO, as the standards are used in TACO as groundwater remediation objectives for the groundwater ingestion exposure route.

TACO selects the more stringent remediation objective calculated by carcinogen and noncarcinogen methods. In addition, the term practical quantitation limit ("PQL") is replaced by the terms lower limit of quantitation ("LLOQ"), as specified in EPA Publication SW-846, and lowest concentration minimum reporting level ("LCMRL"), specified in drinking water methods incorporated by reference at Section 620.125. Analytical methods have updated, and the terminology to describe levels of quantitation in analyses have also updated. Definitions for LLOQ and LCMRL are added to the definitions at Section 620.110. Procedures for quantifying these limits are available in the analytical method procedures.

As the standards calculated using the methods at Part 620, Subpart F and Appendix A are based on the protection of human health from ingesting groundwater, and MCLs are promulgated for drinking water, drinking water methods are appropriate for analyzing Class I potable resource groundwater. When discussing Class I potable resource groundwater, the Board stated the following in its Final Opinion and Order for R89-14(B): In the Matter of Groundwater Quality Standards (35 Ill. Adm. Code 620), dated November 7, 1991:

> "The Board believes that among the most necessary facets of the State's groundwater protection program is the need to protect all drinkable water at a drinkable level. Similarly, the Board does not believe that current actual use should be the sole control of whether potable groundwater is afforded the protection necessary to maintain potability; we simply cannot allow the sullying of a resource that future generations may need. For the same reason the term "Potable Resource Groundwater", rather than "Potable Use Groundwater", is employed in the title of this class."

Part 620.605(b)(2): Deletes the language methodology and equation for calculating HNTACs for carcinogens. This information is updated and moved to Appendix A.

Part 620.Appendix A: Procedures for Determining Human Toxicant Advisory Concentrations for

Class I: Potable Resource Groundwater

The proposal updates the title of Appendix A by removing the term "Threshold" and making "Concentration" plural due to the addition of the HNTAC procedures to Appendix A.

Appendix A(a):

- Specifies the HTTAC calculation is for noncancer effects. The HTTAC equation calculates a drinking water level at which no adverse effects are expected to occur.
- Adjusts the per capita daily water consumption from an average adult rate of 2 liters per day to an average child rate of 0.78 liters per day for a child aged 0 – 6 years. To be protective of more sensitive receptors, such as young children, from adverse health effects from ingesting groundwater, Illinois EPA proposes to utilize child exposure factors when calculating threshold toxicant advisory concentrations for noncancer effects. The proposed use of child exposure factors is consistent with TACO's use of child exposure factors for evaluating the ingestion exposure route for noncarcinogens. In addition to updating the per capita daily water consumption rate, body weight found at Appendix A(b)(2) is updated from an average adult body weight of 70 kilograms to a body weight of 15 kilograms, equivalent of a child aged 0 – 6 years. The source for the child exposure factor values is U.S. EPA Office of Solid Waste and Emergency Response ("OSWER") Directive 9200.1-120, "Subject: Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors," dated February 6, 2014 and included as Attachment 1B. The child exposure factors are also listed at U.S. EPA's Regional Screening Levels for Chemical Contaminants at Superfund Sites ("RSL") User's Guide, available at:

https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide. The RSL databases and equations utilize the most up-to-date methodologies, exposure assumptions, chemical-physical properties, and toxicity values within OSWER's toxicity hierarchy. Illinois EPA is in the process of drafting updates to TACO to align it with RSL methodology and procedures. Adjusting the exposure factors from an adult to a young child protects both children and adults from harmful effects of exposure via ingestion of chemicals in drinking water.

Appendix A(b)(1):

- Adjusts the language to refer to the Acceptable Daily Exposure ("ADE") as representing the maximum amount of the threshold toxicant, if ingested daily by a child aged 0 – 6 years, will result in no adverse effects.

Appendix A(b)(2):

- Adjusts the language to allow for the selection of toxicity values based on various OSWER Directives issued over the years discussing hierarchies and procedures for selecting human health toxicity values in Superfund risk assessments. Thomas C. Hornshaw's prefiled testimony in Attachment 1A, discusses the first revision of the hierarchy of human health toxicity values recommended for use in risk assessments, issued on December 5, 2003 (OWSER Directive 9285.7-53), included in Attachment 1C. OSWER Directive 9285.7-53 recommends the following revised toxicity value hierarchy as follows:

  Tier 1: U.S. EPA Integrated Risk Information System ("IRIS");

  Tier 2: U.S. EPA Provisional Peer-Reviewed Toxicity Values ("PPRTV");

Tier 3: Other Toxicity Values.

The Directive goes on to state:

> "The third tier includes other sources of information. Priority should be given to sources that provide toxicity information based on similar methods and procedures as those used for Tier I and Tier II, contain values which are peer reviewed, are available to the public, and are transparent about the methods and processes used to develop the values."

The Directive further states:

> "Additional sources may be identified for Tier 3. Toxicity values that fall within the third tier in the hierarchy include, but need not be limited to, the following sources:
>
> - The California Environmental Protection Agency ("Cal EPA") toxicity values are peer reviewed and address both cancer and non-cancer effects.
>
> - The Agency for Toxic Substances and Disease Registry ("ATSDR") Minimal Risk Levels ("MRLs") are estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. The ATSDR MRLs are peer reviewed and are available at http://www.atsdr.cdc.gov/mrls.html on the ATSDR website.
>
> - Health Effects Assessment Summary Tables ("HEAST") toxicity values are Tier 3 values. It should be noted that EPA's HEAST has not been updated since 1997."

On May 16, 2013, U.S. EPA's OSWER Human Health Regional Risk Assessors Forum issued, "Tier 3 Toxicity Value White Paper" ("Paper"). The Paper discusses a tentative ranking of Tier 3 toxicity sources implemented by the U.S. EPA Regional Screening Level ("RSL") workgroup, included in Attachment 1C. The Paper lists the Tier 3 ranking for sources as follows:

1. United States Health and Human Services Agency for Toxic Substances and Disease Registry ("ATSDR") dose minimal risk levels ("dose MRL");
2. California EPA, Office of Environmental Health Hazard Assessment ("OEHHA") toxicity values;
3. PPRTV "Appendix" values;
4. Health Effects Assessment Summary Table ("HEAST").

On May 26, 2021, U.S. EPA Office of Land and Emergency Management ("OLEM") issued a Memorandum with the Subject, "Recommendations of the Use of Chronic or Subchronic Noncancer Values for Superfund Human Health Risk Assessments." The Memorandum, included in Attachment 1C, discusses the use of subchronic or intermediate toxicity values in place of chronic values when the subchronic values are based on updated methodologies and/or more recent studies. When subchronic toxicity values are calculated using a human equivalency dose based on a time-weighted average serum concentration, an uncertainty factor of one may be used to extrapolate a chronic dose.

The toxicity value hierarchy discussed above is used by U.S. EPA for human health risk assessments and represents the method of selection by RSL for RSL's toxicity values. In the matter of the proposed amendments to groundwater quality standards (R08-18), the Board's Final Opinion and Order of the Board, dated October 4, 2012, states the following regarding the use of U.S. EPA's toxicity hierarchy to determine a verified oral reference dose:

> "The Board found that the Agency appropriately relied upon the revised toxicity hierarchy of the United States Environmental Protection Agency (USEPA) to account for new scientific data in proposing updates to the Board's groundwater quality standards. *See* R08-18 GQS, slip op. at 9-11. Next, the Board found that the addition to Part 620 of 39 chemical constituents detected in Illinois groundwater that have established toxicity values or groundwater remediation objectives in TACO is consistent with the IGPA (415 ILCS 55 (2010)) and the Act. *Id.* at 11-12."

OTA sets its toxicity values for TACO, and Part 620, to be consistent with the toxicity values listed at RSL, with a few exceptions:

- Cancer toxicity values are not utilized for chemicals that do not meet the definition of a "carcinogen" as stated in Section 620.110 and Section 742.200.
- RSL does not list an oral reference dose for Methyl-tertiary-butyl-ether ("MTBE"). Illinois EPA developed an oral reference dose for MTBE using the processes in Part 620 Appendix A for constituents that do not have a verified oral reference dose. On January 24, 2002, the Board approved Illinois EPA's oral reference dose and adopted a Class I standard for MTBE at the Board's Final Opinion and Order in the Matter of Proposed MTBE and Compliance Determination to Groundwater Quality Standards: 35 Ill. Adm. Code 620 (Illinois Pollution Control Board: R01-14, Rulemaking – Public Water Supply).
- For vanadium (Chemical Abstract Services Registry Number ("CASRN") 7440-62-2), RSL utilizes a toxicity value derived from vanadium pentoxide

(CASRN 1314-62-1), developed by IRIS and issued June 30, 1988. RSL user's guide provides the following statement regarding the basis of the vanadium's oral reference dose ("RfD"):

> "The oral RfD toxicity value for Vanadium, used in this website, is derived from the IRIS oral RfD for Vanadium Pentoxide by factoring out the molecular weight (MW) of the oxide ion. Vanadium Pentoxide (V_2O_5) has a molecular weight of 181.88. The two atoms of Vanadium contribute 56% of the MW. Vanadium Pentoxide's oral RfD of 9E-03 mg/kg-day multiplied by 56% gives a Vanadium oral RfD of 5.04E-03 mg/kg-day."

Illinois EPA utilizes a PPRTV oral RfD toxicity value of 7E-05 mg/kg-day for vanadium (CASRN 7440-62-2), issued on September 30, 2009, included in Attachment 1D.

- Although not listed on the RSL tables, RSL uses toxicity values for perfluorooctanoic acid ("PFOA") and perfluorooctanesulfonic acid ("PFOS"), developed by U.S. EPA Office of Water for U.S. EPA's May 2016 PFOA and PFOS Drinking Water Health Advisories, in its screening level calculator. The 2016 Office of Water Health Effect Support Documents for PFOA and PFOS are included in Attachment 1D.

PFOA and PFOS are constituents proposed to be added to Part 620. The Illinois EPA is proposing Class I standards for PFOS developed using the intermediate dose-MRL of 2E-06 milligrams per kilogram per day (mg/kg-day), calculated by ATSDR and discussed in, "Toxicological Profile for Perfluoroalkyls," released May 2021 and included in Attachment 1D. Illinois EPA

selected the ATSDR value due to the addition of a modifying factor (10) to account for the adverse effect of decreased immune response, and ATSDR's position within the toxicity hierarchy.

PFOA meets Section 620.110's definition of a carcinogen. In 2017, International Agency for Research on Cancer ("IARC") classified PFOA as Group 2B: possibly carcinogenic to humans, meeting the definition of a carcinogen in Section 620.110. Therefore, the Illinois EPA calculated both noncancer and cancer standards to determine the most stringent standard. For noncancer effects, Illinois EPA selected the ATSDR intermediate dose MRL of 3E-06 mg/kg-day for calculating an HTTAC, due to ATSDR's use of updated studies when compared to studies used by U.S. EPA Office of Water to develop an oral RfD for its 2016 PFOA Drinking Water Health Advisory. To calculate a standard based on cancer risks, Illinois EPA utilized California EPA's peer-reviewed oral slope factor ("SF_o") of 143 $(mg/kg\text{-}day)^{-1}$, as its cancer toxicity value to calculate an HNTAC based on a one in one million cancer risk. California EPA's toxicity profile is also included in Attachment 1D. The Illinois EPA selected the California EPA value because of its use of more recent studies than relied upon for determining cancer risks for the U.S. EPA 2016 Drinking Water Health Advisory, and the source of the value is within the toxicity hierarchy recommended by the U.S EPA.

Hexafluoropropylene oxide dimer acid ("HFPO-DA"), perfluorohexanesulfonic acid ("PFHxS"), and perfluorononanoic acid ("PFNA") are not included in the RSL database. Illinois EPA selected the toxicity value for

HFPO-DA from U.S. EPA's Office of Water toxicological profile, titled, "Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3)", finalized on October 25, 2021 and included in Attachment 1D. Illinois EPA selected toxicity values for PFHxS and PFNA from ATSDR's PFAS toxicological profile referenced above.

Appendix A(b)(3)-(6):

Appendix A(b)(3)-(6) are updated to reflect updated procedures used to develop noncancer toxicity values, primarily Benchmark Dose ("BMD") modeling and the development of a Human Equivalent Dose ("HED") using pharmacokinetic ("PBPK") modeling. The proposed updates are based on recommended procedures discussed in U.S. EPA's, "A Review of the Reference Dose and Reference Concentration Processes," dated 2002, and "Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and intraspecies Extrapolation," dated 2014, and are included as Attachment 1E.

Appendix A(d):

Section "d" is added to Appendix A to add the procedures for calculating an HNTAC for carcinogens. These procedures are removed from Part 620.605(b) and placed in Appendix A. In addition, the Illinois EPA proposes updating the procedures for calculating an HNTAC to be consistent with updates to U.S. EPA methods for calculating health-based carcinogen screening levels for Superfund sites. Illinois EPA bases the current methodology for calculating an HNTAC on the groundwater ingestion remediation objective equation for carcinogens found in TACO. The equation currently listed in both TACO and Part 620 has been updated by U.S. EPA to account for

exposure during both childhood and adulthood. The current method in Part 620 accounts only for exposure as an adult and uses outdated exposure factors such as the drinking water ingestion rate, body weight, and exposure duration; all updated by U.S. EPA in 2014. In addition, U.S. EPA developed procedures for calculating risk-based values for carcinogens acting through a mutagenic mode of action. The updated carcinogen procedures are based on "Guidelines for Carcinogenic Risk Assessment," and "Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens," both dated March 2005, by U.S. EPA Risk Assessment Forum, included in Attachment 1F. The proposed updated HNTAC equations and the supporting equations to calculate the age-adjusted resident tap water ingestion rate for carcinogens are provided in Attachment 1G and available on-line at: https://www.epa.gov/risk/regional-screening-levels-rsls-equations. Illinois EPA is drafting updates to its TACO regulations so that they are consistent with the methods and equations described in U.S. EPA's RSL User's Guide.

Appendix A(d)(1):

Introduces an equation used by U.S. EPA for calculating ingestion screening levels for residential tap water, for exposure to a carcinogen acting through a mutagenic mode of action. A mutagenic mode of action is expected to cause irreversible changes to DNA; and, would exhibit a greater carcinogenic effect for exposures occurring in early-life stages than with later life exposures. To account for increased risks from early-life exposure to mutagens, potency adjustments are applied to drinking water exposure factors for children less than 16 years of age. To further explain: a potency factor of 10 is applied to drinking water exposure factors for exposure to a mutagen during ages 0 – 2 years, a potency factor of 3 is applied to drinking water exposure factors for exposure to a mutagen during ages 2 – 6 years and ages 6 – 16 years, and a potency

factor of 1 is applied to drinking water exposure factors for exposure to a mutagen for an adult. U.S. EPA lists the following constituents in Part 620 as mutagens in the RSL User's Guide at: https://www.epa.gov/risk/regional-screening-levels-rsls-users-guide#mutagens

CASRN	Constituent
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
50-32-8	Benzo(a)pyrene
218-01-9	Chrysene
53-70-3	Dibenzo(a,h)anthracene
75-09-2	Dichloromethane (methylene chloride)
193-39-5	Indeno(1,2,3-c,d)pyrene
79-01-6	Trichloroethylene
75-01-4	Vinyl Chloride

As benzo(a)pyrene, dichloromethane, trichloroethylene, and vinyl chloride have established MCLs, standards for these constituents are not calculated using the methods at Appendix A(d)(1). The Class I standards for these 4 mutagens are based on MCLs.

Appendix A(d)(2):

Updates the HNTAC equation to be consistent with updates by U.S. EPA. This includes updates to exposure factors such as body weight, exposure duration, and daily water ingestion rates, specified by OSWER Directive 9200.1-120 (Attachment 1B). The update also includes the above-referenced methodology for calculating an age-adjusted drinking water ingestion rate to account for exposures during both childhood and as an adult.

The proposed updates to Subpart F and Appendix A, result in updated standards for the 39 constituents presently listed in Part 620 that rely on the methods in these sections for developing Class I standards. For those constituents that have calculated standards less than each's applicable LLOQ or LCMRL, the LLOQ or LCMRL is the Class I standard, a table comparing health-based

standards with the applicable LLOQ or LCMRL is included as part of Attachment 1I as supporting documentation for the proposed Class I potable resource standards.

II. Introduction of New Constituents

Illinois EPA proposes the addition and establishment of standards for 10 constituents to Part 620. All have been detected in groundwater in Illinois. 6 of the proposed constituents are per- and polyfluoroalkyls ("PFAS"). Illinois EPA documented detections of proposed per- and polyfluoroalkyls perfluorobutanesulfonic acid ("PFBS"), PFHxS, PFOS, and PFOA in finished water of public water supplies across Illinois as a result of Illinois EPA's statewide investigation into the prevalence and occurrence of PFAS in finished drinking water. With the investigation over 99 percent complete, PFAS have been detected in the finished water of public water supplies that provide drinking water for over 2 million consumers (16.1 percent of the population in Illinois, based on the 2020 census), with over 910,000 of those consumers receiving drinking water from community water supply wells (7.2 percent of the population in Illinois). In addition, approximately 500,000 people are served by non-community public water systems per Illinois Department of Public Health website at: http://dph.illinois.gov/topics-services/environmental-health-protection/non-community-public-water-systems. Non-community public water systems included facilities such as schools, daycares, factories, restaurants, resorts, churches, were not included as part of Illinois EPA's statewide investigation. Further, thousands more utilize groundwater from private potable wells, usually without access to treatment technologies. The above-referenced PFAS were also found in community water supply wells, prompting the issuance of Statewide Health Advisories for the constituents. PFAS does not have any taste, color, or odor

in drinking water. The only way to confirm the presence of PFAS is through proper sampling and analysis.

The Illinois EPA also proposes the addition of another PFAS: HFPO-DA. HFPO-DA is also referred to as "GenX", a trade name for a processing technology that produces fluoropolymers without PFOA; and is a replacement product for PFOA. HFPO-DA is detected in groundwater during sampling for purposes other than the statewide PFAS sampling initiative. In October 2021, U.S. EPA Office of Water issued its final human health toxicity assessment for HFPO-DA. Information regarding U.S. EPA Office of Water's finalization of its human health toxicity assessment is available at: https://www.epa.gov/chemical-research/human-health-toxicity-assessments-genx-chemicals. U.S. EPA Office of Water qualifies as an additional Tier 3 toxicity value source, although it is not specifically mentioned in Tier 3 hierarchy referenced in the 2013 Paper.

PFAS are human-made chemicals often referred to as "forever chemicals", due to the strength of carbon – fluorine bonds. The strength of this bond does not allow for PFAS to break down easily under natural conditions. PFAS are mobile in soil and groundwater and have been shown to bioaccumulate, or build up, in blood and organs over time. Although PFOA and PFOS are no longer manufactured in the United States, they have been replaced with other PFAS alternatives such as PFBS. PFOA and PFOS are still used in manufacturing in other countries, so consumer goods imported to the United States may contain PFOA or PFOS.

PFAS are used in many industrial and consumer processes to make everyday items non-stick, or water-, oil-, or stain-resistant. Examples of items containing PFAS are:

- Food packaging - fast food containers, lunch meat paper, disposable plates, and bowls, and oil-, water- and grease-resistant coatings on food packaging
- Commercial household products - non-stick coated cookware (Teflon), cleaning products, waxes, polishes, and adhesives
- Clothing and fabric textiles - stain- and water-resistant carpeting and upholstery, water repellant clothing, tents, umbrellas, shoes, and leather goods
- Cosmetics and personal care products - shampoos, conditioners, sunscreens, cosmetics, and dental floss
- Building and exterior use products - paints and sealants
- Industrial use - metal plating and finishing, wire coatings, automotive fluids, and the manufacture of artificial turf
- Firefighting foam - aqueous film-forming foam ("AFFF")

PFAS has been measured in indoor air, outdoor air, dust, food, water, and various consumer products. Occupational exposure to PFAS for some individuals, such as those working in PFAS manufacturing facilities, installing or treating carpets, or firefighters using PFAS containing firefighting foams, may be higher than exposure to the general public. Potential routes of PFAS exposure include ingestion, dermal, and inhalation.

The general population is primarily exposed to PFAS through the ingestion of contaminated food, water, dust, and hand-to-mouth contact with PFAS treated products, such as carpets and textiles or cosmetics and lotions containing PFAS. Infants and toddlers have higher exposure to PFAS due to having a single source of nutrition (breast-feeding or formula-feeding), by hand-to-mouth transfer due to being in contact with treated carpets and furniture; and have

greater indoor dust ingestion. Once swallowed, PFAS can enter the bloodstream due to its ability to bind to blood proteins such as albumin, and typically distributes to the liver, kidneys, and blood. Most humans have detections of PFAS in the blood, primarily PFOA and PFOS; but the levels of these PFAS found in human blood are decreasing as PFOA and PFOS is phased out of production. PFAS is also found in umbilical cord blood and breastmilk from highly exposed humans.

Food grown in water or soil contaminated with PFAS, and food packaging treated with PFAS can cause PFAS to transfer to food, directly exposing people to PFAS upon consumption. PFAS biomagnifies through trophic levels, meaning that its concentration increases as it moves upward through the food chain. This becomes a concern for humans because of the variety of foods that we eat including vegetables, fish, livestock, and wildlife.

In addition to the proposed PFAS, Illinois EPA proposes to add three inorganic constituents (aluminum, lithium, and molybdenum), and 1 polycyclic aromatic hydrocarbon (1-methylnapthalene).

Illinois EPA also proposes to include atrazine metabolites desethyl-atrazine ("DEA"), desisopropyl-atrazine ("DIA"), and diaminochlorotriazine ("DACT") as part of the total atrazine standard. The proposed addition of the metabolites is based on recommendations in World Health Organization's ("WHO") document titled, "Atrazine and Its Metabolites in Drinking-Water", published in 2010, and included in Attachment 1H. Toxicity profiles indicating the metabolites had similar properties and modes of action as atrazine formed WHO's basis for the addition of the metabolites. Further, United States Geological Survey ("USGS") in cooperation with Illinois EPA, published, "Herbicides and Their Transformation Products in Source-Water Aquifers Tapped by

Public-Supply Wells in Illinois, 2001-02", to demonstrate the presence of atrazine metabolites in Illinois groundwater. The USGS document is also included in Attachment 1H.

III. Updates to Part 620.310 Preventive Response Activities

The tables located at Section 620.310 are updated to include CASRNs. CASRNs are numerical identifiers unique to each constituent. As constituents can have several identifying names, the use of a CASRN assists in verifying a specific constituent of concern.

Illinois EPA proposes the removal of para-dichlorobenzene and ethylbenzene from the table at Section 620.310(a)(3)(A)(i), as the constituents are now classified as carcinogens. Therefore, the numerical criteria for these constituents at Section 620.310(a)(3)(A)(i) are no longer applicable.

Illinois EPA proposes the addition of the following constituents to the table at Section 620.310(a)(3)(A)(ii), based on proposed additions to Part 620:

CASRN	Constituent
7429-90-5	Aluminum
7439-98-7	Molybdenum
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
90-12-0	1-Methylnaphthalene
375-73-5	PFBS (perfluorobutanesulfonic acid)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-95-1	PFNA (perfluorononanoic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)

Lithium and PFOA are not included in the table because the standards are based on the LLOQ or LCMRL. The terms represent the lowest level an analyte may be quantified by analysis. Standards set at these levels are not included, based on testimony by Richard P. Cobb, P. G., in prefiled testimony dated May 29, 2008 for the Board's rulemaking docket PCB R08-18

(Attachment 1A), where Mr. Cobb discussed the basis for the preventive response activities. Mr. Cobb's testimony stated, in part:

> The Board's final opinion and order on groundwater quality standards indicates that the numerical standard is not meant to be a level to pollute up to and included specific preventive standards prohibiting contamination above detectable levels that threaten a preclusion of use.

Therefore, constituents whose standards are based on the LLOQ or LCMRL are not included in the table.

Illinois EPA proposes to add the following existing Part 620 constituents to the table at Section 620.310(a)(3)(A)(ii), as the Class I standards are noncancer health-based standards, and not based on an LLOQ or LCMRL; therefore, the preventive response activities apply:

CASRN	Constituent
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
98-95-3	Nitrobenzene
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)
118-96-7	TNT (2,4,6-trinitrotoluene)

Illinois EPA also proposes to add the following existing Part 620 carcinogen constituents to the table at Section 620.310(a)(3)(A)(ii), as the Class I standards are health-based standards, and not based on an LLOQ, or LCMRL. Therefore, the preventive response activities apply:

CASRN	Constituent
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
218-01-9	Chrysene
53-70-3	Dibenzo(a,h)anthracene
123-91-1	1,4-Dioxane (*p*-dioxane)
193-39-5	Indeno(1,2,3-c,d)pyrene

Finally, Class I standards for many carcinogens are based on the MCL or MCLG promulgated by U.S. EPA, Office of Water, and Illinois Primary Drinking Water Standards at 35 Ill. Adm. Code 611. The Board Note included at Section 620.310(a)(3)(A), states:

> Constituents that are carcinogens have not been listed in subsection (a)(3)(A) because the standard is set at the PQL and any exceedence thereof is a violation subject to corrective action.

Due to updates in analytical methods that can quantify contaminants at lower levels, many carcinogens whose Class I standards are based on the MCL are no longer set at the practical quantitation limit ("PQL"), now proposed to be referred to as the LLOQ or LCMRL. As a result, preventive response activities also apply to the following carcinogen constituents that do not rely on a limit of quantitation as the Class I standard:

CASRN	**Constituent**	**Class I Standard (mg/L)**	**LLOQ or LCMRL (mg/L)**	**Analytical Method**
71-43-2	Benzene	0.005	0.00003	EPA 524.3
50-32-8	Benzo(a)pyrene	0.0002	0.0001	EPA 525.2
56-23-5	Carbon tetrachloride	0.005	0.000098	EPA 524.3
12798-03-6	Chlordane	0.002	0.0002	EPA 525.2
67-66-3	Chloroform	0.07	0.002	EPA 8260B
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)	0.0002	0.00002	EPA 504.1
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)	0.075	0.000065	EPA 524.3
107-06-2	1,2-Dichloroethane	0.005	0.000051	EPA 524.3
75-09-2	Dichloromethane (methylene chloride)	0.005	0.00025	EPA 524.3
78-87-5	1,2-Dichloropropane	0.005	0.000065	EPA 524.3
117-81-7	Di(2-ethylhexyl)phthalate	0.006	0.0006	EPA 525.2
100-41-4	Ethylbenzene	0.7	0.000085	EPA 524.3
106-93-4	Ethylene dibromide (1,2-dibromoethane)	0.00005	0.00001	EPA 504.1
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)	0.0002	0.00002	EPA 525.2

CASRN	Constituent	Class I Standard (mg/L)	LLOQ or LCMRL (mg/L)	Analytical Method
76-44-8	Heptachlor	0.0004	0.00004	EPA 525.2
1024-57-3	Heptachlor epoxide	0.0002	0.00002	EPA 525.2
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)	0.0005	0.0001	EPA 8082A
87-86-5	Pentachlorophenol	0.001	0.0004	EPA 515.4
127-18-4	Tetrachloroethylene	0.005	0.000081	EPA 524.3
8001-35-2	Toxaphene	0.003	0.001	EPA 525.2
79-01-6	Trichloroethylene	0.005	0.000091	EPA 524.3
75-01-4	Vinyl Chloride	0.002	0.000092	EPA 524.3

The Illinois EPA proposes to add the above carcinogens to the table at Section 620.310(a)(3)(A)(ii), and requests the Board remove the Board Note, as the Board Note is no longer applicable.

One chemical is removed from Section 620.310(a)(3)(A)(ii): MCPP (mecoprop). The updated proposed standard for MCPP is the LLOQ for the constituent. Therefore, preventive response activities are not applicable.

IV. Updates to Class I Standards at Section 620.410

The tables located at Section 620.410 are updated to include CASRNs and notations beneath the tables providing the basis of the standards and as identifiers of carcinogens, including carcinogens operating with a mutagenic mode of action. The addition of notations at the bottom of each table assists the user in determining the basis of the proposed standards. A table listing Class I groundwater quality standards proposed for updates in comparison with the current values is included in Attachment 1I. The table also includes the basis for the current standards and the proposed standards; and provides toxicity values (noncancer oral reference doses ("RfD"), relative source contributions ("RSC"), and cancer oral slope factors ("SF_o")) used for the calculations of

health based standards at Appendix A, including the sources of the RfD and SF_o toxicity values. RSCs generally have default values of 0.2, although the values may be altered by other resources and professional judgement.

For the thirty-nine constituents with current Class I standards based on procedures in Part 620 Subpart F and Appendix A, all have been recalculated using the proposed methods specified in Subpart F and Appendix A. Those constituents are:

CASRN	Constituent
83-32-9	Acenaphthene
67-64-1	Acetone
120-12-7	Anthracene
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
65-85-0	Benzoic acid
78-93-3	2-Butanone (methyl ethyl ketone)
75-15-0	Carbon disulfide
218-01-9	Chrysene
53-70-3	Dibenzo(a,h)anthracene
1918-00-9	Dicamba
75-71-8	Dichlorodifluoromethane
75-34-3	1,1-Dichloroethane
84-66-2	Diethyl phthalate
84-74-2	Di-*n*-butyl phthalate
99-65-0	1,3-Dinitrobenzene
121-14-2	2,4-Dinitrotoluene
606-20-2	2,6-Dinitrotoluene
123-91-1	1,4-Dioxane (*p*-dioxane)
206-44-0	Fluoranthene
86-73-7	Fluorene
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
193-39-5	Indeno(1,2,3-c,d)pyrene
98-82-8	Isopropylbenzene (cumene)
93-65-2	MCPP (Mecoprop)
1634-04-4	MTBE (methyl tertiary-butyl ether)
91-57-6	2-Methylnaphthalene

CASRN	Constituent
95-48-7	2-Methylphenol (*o*-cresol)
91-20-3	Naphthalene
98-95-3	Nitrobenzene
14797-73-0	Perchlorate
129-00-0	Pyrene
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)
118-96-7	TNT (2,4,6-trinitrotoluene)
75-69-4	Trichlorofluoromethane
99-35-4	1,3,5-Trinitrobenzene
7440-62-2	Vanadium

In addition to recalculating health-based standards with the proposed procedures specified in Part 620.Subpart F and Appendix A, the following constituents are recalculated with updated toxicity values:

CASRN	Constituent
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
218-01-9	Chrysene
53-70-3	Dibenzo(a,h)anthracene
121-14-2	2,4-Dinitrotoluene
606-20-2	2,6-Dinitrotoluene
123-91-1	1,4-Dioxane (*p*-dioxane)
193-39-5	Indeno(1,2,3-c,d)pyrene
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)
7440-62-2	Vanadium

Updated toxicological profiles for the constituents are included in Attachment 1I. Illinois EPA notes the toxicological profile for vanadium is not included in Exhibit 9, as it is included in Attachment 1D.

After the recalculation of the health-based standards for the constituents, Illinois EPA compared the updated standards with LLOQs/LCMRLs for groundwater and drinking water

analytical methods. A table is included in Attachment 1I depicting health-based standards compared with LLOQ/LCMRLs.

The ten constituents proposed to be added to Section 620.410 are added to the tables as follows:

Part 620.410(a) Inorganic Chemical Constituents	
CASRN	**Constituent**
7429-90-5	Aluminum
7439-93-2	Lithium
7439-98-7	Molybdenum

Part 620.410(b) Organic Chemical Constituents	
CASRN	**Constituent**
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
90-12-0	1-Methylnaphthalene
375-73-5	PFBS (perfluorobutanesulfonic acid)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-95-1	PFNA (perfluorononanoic acid)
335-67-1	PFOA (perfluorooctanoic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)

The proposed standards for the newly added chemicals are based on proposed procedures at Part 620 Subpart F and Appendix A. Toxicological profiles for the constituents are included in Attachment 1I. Illinois EPA notes the toxicological profiles for the PFAS constituents are not included in Attachment 1I, as they are included in Attachment 1D.

Further updates to Section 620.410 include:

- Updates to the basis of the Class I standards for cobalt, nickel, and zinc from beneficial use for livestock to HTTACs, as human health standards are more stringent. Toxicological profiles for cobalt, nickel, and zinc are included in Attachment 1I.

- Update to the basis of the Class I standard for copper from 50 percent of the U.S. EPA "action level" for copper to beneficial use for livestock. The proposed value is from, "Water Quality Criteria", by National Academy of Sciences, 1972. The livestock value is more stringent, and the Illinois EPA assumes livestock may also utilize potable resource groundwater for its drinking water. Information regarding the basis of the livestock value is included in Attachment 1I.
- Update to the basis of fluoride (sodium fluoride) from the U.S. EPA MCL to beneficial use for livestock. The proposed value is from, "Water Quality Criteria", by National Academy of Sciences, 1972. The livestock value is more stringent. Information regarding the basis of the livestock value is included in Attachment 1I.
- Combination of radium-226 and radium-228 individual standards to a radium (combined 226+228) standard of 5 pCi/L. The value is based on the updated U.S. EPA MCL for radium (combined 226+228). Information verifying the updated MCL is included in Attachment 1I.
- Update to the basis of selenium from the U.S. EPA MCL to beneficial use for irrigation of crops and produce. The proposed value is from, "Water Quality Criteria", by National Academy of Sciences, 1972. The irrigation value is more stringent, and the Illinois EPA assumes that crops may be irrigated with Class I potable resource groundwater. Information regarding the basis of the irrigation value is included in Attachment 1I.

- Update the basis of silver from a maximum allowable concentration ("MAC") listed at 35 Ill. Adm. Code 611.300 to a health-based standard. Silver is no longer listed at 35 Ill. Adm. Code 611.300. The toxicological profile for silver is included in Attachment 1I.
- Add carcinogen designations to four existing constituents and 1 proposed constituent:

CASRN	Constituent	Classification	Source	Year
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)	2B	IARC	1999
100-41-4	Ethylbenzene	2B	IARC	2000
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)	1	IARC	2018
98-82-8	Isopropylbenzene (cumene)	2B	IARC	2018
335-67-1	PFOA (perfluorooctanoic acid)	2B	IARC	2017

IARC: International Agency for Research on Cancer.

The carcinogen designations are available at: https://monographs.iarc.who.int/agents-classified-by-the-iarc/ .

- Deletion of explosive constituents table at Section 620.410(c), with the movement of the constituents to the table at Section 620.410(b).
- Move complex organic chemical mixtures to Section 620.410(c).
- Move atrazine from the table at Section 620.410(b), to a table at Section 620.410(c)(2), complex organic chemical mixtures, with the proposed addition of total atrazine with metabolites.
- Relabel subsection (e) to (d) and (f) to (e) due to the deletion of the explosive constituents at subsection (c).

V. Updates to Class II Standards at Section 620.420

The tables located at Section 620.420 are updated to include CASRNs, notations beneath the tables providing the basis of the standards, and identifiers of carcinogens, plus carcinogens operating with a mutagenic mode of action. The addition of notations at the bottom of each table assists the user in determining the source of the proposed standards. A table listing Class II groundwater quality standards proposed for updates in comparison with the current values is included as Attachment 1J. The table also includes the basis for the current and proposed standards; and provides the chemical-specific data for organics used to determine treatment efficiency and the application of a treatment factor. The source of the chemical-specific data is U.S. EPA RSL chemical-specific parameters table. The Illinois EPA is working to update the Part 742 TACO regulations to be consistent with the chemical-specific data used by the U.S. EPA in its risk assessments.

Richard P. Cobb's prefiled testimony filed May 28, 2008 for the Board's rulemaking docket PCB R08-18 (Attachment 1A), discussed the basis for establishing Class II groundwater quality standards. Mr. Cobb's testimony states in part:

> The final opinion and order of the Board (Docket R89-14(B)), for establishing Class II groundwater quality standards (35 Ill. Adm. Code 620), published November 7, 1991, pages 19 and 20 states that:
>
> 'Part 620.420 establishes standards for Class II: General Resource Groundwaters. Because groundwaters are placed in Class II because they are quality-limited, quantity-limited, or both (see Subpart B discussion above), it is necessary that the standards that apply to these waters reflect this range of possible attributes. Among the factors considered in determining the Class II numbers are the capabilities of treatment technologies to bring Class II waters to qualities suitable for potable use (R3 at 75) ["R3" means the transcript from the Board's May 1991 hearing on this matter, and "at 75" is page 75]. Thus, many Class II

> standards are based on MCL's as modified to reflect treatment capabilities. For some parameters the Class II standards are based on support of a use other than potability (e.g., livestock water, irrigation, industrial use) where a different use requires a more stringent standard (R3 at 1148))'.

Proposed updates to Section 620.420(a)(1) include:

- The addition of lithium and molybdenum, with the proposed Class II standards based the beneficial use for irrigation of crops and produce for both constituents. Information regarding the basis of the irrigation values for lithium and molybdenum are included in Attachment 1J.
- Updates to the Class II standards, based on updates to the Class I standards for fluoride (sodium fluoride) and perchlorate.

Proposed updates to Section 620.420(a)(2) include:

- The addition of aluminum, with a proposed Class II standard based on beneficial use for livestock. Information regarding the basis of the livestock value is included in Attachment 1J.
- The addition of radium (combined 226+228) and silver, both based on proposed Class I standards.
- Updates to the Class II standards, based on updates to the Class I standards, for copper and selenium.

Proposed updates to Section 620.420(b)(1) include the application of treatment factors for all the organic constituents as discussed in Richard P. Cobb's prefiled testimony filed May 28, 2008 for the Board's rulemaking docket PCB R08-18 (Attachment 1A). Mr. Cobb discusses

the decision-making process for the use of a treatment factor (TF) of five when determining Class II standards for organics, by stating:

> Treatment Factor - A five fold treatment factor ("5X") was used to derive a proposed Class II standard for organic compounds with a K_{oc} value greater than (>) ethylbenzene's K_{oc} of 363 liters per kilogram (L/kg) at 20 ^{0} C or a Henry's Law constant greater than methylene chloride's (8.98 X 10^{-2} unit less at 20 ^{0} C). A five fold treatment factor equates to a removal efficiency of 80%. This is a very economical approach, since most of the BATs achieve a 99% removal rate. The Class I standard was proposed where either or both were below the factors detailed above.

The Illinois EPA wishes to clarify the appropriate organic carbon coefficient (K_{oc}) for ethylbenzene is 446 L/kg, per U.S. EPA RSL guidance. In addition, the Henry's Law constant (H') is temperature dependent. The appropriate H' value for methylene chloride at 20 ^{0} C is 0.11, and is calculated by setting the groundwater system temperature to 20 ^{0} C in the RSL calculator

For organic constituents with health-based Class I standards, the Illinois EPA evaluated the chemical-specific data for each constituent and applied a treatment factor of five to the updated Class I standard, when applicable, in Section 620.420(b) and (c).

Further updates to Section 620.420 include:

- Deletion of explosive constituents table at Section 620.420(c), with the movement of the constituents to the table at Section 620.420(b).
- Moving complex organic chemical mixtures to Section 620.420(c).
- Move atrazine from the table at Section 620.420(b), to a table at Section 620.420(c)(2), complex organic chemical mixtures, with the proposed addition of total atrazine with metabolites.

- Relabeling subsection (e) to (d) due to the deletion of the explosive constituents at subsection (c).

VI. Similar-Acting Substances

Part 620 Appendix B(d), states:

> When two or more substances occur together in a mixture, the additivity of the toxicities of some or all of the substances will be considered when determining health-based standards for Class I: Potable Resource Groundwater.

Part 620 Appendix B describes a mixture for the purposes of Appendix B, stating,

> a 'mixture' means two or more substances which are present in Class I: Potable Resource Groundwater which may or may not be related either chemically or commercially, but which are not complex mixtures of related isomers and congeners which are produced as commercial products (for example, PCBs or technical grade chlordane).

To assist users in determining if a mixture of similar-acting substances is present in Class I groundwater, Illinois EPA proposes to add Appendix E. Appendix E provides tables of substances having similar-acting noncancer and cancer effects. The addition of similar-acting substances tables in Appendix E, no longer requires the description of a few of the similar-acting substances currently provided in Part 620 Appendix B(c). Therefore, Illinois EPA proposes to remove the specific similar-acting substances listed at Appendix B(c) and refer users to Appendix E for a complete list of similar-acting substances.

VII. The Addition of Appendix E: Similar-acting Substances

Appendix E consists of two tables:

- Table A: Similar-acting Noncarcinogens
- Table B: Similar-acting Carcinogens

The tables list target organs or health effects, with substances included Section 620.410 listed beneath the applicable target organ or effect. If only one constituent affects a target organ, the effect is not included, as two or more constituents are required to be considered a mixture. The source of a toxicity metadata used to determine constituents with similar-acting effects is the toxicity metadata provided in the RSL calculator; with the exception of: MTBE, HFPO-DA, PFHxS, PFNA, PFOS, PFOA, and vanadium. As noted above under Section I of my testimony, these constituent's toxicity values are not based on values provided in the U.S. EPA RSL database. The target organs or health effects for MTBE, HFPO-DA, PFHxS, PFNA, PFOS, PFOA, and vanadium are based on the toxicological profiles included in Attachment 1D. Tables listing toxicity metadata are included as Attachment 1K.

VIII. Conclusion

This concludes my portion of Illinois EPA's testimony for the proposed amendments to Part 620.

Attachment

1A

1

TITLE 35: ENVIRONMENTAL PROTECTION
SUBTITLE G: WASTE DISPOSAL
CHAPTER I: POLLUTION CONTROL BOARD
SUBCHAPTER f: RISK BASED CLEANUP OBJECTIVES

PART 742
TIERED APPROACH TO CORRECTIVE ACTION OBJECTIVES

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SUBPART B: GENERAL

SUBPART C: EXPOSURE ROUTE EVALUATIONS

SUBPART D: DETERMINING AREA BACKGROUND

SUBPART J: INSTITUTIONAL CONTROLS

SUBPART K: ENGINEERED BARRIERS

SUBPART L: BUILDING CONTROL TECHNOLOGIES

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AUTHORITY: Implementing Sections 22.4, 22.12, Title XVI, and Title XVII and authorized by Sections 27 and 58.5 of the Environmental Protection Act [415 ILCS 5/22.4, 22.12, 27, and 58.5 and Title XVI and Title XVII].

SOURCE: Adopted in R97-12(A) at 21 Ill. Reg. 7942, effective July 1, 1997; amended in R97-12(B) at 21 Ill. Reg. 16391, effective December 8, 1997; amended in R97-12(C) at 22 Ill. Reg. 10847, effective June 8, 1998; amended in R00-19(A) at 25 Ill. Reg. 651, effective January 6, 2001; amended in R00-19(B) at 25 Ill. Reg. 10374, effective August 15, 2001; amended in R00-19(C) at 26 Ill. Reg. 2683, effective February 5, 2002; amended in R06-10 at 31 Ill. Reg. 4063, effective February 23, 2007; amended in R11-09 at 37 Ill. Reg. 7506, effective July 15, 2013.

NOTE: Italics indicates statutory language.

SUBPART A: INTRODUCTION

Section 742.100 Intent and Purpose

a) This Part sets forth procedures for evaluating the risk to human health posed by environmental conditions and developing remediation objectives that achieve acceptable risk levels.

b) The purpose of these procedures is to provide for the adequate protection of human health and the environment based on the risks to human health posed by environmental conditions while incorporating site related information.

Section 742.105 Applicability

a) Any person, including a person required to perform an investigation pursuant to the Illinois Environmental Protection Act [415 ILCS 5] (Act), may elect to proceed under this Part to the extent allowed by State or federal law and regulations and the provisions of this Part and subject to the exceptions listed in subsection (h) below. A person proceeding under this Part may do so to the extent such actions are consistent with the requirements of the program under which site remediation is being addressed.

b) This Part is to be used in conjunction with the procedures and requirements applicable to the following programs:

1) Leaking Underground Storage Tanks (35 Ill. Adm. Code 731 and 734);

2) Site Remediation Program (35 Ill. Adm. Code 740); and

3) RCRA Part B Permits and Closure Plans (35 Ill. Adm. Code 724 and 725).

c) The procedures in this Part may not be used if their use would delay response action to address imminent and substantial threats to human health and the environment. This Part may only be used after actions to address such threats have been completed.

d) This Part may be used to develop remediation objectives to protect surface waters, sediments or ecological concerns, when consistent with the regulations of other programs, and as approved by the Agency.

e) A no further remediation determination issued by the Agency prior to July 1, 1997 pursuant to Section 4(y) of the Act or one of the programs listed in subsection (b) of this Section that approves completion of remedial action relative to a release shall remain in effect in accordance with the terms of that determination.

f) Site specific groundwater remediation objectives determined under this Part for contaminants of concern may exceed the groundwater quality standards established pursuant to the rules promulgated under the Illinois Groundwater Protection Act [415 ILCS 55] as long as done in accordance with Sections 742.805 and 742.900(c)(9). (See 415 ILCS 5/58.5(d)(4))

g) Where contaminants of concern include polychlorinated byphenyls (PCBs), a person may need to evaluate the applicability of regulations adopted under the Toxic Substances Control Act (15 USC 2601).

h) This Part may not be used in lieu of the procedures and requirements applicable to landfills under 35 Ill. Adm. Code 807 or 811 through 814.

i) An evaluation of the indoor inhalation exposure route under this Part addresses the potential of contaminants present in soil gas or groundwater to reach human

receptors within buildings. This Part does not address the remediation or mitigation of any contamination within a building from a source other than soil gas or groundwater, such as the building structure itself and products within the building.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.110 Overview of Tiered Approach

a) This Part presents an approach for developing remediation objectives (see Appendix A, Illustrations A and B) that include an option for exclusion of pathways from further consideration, use of area background concentrations as remediation objectives and three tiers for selecting applicable remediation objectives. An understanding of human exposure routes is necessary to properly conduct an evaluation under this approach. In some cases, applicable human exposure routes can be excluded from further consideration prior to any tier evaluation. Selecting which tier or combination of tiers to be used to develop remediation objectives is dependent on the site-specific conditions and remediation goals. Tier 1 evaluations and Tier 2 evaluations are not prerequisites to conducting Tier 3 evaluations.

b) A Tier 1 evaluation compares the concentration of contaminants detected at a site to the corresponding remediation objectives for residential and industrial/commercial properties contained in Appendix B, Tables A, B, C, D, E, G, H and I. To complete a Tier 1 evaluation, the extent and concentrations of the contaminants of concern, the groundwater class, the land use classification, human exposure routes at the site, and, if appropriate, soil pH, must be known. If remediation objectives are developed based on industrial/commercial property use, then institutional controls under Subpart J are required. For the indoor inhalation exposure route, institutional controls under Subpart J are required to use remediation objectives in Appendix B, Table H or Table I.

c) A Tier 2 evaluation uses the risk based equations from the Soil Screening Level (SSL) model, Risk Based Corrective Action (RBCA) model and modified Johnson and Ettinger (J&E) model documents listed in Appendix C, Tables A, C and L, respectively. In addition to the information that is required for a Tier 1 evaluation, site-specific information is used to calculate Tier 2 remediation objectives. As in Tier 1, Tier 2 evaluates residential and industrial/commercial properties only. If remediation objectives are developed based on industrial/commercial property use, then institutional controls under Subpart J are required. For the indoor inhalation exposure route, institutional controls under Subpart J are required to develop remediation objectives pursuant to Appendix C, Table L.

d) A Tier 3 evaluation allows alternative parameters and factors, not available under a Tier 1 or Tier 2 evaluation, to be considered when developing remediation objectives. Remediation objectives developed for conservation and agricultural properties can only be developed under Tier 3.

e) Remediation objectives may be developed using area background concentrations or any of the three tiers if the evaluation is conducted in accordance with applicable requirements in Subparts D through I. When contaminant concentrations do not exceed remediation objectives developed under one of the tiers or area background procedures under Subpart D, further evaluation under any of the other tiers is not required.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.115 Key Elements

To develop remediation objectives under this Part, the following key elements shall be addressed.

a) Exposure Routes

1) This Part identifies the following as potential exposure routes to be addressed:

A) Outdoor inhalation;

B) Indoor inhalation;

C) Soil ingestion;

D) Groundwater ingestion; and

E) Dermal contact with soil.

2) The evaluation of exposure routes under subsections (a)(1)(A), (a)(1)(B), (a)(1)(C) and (a)(1)(D) is required for all sites when developing remediation objectives or excluding exposure pathways. Evaluation of the dermal contact exposure route is required for use of RBCA equations in Appendix C, Table C or use of formal risk assessment under Section 742.915.

3) The groundwater ingestion exposure route is comprised of two components:

A) Migration from soil to groundwater (soil component); and

B) Direct ingestion of groundwater (groundwater component).

4) The outdoor inhalation route is comprised of two components:

A) Migration from soil through soil gas to outdoor air (soil component); and

B) Migration from soil gas to outdoor air (soil gas component).

5) The indoor inhalation exposure route is comprised of two components:

A) Migration from soil gas to indoor air (soil gas component); and

B) Migration from groundwater through soil gas to indoor air (groundwater component).

b) Contaminants of Concern

The contaminants of concern to be remediated depend on the following:

1) The materials and wastes managed at the site;

2) The extent of the no further remediation determination being requested from the Agency pursuant to a specific program; and

3) The requirements applicable to the specific program, as listed at Section 742.105(b) under which the remediation is being performed.

c) Land Use

The present and post-remediation uses of the site where exposures may occur shall be evaluated. The land use of a site, or portion thereof, shall be classified as one of the following:

1) Residential property;

2) Conservation property;

3) Agricultural property; or

4) Industrial/commercial property.

d) Environmental Media of Concern

This Part provides procedures for developing remediation objectives for the following environmental media:

1) Soil;

2) Soil gas;

3) Groundwater.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.120 Site Characterization

Characterization of the extent and concentrations of contamination at a site shall be performed before beginning development of remediation objectives. The actual steps and methods taken to characterize a site are determined by the requirements applicable to the specific program under which site remediation is being addressed.

SUBPART B: GENERAL

Section 742.200 Definitions

Except as stated in this Section, or unless a different meaning of a word or term is clear from the context, the definition of words or terms in this Part shall be the same as that applied to the same words or terms in the Act.

"Act" means the Illinois Environmental Protection Act [415 ILCS 5].

"ADL" means Acceptable Detection Limit, which is the detectable concentration of a substance that is equal to the lowest appropriate Practical Quantitation Limit (PQL) as defined in this Section.

"Agency" means the Illinois Environmental Protection Agency.

"Agricultural Property" means any real property for which its present or post-remediation use is for growing agricultural crops for food or feed either as harvested crops, cover crops or as pasture. This definition includes, but is not limited to, properties used for confinement or grazing of livestock or poultry and for silviculture operations. Excluded from this definition are farm residences, farm outbuildings and agrichemical facilities.

"Aquifer" means *saturated (with groundwater) soils and geologic materials which are sufficiently permeable to readily yield economically useful quantities of water to wells, springs, or streams under ordinary hydraulic gradients.* (Illinois Groundwater Protection Act [415 ILCS 55/3(a)])

"Area Background" means *concentrations of regulated substances that are consistently present in the environment in the vicinity of a site that are the result of natural conditions or human activities, and not the result solely of releases at the site.* [415 ILCS 5/58.2]

"ASTM" means the American Society for Testing and Materials.

"Board" means the Illinois Pollution Control Board.

"Building" means a man-made structure with an enclosing roof and enclosing walls (except for windows and doors) that is fit for any human occupancy for at least six consecutive months.

"Building Control Technology" means any technology or barrier that affects air flow or air pressure within a building for purposes of reducing or preventing contaminant migration to the indoor air.

"Cancer Risk" means a unitless probability of an individual developing cancer from a defined exposure rate and frequency.

"Cap" means a barrier designed to prevent the infiltration of precipitation or other surface water, or impede the ingestion or inhalation of contaminants.

"Capillary Fringe" means the zone above the water table in which water is held by surface tension. Water in the capillary fringe is under a pressure less than atmospheric.

"Carcinogen" means *a contaminant that is classified as a category A1 or A2 carcinogen by the American Conference of Governmental Industrial Hygienists; a category 1 or 2A/2B carcinogen by the World Health Organization's International Agency for Research on Cancer; a "human carcinogen" or "anticipated human carcinogen" by the United States Department of Health and Human Service National Toxicological Program; or a category A or B1/B2 carcinogen* or as "carcinogenic to humans" or "likely to be carcinogenic to humans" *by the United States Environmental Protection Agency in the integrated risk information system or a final rule issued in a Federal Register notice by the USEPA.* [415 ILCS 5/58.2]

"Class I Groundwater" means groundwater that meets the Class I: Potable Resource Groundwater criteria set forth in 35 Ill. Adm. Code 620.

"Class II Groundwater" means groundwater that meets the Class II: General Resource Groundwater criteria set forth in 35 Ill. Adm. Code 620.

"Conservation Property" means any real property for which present or post-remediation use is primarily for wildlife habitat.

"Construction Worker" means a person engaged on a temporary basis to perform work involving invasive construction activities including, but not limited to, personnel performing demolition, earth-moving, building, and routine and emergency utility installation or repair activities.

"Contaminant of Concern" or "Regulated Substance of Concern" means *any contaminant that is expected to be present at the site based upon past and current land uses and associated releases that are known to the* person conducting a remediation *based upon reasonable inquiry.* [415 ILCS 5/58.2]

"County Highway" means county highway as defined in the Illinois Highway Code [605 ILCS 5].

"District Road" means district road as defined in the Illinois Highway Code [605 ILCS 5].

"Engineered Barrier" means a barrier designed or verified using engineering practices that limits exposure to or controls migration of the contaminants of concern.

"Environmental Land Use Control" means an instrument that meets the requirements of this Part and is placed in the chain of title to real property that limits or places requirements upon the use of the property for the purpose of protecting human health or the environment, is binding upon the property owner, heirs, successors, assigns, and lessees, and runs in perpetuity or until the Agency approves, in writing, removal of the limitation or requirement from the chain of title.

"Exposure Route" means the transport mechanism by which a contaminant of concern reaches a receptor.

"Federally Owned Property" means real property owned in fee by the United States of America on which institutional controls are sought to be placed in accordance with this Subpart.

"Federal Landholding Entity" means that federal department, agency, or instrumentality with the authority to occupy and control the day-to-day use, operation and management of Federally Owned Property.

"Free Product" means a contaminant that is present as a non-aqueous phase liquid for chemicals whose melting point is less than 30°C (e.g., liquid not dissolved in water).

"GIS" means Geographic Information System.

"GPS" means Global Positioning System.

"Groundwater" means underground water which occurs within the saturated zone and geologic materials where the fluid pressure in the pore space is equal to or greater than atmospheric pressure. [415 ILCS 5/3.64]

"Groundwater Quality Standards" means the standards for groundwater as set forth in 35 Ill. Adm. Code 620.

"Hazard Quotient" means the ratio of a single substance exposure level during a specified time period to a reference dose for that substance derived from a similar exposure period.

"Highway" *means any public way for vehicular travel which has been laid out in pursuance of any law of this State, or of the Territory of Illinois, or which has been established by dedication, or used by the public as a highway for 15 years, or which has been or may be laid out and connect a subdivision or platted land with a public highway and which has been dedicated for the use of the owners of the land included in the subdivision or platted land where there has been an acceptance and use under such dedication by such owners, and which has not been vacated in pursuance of law. The term "highway" includes rights of way, bridges, drainage structures, signs, guard rails, protective structures and all other structures and appurtenances necessary or convenient for vehicular traffic. A highway in a rural area may be called a "road", while a highway in a municipal area may be called a "street".* (Illinois Highway Code [605 ILCS 5/2-202])

"Highway Authority" means *the Department* of Transportation *with respect to a State highway;* the Illinois State Toll Highway with respect to a toll highway; *the County Board with respect to a county highway or a county unit district road if a discretionary function is involved and the County Superintendent of Highways if a ministerial function is involved; the Highway Commissioner with respect to a township or district road not in a county unit road district; or the corporate authorities of a municipality with respect to a municipal street.* (Illinois Highway Code [605 ILCS 5/2-213])

"Human Exposure Pathway" means a physical condition which may allow for a risk to human health based on the presence of all of the following: contaminants of concern; an exposure route; and a receptor activity at the point of exposure that could result in contaminant of concern intake.

"Industrial/Commercial Property" means any real property that does not meet the definition of residential property, conservation property or agricultural property.

"Infiltration" means the amount of water entering into the ground as a result of precipitation.

"Institutional Control" means a legal mechanism for imposing a restriction on land use, as described in Subpart J.

"Intrusive activities" means activities that would affect potential flow of contaminants into a building (e.g., breaching the integrity of a foundation due to repairs or installation of utilities).

"Land Use Control Memoranda of Agreement" mean agreements entered into between one or more agencies of the United States and the Illinois Environmental Protection Agency that limit or place requirements upon the use of Federally Owned Property for the purpose of protecting human health or the environment.

"Man-Made Pathways" means *constructed* physical conditions *that may allow for the transport of regulated substances including, but not limited to, sewers, utility lines, utility* or elevator *vaults, building foundations, basements, crawl spaces, drainage ditches, previously excavated and filled areas* or sumps. [415 ILCS 5/58.2]

"Natural Pathways" means *natural* physical conditions that may allow *for the transport of regulated substances including, but not limited to, soil, groundwater, sand seams and lenses, and gravel seams and lenses.* [415 ILCS 5/58.2]

"Person" means an *individual, trust, firm, joint stock company, joint venture, consortium, commercial entity, corporation (including a government corporation), partnership, association, state, municipality, commission, political subdivision of a state, or any interstate body including the United States government and each department, agency, and instrumentality of the United States.* [415 ILCS 5/58.2]

"Point of Human Exposure" means the points at which human exposure to a contaminant of concern may reasonably be expected to occur. The point of human exposure is at the source, unless an institutional control limiting human exposure for the applicable exposure route has been or will be in place, in which case the point of human exposure will be the boundary of the institutional control. Point of human exposure may be at a different location than the point of compliance.

"Populated Area" means:

an area within the boundaries of a municipality that has a population of 10,000 or greater based on the year 2000 or most recent census; or

an area less than three miles from the boundary of a municipality that has a population of 10,000 or greater based on the year 2000 or most recent census.

"Potable" means *generally fit for human consumption in accordance with accepted water supply principles and practices.* (Illinois Groundwater Protection Act [415 ILCS 55/3(h)])

"PQL" means practical quantitation limit or estimated quantitation limit, which is the lowest concentration that can be reliably measured within specified limits of precision and accuracy for a specific laboratory analytical method during routine laboratory operating conditions in accordance with "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846, incorporated by reference in Section 742.210. When applied to filtered water samples, PQL includes the method detection limit or estimated detection limit in accordance with the applicable method revision in: "Methods for the Determination of Organic Compounds in Drinking Water", Supplement II", EPA Publication No. EPA/600/4-88/039; "Methods for the Determination of Organic Compounds in Drinking Water, Supplement III", EPA Publication No. EPA/600/R-95/131, all of which are incorporated by reference in Section 742.210.

"Q_{soil}" means the volumetric flow rate of soil gas from the subsurface into the enclosed building space.

"RBCA" means Risk Based Corrective Action as defined in ASTM E-1739-95, as incorporated by reference in Section 742.210.

"RCRA" means the Resource Conservation and Recovery Act of 1976 (42 USC 6921).

"Reference Concentration" or "RfC" means an estimate of a daily exposure, in units of milligrams of chemical per cubic meter of air (mg/m^3), to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a portion of a lifetime (up to approximately seven years, subchronic) or for a lifetime (chronic).

"Reference Dose" or "RfD" means an estimate of a daily exposure, in units of milligrams of chemical per kilogram of body weight per day (mg/kg/d), to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a portion of a lifetime (up to approximately seven years, subchronic) or for a lifetime (chronic).

"Regulated Substance" means *any hazardous substance as defined under Section 101(14) of the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (P.L. 96-510) and petroleum products including crude oil or any fraction thereof, natural gas, natural gas liquids, liquefied natural gas, or synthetic gas usable for fuel (or mixtures of natural gas and such synthetic gas).* [415 ILCS 5/58.2]

"Rendered inoperable" means having become unable to operate effectively, including, but not limited to, being shut down as part of routine maintenance or due to a malfunction, power failure, or vandalism.

"Residential Property" *means any real property that is used for habitation by individuals, or* where children have the opportunity for exposure to contaminants through ingestion or inhalation (indoor or outdoor) at educational facilities, health care facilities, child care facilities or recreational areas. [415 ILCS 5/58.2]

"Right of Way" means *the land, or interest therein, acquired for or devoted to a highway.* (Illinois Highway Code [605 ILCS 5/2-217])

"Saturated Zone" means a subsurface zone in which all the interstices or voids are filled with water under pressure greater than that of the atmosphere.

"Similar-Acting Chemicals" are chemical substances that have toxic or harmful effect on the same specific organ or organ system (see Appendix A.Tables E and F for a list of similar-acting chemicals with noncarcinogenic and carcinogenic effects).

"Site" means *any single location, place, tract of land or parcel of property, or portion thereof, including contiguous property separated by a public right-of-way.* [415 ILCS 5/58.2]

"Slurry Wall" means a man-made barrier made of geologic material which is constructed to prevent or impede the movement of contamination into a certain area.

"Soil Gas" means the air existing in void spaces in the soil between the groundwater table and the ground surface.

"Soil Saturation Limit" or "C_{sat}" means the contaminant concentration at which the absorptive limits of the soil particles, the solubility limits of the available soil moisture, and saturation of soil pore air have been reached. Above the soil saturation concentration, the assumptions regarding vapor transport to air and/or dissolved phase transport to groundwater (for chemicals that are liquid at ambient soil temperatures) do not apply, and alternative modeling approaches are required.

"Soil Vapor Saturation Limit" or "C_v^{sat}" means the maximum vapor concentration that can exist in the soil pore air at a given temperature and pressure.

"Solubility" means a chemical specific maximum amount of solute that can dissolve in a specific amount of solvent (groundwater) at a specific temperature.

"SPLP" means Synthetic Precipitation Leaching Procedure (Method 1312) as published in "Test Methods for Evaluating Solid Waste, Physical/Chemical

Methods", USEPA Publication No. SW-846, as incorporated by reference in Section 742.210.

"SSL" means Soil Screening Levels as defined in USEPA's Soil Screening Guidance: User's Guide and Technical Background Document, as incorporated by reference in Section 742.210.

"State Highway" means State highway as defined in the Illinois Highway Code [605 ILCS 5].

"Stratigraphic Unit" means a site-specific geologic unit of native deposited material and/or bedrock of varying thickness (e.g., sand, gravel, silt, clay, bedrock, etc.). A change in stratigraphic unit is recognized by a clearly distinct contrast in geologic material or a change in physical features within a zone of gradation. For the purposes of this Part, a change in stratigraphic unit is identified by one or a combination of differences in physical features such as texture, cementation, fabric, composition, density, and/or permeability of the native material and/or bedrock.

"Street" means street as defined in the Illinois Highway Code [605 ILCS 5].

"TCLP" means Toxicity Characteristic Leaching Procedure (Method 1311) as published in "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", USEPA Publication No. SW-846, as incorporated by reference in Section 742.210.

"Toll Highway" means toll highway as defined in the Illinois Highway Code [605 ILCS 5].

"Total Petroleum Hydrocarbon" or "TPH" means the additive total of all petroleum hydrocarbons found in an analytical sample.

"Township Road" means township road as defined in the Illinois Highway Code [605 ILCS 5].

"Unconfined Aquifer" means an aquifer whose upper surface is a water table free to fluctuate under atmospheric pressure.

"Volatile Chemicals" means chemicals with a Dimensionless Henry's Law Constant of greater than 1.9×10^{-2} or a vapor pressure greater than 0.1 Torr (mmHg) at 25°C. For purposes of the indoor inhalation exposure route, elemental mercury is included in this definition.

"Water Table" means the top water surface of an unconfined aquifer at atmospheric pressure.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.205 Severability

If any provision of this Part or its application to any person or under any circumstances is adjudged invalid, such adjudication shall not affect the validity of this Part as a whole or any portion not adjudged invalid.

Section 742.210 Incorporations by Reference

a) The Board incorporates the following material by reference:

Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs), U.S. Environmental Protection Agency, 1600 Clifton Road, Mailstop F32, Atlanta, Georgia 30333, (770) 488-3357 (November 2007).

ASTM International. 100 Barr Harbor Drive, West Conshohocken PA 19428-2959, (610) 832-9585.

ASTM D 2974-00, Standard Test Methods for Moisture, Ash and Organic Matter of Peat and Other Organic Soils, approved August 10, 2000.

ASTM D 2488-00, Standard Practice for Description and Identification of Soils (Visual-Manual Procedure), approved February 10, 2000.

ASTM D 1556-00, Standard Test Method for Density and Unit Weight of Soil in Place by the Sand-Cone Method, approved March 10, 2000.

ASTM D 2167-94, Standard Test Method for Density and Unit Weight of Soil in Place by the Rubber Balloon Method, approved March 15, 1994.

ASTM D 2922-01, Standard Test Methods for Density of Soil and Soil-Aggregate in Place by Nuclear Methods (Shallow Depth), approved June 10, 2001.

ASTM D 2937-00e1, Standard Test Method for Density of Soil in Place by the Drive-Cylinder Method, approved June 10, 2000.

ASTM D 854-02, Standard Test Methods for Specific Gravity of Soil Solids by Water Pycnometer, approved July 10, 2002.

ASTM D 2216-98, Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass, approved February 10, 1998.

ASTM D 4959-00, Standard Test Method for Determination of Water (Moisture) Content of Soil by Direct Heating, approved March 10, 2000.

ASTM D 4643-00, Standard Test Method for Determination of Water (Moisture) Content of Soil by the Microwave Oven Method, approved February 10, 2000.

ASTM D 5084-03, Standard Test Methods for Measurement of Hydraulic Conductivity of Saturated Porous Materials Using a Flexible Wall Permeameter, approved November 1, 2003.

ASTM D 422-63 (2002), Standard Test Method for Particle-Size Analysis of Soils, approved November 10, 2002.

ASTM D 1140-00, Standard Test Methods for Amount of Material in Soils Finer than the No. 200 (75 μm) Sieve, approved June 10, 2000.

ASTM D 3017-01, Standard Test Method for Water Content of Soil and Rock in Place by Nuclear Methods (Shallow Depth), approved June 10, 2001.

ASTM D 4525-90 (2001), Standard Test Method for Permeability of Rocks by Flowing Air, approved May 25, 1990.

ASTM D 2487-00, Standard Classification of Soils for Engineering Purposes (Unified Soil Classification System), approved March 10, 2000.

ASTM D 1945-03, Standard Test Method for Analysis of Natural Gas by Gas Chromatography, approved May 10, 2003.

ASTM D 1946-90, Standard Practice for Analysis of Reformed Gas by Gas Chromatography, approved June 1, 2006.

ASTM E 1527-00, Standard Practice for Environmental Site Assessments: Phase I Environmental Site Assessment Process, approved May 10, 2000. Vol. 11.04.

ASTM E 1739-95 (2002), Standard Guide for Risk-Based Corrective Action Applied at Petroleum Release Sites, approved September 10, 1995.

ASTM E 2121-09, Standard Practice for Installing Radon Mitigation Systems in Existing Low-Rise Residential Buildings, approved November 1, 2009.

ASTM E 2600-10, Standard Practice for Assessment for Vapor Intrusion into Structures on Property Involved in Real Estate Transactions, approved June 2010.

API. American Petroleum Institute, 1220 L Street, NW, Washington DC 20005-4070 (202) 682-8000.

BIOVAPOR-A 1-D Vapor Intrusion Model with Oxygen-Limited Aerobic Biodegradation, Version 2.0 (January 2010).

Barnes, Donald G. and Dourson, Michael. (1988). Reference Dose (RfD): Description and Use in Health Risk Assessments. Regulatory Toxicology and Pharmacology. 8, 471-486.

EPRI. Electric Power Research Institute. 3420 Hillview Avenue, Palo Alto, California 94304. (650) 855-2121.

Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Soil in Illinois: Background PAHs, EPRI, Palo Alto CA, We Energies, Milwaukee WI and IEPA, Springfield IL: 2004. 1011376.

Reference Handbook for Site-Specific Assessment of Subsurface Vapor Intrusion to Indoor Air, Electric Power Research Institute (EPRI), Inc., Program No. 1008492 (March 2005).

GPO. Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20401, (202) 783-3238.

USEPA Guidelines for Carcinogenic Risk Assessment, 51 Fed. Reg. 33992-34003 (September 24, 1986).

"Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", USEPA Publication number SW-846 (Third Edition, Final Update IIIA, April 1998), as amended by Updates I, IIA, III, and IIIA (Document No. 955-001-00000-1).

"Methods for the Determination of Organic Compounds in Drinking Water", EPA Publication No. EPA/600/4-88/039 (December 1988 (Revised July 1991)).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement I", EPA Publication No. EPA/600/4-90/020 (July 1990).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement II", EPA Publication No. EPA/600/R-92/129 (August 1992).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement III", EPA Publication No. EPA/600/R-95/131 (August 1995).

"Guidance for Data Quality Assessment, Practical Methods for Data Analysis, EPA QA/G-9, QAOO Update," EPA/600/R-96/084 (July 2000). Available at www.epa.gov/quality/qs-docs/g9-final.pdf.

"Assessment of Vapor Intrusion in Homes Near the Raymark Superfund Site Using Basement and Sub-Slab Air Samples", EPA Publication No. EPA/600/R-05/147 (March 2006).

"Model Standards and Techniques for Control of Radon in New Residential Buildings" EPA Publication No. EPA/402/R-94/009 (March 1994).

"Radon Reduction Techniques for Existing Detached Houses: Technical Guidance (Third Edition) for Active Soil Depressurization Systems", EPA Publication No. EPA/625/R-93/011 (October 1993).

Illinois Environmental Protection Agency, 1021 N. Grand Ave East, Springfield IL 62701, (217) 785-0830.

"A Summary of Selected Background Conditions for Inorganics in Soil", Publication No. IEPA/ENV/94-161 (August 1994).

IRIS. Integrated Risk Information System, National Center for Environmental Assessment, U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, MS-190, Cincinnati, OH 45268, (513) 569-7254.

"Reference Dose (RfD): Description and Use in Health Risk Assessments", Background Document 1A (March 15, 1993).

"EPA Approach for Assessing the Risks Associated with Chronic Exposures to Carcinogens", Background Document 2 (January 17, 1992).

Johnson, Paul C. (2005). Identification of Application Specific Critical Inputs for the 1991 Johnson and Ettinger Vapor Intrusion Algorithm. Ground Water Monitoring and Remediation. 25(1), 63-78.

Murray, Donald M. and Burmaster, David E. (1995). Residential Air Exchange Rates in the United States: Empirical and Estimated Parametric Distributions by Season and Climatic Region. Risk Analysis. 15(4), 459-465.

Nelson, D.W., and L.E. Sommers (1982). Total carbon, organic carbon, and organic matter. In: A.L. Page (ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. 2nd Edition, pp. 539-579, American Society of Agronomy. Madison, WI.

NTIS. National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161, (703) 487-4600.

"Calculating Upper Confidence Limits for Exposure Point Concentrations at Hazardous Waste Sites," USEPA Office of Emergency and Remedial Response, OSWER 9285.6-10 (December 2002), PB 2003-104982.

"Evaluating the Vapor Intrusion to Indoor Air Pathway from Groundwater and Soils", OSWER Draft Guidance. EPA Publication No. EPA/530D-02/004 (November 2002).

"Exposures Factors Handbook, Vol. I: General Factors", EPA Publication No. EPA/600/P-95/002Fa (August 1997).

"Exposures Factors Handbook, Vol. II: Food Ingestion Factors", EPA Publication No. EPA/600/P-95/002Fb (August 1997).

"Exposures Factors Handbook, Vol. III: Activity Factors", EPA Publication No. EPA/600/P-95/002Fc (August 1997).

"Risk Assessment Guidance for Superfund, Vol. I: Human Health Evaluation Manual, Supplemental Guidance: Standard Default Exposure Factors", OSWER Directive 9285.6-03 (March 1991).

"Rapid Assessment of Exposure to Particulate Emissions from Surface Contamination Sites", EPA Publication No. EPA/600/8-85/002 (February 1985), PB 85-192219.

"Risk Assessment Guidance for Superfund, Vol. I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment) Final", EPA Publication No. EPA/540/R/99/005 (July 2004).

"Risk Assessment Guidance for Superfund, Vol. 1: Human Health Evaluation Manual (Part F, Supplemental Guidance for Inhalation Risk Assessment) Final", EPA Publication No. 540-R-070-002 (January 2009).

"Soil Screening Guidance: Technical Background Document", EPA Publication No. EPA/540/R-95/128, PB 96-963502 (May 1996).

"Soil Screening Guidance: User's Guide", EPA Publication No. EPA/540/R-96/018, PB 96-963505 (April 1996).

"Superfund Exposure Assessment Manual", EPA Publication No. EPA/540/1-88/001 (April 1988).

"Supplemental Guidance for Developing Soil Screening Levels for Superfund Sites", OSWER Directive 9355.4-24 (December 2002).

"User's Guide for Evaluating Subsurface Vapor Intrusion into Buildings", EPA Publication No. EPA/68/W-02/33 (February 2004).

Polynuclear Aromatic Hydrocarbon Background Study, City of Chicago, Illinois, Tetra Tech Em Inc., 200 E. Randolph Drive, Suite 4700, Chicago IL 60601, February 24, 2003.

RCRA Facility Investigation Guidance, Interim Final, developed by USEPA (EPA 530/SW-89-031), 4 volumes (May 1989).

United States Environmental Protection Agency, Office of Environmental Information (2000). "Guidance for Data Quality Assessment, Practical Methods for Data Analysis," EPA QA/G-9, QAOO update. EPA Publication No. EPA/600/R-96-084. (Available at www.epa.gov/oswer/riskassessment/pdf/ucl.pdf).

United States Environmental Protection Agency, Office of Solid Waste and Emergency Response (2003). "Human Health Toxicity Values in Superfund Risk Assessments," OSWER Directive 9285.7-53. (Available at http://www.epa.gov/oswer/riskassessment/pdf/hhmemo.pdf)

United States Environmental Protection Agency, Compendium of Methods for Determination of Toxic Organic Compounds in Ambient Air, Second Edition, EPA Publication No. EPA/625/R-96/010b, January 1999, available at http://www.epa.gov/ttnamti1/files/ambient/airtox/tocomp99.pdf.

United States Environmental Protection Agency, Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846 through Revision IVB (February 2007), available at http://www.epa.gov/sw-846/main.htm.

United States Environmental Protection Agency, CFR Promulgated Test Methods, Methods 3C and 16, Technology Transfer Network, Emission Measurement Center (2007), available at http://www.epa.gov/ttn/emc/promgate.html.

United States Environmental Protection Agency. "Guidelines for Carcinogen Risk Assessment (2005)". U. S. Environmental Protection Agency, Washington, DC, EPA Publication No. EPA/630/P-03/001F, 2005. (Available at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283.)

"Vapor Intrusion Pathway: A Practical Guide", Technical and Regulatory Guidance. Interstate Technology and Regulatory Council (January 2007).

b) CFR (Code of Federal Regulations). Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 (202)783-3238:

40 CFR 761 (1998).

c) This Section incorporates no later editions or amendments.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.215 Determination of Soil Attenuation Capacity

a) The concentrations of organic contaminants of concern remaining in the soil shall not exceed the attenuation capacity of the soil, as determined under subsection (b) of this Section.

b) The soil attenuation capacity is not exceeded if:

1) The sum of the organic contaminant residual concentrations analyzed for the purposes of the remediation program for which the analysis is performed, at each discrete sampling point, is less than the natural organic carbon fraction of the soil. If the information relative to the concentration of other organic contaminants is available, such information shall be included in the sum. The natural organic carbon fraction (f_{oc}) shall be either:

A) A default value of 6000 mg/kg for soils within the top meter and 2000 mg/kg for soils below one meter of the surface; or

B) A site-specific value as measured by the analytical method referenced in Appendix C, Table F, multiplied by 0.58 to estimate the fraction of organic carbon, as stated in, Nelson and Sommers (1982), as incorporated by reference in Section 742.210;

2) The total petroleum hydrocarbon concentration is less than the natural organic carbon fraction of the soil as demonstrated using a method approved by the Agency. The method selected shall be appropriate for the contaminants of concern to be addressed; or

3) Another method, approved by the Agency, shows that the soil attenuation capacity is not exceeded.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.220 Determination of Soil Saturation Limit

a) For any organic contaminant that has a melting point below 30°C, the remediation objective for the outdoor inhalation exposure route developed under Tier 2 shall not exceed the soil saturation limit, as determined under subsection (c).

b) For any organic contaminant that has a melting point below 30°C, the remediation objective under Tier 2 for the soil component of the groundwater ingestion exposure route shall not exceed the soil saturation limit, as determined under subsection (c).

c) The soil saturation limit shall be:

1) The value listed in Appendix A, Table A for that specific contaminant;

2) A value derived from Equation S29 in Appendix C, Table A; or

3) A value derived from another method approved by the Agency.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.222 Determination of Soil Vapor Saturation Limit

a) For any volatile chemical, the soil gas remediation objective for the indoor and outdoor inhalation exposure routes developed under Tier 2 shall not exceed the soil vapor saturation limit, as determined under subsection (b).

b) The soil vapor saturation limit shall be:

1) The value listed in Appendix A, Table K for that specific contaminant;

2) A value derived from Equation J&E5 in Appendix C, Table L; or

3) A value derived from another method approved by the Agency.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.225 Demonstration of Compliance with Soil and Groundwater Remediation Objectives

Compliance with soil and groundwater remediation objectives is achieved if each sample result does not exceed that respective remediation objective unless a person elects to proceed under subsections (c), (d) and (e).

a) Compliance with groundwater remediation objectives developed under Subparts D through F and H through I shall be demonstrated by comparing the contaminant concentrations of discrete samples at each sample point to the applicable groundwater remediation objective. Sample points shall be determined by the program under which remediation is performed.

b) Unless the person elects to composite samples or average sampling results as provided in subsections (c) and (d), compliance with soil remediation objectives developed under Subparts D through G and I shall be demonstrated by comparing the contaminant concentrations of discrete samples to the applicable soil remediation objective.

1) Except as provided in subsections (c) and (d), compositing of samples is not allowed.

2) Except as provided in subsections (c) and (d), averaging of sample results is not allowed.

3) Notwithstanding subsections (c) and (d), compositing of samples and averaging of sample results is not allowed for the construction worker population.

4) The number of sampling points required to demonstrate compliance is determined by the requirements applicable to the program under which remediation is performed.

c) If a person chooses to composite soil samples or average soil sample results to demonstrate compliance relative to the soil component of the groundwater ingestion exposure route, the following requirements apply:

1) A minimum of two sampling locations for every 0.5 acre of contaminated area is required, with discrete samples at each sample location obtained at every two feet of depth, beginning at six inches below the ground surface for surface contamination and at the upper limit of contamination for subsurface contamination and continuing through the zone of contamination. Alternatively, a sampling method may be approved by the Agency based on an appropriately designed site-specific evaluation. Samples obtained at or below the water table shall not be used in compositing or averaging.

2) For contaminants of concern other than volatile chemicals:

A) Discrete samples from the same boring may be composited; or

B) Discrete sample results from the same boring may be averaged.

3) For volatile chemicals:

A) Compositing of samples is not allowed.

B) Discrete sample results from the same boring may be averaged.

4) Composite samples may not be averaged. An arithmetic average may be calculated for discrete samples collected at every two feet of depth through the zone of contamination as specified in subsection (c)(1).

d) If a person chooses to composite soil samples or average soil sample results to demonstrate compliance relative to the outdoor inhalation exposure route or ingestion exposure route, the following requirements apply:

1) A person shall submit a sampling plan for Agency approval, based upon a site-specific evaluation;

2) For volatile chemicals, compositing of samples is not allowed;

3) All samples shall be collected within the contaminated area;

4) Composite samples may not be averaged. Procedures specified in "Calculating Upper Confidence Limits for Exposure Point Concentrations at Hazardous Waste Sites", USEPA Office of Emergency and Remedial Response, OSWER 9285.6-10 (December 2002), as incorporated by reference in Section 742.210, or an alternative procedure approved by the Agency, shall be used to determine sample averages.

e) When averaging under this Section, if no more than 15% of sample results are reported as "non-detect", "no contamination", "below detection limits", or similar terms, such results shall be included in the averaging calculations as one-half the reported analytical detection limit for the contaminant. However, when performing a test for normal or lognormal distribution for the purpose of calculating a 95% Upper Confidence Limit of the mean for a contaminant, a person may substitute for each non-detect value a randomly generated value between, but not including, zero and the reported analytical detection limit. If more than 15% of sample results are "non-detect", procedures specified in "Guidance for Data Quality Assessment, Practical Methods for Data Analysis, EPA QA/G-9, QA00 Update", EPA/600/R-96/084 (July 2000), as incorporated by reference in Section 742.210, or an alternative procedure approved by the Agency shall be used to address the non-detect values, or another statistically valid procedure approved by the Agency may be used to determine an average.

f) All soil samples collected after August 15, 2001 shall be reported on a dry weight basis for the purpose of demonstrating compliance, with the exception of the TCLP and SPLP and the property pH.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.227 Demonstration of Compliance with Soil Gas Remediation Objectives for the Outdoor and Indoor Inhalation Exposure Routes

a) For purposes of the outdoor inhalation exposure route and the indoor inhalation exposure route, compliance with soil gas remediation objectives developed under any tier shall be demonstrated in accordance with this Section by comparing the contaminant concentrations of discrete samples at each sample point to the applicable soil gas remediation objective.

b) This Section applies to exterior soil gas samples for the outdoor inhalation exposure route, near-slab soil gas samples collected outside of an existing building for the indoor inhalation exposure route, and exterior soil gas samples collected at the footprint of a potential building for the indoor inhalation exposure route. Proposals to use sub-slab soil gas data for the indoor inhalation exposure route shall follow Section 742.935(c).

c) Sample points shall be determined by the program under which remediation is performed.

d) When collecting soil gas samples:

1) Use rigid-wall tubing made of nylon or Teflon® or other material approved by the Agency;

2) Use gas-tight, inert containers to hold the sample. For light sensitive or halogenated volatile chemicals, these containers shall be opaque or dark-colored;

3) Purge three volumes before obtaining each discrete soil gas sample;

4) Use a helium tracer or other leak apparatus detection system approved by the Agency; and

5) Limit the flow rate to 200 ml/min.

e) Soil gas samples shall be analyzed using a National Environmental Laboratory Accreditation Program (NELAP) certified laboratory.

f) Soil gas remediation objectives shall be compared to concentrations of soil gas collected at a depth at least 3 feet below ground surface and above the saturated zone.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.230 Agency Review and Approval

a) Documents and requests filed with the Agency under this Part shall be submitted in accordance with the procedures applicable to the specific program under which remediation is performed.

b) Agency review and approval of documents and requests under this Part shall be performed in accordance with the procedures applicable to the specific program under which the remediation is performed.

SUBPART C: EXPOSURE ROUTE EVALUATIONS

Section 742.300 Exclusion of Exposure Route

a) This Subpart sets forth requirements to demonstrate that an actual or potential impact to a receptor or potential receptor from a contaminant of concern can be excluded from consideration from one or more exposure routes. If an evaluation under this Subpart demonstrates the applicable requirements for excluding an exposure route are met, then the exposure route is excluded from consideration and no remediation objective(s) need be developed for that exposure route.

b) No exposure route may be excluded from consideration until characterization of the extent and concentrations of contaminants of concern at a site has been performed. The actual steps and methods taken to characterize a site shall be determined by the specific program requirements under which the site remediation is being addressed.

c) As an alternative to the use of the requirements in this Subpart, a person may use the procedures for evaluation of exposure routes under Tier 3 as set forth in Section 742.925.

(Source: Amended at 25 Ill. Reg. 10374, effective August 15, 2001)

Section 742.305 Contaminant Source and Free Product Determination

No exposure route shall be excluded from consideration relative to a contaminant of concern unless the following requirements are met:

a) The sum of the concentrations of all organic contaminants of concern shall not exceed the attenuation capacity of the soil as determined under Section 742.215;

b) The concentrations of any organic contaminants of concern remaining in the soil shall not exceed the soil saturation limit as determined under Section 742.220;

c) Any soil which contains contaminants of concern shall not exhibit any of the characteristics of reactivity for hazardous waste as determined under 35 Ill. Adm. Code 721.123;

d) Any soil which contains contaminants of concern shall not exhibit a pH less than or equal to 2.0 or greater than or equal to 12.5, as determined by SW-846 Method 9040B: pH Electrometric for soils with 20% or greater aqueous (moisture) content or by SW-846 Method 9045C: Soil pH for soils with less than 20% aqueous (moisture) content as incorporated by reference in Section 742.210;

e) Any soil which contains contaminants of concern in the following list of inorganic chemicals or their salts shall not exhibit any of the characteristics of toxicity for hazardous waste as determined by 35 Ill. Adm. Code 721.124: arsenic, barium, cadmium, chromium, lead, mercury, selenium or silver;

f) If contaminants of concern include polychlorinated biphenyls (PCBs), the concentration of any PCBs in the soil shall not exceed 50 parts per million as determined by SW-846 Methods; and

g) The concentration of any contaminant of concern in soil gas shall not exceed 10% of its Lower Explosive Limit (LEL) as measured by a hand held combustible gas indicator that has been calibrated to manufacturer specifications.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.310 Outdoor Inhalation Exposure Route

The outdoor inhalation exposure route may be excluded from consideration if:

a) The requirements in subsection (a)(1) or (a)(2) are met:

1) An approved engineered barrier is in place that meets the requirements of Subpart K; or

2) The only contaminants of concern are benzene, toluene, ethylbenzene, and total xylenes, and a demonstration of active biodegradation has been made for benzene, toluene, ethylbenzene, and total xylenes such that no outdoor inhalation exposure will occur. This demonstration shall be submitted to the Agency for review and approval;

b) The requirements of Sections 742.300 and 742.305 are met;

c) Safety precautions for the construction worker are taken if the Tier 1 construction worker remediation objectives are exceeded; and

d) An institutional control, in accordance with Subpart J, will be placed on the property.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.312 Indoor Inhalation Exposure Route

The indoor inhalation exposure route may be excluded from consideration if:

a) None of the contaminants of concern are listed on Appendix A, Table J and none of the contaminants of concern are volatile chemicals, as defined in Section 742.200; or

b) The requirements in subsections (b)(1)(A), (B) or (C) and (b)(2) and (b)(3) are met:

1) Exclusion options when the contaminants of concern are volatile chemicals:

A) No building or man-made pathway exists or will be placed above contaminated soil gas or groundwater exceeding Tier 1 remediation objectives for residential property (Appendix B, Table H), provided, however, that there is also no soil or groundwater contamination exceeding Tier 1 remediation objectives for residential property (Appendix B, Table A) or Class I groundwater (Appendix B, Table E) located 5 feet or less, horizontally, from any existing or potential building or man-made pathway; or

B) An approved building control technology is in place or will be placed that meets the requirements of Subpart L; or

C) If the contaminants of concern are benzene, toluene, ethylbenzene, and total xylenes only, a demonstration of active biodegradation has been made for benzene, toluene, ethylbenzene, and total xylenes such that no indoor inhalation exposure will occur. This demonstration shall be submitted to the Agency for review and approval;

2) The requirements of Sections 742.300 and 742.305 are met; and

3) An institutional control, in accordance with Subpart J, will be placed on the property.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.315 Soil Ingestion Exposure Route

The soil ingestion exposure route may be excluded from consideration if:

a) The requirements of Sections 742.300 and 742.305 are met;

b) An approved engineered barrier is in place that meets the requirements of Subpart K;

c) Safety precautions for the construction worker are taken if the Tier 1 construction worker remediation objectives are exceeded; and

d) An institutional control, in accordance with Subpart J, will be placed on the property.

(Source: Amended at 25 Ill. Reg. 10374, effective August 15, 2001)

Section 742.320 Groundwater Ingestion Exposure Route

The groundwater ingestion exposure route may be excluded from consideration if:

a) The requirements of Sections 742.300 and 742.305 are met;

b) The corrective action measures have been completed to remove any free product to the maximum extent practicable;

c) The source of the release is not located within the minimum or designated maximum setback zone or within a regulated recharge area of a potable water supply well;

d) As demonstrated in accordance with Section 742.1015, for any area within the measured and modeled extent of groundwater contamination above what would otherwise be the applicable Tier 1 groundwater remediation objectives, an ordinance adopted by a unit of local government is in place that effectively prohibits the installation of potable water supply wells (and the use of such wells);

e) As demonstrated using Equation R26, in Appendix C, Table C, in accordance with Section 742.810, the concentration of any contaminant of concern in groundwater within the minimum or designated maximum setback zone of an existing potable water supply well will meet the applicable Tier 1 groundwater remediation objective; and

f) As demonstrated using Equation R26, in Appendix C, Table C, in accordance with Section 742.810, the concentration of any contaminant of concern in groundwater discharging into a surface water will meet the applicable surface water quality standard under 35 Ill. Adm. Code 302.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

SUBPART D: DETERMINING AREA BACKGROUNDS

Section 742.400 Area Background

This Subpart provides procedures for determining area background concentrations for contaminants of concern. Except as described in Section 742.415(c) and (d) of this Subpart, area background concentrations may be used as remediation objectives for contaminants of concern at a site.

Section 742.405 Determination of Area Background for Soil

a) Soil sampling results shall be obtained for purposes of determining area background levels in accordance with the following procedures:

1) For volatile chemicals, sample results shall be based on discrete samples;

2) Unless an alternative method is approved by the Agency, for contaminants other than volatile chemicals, sample results shall be based on discrete

samples or composite samples. If a person elects to use composite samples, each 0.5 acre of the area to be sampled shall be divided into quadrants and 5 aliquots of equal volume per quadrant shall be composited into 1 sample;

3) Samples shall be collected from similar depths and soil types, which shall be consistent with the depths and soil types in which maximum levels of contaminants are found in the areas of known or suspected releases; and

4) Samples shall be collected from areas of the site or adjacent to the site that are unaffected by known or suspected releases at or from the site. If the sample results show an impact from releases at or from the site, then the sample results shall not be included in determining area background levels under this Part.

b) Area background shall be determined according to one of the following approaches:

1) Statewide Area Background Approach:

A) The concentrations of inorganic chemicals in background soils listed in Appendix A, Table G may be used as the upper limit of the area background concentration for the site. The first column to the right of the chemical name presents inorganic chemicals in background soils for counties within Metropolitan Statistical Areas. Counties within Metropolitan Statistical Areas are identified in Appendix A, Table G, Footnote a. Sites located in counties outside Metropolitan Statistical Areas shall use the concentrations of inorganic chemicals in background soils shown in the second column to the right of the chemical name.

B) Soil area background concentrations determined according to this statewide area background approach shall be used as provided in Section 742.415(b) of this Part. For each parameter whose sampling results demonstrate concentrations above those in Appendix A, Table G, the person shall develop appropriate soil remediation objectives in accordance with this Part, or may determine area background in accordance with subsection (b)(2).

2) A statistically valid approach for determining area background concentrations appropriate for the characteristics of the data set, and approved by the Agency.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.410 Determination of Area Background for Groundwater

a) Groundwater sampling results shall be obtained for purposes of determining area background in accordance with the following procedures:

1) Samples shall be collected from areas of the site or adjacent to the site that are unaffected by releases at the site;

2) The background monitoring wells shall be sufficient in number to account for the spatial and temporal variability, size, and number of known or suspected off-site releases of contaminants of concern, and the hydrogeological setting of the site;

3) The samples shall be collected in consecutive quarters for a minimum of one year for each well unless another sample schedule is approved by the Agency;

4) The samples shall be collected from the same stratigraphic unit(s) as the groundwater contamination at the site; and

5) The background monitoring wells shall be located hydraulically upgradient from the release(s) of contaminants of concern, unless a person demonstrates to the Agency that the upgradient location is undefinable or infeasible.

b) Area background shall be determined according to one of the following approaches:

1) Prescriptive Approach:

A) If more than 15% of the groundwater sampling results for a chemical obtained in accordance with subsection (a) of this Section are less than the appropriate detection limit for that chemical, the Prescriptive Approach may not be used for that chemical. If 15% or less of the sampling results are less than the appropriate detection limit, a concentration equal to one-half the detection limit shall be used for that chemical in the calculations contained in this Prescriptive Approach.

B) The groundwater sampling results obtained in accordance with subsection (a) of this Section shall be used to determine if the sample set is normally distributed. The Shapiro-Wilk Test of Normality shall be used to determine whether the sample set is normally distributed, if the sample set for the background well(s) contains 50 or fewer samples. Values necessary for the Shapiro-Wilk Test of Normality shall be determined using Appendix A,

Tables C and D. If the computed value of W is greater than the 5% Critical Value in Appendix A, Table D, the sample set shall be assumed to be normally distributed, and the Prescriptive Approach is allowed. If the computed value of W is less than 5% Critical Value in Appendix A, Table D, the sample set shall be assumed to not be normally distributed, and the Prescriptive Approach shall not be used.

C) If the sample set contains at least ten sample results, the Upper Tolerance Limit (UTL) of a normally distributed sample set may be calculated using the mean (x) and standard deviation(s), from:

$$UTL = x + (K \bullet s),$$

where K = the one-sided normal tolerance factor for estimating the 95% upper confidence limit of the 95th percentile of a normal distribution. Values for K shall be determined using Appendix A, Table B.

D) If the sample set contains at least ten sample results, the UTL shall be the upper limit of the area background concentration for the site. If the sample set contains fewer than ten sample results, the maximum value of the sample set shall be the upper limit of the area background concentration for the site.

E) This Prescriptive Approach shall not be used for determining area background for the parameter pH.

2) Another statistically valid approach for determining area background concentrations appropriate for the characteristics of the data set, and approved by the Agency.

Section 742.415 Use of Area Background Concentrations

a) A person may request that area background concentration determined pursuant to Sections 742.405 and 742.410 be used according to the provisions of subsection (b) of this Section. Such request shall address the following:

1) The natural or man-made pathways of any suspected off-site contamination reaching the site;

2) Physical and chemical properties of suspected off-site contaminants of concern reaching the site; and

3) The location and justification of all background sampling points.

b) Except as specified in subsections (c) and (d) of this Section, an area background concentration may be used as follows:

1) To support a request to exclude a chemical as a contaminant of concern from further consideration for remediation at a site due to its presence as a result of background conditions; or

2) As a remediation objective for a contaminant of concern at a site in lieu of an objective developed pursuant to the other procedures of this Part.

c) An area background concentration shall not be used *in the event that the Agency has determined in writing that the background level for a regulated substance poses an acute threat to human health or the environment at the site when considering the post-remedial action land use.* (Section 58.5(b)(3) of the Act)

d) *In the event that the concentration of a regulated substance of concern on the site exceeds a remediation objective adopted by the Board for residential land use, the property may not be converted to residential use unless such remediation objective or an alternative risk-based remediation objective for that regulated substance of concern is first achieved.* If the land use is restricted, there shall be an institutional control in place in accordance with Subpart J. (Section 58.5(b)(2) of the Act)

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

SUBPART E: TIER 1 EVALUAION

Section 742.500 Tier 1 Evaluation Overview

Section 742.500 Tier 1 Evaluation Overview

a) A Tier 1 evaluation compares the concentration of each contaminant of concern detected at a site to the baseline remediation objectives provided in Appendix B, Tables A, B, C, D, E, G, H and I. Use of Tier 1 remediation objectives requires only limited site-specific information: concentrations of contaminants of concern, groundwater classification, land use classification, and, if appropriate, soil pH. (See Appendix B, Illustration A.)

b) Although Tier 1 allows for differentiation between residential and industrial/commercial property use of a site, an institutional control under Subpart J is required where remediation objectives are based on an industrial/commercial property use.

c) For the indoor inhalation exposure route:

1) Appendix B, Tables H and I apply only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls; and

2) Institutional controls under Subpart J are required to use remediation objectives in Appendix B, Table H or Table I.

d) Any given exposure route is not a concern if the concentration of each contaminant of concern detected at the site is below the Tier 1 value of that given route. In such a case, no further evaluation of that route is necessary.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.505 Tier 1 Soil, Soil Gas and Groundwater Remediation Objectives

a) Soil

1) Outdoor Inhalation Exposure Route

A) The Tier 1 soil remediation objectives for this exposure route based upon residential property use are listed in Appendix B, Table A.

B) The Tier 1 soil remediation objectives for this exposure route based upon industrial/commercial property use are listed in Appendix B, Table B. Soil remediation objective determinations relying on this table require use of institutional controls in accordance with Subpart J.

C) For this exposure route, it is acceptable to determine compliance by meeting either the soil or soil gas remediation objectives.

2) Ingestion Exposure Route

A) The Tier 1 soil remediation objectives for this exposure route based upon residential property use are listed in Appendix B, Table A.

B) The Tier 1 soil remediation objectives for this exposure route based upon industrial/commercial property use are listed in Appendix B, Table B. Soil remediation objective determinations relying on this table require use of institutional controls in accordance with Subpart J.

3) Soil Component of the Groundwater Ingestion Route

A) The Tier 1 soil remediation objectives for this exposure route based upon residential property use are listed in Appendix B, Table A.

B) The Tier 1 soil remediation objectives for this exposure route based upon industrial/commercial property use are listed in Appendix B, Table B.

C) The pH-dependent Tier 1 soil remediation objectives for identified ionizable organics or inorganics for the soil component of the groundwater ingestion exposure route (based on the total amount of contaminants present in the soil sample results and groundwater classification) are provided in Appendix B, Tables C and D.

D) Values used to calculate the Tier 1 soil remediation objectives for this exposure route are listed in Appendix B, Table F.

4) Evaluation of the dermal contact with soil exposure route is not required under Tier 1.

b) Soil Gas

1) Outdoor Inhalation Exposure Route

A) The Tier 1 soil gas remediation objectives for this exposure route based upon residential property use are listed in Appendix B, Table G.

B) The Tier 1 soil gas remediation objectives for this exposure route based upon industrial/commercial property use, including the construction worker population, are listed in Appendix B, Table G. Soil gas remediation objective determinations relying on an industrial/commercial scenario require use of institutional controls in accordance with Subpart J.

C) For this exposure route, it is acceptable to determine compliance by meeting either the soil or soil gas remediation objectives.

2) Indoor Inhalation Exposure Route

A) The Tier 1 soil gas remediation objectives for this exposure route are listed in Appendix B, Tables H and I.

B) The Tier 1 soil gas remediation objectives for this exposure route are based on a default water-filled soil porosity value of 0.15 cm^3/cm^3 and the assumed presence of a building with a 10-cm thick, full concrete slab-on-grade.

C) Appendix B, Table H shall be used when any soil or groundwater contamination is located 5 feet or less, vertically or horizontally, from the existing or potential building or man-made pathway. In this scenario, the mode of contaminant transport is both diffusion and advection, which sets the Q_{soil} value at 83.33 cm^3/sec. Appendix B, Table H applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Pursuant to Section 742.1000(a)(9), soil gas remediation objective determinations relying on Appendix B, Table H require the use of institutional controls in accordance with Subpart J.

D) Appendix B, Table I may be used only when all soil and groundwater contamination is located more than 5 feet, vertically and horizontally, from the existing or potential building or man-made pathway. In this scenario, the mode of contaminant transport is diffusion only, which sets the Q_{soil} value at 0.0 cm^3/sec. Appendix B, Table I applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Pursuant to Section 742.1000(a)(7) and (a)(9), soil gas remediation objective determinations relying on Appendix B, Table I require the use of institutional controls in accordance with Subpart J. As an alternative to using Appendix B, Table I, it is permissible to use Appendix B, Table H.

E) To determine whether the Q_{soil} value can be set at 0.0 cm^3/sec, the site evaluator shall demonstrate that all soil and groundwater located 5 feet or less, vertically or horizontally, from the existing or potential building or man-made pathway meets the Tier 1 remediation objectives for residential property listed in Appendix B, Table A, and the Tier 1 remediation objectives for Class I groundwater listed in Appendix B, Table E, respectively.

c) Groundwater

1) The Tier 1 groundwater remediation objectives for the groundwater component of the groundwater ingestion route are listed in Appendix B, Table E.

2) The Tier 1 groundwater remediation objectives for this exposure route are given for Class I and Class II groundwaters, respectively.

3) The evaluation of 35 Ill. Adm. Code 620.615 regarding mixtures of similar-acting chemicals shall be considered satisfied for Class I groundwater at the point of human exposure if:

A) No more than one similar-acting noncarcinogenic chemical as listed in Appendix A, Table E is detected in the groundwater at the site; and

B) No carcinogenic contaminant of concern as listed in Appendix A, Table I is detected in any groundwater sample associated with the site, using analytical procedures capable of achieving either the 1 in 1,000,000 cancer risk concentration or the ADL, whichever is greater.

4) If the conditions of subsection (c)(3) of this Section are not met, the Class I groundwater remediation objectives set forth in Appendix B, Table E shall be corrected for the cumulative effect of mixtures of similar-acting chemicals using the following methodologies:

A) For noncarcinogenic chemicals, the methodologies set forth at Section 742.805(c) or Section 742.915(h) shall be used; and

B) For carcinogenic chemicals, the methodologies set forth at Section 742.805(d) or Section 742.915(h) shall be used.

5) For the groundwater component of the indoor inhalation exposure route, the Tier 1 groundwater remediation objectives are listed in Appendix B, Tables H and I.

A) The Tier 1 groundwater remediation objectives for this exposure route are based on a default water-filled soil porosity value of 0.15 cm^3/cm^3 and the assumed presence of a building with a 10-cm thick, full concrete slab-on-grade.

B) Appendix B, Table H shall be used when any soil or groundwater contamination is located 5 feet or less, vertically or horizontally, from the existing or potential building or man-made pathway. In this scenario, the mode of contaminant transport is both diffusion and advection, which sets the Q_{soil} value at 83.33 cm^3/sec. Appendix B, Table H applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Pursuant to Section 742.1000(a)(9), groundwater remediation objective determinations relying on Appendix B, Table H require the use of institutional controls in accordance with Subpart J.

C) Appendix B, Table I may be used only when all soil and groundwater contamination is located more than 5 feet, vertically and horizontally, from the existing or potential building or man-made pathway. In this scenario, the mode of contaminant transport

is diffusion only, which sets the Q_{soil} value at 0.0 cm^3/sec. Appendix B, Table I applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Pursuant to Section 742.1000(a)(7) and (a)(9), groundwater remediation objective determinations relying on Appendix B, Table I require the use of institutional controls in accordance with Subpart J. As an alternative to using Appendix B, Table I, it is permissible to use Appendix B, Table H.

D) To determine whether the Q_{soil} value can be set at 0.0 cm^3/sec, the site evaluator shall demonstrate that all soil and groundwater located 5 feet or less, vertically or horizontally, from the existing or potential building or man-made pathway meets the Tier 1 remediation objectives for residential property listed in Appendix B, Table A, and the Tier 1 remediation objectives for Class I groundwater listed in Appendix B, Table E, respectively.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.510 Tier 1 Remediation Objectives Tables for the Ingestion, Outdoor Inhalation and Soil Component of the Groundwater Ingestion Exposure Routes

a) Soil remediation objectives are listed in Appendix B, Tables A, B, C and D.

1) Appendix B, Table A is based upon residential property use.

A) The first column to the right of the chemical name lists soil remediation objectives for the soil ingestion exposure route.

B) The second column lists the soil remediation objectives for the outdoor inhalation exposure route.

C) The third and fourth columns list soil remediation objectives for the soil component of the groundwater ingestion exposure route for the respective classes of groundwater:

i) Class I groundwater; and

ii) Class II groundwater.

D) The final column lists the Acceptable Detection Limit (ADL), only when applicable.

2) Appendix B, Table B is based upon industrial/commercial property use.

A) The first and third columns to the right of the chemical name list the soil remediation objectives for the soil ingestion exposure route based on two receptor populations:

i) Industrial/commercial; and

ii) Construction worker.

B) The second and fourth columns to the right of the chemical name list the soil remediation objectives for the outdoor inhalation exposure route based on two receptor populations:

i) Industrial/commercial; and

ii) Construction worker.

C) The fifth and sixth columns to the right of the chemical name list the soil remediation objectives for the soil component of the groundwater ingestion exposure route for two classes of groundwater:

i) Class I groundwater; and

ii) Class II groundwater.

D) The final column lists the acceptable detection limit (ADL), only when applicable.

3) Appendix B, Tables C and D set forth pH specific soil remediation objectives for inorganic and ionizing organic chemicals for the soil component of the groundwater ingestion route.

A) Table C sets forth remediation objectives based on Class I groundwater and Table D sets forth remediation objectives based on Class II groundwater.

B) The first column in Tables C and D lists the chemical names.

C) The second through ninth columns to the right of the chemical names list the pH based soil remediation objectives.

4) For the inorganic chemicals listed in Appendix B, Tables A and B, the soil component of the groundwater ingestion exposure route shall be evaluated using TCLP (SW-846 Method 1311) or SPLP (SW-846 Method 1312), incorporated by reference at Section 742.210 unless a person chooses to evaluate the soil component on the basis of the total amount of

contaminant in a soil sample result in accordance with subsection (a)(5) of this Section.

5) For those inorganic and ionizing organic chemicals listed in Appendix B, Tables C and D, if a person elects to evaluate the soil component of the groundwater ingestion exposure route based on the total amount of contaminant in a soil sample result (rather than TCLP or SPLP analysis), the person shall determine the soil pH at the site and then select the appropriate soil remediation objectives based on Class I and Class II groundwaters from Tables C and D, respectively. If the soil pH is less than 4.5 or greater than 9.0, then Tables C and D cannot be used.

6) Unless one or more exposure routes are excluded from consideration under Subpart C, the most stringent soil remediation objective of the exposure routes (i.e., soil ingestion exposure route, outdoor inhalation exposure route, and soil component of the groundwater ingestion exposure route) shall be compared to the concentrations of soil contaminants of concern measured at the site. When using Appendix B, Table B to select soil remediation objectives for the ingestion exposure route and outdoor inhalation exposure routes, the remediation objective shall be the more stringent soil remediation objective of the industrial/commercial populations and construction worker populations.

7) Confirmation sample results may be averaged or soil samples may be composited in accordance with Section 742.225.

8) If a soil remediation objective for a chemical is less than the ADL, the ADL shall serve as the soil remediation objective.

b) Groundwater remediation objectives for the groundwater component of the groundwater ingestion exposure route are listed in Appendix B, Table E. However, Appendix B, Table E must be corrected for cumulative effect of mixtures of similar-acting noncarcinogenic chemicals as set forth in Section 742.505(c)(3) and (c)(4).

1) The first column to the right of the chemical name lists groundwater remediation objectives for Class I groundwater, and the second column lists the groundwater remediation objectives for Class II groundwater.

2) To use Appendix B, Table E of this Part, the 35 Ill. Adm. Code 620 classification for groundwater at the site shall be determined. The concentrations of groundwater contaminants of concern at the site are compared to the applicable Tier 1 groundwater remediation objectives for the groundwater component of the groundwater ingestion exposure route in Appendix B, Table E.

c) Soil gas remediation objectives for the outdoor inhalation exposure route are listed in Appendix B, Table G.

1) The first column to the right of the chemical name lists the soil gas remediation objectives for residential populations.

2) The second and third columns to the right of the chemical names list the soil gas remediation objectives for the outdoor inhalation exposure route based on two receptor populations:

A) Industrial/commercial; and

B) Construction worker.

d) For contaminants of concern not listed in Appendix B, Tables A, B, E, and G, a person may request site-specific remediation objectives from the Agency or propose site-specific remediation objectives in accordance with 35 Ill. Adm. Code 620, Subpart I of this Part, or both.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.515 Tier 1 Remediation Objectives Tables for the Indoor Inhalation Exposure Route

a) For the indoor inhalation exposure route:

1) Appendix B, Tables H and I apply only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls; and

2) Institutional controls under Subpart J are required to use remediation objectives in Appendix B, Table H or Table I.

b) When the mode of contaminant transport is both diffusion and advection as described in Section 742.505 (i.e., any soil or groundwater contamination is located 5 feet or less, vertically or horizontally, from the existing or potential building or man-made pathway), the remediation objectives for soil gas or groundwater listed in Appendix B, Table H shall be used.

1) The first column to the right of the chemical name lists the soil gas remediation objectives for residential receptors.

2) The second column lists the soil gas remediation objectives for industrial/commercial receptors.

3) The third column lists the groundwater remediation objectives for residential receptors.

4) The fourth column lists the groundwater remediation objectives for industrial/commercial receptors.

c) Only when the mode of contaminant transport is diffusion only as described in Section 742.505 (i.e., all soil and groundwater contamination is located more than 5 feet, vertically and horizontally, from the existing or potential building or man-made pathway), the remediation objectives for soil gas and groundwater listed in Appendix B, Table I may be used.

1) The first column to the right of the chemical name lists the soil gas remediation objectives for residential receptors.

2) The second column lists the soil gas remediation objectives for industrial/commercial receptors.

3) The third column lists the groundwater remediation objectives for residential receptors.

4) The fourth column lists the groundwater remediation objectives for industrial/commercial receptors.

d) If using Appendix B, Table H, compliance is determined by meeting either the soil gas remediation objectives or the groundwater remediation objectives.

e) If using Appendix B, Table I, compliance is determined by meeting both the soil gas remediation objectives and the groundwater remediation objectives.

f) For volatile chemicals not listed in Appendix B, Table H or I, a person may request site-specific remediation objectives from the Agency or propose site-specific remediation objectives in accordance with Subpart I, or both.

g) As an alternative to using Appendix B, Table I pursuant to subsection (c), it is permissible to use Appendix B, Table H pursuant to subsection (b).

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

SUBPART F: TIER 2 GENERAL EVALUATION

Section 742.600 Tier 2 Evaluation Overview

a) Tier 2 remediation objectives are developed through the use of equations which allow site-specific data to be used. (See Appendix C, Illustrations A and B.) The

equations, identified in Appendix C, Tables A, C, and L may be used to develop Tier 2 remediation objectives.

b) Tier 2 evaluation is only required for contaminants of concern and corresponding exposure routes (except where excluded from further consideration under Subpart C) exceeding the Tier 1 remediation objectives. When conducting Tier 2 evaluations, the values used in the calculations must have the appropriate units of measure as identified in Appendix C, Tables B, D, and M.

c) Any development of remediation objectives using site-specific information or equations outside the Tier 2 framework shall be evaluated under Tier 3.

d) Any development of a remediation objective under Tier 2 shall not use a target hazard quotient greater than one at the point of human exposure or a target cancer risk greater than 1 in 1,000,000 at the point of human exposure.

e) In conducting a Tier 2 evaluation, the following conditions shall be met:

1) For each discrete sample, the total soil contaminant concentration of either a single contaminant or multiple contaminants of concern shall not exceed the attenuation capacity of the soil as provided in Section 742.215.

2) Remediation objectives for noncarcinogenic compounds which affect the same target organ, organ system or similar mode of action shall meet the requirements of Section 742.720.

3) The soil remediation objectives based on the outdoor inhalation exposure route and the soil component of the groundwater ingestion exposure routes shall not exceed the soil saturation limit as provided in Section 742.220.

4) The soil gas remediation objectives based on the indoor and outdoor inhalation exposure routes shall not exceed the soil vapor saturation limit provided pursuant to Section 742.222.

f) Tier 2 remediation objectives for the indoor inhalation exposure route shall be calculated for either soil gas or groundwater if a Q_{soil} value of 83.33 cm^3/sec is used.

g) Tier 2 remediation objectives for the indoor inhalation exposure route shall be calculated for both soil gas and groundwater if a Q_{soil} value of 0.0 cm^3/sec is used.

h) If the calculated Tier 2 soil remediation objective for an applicable exposure route is more stringent than the corresponding Tier 1 remediation objective, then the Tier 1 remediation objective applies.

i) If the calculated Tier 2 soil remediation objective for an exposure route is more stringent than the Tier 1 soil remediation objectives for the other exposure routes, then the Tier 2 calculated soil remediation objective applies and Tier 2 soil remediation objectives for the other exposure routes are not required.

j) If the calculated Tier 2 soil remediation objective is less stringent than one or more of the soil remediation objectives for the remaining exposure routes, then the Tier 2 values are calculated for the remaining exposure routes and the most stringent Tier 2 calculated value applies.

k) If a contaminant has both carcinogenic and noncarcinogenic effects for any applicable exposure route or receptor, remediation objectives shall be calculated for each effect and the more stringent remediation objective shall apply. The toxicological-specific information is described in Section 742.705(d).

l) For the indoor inhalation exposure route:

1) Appendix C, Table L applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls; and

2) Institutional controls under Subpart J are required to develop remediation objectives pursuant to Appendix C, Table L.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.605 Land Use

a) Present and post-remediation land use is evaluated in a Tier 2 evaluation. Acceptable exposure factors for the Tier 2 evaluation for residential, industrial/commercial, and construction worker populations are provided in the far right column of Appendix C, Tables B, D, and M. Use of exposure factors different from those in Appendix C, Tables B, D, and M must be approved by the Agency as part of a Tier 3 evaluation.

b) If a Tier 2 evaluation is based on an industrial/commercial property use, then:

1) Construction worker populations shall also be evaluated, except for the indoor inhalation exposure route; and

2) Institutional controls are required in accordance with Subpart J.

c) For the indoor inhalation exposure route, institutional controls under Subpart J are required to develop remediation objectives pursuant to Appendix C, Table L.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.610 Chemical and Site Properties

a) Physical and Chemical Properties of Contaminants

Tier 2 evaluations require information on the physical and chemical properties of the contaminants of concern. The physical and chemical properties used in a Tier 2 evaluation are contained in Appendix C, Table E. If the site has contaminants not included in this table, a person may request the Agency to provide the applicable physical and chemical input values or may propose input values under Subpart I. If a person proposes to apply values other than those in Appendix C, Table E, or those provided by the Agency, the evaluation shall be considered under Tier 3.

b) Soil and Groundwater Parameters

1) A Tier 2 evaluation requires examination of soil and groundwater parameters. The parameters that may be varied, and the conditions under which these parameters are determined as part of Tier 2, are summarized in Appendix C, Tables B, D, and M. If a person proposes to vary site-specific parameters outside of the framework of these tables, the evaluation shall be considered under Tier 3.

2) To determine site-specific physical soil parameters, a minimum of one boring per 0.5 acre of contamination shall be collected. This boring must be deep enough to allow the collection of the required field measurements. The site-specific physical soil parameters must be determined from the portion of the boring representing the stratigraphic units being evaluated. For example, if evaluating the soil component of the groundwater ingestion exposure route, two samples from the boring will be required:

A) A sample of the predominant soil type for the vadose zone; and

B) A sample of the predominant soil type for the saturated zone.

3) A site-specific SSL dilution factor (used in developing soil remediation objectives based upon the protection of groundwater) may be determined by substituting site information in Equation S22 in Appendix C, Table A. To make this demonstration, a minimum of three monitoring wells shall be used to determine the hydraulic gradient. As an alternative, the default dilution factor value listed in Appendix C, Table B may be used. If monitoring wells are used to determine the hydraulic gradient, the soil taken from the borings shall be visually inspected to ensure there are no significant differences in the stratigraphy. If there are similar soil types in

the field, one boring shall be used to determine the site-specific physical soil parameters. If there are significant differences, all of the borings shall be evaluated before determining the site-specific physical soil parameters for the site.

4) Not all of the parameters identified in Appendix C, Tables B, D, and M need to be determined on a site-specific basis. A person may choose to collect partial site-specific information and use default values as listed in Appendix C, Tables B, D, and M for the rest of the parameters.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

SUBPART G: TIER 2 SOIL EVALUATION

Section 742.700 Tier 2 Soil and Soil Gas Evaluation Overview

a) Tier 2 remediation objectives are developed through the use of models which allow site-specific data to be considered. Appendix C, Tables A, C, and L list equations that shall be used under a Tier 2 evaluation to calculate soil remediation objectives prescribed by the SSL, RBCA, and modified J&E models, respectively. (See also Appendix C, Illustration A.)

b) Appendix C, Table A lists equations that are used under the SSL model. (See also Appendix C, Illustration A.) The SSL model has equations to evaluate the following human exposure routes:

1) Soil ingestion exposure route;

2) Outdoor Inhalation exposure route; and

3) Soil component of the groundwater ingestion exposure route.

c) Evaluation of the dermal exposure route is not required under the SSL model.

d) Appendix C, Table C lists equations that are used under the RBCA model. (See also Appendix C, Illustration A.) The RBCA model has equations to evaluate human exposure based on the following:

1) The combined exposure routes of outdoor inhalation of vapors and particulates, soil ingestion and dermal contact with soil;

2) The outdoor inhalation exposure route from subsurface soils;

3) Soil component of the groundwater ingestion exposure route; and

4) Groundwater ingestion exposure route.

e) Appendix C, Table L lists equations that are used under the modified J&E model. The modified J&E model has equations to evaluate human exposure by the indoor inhalation exposure route. The modified model allows for the development of soil gas remediation objectives. For the indoor inhalation exposure route:

1) Appendix C, Table L applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls; and

2) Institutional controls under Subpart J are required to develop soil gas remediation objectives pursuant to Appendix C, Table L.

f) The equations in either Appendix C, Table A, C, or L may be used to calculate remediation objectives for each contaminant of concern under Tier 2, if the following requirements are met:

1) The Tier 2 soil or soil gas remediation objectives for the ingestion and outdoor inhalation exposure routes shall use the applicable equations from the same approach (i.e., SSL equations in Appendix C, Table C). For the indoor inhalation exposure route, only the J&E equations can be used.

2) The equations used to calculate soil remediation objectives for the soil component of the groundwater ingestion exposure route are not dependent on the approach utilized to calculate soil remediation objectives for the other exposure routes. For example, it is acceptable to use the SSL equations for calculating Tier 2 soil remediation objectives for the ingestion and outdoor inhalation exposure routes, and the RBCA equations for calculating Tier 2 soil remediation objectives for the soil component of the groundwater ingestion exposure route.

3) Combining equations from Appendix C, Tables A, C, and L to form a new model is not allowed. In addition, Appendix C, Tables A, C, and L must use their own applicable parameters identified in Appendix C, Tables B, D, and M, respectively.

g) In calculating soil or soil gas remediation objectives for industrial/commercial property use, applicable calculations shall be performed twice: once using industrial/commercial population default values and once using construction worker population default values. The more stringent soil or soil gas remediation objectives derived from these calculations must be used for further Tier 2 evaluations. The indoor inhalation exposure route does not apply to the construction worker population.

h) Tier 2 data sheets provided by the Agency shall be used to present calculated Tier 2 remediation objectives, if required by the particular program for which remediation is being performed.

i) The RBCA equations which rely on the parameter Soil Water Sorption Coefficient (k_s) can only be used for ionizing organics and inorganics by substituting values for k_s from Appendix C, Tables I and J, respectively. This will also require the determination of a site-specific value for soil pH.

j) For the outdoor inhalation exposure route, it is acceptable to use either Section 742.710 to develop a soil remediation objective or Section 742.712 to develop a soil gas remediation objective to determine compliance with the pathway.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.705 Parameters for Soil Remediation Objective Equations

a) Appendix C, Tables B, D, and M list the input parameters for the SSL, RBCA, and J&E equations, respectively. The first column lists each symbol as it is presented in the equation. The next column defines the parameters. The third column shows the units for the parameters. The fourth column identifies where information on the parameters can be obtained (i.e., field measurement, applicable equations, reference source, or default value). The last column identifies how the parameters can be generated.

b) Default Values

Default values are numerical values specified for use in the Tier 2 equations. The fourth column of Appendix C, Tables B, D, and M denotes if the default values are from the SSL model, RBCA model, modified J&E model or some other source. The last column of Appendix C, Tables B, D, and M lists the numerical values for the default values used in the SSL, RBCA, and J&E equations, respectively.

c) Site-specific Information

Site-specific information is a parameter measured, obtained, or determined from the site to calculate Tier 2 remediation objectives. The fourth column of Appendix C, Tables B, D, and M identifies those site-specific parameters that may require direct field measurement. For some parameters, numerical default inputs have been provided in the last column of Appendix C, Tables B, D, and M to substitute for site-specific information. In some cases, information on the receptor or soil type is required to select the applicable numerical default inputs. Site-specific information includes:

1) Physical soil parameters identified in Appendix C, Table F. The second column identifies the location where the sample is to be collected. Acceptable methods for measuring or calculating these soil parameters are identified in the last column of Appendix C, Table F;

2) Institutional controls or engineered barriers, pursuant to Subparts J and K, describe applicable institutional controls and engineered barriers under a Tier 2 evaluation; and

3) Land use classification

d) Toxicological-specific Information

1) Toxicological-specific information is used to calculate Tier 2 remediation objectives for the following parameters, if applicable:

A) Oral Chronic Reference Dose (RfD_o, expressed in mg/kg-d);

B) Oral Subchronic Reference Dose (RfD_s, expressed in mg/kg-d, shall be used for construction worker remediation objective calculations);

C) Oral Slope Factor (SF_o, expressed in $(mg/kg\text{-}d)^{-1}$);

D) Inhalation Unit Risk Factor (URF expressed in $(\mu g/m^3)^{-1}$);

E) Inhalation Chronic Reference Concentration (RfC, expressed in mg/m^3);

F) Inhalation Subchronic Reference Concentration (RfC_s, expressed in mg/m^3, shall be used for construction worker remediation objective calculations);

G) Inhalation Chronic Reference Dose (RfD_i, expressed in mg/kg-d);

H) Inhalation Subchronic Reference Dose (RfD_{is}, expressed in mg/kg-d, shall be used for construction worker remediation objective calculations); and

I) Inhalation Slope Factor (SF_i, expressed in $(mg/kg\text{-}d)^{-1}$);

2) Toxicological information can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210, or the program under which the remediation is being performed.

e) Chemical-specific Information

Chemical-specific information used to calculate Tier 2 remediation objectives is listed in Appendix C, Table E.

f) Calculations
Calculating numerical values for some parameters requires the use of equations listed in Appendix C, Tables A, C, and L. The parameters that are calculated are listed in Appendix C, Tables B, D, and M.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.710 SSL Soil Equations

a) This Section sets forth the equations and parameters used to develop Tier 2 soil remediation objectives for the three exposure routes using the SSL approach.

b) Soil Ingestion Exposure Route

1) Equations S1 through S3 form the basis for calculating Tier 2 remediation objectives for the soil ingestion exposure route using the SSL approach. Equation S1 is used to calculate soil remediation objectives for noncarcinogenic contaminants. Equations S2 and S3 are used to calculate soil remediation objectives for carcinogenic contaminants for residential populations and industrial/commercial and construction worker populations, respectively.

2) For Equations S1 through S3, the SSL default values cannot be modified with site-specific information.

c) Outdoor Inhalation Exposure Route

1) Equations S4 through S16, S26 and S27 are used to calculate Tier 2 soil remediation objectives for the outdoor inhalation exposure route using the SSL approach. To address this exposure route, organic contaminants and mercury must be evaluated separately from fugitive dust using their own equations set forth in subsections (c)(2) and (c)(3), respectively.

2) Organic Contaminants

A) Equations S4 through S10 are used to calculate Tier 2 soil remediation objectives for organic contaminants and mercury based on the outdoor inhalation exposure route. Equation S4 is used to calculate soil remediation objectives for noncarcinogenic organic contaminants in soil for residential and industrial/commercial populations. Equation S5 is used to calculate soil remediation objectives for noncarcinogenic organic

contaminants and mercury in soil for construction worker populations. Equation S6 is used to calculate soil remediation objectives for carcinogenic organic contaminants in soil for residential and industrial/commercial populations. Equation S7 is used to calculate soil remediation objectives for carcinogenic organic contaminants in soil for construction worker populations. Equations S8 through S10, S27 and S28 are used for calculating numerical values for some of the parameters in Equations S4 through S7.

B) For Equation S4, a numerical value for the Volatilization Factor (VF) can be calculated in accordance with subsection (c)(2)(F). The remaining parameters in Equation S4 have either SSL default values listed in Appendix C, Table B or toxicological-specific information (i.e., RfC), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

C) For Equation S5, a numerical value for the Volatilization Factor adjusted for Agitation (VF') can be calculated in accordance with subsection (c)(2)(G). The remaining parameters in Equation S5 have either SSL default values listed in Appendix C, Table B or toxicological-specific information (i.e., RfC), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

D) For Equation S6, a numerical value for VF can be calculated in accordance with subsection (c)(2)(F). The remaining parameters in Equation S6 have either default values listed in Appendix C, Table B or toxicological-specific information (i.e., URF), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

E) For Equation S7, a numerical value for VF' can be calculated in accordance with subsection (c)(2)(G). The remaining parameters in Equation S7 have either default values listed in Appendix C, Table B or toxicological-specific information (i.e., URF), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

F) The VF can be calculated for residential and industrial/commercial populations using one of the following equations based on the information known about the contaminant source and receptor population:

i) Equation S8, in conjunction with Equation S10, is used to calculate VF assuming an infinite source of contamination; or

ii) If the area and depth of the contaminant source are known or can be estimated reliably, mass limit considerations may be used to calculate VF using Equation S26.

G) The VF' can be calculated for the construction worker populations using one of the following equations based on the information known about the contaminant source:

i) Equation S9 is used to calculate VF' assuming an infinite source of contamination; or

ii) If the area and depth of the contaminant source are known or can be estimated reliably, mass limit considerations may be used to calculate VF' using Equation S27.

3) Fugitive Dust

A) Equations S11 through S16 are used to calculate Tier 2 soil remediation objectives using the SSL fugitive dust model for the outdoor inhalation exposure route. Equation S11 is used to calculate soil remediation objectives for noncarcinogenic contaminants in fugitive dust for residential and industrial/commercial populations. Equation S12 is used to calculate soil remediation objectives for noncarcinogenic contaminants in fugitive dust for construction worker populations. Equation S13 is used to calculate soil remediation objectives for carcinogenic contaminants in fugitive dust for residential and industrial/commercial populations. Equation S14 is used to calculate soil remediation objectives for carcinogenic contaminants in fugitive dust for construction worker populations. Equations S15 and S16 are used for calculating numerical quantities for some of the parameters in Equations S11 through S14.

B) For Equation S11, a numerical value can be calculated for the Particulate Emission Factor (PEF) using Equation S15. This equation relies on various input parameters from a variety of

sources. The remaining parameters in Equation S11 have either SSL default values listed in Appendix C, Table B or toxicological-specific information (i.e., RfC), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

C) For Equation S12, a numerical value for the Particulate Emission Factor for Construction Worker (PEF') can be calculated using Equation S16. The remaining parameters in Equation S12 have either SSL default values listed in Appendix C, Table B or toxicological-specific information (i.e., RfC), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

D) For Equation S13, a numerical value for PEF can be calculated using Equation S15. The remaining parameters in Equation S13 have either default values listed in Appendix C, Table B or toxicological-specific information (i.e., URF), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

E) For Equation S14, a numerical value for PEF' can be calculated using Equation S16. The remaining parameters in Equation S14 have either default values listed in Appendix C, Table B or toxicological-specific information (i.e., URF), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

d) Soil Component of the Groundwater Ingestion Exposure Route

The Tier 2 remediation objective for the soil component of the groundwater ingestion exposure route can be calculated using one of the following equations based on the information known about the contaminant source and receptor population:

1) Equation S17 is used to calculate the remediation objective assuming an infinite source of contamination.

A) The numerical quantities for four parameters in Equation S17, the Target Soil Leachate Concentration (C_w), Soil-Water Partition Coefficient (K_d) for non-ionizing organics, Water-Filled Soil Porosity Theta w (θ_w) and Air-Filled Soil Porosity Theta a (θ_a), are calculated using Equations S18, S19, S20 and S21,

respectively. Equations S22, S23, S24 and S25 are also needed to calculate numerical values for Equations S18 and S21. The pH-dependent K_d values for ionizing organics can be calculated using Equation S19 and the pH-dependent Koc values in Appendix C, Table I.

B) The remaining parameters in Equation S17 are Henry's Law Constant (H'), a chemical specific value listed in Appendix C, Table E and Dry Soil Bulk Density (ρ_b), a site-specific based value listed in Appendix C, Table B.

C) The default value for GW_{obj} is the Tier 1 groundwater objective. For chemicals for which there is no Tier 1 groundwater remediation objective, the value for GW_{obj} shall be the concentration determined according to the procedures specified in 35 Ill. Adm. Code 620, Subpart F. As an alternative to using Tier 1 groundwater remediation objectives or concentrations determined according to the procedures specified in 35 Ill. Adm. Code 620, Subpart F, GW_{obj} may be developed using Equations R25 and R26, if approved institutional controls are in place as required in Subpart J.

2) If the area and depth of the contaminant source are known or can be estimated reliably, mass limit considerations may be used to calculate the remediation objective for this exposure route using Equation S28. The parameters in Equation S28 have default values listed in Appendix C, Table B.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.712 SSL Soil Gas Equation for the Outdoor Inhalation Exposure Route

a) This Section sets forth the equation and parameters used to develop Tier 2 soil gas remediation objectives for the outdoor inhalation exposure route using the SSL approach.

b) Equation S30 is used to calculate Tier 2 soil gas remediation objectives for the outdoor inhalation exposure route for residential, industrial/commercial, and construction worker populations.

c) Equations S4 through S16, S26 and S27, which calculate Tier 2 soil remediation objectives as described in Section 742.710(c), form the basis for developing the Tier 2 soil gas remediation objectives for the outdoor inhalation exposure route using the SSL model.

d) The remaining parameters used to calculate Equation S30 are listed in Appendix C, Table B, except for Dimensionless Henry's Law Constant (25°C), a chemical specific value listed in Appendix C, Table E.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.715 RBCA Soil Equations

a) This Section presents the RBCA model and describes the equations and parameters used to develop Tier 2 soil remediation objectives.

b) Ingestion, Outdoor Inhalation, and Dermal Contact

1) The two sets of equations in subsections (b)(2) and (b)(3) shall be used to generate Tier 2 soil remediation objectives for the combined ingestion, outdoor inhalation, and dermal contact with soil exposure routes.

2) Combined Exposure Routes of Soil Ingestion, Outdoor Inhalation of Vapors and Particulates, and Dermal Contact with Soil

A) Equations R1 and R2 form the basis for deriving Tier 2 remediation objectives for the set of equations that evaluates the combined exposure routes of soil ingestion, outdoor inhalation of vapors and particulates, and dermal contact with soil using the RBCA approach. Equation R1 is used to calculate soil remediation objectives for carcinogenic contaminants. Equation R2 is used to calculate soil remediation objectives for noncarcinogenic contaminants. Soil remediation objectives for the outdoor inhalation exposure route from subsurface soils must also be calculated in accordance with the procedures outlined in subsection (b)(3) of this Section and compared to the values generated from Equations R1 or R2. The smaller value (i.e., R1 and R2 compared to R7 and R8, respectively) from these calculations is the Tier 2 soil remediation objective for the combined exposure routes of soil ingestion, outdoor inhalation, and dermal contact with soil.

B) In Equation R1, numerical values are calculated for two parameters:

i) The volatilization factor for surficial soils (VF_{ss}) using Equations R3 and R4; and

ii) The volatilization factor for surficial soils regarding particulates (VF_p) using Equation R5.

C) VF_{ss} uses Equations R3 and R4 to derive a numerical value. Equation R3 requires the use of Equation R6. Both equations must be used to calculate the VF_{ss}. The lowest calculated value from these equations must be substituted into Equation R1.

D) The remaining parameters in Equation R1 have either default values listed in Appendix C, Table D or toxicological-specific information (i.e., SF_o, SF_i), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

E) For Equation R2, the parameters VF_{ss} and VF_p are calculated. The remaining parameters in Equation R2 have either default values listed in Appendix C, Table D or toxicological-specific information (i.e., RfD_o, RfD_i), which can be obtained by following the guidelines in OSWER Directive 9285.7-53, as incorporated by reference in Section 742.210 or requested from the program under which the remediation is being performed.

F) For chemicals other than inorganics which do not have default values for the dermal absorption factor (RAF_d) in Appendix C, Table D a dermal absorption factor of 0.5 shall be used for Equations R1 and R2. For inorganics, dermal absorption may be disregarded (i.e., $RAF_d = 0$).

3) Outdoor Inhalation Exposure Route from Subsurface Soils (soil below one meter)

A) Equations R7 and R8 form the basis for deriving Tier 2 remediation objectives for the outdoor inhalation exposure route from subsurface soils using the RBCA approach. Equation R7 is used to calculate soil remediation objectives for carcinogenic contaminants. Equation R8 is used to calculate soil remediation objectives for noncarcinogenic contaminants.

B) For Equation R7, the carcinogenic risk-based screening level for air ($RBSL_{air}$) and the volatilization factor for soils below one meter to ambient air (VF_{samb}) have numerical values that are calculated using Equations R9 and R11, respectively. Both equations rely on input parameters from a variety of sources.

C) The noncarcinogenic risk-based screening level for air ($RBSL_{air}$) and the volatilization factor for soils below one meter to ambient air (VF_{samb}) in Equation R8 have numerical values that can be calculated using Equations R10 and R11, respectively.

c) Soil Component of the Groundwater Ingestion Exposure Route

1) Equation R12 forms the basis for deriving Tier 2 remediation objectives for the soil component of the groundwater ingestion exposure route using the RBCA approach. The parameters, groundwater at the source (GW_{source}) and Leaching Factor (LF_{sw}), have numerical values that are calculated using Equations R13 and R14, respectively.

2) Equation R13 requires numerical values that are calculated using Equation R15.

3) Equation R14 requires numerical values that are calculated using Equations R21, R22, and R24. For non-ionizing organics, the Soil Water Sorption Coefficient (k_s) shall be calculated using Equation R20. For ionizing organics and inorganics, the values for k_s are listed in Appendix C, Tables I and J, respectively. The pH-dependent k_s values for ionizing organics can be calculated using Equation R20 and the pH-dependent K_{oc} values in Appendix C, Table I. The remaining parameters in Equation R14 are field measurements or default values listed in Appendix C, Table D.

d) The default value for GW_{comp} is the Tier 1 groundwater remediation objective. For chemicals for which there is no Tier 1 groundwater remediation objective, the value for GW_{comp} shall be the concentration determined according to the procedures specified in 35 Ill. Adm. Code 620, Subpart F. As an alternative to using the above concentrations, GW_{comp} may be developed using Equations R25 and R26, if approved institutional controls are in place as may be required in Subpart J.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.717 J&E Soil Gas Equations for the Indoor Inhalation Exposure Route

a) This Section sets forth the equations and parameters to be used to develop Tier 2 soil gas remediation objectives for the indoor inhalation exposure route using the modified J&E model.

b) Equations J&E1 and J&E2 calculate, for carcinogens and noncarcinogens, respectively, an acceptable concentration of the contaminant of concern in indoor air that adequately protects humans who inhale this air. Equation J&E3 converts indoor air concentrations from parts per million volume to milligrams per cubic meter.

c) Equation J&E4 calculates an acceptable concentration of the contaminant of concern in the soil gas at the source of contamination. This calculation is made using:

1) an attenuation factor developed in accordance with Equations J&E7 through 18; and

2) the acceptable concentration of the contaminant of concern in indoor air calculated in accordance with Equation J&E1 (for carcinogens) or J&E2 (for noncarcinogens).

d) The attenuation factor (Equation J&E7 or J&E8) accounts for the following processes:

1) Migration of contaminants from the source upwards through the vadose zone;

2) Migration of contaminants through the earthen filled cracks in the building's full concrete slab-on-grade or full concrete basement floor and walls; and

3) Mixing of the contaminants with air inside the building.

e) Equation J&E7 must be used when the mode of contaminant transport is both diffusion and advection. In this scenario, the Q_{soil} value equals 83.33 cm^3/sec as described in Section 742.505.

f) Equation J&E8 may be used only when the mode of contaminant transport is diffusion only. In this scenario, the Q_{soil} value equals 0.0 cm^3/sec as described in Section 742.505. As an alternative to using Equation J&E8 pursuant to this subsection, it is permissible to use Equation J&E7, in which case the Q_{soil} value equals 83.33 cm^3/sec as described in Section 742.505.

g) Equations J&E9a through J&E18 calculate input parameters for either Equation J&E7 or J&E8 (the equations used to calculate an attenuation factor). These equations assume there are "n" different soil layers between the source of the contamination and the floor of the building. Equations J&E11, 16, 17 and 18 shall be used to calculate the needed parameters for each of the n layers (the general soil layer is referred to as soil layer "i" and i = 1, 2, . . . n). Equations J&E16, 17, and 18 shall also be used to calculate needed parameters for the soil in the cracks of the building's full concrete slab-on-grade or full concrete basement floor and walls (it is through these cracks that contaminated soil gas is assumed to flow from the subsurface into the building). As reflected in Equation J&E14, the only crack assumed to be present is the floor-wall seam gap. To calculate the surface area of the enclosed space at or below grade, Equation J&E12a shall be

used for a building with a full concrete slab-on-grade and Equation J&E12b shall be used for a building with a full concrete basement floor and walls.

h) The default representative subsurface temperature for Henry's Law Constant is 13°C. This value shall be used, as appropriate, in all calculations needed to represent the system by which contaminants migrate through the subsurface.

i) The calculated soil gas remediation objective shall be compared with the soil vapor saturation limit (C_v^{sat}, Equation J&E5) for each volatile chemical. The calculated C_v^{sat} shall use the default representative subsurface temperature specified in subsection (h). If the calculated soil gas remediation objective is greater than C_v^{sat}, then C_v^{sat} is used as the soil gas remediation objective.

j) The calculated soil gas remediation objective shall be compared to concentrations of soil gas collected at a depth at least 3 feet below ground surface and above the saturated zone. If a valid sample cannot be collected, a soil gas sampling plan shall be approved by the Agency under Tier 3.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.720 Chemicals with Cumulative Noncarcinogenic Effects

Appendix A, Table E lists the groups of chemicals from Appendix B, Tables A and B that have remediation objectives based on noncarcinogenic toxicity and that affect the same target organ. If more than one chemical detected at a site affects the same target organ (i.e., has the same critical effect as defined by the RfD), the initially calculated remediation value for each chemical in the group shall be corrected for cumulative effects by one of the following two methods:

a) Calculate the weighted average using the following equations:

$$W_{ave} = \frac{x_1}{CUO_{x_1}} + \frac{x_2}{CUO_{x_2}} + \frac{x_3}{CUO_{x_3}} + \ldots + \frac{x_a}{CUO_{x_a}}$$

where:

W_{ave}= Weighted Average

x_1 through x_a = Concentration of each individual contaminant at the location of concern. Note that, depending on the target organ/mode of action, the actual number of contaminants will range from 2 to 14.

$CUOx_a$ = A Tier 2 remediation objective must be developed

for each x_a.

If the value of the weighted average calculated in accordance with the equations above is less than or equal to 1.0, then the remediation objectives are met for those chemicals.

If the value of the weighted average calculated in accordance with the equations above is greater than 1.0, then additional remediation must be carried out until the level of contaminants remaining in the remediated area has a weighted average calculated in accordance with the equation above less than or equal to one.

b) Divide each individual chemical's remediation objective by the number of chemicals in that specific target organ group that were detected at the site. Each of the contaminant concentrations at the site is then compared to the remediation objectives that have been adjusted to account for this potential additivity. For the noncarcinogenic contaminants listed in Appendix A, Table E, a respective soil remediation objective need be no lower than the respective value listed in Appendix B, Table A or B.

SUBPART H: TIER 2 GROUNDWATER EVALUATION

Section 742.800 Tier 2 Groundwater Evaluation Overview

If the contaminant concentrations in the groundwater exceed the applicable Tier 1 remediation objectives, a person has the following options:

a) Demonstrate that the groundwater ingestion exposure route is excluded from consideration pursuant to Subpart C;

b) Demonstrate that the groundwater contamination is at or below area background concentrations in accordance with Subpart D and, if necessary, an institutional control restricting usage of the groundwater is in place in accordance with Subpart J;

c) Remediate to Tier 1 remediation objectives;

d) Propose and obtain approval of Tier 2 groundwater remediation objectives in accordance with Section 742.805 and remediate to that level, if necessary;

e) Conduct a Tier 3 evaluation in accordance with Subpart I; or

f) Obtain approval from the Board to:

1) Reclassify the groundwater pursuant to 35 Ill. Adm. Code 620.260; or

2) Use an adjusted standard pursuant to Section 28.1 of the Act. [415 ILCS 5/28.1].

Section 742.805 Tier 2 Groundwater Remediation Objectives

a) To develop a groundwater remediation objective under this Section that exceeds the applicable Tier 1 groundwater remediation objective, or for which there is no Tier I groundwater remediation objective, a person may request approval from the Agency if the person has performed the following:

1) Identified the horizontal and vertical extent of groundwater for which the Tier 2 groundwater remediation objective is sought;

2) Taken corrective action, to the maximum extent practicable to remove any free product;

3) Using Equation R26 in accordance with Section 742.810, demonstrated that the concentration of any contaminant of concern in groundwater will meet:

A) The applicable Tier 1 groundwater remediation objective at the point of human exposure; or

B) For any contaminant of concern for which there is no Tier 1 groundwater remediation objective, the concentration determined according to the procedures specified in 35 Ill. Adm. Code 620 at the point of human exposure. A person may request the Agency to provide these concentrations or may propose these concentrations under Subpart I;

4) Using Equation R26 in accordance with Section 742.810, demonstrated that the concentration of any contaminant of concern in groundwater within the minimum or designated maximum setback zone of an existing potable water supply well will meet the applicable Tier 1 groundwater remediation objective or, if there is no Tier 1 groundwater remediation objective, the concentration determined according to the procedures specified in 35 Ill. Adm. Code 620. A person may request the Agency to provide these concentrations or may propose these concentrations under Subpart I;

5) Using Equation R26 in accordance with Section 742.810, demonstrated that the concentration of any contaminant of concern in groundwater discharging into a surface water will meet the applicable water quality standard under 35 Ill. Adm. Code 302;

6) Demonstrated that the source of the release is not located within the minimum or designated maximum setback zone or within a regulated recharge area of an existing potable water supply well; and

7) If the selected corrective action includes an engineered barrier as set forth in Subpart K to minimize migration of contaminants of concern from the soil to the groundwater, demonstrated that the engineered barrier will remain in place for post-remediation land use through an institutional control as set forth in Subpart J.

b) A groundwater remediation objective that exceeds the water solubility of that chemical (refer to Appendix C, Table E for solubility values) is not allowed.

c) The contaminants of concern for which a Tier 1 remediation objective has been developed shall be included in any mixture of similar-acting chemicals under consideration in Tier 2. The evaluation of 35 Ill. Adm. Code 620.615 regarding mixtures of similar-acting chemicals shall be considered satisfied for Class I groundwater at the point of human exposure if either of the following requirements are achieved:

1) Calculate the weighted average using the following equations:

$$W_{ave} = \frac{x_1}{CUOx_1} + \frac{x_2}{CUOx_2} + \frac{x_3}{CUOx_3} + \ldots + \frac{x_a}{CUOx_a}$$

where:

W_{ave} = Weighted Average

x_1 through x_a = Concentration of each individual contaminant at the location of concern. Note that, depending on the target organ, the actual number of contaminants will range from 2 to 33.

$CUOx_a$ = A Tier 1 or Tier 2 remediation objective must be developed for each x_a.

A) If the value of the weighted average calculated in accordance with the equations above is less than or equal to 1.0, then the remediation objectives are met for those chemicals.

B) If the value of the weighted average calculated in accordance with the equations above is greater than 1.0, then additional remediation must be carried out until the level of contaminants remaining in the remediated area has a weighted average calculated in accordance with the equation above less than or equal to one; or

2) Divide each individual chemical's remediation objective by the number of chemicals in that specific target organ group that were detected at the site. Each of the contaminant concentrations at the site is then compared to the remediation objectives that have been adjusted to account for this potential additivity.

d) The evaluation of 35 Ill. Adm. Code 620.615 regarding mixtures of similar-acting chemicals is considered satisfied if the cumulative risk from any contaminants of concern listed in Appendix A, Table I, plus any other contaminants of concern detected in groundwater and listed in Appendix A, Table F as affecting the same target organ/organ system as the contaminants of concern detected from Appendix A, Table I, does not exceed 1 in 10,000.

e) Groundwater remediation objectives for the indoor inhalation exposure route shall be developed in accordance with Section 742.812. For the indoor inhalation exposure route:

1) Appendix C, Table L applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls; and

2) Institutional controls under Subpart J are required to develop groundwater remediation objectives pursuant to Appendix C, Table L.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.810 RBCA Calculations to Predict Impacts from Remaining Groundwater Contamination

a) Equation R26 predicts the contaminant concentration along the centerline of a groundwater plume emanating from a vertical planar source in the aquifer (dimensions S_w wide and S_d deep). This model accounts for both three-dimensional dispersion (x is the direction of groundwater flow, y is the other horizontal direction, and z is the vertical direction) and biodegradation.

1) The parameters in this equation are:

$X =$ distance from the planar source to the location of concern, along the centerline of the groundwater plume (i.e., y=0, z=0)

$C_x =$ the concentration of the contaminant at a distance X from the source, along the centerline of the plume

C_{source} = the greatest potential concentration of the contaminant of concern in the groundwater at the source of the contamination, based on the concentrations of contaminants in groundwater due to the release and the projected concentration of the contaminant migrating from the soil to the groundwater. As indicated above, the model assumes a planar source discharging groundwater at a concentration equal to C_{source}.

α_x = dispersivity in the x direction (i.e., Equation R16)

α_y = dispersivity in the y direction (i.e., Equation R17)

α_z = dispersivity in the z direction (i.e., Equation R18)

U = specific discharge (i.e., actual groundwater flow velocity through a porous medium; takes into account the fact that the groundwater actually flows only through the pores of the subsurface materials) where the aquifer hydraulic conductivity (K), the hydraulic gradient (I) and the total soil porosity θ_T must be known (i.e., Equation R19)

λ= first order degradation constant obtained from Appendix C, Table E or from measured groundwater data

S_w = width of planar groundwater source in the y direction

S_d = depth of planar groundwater source in the z direction

2) The following parameters are determined through field measurements: U, K, I, θ_T, S_w, S_d.

A) The determination of values for U, K, I and θ_T can be obtained through the appropriate laboratory and field techniques;

B) From the immediate down-gradient edge of the source of the groundwater contamination values for S_w and S_d shall be determined. S_w is defined as the width of groundwater at the source which exceeds the Tier 1 groundwater remediation objective. S_d is defined as the depth of groundwater at the source which exceeds the Tier 1 groundwater remediation objective; and

C) Total soil porosity can also be calculated using Equation R23.

b) Once values are obtained for all the input parameters identified in subsection (a) of this Section, the contaminant concentration C_x along the centerline of the plume at a distance X from the source shall be calculated so that X is the distance from the down-gradient edge of the source of the contamination at the site to the point where the contaminant concentration is equal to the Tier 1 groundwater remediation objective or concentration determined according to the procedures specified in 35 Ill. Adm. Code 620, Subpart F.

1) If there are any potable water supply wells located within the calculated distance X, then the Tier 1 groundwater remediation objective or concentration shall be met at the edge of the minimum or designated maximum setback zone of the nearest potable water supply down-gradient of the source. To demonstrate that a minimum or maximum setback zone of a potable water supply well will not be impacted above the applicable Tier 1 groundwater remediation objective or concentration determined according to the procedures specified in 35 Ill. Adm. Code 620, Subpart F, X shall be the distance from the C_{source} location to the edge of the setback zone.

2) To demonstrate that no surface water is adversely impacted, X shall be the distance from the down-gradient edge of the source of the contamination site to the nearest surface water body. This calculation must show that the contaminant in the groundwater at this location (C_x) does not exceed the applicable water quality standard.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.812 J&E Groundwater Equations for the Indoor Inhalation Exposure Route

Groundwater remediation objectives for the indoor inhalation exposure route are calculated using the modified J&E model as described in Section 742.717, except as follows:

a) In Equation J&E9a, the total number of layers of soil that contaminants migrate through from the source to the building shall include a capillary fringe layer.

b) The thickness of the capillary fringe layer is 37.5 cm.

c) The volumetric water content of the capillary fringe shall be 90 % of the total porosity of the soil that comprises the capillary fringe.

d) Equations J&E7 and J&E8 calculate an acceptable groundwater remediation objective.

1) This calculation is made using:

A) the soil gas remediation objective calculated in accordance with Equation J&E4; and

B) the assumption that this gas is in equilibrium with any contamination in the groundwater.

2) Equation J&E7 must be used when the mode of contaminant transport is both diffusion and advection. In this scenario, the Q_{soil} value equals 83.33 cm^3/sec as described in Section 742.505.

3) Equation J&E8 may be used only when the mode of contaminant transport is diffusion only. In this scenario, the Q_{soil} value equals 0.0 cm^3/sec as described in Section 742.505. As an alternative to using Equation J&E8 pursuant to this subsection, it is permissible to use Equation J&E7, in which case the Q_{soil} value equals 83.33 cm^3/sec as described in Section 742.505.

e) A groundwater remediation objective that exceeds the water solubility of that chemical (refer to Appendix C, Table E for solubility values) is not allowed. If the calculated groundwater remediation objective is greater than the water solubility of that chemical, then the solubility is used as the groundwater remediation objective.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

SUBPART I: TIER 3 EVALUATION

Section 742.900 Tier 3 Evaluation Overview

a) Tier 3 sets forth a flexible framework to develop remediation objectives outside of the requirements of Tiers 1 and 2. Although Tier 1 and Tier 2 evaluations are not prerequisites to conduct Tier 3 evaluations, data from Tier 1 and Tier 2 can assist in developing remediation objectives under a Tier 3 evaluation.

b) The level of detail required to adequately characterize a site depends on the particular use of Tier 3. Tier 3 can require additional investigative efforts beyond those described in Tier 2 to characterize the physical setting of the site. However, in situations where remedial efforts have simply reached a physical obstruction additional investigation may not be necessary for a Tier 3 submittal.

c) Situations that can be considered for a Tier 3 evaluation include, but are not limited to:

1) Modification of parameters not allowed under Tier 2;

2) Use of models different from those used in Tier 2;

3) Use of additional site data, such as results of indoor air sampling, to improve or confirm predictions of exposed receptors to contaminants of concern;

4) Analysis of site-specific risks using formal risk assessment, probabilistic data analysis, and sophisticated fate and transport models (e.g., requesting a target hazard quotient greater than 1 or a target cancer risk greater than 1 in 1,000,000);

5) Requests for site-specific remediation objectives because an assessment indicates further remediation is not practical;

6) Incomplete human exposure pathways not excluded under Subpart C;

7) Use of toxicological-specific information not available from the sources listed in Tier 2;

8) Land uses which are substantially different from the assumed residential or industrial/commercial property uses of a site (e.g., a site will be used for recreation in the future and cannot be evaluated in Tier 1 or 2);

9) Requests for site-specific remediation objectives that exceed Tier 1 groundwater remediation objectives so long as the following is demonstrated:

A) *To the extent practical, the exceedance of the groundwater quality standard has been minimized and beneficial use appropriate to the groundwater that was impacted has been returned; and*

B) *Any threat to human health or the environment has been minimized* [415 ILCS 5/58.5(d)(4)(A)]; and

10) Use of building control technologies, other than those described in Subpart L, to prevent completion of the indoor inhalation exposure route.

d) For requests of a target cancer risk ranging between 1 in 1,000,000 and 1 in 10,000 at the point of human exposure or a target hazard quotient greater than 1 at the point of human exposure, the requirements of Section 742.915 shall be followed. Requests for a target cancer risk exceeding 1 in 10,000 at the point of human exposure are not allowed.

e) Requests for approval of a Tier 3 evaluation must be submitted to the Agency for review under the specific program under which remediation is performed. When reviewing a submittal under Tier 3, the Agency shall consider *whether the interpretations and conclusions reached are supported by the information gathered* [415 ILCS 58.7(e)(1)]. The Agency shall approve a Tier 3 evaluation if

the person submits the information required under this Part and establishes through such information that public health is protected and that specified risks to human health and the environment have been minimized.

f) If contaminants of concern include polychlorinated biphenyls (PCBs), requests for approval of a Tier 3 evaluation must additionally address the applicability of 40 CFR 761.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.905 Modifications of Parameters

Any proposed changes to Tier 2 parameters which are not provided for in Tier 2 shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

a) The justification for the modification; and

b) The technical and mathematical basis for the modification.

Section 742.910 Alternative Models

Any proposals for the use of models other than those specified in Tier 2 shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

a) Physical and chemical properties of contaminants of concern;

b) Contaminant movement properties;

c) Contaminant availability to receptors;

d) Receptor exposure to the contaminants of concern;

e) Mathematical and technical justification for the model proposed;

f) A licensed copy of the model, if the Agency does not have a licensed copy of the model currently available for use; and

g) Demonstration that the models were correctly applied.

Section 742.915 Formal Risk Assessments

A comprehensive site-specific risk assessment shall demonstrate that contaminants of concern at a site do not pose a significant risk to any human receptor. All site-specific risk assessments shall be submitted to the Agency for review and approval. A submittal under this Section shall address the following factors:

a) Whether the risk assessment procedure used is nationally recognized and accepted including, but not limited to, those procedures incorporated by reference in Section 742.210;

b) Whether the site-specific data reflect actual site conditions;

c) The adequacy of the investigation of present and post-remediation exposure routes and risks to receptors identified at the site;

d) The appropriateness of the sampling and analysis;

e) The adequacy and appropriateness of toxicity information;

f) The extent of contamination;

g) Whether the calculations were accurately performed;

h) Similar-acting chemicals shall be specifically addressed. At a minimum, the chemicals subject to this requirement are identified in Appendix A, Tables E and F; and

i) Proposals seeking to modify the target risk consistent with Section 742.900(d) shall address the following factors:

1) the presence of sensitive populations;

2) the number of receptors potentially impacted;

3) the duration of risk at the differing target levels; and

4) the characteristic of the chemicals of concern.

SOURCE: Amended at 21 Ill. Reg. 16391, effective December 8, 1997.

Section 742.920 Impractical Remediation

Any request for site-specific remediation objectives due to impracticality of remediation shall be submitted to the Agency for review and approval. Any request for site-specific remediation objectives due to impracticality of remediation that involves the indoor inhalation exposure route

shall follow Section 742.935 in lieu of this Section. A submittal under this Section shall include the following information:

a) The reasons why the remediation is impractical;

b) The current extent and modeled migration of contamination;

c) Geology, including soil types and parameters;

d) The potential impact to groundwater;

e) Results and locations of sampling events;

f) Map of the area, including all utilities and structures; and

g) Present and post-remediation uses of the area of contamination, including human receptors at risk.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.925 Exposure Routes

Technical information may demonstrate that there is no actual or potential impact of contaminants of concern to receptors from a particular exposure route. In these instances, a demonstration excluding an exposure route shall be submitted to the Agency for review and approval. A demonstration that involves the indoor inhalation exposure route shall follow Section 742.935 in lieu of this Section. A submittal under this Section shall include the following information:

a) A description of the route evaluated;

b) A description of the site and physical site characteristics;

c) A discussion of the result and possibility of the route becoming active in the future; and

d) Technical support that may include, but is not limited to, the following:

1) a discussion of the natural or man-made barriers to that exposure route;

2) calculations and modeling;

3) physical and chemical properties of contaminants of concern; and

4) contaminant migration properties.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.930 Derivation of Toxicological Data

If toxicological-specific information is not available for one or more contaminants of concern from the sources incorporated by reference in Section 742.210, the derivations of toxicological-specific information shall be submitted for Agency review and approval.

Section 742.935 Indoor Inhalation Exposure Route

a) Exclusion of Exposure Route

Site information may demonstrate that there is no actual or potential impact of contaminants of concern to receptors from the indoor inhalation exposure route. In these instances, a demonstration excluding the exposure route shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

1) A description of the site, physical site characteristics, existing and planned buildings, and existing and planned man-made pathways; and

2) A discussion of the possibility of the route becoming active in the future.

b) Exclusion of Exposure Route Using Building Control Technologies

Any proposals to use building control technologies as a means to prevent or mitigate human exposures under the indoor inhalation exposure route that differ from the requirements of Subpart L shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

1) A description of the site and physical site characteristics;

2) The current extent and modeled migration of contamination;

3) Geology, including soil types and parameters;

4) Results and locations of sampling events;

5) Scaled map of the area, including all buildings and man-made pathways;

6) A description of building characteristics and methods of construction, including a description of man-made pathways;

7) Present and post-remediation uses of the land that are at issue due to the area of contamination, including human receptors at risk;

8) A description of any building control technologies currently in place or proposed for installation that can reduce or eliminate the potential for completion of the exposure route, including design and construction specifications;

9) Information regarding the effectiveness of any building control technologies currently in place or proposed for installation and a schedule for performance testing to show the effectiveness of the control technology. For buildings not yet constructed, an approved building control technology shall be in place and operational prior to human occupancy;

10) Identification of documents reviewed and the criteria used in the documents for determining whether building control technologies are effective and how those criteria compare to existing or potential buildings or man-made pathways at the site; and

11) A description as to how the effectiveness of the building control technologies will be operated and maintained for the life of the buildings and man-made pathways, or until soil gas and groundwater contaminant concentrations have reached remediation objectives that are approved by the Agency. This includes provisions for potential extended system inoperability due to power failure or other disruption.

c) Calculations and Modeling Used to Establish Soil Gas Remediation Objectives

The calculations and modeling shall account for contaminant transport through the mechanisms of diffusion and advection. Proposals to use soil gas data, including sub-slab samples, to establish remediation objectives for the indoor inhalation exposure route that differ from the requirements of Section 742.227 shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

1) Scaled map of the area, showing all buildings and man-made pathways (current and planned);

2) The current extent and modeled migration of contamination;

3) Geology, including soil types and parameters;

4) Depth to groundwater (including seasonal variation) and flow direction;

5) Location of soil gas sampling points;

6) A discussion of soil gas sampling procedures that, at a minimum, addresses the following:

A) sampling equipment;

B) soil gas collection protocol, including field tests and weather conditions; and

C) laboratory analytical methods.

d) Calculations and Modeling Used to Establish Soil Remediation Objectives

The calculations and modeling shall account for contaminant transport through the mechanisms of diffusion and advection. Any proposals to use soil data in lieu of soil gas data to establish remediation objectives for the indoor inhalation exposure route shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

1) Scaled map of the area, showing all buildings and man-made pathways (current and planned);

2) The current extent and modeled migration of contamination;

3) Geology, including soil types and parameters;

4) Location of soil sampling points;

5) A discussion of soil sampling procedures that, at a minimum, addresses the following:

A) sampling equipment;

B) soil collection protocol, including field tests and weather conditions; and

C) laboratory analytical methods;

6) Mathematical and technical justification for the model proposed; and

7) Demonstration that the model was correctly applied.

e) Calculations and Modeling Used to Establish Groundwater Remediation Objectives

The calculations and modeling shall account for contaminant transport through the mechanisms of diffusion and advection. Proposals to use groundwater data to establish remediation objectives for the indoor inhalation exposure route that differ from the requirements of Sections 742.805 and 742.812 shall be submitted to the Agency for review and approval. A submittal under this Section shall include the following information:

1) Scaled map of the area, showing all buildings and man-made pathways (current and planned);

2) The current extent and modeled migration of contamination;

3) Geology, including soil types and parameters and the thickness of the capillary fringe;

4) Depth to groundwater (including seasonal variation) and flow direction;

5) Results and locations of groundwater sampling events;

6) Mathematical and technical justification for the model proposed; and

7) Demonstration that the model was correctly applied.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

SUBPART J: INSTITUTIONAL CONTROLS

Section 742.1000 Institutional Controls

a) Institutional controls in accordance with this Subpart must be placed on the property when remediation objectives are based on any of the following assumptions:

1) Industrial/Commercial property use;

2) Target cancer risk greater than 1 in 1,000,000;

3) Target hazard quotient greater than 1;

4) Engineered barriers;

5) The point of human exposure is located at a place other than at the source;

6) Exclusion of exposure routes;

7) A diffusion only mode of contaminant transport for the indoor inhalation exposure route;

8) Use of an indoor inhalation building control technology;

9) For the indoor inhalation exposure route, the presence of a building with a full concrete slab-on-grade or a full concrete basement floor and walls; or

10) Any combination of the above.

b) The Agency shall not approve any remediation objective under this Part that is based on the use of institutional controls unless the person has proposed institutional controls meeting the requirements of this Subpart and the requirements of the specific program under which the institutional control is proposed. A proposal for approval of institutional controls shall provide identification of the selected institutional controls from among the types recognized in this Subpart.

c) The following instruments may be institutional controls subject to the requirements of this Subpart J and the requirements of the specific program under which the institutional control is proposed:

1) No Further Remediation Letters;

2) Environmental Land Use Controls;

3) Land Use Control Memoranda of Agreement;

4) Ordinances adopted and administered by a unit of local government;

5) Agreements between a property owner (or, in the case of a petroleum leaking underground storage tank, the owner or operator of the tank) and a highway authority with respect to any contamination remaining under highways; and

6) Agreements between a highway authority that is also the property owner (or, in the case of a petroleum leaking underground storage tank, the owner or operator of the tank) and the Agency with respect to any contamination remaining under the highways.

d) No Further Remediation Letters and Environmental Land Use Controls that meet the requirements of this Subpart and the recording requirements of the program under which remediation is being performed are transferred with the property.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.1005 No Further Remediation Letters

a) A No Further Remediation Letter issued by the Agency under 35 Ill. Adm. Code 732 or 740 may be used as an institutional control under this Part if the requirements of subsection (b) of this Section are met.

b) A request for approval of a No Further Remediation Letter as an institutional control shall meet the requirements applicable to the specific program under which the remediation is performed.

(Source: Amended at 25 Ill. Reg. 10374, effective August 15, 2001)

Section 742.1010 Environmental Land Use Controls

a) An Environmental Land Use Control (ELUC) is an institutional control that may be used under this Part to impose land use limitations or requirements related to environmental contamination. ELUCs are only effective when approved by the Agency in accordance with this Part. Activities or uses that may be limited or required include, but are not limited to, prohibition of use of groundwater for potable purposes, restriction to industrial/commercial uses, operation or maintenance of engineered barriers, indoor inhalation building control technologies, or worker safety plans. ELUCs may be used in the following circumstances:

1) When No Further Remediation Letters are not available, including but not limited to when contamination has migrated off-site or outside the remediation site; or

2) When No Further Remediation Letters are not issued under the program for which a person is undergoing remediation.

b) Recording requirements:

1) An ELUC approved by the Agency pursuant to this Section must be recorded in the Office of the Recorder or Registrar of Titles for the county in which the property that is the subject of the ELUC is located. A copy of the ELUC demonstrating that it has been recorded must be submitted to the Agency before the Agency will issue a no further remediation determination.

2) An ELUC approved under this Section will not become effective until officially recorded in the chain of title for the property that is the subject of the ELUC in accordance with subsection (b)(1) of this Section.

3) Reference to the recorded ELUC must be made in the instrument memorializing the Agency's no further remediation determination. Recording of the no further remediation determination and confirmation of

recording must be in accordance with the requirements of the program under which the determination was issued.

4) The requirements of this Section do not apply to Federally Owned Property for which the Federal Landholding Entity does not have the authority under federal law to record land use limitations on the chain of title.

5) The requirements of this Section apply only to those sites for which a request for a no further remediation determination has not yet been made to the Agency by January 6, 2001.

c) Duration:

1) Except as provided in this subsection (c), an ELUC shall remain in effect in perpetuity.

2) *At no time shall any site for which an ELUC has been imposed as a result of remediation activities under this Part be used in a manner inconsistent with the land use limitation unless attainment of objectives appropriate for the new land use is achieved and a new* no further remediation determination has been *obtained and recorded in accordance with* the program under which the ELUC was first imposed or the Site Remediation Program (35 Ill. Adm. Code 740) [415 ILCS 58.8(c)]. In addition, the appropriate release or modification of the ELUC must be prepared by the Agency and filed on the chain of title for the property that is the subject of the ELUC.

A) For a Leaking Underground Storage Tank (LUST) site under 35 Ill. Adm. Code 731 or 734 or a Site Remediation Program site under 35 Ill. Adm. Code 740, an ELUC may be released or modified only if the NFR Letter is also modified under the Site Remediation Program to reflect the change;

B) For a RCRA site under 35 Ill. Adm. Code 721-730, an ELUC may be released or modified only if there is also an amended certification of closure or a permit modification.

3) In addition to any other remedies that may be available, a failure to comply with the limitations or requirements of an ELUC may result in voidance of an Agency no further remediation determination in accordance with the program under which the determination was made. The failure to comply with the limitations or requirements of an ELUC may also be grounds for an enforcement action pursuant to Title VIII of the Act.

d) An ELUC submitted to the Agency must match the form and contain the same substance, except for variable elements (e.g., name of property owner), as the model in Appendix F and must contain the following elements:

1) Name of property owners and declaration of property ownership;

2) Identification of the property to which the ELUC applies by common address, legal description, and Real Estate Tax Index/Parcel Index Number;

3) A reference to the Bureau of Land LPC numbers or 10-digit identification numbers under which the remediation was conducted;

4) A statement of the reason for the land use limitation or requirement relative to protecting human health and the surrounding environment from soil, groundwater, and/or other environmental contamination;

5) The language instituting such land use limitations or requirements;

6) A statement that the limitations or requirements apply to the current owners, occupants, and all heirs, successors, assigns, and lessees;

7) A statement that the limitations or requirements apply in perpetuity or until:

A) The Agency determines that there is no longer a need for the ELUC;

B) The Agency, upon written request, issues to the site that received the no further remediation determination that relies on the ELUC a new no further remediation determination approving modification or removal of the limitations or requirements;

C) The new no further remediation determination is filed on the chain of title of the site subject to the no further remediation determination; and

D) A release or modification of the land use limitation is filed on the chain of title for the property that is the subject of the ELUC;

8) Scaled site maps showing:

A) The legal boundary of the property to which the ELUC applies;

B) The horizontal and vertical extent of contaminants of concern above applicable remediation objectives for soil, groundwater, and soil gas to which the ELUC applies;

C) Any physical features to which an ELUC applies (e.g., engineered barriers, monitoring wells, caps, indoor inhalation building control technologies); and

D) The nature, location of the source, and direction of movement of the contaminants of concern;

9) A statement that any information regarding the remediation performed on the property for which the ELUC is necessary may be obtained from the Agency through a request under the Freedom of Information Act [5 ILCS 140] and rules promulgated thereunder; and

10) The dated, notarized signatures of the property owners or authorized agent.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.1012 Federally Owned Property: Land Use Control Memoranda of Agreement

a) A Land Use Control Memorandum of Agreement (LUC MOA) between one or more agencies of the federal government and the Illinois Environmental Protection Agency is the institutional control that shall be used under this Part to impose land use limitations or restrictions related to environmental contamination on Federally Owned Property. A LUC MOA may be used only for Federally Owned Property. Each LUC MOA, at a minimum, must require that the Federal Landholding Entities responsible for the Federally Owned Property do the following:

1) Provide adequate identification of the location on the Federally Owned Property of each site with land use limitations or requirements. Such identification shall be by means of common address, notations in any available facility master land use plan, site specific GIS or GPS coordinates, plat maps, or any other means which identifies the site in question with particularity;

2) Implement periodic site inspection procedures to ensure adequate oversight by the Federal Landholding Entities of such land use limitation or requirement;

3) Implement procedures for the Federal Landholding Entities to periodically advise the Agency of continued compliance with the maintenance of the land use control and site inspection requirements included in the LUC MOA;

4) Implement procedures for the Federal Landholding Entities to notify the Agency of any planned or emergency changes in land use that may adversely impact any site with land use limitations or requirements; and

5) Notify the Agency at least 60 days in advance of a conveyance by deed or fee simple title, by the Federal Landholding Entities, of a site with land use limitations or requirements, to any entity that will not remain or become a Federal Landholding Entity, and provide the Agency with information about how the Federal Landholding Entities will ensure that the requirements of Section 742.1010 are to be satisfied upon conveyance of that site.

a) Any LUC MOA entered into pursuant to this Section remains effective only so long as title to the affected property is retained by the United States.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.1015 Ordinances

a) An ordinance adopted by a unit of local government that effectively prohibits the installation of potable water supply wells (and the use of such wells) may be used as an institutional control to meet the requirements of Section 742.320(d) or 742.805(a)(3) if the requirements of this Section are met. A model ordinance is found in Appendix G. Ordinances prohibiting the installation of potable water supply wells (and the use of such wells) that do not expressly prohibit the installation of potable water supply wells (and the use of such wells) by units of local government may be acceptable as institutional controls if the requirements of this Section are met and a Memorandum of Understanding (MOU) is entered into under subsection (i) of this Section. For purposes of this Section, a unit of local government is considered to be expressly prohibited from installing and using potable water supply wells only if the unit of local government is included in the prohibition provision by name. The prohibition required by this Section shall satisfy the following requirements at a minimum:

1) The prohibition shall not allow exceptions for potable water well installation and use other than for the adopting unit of local government;

2) The prohibition shall apply at all depths and shall not be limited to particular aquifers or other geologic formations;

3) If the prohibition does not apply everywhere within the boundaries of the unit of local government, the limited area to which the prohibition applies shall be easily identifiable and clearly defined by the ordinance (e.g., narrative descriptions accompanied by maps with legends or labels showing prohibition boundaries, or narrative descriptions using fixed, common reference points such as street names). Boundaries of prohibitions limited by area shall be fixed by the terms of the ordinance and shall not be subject to change without amending the ordinance in which the prohibition has been adopted (e.g., no boundaries defined with reference to zoning districts or the availability of the public water supply); and

4) The prohibition shall not in any way restrict or limit the Agency's approval of the use of the ordinance as an institutional control pursuant to this Part (e.g., no restrictions based on remediation program participation, no restrictions on persons performing remediation within the prohibition area who may use the ordinance).

b) A request for approval of a local ordinance as an institutional control shall provide the following:

1) A copy of the ordinance restricting groundwater use certified by an official of the unit of local government in which the site is located that it is a true and accurate copy of the ordinance, unless the Agency and the unit of local government have entered an agreement under subsection (i) of this Section, in which case the request may alternatively reference the MOU. The ordinance must demonstrate that potable use of groundwater from potable water supply wells is prohibited;

2) A scaled map or maps delineating the area and extent of groundwater contamination modeled above the applicable remediation objectives including any measured data showing concentrations of contaminants of concern in which the applicable remediation objectives are exceeded;

3) A scaled map delineating the boundaries of all properties under which groundwater is located that exceeds the applicable groundwater remediation objectives;

4) Information identifying the current owners of each property identified in subsection (b)(3); and

5) A copy of the proposed written notification to the unit of local government that adopted the ordinance and to the current owners identified in subsection (b)(4) that includes the following information:

A) The name and address of the unit of local government that adopted the ordinance;

B) The ordinance's citation;

C) A description of the property being sent notice by adequate legal description, reference to a plat showing the boundaries of the property, or accurate street address;

D) Identification of the party requesting to use the groundwater ordinance as an institutional control, and a statement that the party

has requested approval from the Agency to use the ordinance as an institutional control;

E) A statement that use of the ordinance as an institutional control allows contamination above groundwater ingestion remediation objectives to remain in groundwater beneath the affected properties, and that the ordinance strictly prohibits human and domestic consumption of the groundwater;

F) A statement as to the nature of the release and response action with the site name, site address, and Agency site number or Illinois inventory identification number; and

G) A statement that more information about the remediation site may be obtained by contacting the party requesting the use of the groundwater ordinance as an institutional control or by submitting a FOIA request to the Agency.

c) Written notification proposed pursuant to subsection (b)(5) must be sent to the unit of local government that adopted the ordinance, as well as to all current property owners identified in subsection (b)(4). Written proof that the notification was sent to the unit of local government and the property owners shall be submitted to the Agency within 45 days from the date the Agency's no further remediation determination is recorded. Such proof may consist of the return card from certified mail, return receipt requested, a notarized certificate of service, or a notarized affidavit.

d) Unless the Agency and the unit of local government have entered into a MOU under subsection (i), the current owner or successors in interest of a site who have received approval of use of an ordinance as an institutional control under this Section shall:

1) Monitor activities of the unit of local government relative to variance requests or changes in the ordinance relative to the use of potable groundwater at properties identified in subsection (b)(3); and

2) Notify the Agency of any approved variance requests or ordinance changes within 30 days after the date such action has been approved.

e) The information required in subsections (b)(1) through (b)(5) and the Agency letter approving the groundwater remediation objective shall be submitted to the unit of local government. Proof that the information has been filed with the unit of local government shall be provided to the Agency.

f) Any ordinance or MOU used as an institutional control pursuant to this Section shall be recorded in the Office of the Recorder or Registrar of Titles of the county

in which the site is located together with the instrument memorializing the Agency's no further remediation determination pursuant to the specific program within 45 days after receipt of the Agency's no further remediation determination.

g) An institutional control approved under this Section shall not become effective until officially recorded in accordance with subsection (f). The person receiving the approval shall obtain and submit to the Agency within 30 days after recording a copy of the institutional control demonstrating that it has been recorded.

h) The following shall be grounds for voidance of the ordinance as an institutional control and the instrument memorializing the Agency's no further remediation determination:

1) Modification of the ordinance by the unit of local government to allow potable use of groundwater;

2) Approval of a site-specific request, such as a variance, to allow potable use of groundwater at a site identified in subsection (b)(3);

3) Violation of the terms of an institutional control recorded under Section 742.1005 or Section 742.1010; or

4) Failure to provide notification and proof of such notification pursuant to subsection (c).

i) The Agency and a unit of local government may enter into a MOU under this Section if the unit of local government has adopted an ordinance satisfying subsection (a) and if the requirements of this subsection are met. The MOU submitted to the Agency must match the form and contain the same substance as the model in Appendix H and shall include the following:

1) Identification of the authority of the unit of local government to enter the MOU;

2) Identification of the legal boundaries, or equivalent, under which the ordinance is applicable;

3) A certified copy of the ordinance;

4) A commitment by the unit of local government to notify the Agency of any variance requests or proposed ordinance changes at least 30 days prior to the date the local government is scheduled to take action on the request or proposed change;

5) A commitment by the unit of local government to maintain a registry of all sites within the unit of local government that have received no further remediation determinations pursuant to specific programs; and

6) If the ordinance does not expressly prohibit the installation of potable water supply wells (and the use of such wells) by units of local government, a commitment by the unit of local government:

A) To review the registry of sites established under subsection (i)(5) prior to siting potable water supply wells within the area covered by the ordinance;

B) To determine whether the potential source of potable water may be or has been affected by contamination left in place at those sites; and

C) To take whatever steps are necessary to ensure that the potential source of potable water is protected from the contamination or treated before it is used as a potable water supply.

j) A groundwater ordinance may not be used to exclude the indoor inhalation exposure route.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.1020 Highway Authority Agreements and Highway Authority Agreement Memoranda of Agreement

a) An agreement with a highway authority may be used as an institutional control where the requirements of this Section are met and the Agency has determined that no further remediation is required as to the property(ies) to which the agreement is to apply. Highway Authority Agreements submitted to the Agency, except for those agreements with the Illinois Department of Transportation, must match the form and contain the same substance, except for variable elements, as the model in Appendix D.

b) As part of the agreement the highway authority shall agree to:

1) Prohibit the use of groundwater under the highway right of way that is contaminated above residential Tier 1 remediation objectives from the release as a potable supply of water; and

2) Limit access to soil contamination under the highway right of way that is contaminated above residential Tier 1 or construction worker remediation objectives, whichever is less, from the release. Access to soil contamination may be allowed if, during and after any access, public health and the environment are protected.

c) The agreement shall provide the following:

1) Fully executed signature blocks by the highway authority and the owner of the property (or, in the case of a petroleum leaking underground storage tank, the owner or operator of the tank) from which the release occurred;

2) A scaled map delineating the area and extent of soil and groundwater contamination above the applicable Tier 1 remediation objectives or a statement that either soil or groundwater is not contaminated above the applicable Tier 1 residential remediation objectives;

3) Information showing the concentration of contaminants of concern within the zone in which the applicable Tier 1 remediation objectives are exceeded;

4) A stipulation of the information required by subsections (c)(2) and (3) of this Section in the agreement if it is not practical to obtain the information by sampling the highway right-of-way; and

5) Information identifying the highway authority having jurisdiction.

d) Highway Authority Agreements must be referenced in the instrument that is to be recorded on the chain of title for the remediation property.

e) Violation of the terms of an Agreement approved by the Agency as an institutional control under this Section shall be grounds for voidance of the Agreement as an institutional control and the instrument memorializing the Agency's no further remediation determination.

f) Failure to provide all of the information required in subsections (b) and (c) of this Section will be grounds for denial of the Highway Authority Agreement as an institutional control.

g) In instances in which the highway authority is also the property owner of the site, a Highway Authority Agreement may not be used. In such cases, the highway authority shall instead enter into a Highway Authority Agreement Memorandum of Agreement (HAA MOA) between the highway authority and the Agency. An HAA MOA may be used as an institutional control where the requirements of this Section are met and the Agency has determined that no further remediation is required as to the property(ies) to which the agreement is to apply. HAA MOAs submitted to the Agency must match the form and contain the same substance, except for variable elements, as the model in Appendix E.

h) As part of the HAA MOA the highway authority shall agree to:

1) Prohibit the use of groundwater under the highway right of way that is contaminated above residential Tier 1 or construction worker remediation objectives, whichever are less, from the release as a potable supply of water; and

2) Limit access to soil contamination under the highway right of way that is contaminated above residential Tier 1 or construction worker remediation objectives, whichever are less, from the release. Access to soil

contamination may be allowed if, during and after any access, public health and the environment are protected.

i) The HAA MOA shall provide the following:

1) Information identifying the site by common address or legal description or both;

2) The Illinois Emergency Management Agency's (IEMA) incident number for the site, if one has been assigned;

3) A scaled map delineating the current and estimated future area and extent of soil and groundwater contamination above the applicable Tier 1 or construction worker remediation objectives, whichever are less, or a statement that either soil or groundwater is not contaminated above the applicable Tier 1 residential remediation objectives;

4) Information prepared by the highway authority that lists each contaminant of concern that exceeds its Tier 1 residential or construction worker remediation objective, its Tier 1 residential remediation objective, and its concentrations within the zone where Tier 1 residential or construction worker remediation objectives, whichever is less, are exceeded;

5) A scaled map prepared by the highway authority showing the area of the highway authority's right of way that is governed by the HAA MOA;

6) If samples have not been collected within the right of way because of impracticability, a stipulation by the parties that, based on modeling, soil and groundwater contamination exceeding Tier 1 residential or construction worker remediation objectives, whichever is less, does not and will not extend beyond the boundaries of the right-of-way;

7) A stipulation by the highway authority that it has jurisdiction over the right of way that gives it sole control over the use of the groundwater and access to the soil located within or beneath the right of way;

8) A stipulation by the highway authority that it agrees to limit access by itself and others to soil within the right of way exceeding Tier 1 residential or construction worker remediation objectives, whichever is less. Access may only be allowed if human health (including worker safety) and the environment are protected during and after any access. The highway authority may construct, reconstruct, improve, repair, maintain, and operate a highway upon the right of way, or allow others to do the same by permit. The highway authority and others using or working in the right of way under permit have the right to remove soil or groundwater from the right of way and dispose of the same in accordance with applicable environmental laws and regulations. The highway authority agrees to issue all permits for work in the right of way, and make all existing permits for work in the right of way, subject to the following or substantially similar conditions:

A) As a condition of this permit the permittee shall request the office issuing this permit to identify sites in the right of way where a HAA MOA governs access to soil that exceeds the Tier 1 residential remediation objectives of 35 Ill. Adm. Code 742; and

B) The permittee shall take all measures necessary to protect human health (including worker safety) and the environment during and after any access to such soil;

9) A stipulation that the HAA MOA shall be referenced in the Agency's no further remediation determination issued for the release(s);

10) A stipulation that the highway authority shall notify the Agency of any transfer of jurisdiction over the right of way at least 30 days prior to the date the transfer takes effect. The HAA MOA shall be null and void upon the transfer unless the transferee agrees to be bound by the agreement as if the transferee were an original party to the agreement. The transferee's agreement to be bound by the terms of the agreement shall be memorialized at the time of transfer as a rider to this agreement that references the HAA MOA and is signed by the highway authority, or subsequent transferor, and the transferee;

11) A stipulation that the HAA MOA will become effective on the date the Agency issues a no further remediation determination for the release(s). It shall remain effective until the right of way is demonstrated to be suitable for unrestricted use and the Agency issues a new no further remediation determination to reflect there is no longer a need for the HAA MOA, or until the agreement is otherwise terminated or voided;

12) A stipulation that in addition to any other remedies that may be available, the Agency may bring suit to enforce the terms of the HAA MOA or may, at its sole discretion, declare the HAA MOA null and void if the highway authority or a transferee violates any term of the HAA MOA. The highway authority or transferee shall be notified in writing of any such declaration; and

13) A fully executed signature block by the highway authority and a block for the Agency's Director.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

SUBPART K: ENGINEERED BARRIERS

Section 742.1100 Engineered Barriers

a) Any person who develops remediation objectives under this Part based on engineered barriers shall meet the requirements of this Subpart and the requirements of Subpart J relative to institutional controls.

b) The Agency shall not approve any remediation objective under this Part that is based on the use of engineered barriers unless the person has proposed engineered barriers meeting the requirements of this Subpart.

c) The use of engineered barriers can be recognized in calculating remediation objectives only if the engineered barriers are intended for use as part of the final corrective action.

d) Any no further remediation determination based upon the use of engineered barriers shall require effective maintenance of the engineered barrier. The maintenance requirements shall be included in an institutional control under Subpart J. This institutional control shall address provisions for temporary breaches of the barrier by requiring the following if intrusive construction work is to be performed in which the engineered barrier is to be temporarily breached:

1) The construction workers shall be notified by the site owner/operator in advance of intrusive activities. Such notification shall enumerate the contaminant of concern known to be present; and

2) The site owner/operator shall require construction workers to implement protective measures consistent with good industrial hygiene practice.

e) Failure to maintain an engineered barrier in accordance with that no further remediation determination shall be grounds for voidance of the determination and the instrument memorializing the Agency's no further remediation determination.

Section 742.1105 Engineered Barrier Requirements

a) Natural attenuation, access controls, and point of use treatment shall not be considered engineered barriers. Engineered barriers may not be used to prevent direct human exposure to groundwater without the use of institutional controls.

b) For purposes of determining remediation objectives under Tier 1, engineered barriers are not recognized.

c) The following engineered barriers are recognized for purposes of calculating remediation objectives that exceed residential remediation objectives:

1) For the soil component of the groundwater ingestion exposure route, the following engineered barriers are recognized if they prevent completion of the exposure pathway:

A) Caps or walls constructed of compacted clay, asphalt, concrete or other material approved by the Agency; and

B) Permanent structures such as buildings and highways.

2) For the soil ingestion exposure route, the following engineered barriers are recognized if they prevent completion of the exposure pathway:

A) Caps or walls constructed of compacted clay, asphalt, concrete, or other material approved by the Agency;

B) Permanent structures such as buildings and highways; and

C) Soil, sand, gravel, or other geologic materials that:

i) Cover the contaminated media;

ii) Meet the soil remediation objectives under Subpart E for residential property for contaminants of concern; and

iii) Are a minimum of three feet in depth.

3) For the outdoor inhalation exposure route, the following engineered barriers are recognized if they prevent completion of the exposure pathway:

A) Caps or walls constructed of compacted clay, asphalt, concrete, or other material approved by the Agency;

B) Permanent structures such as buildings and highways; and

C) Soil, sand, gravel, or other geologic materials that:

i) Cover the contaminated media;

ii) Meet the soil remediation objectives under Subpart E for residential property for contaminants of concern; and

iii) Are a minimum of ten feet in depth and not within ten feet of any manmade pathway.

4) For the ingestion of groundwater exposure route, the following engineered barriers are recognized if they prevent completion of the exposure pathway:

A) Slurry walls; and

B) Hydraulic control of groundwater.

d) Unless otherwise prohibited under Section 742.1100, any other type of engineered barrier may be proposed if it will be as effective as the options listed in subsection (c).

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

SUBPART L: BUILDING CONTROL TECHNOLOGIES

Section 742.1200 Building Control Technologies

a) Any person who develops remediation objectives under this Part based on building control technologies shall meet the requirements of this Subpart and the requirements of Subpart J relative to institutional controls.

b) The Agency shall not approve any remediation objective under this Part that is based on the use of building control technologies unless the person has proposed building control technologies meeting the requirements of the following:

1) This Subpart L or Subpart I; and

2) Subpart J relative to institutional controls.

c) The use of building control technologies can be recognized in determining remediation objectives only if the building control technologies are intended for use as part of the final corrective action.

d) An approved building control technology shall be in place and operational prior to human occupancy.

e) Any no further remediation determination based upon the use of building control technologies shall require effective maintenance of the building control technology. The maintenance requirements shall be included in an institutional control under Subpart J. This institutional control shall address provisions for inoperability by requiring the following if the building control technology is rendered inoperable:

1) The site owner/operator shall notify building occupants and workers in advance of intrusive activities. The notification shall enumerate the contaminant of concern known to be present;

2) The site owner/operator shall require building occupants and workers to implement protective measures consistent with good industrial hygiene practice; and

3) For a school, the school administrator shall notify the Agency, the school board, and every parent or legal guardian for all enrolled students when a building control technology is rendered inoperable for a period of five consecutive calendar days during the school year when school is in session. For purposes of the preceding sentence, any occurrence of inoperability, regardless of its duration, results in the date of the occurrence constituting a day of inoperability. For purposes of this subsection (e)(3), the term "school" means any public educational facility in Illinois, including grounds and/or campus, consisting of students, comprising one or more grade groups or other identifiable groups, organized as one unit with one or more teachers to give instruction of a defined type. Public educational facility includes, but is not limited to, primary and secondary (kindergarten-12th grade), charter, vocational, alternative, and special education schools. Public educational facility does not include junior colleges, colleges, or universities. For purposes of this subsection (e)(3), the term "school administrator" means the school's principal, or similar administrator responsible for the school's operations, or his or her designee.

f) Failure to install or maintain a building control technology in accordance with a no further remediation determination shall be grounds for voidance of the determination and the instrument memorializing the Agency's no further remediation determination.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.1205 Building Control Technology Proposals

A proposal to use a building control technology under this Subpart shall include the following information:

a) A description of the site and physical site characteristics;

b) The current extent and modeled migration of contamination;

c) Geology, including soil types and parameters;

d) Results and locations of sampling events;

e) Scaled map of the area, including all buildings and man-made pathways;

f) A description of building characteristics and methods of construction, including a description of man-made pathways; and

g) Present and post-remediation uses of the land that are at issue due to the area of contamination, including human receptors at risk.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.1210 Building Control Technology Requirements

a) Natural attenuation, access controls, and point of use treatment shall not be considered building control technologies.

b) For purposes of determining compliance with remediation objectives under Tier 1, building control technologies are not recognized.

c) The following building control technologies are recognized for purposes of pathway exclusion under Section 742.312.

1) Sub-slab depressurization (SSD) systems meeting the following requirements:

A) A suction pit is installed that is at least two cubic feet and extends at least 6 inches below the slab (larger suction pits may be excavated as needed to achieve the performance criteria in subsection (c)(1)(B));

B) A PVC pipe of at least 3 inches in diameter extends from the suction pit to the intake side of an in-line fan capable of achieving a static vacuum of at least 0.25 inches water column (wc) at the suction point and measureable vacuum at the farthest edges of the area served by the suction pit under worst case conditions (all exhaust fans and heating systems running, during cold weather) as determined by a differential pressure reading of at least -0.003 inches wc below the slab or visible downward flow of air at test holes using chemical or smoke sticks;

C) All visible cracks and joints in the slab (including the place where the pipe exits the slab) and foundation walls are sealed;

D) The pipe exhausts outside the building at least 10 feet above ground and at least 10 feet from any door or window; and

E) Additional suction pits meeting the requirements of subsection (c)(1)(A) shall be installed as necessary to achieve measureable vacuum below the slab in all areas, including in any area where subsurface or foundation conditions (e.g., a sub-slab grade beam) prevent adequate suction field extension.

2) Sub-membrane depressurization (SMD) systems meeting the following requirements:

A) A non-woven geotextile is installed on the exposed earthen material;

B) A cross-laminated polyethylene membrane liner at least 0.10 mm (or 4 mil) thick is placed over the geotextile and sealed to foundation walls using a low volatile adhesive that is recommended by the liner manufacturer (e.g., acrylic latex adhesive);

C) A 3 inch diameter PVC pipe extends from a hole cut in the liner to the intake side of an in-line fan capable of achieving a static vacuum of at least 0.25 inches water column (wc) at the riser pipe and measureable vacuum at the farthest edges of the liner under worst case conditions (all exhaust fans running during cold weather) as determined by a differential pressure reading of at least -0.003 inches wc below the liner or visible downward flow of air in test holes using chemical or smoke sticks;

D) The pipe is sealed to the liner;

E) The pipe exhausts outside the building at least 10 feet above ground and at least 10 feet from any door or window; and

F) No leaks based on smoke stick tests along the entire perimeter of the liner (i.e., at all sealed edges) with the fan running. Where leaks are identified, appropriate repairs are undertaken and smoke stick testing repeated until no leaks are detected.

3) Membrane barrier systems when placed below concrete slabs meeting the following requirements:

A) The membrane is impermeable to volatile chemicals and is not less than 1.5 mm (or 60 mil) thick;

B) The membrane is sealed to foundation walls and any penetrating pipes according to membrane manufacturer/installer recommendations;

C) The membrane is installed in accordance with the manufacturer's requirements and by an applicator trained and approved by the manufacturer;

D) A smoke test of the membrane system (where smoke is injected below the installed liner prior to slab installation), in accordance with the manufacturer's requirements, is performed to ensure no leaks exist. Where leaks are identified, appropriate repairs are undertaken and smoke testing repeated until no leaks are detected;

E) The membrane is puncture resistant to slab installation construction activities and protected by sand layers or geotextiles as recommended by the manufacturer; and

F) Construction activities following membrane installation do not damage, puncture or tear the membrane or otherwise compromise its ability to prevent the migration of volatile chemicals.

4) Vented raised floors meeting the following requirements:

A) An interconnected void system below the slab sufficient to allow free movement of air and communication of negative pressures to all points below the slab;

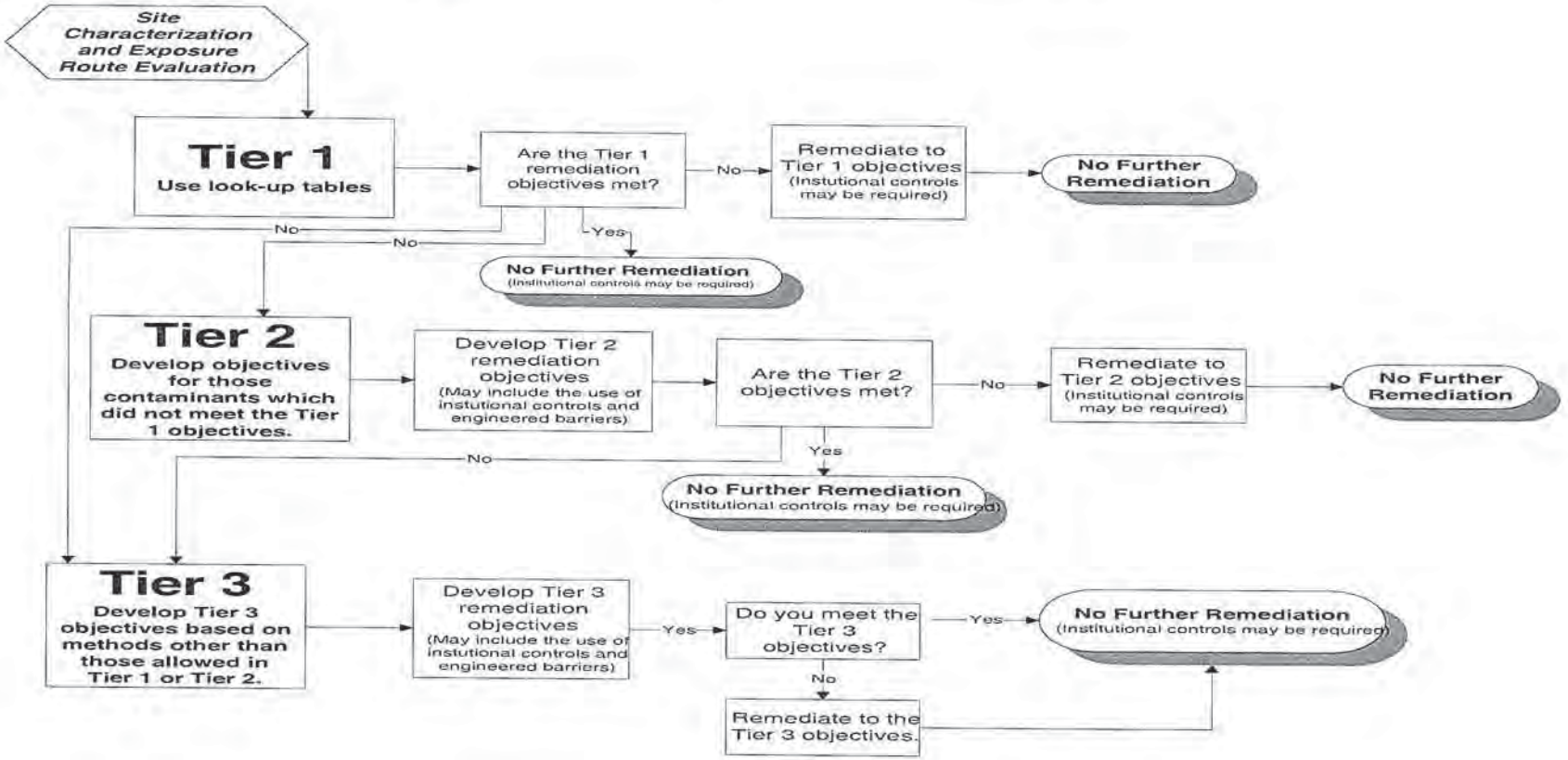
B) Sealing of all construction joints, open cracks, and penetrations through the slab (e.g., for utilities and riser pipes) with a low volatile caulk; and

C) At least one 3 inch diameter riser pipe venting to the atmosphere above the roof line (at least 10 feet from any doors or windows) for each 5000 square feet of membrane area, with the capability of converting passively vented floor systems to actively vented or SSD systems meeting the performance requirements of subsection (c)(1).

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

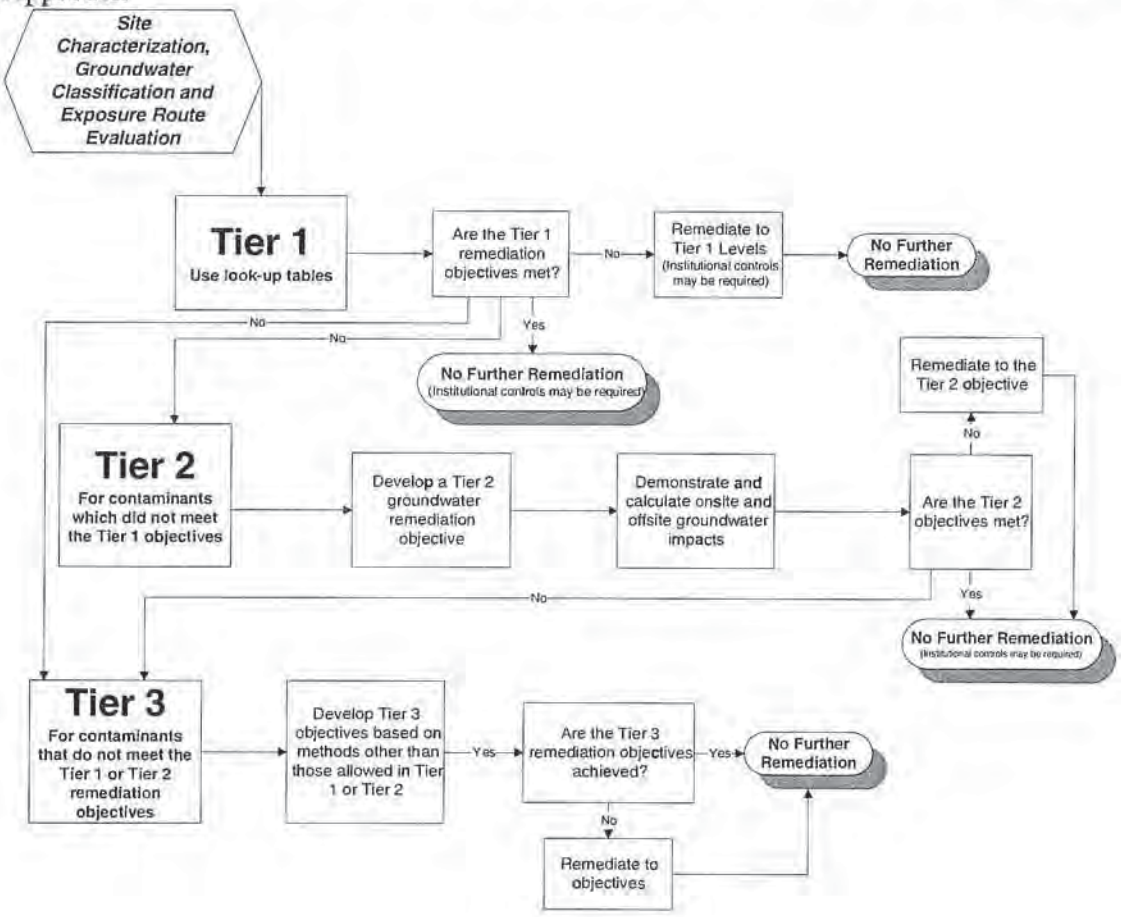
Section 742.APPENDIX A: General

Section 742.Illustration A: Developing Soil Remediation Objectives Under the Tiered Approach



Section 742.APPENDIX A: General

Section 742.Illustration B: Developing Groundwater Remediation Objectives Under the Tiered Approach



Section 742.APPENDIX A: General

Section 742.TABLE A: Soil Saturation Limits (C_{sat}) for Chemicals Whose Melting Point is Less Than 30° C

CAS No.	Chemical Name	For the Outdoor Inhalation Exposure Route[a] C_{sat} (mg/kg)	For the Soil Component of the Groundwater Ingestion Exposure Route[b] C_{sat} (mg/kg)
67-64-1	Acetone	1.00E+05	2.00E+05
71-43-2	Benzene	8.00E+02	5.80E+02
111-44-4	Bis(2-chloroethyl)ether	3.00E+03	3.90E+03
117-81-7	Bis(2-ethylhexyl)phthalate	2.00E+02	6.80E+01
75-27-4	Bromodichloromethane (Dichlorobromomethane)	2.80E+03	2.00E+03
75-25-2	Bromoform	2.00E+03	1.20E+03
71-36-3	Butanol	1.00E+04	1.60E+04
78-93-3	2-Butanone (MEK)	2.50E+04	4.50E+04
85-68-7	Butyl benzyl phthalate	1.00E+03	3.40E+02
75-15-0	Carbon disulfide	8.50E+02	5.20E+02
56-23-5	Carbon tetrachloride	1.20E+03	5.60E+02
108-90-7	Chlorobenzene (Monochlorobenzene)	6.20E+02	2.90E+02
124-48-1	Chlorodibromomethane (Dibromochloromethane)	1.40E+03	8.90E+02
67-66-3	Chloroform	3.40E+03	2.50E+03
95-57-8	2-Chlorophenol [c] (ionizable organic)	1.00E+04	7.10E+03
75-99-0	Dalapon	1.20E+05	1.90E+05
96-12-8	1,2-Dibromo-3-chloropropane	6.90E+02	4.30E+02
106-93-4	1,2-Dibromoethane (Ethylene dibromide)	1.60E+03	1.20E+03

CAS No.	Chemical Name	For the Outdoor Inhalation Exposure Route[a] C_{sat} (mg/kg)	For the Soil Component of the Groundwater Ingestion Exposure Route[b] C_{sat} (mg/kg)
84-74-2	Di-*n*-butyl phthalate	2.60E+03	8.80E+02
95-50-1	1,2-Dichlorobenzene (o-Dichlorobenzene)	5.60E+02	2.10E+02
75-71-8	Dichlorodifluoromethane	8.70E+02	4.30E+02
75-34-3	1,1-Dichloroethane	1.70E+03	1.40E+03
107-06-2	1,2-Dichloroethane (Ethylene dichloride)	1.90E+03	2.10E+03
75-35-4	1,1-Dichloroethylene	1.40E+03	9.10E+02
156-59-2	*cis*-1,2-Dichloroethylene	1.30E+03	1.00E+03
156-60-5	*trans*-1,2-Dichloroethylene	3.00E+03	2.10E+03
78-87-5	1,2-Dichloropropane	1.20E+03	8.70E+02
542-75-6	1,3-Dichloropropene (1,3-Dichloropropylene, *cis* + *trans*)	1.00E+03	8.50E+02
84-66-2	Diethyl phthalate	2.20E+03	9.20E+02
105-67-9	2,4-Dimethylphenol	1.00E+04	4.70E+03
117-84-0	Di-*n*-octyl phthalate	1.60E+01	5.20E+00
123-91-1	p-Dioxane	1.00E+05	2.00E+05
100-41-4	Ethylbenzene	3.50E+02	1.50E+02
77-47-4	Hexachlorocyclopentadiene	1.30E+02	4.40E+01
78-59-1	Isophorone	3.00E+03	3.00E+03
98-82-8	Isopropylbenzene (Cumene)	9.40E+02	4.00E+02
7439-97-6	Mercury (elemental)	3.10E+00	N/A
74-83-9	Methyl bromide (Bromomethane)	3.10E+03	3.60E+03
1634-04-4	Methyl tertiary-butyl ether	8.40E+03	1.10E+04
75-09-2	Methylene chloride (Dichloromethane)	2.50E+03	3.00E+03

CAS No.	Chemical Name	For the Outdoor Inhalation Exposure Route[a] C_{sat} (mg/kg)	For the Soil Component of the Groundwater Ingestion Exposure Route[b] C_{sat} (mg/kg)
98-95-3	Nitrobenzene	7.10E+02	5.90E+02
621-64-7	n-Nitrosodi-n-propylamine	1.90E+03	2.30E+03
100-42-5	Styrene	6.30E+02	2.60E+02
127-18-4	Tetrachloroethylene (Perchloroethylene)	8.00E+02	3.10E+02
108-88-3	Toluene	5.80E+02	2.90E+02
120-82-1	1,2,4-Trichlorobenzene	3.40E+02	1.20E+02
71-55-6	1,1,1-Trichloroethane	1.30E+03	6.70E+02
79-00-5	1,1,2-Trichloroethane	1.80E+03	1.30E+03
79-01-6	Trichloroethylene	1.20E+03	6.50E+02
75-69-4	Trichlorofluoromethane	1.80E+03	8.90E+02
108-05-4	Vinyl acetate	2.60E+03	4.20E+03
75-01-4	Vinyl chloride	2.60E+03	2.90E+03
108-38-3	m-Xylene	4.10E+02	1.60E+02
95-47-6	o-Xylene	3.70E+02	1.50E+02
106-42-3	p-Xylene	3.30E+02	1.40E+02
1330-20-7	Xylenes (total)	2.80E+02	1.10E+02

[a] Soil Saturation Limits calculated using an f_{oc} of 0.006 g/g and a system temperature of 25°C.

[b] Soil Saturation Limits calculated using an f_{oc} of 0.002 g/g and a system temperature of 25°C.

[c] C_{sat} for pH of 6.8. If soil pH is other than 6.8, a site-specific C_{sat} should be calculated using equations S19 and S29 and the pH-specific K_{oc} values in Appendix C Table I.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX A: General

Section 742.TABLE B: Tolerance Factor (K)

Tolerance factors (K) for one-sided normal tolerance intervals with probability level (confidence factor) Y = 0.95 and coverage P = 95%. n = number of samples collected.

n	K
3	7.655
4	5.145
5	4.202
6	3.707
7	3.399
8	3.188
9	3.031
10	2.911
11	2.815
12	2.736
13	2.670
14	2.614
15	2.566
16	2.523
17	2.486
18	2.543
19	2.423
20	2.396
21	2.371
22	2.350
23	2.329
24	2.309
25	2.292
30	2.220
35	2.166
40	2.126
45	2.092
50	2.065
55	2.036
60	2.017
65	2.000
70	1.986
75	1.972
100	1.924
125	1.891
150	1.868
175	1.850

200	1.836
225	1.824
250	1.814
275	1.806
300	1.799
325	1.792
350	1.787
375	1.782
400	1.777
425	1.773
450	1.769

n	K
475	1.766
500	1.763
525	1.760
550	1.757
575	1.754
600	1.752
625	1.750
650	1.748
675	1.746
700	1.744
725	1.742
750	1.740
775	1.739
800	1.737
825	1.736
850	1.734
875	1.733
900	1.732
925	1.731
950	1.729
975	1.728
1000	1.727

Section 742.APPENDIX A: General

Section 742.TABLE C: Coefficients $\{A_{N-I+1}\}$ for W Test of Normality, for N=2(1)50

i/n	2	3	4	5	6	7	8	9	10	
1	0.7071	0.7071	0.6872	0.6646	0.6431	0.6233	0.6052	0.5888	0.5739	
2	---	.0000	.1677	.2413	.2806	.3031	.3164	.3244	.3291	
3	---	---	---	.0000	.0875	.1401	.1743	.1976	.2141	
4	---	---	---	---	---	.0000	.0561	.0947	.1224	
5	---	---	---	---	---	---	---	.0000	.0399	
i/n	11	12	13	14	15	16	17	18	19	20
1	0.5601	0.5475	0.5359	0.5251	0.5150	0.5056	0.4968	0.4886	0.4808	0.4734
2	.3315	.3325	.3325	.3318	.3306	.3290	.3273	.3253	.3232	.3211
3	.2260	.2347	.2412	.2460	.2495	.2521	.2540	.2553	.2561	.2565
4	.1429	.1586	.1707	.1802	.1878	.1939	.1988	.2027	.2059	.2085
5	.0695	.0922	.1099	.1240	.1353	.1447	.1524	.1587	.1641	.1686
6	0.0000	0.0303	0.0539	0.0727	0.0880	0.1005	0.1109	0.1197	0.1271	0.1334
7	---	---	.0000	.0240	.0433	.0593	.0725	.0837	.0932	.1013
8	---	---	---	---	.0000	.0196	.0359	.0496	.0612	.0711
9	---	---	---	---	---	---	.0000	.0163	.0303	.0422
10	---	---	---	---	---	---	---	---	.0000	.0140

i/n	21	22	23	24	25	26	27	28	29	30
1	0.4643	0.4590	0.4542	0.4493	0.4450	0.4407	0.4366	0.4328	0.4291	0.4254
2	.3185	.3156	.3126	.3098	.3069	.3043	.3018	.2992	.2968	.2944
3	.2578	.2571	.2563	.2554	.2543	.2533	.2522	.2510	.2499	.2487
4	.2119	.2131	.2139	.2145	.2148	.2151	.2152	.2151	.2150	.2148
5	.1736	.1764	.1787	.1807	.1822	.1836	.1848	.1857	.1864	.1870
6	0.1399	0.1443	0.1480	0.1512	0.1539	0.1563	0.1584	0.1601	0.1616	0.1630
7	.1092	.1150	.1201	.1245	.1283	.1316	.1346	.1372	.1395	.1415
8	.0804	.0878	.0941	.0997	.1046	.1089	.1128	.1162	.1192	.1219
9	.0530	.0618	.0696	.0764	.0823	.0876	.0923	.0965	.1002	.1036
10	.0263	.0368	.0459	.0539	.0610	.0672	.0728	.0778	.0822	.0862
11	0.0000	0.0122	0.0228	0.0321	0.0403	0.0476	0.0540	0.0598	0.0650	0.0697
12	---	---	.0000	.0107	.0200	.0284	.0358	.0424	.0483	.0537
13	---	---	---	---	.0000	.0094	.0178	.0253	.0320	.0381
14	---	---	---	---	---	---	.0000	.0084	.0159	.0227
15	---	---	---	---	---	---	---	---	.0000	.0076

i/n	31	32	33	34	35	36	37	38	39	40
1	0.4220	0.4188	0.4156	0.4127	0.4096	0.4068	0.4040	0.4015	0.3989	0.3964
2	.2921	.2898	.2876	.2854	.2834	.2813	.2794	.2774	.2755	.2737
3	.2475	.2463	.2451	.2439	.2427	.2415	.2403	.2391	.2380	.2368
4	.2145	.2141	.2137	.2132	.2127	.2121	.2116	.2110	.2104	.2098
5	.1874	.1878	.1880	.1882	.1883	.1883	.1883	.1881	.1880	.1878
i/n	31	32	33	34	35	36	37	38	39	40
6	0.1641	0.1651	0.1660	0.1667	0.1673	0.1678	0.1683	0.1686	0.1689	0.1691
7	.1433	.1449	.1463	.1475	.1487	.1496	.1503	.1513	.1520	.1526
8	.1243	.1265	.1284	.1301	.1317	.1331	.1344	.1356	.1366	.1376
9	.1066	.1093	.1118	.1140	.1160	.1179	.1196	.1211	.1225	.1237
10	.0899	.0931	.0961	.0988	.1013	.1036	.1056	.1075	.1092	.1108
11	0.0739	0.0777	0.0812	0.0844	0.0873	0.0900	0.0924	0.0947	0.0967	0.0986
12	.0585	.0629	.0669	.0706	.0739	.0770	.0798	.0824	.0848	.0870
13	.0435	.0485	.0530	.0572	.0610	.0645	.0677	.0706	.0733	.0759
14	.0289	.0344	.0395	.0441	.0484	.0523	.0559	.0592	.0622	.0651
15	.0144	.0206	.0262	.0314	.0361	.0404	.0444	.0481	.0515	.0546

16	0.0000	0.0068	0.0131	0.0187	0.0239	0.0287	0.0331	0.0372	0.0409	0.0444
17	---	---	.0000	.0062	.0119	.0172	.0220	.0264	.0305	.0343
18	---	---	---	---	.0000	.0057	.0110	.0158	.0203	.0244
19	---	---	---	---	---	---	.0000	.0053	.0101	.0146
20	---	---	---	---	---	---	---	---	.0000	.0049
i/n	41	42	43	44	45	46	47	48	49	50
1	0.3940	0.3917	0.3894	0.3872	0.3850	0.3830	0.3808	0.3789	0.3770	0.3751
2	.2719	.2701	.2684	.2667	.2651	.2635	.2620	.2604	.2589	.2574
3	.2357	.2345	.2334	.2323	.2313	.2302	.2291	.2281	.2271	.2260
4	.2091	.2085	.2078	.2072	.2065	.2058	.2052	.2045	.2038	.2032
5	.1876	.1874	.1871	.1868	.1865	.1862	.1859	.1855	.1851	.1847
i/n	41	42	43	44	45	46	47	48	49	50
6	0.1693	0.1694	0.1695	0.1695	0.1695	0.1695	0.1695	0.1693	0.1692	0.1691
7	.1531	.1535	.1539	.1542	.1545	.1548	.1550	.1551	.1553	.1554
8	.1384	.1392	.1398	.1405	.1410	.1415	.1420	.1423`	.1427	.1430
9	.1249	.1259	.1269	.1278	.1286	.1293	.1300	.1306	.1312	.1317
10	.1123	.1136	.1149	.1160	.1170	.1180	.1189	.1197	.1205	.1212

11	0.1004	0.1020	0.1035	0.1049	0.1062	0.1073	0.1085	0.1095	0.1105	0.1113
12	.0891	.0909	.0927	.0943	.0959	.0972	.0986	.0998	.1010	.1020
13	.0782	.0804	.0824	.0842	.0860	.0876	.0892	.0906	.0919	.0932
14	.0677	.0701	.0724	.0745	.0775	.0785	.0801	.0817	.0832	.0846
15	.0575	.0602	.0628	.0651	.0673	.0694	.0713	.0731	.0748	.0764
16	0.0476	0.0506	0.0534	0.0560	0.0584	0.0607	0.0628	0.0648	0.0667	0.0685
17	.0379	.0411	.0442	.0471	.0497	.0522	.0546	.0568	.0588	.0608
18	.0283	.0318	.0352	.0383	.0412	.0439	.0465	.0489	.0511	.0532
19	.0188	.0227	.0263	.0296	.0328	.0357	.0385	.0411	.0436	.0459
20	.0094	.0136	.0175	.0211	.0245	.0277	.0307	.0335	.0361	.0386
21	0.0000	0.0045	0.0087	0.0126	0.0163	0.0197	0.0229	0.0259	0.0288	0.0314
22	---	---	.0000	.0042	.0081	.0118	.0153	.0185	.0215	.0244
23	---	---	---	---	.0000	.0039	.0076	.0111	.0143	.0174
24	---	---	---	---	---	---	.0000	.0037	.0071	.0104
25	---	---	---	---	---	---	---	---	.0000	.0035

Section 742.APPENDIX A: General

Section 742.TABLE D: Percentage Points of the W Test for n=3(1)50

N	0.01	0.05
3	0.753	0.767
4	0.687	0.748
5	0.686	0.762
6	0.713	0.788
7	0.730	0.803
8	0.749	0.818
9	0.764	0.829
10	0.781	0.842
11	0.792	0.850
12	0.805	0.859
13	0.814	0.866
14	0.825	0.874
15	0.835	0.881
16	0.844	0.887
17	0.851	0.892
18	0.858	0.897
19	0.863	0.901
20	0.868	0.905
21	0.873	0.908
22	0.878	0.911
23	0.881	0.914
24	0.884	0.916
25	0.888	0.918
26	0.891	0.920
27	0.894	0.923
28	0.896	0.924
29	0.898	0.926
30	0.900	0.927
31	0.902	0.929
32	0.904	0.930
33	0.906	0.931
34	0.908	0.933

N	0.01	0.05
35	0.910	0.934

(Source: Amended at 25 Ill. Reg. 10374, effective August 15,2001)

Section 742.APPENDIX A General

Section 742.TABLE E Similar-Acting Noncarcinogenic Chemicals

Adrenal Gland
Isopropylbenzene

Cholinesterase Inhibition
Aldicarb
Carbofuran

Circulatory System
Alachlor
Antimony (ingestion only)
Benzene
Cobalt (ingestion only)
2,4-D
cis-1,2-Dichloroethylene (ingestion only)
2,4-Dimethylphenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Ensosulfan
Fluoranthene
Fluorene
Methylene Chloride (inhalation only)
Nickel (Res. & I/C only) (inhalation only)
Nitrate as N
Nitrobenzene (ingestion only)
Selenium
Simazine
Styrene (ingestion only)
1,3,5-Trinitrobenzene
Zinc

Decreased Body Weight Gain
Atrazine
Bis(2-chloroethyl)ether
Cyanide
1,2-Dichlorobenzene (inhalation only)
Diethyl phthalate (ingestion only)
Ensosulfan
2-Methylphenol (o-cresol)
Naphthalene (ingestion only)
Nickel (ingestion only)
n-Nitrosodiphenylamine
Phenol (ingestion only)
Simazine
Tetrachloroethylene (ingestion only)

1,1,1-Trichloroethane (ingestion only)
Vinyl acetate (ingestion only)
Xylenes (Res. & I/C only) (ingestion only)

Endocrine System
Cyanide
1,2-Dibromoethane (ingestion only)
Di-n-octyl phthalate (ingestion only)
Nitrobenzene
1,2,4-Trichlorobenzene (ingestion only)

Eye
2,4-Dinitrophenol
n-Nitrosodiphenylamine
Polychlorinated biphenyls (PCBs)
Trichloroethylene

Gastrointestinal System
Beryllium (ingestion only)
Copper
1,3-Dichloropropene (*cis* + *trans*) (ingestion only)
Endothall
Fluoride
Hexachlorocyclopentadiene (ingestion only)
Iron
Methyl bromide (ingestion only)
Methyl tertiary-butyl ether (ingestion only)

Immune System
4-Chloroaniline
2,4-Dichlorophenol
Mercury (ingestion only)
Polychlorinated biphenyls (PCBs)

Kidney
Acetone (ingestion only)
Aldrin (CW only)
Barium
Bromodichloromethane (ingestion only)
Cadmium
2,4-D
Dalapon
1,1-Dichloroethane
1,2-Dichloroethane (CW only) (ingestion only)
Ensosulfan
Ethylbenzene (ingestion only)
Fluoranthene

gamma-HCH (gamma-BHC)
Hexachloroethane (ingestion only)
Isopropylbenzene
Mecoprop (MCPP)
Methyl tertiary-butyl ether (inhalation only)
Pentachlorophenol
Pyrene
Toluene (ingestion only)
2,4,5-Trichlorophenol
Vinyl acetate (ingestion only)

Liver
Acenapthene
Aldrin (Res. & I/C only)
Bis(2-ethylhexyl)phthalate (Res. & I/C only) (ingestion only)
Bromoform
Butyl Benzyl Phtalate (ingestion only)
Carbon Tetrachloride
Chlordane
Chlorobenzene (ingestion only)
Chlorodibromomethane (ingestion only)
Chloroform
2,4-D
DDT
1,2-Dibromoethane (ingestion only)
1,2-Dichlorobenezene (CW only) (ingestion only)
1,4-Dichlorobenzene
Dichlorodifluoromethane
1,2-Dichloroethane (inhalation only)
1,1-Dichloroethylene
trans-1,2-Dichloroethylene
1,2-Dichloropropane (ingestion only)
Dieldrin (Res. & I/C only)
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Di-n-octyl phthalate (ingestion only)
p-Dioxane
Endrin
Ethylbenzene (ingestion only)
Fluoranthene
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
alpha-HCH (alpha-BHC
gamma-HCH (gamma-BHC)
High Melting Explosive, Octogen (HMX)
Isophorone (inhalation only)

Methyl tertiary-butyl ether
Methylene Chloride (ingestion only)
Pentachlorophenol
Phenol (inhalation only)
Picloram
Styrene (ingestion only)
Tetrachloroethylene (ingestion only)
Toxaphene (CW only)
2,4,5-TP (Silvex)
1,2,4-Trichlorobenzene (inhalation only)
1,1,1-Trichloroethane (inhalation only)
1,1,2-Trichloroethane (ingestion only)
2,4,5-Trichlorophenol
2,4,6-Trinitrotoluene (TNT)
Vinyl Chloride

Mortality
Di-n-butyl phthalate (ingestion only)
Xylenes (Res. & I/C only) (ingestion only)

Nervous System
Butanol (ingestion only)
Carbon disulfide (inhalation only)
Cyanide
Dieldrin (CW only)
2,4-Dimethylphenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Endrin
Hexachloroethane (inhalation only) (CW only)
Manganese
Mercury (inhalation only)
2-Methylphenol (o-cresol)
Phenol (inhalation only)
Selenium
Styrene (inhalation only)
Tetrachloroethylene (inhalation only)
Toluene (inhalation only)
Trichloroethylene
Xylenes (CW only) (ingestion only)
Xylenes (inhalation only)

Reproductive System
Arsenic (inhalation only)
Bis(2-ethylhexyl)phthalate (CW only) (ingestion only)
Boron
2-Butanone

Carbofuran
Carbon disulfide (ingestion only)
2-Chlorophenol
1,2-Dibromo-3-chloropropane
1,2-Dibromoethane (ingestion only)
Dicamba
Dinoseb
Ethylbenzene (inhalation only)
Isophorone (inhalation only)
Methoxychlor
Royal Demolition Explosive , Cyclonite (RDX)
2,4,6-Trichlorophenol

Respiratory System
Antimony (inhalation only)
Benzoic Acid (inhalation only)
Berryllium (inhalation only)
Cadmium (inhalation only)
Chromium (hex) (inhalation only)
Cobalt (inhalation only)
1,2-Dibromoethane (inhalation only)
trans-1,2-Dichloroethylene (inhalation only)
1,2-Dichloropropane (inhalation only)
1,3-Dichloropropene (*cis* + *trans*) (inhalation only)
Hexachlorocyclopentadiene (inhalation only)
Methyl bromide (inhalation only)
Naphtalene (inhalation only)
Nickel (inhalation only)
Nitrobenezene (inhalation only)
Vinyl acetate (inhalation only)

Skin
Arsenic (ingestion only)
Polychlorinated biphenyls (PCBs)
Selenium
Silver

Spleen
1,3-Dinotrobenzene
1,3,5-Trinitrobenzene

Notes:
Res. = Residential receptor
I/C = Industrial/Commercial receptor
CW = Construction Worker receptor

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX A: General

Section 742.TABLE F: Similar-Acting Carcinogenic Chemicals

Bladder
1,3-Dichloropropene (*cis* + *trans*) (ingestion only)
n-Nitrosodiphenylamine

Circulatory System
Benzene
1,2-Dibromoethane
1,2-Dichloroethane
Pentachlorophenol
2,4,6-Trichlorophenol

Gall Bladder
p-Dioxane (inhalation only)

Gastrointestinal System
Benzo(a)anthracene (ingestion only)
Benzo(b)fluoranthene (ingestion only)
Benzo(k)flouranthene (ingestion only)
Benzo(a)pyrene (ingestion only)
Bromoform
Chrysene (ingestion only)
Dibenzo(a,h)anthracene (ingestion only)
1,2-Dibromoethane (ingestion only)
Indeno(1,2,3-cd)pyrene (ingestion only)

Kidney
Bromodichloromethane (ingestion only)
Chloroform (ingestion only)
1,2-Dibromo-3-chloropropane (ingestion only)
Nitrobenzene

Liver
Aldrin
Bis(2-chloroethyl)ether
Bis(2-ethylhexyl)phthalate
Carbazole
Carbon Tetrachloride

Respiratory System (continued)
Benzo(k)flouranthene (inhalation only)
Benzo(a)pyrene (inhalation only)
Beryllium
Cadmium

Liver (continued)
Chlordane
Chloroform
DDD
DDE
DDT
1,2-Dichloropropane
Dieldrin
2,4-Dinitrotoluene
2,6-Dinitrotoluene
p-Dioxane
Heptachlor
Heptachlor epoxide
Hexachlorobenzene
alpha-HCH (alpha-BHC)
gamma-HCH (gamma-BHC)
Methylene Chloride
Nitrobenzene
n-Nitrosodiphenylamine (inhalation only)
n-Nitrosodi-n-propylamine
Pentachlorophenol
Polychlorinated biphenyls (PCBs)
Tetrachloroethylene
Toxaphene
Trichloroethylene
Vinyl Chloride (I/C & CW)
Vinyl Chloride (Res.)

Mammary Gland
3,3'-Dichlorobenzidine
2,4-Dinitrotoluene
2,6-Dinitrotoluene

Respiratory System
Arsenic (inhalation only)
Benzo(a)anthracene (inhalation only)
Benzo(b)fluoranthene (inhalation only)

Chromium (hexavalent ion)
Chrysene (inhalation only)
Cobalt
Dibenzo(a,h)anthracene (inhalation only)
1,2-Dibromo-3-chloropropane (inhalation only)
1,2-Dibromoethane (inhalation only)
1,3-Dichloropropene (*cis* + *trans*) (inhalation only)
p-Dioxane (inhalation only)
Trichloroethylene

Notes:
Res. = Residential receptor
I/C = Industrial/Commercial receptor
CW = Construction Worker receptor

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX A General

Section 742.TABLE G Concentrations of Inorganic Chemicals in Background Soils

Chemical Name	Counties Within Metropolitan Statistical Areas (mg/kg)	Counties Outside Metropolitan Statistical Areas (mg/kg)
Aluminum	9,500	9,200
Antimony	4.0	3.3
Arsenic	13.0	11.3
Barium	110`	122
Beryllium	0.59	0.56
Cadmium	0.6	0.50
Calcium	9,300	5,525
Chromium	16.2	13.0
Cobalt	8.9	8.9
Copper	19.6	12.0
Cyanide	0.51	0.50
Iron	15,900	15,000
Lead	36.0	20.9
Magnesium	4,820	2,700
Manganese	636	630
Mercury	0.06	0.05
Nickel	18.0	13.0
Potassium	1,268	1,100
Selenium	0.48	0.37
Silver	0.55	0.50
Sodium	130	130.0
Sulfate	85.5	110
Sulfide	3.1	2.9
Thallium	0.32	0.42
Vanadium	25.2	25.0
Zinc	95.0	60.2

BOARD NOTE: Counties within Metropolitan Statistical Areas: Boone, Champaign, Clinton, Cook, DuPage, Grundy, Henry, Jersey, Kane, Kankakee, Kendall, Lake, Macon, Madison, McHenry, McLean, Menard, Monroe, Peoria, Rock Island, Sangamon, St. Clair, Tazewell, Will, Winnebago and Woodford.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX A: General

Section 742.TABLE H Concentrations of Polynuclear Aromatic Hydrocarbon Chemicals in Background Soils

Chemical Name	Chicago[a] mg/kg	Metropolitan Areas[b] (mg/kg)	Non-Metropolitan Areas[c] (mg/kg)
2-Methylnaphthalene	-----	0.14	0.29
Acenaphthene	0.09	0.13	0.04
Acenaphthylene	0.03	0.07	0.04
Anthracene	0.25	0.40	0.14
Benzo(a)anthracene	1.1	1.8	0.72
Benzo(a)pyrene	1.3	2.1	0.98
Benzo(b)fluoranthene	1.5	2.1	0.70
Benzo(g,h,i)perylene	0.68	1.7	0.84
Benzo(k)fluoranthene	0.99	1.7	0.63
Chrysene	1.2	2.7	1.1
Dibenzo(a,h)anthracene	0.20	0.42	0.15
Fluoranthene	2.7	4.1	1.8
Fluorene	0.10	0.18	0.04
Indeno(1,2,3-c,d)pyrene	0.86	1.6	0.51
Naphthalene	0.04	0.20	0.17
Phenanthrene	1.3	2.5	0.99
Pyrene	1.9	3.0	1.2

[a] Chicago means within the corporate limits of the City of Chicago.

[b] Metropolitan area means a populated area, as defined in Section 742.200, (other than the City of Chicago) that is located within any county in a Metropolitan Statistical Area listed in Appendix A, Table G, footnote a.

[c] Non-Metropolitan area means a populated area, as defined in Section 742.200, that is not located within any county in a Metropolitan Statistical Area listed in Appendix A, Table G, footnote a.

(Source: Appendix A, Table H renumbered to Appendix A, Table I and new Appendix A, Table H Added at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX A General

Section 742.TABLE I Chemicals Whose Tier 1 Class I Groundwater Remediation Objective Exceeds the 1 in 1,000,000 Cancer Risk Concentration

Chemical	**Class I Groundwater Remediation Objective (mg/L)**	**1 in 1,000,000 Cancer Risk Concentration (mg/L)**	**ADL (mg/L)**
Aldrin	0.014	0.000005	0.014
Benzo(a)pyrene	0.0002	0.000012	0.00023
Bis(2-chloroethyl)ether	0.01	0.000077	0.01
Bis(2-ethylhexyl)phthalate (Di(2-ethylhexyl)phthalate)	0.006	0.0061	0.0027
Carbon Tetrachloride	0.005	0.00066	0.0001
Chlordane	0.002	0.000066	0.00014
DDD	0.014	0.00023	0.014
DDE	0.01	0.00023	0.01
DDT	0.006	0.00023	0.006
Dibenzo(a,h)anthracene	0.0003	0.000012	0.0003
1,2-Dibromo-3-chloropropane	0.0002	0.000061	0.001
1,2-Dibromoethane	0.00005	0.00002	0.001
3,3'-Dichlorobenzidine	0.02	0.00019	0.02
1,2-Dichloroethane	0.005	0.00094	0.0003
Dieldrin	0.009	0.0000053	0.009
2,6-Dinitrotoluene	0.00031	0.0001	0.00031
Heptachlor	0.0004	0.000019	0.013
Heptachlor epoxide	0.0002	0.0000094	0.015
Hexachlorobenzene	0.00006	0.000053	0.00006
Alpha-HCH	0.00011	0.000014	0.000111
Tetrachloroethylene	0.005	0.0016	0.0004
Toxaphene	0.003	0.000077	0.00086
Vinyl chloride	0.002	0.000045	0.0002
Ionizable Organics			
N-Nitrosodi-n-propylamine	0.0018	0.000012	0.0018
Pentachlorophenol	0.001	0.00071	0.000076
2,4,6-Trichlorophenol	0.01	0.007	0.01
Inorganics			
Arsenic	0.05	0.000057	0.001

(Source: Appendix A, Table I renumbered from Appendix A, Table H and amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.Table J: List of TACO Volatile Chemicals for the Indoor Inhalation Exposure Route

CAS No.	Chemical
67-64-1	Acetone
71-43-2	Benzene
111-44-4	Bis(2-chloroethyl)ether
75-27-4	Bromodichloromethane
75-25-2	Bromoform
71-36-3	Butanol
78-93-3	2-Butanone (MEK)
75-15-0	Carbon disulfide
56-23-5	Carbon tetrachloride
108-90-7	Chlorobenzene
124-48-1	Chlorodibromomethane
67-66-3	Chloroform
95-57-8	2-Chlorophenol
75-99-0	Dalapon
96-12-8	1,2-dibromo-3-chloropropane
106-93-4	1,2-Dibromoethane
95-50-1	1,2-Dichlorobenzene
106-46-7	1,4-Dichlorobenzene
75-71-8	Dichlorodifluoromethane
75-34-3	1,1-Dichloroethane
107-06-2	1,2-Dichloroethane
75-35-4	1,1-Dichloroethylene
156-59-2	*cis*-1,2-Dichloroethylene
156-60-5	*trans*-1,2-Dichloroethylene
78-87-5	1,2-Dichloropropane
542-75-6	1,3-Dichloropropylene (*cis* + *trans*)
123-91-1	p-Dioxane
100-41-4	Ethylbenzene
76-44-8	Heptachlor
118-74-1	Hexachlorobenzene
77-47-4	Hexachlorocyclopentadiene
67-72-1	Hexachloroethane
78-59-1	Isophorone
98-82-8	Isopropylbenzene (Cumene)
7439-97-6	Mercury
74-83-9	Methyl bromide
1634-04-4	Methyl tertiary-butyl ether
75-09-2	Methylene chloride
93-65-2	2-Methylnaphthalene
95-48-7	2-Methylphenol (o-cresol)
91-20-3	Naphthalene

CAS No.	Chemical
98-95-3	Nitrobenzene
621-64-7	n-Nitrosodi-n-propylamine
108-95-2	Phenol
1336-36-3	Polychlorinated biphenyls (PCBs)
100-42-5	Styrene
127-18-4	Tetrachloroethylene
108-88-3	Toluene
120-82-1	1,2,4-Trichlorobenzene
71-55-6	1,1,1-Trichloroethane
79-00-5	1,1,2-Trichloroethane
79-01-6	Trichloroethylene
75-69-4	Trichlorofluoromethane
108-05-4	Vinyl acetate
75-01-4	Vinyl chloride
108-38-3	m-Xylene
95-47-6	o-Xylene
106-42-3	p-Xylene
1330-20-7	Xylenes (total)

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX A: General

Section 742.TABLE K: Soil Vapor Saturation Limits (C_v^{sat}) for Volatile Chemicals

CAS No.	Chemical Name	C_v^{sat} (mg/m^3)
67-64-1	Acetone	7.50E+05
71-43-2	Benzene	4.20E+05
111-44-4	Bis(2-chloroethyl)ether	1.20E+04
75-27-4	Bromodichloromethane	4.50E+05
75-25-2	Bromoform	7.80E+04
71-36-3	Butanol	2.90E+04
78-93-3	2-Butanone (MEK)	3.80E+05
75-15-0	Carbon disulfide	1.50E+06
56-23-5	Carbon tetrachloride	1.00E+06
108-90-7	Chlorobenzene	7.40E+04
124-48-1	Chlorodibromomethane	5.70E+04
67-66-3	Chloroform	1.30E+06
95-57-8	2-Chlorophenol (ionizable organic)	1.70E+04
75-99-0	Dalapon	1.50E+03
96-12-8	1,2-Dibromo-3-chloropropane	7.80E+03
106-93-4	1,2-Dibromoethane	1.40E+05
95-50-1	1,2-Dichlorobenzene	1.10E+04
106-46-7	1,4-Dichlorobenzene	8.40E+03
75-71-8	Dichlorodifluoromethane	3.30E+07
75-34-3	1,1-Dichloroethane	1.30E+06
107-06-2	1,2-Dichloroethane	4.40E+05
75-35-4	1,1-Dichloroethylene	3.30E+06
156-59-2	*cis*-1,2-Dichloroethylene	1.10E+06
156-60-5	*trans*-1,2-Dichloroethylene	1.80E+06
78-87-5	1,2-Dichloropropane	3.20E+05

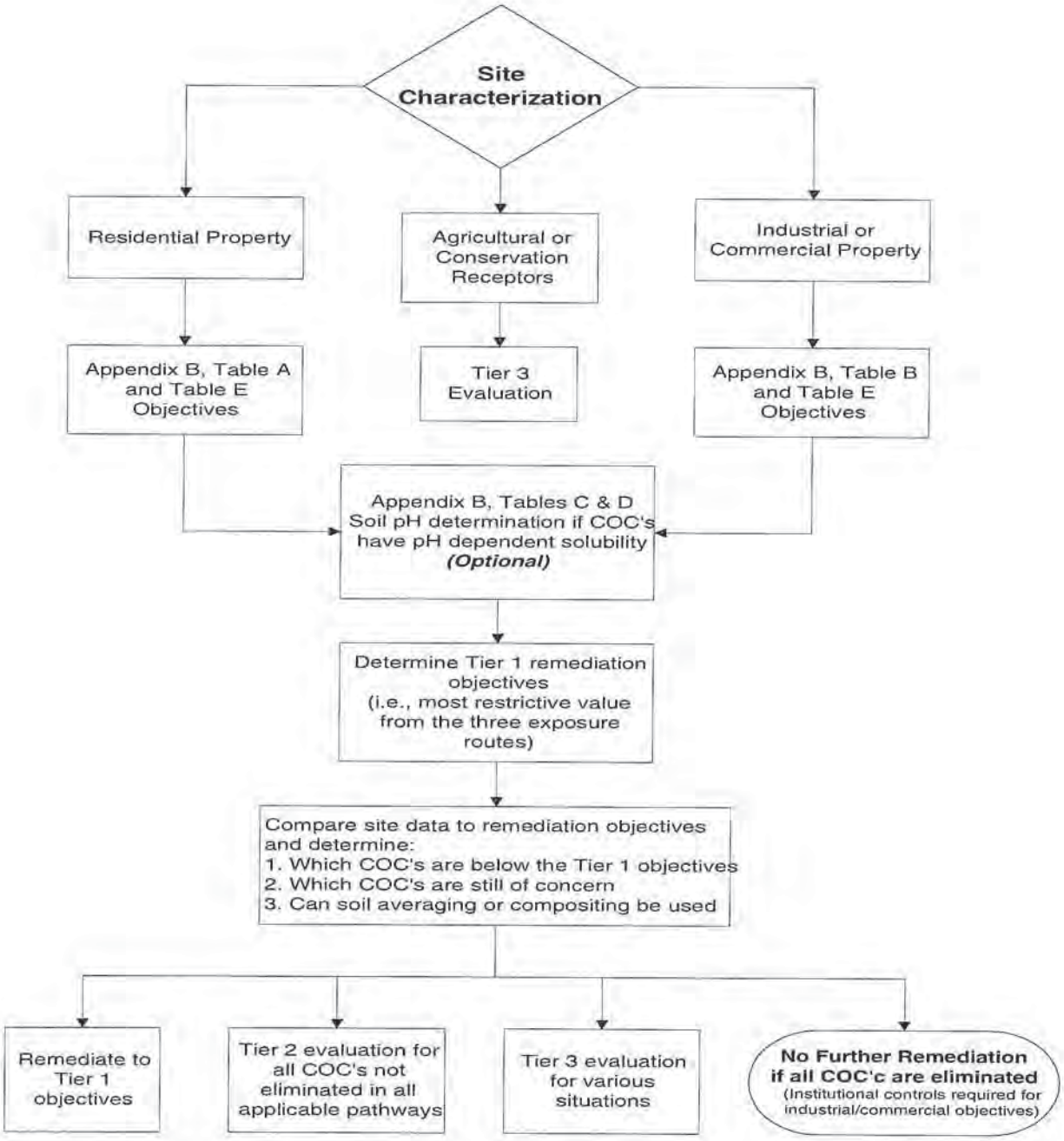
CAS No.	Chemical Name	C_v^{sat} (mg/m^3)
542-75-6	1,3-Dichloropropylene (*cis* + *trans*)	2.10E+05
123-91-1	p-Dioxane	1.90E+05
100-41-4	Ethylbenzene	5.90E+04
76-44-8	Heptachlor	8.30E+00
118-74-1	Hexachlorobenzene	2.80E-01
77-47-4	Hexachlorocyclopentadiene	9.10E+02
67-72-1	Hexachloroethane	2.80E+03
78-59-1	Isophorone	3.40E+03
98-82-8	Isopropylbenzene (Cumene)	3.00E+04
7439-97-6	Mercury (elemental)	2.20E+01
74-83-9	Methyl bromide	8.60E+06
1634-04-4	Methyl tertiary-butyl ether	1.20E+06
75-09-2	Methylene chloride	2.00E+06
93-65-2	2-Methylnaphthalene	5.30E+02
1634-04-4	2-Methylphenol (o-cresol)	1.80E+03
91-20-3	Naphthalene	6.20E+02
98-95-3	Nitrobenzene	1.70E+03
621-64-7	n-Nitrosodi-n-propylamine	9.50E+02
108-95-2	Phenol	1.50E+03
1336-36-3	Polychlorinated biphenyls (PCBs)	9.00E+00
100-42-5	Styrene	3.40E+04
127-18-4	Tetrachloroethylene	1.80E+05
108-88-3	Toluene	1.40E+05
120-82-1	1,2,4-Trichlorobenzene	4.30E+03
71-55-6	1,1,1-Trichloroethane	8.70E+05
79-00-5	1,1,2-Trichloroethane	1.70E+05
79-01-6	Trichloroethylene	5.30E+05

CAS No.	Chemical Name	C_v^{sat} (mg/m^3)
75-69-4	Trichlorofluoromethane	6.30E+06
108-05-4	Vinyl acetate	4.30E+05
75-01-4	Vinyl chloride	1.10E+07
108-38-3	m-Xylene	5.20E+04
95-47-6	o-Xylene	4.10E+04
106-42-3	p-Xylene	5.50E+04
1330-20-7	Xylenes (total)	4.90E+04

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.Illustration A Tier 1 Evaluation



(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.TABLE A Tier 1 Soil Remediation Objectives[a] for Residential Properties

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
83-32-9	Acenaphthene	4,700[b]	---[c]	570[b]	2,900	*
67-64-1	Acetone	70,000[b]	100,000[d]	25[b]	25	*
15972-60-8	Alachlor[o]	8[e]	---[c]	0.04	0.2	NA
116-06-3	Aldicarb[o]	78[b]	---[c]	0.013	0.07	NA
309-00-2	Aldrin	0.04[e]	3[e]	0.5[e]	2.5	0.94
120-12-7	Anthracene	23,000[b]	---[c]	12,000[b]	59,000	*
1912-24-9	Atrazine[o]	2700[b]	---[c]	0.066	0.33	NA
71-43-2	Benzene	12[e]	0.8[e]	0.03	0.17	*
56-55-3	Benzo(*a*)anthracene	0.9[e,w]	---[c]	2	8	*
205-99-2	Benzo(*b*)fluoranthene	0.9[e,w]	---[c]	5	25	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
207-08-9	Benzo(*k*)fluroanthene	9[e]	---[c]	49	250	*
50-32-8	Benzo(*a*)pyrene	0.09[e, w]	---[c]	8	82	*
111-44-4	Bis(2-chloroethyl)ether	0.6[e]	0.2[e,]	0.0004[e,]	0.0004	0.66
117-81-7	Bis(2-ethylhexyl)phthalate	46[e]	31,000[d]	3,600	31,000[d]	*
75-27-4	Bromodichloromethane (Dichlorobromomethane)	10[e]	3,000[d]	0.6	0.6	*
75-25-2	Bromoform	81[e]	53[e]	0.8	0.8	*
71-36-3	Butanol	7,800[b]	10,000[d]	17[b]	17	NA
85-68-7	Butyl benzyl phthalate	16,000[b]	930[d]	930[d]	930[d]	*
86-74-8	Carbazole	32[e]	---[c]	0.6[e]	2.8	NA
1563-66-2	Carbofuran[o]	390[b]	---[c]	0.22	1.1	NA
75-15-0	Carbon disulfide	7,800[b]	720[d, x]	32[b]	160	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
56-23-5	Carbon tetrachloride	5[e]	0.3[e]	0.07	0.33	*
57-74-9	Chlordane	1.8[e]	72[e, x]	10	48	*
106-47-8	4-Chloroaniline (*p*-Chloroaniline)	310[b]	---[c]	0.7[b]	0.7	*
108-90-7	Chlorobenzene (Monochlorobenzene)	1,600[b]	130[b, x]	1	6.5	*
124-48-1	Chlorodibromomethane (Dibromochloromethane)	1,600[b]	1,300[d]	0.4	0.4	*
67-66-3	Chloroform	100[e]	0.3[e]	0.6	2.9	*
218-01-9	Chrysene	88[e]	---[c]	160	800	*
94-75-7	2,4-D[o]	780[b]	---[c]	1.5	7.7	*
75-99-0	Dalapon[o]	2,300[b]	---[c]	0.85	8.5	*
72-54-8	DDD	3[e]	---[c]	16[e]	80	*
72-55-9	DDE	2[e]	---[c]	54[e]	270	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
50-29-3	DDT	2[e]	---[g, x]	32[e]	160	*
53-70-3	Dibenzo(*a,h*)anthracene	0.09[e, w]	---[c]	2	7.6	*
96-12-8	1,2-Dibromo-3-chloropropane	0.46[e]	11[b, x]	0.002	0.02	*
106-93-4	1,2-Dibromoethane (Ethylene dibromide)	0.32[e]	0.06[e]	0.0004	0.004	0.005
84-74-2	Di-*n*-butyl phthalate	7,800[b]	2,300[d]	2,300[d]	2,300[d]	*
95-50-1	1,2-Dichlorobenzene (*o* – Dichlorobenzene)	7,000[b]	560[d, x]	17	43	*
106-46-7	1,4-Dichlorobenzene (*p* – Dichlorobenzene)	---[c]	11,000[b, x]	2	11	*
91-94-1	3,3'-Dichlorobenzidine	1[e]	---[c]	0.007[e,	0.033	1.3
75-34-3	1,1-Dichloroethane	7,800[b]	1,300[b, x]	23[b]	110	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
107-06-2	1,2-Dichloroethane (Ethylene dichloride)	7[e]	0.4[e]	0.02	0.1	*
75-35-4	1,1-Dichloroethylene	3,900[b]	290[b, x]	0.06	0.3	*
156-59-2	*cis*-1,2-Dichloroethylene	780[b]	1,200[d]	0.4	1.1	*
156-60-5	*trans*-1,2-Dichloroethylene	1,600[b]	3,100[d]	0.7	3.4	*
78-87-5	1,2-Dichloropropane	9[e]	15[b, x]	0.03	0.15	*
542-75-6	1,3-Dichloropropene (1,3-Dichloropropylene, *cis* + *trans*)	6.4[e]	1.1[e, x]	0.004[e]	0.02	0.005
60-57-1	Dieldrin[n]	0.04[e]	1[e]	0.004[e]	0.02	0.603
84-66-2	Diethyl phthalate	63,000[b]	2,000[d]	470[b]	470	*
105-67-9	2,4-Dimethylphenol	1,600[b]	---[c]	9[b]	9	*
121-14-2	2,4-Dinitrotoluene	0.9[e]	---[c]	0.0008[e,	0.0008	0.250

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
606-20-2	2,6-Dinitrotoluene	0.9[e]	---[c]	0.0007[e]	0.0007	0.260
117-84-0	Di-*n*-octyl phthalate	1,600[b]	10,000[d]	10,000[d]	10,000[d]	*
115-29-7	Endosulfan[o]	470[b]	---[c]	18[b]	90	*
145-73-3	Endothall[o]	1,600[b]	---[c]	0.4	0.4	NA
72-20-8	Endrin	23[b]	---[c]	1	5	*
100-41-4	Ethylbenzene	7,800[b]	400[d, x]	13	19	*
206-44-0	Fluoranthene	3,100[b]	---[c]	4,300[b]	21,000	*
86-73-7	Fluorene	3,100[b]	---[c]	560[b]	2,800	*
76-44-8	Heptachlor	0.1[e]	0.1[e]	23	110	0.871
1024-57-3	Heptachlor epoxide	0.07[e]	5[e]	0.7	3.3	1.005
118-74-1	Hexachlorobenzene	0.4[e]	1[e]	2	11	*
319-84-6	*Alpha*-HCH (*alpha*-BHC)	0.1[e]	0.8[e]	0.0005[e,]	0.003	0.0074

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
58-89-9	*Gamma*-HCH (Lindane)[n]	0.5[e]	---[c, x]	0.009	0.047	*
77-47-4	Hexachlorocyclopentadiene	550[b]	10[b, x]	400	2,200[d]	*
67-72-1	Hexachloroethane	78[b]	---[c]	0.5[b]	2.6	*
193-39-5	Indeno(1,2,3-*c,d*)pyrene	0.9[e,w]	---[c]	14	69	*
78-59-1	Isophorone	15,600[b]	4,600[d]	8[b]	8	*
72-43-5	Methoxychlor[o]	390[b]	---[c]	160	780	*
74-83-9	Methyl bromide (Bromomethane)	110[b]	10[b, x]	0.2[b]	1.2	*
1634-04-4	Methyl tertiary-butyl ether	780[b]	8,800[d, x]	0.32	0.32	*
75-09-2	Methylene chloride (Dichloromethane)	85[e]	13[e]	0.02[e]	0.2	*
95-48-7	2-Methylphenol (*o* – Cresol)	3,900[b]	---[c]	15[b]	15	*
91-20-3	Naphthalene	1,600[b]	170[b, x]	12[b]	18	*
98-95-3	Nitrobenzene	39[b]	92[b, x]	0.1[b,	0.1	0.26

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
86-30-6	*N*-Nitrosodiphenylamine	130[e]	---[c]	1[e]	5.6	*
621-64-7	*N*-Nitrosodi-*n*-propylamine	0.09[e,]	---[c]	0.00005[e,]	0.00005	0.0018
108-95-2	Phenol	23,000[b]	---[c]	100[b]	100	*
1918-02-1	Picloram[o]	5,500[b]	---[c]	2	20	NA
1336-36-3	Polychlorinated biphenyls (PCBs)[n]	1[h]	---[c,h]	---[h]	---[h]	*
129-00-0	Pyrene	2,300[b]	---[c]	4,200[b]	21,000	*
122-34-9	Simazine[o]	390[b]	---[c]	0.04	0.37	NA
100-42-5	Styrene	16,000[b]	1,500[d, x]	4	18	*
127-18-4	Tetrachloroethylene (Perchloroethylene)	12[e]	11[e]	0.06	0.3	*
108-88-3	Toluene	16,000[b]	650[d, x]	12	29	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
8001-35-2	Toxaphene[n]	0.6[e]	89[e]	31	150	*
120-82-1	1,2,4-Trichlorobenzene	780[b]	3,200[b, x]	5	53	*
71-55-6	1,1,1-Trichloroethane	---[c]	1,200[d]	2	9.6	*
79-00-5	1,1,2-Trichloroethane	310[b]	1,800[d]	0.02	0.3	*
79-01-6	Trichloroethylene	58[e]	5[e]	0.06	0.3	*
108-05-4	Vinyl acetate	78,000[b]	1,000[b, x]	170[b]	170	*
75-01-4	Vinyl chloride	0.46[e]	0.28[e]	0.01	0.07	*
108-38-3	m-Xylene	16,000[b]	420[d, x]	210	210	*
95-47-6	o-Xylene	16,000[b]	410[d, x]	190	190	*
106-42-3	p-Xylene	16,000[b]	460[d, x]	200	200	*

		Exposure Route-Specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	Class II (mg/kg)	ADL (mg/kg)
1330-20-7	Xylenes (total)	16,000[b]	320[d, x]	150	150	*
	Ionizable Organics					
65-85-0	Benzoic Acid	310,000[b]	---[c]	400[b,i]	400[i]	*
95-57-8	2-Chlorophenol	390[b]	53,000[d]	4[b,i]	4[i]	*
120-83-2	2,4-Dichlorophenol	230[b]	---[c]	1[b,i]	1[i]	*
51-28-5	2,4-Dinitrophenol	160[b]	---[c]	0.2[b,]	0.2	3.3
88-85-7	Dinoseb[o]	78[b]	---[c]	0.34[b,i]	3.4[i]	*
87-86-5	Pentachlorophenol	3[e,j]	---[c]	0.03[i]	0.14[i]	*
93-72-1	2,4,5-TP (Silvex)	630[b]	---[c]	11[i]	55[i]	*
95-95-4	2,4,5-Trichlorophenol	7,800[b]	---[c]	270[b,i]	1,400[i]	*
88-06-2	2,4,6 Trichlorophenol	58[e]	200[e]	0.2[e, i]	0.77[i]	0.66

		Exposure Route-specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
	Inorganics					
7440-36-0	Antimony	31[b]	---[c]	0.006[m]	0.024[m]	*
7440-38-2	Arsenic[l,n]	---[t]	750[e]	0.05[m]	0.2[m]	*
7440-39-3	Barium	5,500[b]	690,000[b]	2.0[m]	2.0[m]	*
7440-41-7	Beryllium	160[b]	1,300[e]	0.004[m]	0.5[m]	*
7440-42-8	Boron	16,000[b]	---[c]	2.0[m]	2.0[m]	*
7440-43-9	Cadmium[l,n]	78[b, r]	1,800[e]	0.005[m]	0.05[m]	*
7440-70-2	Calcium[n]	---[g]	---[c]	---[c]	---[c]	*
16887-00-6	Chloride	---[c]	---[c]	200[m]	200[m]	*
7440-47-3	Chromium, total	230[b]	270[e]	0.1[m]	1.0[m]	*
16065-83-1	Chromium, ion, trivalent	120,000[b]	---[c]	---[g]	---[g]	*
18540-29-9	Chromium, ion, hexavalent	230[b]	270[e]	---	---	*

		Exposure Route-specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
7440-48-4	Cobalt	4,700[b]	---[c]	1.0[m]	1.0[m]	*
7440-50-8	Copper[n]	2,900[b]	---[c]	0.65[m]	0.65[m]	*
57-12-5	Cyanide (amenable)	1,600[b]	---[c]	0.2[q,m]	0.6[q,m]	*
7782-41-4	Fluoride	4,700[b]	---[c]	4.0[m]	4.0[m]	*
15438-31-0	Iron	---[c]	---[c]	5.0[m]	5.0[m]	*
7439-92-1	Lead	400[k]	---[c]	0.0075[m]	0.1[m]	*
7439-95-4	Magnesium[n]	325,000	---[c]	---[c]	---[c]	*
7439-96-5	Manganese	1,600 [b,v]	69,000[b, x]	0.15[m]	10.0[m]	*
7439-97-6	Mercury[l,n,s]	23[b]	10[b, x]	0.002[m]	0.01[m]	*
7440-02-0	Nickel[l]	1,600[b]	13,000[e]	0.1[m]	2.0[m]	*
14797-55-8	Nitrate as N[p]	130,000[b]	---[c]	10.0[q, m]	100[q]	*
7723-14-0	Phosphorus[n]	---[g]	---[c]	---[c]	---[c]	*

		Exposure Route-specific Values for Soils		Soil Component of the Groundwater Ingestion Exposure Route Values		
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
7440-09-7	Potassium[n]	---[g]	---[c]	---[c]	---[c]	*
7782-49-2	Selenium[l,n]	390[b]	---[c]	0.05[m]	0.05[m]	*
7440-22-4	Silver	390[b]	---[c]	0.05[m]	---[c]	*
7440-23-5	Sodium[n]	---[g]	---[c]	---[c]	---[c]	*
14808-79-8	Sulfate	---[c]	---[c]	400[m]	400[m]	*
7440-28-0	Thallium	6.3[b,u]	---[c]	0.002[m]	0.02[m]	*
7440-62-2	Vanadium	550[b]	---[c]	0.049[m]	0.1[m]	*
7440-66-6	Zinc[l]	23,000[b]	---[c]	5.0[m]	10[m]	*

"*" indicates that the ADL is less than or equal to the specified remediation objective.
NA means not available; no PQL or EQL available in USEPA analytical methods.

Chemical Name and Soil Remediation Objective Notations

[a] Soil remediation objectives based on human health criteria only.
[b] Calculated values correspond to a target hazard quotient of 1.
[c] No toxicity criteria available for the route of exposure.
[d] Soil saturation concentration ($C_{[sat]}$) = the concentration at which the absorptive limits of the soil particles, the solubility limits of the available soil moisture, and saturation of soil pore air have been reached. Above the soil saturation concentration, the assumptions regarding vapor transport to air and/or dissolved phase transport to groundwater (for chemicals which are liquid at ambient soil temperatures) have been violated, and alternative modeling approaches are required.
[e] Calculated values correspond to a cancer risk level of 1 in 1,000,000.
[g] Chemical-specific properties are such that this route is not of concern at any soil contaminant concentration.
[h] 40 CFR 761 contains applicability requirements and methodologies for the development of PCB remediation objectives. Requests for approval of a Tier 3 evaluation must address the applicability of 40 CFR 761.
[l] Soil remediation objective for pH of 6.8. If soil pH is other than 6.8, refer to Appendix B, Tables C and D of this Part.
[j] Ingestion soil remediation objective adjusted by a factor of 0.5 to account for dermal route.
[k] A preliminary remediation goal of 400 mg/kg has been set for lead based on *Revised Interim Soil Lead Guidance for CERCLA Sites and RCRA Corrective Action Facilities*, OSWER Directive #9355.4-12.
[l] Potential for soil-plant-human exposure.
[m] The person conducting the remediation has the option to use: 1) TCLP or SPLP test results to compare with the remediation objectives listed in this Table; 2) where applicable, the total amount of contaminant in the soil sample results to compare with pH specific remediation objectives listed in Appendix B, Table C or D of this Part (see Section 742.510); or 3) the appropriate background value listed in Appendix A, Table G. If the person conducting the remediation wishes to calculate soil remediation objectives based on background concentrations, this should be done in accordance with Subpart D of this Part.
[n] The Agency reserves the right to evaluate the potential for remaining contaminant concentrations to pose significant threats to crops, livestock, or wildlife.
[o] For agrichemical facilities, remediation objectives for surficial soils which are based on field application rates may be more appropriate for currently registered pesticides. Consult the Agency for further information.
[p] For agrichemical facilities, soil remediation objectives based on site-specific background concentrations of Nitrate as N may be more appropriate. Such determinations shall be conducted in accordance with the procedures set forth in Subparts D and I of this Part.
[q] The TCLP extraction must be done using water at a pH of 7.0.
[r] Value based on dietary Reference Dose.

[s] Value for Ingestion based on Reference Dose for Mercuric chloride (CAS No. 7487-94-7); value for Inhalation based on Reference Concentration for elemental Mercury (CAS No. 7439-97-6). Inhalation remediation objective only applies at sites where elemental mercury is a contaminant of concern.

[t] For the ingestion route for arsenic, see 742.Appendix A, Table G.

[u] Value based on Reference Dose for Thallium sulfate (CAS No. 7446-18-6).

[v] Value based on Reference Dose adjusted for dietary intake.

[w] For sites located in any populated area as defined in Section 742.200, Appendix A, Table H may be used.

[x] The remediation objectives for these chemicals must also include the construction worker inhalation objective in Appendix B, Table B.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.Table B Tier 1 Soil Remediation Objectives[a] for Industrial/Commercial Properties

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
83-32-9	Acenaphthene	120,000[b]	-----[c]	120,000[b]	-----[c]	570[b]	2,900	*
67-64-1	Acetone	----[g]	100,000[d]	----[g]	100,000[d]	25[b]	25	*
15972-60-8	Alachlor[o]	72[e]	-----[c]	1,600[e]	-----[c]	0.04	0.2	NA
116-06-3	Aldicarb[o]	2,000[b]	-----[c]	200[b]	-----[c]	0.013	0.07	NA
309-00-2	Aldrin	0.3[e]	6.6[e]	6.1[b]	9.3[e]	0.5[e]	2.5	0.94
120-12-7	Anthracene	610,000[b]	-----[c]	610,000[b]	-----[c]	12,000[b]	59,000	*
1912-24-9	Atrazine[o]	72,000[b]	-----[c]	7,100[b]	-----[c]	0.066	0.33	NA
71-43-2	Benzene	100[e]	1.6[e]	2,300[e]	2.2[e]	0.03	0.17	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
56-55-3	Benzo(*a*)anthracene	8[e]	-----[c]	170[e]	-----[c]	2	8	*
205-99-2	Benzo(*b*)fluoranthene	8[e]	-----[c]	170[e]	-----[c]	5	25	*
207-08-9	Benzo(*k*)fluroanthene	78[e]	-----[c]	1,700[e]	-----[c]	49	250	*
50-32-8	Benzo(*a*)pyrene	0.8[e,x]	-----[c]	17[e]	-----[c]	8	82	*
111-44-4	Bis(2-chloroethyl)ether	5[e]	0.47[e]	75[e]	0.66[e]	0.0004[e,]	0.0004	0.66
117-81-7	Bis(2-ethylhexyl)phthalate	410[e]	31,000[d]	4,100[b]	31,000[d]	3,600	31,000[d]	*
75-27-4	Bromodichloromethane (Dichlorobromomethane)	92[e]	3,000[d]	2,000[e]	3,000[d]	0.6	0.6	*
75-25-2	Bromoform	720[e]	100[e]	16,000[e]	140[e]	0.8	0.8	*
71-36-3	Butanol	200,000[b]	10,000[d]	200,000[b]	10,000[d]	17[b]	17	NA
85-68-7	Butyl benzyl phthalate	410,000[b]	930[d]	410,000[b]	930[d]	930[d]	930[d]	*
86-74-8	Carbazole	290[e]	-----[c]	6,200[e]	-----[c]	0.6[e]	2.8	NA

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
1563-66-2	Carbofuran[o]	10,000[b]	-----[c]	1,000[b]	-----[c]	0.22	1.1	NA
75-15-0	Carbon disulfide	200,000[b]	720[d]	20,000[b]	9.0[b]	32[b]	160	*
56-23-5	Carbon tetrachloride	44[e]	0.64[e]	410[b]	0.90[e]	0.07	0.33	*
57-74-9	Chlordane	16[e]	140[e]	100[b]	22[b]	10	48	*
106-47-8	4 – Chloroaniline (*p*-Chloroaniline)	8,200[b]	-----[c]	820[b]	-----[c]	0.7[b]	0.7	*
108-90-7	Chlorobenzene (Monochlorobenzene)	41,000[b]	210[b]	4,100[b]	1.3[b]	1	6.5	*
124-48-1	Chlorodibromomethane (Dibromochloromethane)	41,000[b]	1,300[d]	41,000[b]	1,300[d]	0.4	0.4	*
67-66-3	Chloroform	940[e]	0.54[e]	2,000[b]	0.76[e]	0.6	2.9	*
218-01-9	Chrysene	780[e]	-----[c]	17,000[e]	-----[e]	160	800	*
94-75-7	2,4-D[o]	20,000[b]	-----[c]	2,000[b]	-----[c]	1.5	7.7	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
75-99-0	Dalapon[o]	61,000[b]	-----[c]	6,100[b]	-----[c]	0.85	8.5	*
72-54-8	DDD	24[e]	-----[c]	520[e]	-----[c]	16[e]	80	*
72-55-9	DDE	17[e]	-----[c]	370[e]	-----[c]	54[e]	270	*
50-29-3	DDT	17[e]	1,500[e]	100[b]	2,100[e]	32[e]	160	*
53-70-3	Dibenzo(*a,h*)anthracene	0.8[e]	-----[c]	17[e]	-----[c]	2	7.6	*
96-12-8	1,2-Dibromo-3-chloropropane	4[e]	17[b]	89[e]	0.11[b]	0.002	0.02	*
106-93-4	1,2-Dibromoethane (Ethylene dibromide)	2.9[e]	0.12[e]	62[e]	0.16[e]	0.0004	0.004	0.005
84-74-2	Di-*n*-butyl phthalate	200,000[b]	2,300[d]	200,000[b]	2,300[d]	2,300[d]	2,300[d]	*
95-50-1	1,2-Dichlorobenzene (*o* – Dichlorobenzene)	180,000[b]	560[d]	18,000[b]	310[b]	17	43	*
106-46-7	1,4-Dichlorobenzene (*p* – Dichlorobenzene)	-----[c]	17,000[b]	-----[c]	340[b]	2	11	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
91-94-1	3,3'-Dichlorobenzidine	13[e]	-----[c]	280[e]	-----[c]	0.007[e,]	0.033	1.3
75-34-3	1,1-Dichloroethane	200,000[b]	1,700[d]	200,000[b]	130[b]	23[b]	110	*
107-06-2	1,2-Dichloroethane (Ethylene dichloride)	63[e]	0.70[e]	1,400[e]	0.99[e]	0.02	0.1	*
75-35-4	1,1-Dichloroethylene	100,000[b]	470[b]	10,000[b]	3.0[b]	0.06	0.3	*
156-59-2	*cis*-1,2-Dichloroethylene	20,000[b]	1,200[d]	20,000[b]	1,200[d]	0.4	1.1	*
156-60-5	*Trans*-1,2-Dichloroethylene	41,000[b]	3,100[d]	41,000[b]	3,100[d]	0.7	3.4	*
78-87-5	1,2-Dichloropropane	84[e]	23[b]	1,800[e]	0.50[b]	0.03	0.15	*
542-75-6	1,3-Dichloropropene (1,3-Dichloropropylene, *cis* + *trans*)	57[e]	2.1[e]	1,200[e]	0.39[b]	0.004[e]	0.02	0.005
60-57-1	Dieldrin[n]	0.4[e]	2.2[e]	7.8[e]	3.1[e]	0.004[e]	0.02	0.603
84-66-2	Diethyl phthalate	1,000,000[b]	2,000[d]	1,000,000[b]	2,000[d]	470[b]	470	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
105-67-9	2,4-Dimethylphenol	41,000[b]	-----[c]	41,000[b]	-----[c]	9[b]	9	*
121-14-2	2,4-Dinitrotoluene	8.4[e]	-----[c]	180[e]	-----[c]	0.0008[e,]	0.0008	0.250
606-20-2	2,6-Dinitrotoluene	8.4[e]	-----[c]	180[e]	-----[c]	0.0007[e,]	0.0007	0.260
117-84-0	Di-*n*-octyl phthalate	41,000[e]	10,000[d]	4,100[b]	10,000[d]	10,000[d]	10,000[d]	*
115-29-7	Endosulfan[o]	12,000[b]	-----[c]	1,200[b]	-----[c]	18[b]	90	*
145-73-3	Endothall[o]	41,000[c]	-----[c]	4,100[b]	-----[c]	0.4	0.4	NA
72-20-8	Endrin	610[b]	-----[c]	61[b]	-----[c]	1	5	*
100-41-4	Ethylbenzene	200,000[b]	400[d]	20,000[b]	58[b]	13	19	*
206-44-0	Fluoranthene	82,000[b]	-----[c]	82,000[b]	-----[c]	4,300[b]	21,000	*
86-73-7	Fluorene	82,000[b]	-----[c]	82,000[b]	-----[c]	560[b]	2,800	*
76-44-8	Heptachlor	1[e]	11[e]	28[e]	16[e]	23	110	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
1024-57-3	Heptachlor epoxide	0.6[e]	9.2[e]	2.7[b]	13[e]	0.7	3.3	1.005
118-74-1	Hexachlorobenzene	4[e]	1.8[e]	78[e]	2.6[e]	2	11	*
319-84-6	*Alpha*-HCH (*alpha*-BHC)	0.9[e]	1.5[e]	20[e]	2.1[e]	0.0005[e,]	0.003	0.0074
58-89-9	*Gamma*-HCH (Lindane)[n]	4[e]	-----[c]	96[e]	-----[c]	0.009	0.047	*
77-47-4	Hexachlorocyclopentadiene	14,000[b]	16[b]	14,000[b]	1.1[b]	400	2,200[d]	*
67-72-1	Hexachloroethane	2,000[b]	-----[c]	2,000[b]	-----[c]	0.5[b]	2.6	*
193-39-5	Indeno(1,2,3-*c,d*)pyrene	8[e]	-----[c]	170[e]	-----[c]	14	69	*
78-59-1	Isophorone	410,000[b]	4,600[d]	410,000[b]	4,600[d]	8[b]	8	*
72-43-5	Methoxychlor[o]	10,000[b]	-----[c]	1,000[b]	-----[c]	160	780	*
74-83-9	Methyl bromide (Bromomethane)	2,900[b]	15[b]	1,000[b]	3.9[b]	0.2[b]	1.2	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
1634-04-4	Methyl tertiary-butyl ether	20,000[b]	8,800[d]	2,000[b]	140[b]	0.32	0.32	*
75-09-2	Methylene chloride (Dichloromethane)	760[e]	24[e]	12,000[b]	34[e]	0.02[e]	0.2	*
95-48-7	2-Methylphenol (*o* – Cresol)	100,000[b]	-----[c]	100,000[b]	-----[c]	15[b]	15	*
86-30-6	*N*-Nitrosodiphenylamine	1,200[e]	-----[c]	25,000[e]	-----[c]	1[e]	5.6	*
621-64-7	*N*-Nitrosodi-*n*-propylamine	0.8[e]	-----[c]	18[e]	-----[c]	0.00005[e]	0.00005	0.0018
91-20-3	Naphthalene	41,000[b]	270[b]	4,100[b]	1.8[b]	12[b]	18	*
98-95-3	Nitrobenzene	1,000[b]	140[b]	1,000[b]	9.4[b]	0.1[b]	0.1	0.26
108-95-2	Phenol	610,000[b]	-----[c]	61,000[b]	-----[c]	100[b]	100	*
1918-02-1	Picloram[o]	140,000[b]	-----[c]	14,000[b]	-----[c]	2	20	NA
1336-36-3	Polychlorinated biphenyls (PCBs)[n]	1[h]	-----[c,h]	1[h]	-----[c,h]	-----[h]	-----[h]	*
129-00-0	Pyrene	61,000[b]	-----[c]	61,000[b]	-----[c]	4,200[b]	21,000	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
122-34-9	Simazine[o]	10,000[b]	-----[c]	1,000[b]	-----[c]	0.04	0.37	NA
100-42-5	Styrene	410,000[b]	1,500[d]	41,000[b]	430[b]	4	18	*
127-18-4	Tetrachloroethylene (Perchloroethylene)	110[e]	20[e]	2,400[e]	28[e]	0.06	0.3	*
108-88-3	Toluene	410,000[b]	650[d]	410,000[b]	42[b]	12	29	*
8001-35-2	Toxaphene[n]	5.2[e]	170[e]	110[e]	240[e]	31	150	*
120-82-1	1,2,4-Trichlorobenzene	20,000[b]	3,200[d]	2,000[b]	920[b]	5	53	*
71-55-6	1,1,1-Trichloroethane	-----[c]	1,200[d]	-----[c]	1,200[d]	2	9.6	*
79-00-5	1,1,2-Trichloroethane	8,200[b]	1,800[d]	8,200[b]	1,800[d]	0.02	0.3	*
79-01-6	Trichloroethylene	520[e]	8.9[e]	1,200[b]	12[e]	0.06	0.3	*
108-05-4	Vinyl acetate	1,000,000[b]	1,600[b]	200,000[b]	10[b]	170[b]	170	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
75-01-4	Vinyl chloride	7.9[e]	1.1[e]	170[c]	1.1[b]	0.01	0.07	*
108-38-3	m-Xylene	410,000[b]	420[d]	41,000[b]	6.4[b]	210	210	*
95-47-6	o-Xylene	410,000[b]	410[d]	41,000[b]	6.5[b]	190	190	*
106-42-3	p-Xylene	410,000[b]	460[d]	41,000[b]	5.9[b]	200	200	*
1330-20-7	Xylenes (total)	410,000[b]	320[d]	41,000[b]	5.6[b]	150	150	*
	Ionizable Organics							
65-85-0	Benzoic Acid	1,000,000[b]	-----[c]	820,000[b]	-----[c]	400[b,i]	400[i]	*
95-57-8	2-Chlorophenol	10,000[b]	53,000[d]	10,000[b]	53,000[d]	4[b, i]	20[i]	*
120-83-2	2,4-Dichlorophenol	6,100[b]	-----[c]	610[b]	-----[c]	1[b, i]	1[i]	*
51-28-5	2,4-Dinitrophenol	4,100[b]	-----[c]	410[b]	-----[c]	0.2[b, i]	0.2[i]	3.3
88-85-7	Dinoseb[o]	2,000[b]	-----[c]	200[b]	-----[c]	0.34[b, i]	3.4[i]	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/kg)	ClassII (mg/kg)	ADL (mg/kg)
87-86-5	Pentachlorophenol	24[e,j]	-----[c]	520[e,j]	-----[c]	0.03[i]	0.14[i]	*
93-72-1	2,4,5-TP (Silvex)	16,000[b]	-----[c]	1,600[b]	-----[c]	11[i]	55[i]	*
95-95-4	2,4,5-Trichlorophenol	200,000[b]	-----[c]	200,000[b]	-----[c]	270[b, i]	1,400[i]	*
88-06-2	2,4,6- Trichlorophenol	520[e]	390[e]	11,000[e]	540[e]	0.2[e, i]	0.77[i]	0.66

CAS No.	Chemical Name	Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
		Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
	Inorganics							
7440-36-0	Antimony	820[b]	-----[c]	82[b]	-----[c]	0.006[m]	0.024[m]	*
7440-38-2	Arsenic[l,n]	---[t]	1,200[e]	61[b]	25,000[e]	0.05[m]	0.2[m]	*
7440-39-3	Barium	140,000[b]	910,000[b]	14,000[b]	870,000[b]	2.0[m]	2.0[m]	*
7440-41-7	Beryllium	4,100[b]	2,100[e]	410[b]	44,000[e]	0.004[m]	0.5[m]	*
7440-42-8	Boron	410,000[b]	---[c]	41,000[b]	---[c]	2.0[m]	2.0[m]	*
7440-43-9	Cadmium[l,n]	2,000[b,r]	2,800[e]	200[b,r]	59,000[e]	0.005[m]	0.05[m]	*
7440-70-2	Calcium[n]	---[g]	---[c]	---[g]	---[c]	---[c]	---[c]	*
16887-00-6	Chloride	--------[c]	-----[c]	-----[c]	-----[c]	200[m]	200[m]	*
7440-47-3	Chromium, total	6,100[b]	420[e]	4,100[b]	690[b]	0.1[m]	1.0[m]	*
16065-83-1	Chromium, ion, trivalent	1,000,000[b]	-----[c]	310,000[b]	-----[c]	-----[g]	-----[g]	*
18540-29-9	Chromium, ion, hexavalent	6,100[b]	420[e]	4,100[b]	690[b]	-----	-----	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
7440-48-4	Cobalt	120,000[b]	-----[c]	12,000[b]	-----[c]	1.0[m]	1.0[m]	*
7440-50-8	Copper[n]	82,000[b]	-----[c]	8,200[b]	-----[c]	0.65[m]	0.65[m]	*
57-12-5	Cyanide (amenable)	41,000[b]	-----[c]	4,100[b]	-----[c]	0.2[q,m]	0.6[q,m]	*
7782-41-4	Fluoride	120,000[b]	-----[c]	12,000[b]	-----[c]	4.0[m]	4.0[m]	*
15438-31-0	Iron	-----[c]	-----[c]	-----[c]	-----[c]	5.0[m]	5.0[m]	*
7439-92-1	Lead	800[y]	-----[c]	700[y]	-----[c]	0.0075[m]	0.1[m]	*
7439-95-4	Magnesium[n]	---[g]	---[c]	730,000	---[c]	---[c]	---[c]	*
7439-96-5	Manganese	41,000 [b,w]	91,000[b]	4,100 [b,w]	8,700[b]	0.15[m]	10.0[m]	*
7439-97-6	Mercury[l,n,s]	610[b]	16[b]	61[b]	0.1[b]	0.002[m]	0.01[m]	*
7440-02-0	Nickel[l]	41,000[b]	21,000[e]	4,100[b]	440,000[e]	0.1[m]	2.0[m]	*
14797-55-8	Nitrate as N[p]	1,000,000[b]	-----[c]	330,000[b]	-----[c]	10.0[q, m]	100[q]	*
7723-14-0	Phosphorus[n]	---[g]	---[c]	---[g]	---[c]	---[c]	---[c]	*

		Exposure Route-Specific Values for Soils				Soil Component of the Groundwater Ingestion Exposure Route Values		
		Industrial-Commercial		Construction Worker				
CAS No.	Chemical Name	Ingestion (mg/kg)	Inhalation (mg/kg)	Ingestion (mg/kg)	Inhalation (mg/kg)	Class I (mg/L)	Class II (mg/L)	ADL (mg/kg)
7440-09-7	Potassium[n]	---[g]	---[c]	---[g]	---[c]	---[c]	---[c]	*
7782-49-2	Selenium[l,n]	10,000[b]	-----[c]	1,000[b]	-----[c]	0.05[m]	0.05[m]	*
7440-22-4	Silver	10,000[b]	-----[c]	1,000[b]	-----[c]	0.05[m]	-----	*
7440-23-5	Sodium[n]	---[g]	---[c]	---[g]	---[c]	---[c]	---[c]	*
14808-79-8	Sulfate	-----[c]	-----[c]	-----[c]	-----[c]	400[m]	400[m]	*
7440-28-0	Thallium	160[b,u]	-----[c]	160[b,u]	-----[c]	0.002[m]	0.02[m]	*
7440-62-2	Vanadium	14,000[b]	-----[c]	1,400[b]	-----[c]	0.049[m]	0.1[m]	*
7440-66-6	Zinc[l]	610,000[b]	-----[c]	61,000[b]	-----[c]	5.0[m]	10[m]	*

"*" indicates that the ADL is less than or equal to the specified remediation objective.

NA means Not Available; no PQL or EQL available in USEPA analytical methods.

Chemical Name and Soil Remediation Objective Notations (2nd, 5th thru 8th Columns)

[a] oil remediation objectives based on human health criteria only.
[b] Calculated values correspond to a target hazard quotient of 1.
[c] No toxicity criteria available for this route of exposure.

[d] Soil saturation concentration ($C_{[sat]}$) = the concentration at which the absorptive limits of the soil particles, the solubility limits of the available soil moisture, and saturation of soil pore air have been reached. Above the soil saturation concentration, the assumptions regarding vapor transport to air and/or dissolved phase transport to groundwater (for chemicals which are liquid at ambient soil temperatures) have been violated, and alternative modeling approaches are required.

[e] Calculated values correspond to a cancer risk level of 1 in 1,000,000.

[g] Chemical-specific properties are such that this route is not of concern at any soil contaminant concentration.

[h] 40 CFR 761 contains applicability requirements and methodologies for the development of PCB remediation objectives. Requests for approval of a Tier 3 evaluation must address the applicability of 40 CFR 761.

[i] Soil remediation objective for pH of 6.8. If soil pH is other than 6.8, refer to Appendix B, Tables C and D in this Part.

[j] Ingestion soil remediation objective adjusted by a factor of 0.5 to account for dermal route.

[l] Potential for soil-plant-human exposure.

[m] The person conducting the remediation has the option to use: (1) TCLP or SPLP test results to compare with the remediation objectives listed in this Table; (2) the total amount of contaminant in the soil sample results to compare with pH specific remediation objectives listed in Appendix B, Table C or D of this Part (see Section 742.510); or (3) the appropriate background value listed in Appendix A, Table G. If the person conducting the remediation wishes to calculate soil remediation objectives based on background concentrations, this should be done in accordance with Subpart D of this Part.

[n] The Agency reserves the right to evaluate the potential for remaining contaminant concentrations to pose significant threats to crops, livestock, or wildlife.

[o] For agrichemical facilities, remediation objectives for surficial soils which are based on field application rates may be more appropriate for currently registered pesticides. Consult the Agency for further information.

[p] For agrichemical facilities, soil remediation objectives based on site-specific background concentrations of Nitrate as N may be more appropriate. Such determinations shall be conducted in accordance with the procedures set forth in Subparts D and I of this Part.

[q] The TCLP extraction must be done using water at a pH of 7.0.

[r] Value based on dietary Reference Dose.

[s] Value for Ingestion based on Reference Dose for Mercuric chloride (CAS No. 7487-94-7); value for Inhalation based on Reference Concentration for elemental Mercury (CAS No. 7439-97-6). Inhalation remediation objective only applies at sites where elemental mercury is a contaminant of concern.

[t] For the ingestion route for arsenic for industrial/commercial, see 742.Appendix A, Table G.

[u] Value based on Reference Dose for Thallium sulfate (CAS No. 7446-18-6).

[w] Value based on Reference Dose adjusted for dietary intake.

[x] For any populated areas as defined in Section 742.200, Appendix A, Table H may be used.

[y] Value based on maintaining fetal blood lead below 10 ug/d1, using the USEPA adults Blood Lead Model.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.Table C pH Specific Soil Remediation Objectives for Inorganics and Ionizing Organics for the Soil Component of the Groundwater Ingestion Route (Class I Groundwater)

Chemical (totals) (mg/kg)	pH 4.5 to 4.74	pH 4.75 to 5.24	pH 5.25 to 5.74	pH 5.75 to 6.24	pH 6.25 to 6.64	pH 6.65 to 6.89	pH 6.9 to 7.24	pH 7.25 to 7.74	pH 7.75 to 8.24	pH 8.25 to 8.74	pH 8.75 to 9.0
Inorganics											
Antimony	5	5	5	5	5	5	5	5	5	5	5
Arsenic	25	26	27	28	29	29	29	30	31	32	33
Barium	260	490	850	1,200	1,500	1,600	1,700	1,800	2,100	—[a]	—[a]
Beryllium	1.1	2.1	3.4	6.6	22	63	140	1,000	8,000	—[a]	—[a]
Cadmium	1.0	1.7	2.7	3.7	5.2	7.5	11	59	430	—[a]	—[a]
Chromium (+6)	70	62	54	46	40	38	36	32	28	24	21
Copper	330	580	2,100	11,000	59,000	130,000	200,000	330,000	330,000	—[a]	—[a]
Cyanide	40	40	40	40	40	40	40	40	40	40	40
Lead	23	23	23	23	107	107	107	107	107	107	282
Mercury	0.01	0.01`	0.03	0.15	0.89	2.1	3.3	6.4	8.0	—[a]	—[a]
Nickel	20	36	56	76	100	130	180	700	3,800	—[a]	—[a]
Selenium	24	17	12	8.8	6.3	5.2	4.5	3.3	2.4	1.8	1.3
Silver	0.24	0.33	0.62	1.5	4.4	8.5	13	39	110	—[a]	—[a]

Chemical (totals) (mg/kg)	pH 4.5 to 4.74	pH 4.75 to 5.24	pH 5.25 to 5.74	pH 5.75 to 6.24	pH 6.25 to 6.64	pH 6.65 to 6.89	pH 6.9 to 7.24	pH 7.25 to 7.74	pH 7.75 to 8.24	pH 8.25 to 8.74	pH 8.75 to 9.0
Thallium	1.6	1.8	2.0	2.4	2.6	2.8	3.0	3.4	3.8	4.4	4.9
Vanadium	980	980	980	980	980	980	980	980	980	980	980
Zinc	1,000	1,800	2,600	3,600	5,100	6,200	7,500	16,000	53,000	—[a]	—[a]
Organics											
Benzoic Acid	440	420	410	400	400	400	400	400	400	400	400
2-Chlorophenol	4.0	4.0	4.0	4.0	3.9	3.9	3.9	3.6	3.1	2.2	1.5
2,4-Dichlorophenol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.86	0.69	0.56	0.48
Dinoseb	8.4	4.5	1.9	0.82	0.43	0.34	0.31	0.27	0.25	0.25	0.25
Pentachlorophenol	0.54	0.32	0.15	0.07	0.04	0.03	0.02	0.02	0.02	0.02	0.02
2,4,5-TP (Silvex)	26	16	12	11	11	11	11	11	11	11	11
2,4,5-Trichlorophenol	400	390	390	370	320	270	230	130	64	36	26
2,4,6-Trichlorophenol	0.37	0.36	0.34	0.29	0.20	0.15	0.13	0.09	0.07	0.07	0.07

[a] No data available for this pH range.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.Table D pH Specific Soil Remediation Objectives for Inorganics and Ionizing Organics for the Soil Component of the Groundwater Ingestion Route (Class II Groundwater)

Chemical (totals) (mg/kg)	pH 4.5 to 4.74	pH 4.75 to 5.24	pH 5.25 to 5.74	pH 5.75 to 6.24	pH 6.25 to 6.64	pH 6.65 to 6.89	pH 6.9 to 7.24	pH 7.25 to 7.74	pH 7.75 to 8.24	pH 8.25 to 8.74	pH 8.75 to 9.0
Inorganics											
Antimony	20	20	20	20	20	20	20	20	20	20	20
Arsenic	100	100	100	110	110	120	120	120	120	130	130
Barium	260	490	850	1,200	1,500	1,600	1,700	1,800	2,100	—[a]	—[a]
Beryllium	140	260	420	820	2,800	7,900	17,000	130,000	1,000,000	—[a]	—[a]
Cadmium	10	17	27	37	52	75	110	590	4,300	—[a]	—[a]
Chromium (+6)	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data	No Data
Copper	330	580	2,100	11,000	59,000	130,000	200,000	330,000	330,000	—[a]	—[a]
Cyanide	120	120	120	120	120	120	120	120	120	120	120
Lead	300	300	300	300	1,420	1,420	1,420	1,420	1,420	1,420	3,760
Mercury	0.05	0.06	0.14	0.75	4.4	10	16	32	40	—[a]	—[a]
Nickel	400	730	1,100	1,500	2,000	2,600	3,500	14,000	76,000	—[a]	—[a]
Selenium	24	17	12	8.8	6.3	5.2	4.5	3.3	2.4	1.8	1.3
Thallium	16	18	20	24	26	28	30	34	38	44	49

Chemical (totals) (mg/kg)	pH 4.5 to 4.74	pH 4.75 to 5.24	pH 5.25 to 5.74	pH 5.75 to 6.24	pH 6.25 to 6.64	pH 6.65 to 6.89	pH 6.9 to 7.24	pH 7.25 to 7.74	pH 7.75 to 8.24	pH 8.25 to 8.74	pH 8.75 to 9.0
Zinc	2,000	3,600	5,200	7,200	10,000	12,000	15,000	32,000	110,000	— [a]	— [a]
Organics											
Benzoic Acid	440	420	410	400	400	400	400	400	400	400	400
2-Chlorophenol	20	20	20	20	20	20	19	3.6	3.1	2.2	1.5
2,4-Dichlorophenol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.86	0.69	0.56	0.48
Dinoseb	84	45	19	8.2	4.3	3.4	3.1	2.7	2.5	2.5	2.5
Pentachlorophenol	2.7	1.6	0.75	0.33	0.18	0.15	0.12	0.11	0.10	0.10	0.10
2,4,5-TP (Silvex)	130	79	62	57	55	55	55	55	55	55	55
2,4,5-Trichlorophenol	2,000	2,000	1,900	1,800	1,600	1,400	1,200	640	64	36	26
2,4,6-Trichlorophenol	1.9	1.8	1.7	1.4	1.0	0.77	0.13	0.09	0.07	0.07	0.07

[a] No data available for this pH range.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.TABLE E Tier 1 Groundwater Remediation Objectives for the Groundwater Component of the Groundwater Ingestion Route

		Groundwater Remediation Objective	
CAS No.	Chemical Name Organics	Class I (mg/L)	Class II (mg/L)
83-32-9	Acenaphthene	0.42	2.1
67-64-1	Acetone	6.3	6.3
15972-60-8	Alachlor	0.002[c]	0.01[c]
116-06-3	Aldicarb	0.003[c]	0.015[c]
309-00-2	Aldrin	0.014[a]	0.07
120-12-7	Anthracene	2.1	10.5
1912-24-9	Atrazine	0.003[c]	0.015[c]
71-43-2	Benzene	0.005[c]	0.025[c]
56-55-3	Benzo(*a*)anthracene	0.00013[a]	0.00065
205-99-2	Benzo(*b*)fluoranthene	0.00018[a]	0.0009
207-08-9	Benzo(*k*)fluroanthene	0.00017[a]	0.00085
50-32-8	Benzo(*a*)pyrene	0.0002[a,c]	0.002[c]
65-85-0	Benzoic Acid	28	28
111-44-4	Bis(2-chloroethyl)ether	0.01[a]	0.01
117-81-7	Bis(2-ethylhexyl)phthalate (Di(2-ethylhexyl)phthalate)	0.006[c]	0.06[c]
75-27-4	Bromodichloromethane (Dichlorobromomethane)	0.0002[a]	0.0002
75-25-2	Bromoform	0.001[a]	0.001
71-36-3	Butanol	0.7	0.7
85-68-7	Butyl benzyl phthalate	1.4	7.0
86-74-8	Carbazole	---	---
1563-66-2	Carbofuran	0.04[c]	0.2[c]
75-15-0	Carbon disulfide	0.7	3.5
56-23-5	Carbon tetrachloride	0.005[c]	0.025[c]
57-74-9	Chlordane	0.002[c]	0.01[c]

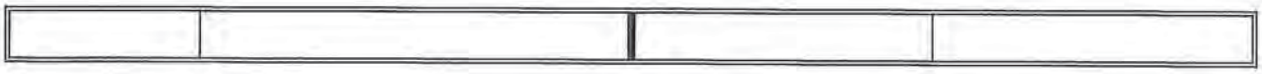
		Groundwater Remediation Objective	
CAS No.	Chemical Name	Class I (mg/L)	Class II (mg/L)
106-47-8	4-Chloroaniline (ρ-Chloroaniline)	0.028	0.028
108-90-7	Chlorobenzene (Monochlorobenzene)	0.1[c]	0.5[c]
124-48-1	Chlorodibromomethane (Dibromochloromethane)	0.14	0.14
67-66-3	Chloroform	0.0002[a]	0.001
95-57-8	2-Chlorophenol (pH 4.9-7.3)	0.035	0.175
	2-Chlorophenol (pH 7.4-8.0)	0.035	0.035
218-01-9	Chrysene	0.0015[a]	0.0075
94-75-7	2,4-D	0.07[c]	0.35[c]
75-99-0	Dalapon	0.2[c]	2.0[c]
72-54-8	DDD	0.014[a]	0.07
72-55-9	DDE	0.01[a]	0.05
50-29-3	DDT	0.006[a]	0.03
53-70-3	Dibenzo(*a,h*)anthracene	0.0003[a]	0.0015
96-12-8	1,2-Dibromo-3-chloropropane	0.0002[c]	0.002 [c]
106-93-4	1,2-Dibromoethane (Ethylene dibromide)	0.00005[c]	0.0005[c]
84-74-2	Di-*n*-butyl phthalate	0.7	3.5
95-50-1	1,2-Dichlorobenzene (*o* – Dichlorobenzene)	0.6[c]	1.5[c]
106-46-7	1,4-Dichlorobenzene (*p* – Dichlorobenzene)	0.075[c]	0.375[c]
91-94-1	3,3'-Dichlorobenzidine	0.02[a]	0.1
75-34-3	1,1-Dichloroethane	0.7	3.5
107-06-2	1,2-Dichloroethane (Ethylene dichloride)	0.005[c]	0.025[c]
75-35-4	1,1-Dichloroethylene[b]	0.007[c]	0.035[c]
156-59-2	*cis*-1,2-Dichloroethylene	0.07[c]	0.2[c]
156-60-5	*trans*-1,2-Dichloroethylene	0.1[c]	0.5[c]
120-83-2	2,4-Dichlorophenol	0.021	0.021

78-87-5	1,2-Dichloropropane	0.005[c]	0.025[c]
542-75-6	1,3-Dichloropropene (1,3-Dichloropropylene, *cis* + *trans*)	0.001[a]	0.005

		Groundwater Remediation Objective	
CAS No.	Chemical Name	Class I (mg/L)	Class II (mg/L)
60-57-1	Dieldrin	0.009[a]	0.045
84-66-2	Diethyl phthalate	5.6	5.6
105-67-9	2,4-Dimethylphenol	0.14	0.14
51-28-5	2,4-Dinitrophenol	0.014	0.014
121-14-2	2,4-Dinitrotoluene	0.00002[a]	0.00002
606-20-2	2,6-Dinitrotoluene	0.00031[a]	0.00031
88-85-7	Dinoseb	0.007[c]	0.07[c]
117-84-0	Di-*n*-octyl phthalate	0.14	0.7
115-29-7	Endosulfan	0.042	0.21
145-73-3	Endothall	0.1[c]	0.1[c]
72-20-8	Endrin	0.002[c]	0.01[c]
100-41-4	Ethylbenzene	0.7[c]	1.0[c]
206-44-0	Fluoranthene	0.28	1.4
86-73-7	Fluorene	0.28	1.4
76-44-8	Heptachlor	0.0004[c]	0.002[c]
1024-57-3	Heptachlor epoxide	0.0002[c]	0.001[c]
118-74-1	Hexachlorobenzene	0.00006[a]	0.0003
319-84-6	*alpha*-HCH (*alpha*-BHC)	0.00011[a]	0.00055
58-89-9	*Gamma*-HCH (Lindane)	0.0002[c]	0.001[c]
77-47-4	Hexachlorocyclopentadiene	0.05[c]	0.5[c]
67-72-1	Hexachloroethane	0.007	0.035
193-39-5	Indeno(1,2,3-*c,d*)pyrene	0.00043[a]	0.00215
78-59-1	Isophorone	1.4	1.4
72-43-5	Methoxychlor	0.04[c]	0.2[c]
74-83-9	Methyl bromide (Bromomethane)	0.0098	0.049
1634-04-4	Methyl tertiary-butyl ether	0.07	0.07
75-09-2	Methylene chloride (Dichloromethane)	0.005[c]	0.05[c]
95-48-7	2-Methylphenol (*o*-Cresol)	0.35	0.35
91-20-3	Naphthalene	0.14	0.22

98-95-3	Nitrobenzene[b]	0.0035	0.0035

		Groundwater Remediation Objective	
CAS No.	Chemical Name	Class I (mg/L)	Class II (mg/L)
86-30-6	*N*-Nitrosodiphenylamine	0.0032 [a]	0.016
621-64-7	*N*-Nitrosodi-*n*-propylamine	0.0018 [a]	0.0018
87-86-5	Pentachlorophenol	0.001 [c]	0.005 [c]
108-95-2	Phenol	0.1 [c]	0.1 [c]
1918-02-1	Picloram	0.5 [c]	5.0 [c]
1336-36-3	Polychlorinated biphenyls (PCBs)	0.0005 [c]	0.0025 [c]
129-00-0	Pyrene	0.21	1.05
122-34-9	Simazine	0.004 [c]	0.04 [c]
100-42-5	Styrene	0.1 [c]	0.5 [c]
93-72-1	2,4,5-TP (Silvex)	0.05 [c]	0.25 [c]
127-18-4	Tetrachloroethylene (Perchloroethylene)	0.005 [c]	0.025 [c]
108-88-3	Toluene	1.0 [c]	2.5 [c]
8001-35-2	Toxaphene	0.003 [c]	0.015 [c]
120-82-1	1,2,4-Trichlorobenzene	0.07 [c]	0.7 [c]
71-55-6	1,1,1-Trichloroethane [b]	0.2 [c]	1.0 [c]
79-00-5	1,1,2-Trichloroethane	0.005 [c]	0.05 [c]
79-01-6	Trichloroethylene	0.005 [c]	0.025 [c]
95-95-4	2,4,5-Trichlorophenol (pH 4.9-7.8)	0.7	3.5
	2,4,5-Trichlorophenol (pH 7.9-8.0)	0.7	0.7
88-06-2	2,4,6-Trichlorophenol (pH 4.9-6.8)	0.01 [a]	0.05
	2,4,6-Trichlorophenol (pH 6.9-8.0)	0.01	0.01
108-05-4	Vinyl acetate	7.0	7.0
75-01-4	Vinyl chloride	0.002 [c]	0.01 [c]
1330-20-7	Xylenes (total)	10.0 [c]	10.0 [c]



CAS No.	Chemical Name	Groundwater Remediation Objective	
		Class I (mg/L)	Class II (mg/L)
	Inorganics		
7440-36-0	Antimony	0.006[c]	0.024[c]
7440-38-2	Arsenic	0.05[c]	0.2[c]
7440-39-3	Barium	2.0[c]	2.0[c]
7440-41-7	Beryllium	0.004[c]	0.5[c]
7440-42-8	Boron	2.0[c]	2.0[c]
7440-43-9	Cadmium	0.005[c]	0.05[c]
7440-70-2	Calcium	---[d]	---[d]
16887-00-6	Chloride	200[c]	200[c]
7440-47-3	Chromium, total	0.1[c]	1.0[c]
18540-29-9	Chromium, ion, hexavalent	---	---
7440-48-4	Cobalt	1.0[c]	1.0[c]
7440-50-8	Copper	0.65[c]	0.65[c]
57-12-5	Cyanide	0.2[c]	0.6[c]
7782-41-4	Fluoride	4.0[c]	4.0[c]
15438-31-0	Iron	5.0[c]	5.0[c]
7439-92-1	Lead	0.0075[c]	0.1[c]
7439-95-4	Magnesium	---[d]	---[d]
7439-96-5	Manganese	0.15[c]	10.0[c]
7439-97-6	Mercury	0.002[c]	0.01[c]
7440-02-0	Nickel	0.1[c]	2.0[c]
14797-55-8	Nitrate as N	10.0[c]	100[c]
7723-14-0	Phosphorus	---[d]	---[d]
7440-09-7	Potassium	---[d]	---[d]
7782-49-2	Selenium	0.05[c]	0.05[c]

		Groundwater Remediation Objective	
CAS No.	Chemical Name	Class I (mg/L)	Class II (mg/L)
7440-22-4	Silver	0.05[c]	---
7440-23-5	Sodium	---[d]	---[d]
14808-79-8	Sulfate	400[c]	400[c]
7440-28-0	Thallium	0.002[c]	0.02[c]
7440-62-2	Vanadium[b]	0.049	0.1
7440-66-6	Zinc	5.0[c]	10[c]

Chemical Name and Groundwater Remediation Objective Notations

[a] The groundwater remediation objective is equal to the ADL for carcinogens according to the procedures specified in 35 Ill. Adm. Code 620.

[b] Oral Reference Dose and/or Reference Concentration under review by USEPA. Listed values subject to change.

[c] Value listed is also the Groundwater Quality Standard for this chemical pursuant to 35 Ill. Adm. Code 620.410 for Class I Groundwater or 35 Ill. Adm. Code 620.420 for Class II Groundwater.

[d] This chemical is included in the Total Dissolved Solids (TDS) Groundwater Quality Standard of 1,200 mg/l pursuant to 35 Ill. Adm. Code 620.410 for Class I Groundwater or 35 Ill. Adm. Code 620.420 for Class II Groundwater.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B Tier 1 Illustrations and Tables

Section 742.TABLE F Values Used to Calculate the Tier 1 Soil Remediation Objectives for the Soil Component of the Groundwater Ingestion Route

		GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
CAS No.	Chemical Name Organics	Class I (mg/L)	Class II (mg/L)
83-32-9	Acenaphthene	2.0[b]	10
67-64-1	Acetone	6.3	6.3
15972-60-8	Alachlor	0.002[c]	0.01[c]
116-06-3	Aldicarb	0.003[c]	0.015[c]
309-00-2	Aldrin	5.0E-6[b]	2.5E-5
120-12-7	Anthracene	10[b]	50
1912-24-9	Atrazine	0.003[c]	0.015[c]
71-43-2	Benzene	0.005[c]	0.025[c]
56-55-3	Benzo(*a*)anthracene	0.0001[b]	0.0005
205-99-2	Benzo(*b*)fluoranthene	0.0001[b]	0.0005
207-08-9	Benzo(*k*)fluroanthene	0.001[b]	0.005
50-32-8	Benzo(*a*)pyrene	0.0002[a,c]	0.002[c]
65-85-0	Benzoic Acid	100[b]	100
111-44-4	Bis(2-chloroethyl)ether	8.0E-5[b]	8.0E-5
117-81-7	Bis(2-ethylhexyl)phthalate (Di(2-ethylhexyl)phthalate)	0.006[a,c]	0.06[c]
75-27-4	Bromodichloromethane (Dichlorobromomethane)	0.1[b]	0.1
75-25-2	Bromoform	0.1[b]	0.01
71-36-3	Butanol	4.0[b]	4.0
85-68-7	Butyl benzyl phthalate	7.0[b]	35
86-74-8	Carbazole	0.004[b]	0.02
1563-66-2	Carbofuran	0.04[c]	0.2[c]
75-15-0	Carbon disulfide	4.0[b]	20
56-23-5	Carbon tetrachloride	0.005[c]	0.025[c]
57-74-9	Chlordane	0.002[c]	0.01[c]

CAS No.	Chemical Name	GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
		Class I (mg/L)	Class II (mg/L)
106-47-8	4-Chloroaniline (ρ-Chloroaniline)	0.1[b]	0.1
108-90-7	Chlorobenzene (Monochlorobenzene)	0.1[c]	0.5[c]
124-48-1	Chlorodibromomethane (Dibromochloromethane)	0.06[b]	0.06
67-66-3	Chloroform	0.1[b]	0.5
95-57-8	2-Chlorophenol (pH 4.9-7.3)	0.2[b]	1.0
	2-Chlorophenol (pH 7.4-8.0)	0.2	0.2
218-01-9	Chrysene	0.1[b]	0.05
94-75-7	2,4-D	0.07[c]	0.35[c]
75-99-0	Dalapon	0.2[c]	2.0[c]
72-54-8	DDD	0.0004[b]	0.002
72-55-9	DDE	0.0003[b]	0.0015
50-29-3	DDT	0.0003[b]	0.0015
53-70-3	Dibenzo(*a,h*)anthracene	1.0E-5[b]	5.0E-5
96-12-8	1,2-Dibromo-3-chloropropane	0.0002[c]	0.002 [c]
106-93-4	1,2-Dibromoethane (Ethylene dibromide)	0.00005[a,c]	0.0005[c]
84-74-2	Di-*n*-butyl phthalate	4.0[b]	20
95-50-1	1,2-Dichlorobenzene (*o* – Dichlorobenzene)	0.6[c]	1.5[c]
106-46-7	1,4-Dichlorobenzene (*p* – Dichlorobenzene)	0.075[c]	0.375[c]
91-94-1	3,3'-Dichlorobenzidine	0.0002[b]	0.001
75-34-3	1,1-Dichloroethane	4.0[b]	20
107-06-2	1,2-Dichloroethane (Ethylene dichloride)	0.005[c]	0.025[c]
75-35-4	1,1-Dichloroethylene	0.007[c]	0.035[c]
156-59-2	*cis*-1,2-Dichloroethylene	0.07[c]	0.2[c]
156-60-5	*trans*-1,2-Dichloroethylene	0.1[c]	0.5[c]

120-83-2	2,4-Dichlorophenol	0.1[b]	0.1
78-97-5	1,2-Dichloropropane	0.005[c]	0.025[c]
542-75-6	1,3-Dichloropropene (1,3-Dichloropropylene, *cis* + *trans*)	0.0005[b]	0.0025

CAS No.	Chemical Name	GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
		Class I (mg/L)	Class II (mg/L)
60-57-1	Dieldrin	5.0E-6[b]	2.5E-5
84-66-2	Diethyl phthalate	30[b]	30
105-67-9	2,4-Dimethylphenol	0.7[b]	0.7
51-28-5	2,4-Dinitrophenol	0.04[b]	0.04
121-14-2	2,4-Dinitrotoluene	0.0001[b]	0.0001
606-20-2	2,6-Dinitrotoluene	0.0001	0.0001
88-85-7	Dinoseb	0.007[c]	0.07[c]
117-84-0	Di-*n*-octyl phthalate	0.7[b]	3.5
115-29-7	Endosulfan	0.2[b]	1.0
145-73-3	Endothall	0.1[c]	0.1[c]
72-20-8	Endrin	0.002[c]	0.01[c]
100-41-4	Ethylbenzene	0.7[c]	1.0[c]
206-44-0	Fluoranthene	1.0[b]	5.0
86-73-7	Fluorene	1.0[b]	5.0
76-44-8	Heptachlor	0.0004[c]	0.002[c]
1024-57-3	Heptachlor epoxide	0.0002[c]	0.001[c]
118-74-1	Hexachlorobenzene	0.001[b]	0.005
319-84-6	*alpha*-HCH (*alpha*-BHC)	1.0E-5[b]	5.0E-5
58-89-9	*Gamma*-HCH (Lindane)	0.0002[c]	0.001[c]
77-47-4	Hexachlorocyclopentadiene	0.05[c]	0.5[c]
67-72-1	Hexachloroethane	0.007	0.035
193-39-5	Indeno(1,2,3-*c,d*)pyrene	0.0001[b]	0.0005
78-59-1	Isophorone	1.4	1.4
72-43-5	Methoxychlor	0.04[c]	0.2[c]
74-83-9	Methyl bromide (Bromomethane)	0.05[b]	0.25
1634-04-4	Methyl tertiary-butyl ether	0.07	0.07
75-09-2	Methylene chloride (Dichloromethane)	0.005[c]	0.05[c]
95-48-7	2-Methylphenol (*o*-Cresol)	2.0[b]	2.0

91-20-3	Naphthalene	0.14	0.22
98-95-3	Nitrobenzene	0.02[b]	0.02

CAS No.	Chemical Name	GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
		Class I (mg/L)	Class II (mg/L)
86-30-6	*N*-Nitrosodiphenylamine	0.02[b]	0.1
621-64-7	*N*-Nitrosodi-*n*-propylamine	1.0E-5[b]	1.0E-5
87-86-5	Pentachlorophenol	0.001[a,c]	0.005[c]
108-95-2	Phenol	0.1[c]	0.1[c]
1918-02-1	Picloram	0.5[c]	5.0[c]
1336-36-3	Polychlorinated biphenyls (PCBs)	---	---
129-00-0	Pyrene	1.0[b]	5.0
122-34-9	Simazine	0.004[c]	0.04[c]
100-42-5	Styrene	0.1[c]	0.5[c]
93-72-1	2,4,5-TP (Silvex)	0.05[c]	0.25[c]
127-18-4	Tetrachloroethylene (Perchloroethylene)	0.005[c]	0.025[c]
108-88-3	Toluene	1.0[c]	2.5[c]
8001-35-2	Toxaphene	0.003[c]	0.015[c]
120-82-1	1,2,4-Trichlorobenzene	0.07[c]	0.7[c]
71-55-6	1,1,1-Trichloroethane	0.2[c]	1.0[c]
79-00-5	1,1,2-Trichloroethane	0.005[c]	0.05[c]
79-01-6	Trichloroethylene	0.005[c]	0.025[c]
95-95-4	2,4,5-Trichlorophenol (pH 4.9-7.8)	4.0[b]	20
	2,4,5-Trichlorophenol (pH 7.9-8.0)	4.0	4.0
88-06-2	2,4,6-Trichlorophenol (pH 4.9-6.8)	0.008[b]	0.04
	2.4.6-Trichlorophenol (pH 6.9-8.0)	0.008	0.008
108-05-4	Vinyl acetate	40[b]	40
75-01-4	Vinyl chloride	0.002[c]	0.01[c]
1330-20-7	Xylenes (total)	10.0[c]	10.0[c]

CAS No.	Chemical Name	GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
		Class I (mg/L)	Class II (mg/L)
	Inorganics		
7440-36-0	Antimony	0.006[c]	0.024[c]
7440-38-2	Arsenic	0.05[c]	0.2[c]
7440-39-3	Barium	2.0[c]	2.0[c]
7440-41-7	Beryllium	0.004[c]	0.5[c]
7440-42-8	Boron	2.0[c]	2.0[c]
7440-43-9	Cadmium	0.005[c]	0.05[c]
7440-70-2	Calcium	---	---
16887-00-6	Chloride	200[c]	200[c]
7440-47-3	Chromium, total	0.1[c]	1.0[c]
18540-29-9	Chromium, ion, hexavalent	---	---
7440-48-4	Cobalt	1.0[c]	1.0[c]
7440-50-8	Copper	0.65[c]	0.65[c]
57-12-5	Cyanide	0.2[c]	0.6[c]
7782-41-4	Fluoride	4.0[c]	4.0[c]
15438-31-0	Iron	5.0[c]	5.0[c]
7439-92-1	Lead	0.0075[c]	0.1[c]
7439-95-4	Magnesium	---	---
7439-96-5	Manganese	0.15[c]	10.0[c]
7439-97-6	Mercury	0.002[c]	0.01[c]
7440-02-0	Nickel	0.1[c]	2.0[c]
14797-55-8	Nitrate as N	10.0[c]	100[c]

CAS No.	Chemical Name	GW_{obj} Concentration used to Calculate Tier 1 Soil Remediation Objectives[a]	
		Class I (mg/L)	Class II (mg/L)
7723-14-0	Phosphorus	---	---
7440-09-7	Potassium	---	---
7782-49-2	Selenium	0.05[c]	0.05[c]
7440-22-4	Silver	0.05[c]	---
7440-23-5	Sodium	---	---
14808-79-8	Sulfate	400[c]	400[c]
7440-28-0	Thallium	0.002[c]	0.02[c]
7440-62-2	Vanadium	0.049	0.1
7440-66-6	Zinc	5.0[c]	10[c]

Chemical Name and Groundwater Remediation Objective Notations

[a] The Equation S17 is used to calculate the Soil Remediation Objective for the Soil Component of the Groundwater Ingestion Route; this equation requires calculation of the Target Soil Leachate Concentration (C_w) from Equation S18: $C_w = DF \times GW_{obj}$.

[b] Value listed is the Water Health Based Limit (HBL) for this chemical from Soil Screening Guidance: User's Guide, incorporated by reference at Section 742.210. The HBL is equal to the non-zero MCLG (if available); the MCL (if available); or, for carcinogens, a cancer risk of 1.0E-6, and for noncarcinogens is equal to a Hazard Quotient of 1.0. NOTE: These GW_{obj} concentrations are not equal to the Tier 1 Groundwater Remediation Objectives for the Direct Ingestion of Groundwater Component of the Groundwater Ingestion Route, listed in Section 742.Appendix B, Table E.

[c] Value listed is also the Groundwater Quality Standard for this chemical pursuant to 35 Ill. Adm. Code 620.410 for Class I Groundwater or 35 Ill. Adm. Code 620.420 for Class II Groundwater.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX B: Tier 1 Illustrations and Tables

Section 742.TABLE G: Tier 1 Soil Gas Remediation Objectives for the Outdoor Inhalation Exposure Route[a]

CAS No.	Chemical Name	Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Construction Worker (mg/m^3)
67-64-1	Acetone	750,000[e]	750,000[e]	750,000[e]
71-43-2	Benzene	420[c]	800[c]	1,100[c]
111-44-4	Bis(2-chloroethyl)ether	1.3[c]	2.4[c]	3.4[c]
75-27-4	Bromodichloromethane	450,000[e]	450,000[e]	450,000[e]
75-25-2	Bromoform	1,800[c]	3,500[c]	4,900[c]
71-36-3	Butanol	29,000[e]	29,000[e]	29,000[e]
78-93-3	2-Butanone (MEK)	380,000[e]	380,000[e]	15,000[b]
75-15-0	Carbon disulfide	1,500,000[e]	1,500,000[e]	48,000[b]
56-23-5	Carbon tetrachloride	290[c]	550[c]	770[c]
108-90-7	Chlorobenzene	36,000[b]	57,000[b]	3,700[b]
124-48-1	Chlorodibromomethane	57,000[e]	57,000[e]	150[b]
67-66-3	Chloroform	110[c]	200[c]	290[c]
95-57-8	2-Chlorophenol	17,000[e]	17,000[e]	17,000[e]
75-99-0	Dalapon	1,500[e]	1,500[e]	1,500[e]
96-12-8	1,2-Dibromo-3-chloropropane	0.14[c]	0.27[c]	0.38[c]
106-93-4	1,2-Dibromoethane	2.9[c]	5.6[c]	7.9[c]
95-50-1	1,2-Dichlorobenzene	11,000[e]	11,000[e]	6,700[b]
106-46-7	1,4-Dichlorobenzene	8,400[e]	8,400[e]	6,400[b]
75-71-8	Dichlorodifluoromethane	890,000[b]	1,400,000[b]	92,000[b]
75-34-3	1,1-Dichloroethane	870,000[b]	1,300,000[e]	90,000[b]
107-06-2	1,2-Dichloroethane	67[c]	130[c]	180[c]
75-35-4	1,1-Dichloroethylene	520,000[b]	820,000[b]	5,300[b]

CAS No.	Chemical Name	Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Construction Worker (mg/m^3)
156-59-2	*cis*-1,2-Dichloroethylene	1,100,000[e]	1,100,000[e]	1,100,000[e]
156-60-5	*trans*-1,2-Dichloroethylene	120,000[b]	190,000[b]	12,000[b]
78-87-5	1,2-Dichloropropane	240[c]	470[c]	110[c]
542-75-6	1,3-Dichloropropylene (*cis* + *trans*)	1,900[c]	3,700[c]	1,400[c]
123-91-1	p-Dioxane	16[c]	30[c]	42[c]
100-41-4	Ethylbenzene	59,000[e]	59,000[e]	8,500[b]
76-44-8	Heptachlor	0.40[c]	0.76[c]	1.1[c]
118-74-1	Hexachlorobenzene	0.26[c]	0.28[e]	0.28[e]
77-47-4	Hexachlorocyclopentadiene	85[b]	140[b]	440[b]
67-72-1	Hexachloroethane	2,800[e]	2,800[e]	2,800[e]
78-59-1	Isophorone	3,400[e]	3,400[e]	1,500[b]
98-82-8	Isopropylbenzene (Cumene)	30,000[e]	30,000[e]	30,000[e]
7439-97-6	Mercury[f]	22[e]	22[e]	0.62[b]
74-83-9	Methyl bromide	12,000[b]	19,000[b]	2,400[b]
1634-04-4	Methyl tertiary-butyl ether	1,200,000[e]	1,200,000[e]	23,000[b]
75-09-2	Methylene chloride	6,100[c]	12,000[c]	5,100[b]
91-57-6	2-Methylnaphthalene	530[e]	530[e]	530[e]
95-48-7	2-Methylphenol (o-cresol)	1,800[e]	1,800[e]	410[b]
91-20-3	Naphthalene	560[b]	620[e]	5.8[b]
98-95-3	Nitrobenzene	6.5[c]	12[c]	10[b]
621-64-7	n-Nitrosodi-n-propylamine	0.056[c]	0.11[c]	0.15[c]
108-95-2	Phenol	1,500[e]	1,500[e]	79[b]
1336-36-3	Polychlorinated biphenyls (PCBs)	---[d]	---[d]	---[d]
100-42-5	Styrene	34,000[e]	34,000[e]	16,000[b]
127-18-4	Tetrachloroethylene	360[c]	690[c]	970[c]
108-88-3	Toluene	140,000[e]	140,000[e]	50,000[b]
120-82-1	1,2,4-Trichlorobenzene	1,000[b]	1,600[b]	110[b]
71-55-6	1,1,1-Trichloroethane	870,000[e]	870,000[e]	89,000[b]

CAS No.	Chemical Name	Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Construction Worker (mg/m^3)
79-00-5	1,1,2-Trichloroethane	170,000[e]	170,000[e]	170,000[e]
79-01-6	Trichloroethylene	1,700[c]	3,300[c]	1,500[b]
75-69-4	Trichlorofluoromethane	2,100,000[b]	3,400,000[b]	220,000[b]
108-05-4	Vinyl acetate	160,000[b]	250,000[b]	1,600[b]
75-01-4	Vinyl chloride	780[c]	3,000[c]	3,000[b]
108-38-3	m-Xylene	52,000[e]	52,000[e]	3,100[b]
95-47-6	o-Xylene	41,000[e]	41,000[e]	2,600[b]
106-42-3	p-Xylene	55,000[e]	55,000[e]	3,300[b]
1330-20-7	Xylenes (total)	49,000[e]	49,000[e]	2,900[b]

Chemical Name and Remediation Objective Notations

[a] For the outdoor inhalation exposure route, it is acceptable to determine compliance by meeting either the soil or soil gas remediation objectives. The soil remediation objectives for the outdoor inhalation route are located in Appendix B, Tables A and B.

[b] Calculated values correspond to a target hazard quotient of 1.

[c] Calculated values correspond to a cancer risk level of 1 in 1,000,000.

[d] PCBs are a mixture of different congeners. The appropriate values to use for the physical/chemical and toxicity parameters depend on the congeners present at the site. Persons remediating sites should consult with IEPA Bureau of Land (BOL) if calculation of Tier 2 or 3 remediation objectives is desired.

[e] The value shown is the C_v^{sat} value of the chemical in soil gas. The C_v^{sat} of the chemical becomes the remediation objective if the calculated value exceeds the C_v^{sat} value or if there are no toxicity criteria available for the inhalation route of exposure.

[f] Value for the inhalation exposure route is based on Reference Concentration for elemental Mercury (CAS No. 7439-97-6). Inhalation remediation objectives only apply at sites where elemental Mercury is a contaminant of concern.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX B: Tier 1 Illustrations and Tables

Section 742.TABLE H: Tier 1 Soil Gas and Groundwater Remediation Objectives for the Indoor Inhalation Exposure Route – Diffusion and Advection[j]

Q_{soil} equals 83.33 cm^3/sec[a]

		Soil Gas		Groundwater	
CAS No.	Chemical Name	Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
67-64-1	Acetone	750,000[f]	750,000[f]	1,000,000[g]	1,000,000[g]
71-43-2	Benzene	0.37[c]	2.8[c]	0.11[c]	0.41[c]
111-44-4	Bis(2-chloroethyl)ether	0.014[c]	0.087[c]	0.083[c]	0.43[c]
75-27-4	Bromodichloromethane	450,000[f]	450,000[f]	6,700[g]	6,700[g]
75-25-2	Bromoform	11[c]	52[c]	3.1[c]	12[c]
71-36-3	Butanol	29,000[f]	29,000[f]	74,000[g]	74,000[g]
78-93-3	2-Butanone (MEK)	6,400[b]	40,000[b]	10,000[b]	48,000[b]
75-15-0	Carbon disulfide	780[b]	5,300[b]	67[b]	210[b]
56-23-5	Carbon tetrachloride	0.21[c]	1.5[c]	0.020[c]	0.076[c]
108-90-7	Chlorobenzene	69[b]	420[b]	26[b]	82[b]
124-48-1	Chlorodibromomethane	57,000[f]	57,000[f]	2,600[g]	2,600[g]
67-66-3	Chloroform	0.11[c]	0.92[c]	0.07[i]	0.15[c]
95-57-8	2-Chlorophenol	17,000[f]	17,000[f]	22,000[g]	22,000[g]
75-99-0	Dalapon[e]	1,500[f]	1,500[f]	900,000[g]	900,000[g]
96-12-8	1,2-Dibromo-3-chloropropane[e]	0.0012[c]	0.0062[c]	0.00065[c]	0.0027[c]
106-93-4	1,2-Dibromoethane	0.0078[c]	0.048[c]	0.0035[c]	0.014[c]
95-50-1	1,2-Dichlorobenzene	290[b]	1,700[b]	140[b]	160[g]
106-46-7	1,4-Dichlorobenzene	1,200[b]	6,800[b]	79[g]	79[g]
75-71-8	Dichlorodifluoromethane	270[b]	1,700[b]	3.0[b]	9.2[b]
75-34-3	1,1-Dichloroethane	690[b]	4,200[b]	180[b]	580[b]

CAS No.	Chemical Name	Soil Gas		Groundwater	
		Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
107-06-2	1,2-Dichloroethane	0.099[c]	0.81[c]	0.054[c]	0.22[c]
75-35-4	1,1-Dichloroethylene	240[b]	1,600[b]	24[b]	74[b]
156-59-2	*cis*-1,2-Dichloroethylene	1,100,000[f]	1,100,000[f]	3,500[g]	3,500[g]
156-60-5	*trans*-1,2-Dichloroethylene	85[b]	510[b]	16[b]	51[b]
78-87-5	1,2-Dichloropropane	0.31[c]	2.3[c]	0.12[c]	0.48[c]
542-75-6	1,3-Dichloropropylene (*cis* + *trans*)	0.90[c]	6.2[c]	0.14[c]	0.52[c]
123-91-1	p-Dioxane	0.22[c]	2.3[c]	2.9[c]	25[c]
100-41-4	Ethylbenzene	1,3[c]	9.3[c]	0.37[c]	1.4[c]
76-44-8	Heptachlor	0.0063[c]	0.032[c]	0.0025[c]	0.0096[c]
118-74-1	Hexachlorobenzene	0.0087[c]	0.057[c]	0.0059[c]	0.0062[g]
77-47-4	Hexachlorocyclopentadiene	0.58[b]	2.6[b]	0.084[b]	0.26[b]
67-72-1	Hexachloroethane	2,800[f]	2,800[f]	50[g]	50[g]
78-59-1	Isophorone	2,900[b]	3,400[f]	12,000[g]	12,000[g]
98-82-8	Isopropylbenzene (Cumene)	600[b]	3,500[b]	2.7[b]	8.4[b]
7439-97-6	Mercury[h]	0.42[b]	2.5[b]	0.053[b]	0.060[g]
74-83-9	Methyl bromide	6.9[b]	42[b]	1.5[b]	4.8[b]
1634-04-4	Methyl tertiary-butyl ether	3,700[b]	24,000[b]	1,900[b]	6,800[b]
75-09-2	Methylene chloride	5.6[c]	45[c]	2.1[c]	8.2[c]
91-57-6	2-Methylnaphthalene	530[f]	530[f]	25[g]	25[g]
95-48-7	2-Methylphenol (o-cresol)	600[b]	1,800[f]	26,000[g]	26,000[g]
91-20-3	Naphthalene	0.11[c]	0.75[c]	0.075[c]	0.32[c]
98-95-3	Nitrobenzene	0.077[c]	0.57[c]	0.34[c]	2.0[c]
621-64-7	n-Nitrosodi-n-propylamine	0.0016[c]	0.012[c]	0.044[c]	0.27[c]
108-95-2	Phenol	140[b]	1,300[b]	28,000[b]	83,000[g]

CAS No.	Chemical Name	Soil Gas		Groundwater	
		Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
1336-36-3	Polychlorinated biphenyls (PCBs)	---[d]	---[d]	---[d]	---[d]
100-42-5	Styrene	1,400[b]	8,500[b]	310[g]	310[g]
127-18-4	Tetrachloroethylene	0.55[c]	4.0[c]	0.091[c]	0.34[c]
108-88-3	Toluene	6,200[b]	40,000[b]	530[g]	530[g]
120-82-1	1,2,4-Trichlorobenzene	5.4[b]	25[b]	1.8[b]	5.9[b]
71-55-6	1,1,1-Trichloroethane	6,600[b]	41,000[b]	1,000[b]	1,300[g]
79-00-5	1,1,2-Trichloroethane	170,000[f]	170,000[f]	4,400[g]	4,400[g]
79-01-6	Trichloroethylene	1.5[c]	12[c]	0.34[c]	1.3[c]
75-69-4	Trichlorofluoromethane	860[b]	5,600[b]	26[b]	82[b]
108-05-4	Vinyl acetate	250[b]	1,600[b]	160[b]	550[b]
75-01-4	Vinyl chloride	0.29[c]	4.8[c]	0.028[c]	0.21[c]
108-38-3	m-Xylene	140[b]	850[b]	43[b]	130[b]
95-47-6	o-Xylene	120[b]	790[b]	40[b]	130[b]
106-42-3	p-Xylene	130[b]	820[b]	38[b]	120[b]
1330-20-7	Xylenes (total)[e]	140[b]	840[b]	30[b]	93[b]

Chemical Name and Remediation Objective Notations

[a] Compliance is determined by meeting either the soil gas remediation objectives or the groundwater remediation objectives. See Sections 742.505 and 742.515.

[b] Calculated values correspond to a target hazard quotient of 1.

[c] Calculated values correspond to a cancer risk level of 1 in 1,000,000.

[d] PCBs are a mixture of different congeners. The appropriate values to use for the physical/chemical and toxicity parameters depend on the congeners present at the site. Persons remediating sites should consult with BOL if calculation of Tier 2 or 3 remediation objectives is desired.

[e] Groundwater remediation objective calculated at 25°C. For Dalapon and 1,2-Dibromo-3-chloropropane, the critical temperature (T_c) and enthalpy of vaporization at the normal boiling point ($H_{v,b}$) are not available. For Xylenes (total), the enthalpy of vaporization at the normal boiling point ($H_{v,b}$) is not available.

[f] The value shown is the C_v^{sat} value of the chemical in soil gas. The C_v^{sat} of the chemical becomes the remediation objective if the calculated value exceeds the C_v^{sat} value or if there are no toxicity criteria available for the inhalation route of exposure.

[g] The value shown is the solubility of the chemical in water. The solubility of the chemical becomes the remediation objective if the calculated value exceeds the solubility or if there are no toxicity criteria available for the ingestion route of exposure.

[h] Value for the inhalation exposure route is based on Reference Concentration for elemental Mercury (CAS No. 7439-97-6). Inhalation remediation objectives only apply at sites where elemental Mercury is a contaminant of concern.

[i] The value shown is the Groundwater Remediation Objective listed in Appendix B, Table E.

[j] Calculated values for the remediation objectives in this table are based on the assumption that the existing or potential building has a full concrete slab-on-grade, though the remediation objectives in this table are also considered protective of occupants of buildings with full concrete basement floors and walls. This table applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Institutional controls under Subpart J are required to use remediation objectives in this table. This table does not apply when the existing or potential building has neither a full concrete slab-on-grade nor a full concrete basement floor and walls, such as a building with an earthen crawl space, an earthen floor, a stone foundation, a partial concrete floor, or a sump. In such cases, site evaluators have the option of excluding the indoor inhalation exposure route under Section 742.312, meeting the building control technology requirements under Subpart L, or proposing an alternative approach under Tier 3.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX B: Tier 1 Illustrations and Tables

Section 742.TABLE I: Tier 1 Soil Gas and Groundwater Remediation Objectives for the Indoor Inhalation Exposure Route – Diffusion Only[j]

Q_{soil} equals 0.0 cm^3/sec[a,b]

		Soil Gas		Groundwater	
CAS No.	Chemical Name	Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
67-64-1	Acetone	750,000[g]	750,000[g]	1,000,000[h]	1,000,000[h]
71-43-2	Benzene	41[d]	300[d]	0.41[d]	2.6[d]
111-44-4	Bis(2-chloroethyl)ether	1.9[d]	14[d]	6.6[d]	48[d]
75-27-4	Bromodichloromethane	450,000[g]	450,000[g]	6,700[h]	6,700[h]
75-25-2	Bromoform	1,800[d]	13,000[d]	170[d]	1,300[d]
71-36-3	Butanol	29,000[g]	29,000[g]	74,000[h]	74,000[h]
78-93-3	2-Butanone (MEK)	380,000[g]	380,000[g]	220,000[h]	220,000[h]
75-15-0	Carbon disulfide	81,000[c]	500,000[c]	170[c]	820[c]
56-23-5	Carbon tetrachloride	24[d]	180[d]	0.052[d]	0.31[d]
108-90-7	Chlorobenzene	8,300[c]	51,000[c]	130[c]	470[h]
124-48-1	Chlorodibromomethane	57,000[g]	57,000[g]	2,600[h]	2,600[h]
67-66-3	Chloroform	12[d]	87[d]	0.17[d]	1.1[d]
95-57-8	2-Chlorophenol	17,000[g]	17,000[g]	22,000[h]	22,000[h]
75-99-0	Dalapon[f]	1,500[g]	1,500[g]	900,000[h]	900,000[h]
96-12-8	1,2-Dibromo-3-chloropropane[f]	0.17[d]	1.3[d]	0.029[d]	0.21[d]
106-93-4	1,2-Dibromoethane	1.1[d]	7.9[d]	0.073[d]	0.52[d]
95-50-1	1,2-Dichlorobenzene	11,000[g]	11,000[g]	160[h]	160[h]
106-46-7	1,4-Dichlorobenzene	8,400[g]	8,400[g]	79[h]	79[h]
75-71-8	Dichlorodifluoromethane	32,000[c]	200,000[c]	6.8[c]	33[c]
75-34-3	1,1-Dichloroethane	81,000[c]	500,000[c]	750[c]	4,100[c]

CAS No.	Chemical Name	Soil Gas		Groundwater	
		Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
107-06-2	1,2-Dichloroethane	10[d]	76[d]	0.50[d]	3.5[d]
75-35-4	1,1-Dichloroethylene	27,000[c]	160,000[c]	61[c]	300[c]
156-59-2	*cis*-1,2-Dichloroethylene	1,100,000[g]	1,100,000[g]	3,500[h]	3,500[h]
156-60-5	*trans*-1,2-Dichloroethylene	10,000[c]	63,000[c]	58[c]	310[c]
78-87-5	1,2-Dichloropropane	36[d]	260[d]	0.67[d]	4.5[d]
542-75-6	1,3-Dichloropropylene (*cis* + *trans*)	110[d]	830[d]	0.42[d]	2.6[d]
123-91-1	p-Dioxane	15[d]	110[d]	140[d]	1,000[d]
100-41-4	Ethylbenzene	150[d]	1,100[d]	1.3[d]	8.1[d]
76-44-8	Heptachlor	0.97[d]	7.1[d]	0.058[d]	0.18[h]
118-74-1	Hexachlorobenzene	0.28[g]	0.28[g]	0.0062[h]	0.0062[h]
77-47-4	Hexachlorocyclopentadiene	86[c]	530[c]	0.29[c]	1.5[c]
67-72-1	Hexachloroethane	2,800[g]	2,800[g]	50[h]	50[h]
78-59-1	Isophorone	3,400[g]	3,400[g]	12,000[h]	12,000[h]
98-82-8	Isopropylbenzene (Cumene)	30,000[g]	30,000[g]	6.2[c]	30[c]
7439-97-6	Mercury[i]	22[g]	22[g]	0.060[h]	0.060[h]
74-83-9	Methyl bromide	830[c]	5,100[c]	6.1[c]	33[c]
1634-04-4	Methyl tertiary-butyl ether	420,000[c]	1,200,000[g]	30,000[c]	51,000[h]
75-09-2	Methylene chloride	590[d]	4,400[d]	12[d]	84[d]
91-57-6	2-Methylnaphthalene	530[g]	530[g]	25[h]	25[h]
95-48-7	2-Methylphenol (o-cresol)	1,800[g]	1,800[g]	26,000[h]	26,000[h]
91-20-3	Naphthalene	14[d]	100[d]	1.8[d]	13[d]
98-95-3	Nitrobenzene	9.0[d]	66[d]	23[d]	170[d]
621-64-7	n-Nitrosodi-n-propylamine	0.18[d]	1.3[d]	3.3[d]	24[d]
108-95-2	Phenol	1,500[g]	1,500[g]	83,000[h]	83,000[h]
1336-36-3	Polychlorinated biphenyls	---[e]	---[e]	---[e]	---[e]

CAS No.	Chemical Name	Soil Gas		Groundwater	
		Residential (mg/m^3)	Industrial/Commercial (mg/m^3)	Residential (mg/L)	Industrial/Commercial (mg/L)
	(PCBs)				
100-42-5	Styrene	34,000[g]	34,000[g]	310[h]	310[h]
127-18-4	Tetrachloroethylene	66[d]	490[d]	0.26[d]	1.6[d]
108-88-3	Toluene	140,000[g]	140,000[g]	530[h]	530[h]
120-82-1	1,2,4-Trichlorobenzene	800[c]	4,300[g]	35[h]	35[h]
71-55-6	1,1,1-Trichloroethane	770,000[c]	870,000[g]	1,300[h]	1,300[h]
79-00-5	1,1,2-Trichloroethane	170,000[g]	170,000[g]	4,400[h]	4,400[h]
79-01-6	Trichloroethylene	180[d]	1,300[d]	1.1[d]	6.7[d]
75-69-4	Trichlorofluoromethane	97,000[c]	600,000[c]	62[c]	300[c]
108-05-4	Vinyl acetate	28,000[c]	170,000[c]	2,500[c]	15,000[c]
75-01-4	Vinyl chloride	30[d]	440[d]	0.065[d]	0.75[d]
108-38-3	m-Xylene	17,000[d]	52,000[c]	160[c]	160[h]
95-47-6	o-Xylene	14,000[d]	41,000[c]	170[c]	180[h]
106-42-3	p-Xylene	16,000[d]	55,000[c]	140[c]	160[h]
1330-20-7	Xylenes (total)[f]	17,000[d]	49,000[c]	96[c]	110[h]

Chemical Name and Remediation Objective Notations

[a] Compliance is determined by meeting both the soil gas remediation objectives and the groundwater remediation objectives. See Sections 742.505 and 742.515.

[b] Remediation objectives relying on this table require use of institutional controls in accordance with Subpart J.

[c] Calculated values correspond to a target hazard quotient of 1.

[d] Calculated values correspond to a cancer risk level of 1 in 1,000,000.

[e] PCBs are a mixture of different congeners. The appropriate values to use for the physical/chemical and toxicity parameters depend on the congeners present at the site. Persons remediating sites should consult with BOL if calculation of Tier 2 or 3 remediation objectives is desired

[f] Groundwater remediation objective calculated at 25°C. For Dalapon and 1,2-Dibromo-3-chloropropane, the critical temperature (T_c) and enthalpy of vaporization at the normal boiling point ($H_{v,b}$) are not available. For Xylenes (total), the enthalpy of vaporization at the normal boiling point ($H_{v,b}$) is not available.

[g] The value shown is the C_v^{sat} value of the chemical in soil gas. The C_v^{sat} of the chemical becomes the remediation objective if the calculated value exceeds the C_v^{sat} value or if there are no toxicity criteria available for the inhalation route of exposure.

[h] The value shown is the solubility of the chemical in water. The solubility of the chemical becomes the remediation objective if the calculated value exceeds the solubility or if there are no toxicity criteria available for the inhalation route of exposure.

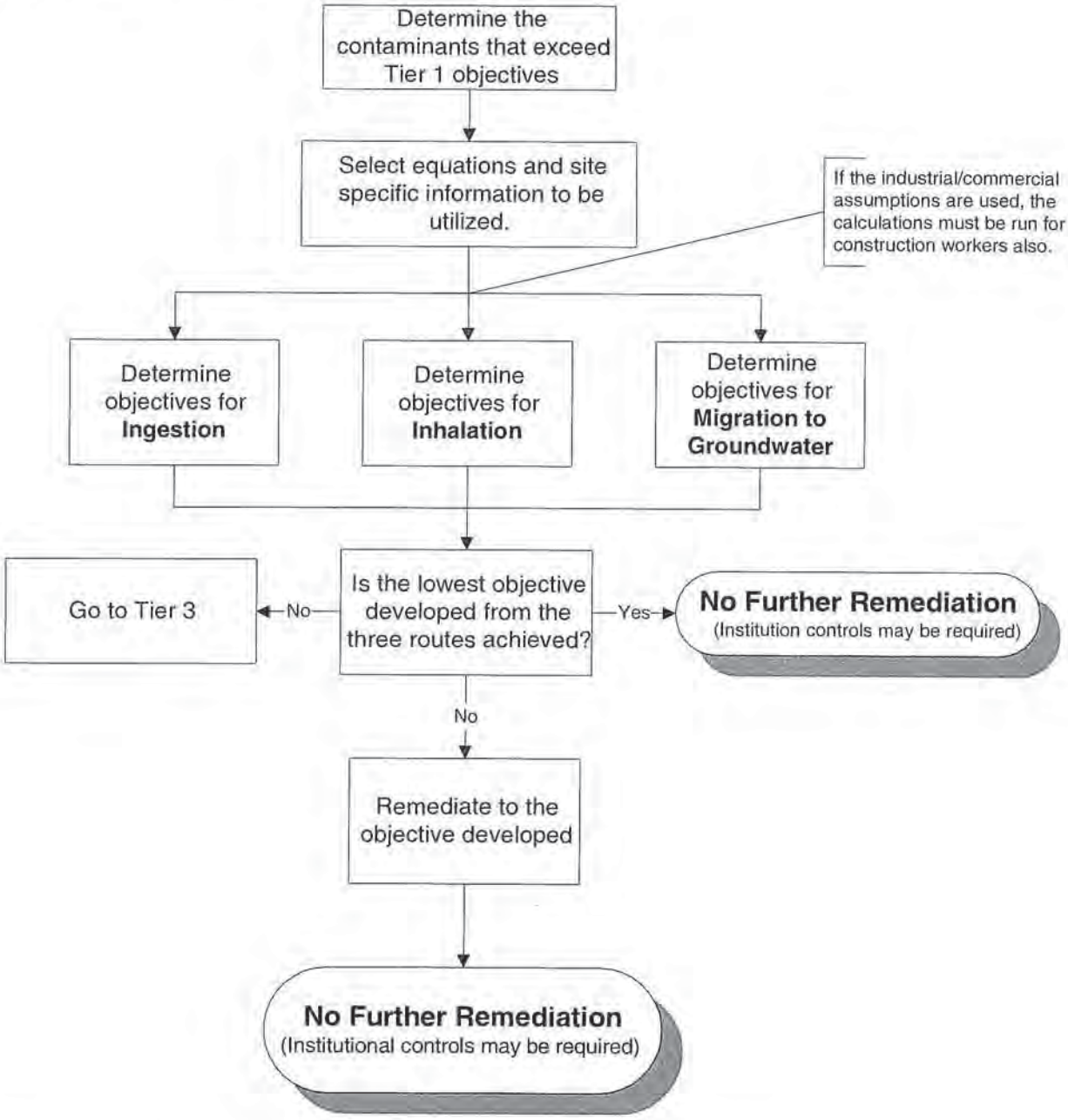
[i] Value for the inhalation exposure route is based on Reference Concentration for elemental Mercury (CAS No. 7439-97-6). Inhalation remediation objectives only apply at sites where elemental Mercury is a contaminant of concern.

[j] Calculated values for the remediation objectives in this table are based on the assumption that the existing or potential building has a full concrete slab-on-grade, though the remediation objectives in this table are also considered protective of occupants of buildings with full concrete basement floors and walls. This table applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Institutional controls under Subpart J are required to use remediation objectives in this table. This table does not apply when the existing or potential building has neither a full concrete slab-on-grade nor a full concrete basement floor and walls, such as a building with an earthen crawl space, an earthen floor, a stone foundation, a partial concrete floor, or a sump. In such cases, site evaluators have the option of excluding the indoor inhalation exposure route under Section 742.312, meeting the building control technology requirements under Subpart L, or proposing an alternative approach under Tier 3.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

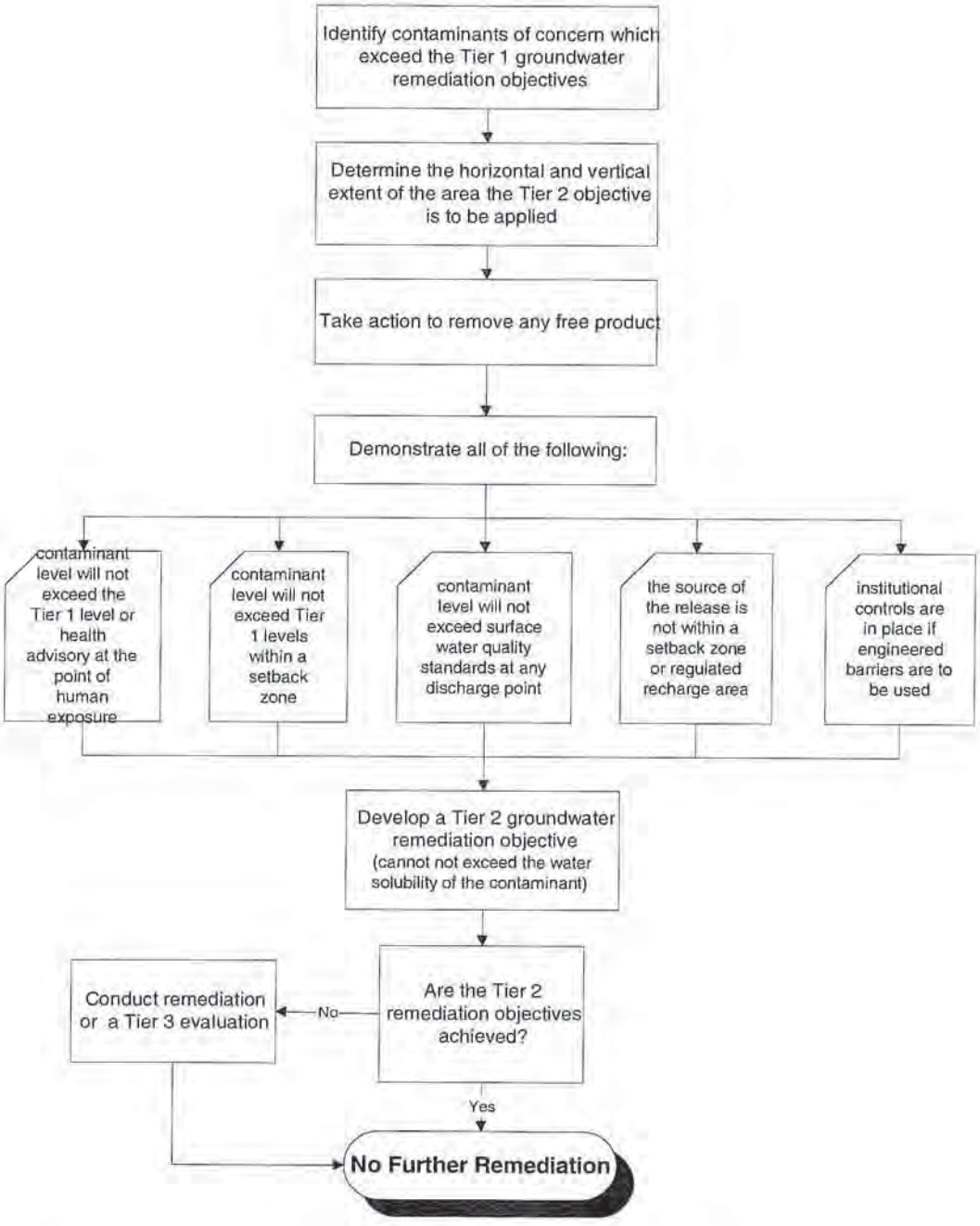
Section 742.Illustration A Tier 2 Evaluation for Soil



(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

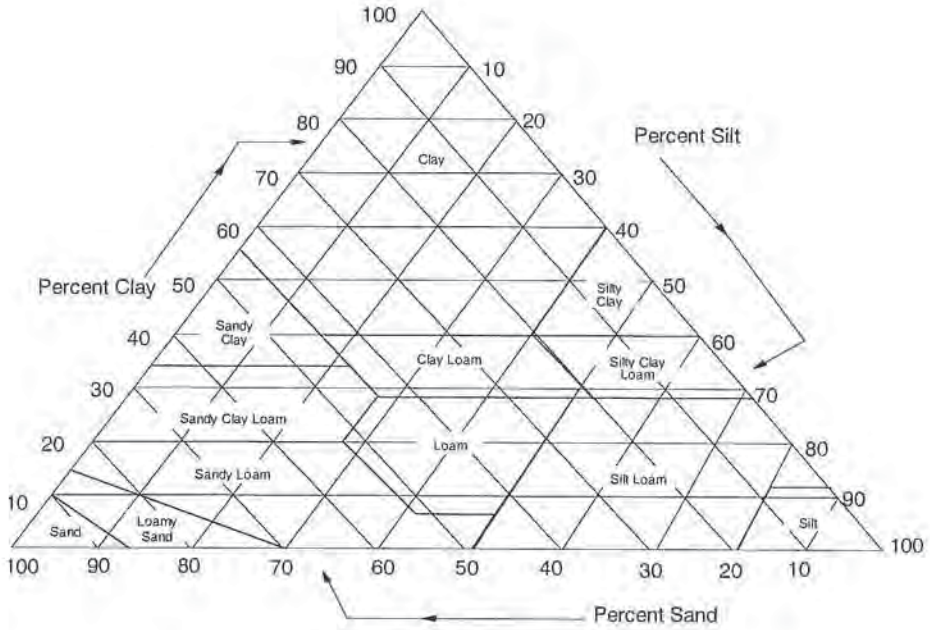
Section 742.Illustration B Tier 2 Evaluation for Groundwater



(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

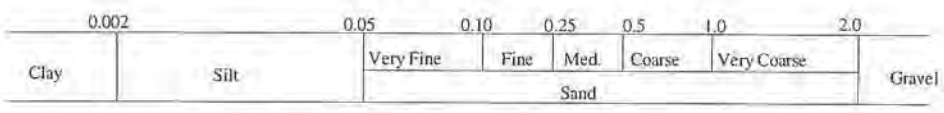
Section 742.Illustration C U.S. Department of Agriculture Soil Texture Classification



Criteria Used with the Field Method for Determining Soil Texture Classes

Criterion	Sand	Sandy loam	Loam	Silt loam	Clay loam	Clay
1. Individual grains visible to eye	Yes	Yes	Some	Few	No	No
2. Stability of dry clods	Do not form	Do not form	Easily broken	Moderately easily broken	Hard and stable	Very hard and stable
3. Stability of wet clods	Unstable	Slightyl stable	Moderately stable	Stable	Very stable	Very stable
4. Stability of "ribbon" when wet soil rubbed between thumb and fingers	Does not form	Does not form	Does not form	Broken appearance	Thin, will break	Very long, flexible

Particle Size, mm



(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C: Tier 2 Illustrations and Tables

Section 742.Table A: SSL Equations

Equations for Soil Ingestion Exposure Route	Remediation Objectives for Noncarcinogenic Contaminants (mg/kg)	$\frac{THQ \bullet BW \bullet AT \bullet 365\frac{d}{yr}}{\frac{1}{RfD_O} \bullet 10^{-6}\frac{kg}{mg} \bullet EF \bullet ED \bullet IR_{soil}}$	**S1**
	Remediation Objectives for Carcinogenic Contaminants - Residential (mg/kg)	$\frac{TR \bullet AT_c \bullet 365\frac{d}{yr}}{SF_o \bullet 10^{-6}\frac{kg}{mg} \bullet EF \bullet IF_{soil-adj}}$	**S2**
	Remediation Objectives for Carcinogenic Contaminants - Industrial/ Commercial, Construction Worker (mg/kg)	$\frac{TR \bullet BW \bullet AT_c \bullet 365\frac{d}{yr}}{SF_o \bullet 10^{-6}\frac{kg}{mg} \bullet EF \bullet ED \bullet IR_{soil}}$	**S3**

Equations for Inhalation Exposure Route (Organic Contaminants and Mercury)	Remediation Objectives for Noncarcinogenic Contaminants - Residential, Industrial/ Commercial (mg/kg)	$\frac{THQ \bullet AT \bullet 365\frac{d}{yr}}{EF \bullet ED \bullet \left(\frac{1}{RfC} \bullet \frac{1}{VF}\right)}$	**S4**
	Remediation Objectives for Noncarcinogenic Contaminants - Construction Worker (mg/kg)	$\frac{THQ \bullet AT \bullet 365\frac{d}{yr}}{EF \bullet ED \bullet \left(\frac{1}{RfC} \bullet \frac{1}{VF'}\right)}$	**S5**
	Remediation Objectives for Carcinogenic Contaminants - Residential, Industrial/ Commercial (mg/kg)	$\frac{TR \bullet AT_c \bullet 365\frac{d}{yr}}{URF \bullet 1{,}000\frac{ug}{mg} \bullet EF \bullet ED \bullet \frac{1}{VF}}$	**S6**
	Remediation Objectives for Carcinogenic Contaminants - Construction Worker (mg/kg)	$\frac{TR \bullet AT_c \bullet 365\frac{d}{yr}}{URF \bullet 1{,}000\frac{ug}{mg} \bullet EF \bullet ED \bullet \frac{1}{VF'}}$	**S7**

	Equation for Derivation of the Volatilization Factor - Residential, Industrial/ Commercial, VF (m^3/kg)	$VF = \frac{Q}{C} \bullet \frac{\left(3.14 \bullet D_A \bullet T\right)^{1/2}}{\left(2 \bullet \rho_b \bullet D_A\right)} \bullet 10^{-4} \frac{m^2}{cm^2}$	**S8**
	Equation for Derivation of the Volatilization Factor - Construction Worker, VF′ (m^3/kg)	$VF' = \frac{VF}{10}$	**S9**
	Equation for Derivation of Apparent Diffusivity, D_A (cm^2/s)	$D_A = \frac{\left(\theta_a^{3.33} \bullet D_i \bullet H'\right) + \left(\theta_w^{3.33} \bullet D_w\right)}{\eta^2} \bullet \frac{1}{\left(\rho_b \bullet K_d\right) + \theta_w + \left(\theta_a \bullet H'\right)}$	**S10**
Equations for Inhalation Exposure Route (Fugitive Dusts)	Remediation Objectives for Noncarcinogenic Contaminants - Residential, Industrial/Commercial (mg/kg)	$\frac{THQ \bullet AT \bullet 365 \frac{d}{yr}}{EF \bullet ED \bullet \left(\frac{1}{RfC} \bullet \frac{1}{PEF}\right)}$	**S11**

	Remediation Objectives for Noncarcinogenic Contaminants - Construction Worker (mg/kg)	$\frac{THQ \bullet AT \bullet 365\frac{d}{yr}}{EF \bullet ED \bullet \left(\frac{1}{RfC} \bullet \frac{1}{PEF'}\right)}$	**S12**
	Remediation Objectives for Carcinogenic Contaminants - Residential, Industrial/ Commercial (mg/kg)	$\frac{TR \bullet AT_c \bullet 365\frac{d}{yr}}{URF \bullet 1{,}000\frac{ug}{mg} \bullet EF \bullet ED \bullet \frac{1}{PEF}}$	**S13**
	Remediation Objectives for Carcinogenic Contaminants - Construction Worker (mg/kg)	$\frac{TR \bullet AT_c \bullet 365\frac{d}{yr}}{URF \bullet 1{,}000\frac{ug}{mg} \bullet EF \bullet ED \bullet \frac{1}{PEF'}}$	**S14**
	Equation for Derivation of Particulate Emission Factor, PEF (m^3/kg)	$PEF = \frac{Q}{C} \bullet \frac{3{,}600\frac{s}{hr}}{0.036 \bullet (1-V) \bullet \left(\frac{U_m}{U_t}\right)^3 \bullet F(x)}$	**S15**

	Equation for Derivation of Particulate Emission Factor, PEF′ - Construction Worker (m^3/kg)	$PEF' = \frac{PEF}{10}$ NOTE: PEF must be the industrial/commercial value	**S16**
Equations for the Soil Component of the Groundwater Ingestion Exposure Route	Remediation Objective (mg/kg)	$C_w \bullet \left[K_d + \frac{(\theta_w + \theta_a \bullet H')}{\rho_b} \right]$ NOTE: This equation can only be used to model contaminant migration not in the water bearing unit.	**S17**
	Target Soil Leachate Concentration, C_w (mg/L)	$C_w = DF \bullet GW_{obj}$	**S18**

	Soil-Water Partition Coefficient, K_d (cm^3/g)	$K_d = K_{oc} \bullet f_{oc}$	**S19**
	Water-Filled Soil Porosity, $\underline{\theta}_w$ (L_{water}/L_{soil})	$\theta_w = \eta \bullet \left(\frac{I}{K_s}\right)^{1/(2b+3)}$	**S20**
	Air-Filled Soil Porosity, $\underline{\theta}_a$ (L_{air}/L_{soil})	$\theta_a = \eta - \theta_w$	**S21**
	Dilution Factor, DF (unitless)	$DF = 1 + \frac{K \bullet i \bullet d}{I \bullet L}$	**S22**
	Groundwater Remediation Objective for Carcinogenic Contaminants, GW_{obj} (mg/L)	$\frac{TR \bullet BW \bullet AT_c \bullet 365 \frac{d}{yr}}{SF_o \bullet IR_w \bullet EF \bullet ED}$	**S23**
	Total Soil Porosity, η (L_{pore}/L_{soil})	$\eta = 1 - \frac{\rho_b}{\rho_s}$	**S24**
	Equation for Estimation of Mixing Zone Depth, d (m)	$d = \left(0.0112 \bullet L^2\right)^{0.5} + d_a\left[1 - \exp\frac{(-L \bullet I)}{\left(K \bullet i \bullet d_a\right)}\right]$	**S25**

Mass-Limit Equations for Inhalation Exposure Route and Soil Component of the Groundwater Ingestion Exposure Route	Mass-Limit Volatilization Factor for the Inhalation Exposure Route - Residential, Industrial/ Commercial, VF (m^3/kg)	$$VF_{M-L} = \frac{Q}{C} \bullet \frac{\left[T_{M-L} \bullet \left(3.15 \bullet 10^7 \frac{s}{yr}\right)\right]}{\rho_b \bullet d_s \bullet 10^6 \frac{cm^3}{m^3}}$$ NOTE: This equation may be used when vertical thickness of contamination is known or can be estimated reliably.	**S26**
	Mass-Limit Volatilization Factor for Inhalation Exposure Route - Construction Worker, VF′ - (m^3/kg)	$$VF'_{M-L} = \frac{VF_{M-L}}{10}$$	**S27**
	Mass-Limit Remediation Objective for Soil Component of the Groundwater Ingestion Exposure Route (mg/kg)	$$\frac{\left(C_W \bullet I_{M-L} \bullet ED_{M-L}\right)}{\rho_b \bullet d_s}$$ NOTE: This equation may be used when vertical thickness is known or can be estimated reliably.	**S28**

Equation for Derivation of the Soil Saturation Limit, C_{sat}	$C_{sat} = \frac{S}{\rho_b} \bullet \left[\left(K_d \bullet \rho_b \right) + \theta_w + \left(H' \bullet \theta_a \right) \right]$	**S29**
Equation for the soil gas component of the Outdoor Inhalation Exposure Route	$RO_{soil\ gas} = \frac{RO_{soil} \times H \times \rho_b \times 1000}{H' \times \theta_a + \theta_w + K_d \times \rho_b}$	**S30**

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C: Tier 2 Illustrations and Tables

Section 742.Table B: SSL Parameters

Symbol	Parameter	Units	Source	Parameter Value(s)
AT	Averaging Time for Noncarcinogens in Ingestion Equation	yr		Residential = 6 Industrial/Commercial = 25 Construction Worker = 0.115
AT	Averaging Time for Noncarcinogens in Inhalation Equation	yr		Residential = 30 Industrial/Commercial = 25 Construction Worker = 0.115
AT_c	Averaging Time for Carcinogens	yr	SSL	70
BW	Body Weight	kg		Residential = 15, noncarcinogens 70, carcinogens Industrial/Commercial = 70 Construction Worker = 70
C_{sat}	Soil Saturation Concentration	mg/kg	Appendix A, Table A or Equation S29 in Appendix C, Table A	Chemical-Specific or Calculated Value
C_w	Target Soil Leachate Concentration	mg/L	Equation S18 in Appendix C, Table A	Groundwater Standard, Health Advisory concentration, or Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
d	Mixing Zone Depth	m	SSL or Equation S25 in Appendix C, Table A	2 m or Calculated Value
d_a	Aquifer Thickness	m	Field Measurement	Site-Specific
d_s	Depth of Source (Vertical thickness of contamination)	m	Field Measurement or Estimation	Site-Specific
D_A	Apparent Diffusivity	cm^2/s	Equation S10 in Appendix C, Table A	Calculated Value
D_i	Diffusivity in Air	cm^2/s	Appendix C, Table E	Chemical-Specific
D_w	Diffusivity in Water	cm^2/s	Appendix C, Table E	Chemical-Specific
DF	Dilution Factor	unitless	Equation S22 in Appendix C, Table A	20 or Calculated Value
ED	Exposure Duration for Ingestion of Carcinogens	yr		Industrial/Commercial = 25 Construction Worker = 1
ED	Exposure Duration for Inhalation of Carcinogens	yr		Residential = 30 Industrial/Commercial = 25 Construction Worker = 1

Symbol	Parameter	Units	Source	Parameter Value(s)
ED	Exposure Duration for Ingestion of Noncarcinogens	yr		Residential = 6 Industrial/Commercial = 25 Construction Worker = 1
ED	Exposure Duration for Inhalation of Noncarcinogens	yr		Residential = 30 Industrial/Commercial = 25 Construction Worker = 1
ED	Exposure Duration for the Direct Ingestion of Groundwater	yr		Residential = 30 Industrial/Commercial = 25 Construction Worker = 1
$ED_{M\text{-}L}$	Exposure Duration for Migration to Groundwater Mass-Limit Equation S28	yr	SSL	70
EF	Exposure Frequency	d/yr		Residential = 350 Industrial/Commercial = 250 Construction Worker = 30
F(x)	Function dependent on U_m/U_t	unitless	SSL	0.194
f_{oc}	Organic Carbon Content of Soil	g/g	SSL or Field Measurement (See Appendix C, Table F)	Surface Soil = 0.006 Subsurface soil = 0.002, or Site-Specific

Symbol	Parameter	Units	Source	Parameter Value(s)
GW_{obj}	Groundwater Remediation Remediation Objective	mg/L	Appendix B, Table E, 35 IAC 620.Subpart F, or Equation S23 in Appendix C, Table A	Chemical-Specific or Calculated
H'	Henry's Law Constant	unitless	Appendix C, Table E	Chemical-Specific
i	Hydraulic Gradient	m/m	Field Measurement (See Appendix C, Table F)	Site-Specific
I	Infiltration Rate	m/yr	SSL	0.3
I_{M-L}	Infiltration Rate for Migration to Groundwater Mass-Limit Equation S28	m/yr	SSL	0.18
$IF_{soil-adj}$ (residential)	Age Adjusted Soil Ingestion Factor for Carcinogens	(mg-yr)/(kg-d)	SSL	114
IR_{soil}	Soil Ingestion Rate	mg/d		Residential = 200 Industrial/Commercial = 50 Construction Worker = 480
IR_W	Daily Water Ingestion Rate	L/d		Residential = 2 Industrial/Commercial = 1

Symbol	Parameter	Units	Source	Parameter Value(s)
K	Aquifer Hydraulic Conductivity	m/yr	Field Measurement (See Appendix C, Table F)	Site-Specific
K_d (Non-ionizing organics)	Soil-Water Partition Coefficient	cm^3/g or L/kg	Equation S19 in Appendix C, Table A	Calculated Value
K_d (Ionizing organics)	Soil-Water Partition Coefficient	cm3/g or L/kg	Equation S19 in Appendix C, Table A	Chemical and pH-Specific (see Appendix C, Table I)
K_d (Inorganics)	Soil-Water Partition Coefficient	cm3/g or L/kg	Appendix C, Table J	Chemical and pH-Specific
K_{oc}	Organic Carbon Partition Coefficient	cm^3/g or L/kg	Appendix C, Table E or Appendix C, Table I	Chemical-Specific
K_s	Saturated Hydraulic Conductivity	m/yr	Appendix C, Table K Appendix C, Illustration C	Site-Specific
L	Source Length Parallel to Groundwater Flow	m	Field Measurement	Site-Specific
PEF	Particulate Emission Factor	m^3/kg	SSL or Equation S15 in Appendix C, Table A	Residential = $1.32 \bullet 10^9$ or Site-Specific Industrial/Commercial = $1.24 \bullet 10^9$ or Site-Specific

Symbol	Parameter	Units	Source	Parameter Value(s)
PEF′	Particulate Emission Factor adjusted for Agitation (construction worker)	m^3/kg	Equation S16 in Appendix C, Table A using PEF (industrial/commercial)	$1.24 \bullet 10^8$ or Site-Specific
Q/C (used in VF equations)	Inverse of the mean concentration at the center of a square source	$(g/m^2\text{-}s)/(kg/m^3)$	Appendix C, Table H	Residential = 68.81 Industrial/Commercial = 85.81 Construction Worker = 85.81
Q/C (used in PEF equations)	Inverse of the mean concentration at the center of a square source	$(g/m^2\text{-}s)/(kg/m^3)$	SSL or Appendix C, Table H	Residential = 90.80 Industrial/Commercial = 85.81 Construction Worker = 85.81
RfC	Inhalation Reference Concentration	mg/m^3	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological-Specific (Note: for Construction Workers use subchronic reference concentrations)
RfD_o	Oral Reference Dose	mg/(kg-d)	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological-Specific (Note: for Construction Worker use subchronic reference doses)
RO_{soil}	Soil remediation objective	mg/kg	Equation S30 in Appendix C, Table A	Calculated value

Symbol	Parameter	Units	Source	Parameter Value(s)
$RO_{soil\ gas}$	Soil gas remediation objective	mg/m^3	Equation S30 in Appendix C, Table A	Calculated value
S	Solubility in Water	mg/L	Appendix C, Table E	Chemical-Specific
SF_o	Oral Slope Factor	$(mg/kg\text{-}d)^{-1}$	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological-Specific
T	Exposure Interval	s		Residential = $9.5 \bullet 10^8$ Industrial/Commercial = $7.9 \bullet 10^8$ Construction Worker = $3.6 \bullet 10^6$
$T_{M\text{-}L}$	Exposure Interval for Mass-Limit Volatilization Factor Equation S26	yr	SSL	30
THQ	Target Hazard Quotient	unitless	SSL	1
TR	Target Cancer Risk	unitless		Residential = 10^{-6} at the point of human exposure Industrial/Commercial = 10^{-6} at the point of human exposure Construction Worker = 10^{-6} at the point of human exposure
U_m	Mean Annual Windspeed	m/s	SSL	4.69

Symbol	Parameter	Units	Source	Parameter Value(s)
URF	Inhalation Unit Risk Factor	$(ug/m^3)^{-1}$	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological-Specific
U_t	Equivalent Threshold Value of Windspeed at 7 m	m/s	SSL	11.32
V	Fraction of Vegetative Cover	unitless	SSL or Field Measurement	0.5 or Site-Specific
VF	Volatilization Factor	m^3/kg	Equation S8 in Appendix C, Table A	Calculated Value
VF′	Volatilization Factor adjusted for Agitation	m^3/kg	Equation S9 in Appendix C, Table A	Calculated Value
VF_{M-L}	Mass-Limit Volatilization Factor	m^3/kg	Equation S26 in Appendix C, Table A	Calculated Value
VF'_{M-L}	Mass-Limit Volatilization Factor adjusted for Agitation	m^3/kg	Equation S27 in Appendix C, Table A	Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
η	Total Soil Porosity	L_{pore}/L_{soil}	SSL or Equation S24 in Appendix C, Table A	0.43, or Gravel = 0.25 Sand = 0.32 Silt = 0.40 Clay = 0.36, or Calculated Value
θ_a	Air-Filled Soil Porosity	L_{air}/L_{soil}	SSL or Equation S21 in Appendix C, Table A	Surface Soil (top 1 meter) = 0.28 Subsurface Soil (below 1 meter) = 0.13, or Gravel = 0.05 Sand = 0.14 Silt - 0.24 Clay = 0.19, or Calculated Value
θ_w	Water-Filled Soil Porosity	L_{water}/L_{soil}	SSL or Equation S20 in Appendix C, Table A	Surface Soil (top 1 meter) = 0.15 Subsurface Soil (below 1 meter) = 0.30, or Gravel = 0.20 Sand = 0.18 Silt = 0.16 Clay = 0.17, or Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
ρ_b	Dry Soil Bulk Density	kg/L or g/cm^3	SSL or Field Measurement (See Appendix C, Table F)	1.5, or Gravel = 2.0 Sand = 1.8 Silt = 1.6 Clay = 1.7, or Site-Specific
ρ_s	Soil Particle Density	g/cm^3	SSL or Field Measurement (See Appendix C, Table F)	2.65, or Site-Specific
ρ_w	Water Density	g/cm^3	SSL	1
1/(2b+3)	Exponential in Equation S20	unitless	Appendix C, Table K Appendix C, Illustration C	Site-Specific

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

Section 742.Table C RBCA Equations

Equations for the combined exposures routes of soil ingestion inhalation of vapors and particulates, and dermal contact with soil	Remediation Objectives for Carcinogenic Contaminants (mg/kg)	$$\frac{TR \bullet BW \bullet AT_C \bullet 365\frac{d}{yr}}{EF \bullet ED \bullet \left\{\left[SF_o \bullet 10^{-6}\frac{kg}{mg} \bullet \left(\left(IR_{soil} \bullet RAF_o\right)+\left(SA \bullet M \bullet RAF_d\right)\right)\right]+\left[SF_i \bullet IR_{air} \bullet \left(VF_{ss}+VF_p\right)\right]\right\}}$$	**R1**
	Remediation Objectives for Non-carcinogenic Contaminants (mg/kg)	$$\frac{THQ \bullet BW \bullet AT_n \bullet 365\frac{d}{yr}}{EF \bullet ED \bullet \left[\frac{10^{-6}\frac{kg}{mg}\left[\left(IR_{soil} \bullet RAF_o\right)+\left(SA \bullet M \bullet RAF_d\right)\right]}{RfD_o}+\frac{IR_{air} \bullet \left(VF_{ss}+VF_p\right)}{RfD_i}\right]}$$	**R2**
	Volatilization Factor for Surficial Soils, VF_{ss} (kg/m^3) Whichever is less between R3 and R4	$$VF_{ss}=\frac{2 \bullet W \bullet \rho_s \bullet 10^3\frac{cm^3 \cdot kg}{m^3 \cdot g}}{U_{air} \bullet \delta_{air}} \bullet \sqrt{\frac{D_s^{eff} \bullet H'}{\pi \bullet \left[\theta_{ws}+\left(k_s \bullet \rho_s\right)+\left(H' \bullet \theta_{as}\right)\right] \bullet \tau}}$$	**R3**
		$$VF_{ss}=\frac{W \bullet \rho_s \bullet d \bullet 10^3\frac{cm^3 \cdot kg}{m^3 \cdot g}}{U_{air} \bullet \delta_{air} \bullet \tau}$$	**R4**

	Volatilization Factor for Surficial Soils Regarding Particulates, VF_p (kg/m^3)	$$VF_p = \frac{P_e \bullet W \bullet 10^3 \frac{cm^3 \cdot kg}{m^3 \cdot g}}{U_{air} \bullet \delta_{air}}$$	**R5**
	Effective Diffusion Coefficient in Soil Based on Vapor-Phase Concentration D_s^{eff} (cm^2/s)	$$D_s^{eff} = \frac{D^{air} \bullet \theta_{as}^{3.33}}{\theta_T^2} + \frac{D^{water} \bullet \theta_{ws}^{3.33}}{H' \bullet \theta_T^2}$$	**R6**
Equations for the ambient vapor inhalation (outdoor) route fromsubsurface soils	Remediation Objectives for Carcinogenic Contaminants (mg/kg)	$$\frac{RBSL_{air} \bullet 10^{-3}}{VF_{samb}}$$	**R7**
	Remediation Objectives for Non-carcinogenic Contaminants (mg/kg)	$$\frac{RBSL_{air} \bullet 10^{-3}}{VF_{samb}}$$	**R8**

	Carcinogenic Risk-Based Screening Level for Air, $RBSL_{air}$ (ug/m^3)	$$RBSL_{air} = \frac{TR \bullet BW \bullet AT_c \bullet 365 \frac{d}{yr} \bullet 10^3 \frac{ug}{mg}}{SF_i \bullet IR_{air} \bullet EF \bullet ED}$$	**R9**
	Noncarcinogenic Risk-Based Screening Level for Air, $RBSL_{air}$ (ug/m^3)	$$RBSL_{air} = \frac{THQ \bullet RfD_i \bullet BW \bullet AT_n \bullet 365 \frac{d}{yr} \bullet 10^3 \frac{ug}{mg}}{IR_{air} \bullet EF \bullet ED}$$	**R10**
	Volatilization Factor - Subsurface Soil to Ambient Air, VF_{samb} $(mg/m^3)/(mg/kg_{soil})$	$$VF_{samb} = \frac{H' \bullet \rho_s \bullet 10^3 \frac{cm^3 \cdot kg}{m^3 \cdot g}}{\left[\theta_{ws} + \left(k_s \bullet \rho_s\right) + \left(H' \bullet \theta_{as}\right)\right] \bullet \left[1 + \frac{\left(U_{air} \bullet \delta_{air} \bullet L_s\right)}{\left(D_s^{eff} \bullet W\right)}\right]}$$	**R11**

Equations for the Soil Component of the Groundwater Ingestion Exposure Route	Remediation Objective (mg/kg)	$\frac{GW_{source}}{LF_{sw}}$ NOTE: This equation can only be used to model contaminant migration not in the water bearing unit.	**R12**
	Groundwater at the source, GW_{source} (mg/L)	$GW_{source} = \frac{GW_{comp}}{C_{(x)}/C_{source}}$	**R13**
	Leaching Factor, LF_{sw} $(mg/L_{water})/(mg/kg_{soil})$	$LF_{sw} = \frac{\rho_s \bullet \frac{cm^3 \cdot kg}{L \cdot g}}{\left[\theta_{ws} + (k_s \bullet \rho_s) + (H' \bullet \theta_{as})\right] \bullet \left[1 + \frac{(U_{gw} \bullet \delta_{gw})}{(I \bullet W)}\right]}$	**R14**
	Steady-State Attenuation Along the Centerline of a Dissolved Plume, $C_{(x)}/C_{source}$	$C_{(x)}/C_{source} = \exp\left[\left(\frac{X}{2\alpha_x}\right) \bullet \left(1 - \sqrt{1 + \frac{4\lambda \bullet \alpha_x}{U}}\right)\right] \bullet erf\left[\frac{S_w}{4 \bullet \sqrt{\alpha_y \bullet X}}\right] \bullet erf\left[\frac{S_d}{2 \bullet \sqrt{\alpha_z \bullet X}}\right]$ NOTE: 1. This equation does not predict the contaminant flow within bedrock and may not accurately predict downgradient concentrations in the presence of a confining layer. 2. If the value of the First Order Degradation Constant (λ) is not readily available, then set $\lambda = 0$.	**R15**
	Longitudinal Dispersivity, α_x (cm)	$\alpha_x = 0.10 \bullet X$	**R16**

Transverse Dispersivity, α_y (cm)	$\alpha_y = \frac{\alpha_x}{3}$	**R17**
Vertical Dispersivity, α_z (cm)	$\alpha_z = \frac{\alpha_x}{20}$	**R18**
Specific Discharge, U (cm/d)	$U = \frac{K \bullet i}{\theta_T}$	**R19**
Soil-Water Sorption Coefficient, k_s	$k_s = K_{oc} \bullet f_{oc}$	**R20**
Volumetric Air Content in Vadose Zone Soils, θ_{as} (cm^3_{air}/cm^3_{soil})	$\theta_{as} = \theta_T - \frac{(w \bullet \rho_s)}{\rho_w}$	**R21**
Volumetric Water Content in Vadose Zone Soils, θ_{ws} (cm^3_{water}/cm^3_{soil})	$\theta_{ws} = \frac{w \bullet \rho_s}{\rho_w}$	**R22**
Total Soil Porosity, θ_T (cm^3/cm^3_{soil})	$\theta_T = \theta_{as} + \theta_{ws}$	**R23**

	Groundwater Darcy Velocity, U_{gw} (cm/yr)	$U_{gw} = K \bullet i$	**R24**
Equations for the Groundwater Ingestion Exposure Route	Remediation Objective for Carcinogenic Contaminants (mg/L)	$\frac{TR \bullet BW \bullet AT_r \bullet 365 \frac{d}{yr}}{SF_o \bullet IR_w \bullet EF \bullet ED}$	**R25**
	Dissolved Hydrocarbon Concentration along Centerline, $C_{(x)}$ (mg/L_{water})	$C_{(x)} = C_{source} \bullet \exp\left[\left(\frac{X}{2\alpha_x}\right) \bullet \left(1 - \sqrt{1 + \frac{4\lambda \bullet \alpha_x}{U}}\right)\right] \bullet erf\left[\frac{S_w}{4 \bullet \sqrt{\alpha_y \bullet X}}\right] \bullet erf\left[\frac{S_d}{2 \bullet \sqrt{\alpha_z \bullet X}}\right]$ NOTE: 1. This equation does not predict the contaminant flow within bedrock and may not accurately predict downgradient concentrations in the presence of a confining layer. 2. If the value of the First Order Degradation Constant (λ) is not readily available, then set $\lambda = 0$.	**R26**

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

Section 742.Table D RBCA Parameters

Symbol	Parameter	Units	Source	Parameter Value(s)
AT_c	Averaging Time for Carcinogens	yr	RBCA	70
AT_n	Averaging Time for Noncarcinogens	yr	RBCA	Residential = 30 Industrial/Commercial = 25 Construction Worker = 0.115
BW	Adult Body Weight	kg	RBCA	70
C_{source}	The greatest potential concentration of the contaminant of concern in the groundwater at the source of the contamination, based on the concentrations of contaminants in groundwater due to the release and the projected concentration of the contaminant migrating from the soil to the groundwater.	mg/L	Field Measurement	Site-Specific
$C_{(x)}$	Concentration of Contaminant in Groundwater at Distance X from the source	mg/L	Equation R26 in Appendix C, Table C	Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
$C_{(x)}/C_{source}$	Steady-State Attenuation Along the Centerline of a Dissolved Plume	unitless	Equation R15 in Appendix C, Table C	Calculated Value
d	Lower Depth of Surficial Soil Zone	cm	Field Measurement	100 or Site-Specific (not to exceed 100)
D^{air}	Diffusion Coefficient in Air	cm^2/s	Appendix C, Table E	Chemical-Specific
D^{water}	Diffusion Coefficient in Water	cm^2/s	Appendix C, Table E	Chemical-Specific
D_s^{eff}	Effective Diffusion Coefficient in Soil Based on Vapor-Phase Concentration	cm^2/s	Equation R6 in Appendix C, Table C	Calculated Value
ED	Exposure Duration	yr	RBCA	Residential = 30 Industrial/Commercial = 25 Construction Worker = 1
EF	Exposure Frequency	d/yr	RBCA	Residential = 350 Industrial/Commercial = 250 Construction Worker = 30
erf	Error Function	unitless	Appendix C, Table G	Mathematical Function

Symbol	Parameter	Units	Source	Parameter Value(s)
f_{oc}	Organic Carbon Content of Soil	g/g	RBCA or Field Measurement (See Appendix C, Table F)	Surface Soil = 0.006 Subsurface Soil = 0.002 or Site-Specific
GW_{comp}	Groundwater Objective at the Compliance Point	mg/L	Appendix B, Table E, 35 IAC 620.Subpart F, or Equation R25 in Appendix C, Table C	Site-Specific
GW_{source}	Groundwater Concentration at the Source	mg/L	Equation R13 in Appendix C, Table C	Calculated Value
H'	Henry's Law Constant	cm^3_{water}/cm^3_{air}	Appendix C, Table E	Chemical-Specific
i	Hydraulic Gradient	cm/cm (unitless)	Field Measurement (See Appendix C, Table F)	Site-Specific
I	Infiltration Rate	cm/yr	RBCA	30
IR_{air}	Daily Outdoor Inhalation Rate	m^3/d	RBCA	20
IR_{soil}	Soil Ingestion Rate	mg/d	RBCA	Residential = 100 Industrial/Commercial = 50 Construction Worker = 480
IR_w	Daily Water Ingestion Rate	L/d	RBCA	Residential = 2 Industrial/Commercial = 1

Symbol	Parameter	Units	Source	Parameter Value(s)
K	Aquifer Hydraulic Conductivity	cm/d for Equations R15, R19 and R26 cm/yr for Equation R24	Field Measurement (See Appendix C, Table F)	Site-Specific
K_{oc}	Organic Carbon Partition Coefficient	cm^3/g or L/kg	Appendix C, Table E or Appendix C, Table I	Chemical-Specific
k_s (non-ionizing organics)	Soil Water Sorption Coefficient	cm^3_{water}/g_{soil}	Equation R20 in Appendix C,Table C	Calculated Value
k_s (ionizing organics)	Soil Water Sorption Coefficient	cm^3_{water}/g_{soil}	Equation R20 in Appendix C, Table C	Chemical and pH-Specific (See Appendix C, Table I)
k_s (inorganics)	Soil Water Sorption Coefficient	cm^3_{water}/g_{soil}	Appendix C, Table J	Chemical and pH-Specific
L_s	Depth to Subsurface Soil Sources	cm	RBCA	100
LF_{sw}	Leaching Factor	$(mg/L_{water})/(mg/kg_{soil})$	Equation R14 in Appendix C, Table C	Calculated Value
M	Soil to Skin Adherence Factor	mg/cm^2	RBCA	0.5

Symbol	Parameter	Units	Source	Parameter Value(s)
Pe	Particulate Emission Rate	g/cm^2-s	RBCA	$6.9 \bullet 10^{-14}$
RAF_d	Dermal Relative Absorption Factor	unitless	RBCA	0.5
RAF_d (PNAs)	Dermal Relative Absorption Factor	unitless	RBCA	0.05
RAF_d (inorganics)	Dermal Relative Absorption Factor	unitless	RBCA	0
RAF_o	Oral Relative Absorption Factor	unitless	RBCA	1.0
$RBSL_{air}$	Carcinogenic Risk-Based Screening Level for Air	ug/m^3	Equation R9 in Appendix C, Table C	Chemical-, Media-, and Exposure Route-Specific
$RBSL_{air}$	Noncarcinogenic Risk-Based Screening Level for Air	ug/m^3	Equations R10 in Appendix C, Table C	Chemical-, Media-, and Exposure Route-Specific
RfD_i	Inhalation Reference Dose	mg/kg-d	IEPA (IRIS/HEAST[a])	Toxicological-Specific
RfD_o	Oral Reference Dose	mg/(kg-d)	IEPA (IRIS/HEAST[a])	Toxicological-Specific (Note: for Construction Worker use subchronic reference doses)
SA	Skin Surface Area	cm^2/d	RBCA	3,160

Symbol	Parameter	Units	Source	Parameter Value(s)
S_d	Source Width Perpendicular to Groundwater Flow Direction in Vertical Plane	cm	Field Measurement	For Migration to Groundwater Route: Use 200 or Site-Specific For Groundwater remediation objective: Use Site-Specific
S_w	Source Width Perpendicular to Groundwater Flow Direction in Horizontal Plane	cm	Field Measurement	Site-Specific
SF_i	Inhalation Cancer Slope Factor	$(mg/kg\text{-}d)^{-1}$	IEPA (IRIS/HEAST[a])	Toxicological-Specific
SF_o	Oral Slope Factor	$(mg/kg\text{-}d)^{-1}$	IEPA (IRIS/HEAST[a])	Toxicological-Specific
THQ	Target Hazard Quotient	unitless	RBCA	1
TR	Target Cancer Risk	unitless	RBCA	Residential = 10^{-6} at the point of human exposure Industrial/Commercial = 10^{-6} at the point of human exposure Construction Worker = 10^{-6} at the point of human exposure
U	Specific Discharge	cm/d	Equation R19 in Appendix C, Table C	Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
U_{air}	Average Wind Speed Above Ground Surface in Ambient Mixing Zone	cm/s	RBCA	225
U_{gw}	Groundwater Darcy Velocity	cm/yr	Equation R24 in Appendix C, Table C	Calculated Value
VF_p	Volatilization Factor for Surficial Soils Regarding Particulates	kg/m^3	Equation R5 in Appendix C, Table C	Calculated Value
VF_{samb}	Volatilization Factor (Subsurface Soils to Ambient Air)	$(mg/m^3_{air})/(mg/kg_{soil})$ or kg/m^3	Equation R11 in Appendix C, Table C	Calculated Value
VF_{ss}	Volatilization Factor for Surficial Soils	kg/m^3	Use Equations R3 and R4 in Appendix C, Table C	Calculated Value from Equation R3 or R4 (whichever is less)
W	Width of Source Area Parallel to Direction to Wind or Groundwater Movement	cm	Field Measurement	Site-Specific

Symbol	Parameter	Units	Source	Parameter Value(s)
w	Average Soil Moisture Content	g_{water}/g_{soil}	RBCA or Field Measurement (See Appendix C, Table F)	0.1, or Surface Soil (top 1 meter) = 0.1 Subsurface Soil (below 1 meter) = 0.2, or Site-Specific
X	Distance along the Centerline of the Groundwater Plume Emanating from a Source. The x direction is the direction of groundwater flow	cm	Field Measurement	Site-Specific
α_x	Longitudinal Dispersitivity	cm	Equation R16 in Appendix C, Table C	Calculated Value
α_y	Transverse Dispersitivity	cm	Equation R17 in Appendix C, Table C	Calculated Value
α_z	Vertical Dispersitivity	cm	Equation R18 in Appendix C, Table C	Calculated Value
δ_{air}	Ambient Air Mixing Zone Height	cm	RBCA	200

Symbol	Parameter	Units	Source	Parameter Value(s)
δ_{gw}	Groundwater Mixing Zone Thickness	cm	RBCA	200
θ_{as}	Volumetric Air Content in Vadose Zone Soils	cm^3_{air}/cm^3_{soil}	RBCA or Equation R21 in Appendix C, Table C	Surface Soil (top 1 meter) = 0.28 Subsurface Soil (below 1 meter)= 0.13, Or Gravel = 0.05 Sand = 0.14 Silt = 0.16 Clay = 0.17, or Calculated Value
θ_{ws}	Volumetric Water Content in Vadose Zone Soils	cm^3_{water}/cm^3_{soil}	RBCA or Equation R22 in Appendix C, Table C	Surface Soil (top 1 meter) = 0.15 Subsurface Soil (below 1 meter) = 0.30, or Gravel = 0.20 Sand = 0.18 Silt = 0.16 Clay = 0.17, or Calculated Value

Symbol	Parameter	Units	Source	Parameter Value(s)
θ_T	Total Soil Porosity	cm^3/cm^3_{soil}	RBCA or Equation R23 in Appendix C, Table C	0.43, or Gravel = 0.25 Sand = 0.32 Silt = 0.40 Clay = 0.36, or Calculated Value
λ	First Order Degradation Constant	d^{-1}	Appendix C, Table E	Chemical-Specific
π	pi			3.1416
ρ_b	Soil Bulk Density	g/cm^3	RBCA or Field Measurement (See Appendix C, Table F)	1.5, or Gravel = 2.0 Sand = 1.8 Silt = 1.6 Clay = 1.7, or Site-Specific
ρ_w	Water Density	g/cm^3	RBCA	1
τ	Averaging Time for Vapor Flux	s	RBCA	$9.46 \bullet 10^8$

[a] HEAST = Health Effects Assessment Summary Tables. USEPA, Office of Solid Waste and Emergency Response. EPA/540/R-95/036. Updated Quarterly.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C: Tier 2 Illustrations and Tables

Section 742.Table E: Default Physical and Chemical Parameters[e]

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
Neutral Organics									
83-32-9	Acenaphthene	3.60E+00	4.76E-02	7.69E-06	6.60E-03	-------[b]	6.30E+03	3.40E-03	2.50E-03
67-64-1	Acetone	1.00E+06	1.24E-01	1.14E-05	1.60E-03	9.73E-04	7.80E-01	4.95E-02	2.30E+02
15972-60-8	Alachlor	2.40E+02	2.13E-02	5.28E-06	3.40E-06	-------[b]	3.20E+03	No Data	2.20E-05
116-06-3	Aldicarb	6.03E+03	3.18E-02	7.24E-06	5.90E-08	-------[b]	1.29E+01	1.09E-03	3.47E-05
309-00-2	Aldrin	1.70E-02	1.96E-02	4.86E-06	7.00E-03	-------[b]	2.50E+05	5.90E-04	6.00E-06
120-12-7	Anthracene	4.30E-02	3.85E-02	7.74E-06	2.70E-03	-------[b]	2.50E+04	7.50E-04	2.70E-06
1912-24-9	Atrazine	7.00E+01	2.59E-02	6.67E-06	9.68E-08	-------[b]	3.63E+02	No Data	2.70E-07
71-43-2	Benzene	1.80E+03	8.80E-02	1.02E-05	2.30E-01	1.34E-01	5.00E+01	9.00E-04	9.50E+01
56-55-3	Benzo(a) anthracene	9.40E-03	5.10E-02	9.00E-06	1.39E-04	-------[b]	4.00E+05	5.10E-04	1.10E-07
205-99-2	Benzo(b) fluoranthene	1.50E-03	2.23E-02	5.56E-06	4.55E-03	-------[b]	1.05E+06	5.70E-04	5.00E-07
207-08-9	Benzo(k) fluoranthene	8.00E-04	2.23E-02	5.56E-06	3.40E-05	-------[b]	1.00E+06	1.60E-04	2.00E-09

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
65-85-0	Benzoic Acid	3.40E+03	7.02E-02	7.97E-06	1.56E-06	-------[b]	1.21E+00[d]	No Data	7.00E-04
50-32-8	Benzo(a)pyrene	1.60E-03	4.30E-02	9.49E-06	4.50E-05	-------[b]	7.90E+05	6.50E-04	5.50E-09
111-44-4	Bis(2-chloroethyl)ether	1.72E+04	4.13E-02	7.53E-06	7.40E-04	2.94E-04	1.26E+01	1.90E-03	1.55E+00
117-81-7	Bis(2-ethylhexyl) phthalate	3.40E-01	3.51E-02	3.66E-06	4.10E-06	-------[b]	1.00E+05	1.80E-03	6.80E-08
75-27-4	Bromodichloro-methane	6.70E+03	5.61E-02	1.06E-05	6.60E-02	3.71E-02	5.00E+01	No Data	5.00E+01
75-25-2	Bromoform	3.10E+03	1.49E-02	1.03E-05	2.19E-02	1.06E-02	9.12E+01	1.90E-03	5.51E+00
71-36-3	Butanol	7.40E+04	8.00E-02	9.30E-06	3.61E-04	1.55E-04	6.00E+00	1.28E-02	7.00E+00
78-93-3	2-Butanone (MEK)	2.20E+05	8.08E-02	9.8E-06	2.30E-03	1.32E-03	2.00E+00	4.95E-02	9.50E+01
85-68-7	Butyl Benzyl Phthalate	2.70E+00	1.99E-02	4.89E-06	5.30E-05	-------[b]	6.30E+04	3.85E-03	8.30E-06
86-74-8	Carbazole	1.20E+00	4.17E-02	7.45E-06	3.60E-06	-------[b]	4.00E+03	No Data	7.00E-04
1563-66-2	Carbofuran	3.20E+02	2.37E-02	5.95E-06	1.27E-07	-------[b]	1.91E+02	No Data	4.85E-06
75-15-0	Carbon Disulfide	1.20E+03	1.04E-01	1.00E-05	1.23E+00	8.06E-01	6.30E+01	No Data	3.60E+02
56-23-5	Carbon Tetrachloride	7.90E+02	7.80E-02	8.80E-06	1.23E+00	7.48E-01	2.00E+02	1.90E-03	1.20E+02
57-74-9	Chlordane	5.60E-02	1.79E-02	4.37E-06	2.00E-03	-------[b]	2.50E+05	2.50E-04	9.80E-06

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
106-47-8	p-Chloroaniline	5.30E+03	6.99E-02	1.01E-05	4.76E-05	-------[b]	6.31E+01	No Data	1.23E-02
108-90-7	Chlorobenzene	4.70E+02	7.30E-02	8.70E-06	1.50E-01	7.93E-02	2.00E+02	2.30E-03	1.20E+01
124-48-1	Chlorodibromo-methane	2.60E+03	3.66E-02	1.05E-05	3.20E-02	2.07E-02	6.92E+01	3.85E-03	4.90E+00
67-66-3	Chloroform	7.90E+03	1.04E-01	1.00E-05	1.50E-01	9.18E-02	5.00E+01	3.90E-04	2.00E+02
95-57-8	2-Chlorophenol	2.20E+04	6.61E-02	9.46E-06	1.60E-02	7.28E-03	5.93E+01[d]	No Data	2.34E+00
218-01-9	Chrysene	6.30E-03	2.44E-02	6.21E-06	3.90E-03	-------[b]	4.00E+05	3.50E-04	6.20E-09
94-75-7	2,4-D	6.77E+02	5.88E-02	6.49E-06	4.18E-07	-------[b]	5.75E+02	3.85E-03	6.00E-07
72-54-8	4,4'-DDD	9.00E-02	2.27E-02	5.79E-06	1.60E-04	-------[b]	7.90E+05	6.20E-05	6.70E-07
72-55-9	4,4'-DDE	1.20E-01	2.38E-02	5.87E-06	8.60E-04	-------[b]	4.00E+05	6.20E-05	6.00E-06
50-29-3	4,4'-DDT	2.50E-02	1.99E-02	4.95E-06	3.30E-04	-------[b]	2.00E+06	6.20E-05	1.60E-07
75-99-0	Dalapon	9.00E+05	6.08E-02	9.45E-06	2.64E-06	NA	4.80E+00	5.78E-03	1.90E-01
53-70-3	Dibenzo(a,h) anthracene	2.50E-03	2.11E-02	5.24E-06	6.10E-07	-------[b]	2.50E+06	3.70E-04	1.00E-10
96-12-8	1,2-Dibromo-3-chloropropane	1.20E+03	2.68E-02	7.02E-06	6.20E-03[c]	NA	7.90E+01	1.93E-03	5.80E-01
106-93-4	1,2-Dibromoethane	4.00E+03	4.37E-02	8.44E-06	3.00E-02	1.54E-02	5.00E+01	5.78E-03	1.30E+01

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
84-74-2	Di-n-butyl Phthalate	1.10E+01	4.38E-02	7.86E-06	7.40E-05	-------[a]	4.00E+04	3.01E-02	7.30E-05
1918-00-9	Dicamba	4.50E+03	2.37E-02	5.95E-06	2.18E-09	-------[a]	2.95E+00	No Data	3.38E-05
95-50-1	1,2-Dichlorobenzene	1.56E+02	6.90E-02	7.90E-06	7.79E-02	3.56E-02	5.75E+02	1.90E-03	1.36E+00
106-46-7	1,4-Dichlorobenzene	7.90E+01	6.90E-02	7.90E-06	9.80E-02	4.69E-02	7.90E+02	1.90E-03	1.00E+00
91-94-1	3,3-Dichloro-benzidine	3.10E+00	2.59E-02	6.74E-06	1.60E-07	-------[a]	2.82E+03	1.90E-03	3.71E-08
75-71-8	Dichlorodifluoro-methane	2.80E+02	7.60E-02	1.08E-05	1.41E+01	8.14E+00	6.17E+01	1.92E-03	4.85E+03
75-34-3	1,1-Dichloroethane	5.10E+03	7.42E-02	1.05E-05	2.30E-01	1.42E-01	3.20E+01	1.90E-03	2.30E+02
107-06-2	1,2-Dichloroethane	8.50E+03	1.04E-02	9.90E-06	4.00E-02	2.29E-02	2.00E+01	1.90E-03	7.90E+01
75-35-4	1,1-Dichloroethylene	2.30E+03	9.00E-02	1.04E-05	1.10E+00	7.10E-01	5.00E+01	5.30E-03	6.00E+02
156-59-2	*cis*-1,2-Dichloroethylene	3.50E+03	8.86E-02	1.13E-05	1.70E-01	1.00E-01	4.00E+01	2.40E-04	2.00E+02
156-60-5	*trans*-1,2-Dichloroethylene	6.30E+03	7.03E-02	1.19E-05	3.90E-01	2.43E-01	5.00E+01	2.40E-04	3.30E+02
120-83-2	2,4-Dichlorophenol	4.50E+03	4.89E-02	8.77E-06	1.30E-04	-------[a]	7.32E+02[d]	2.70E-04	6.70E-02

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
78-87-5	1,2-Dichloropropane	2.80E+03	7.82E-02	8.73E-06	1.10E-01	6.52E-02	5.00E+01	2.70E-04	5.20E+01
542-75-6	1,3-Dichloro-propylene (*cis + trans*)	2.80E+03	6.26E-02	1.00E-05	7.40E-01	3.98E-01	2.00E+01	6.10E-02	3.40E+01
60-57-1	Dieldrin	2.00E-01	1.92E-02	4.74E-06	6.2E-04	-------[a]	2.50E+04	3.20E-04	5.9E-06
84-66-2	Diethyl Phthalate	1.10E+03	2.49E-02	6.35E-06	1.80E-05	-------[a]	3.20E+02	6.19E-03	1.60E-03
105-67-9	2,4-Dimethylphenol	7.90E+03	6.43E-02	8.69E-06	8.20E-05	-------[a]	2.00E+02	4.95E-02	9.80E-02
75-71-8	1,3-Dinitrobenzene	8.60E+02	4.55E-02	8.46E-06	2.30E-07	-------[a]	3.20E+01	1.92E-03	9.00E-04
51-28-5	2,4-Dinitrophenol	2.79E+03	2.73E-02	9.06E-06	1.82E-05	-------[a]	3.24E+01	1.32E-03	5.10E-03
121-14-2	2,4-Dinitrotoluene	2.70E+02	2.03E-01	7.06E-06	3.80E-06	-------[a]	8.90E+01	1.92E-03	1.47E-04
606-20-2	2,6-Dinitrotoluene	1.82E+02	3.70E-02	7.76E-06	3.06E-05	-------[a]	4.90E+01	1.92E-03	5.67E-04
88-85-7	Dinoseb	5.20E+01	2.45E-02	6.25E-06	1.87E-05	-------[a]	9.17E+01[d]	2.82E-03	7.50E-05
117-84-0	Di-n-octyl Phthalate	2.00E-02	1.73E-02	4.17E-06	2.74E-03	-------[a]	1.30E+05	1.90E-03	2.60E-06
123-91-1	p-Dioxane	1.00E+06	2.29E-01	1.02E-05	1.97E-04	1.07E-04	7.20E-01	1.92E-03	3.81E+01
115-29-7	Endosulfan	5.10E-01	1.85E-02	4.55E-06	4.51E-04	-------[a]	5.00E+03	7.63E-02	1.00E-05

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
145-73-3	Endothall	2.10E+04	2.91E-02	8.07E-06	1.58E-14	-------[a]	7.59E+01	No Data	1.57E-10
72-20-8	Endrin	2.50E-01	1.92E-02	4.74E-6	3.08E-04	-------[a]	3.20E+04	3.20E-04	3.00E-06
100-41-4	Ethylbenzene	1.70E+02	7.50E-02	7.80E-06	3.24E-01	1.64E-01	3.20E+02	3.00E-03	9.60E+00
206-44-0	Fluoranthene	2.06E-01	2.51E-02	6.35E-06	6.60E-04	-------[a]	7.40E+04	1.90E-04	1.23E-08
86-73-7	Fluorene	2.00E+00	4.40E-02	7.88E-06	2.62E-03	-------[a]	1.30E+04	6.91E-04	6.30E-04
76-44-8	Heptachlor	1.80E-01	2.23E-02	5.69E-06	6.07E-02	1.73E-02	3.00E+03	1.30E-01	4.00E-04
1024-57-3	Heptachlor epoxide	2.00E-01	2.19E-02	5.57E-06	3.90E-04	-------[a]	2.00E+05	6.30E-04	1.90E-05
118-74-1	Hexachloro-benzene	6.20E-03	5.42E-02	5.91E-06	5.33E-02	1.35E-02	2.00E+04	1.70E-04	1.80E-05
319-84-6	Alpha-HCH (alpha-BHC)	2.00E+00	2.04E-02	5.04E-06	4.51E-04	-------[a]	5.00E+03	2.50E-03	4.50E-05
58-89-9	Gamma-HCH (Lindane)	7.30E+00	2.75E-02	7.34E-06	5.74E-04	-------[a]	3.00E+03	2.90E-03	4.10E-04
2691-41-0	High Melting Explosive, Octogen (HMX)	5.00E+00	2.69E-02	7.15E-06	8.67E-10	3.55E-08	1.40E+00	No Data	3.30E-14
77-47-4	Hexachlorocyclo-Pentadiene	1.80E+00	2.79E-02	7.21E-06	1.11E+00	4.22E-01	1.20E+04	1.20E-02	5.96E-02
67-72-1	Hexachloroethane	5.00E+01	2.50E-03	6.80E-06	1.59E-01	7.26E-02	1.50E+03	1.92E-03	2.10E-01

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
193-39-5	Indeno(1,2,3-c,d)pyrene	2.20E-05	2.25E-02	5.66E-06	6.56E-05	-------[a]	3.10E+06	4.70E-04	1.00E-10
78-59-1	Isophorone	1.20E+04	6.23E-02	6.76E-06	2.72E-04	1.12E-04	2.50E+01	1.24E-02	4.38E-01
98-82-8	Isopropylbenzene (Cumene)	6.10E+01	6.50E-02	7.10E-06	4.92E+01	2.10E+01	1.02E+03	4.33E-02	4.50E+00
93-65-2	Mecoprop (MCPP)	8.95E+02	2.40E-02	6.05E-06	7.70E-09	-------[a]	1.84E+01[d]	3.85E-03	2.44E-05
7439-97-6	Mercury	6.00E-02	7.14E-02	3.01E-05	4.51E-01	1.59E-01	8.70E+03	No Data	2.00E-03
72-43-5	Methoxychlor	4.50E-02	1.84E-02	4.46E-06	6.56E-04	-------[a]	5.00E+04	1.90E-03	6.00E-07
74-83-9	Methyl Bromide	1.50E+04	7.28E-02	1.21E-05	2.56E-01	1.79E-01	1.00E+01	1.82E-02	1.62E+03
1634-04-4	Methyl tertiary-butyl ether	5.10E+04	8.59E-02	1.10E-05	2.42E-02	1.50E-02	1.00E+01	No Data	2.50E+02
75-09-2	Methylene Chloride	1.30E+04	1.01E-01	1.17E-05	9.02E-02	5.70E-02	1.30E+01	1.20E-02	4.30E+02
93-65-2	2-Methyl-naphthalene	2.50E+01	5.22E-02	7.75E-06	2.10E-02	6.95E-03	1.60E+03	No Data	6.80E-02
95-48-7	2-Methylphenol (o-cresol)	2.60E+04	7.40E-02	8.30E-06	4.92E-05	2.00E-05	4.20E+01	4.95E-02	2.99E-01
91-20-3	Naphthalene	3.10E+01	5.90E-02	7.50E-06	1.97E-02	8.29E-03	5.00E+02	2.70E-03	8.50E-02
98-95-3	Nitrobenzene	2.09E+03	7.60E-02	8.60E-06	9.84E-04	3.99E-04	4.00E+01	1.76E-03	2.40E-01

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
86-30-6	N-Nitrosodiphenyl-amine	3.50E+01	2.83E-02	7.19E-06	2.10E-04	-------[a]	1.00E+03	1.00E-02	6.70E-04
621-64-7	N-Nitrosodi-n-propylamine	9.89E+03	5.87E-02	8.17E-06	9.20E-05	5.48E-05	1.45E+01	1.90E-03	1.30E-01
87-86-5	Pentachloro-phenol	2.00E+03	5.60E-02	6.10E-06	9.84E-07	-------[a]	2.77E+03[d]	4.50E-04	3.20E-05
108-95-2	Phenol	8.30E+04	8.20E-02	9.10E-06	1.64E-05	6.67E-06	2.00E+01	9.90E-02	2.80E-01
1918-02-1	Picloram	4.30E+02	2.26E-02	5.64E-06	2.19E-12	-------[a]	2.00E+00	No Data	7.21E-11
1336-36-3	Polychlorinated biphenyls (PCBs)	-------[a]	-------[a]	-------[a]	-------[a]	-------[a]	-------[a]	-------[a]	-------[a]
129-00-0	Pyrene	1.40E+00	2.77E-02	7.24E-06	4.51E-04	-------[a]	6.31E+04	1.80E-04	4.60E-06
121-82-4	Royal Demolition Explosive, Cyclonite (RDX)	5.97E+01	3.11E-02	8.49E-06	2.01E-11	-------[a]	7.20E+00	No Data	4.10E-09
122-34-9	Simazine	6.20E+00	2.48E-02	6.28E-06	3.80E-08	-------[a]	1.32E+02	No Data	2.21E-08
100-42-5	Styrene	3.10E+02	7.10E-02	8.00E-06	1.11E-01	5.48E-03	3.16E+02	3.30E-03	6.10E+00
93-72-1	2,4,5-TP (Silvex)	7.10E+01	2.30E-02	5.83E-06	3.71E-07	-------[a]	5.50E+03	No Data	9.97E-06
127-18-4	Tetrachloro-ethylene	2.00E+02	7.20E-02	8.20E-06	7.38E-01	4.00E-01	6.31E+02	9.60E-04	1.90E+01
108-88-3	Toluene	5.30E+02	8.70E-02	8.60E-06	2.71E-01	1.49E-01	1.58E+02	1.10E-02	2.80E+01

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
8001-35-2	Toxaphene	7.40E-01	2.16E-02	5.51E-06	2.46E-04	-------[a]	5.01E+04	No Data	9.80E-07
120-82-1	1,2,4-Trichlorobenzene	3.50E+01	3.00E-02	8.23E-06	5.74E-02	2.38E-02	1.58E+03	1.90E-03	4.30E-01
71-55-6	1,1,1-Trichloroethane	1.30E+03	7.80E-02	8.80E-06	6.97E-01	4.21E-01	1.26E+02	1.30E-03	1.20E+02
79-00-5	1,1,2-Trichloroethane	4.40E+03	7.80E-02	8.80E-06	3.73E-02	1.98E-02	5.01E+01	9.50E-04	2.30E+01
79-01-6	Trichloroethylene	1.50E+03	7.90E-02	9.10E-06	4.10E-01	2.41E-01	1.00E+02	4.20E-04	7.30E+01
75-69-4	Trichlorofluoro-methane	1.10E+03	8.70E-02	9.70E-06	3.98E+00	2.69E+00	1.30E+02	9.63E-04	8.00E+02
95-95-4	2,4,5-Trichlorophenol	1.20E+03	2.91E-02	7.03E-06	1.78E-04	-------[a]	2.68E+03[d]	3.80E-04	2.40E-02
88-06-2	2,4,6-Trichlorophenol	8.00E+02	2.61E-02	6.36E-06	3.53E-04	-------[a]	8.78E+02[d]	3.80E-04	2.00E-02
108-05-4	Vinyl Acetate	2.00E+04	8.50E-02	9.20E-06	2.09E-02	1.18E-02	4.57E+00	No Data	9.00E+01
99-35-4	1,3,5-Trinitrobenzene	2.80E+02	2.41E-02	6.08E-06	3.30E-10	-------[a]	1.60E+01	No Data	6.40E-06
118-96-7	2,4,6-Trinitrotoluene (TNT)	1.24E+02	2.94E-02	7.90E-06	4.87E-09	-------[a]	3.72E+01	1.92E-03	2.02E-06
57-01-4	Vinyl Chloride	8.80E+03	1.06E-01	1.23E-06	1.11E+00	8.14E-01	1.58E+01	2.40E-04	3.00E+03
108-38-3	m-Xylene	1.60E+02	7.00E-02	7.80E-06	2.99E-01	1.52E-01	3.98E+02	1.90E-03	8.50E+00

CAS No.	Chemical	Solubility in Water (S) (mg/L)	Diffusivity in Air (D_i) (cm^2/s)	Diffusivity in Water (D_w) (cm^2/s)	Dimensionless Henry's Law Constant (H') (25°C)	Dimensionless Henry's Law Constant (H') (13°C) For the indoor inhalation exposure route	Organic Carbon Partition Coefficient (K_{oc}) (L/kg)	First Order Degradation Constant (λ) (d^{-1})	Vapor Pressure (mm/Hg)
95-47-6	o-Xylene	1.80E+02	8.70E-02	1.00E-05	2.13E-01	1.07E-01	3.16E+02	1.90E-03	6.60E+00
106-42-3	p-Xylene	1.60E+02	7.69E-02	8.44E-06	3.16E-01	1.59E-01	3.16E+02	1.90E-03	8.90E+00
1330-20-7	Xylenes (total)	1.10E+02	7.35E-02	9.23E-06	2.71E-01	NA	3.98E+02	1.90E-03	8.00E+00

Chemical Abstracts Service (CAS) registry number. This number in the format xxx-xx-x, is unique for each chemical and allows efficient searching on computerized data bases.

[a] Soil remediation objectives are determined pursuant to 40 CFR 761, as incorporated by reference at Section 742.210(b) (the USEPA "PCB Spill Cleanup Policy"), for most sites; persons remediating sites should consult with BOL if calculation of Tier 2 or 3 remediation objectives is desired. PCBs are a mixture of different congeners. The appropriate values to use for the physical/chemical parameters depend on congeners present at the site.

[b] Dimensionless Henry's Law Constant at 13°C is not calculated because the chemical is not volatile and does not require evaluation under the indoor inhalation exposure route.

[c] Dimensionless Henry's Law Constant = 20°C

[d] These chemicals are ionizing and its K_{oc} value will change with pH. The K_{oc} values listed in this table is the effective K_{oc} at pH of 6.8. If the site-specific pH is values other than 6.8, the K_{oc} value listed in Section 742, Appendix C, Table I should be used.

[e] The values in this table were taken from the following sources (in order of preference): SCDMS online database (http://www.epa.gov/superfund/sites/npl/hrsres/tools/scdm.htm); CHEMFATE online database (http://www.srcinc.com/what-we-do/databaseforms.aspx?id=381); PhysProp online database (http://www.srcinc.com/what-we-do/databaseforms.aspx?id-386); Water9

(http://www.epa.gov/ttn/chief/software/water/) for diffusivity values; and Handbook of Environmental Degradation Rates by P.H. Howard (1991) for first order degradation constant values.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C: Tier 2 Illustrations and Tables

Section 742.Table F: Methods for Determining Physical Soil Parameters

Methods for Determining Physical Soil Parameters		
Parameter	Sampling Location[a]	Method
ρ_b (soil bulk density)	Surface	ASTM - D 1556-90 Sand Cone Method[b]
		ASTM - D 2167-94 Rubber Balloon Method[b]
		ASTM - D 2922-91 Nuclear Method[b]
	Subsurface	ASTM - D 2937-94 Drive Cylinder Method[b]
ρ_s (soil particle density)	Surface or Subsurface	ASTM - D 854-92 Specific Gravity of Soil[b]
w (moisture content)	Surface or Subsurface	ASTM - D 4959-89 (Reapproved 1994) Standard[b]
		ASTM - D 4643-93 Microwave Oven[b]
		ASTM - D2216-92 Laboratory Determination[b]
		ASTM - D3017-88 (Reapproved 1993) Nuclear Method[b]
		Equivalent USEPA Method (e.g., sample preparation procedures described in methods 3541 or 3550)
f_{oc} (fraction organic carbon content)	Surface or Subsurface	ASTM - D 2974-00 Moisture, Ash, and Organic Matter appropriately adjusted to estimate the fraction of organic carbon as stated in Nelson and Sommers (1982)[b]

Methods for Determining Physical Soil Parameters		
Parameter	Sampling Location[a]	Method
η or θ_T (total soil porosity)	Surface or Subsurface (calculated)	Equation S24 in Appendix C, Table A for SSL Model, or Equation R23 in Appendix C, Table C for RBCA Model, or Equation J&E 16 in Appendix C, Table L for J&E Model
θ_a or θ_{as} (air-filled soil porosity)	Surface or Subsurface (calculated)	Equation S21 in Appendix C, Table A for SSL Model, or Equation R21 in Appendix C, Table C for RBCA Model, or Equation J&E 18 in Appendix C, Table L for J&E Model
θ_w or θ_{ws} (water-filled soil porosity)	Surface or Subsurface (calculated)	Equation S20 in Appendix C, Table A for SSL Model, or Equation R22 in Appendix C, Table C for RBCA Model, or Equation J&E 17 in Appendix C, Table L for J&E Model
K (hydraulic conductivity)	Surface or Subsurface	ASTM - D 5084-90 Flexible Wall Permeameter[b]
		Pump Test
		Slug Test
i (hydraulic gradient)	Surface or Subsurface	Field Measurement

[a] This is the location where the sample is collected

[b] As incorporated by reference in Section 742.120.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C: Tier 2 Tables and Illustrations

Section 742.Table G: Error Function (erf)

$$erf(\beta) = \frac{2}{\sqrt{\pi}} \int_0^{\beta} e^{-\varepsilon^2} \, d\varepsilon$$

β	$erf(\beta)$
0	0
0.05	0.056372
0.1	0.112463
0.15	0.167996
0.2	0.222703
0.25	0.276326
0.3	0.328627
0.35	0.379382
0.4	0.428392
0.45	0.475482
0.5	0.520500
0.55	0.563323
0.6	0.603856
0.65	0.642029
0.7	0.677801
0.75	0.711156
0.8	0.742101
0.85	0.770668
0.9	0.796908
0.95	0.820891
1.0	0.842701
1.1	0.880205
1.2	0.910314
1.3	0.934008
1.4	0.952285
1.5	0.966105
1.6	0.976348
1.7	0.983790
1.8	0.989091
1.9	0.992790
2.0	0.995322
2.1	0.997021
2.2	0.998137
2.3	0.998857
2.4	0.999311
2.5	0.999593
2.6	0.999764
2.7	0.999866
2.8	0.999925
2.9	0.999959
3.0	0.999978

Section 742.APPENDIX C Tier 2 Illustrations and Tables

Section 742.Table H Q/C Values by Source Area

Source (Acres)	Area Q/C Value (g/m^2-s per kg/m^3)
0.5	97.78
1	85.81
2	76.08
5	65.75
10	59.16
30	50.60

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C TABLE I: K_{oc} Values for Ionizing Organics as a Function of pH (cm^3/g or L/kg or cm^3_{water}/g_{soil})

pH	Benzoic Acid	2-Chloro-phenol	2,4-Dichloro-phenol	Pentachloro-phenol	2,4,5-Trichloro-phenol	2,4,6-Trichloro-phenol	Dinoseb	2,4,5-TP (Silvex)
4.5	1.07E+01	3.98E+02	1.59E+02	1.34E+04	2.37E+03	1.06E+03	3.00E+04	1.28E+04
4.6	9.16E+00	3.98E+02	1.59E+02	1.24E+04	2.37E+03	1.05E+03	2.71E+04	1.13E+04
4.7	7.79E+00	3.98E+02	1.59E+02	1.13E+04	2.37E+03	1.05E+03	2.41E+04	1.01E+04
4.8	6.58E+00	3.98E+02	1.59E+02	1.02E+04	2.37E+03	1.05E+03	2.12E+04	9.16E+03
4.9	5.54E+00	3.98E+02	1.59E+02	9.05E+03	2.37E+03	1.04E+03	1.85E+04	8.40E+03
5.0	4.62E+00	3.98E+02	1.59E+02	7.96E+03	2.36E+03	1.03E+03	1.59E+04	7.76E+03
5.1	3.86E+00	3.98E+02	1.59E+02	6.93E+03	2.36E+03	1.02E+03	1.36E+04	7.30E+03
5.2	3.23E+00	3.98E+02	1.59E+02	5.97E+03	2.35E+03	1.01E+03	1.15E+04	6.91E+03
5.3	2.70E+00	3.98E+02	1.59E+02	5.10E+03	2.34E+03	9.99E+02	9.66E+03	6.60E+03
5.4	2.27E+00	3.98E+02	1.58E+02	4.32E+03	2.33E+03	9.82E+02	8.10E+03	6.36E+03
5.5	1.92E+00	3.97E+02	1.58E+02	3.65E+03	2.32E+03	9.62E+02	6.77E+03	6.16E+03
5.6	1.63E+00	3.97E+02	1.58E+02	3.07E+03	2.31E+03	9.38E+02	5.65E+03	6.00E+03
5.7	1.40E+00	3.97E+02	1.58E+02	2.58E+03	2.29E+03	9.10E+02	4.73E+03	5.88E+03
5.8	1.22E+00	3.97E+02	1.58E+02	2.18E+03	2.27E+03	8.77E+02	3.97E+03	5.78E+03
5.9	1.07E+00	3.97E+02	1.57E+02	1.84E+03	2.24E+03	8.39E+02	3.35E+03	5.70E+03
6.0	9.50E-01	3.96E+02	1.57E+02	1.56E+03	2.21E+03	7.96E+02	2.84E+03	5.64E+03
6.1	8.54E-01	3.96E+02	1.57E+02	1.33E+03	2.17E+03	7.48E+02	2.43E+03	5.59E+03
6.2	7.78E-01	3.96E+02	1.56E+02	1.15E+03	2.12E+03	6.97E+02	2.10E+03	5.55E+03
6.3	7.19E-01	3.95E+02	1.55E+02	9.98E+02	2.06E+03	6.44E+02	1.83E+03	5.52E+03

pH	Benzoic Acid	2-Chloro-phenol	2,4-Dichloro-phenol	Pentachloro-phenol	2,4,5-Trichloro-phenol	2,4,6-Trichloro-phenol	Dinoseb	2,4,5-TP (Silvex)
6.4	6.69E-01	3.94E+02	1.54E+02	8.77E+02	1.99E+03	5.89E+02	1.62E+03	5.50E+03
6.5	6.31E-01	3.93E+02	1.53E+02	7.81E+02	1.91E+03	5.33E+02	1.45E+03	5.48E+03
6.6	6.00E-01	3.92E+02	1.52E+02	7.03E+02	1.82E+03	4.80E+02	1.32E+03	5.46E+03
6.7	5.74E-01	3.90E+02	1.50E+02	6.40E+02	1.71E+03	4.29E+02	1.21E+03	5.45E+03
6.8	5.55E-01	3.88E+02	1.47E+02	5.92E+02	1.60E+03	3.81E+02	1.12E+03	5.44E+03
6.9	5.39E-01	3.86E+02	1.45E+02	5.52E+02	1.47E+03	3.38E+02	1.05E+03	5.43E+03
7.0	5.28E-01	3.83E+02	1.41E+02	5.21E+02	1.34E+03	3.00E+02	9.96E+02	5.43E+03
7.1	5.18E-01	3.79E+02	1.38E+02	4.96E+02	1.21E+03	2.67E+02	9.52E+02	5.42E+03
7.2	5.10E-01	3.75E+02	1.33E+02	4.76E+02	1.07E+03	2.39E+02	9.18E+02	5.42E+03
7.3	5.04E-01	3.69E+02	1.28E+02	4.61E+02	9.43E+02	2.15E+02	8.90E+02	5.42E+03
7.4	4.99E-01	3.62E+02	1.21E+02	4.47E+02	8.19E+02	1.95E+02	8.68E+02	5.41E+03
7.5	4.95E-01	3.54E+02	1.14E+02	4.37E+02	7.03E+02	1.78E+02	8.50E+02	5.41E+03
7.6	4.92E-01	3.44E+02	1.07E+02	4.29E+02	5.99E+02	1.64E+02	8.36E+02	5.41E+03
7.7	4.86E-01	3.33E+02	9.84E+01	4.23E+02	5.07E+02	1.53E+02	8.25E+02	5.41E+03
7.8	4.86E-01	3.19E+02	8.97E+01	4.18E+02	4.26E+02	1.44E+02	8.17E+02	5.41E+03
7.9	4.85E-01	3.04E+02	8.07E+01	4.14E+02	3.57E+02	1.37E+02	8.10E+02	5.41E+03
8.0	4.85E-01	2.86E+02	7.17E+01	4.10E+02	2.98E+02	1.31E+02	8.04E+02	5.41E+03
8.1	4.84E-01	2.67E+02	6.30E+01	4.09E+02	2.49E+02	1.26E+02	8.00E+02	5.40E+03
8.2	4.84E-01	2.46E+02	5.47E+01	4.07E+02	2.08E+02	1.22E+02	7.97E+02	5.40E+03
8.3	4.83E-01	2.24E+02	4.40E+01	4.05E+02	1.75E+02	1.19E+02	7.93E+02	5.40E+03

pH	Benzoic Acid	2-Chloro-phenol	2,4-Dichloro-phenol	Pentachloro-phenol	2,4,5-Trichloro-phenol	2,4,6-Trichloro-phenol	Dinoseb	2,4,5-TP (Silvex)
8.4	4.83E-01	2.02E+02	4.00E+01	4.04E+02	1.48E+02	1.17E+02	7.91E+02	5.40E+03
8.5	4.82E-01	1.80E+02	3.38E+01	4.03E+02	1.25E+02	1.15E+02	7.89E+02	5.40E+03
8.6	4.82E-01	1.58E+02	2.84E+01	4.02E+02	1.08E+02	1.13E+02	7.88E+02	5.40E+03
8.7	4.82E-01	1.37E+02	2.38E+01	4.02E+02	9.31E+02	1.12E+02	7.87E+02	5.40E+03
8.8	4.81E-01	1.18E+02	1.99E+01	4.01E+02	8.16E+02	1.11E+02	7.86E+02	5.40E+03
8.9	4.81E-01	1.00E+02	1.66E+01	4.01E+02	7.23E+01	1.10E+02	7.85E+02	5.40E+03
9.0	4.80E-01	8.47E+01	1.39E+01	4.00E+02	6.48E+01	1.09E+02	7.85E+02	5.40E+03

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C: Tier 2 Illustrations and Tables

Section 742.TABLE J Values to be Substituted for k_d or k_s when Evaluating Inorganics as a Function of pH (cm^3/g or L/kg or cm^3_{water}/g_{soil})

pH	As	Ba	Be	Cd	Cr (+3)	Cr (+6)	Hg	Ni	Ag	Se	Tl	Zn	Pb
4.9	2.5E+01	1.1E+01	2.3E+01	1.5E+01	1.2E+03	3.1E+01	4.0E-02	1.6E+01	1.0E-01	1.8E+01	4.4E+01	1.6E+01	1.5E+01
5.0	2.5E+01	1.2E+01	2.6E+01	1.7E+01	1.9E+03	3.1E+01	6.0E-02	1.8E+01	1.3E-01	1.7E+01	4.5E+01	1.8E+01	1.5E+01
5.1	2.5E+01	1.4E+01	2.8E+01	1.9E+01	3.0E+03	3.0E+01	9.0E-02	2.0E+01	1.6E-01	1.6E+01	4.6E+01	1.9E+01	1.5E+01
5.2	2.6E+01	1.5E+01	3.1E+01	2.1E+01	4.9E+03	2.9E+01	1.4E-01	2.2E+01	2.1E-01	1.5E+01	4.7E+01	2.1E+01	1.5E+01
5.3	2.6E+01	1.7E+01	3.5E+01	2.3E+01	8.1E+03	2.8E+01	2.0E-01	2.4E+01	2.6E-01	1.4E+01	4.8E+01	2.3E+01	1.5E+01
5.4	2.6E+01	1.9E+01	3.8E+01	2.5E+01	1.3E+04	2.7E+01	3.0E-01	2.6E+01	3.3E-01	1.3E+01	5.0E+01	2.5E+01	1.5E+01
5.5	2.6E+01	2.1E+01	4.2E+01	2.7E+01	2.1E+04	2.7E+01	4.6E-01	2.8E+01	4.2E-01	1.2E+01	5.1E+01	2.6E+01	1.5E+01
5.6	2.6E+01	2.2E+01	4.7E+01	2.9E+01	3.5E+04	2.6E+01	6.9E-01	3.0E+01	5.3E-01	1.1E+01	5.2E+01	2.8E+01	1.5E+01
5.7	2.7E+01	2.4E+01	5.3E+01	3.1E+01	5.5E+04	2.5E+01	1.0E-00	3.2E+01	6.7E-01	1.1E+01	5.4E+01	3.0E+01	1.5E+01
5.8	2.7E+01	2.6E+01	6.0E+01	3.3E+01	8.7E+04	2.5E+01	1.6E-00	3.4E+01	8.4E-01	9.8E+00	5.5E+01	3.2E+01	1.5E+01

pH	As	Ba	Be	Cd	Cr (+3)	Cr (+6)	Hg	Ni	Ag	Se	Tl	Zn	Pb
5.9	2.7E+01	2.8E+01	6.9E+01	3.5E+01	1.3E+05	2.4E+01	2.3E-00	3.6E+01	1.1E+00	9.2E+00	5.6E+01	3.4E+01	1.5E+01
6.0	2.7E+01	3.0E+01	8.2E+01	3.7E+01	2.0E+05	2.3E+01	3.5E-00	3.8E+01	1.3E+00	8.6E+00	5.8E+01	3.6E+01	1.5E+01
6.1	2.7E+01	3.1E+01	9.9E+01	4.0E+01	3.0E+05	2.3E+01	5.1E-00	4.0E+01	1.7E+00	8.0E+00	5.9E+01	3.9E+01	1.5E+01
6.2	2.8E+01	3.3E+01	1.2E+02	4.2E+01	4.2E+05	2.2E+01	7.5E-00	4.2E+01	2.1E+00	7.5E+00	6.1E+01	4.2E+01	1.5E+01
6.3	2.8E+01	3.5E+01	1.6E+02	4.4E+01	5.8E+05	2.2E+01	1.1E+01	4.5E+01	2.7E+00	7.0E+00	6.2E+01	4.4E+01	1.5E+01
6.4	2.8E+01	3.6E+01	2.1E+02	4.8E+01	7.7E+05	2.1E+01	1.6E+01	4.7E+01	3.4E+00	6.5E+00	6.4E+01	4.7E+01	7.1E+02
6.5	2.8E+01	3.7E+01	2.8E+02	5.2E+01	9.9E+05	2.0E+01	2.2E+01	5.0E+01	4.2E+00	6.1E+00	6.6E+01	5.1E+01	7.1E+02
6.6	2.8E+01	3.9E+01	3.9E+02	5.7E+01	1.2E+06	2.0E+01	3.0E+01	5.4E+01	5.3E+00	5.7E+00	6.7E+01	5.4E+01	7.1E+02
6.7	2.9E+01	4.0E+01	5.5E+02	6.4E+01	1.5E+06	1.9E+01	4.0E+01	5.8E+01	6.6E+00	5.3E+00	6.9E+01	5.8E+01	7.1E+02
6.8	2.9E+01	4.1E+01	7.9E+02	7.5E+01	1.8E+06	1.9E+01	5.2E+01	6.5E+01	8.3E+00	5.0E+00	7.1E+01	6.2E+01	7.1E+02
6.9	2.9E+01	4.2E+01	1.1E+03	9.1E+01	2.1E+06	1.8E+01	6.6E+01	7.4E+01	1.0E+01	4.7E+00	7.3E+01	6.8E+01	7.1E+02
7.0	2.9E+01	4.2E+01	1.7E+03	1.1E+02	2.5E+06	1.8E+01	8.2E+01	8.8E+01	1.3E+01	4.3E+00	7.4E+01	7.5E+01	7.1E+02

pH	As	Ba	Be	Cd	Cr (+3)	Cr (+6)	Hg	Ni	Ag	Se	Tl	Zn	Pb
7.1	2.9E+01	4.3E+01	2.5E+03	1.5E+02	2.8E+06	1.7E+01	9.9E+01	1.1E+02	1.6E+01	4.1E+00	7.6E+01	8.3E+01	7.1E+02
7.2	3.0E+01	4.4E+01	3.8E+03	2.0E+02	3.1E+06	1.7E+01	1.2E+02	1.4E+02	2.0E+01	3.8E+00	7.8E+01	9.5E+01	7.1E+02
7.3	3.0E+01	4.4E+01	5.7E+03	2.8E+02	3.4E+06	1.6E+01	1.3E+02	1.8E+02	2.5E+01	3.5E+00	8.0E+01	1.1E+02	7.1E+02
7.4	3.0E+01	4.5E+01	8.6E+03	4.0E+02	3.7E+06	1.6E+01	1.5E+02	2.5E+02	3.1E+01	3.3E+00	8.2E+01	1.3E+02	7.1E+02
7.5	3.0E+01	4.6E+01	1.3E+04	5.9E+02	3.9E+06	1.6E+01	1.6E+02	3.5E+02	3.9E+01	3.1E+00	8.5E+01	1.6E+02	7.1E+02
7.6	3.1E+01	4.6E+01	2.0E+04	8.7E+02	4.1E+06	1.5E+01	1.7E+02	4.9E+02	4.8E+01	2.9E+00	8.7E+01	1.9E+02	7.1E+02
7.7	3.1E+01	4.7E+01	3.0E+04	1.3E+03	4.2E+06	1.5E+01	1.8E+02	7.0E+02	5.9E+01	2.7E+00	8.9E+01	2.4E+02	7.1E+02
7.8	3.1E+01	4.9E+01	4.6E+04	1.9E+03	4.3E+06	1.4E+01	1.9E+02	9.9E+02	7.3E+01	2.5E+00	9.1E+01	3.1E+02	7.1E+02
7.9	3.1E+01	5.0E+01	6.9E+04	2.9E+03	4.3E+06	1.4E+01	1.9E+02	1.4E+03	8.9E+01	2.4E+00	9.4E+01	4.0E+02	7.1E+02
8.0	3.1E+01	5.2E+01	1.0E+05	4.3E+03	4.3E+06	1.4E+01	2.0E+02	1.9E+03	1.1E+02	2.2E+00	9.6E+01	5.3E+02	7.1E+02
8.1	3.2E+01	---[a]	---[a]	---[a]	---[a]	1.3E+01	---[a]	---[a]	---[a]	2.1E+00	1.0E+02	---[a]	7.1E+02
8.2	3.2E+01	---[a]	---[a]	---[a]	---[a]	1.3E+01	---[a]	---[a]	---[a]	1.9E+00	1.0E+02	---[a]	7.1E+02

pH	As	Ba	Be	Cd	Cr (+3)	Cr (+6)	Hg	Ni	Ag	Se	Tl	Zn	Pb
8.3	3.2E+01	---[a]	---[a]	---[a]	---[a]	1.3E+01	---[a]	---[a]	---[a]	1.8E+00	1.0E+02	---[a]	7.1E+02
8.4	3.2E+01	---[a]	---[a]	---[a]	---[a]	1.2E+01	---[a]	---[a]	---[a]	1.7E+00	1.1E+02	---[a]	7.1E+02
8.5	3.2E+01	---[a]	---[a]	---[a]	---[a]	1.2E+01	---[a]	---[a]	---[a]	1.6E+00	1.1E+02	---[a]	7.1E+02
8.6	3.3E+01	---[a]	---[a]	---[a]	---[a]	1.2E+01	---[a]	---[a]	---[a]	1.5E+00	1.1E+02	---[a]	7.1E+02
8.7	3.3E+01	---[a]	---[a]	---[a]	---[a]	1.2E+01	---[a]	---[a]	---[a]	1.4E+00	1.2E+02	---[a]	7.1E+02
8.8	3.3E+01	---[a]	---[a]	---[a]	---[a]	1.1E+01	---[a]	---[a]	---[a]	1.3E+00	1.2E+02	---[a]	1.9E+03
8.9	3.3E+01	---[a]	---[a]	---[a]	---[a]	1.1E+01	---[a]	---[a]	---[a]	1.2E+00	1.2E+02	---[a]	1.9E+03
9.0	3.3E+01	---[a]	---[a]	---[a]	---[a]	1.0E+01	---[a]	---[a]	---[a]	1.1E+00	1.2E+02	---[a]	1.9E+03

[a] No data available for this pH.

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C Tier 2 Illustrations and Tables

Section 742.TABLE K Parameter Estimates for Calculating Water-Filled Soil Porosity (θ_w)

Soil Texture[a]	Saturated Hydraulic Conductivity, K_s (m/yr)	$1/(2b+3)^b$
Sand	1,830	0.090
Loamy Sand	540	0.085
Sandy Loam	230	0.080
Silt Loam	120	0.074
Loam	60	0.073
Sandy Clay Loam	40	0.058
Silt Clay Loam	13	0.054
Clay Loam	20	0.050
Sandy Clay	10	0.042
Silt Clay	8	0.042
Clay	5	0.039

[a] The appropriate texture classification is determined by a particle size analysis by ASTM D2488-93 as incorporated by reference in Section 742.210 and the U.S. Department of Agriculture Soil Textural Triangle shown in Appendix C, Illustration C.

[b] Where b is the soil-specific exponential parameter (unitless)

(Source: Amended at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX C: Tier 2 Tables

Section 742.Table L: J&E Equations[a]

Indoor air remediation objectives (mg/m^3)	For carcinogenic contaminants	$RO_{indoor\ air} = \frac{TR \times AT_c \times 365 \frac{days}{yr}}{ED \times EF \times URF \times 1000 \frac{\mu g}{mg}}$	**J&E1**
	For noncarcinogenic contaminants	$RO_{indoor\ air} = \frac{THQ \times AT_{nc} \times 365 \frac{days}{yr} \times RfC}{ED \times EF}$	**J&E2**
To convert mg/m^3 from parts per million volume		$mg/m^3 = \frac{ppmv \times MW}{24.45}$ Note: 24.45 equals the molar volume of air in liters at normal temperature (25°C) and pressure (760 mm Hg).	**J&E3**

Soil gas remediation objective (mg/m^3)		$RO_{soil\ gas} = \frac{RO_{indoor\ air}}{\alpha}$	**J&E4**
Soil Vapor Saturation Limit (mg/m^3-air)		$C_v^{sat} = \frac{P \times MW}{R \times T} \times 10^6$	**J&E5**
Groundwater remediation objectives		$RO_{gw} = \frac{RO_{soil\ gas}}{H'_{TS} \times 1000 \frac{L}{m^3}}$	**J&E6**
Attenuation factor	Attenuation factor when the mode of contaminant transport is both diffusion and advection Q_{soil} = 83.33 cm^3/sec	$\alpha = \frac{\left[\left(\frac{D_T^{eff} \times A_B}{Q_{bldg} \times L_T}\right) \times \exp\left(\frac{Q_{soil} \times L_{crack}}{D_{crack}^{eff} \times A_{crack}}\right)\right]}{\left[\exp\left(\frac{Q_{soil} \times L_{crack}}{D_{crack}^{eff} \times A_{crack}}\right) + \left(\frac{D_T^{eff} \times A_B}{Q_{bldg} \times L_T}\right) + \left(\frac{D_T^{eff} \times A_B}{Q_{soil} \times L_T}\right)\left[\exp\left(\frac{Q_{soil} \times L_{crack}}{D_{crack}^{eff} \times A_{crack}}\right) - 1\right]\right]}$	**J&E7**

	Attenuation factor when the mode of contaminant transport is diffusion only Q_{soil}= 0 cm^3/sec	$$\alpha = \frac{\left(\frac{D_T^{eff} \times A_B}{Q_{bldg} \times L_T}\right)}{\left[1+\left(\frac{D_T^{eff} \times A_B}{Q_{bldg} \times L_T}\right)+\left(\frac{D_T^{eff} \times A_B \times L_{crack}}{L_T \times D_{crack}^{eff} \times A_{crack}}\right)\right]}$$	**J&E8**
Total overall effective diffusion coefficient for vapor transport in porous media for multiple soil layers (cm^2/s)		$$D_T^{eff} = \frac{L_T}{\sum_{i=1}^{n} L_i / D_i^{eff}}$$	**J&E9a**
	In Equation J&E9a, the following condition must be satisfied:	$$\sum_{i=1}^{n} L_i = L_T$$	**J&E9b**
Source to building separation (cm)		$$L_T = D_{source} - L_F$$	**J&E10**
Effective diffusion coefficient for each soil layer (cm^2/s)		$$D_i^{eff} = D_i\left(\frac{\theta_{a,i}^{3.33}}{\theta_{T,i}^2}\right)+\left(\frac{D_w}{H'_{TS}}\right)\left(\frac{\theta_{w,i}^{3.33}}{\theta_{T,i}^2}\right)$$	**J&E11**

Surface area of enclosed space at or below grade (cm^2)	For a building with a full concrete slab-on-grade	$A_B = (L_B \times W_B)$	**J&E12a**
Surface area of enclosed space at or below grade (cm^2)	For a building with a full concrete basement floor and walls	$A_B = (L_B \times W_B) + (2 \times L_F \times L_B) + (2 \times L_F \times W_B)$	**J&E12b**
Building ventilation rate (cm^3/s)		$Q_{bldg} = \left(\frac{L_B \times W_B \times H_B \times ER}{3600 \frac{sec}{hr}} \right)$	**J&E13**
Area of total cracks (cm^2)		$A_{crack} = 2 \times (L_B + W_B) \times w$	**J&E14**
Effective diffusion coefficient through the cracks (cm^2/s)		$D_{crack}^{eff} = D_i \left(\frac{\theta_{a,crack}^{3.33}}{\theta_{T,crack}^2} \right) + \left(\frac{D_w}{H_{TS}'} \right) \left(\frac{\theta_{w,crack}^{3.33}}{\theta_{T,crack}^2} \right)$	**J&E15**

Total porosity		$\theta_{T_i} = 1 - \frac{\rho_{bi}}{\rho_s}$	**J&E16**
Water-filled soil porosity		$\theta_w = (W)\left(\frac{\rho_b}{\rho_w}\right)$	**J&E17**
Air-filled soil porosity		$\theta_a = \theta_T - \theta_w$	**J&E18**

[a] This table contains equations based on the assumption that the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. This table applies only when the existing or potential building has a full concrete slab-on-grade or a full concrete basement floor and walls. Institutional controls under Subpart J are required to develop remediation objectives pursuant to this table. This table does not apply when the existing or potential building has neither a full concrete slab-on-grade nor a full concrete basement floor and walls, such as a building with an earthen crawl space, an earthen floor, a stone foundation, a partial concrete floor, or a sump. In such cases, site evaluators have the option of excluding the indoor inhalation exposure route under Section 742.312, meeting the building control technology requirements under Subpart L, or proposing an alternative approach under Tier 3.

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX C: Tier 2 Tables

Section 742.Table M: J&E Parameters

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
A_B	Surface area of enclosed space at or below grade	cm^2	Equation J&E 12a or 12b, Appendix C, Table L	Residential = 1×10^6 Industrial/Commercial = 4.0×10^6
A_{crack}	Area of total cracks	cm^2	Equation J&E 14, Appendix C, Table L	Calculated Value
AT_c	Averaging time for carcinogens	year	SSL, May 1996	70
AT_{nc}	Averaging time for noncarcinogens	year	AT_{nc}= ED	Residential = 30 Industrial/Commercial = 25
C_v^{sat}	Soil vapor saturation limit	mg/m^3-air	Equation J&E 5, Appendix C, Table L	Chemical-Specific or Calculated Value
D_{crack}^{eff}	Effective diffusion coefficient through the cracks	cm^2 /s	Equation J&E 15, Appendix C, Table L	Calculated Value
D_i	Diffusivity in air	cm^2 /s	Appendix C, Table E	Chemical-Specific

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
D_i^{eff}	Effective diffusion coefficient for each soil layer	cm^2 /s	Equation J&E 11, Appendix C, Table L	Calculated Value
D_{source}	Distance from ground surface to top of contamination	cm	Field Measurement	Soil Gas Contamination = 152.4 Groundwater Contamination = 304.8 Site-Specific
D_T^{eff}	Total overall effective diffusion coefficient	cm^2 /s	Equation J&E 9a, Appendix C, Table L	Calculated Value
D_w	Diffusivity in water	cm^2 /s	Appendix C, Table E	Chemical-Specific
ED	Exposure duration	year	Residential: SSL, May 1996 Industrial/Commercial: SSL 2002	Residential = 30 Industrial/Commercial = 25
EF	Exposure frequency	day/year	Residential: SSL, May 1996 Industrial/Commercial: SSL 2002	Residential = 350 Industrial/Commercial = 250

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
ER	Air exchange rate	exchanges per hour	Illinois EPA	Residential = 0.53 Industrial/Commercial = 0.93
f_{oc}	Fraction organic carbon content	g/g	SSL, May 1996, or Field Measurement Appendix C, Table F	0.002 or Site-Specific
H_B	Height of building	cm	Illinois EPA	Slab on Grade Residential = 244 Industrial/Commercial = 305 or Site-Specific in Tier 3 Basement Residential = 427 Industrial/Commercial = 488 or Site-Specific in Tier 3
H'_{TS}	Dimensionless Henry's law constant at the system (soil) temperature 13°C	unitless	Appendix C, Table E	Chemical-Specific

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
L_B	Length of building	cm	Illinois EPA	Residential = 1000 Industrial/Commercial = 2000 or Site-Specific in Tier 3
L_{crack}	Slab thickness	cm	US EPA, Users Guide 2004	10
L_F	Distance from ground surface to bottom of slab	cm	US EPA, Users Guide 2004	10 (slab on grade) 200 (basement)
L_i	Thickness of soil layer i	cm	Field Measurement For capillary fringe, USEPA, 2004	Site-Specific For capillary fringe, 37.5 cm
L_T	Distance from bottom of slab to top of contamination	cm	Field Measurement or Equation J&E 10, Appendix C, Table L	142.4 or Site-Specific
MW	Molecular weight	g/mole	Illinois EPA	Chemical-Specific

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
n	Total number of layers of different types of soil vapors migrate through from source to building (if source is groundwater, include a capillary fringe layer of 37.5 cm as one of the layers)	unitless	Field measurement	Site-Specific
P	Vapor Pressure	atm	Appendix C, Table E	Chemical-Specific
Q_{bldg}	Building ventilation rate	cm^3/s	Equation J&E 13, Appendix C, Table L	Slab on Grade Residential = 3.59×10^4 Industrial/Commercial = 3.15×10^5 or Site-Specific in Tier 3 Basement Residential = 6.28×10^4 Industrial/Commercial = 5.04×10^5 or Site-Specific in Tier 3

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
Q_{soil}	Volumetric flow rate of soil gas into the enclosed space	cm^3/s	US EPA, Users Guide for Evaluating Subsurface Vapor Intrusion into Buildings 2004	If L_T is less than 5 feet (152 cm), Q_{soil} equals 83.33 If L_T is 5 feet (152 cm) or greater, Q_{soil} equals zero An input value of zero requires an institutional control. See Section 742.505(b) and (c).
R	Ideal gas constant	atm-L/mol-K	US EPA, Users Guide 2004	0.08206
RfC	Reference concentration	ug/m^3	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological-Specific
RO_{gw}	Groundwater remediation objective	mg/L	Appendix B, Table E, or Equation J&E 6, Appendix C, Table L	Chemical-Specific or Calculated Value
$RO_{indoor\ air}$	Indoor air remediation objective	mg/m^3	Equations J&E 1 and 2, Appendix C, Table L	Calculated Value

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
$RO_{soil\ gas}$	Soil gas remediation objective	mg/m^3	Equation J&E 4, Appendix C, Table L	Calculated Value
S	Solubility in water	mg/L	Appendix C, Table E	Chemical-Specific
T	Temperature	K	US EPA, Users Guide 2004	286 (converted from 13°C)
THQ	Target hazard quotient for a chemical	unitless	SSL, May 1996	1
TR	Target risk or the increased chance of developing cancer over a lifetime due to exposure to a chemical	unitless	SSL, May 1996	Residential = 10^{-6} at the point of human exposure Industrial/Commercial = 10^{-6} at the point of human exposure
URF	Unit risk factor	$(ug/m^3)^{-1}$	Illinois EPA: http://www.epa.state.il.us/land/taco/toxicity-values.xls	Toxicological- Specific

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
w	Floor-wall seam gap	cm	US EPA, Users Guide 2004	0.1
W	Moisture content	g of water/g of soil	Field Measurement, Appendix C, Table F	Site-Specific
W_B	Width of building	cm	Illinois EPA	Residential = 1000 Industrial/Commercial = 2000 or Site-Specific in Tier 3
α	Attenuation factor	unitless	Equations J&E 7 or 8, Appendix C, Table L	Site-Specific
θ_a	Air-filled soil porosity	cm^3/cm^3	SSL, May 1996 or Equation J&E 18, Appendix C, Table L	0.28 or Calculated Value
$\theta_{a,crack}$	Air-filled porosity for soil in cracks	cm^3/cm^3	SSL, May 1996 or Equation J&E 18, Appendix C, Table L	0.13
$\theta_{a,i}$	Air-filled porosity of soil layer i	cm^3/cm^3	SSL, May 1996 or Equation J&E 18, Appendix C, Table L	0.13 or Calculated Value For capillary fringe, $\theta_{a,i} = 0.1\ \theta_{T,i}$

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
$\theta_{T,crack}$	Total porosity for soil in cracks	cm^3/cm^3	SSL, May 1996 or Equation J&E 16, Appendix C, Table L	0.43
$\theta_{T,i}$	Total porosity of soil layer i	cm^3/cm^3	SSL, May 1996 or Equation J&E 16, Appendix C, Table L	0.43 or Calculated Value
θ_w	Water-filled soil porosity	cm^3/cm^3	SSL, May 1996 or Equation J&E 17, Appendix C, Table L	0.15 or Calculated Value
$\theta_{w,crack}$	Water-filled porosity for soil in cracks	cm^3/cm^3	SSL, May 1996 or Equation J&E 17, Appendix C, Table L	0.15
$\theta_{w,i}$	Water-filled porosity of soil layer i	cm^3/cm^3	SSL, May 1996 or Equation J&E 17, Appendix C, Table L For capillary fringe, US EPA, Users Guide 2004	0.15 or Calculated Value For capillary fringe = 0.375 or 0.9 $\theta_{T,i}$

Symbol	Parameter	Units	Source	Tier 1 or Calculated Value
ρ_b	Dry soil bulk density	g/cm^3	SSL, May 1996 or Field Measurement, Appendix C, Table F	1.5 or Calculated Value
$\rho_{s,i}$	Soil particle density	g/cm^3	SSL, May 1996 or Field Measurement, Appendix C, Table F	2.65 or Calculated Value
ρ_w	Density of water	g/cm^3	Illinois EPA	1

(Source: Added at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX D Highway Authority Agreement

HIGHWAY AUTHORITY AGREEMENT

This Agreement is entered into this ____ day of _____, 200_ pursuant to 35 Ill. Adm. Code 742.1020 by and between the (1)_____ ("Property Owner") *[or, in the case of a petroleum leaking underground storage tank, the owner/operator of the tank ("Owner/Operator")]* and (2) *Name of Entity in Control of the Right-of-Way* ("Highway Authority"), collectively known as the "Parties."

[Use this paragraph for sites with petroleum leaking underground storage tank(s)] **WHEREAS,** _____ is the owner or operator of one or more leaking underground storage tanks presently or formerly located at *common address or description of Site location* ("the Site");

[Use this paragraph for sites that do not have petroleum leaking underground storage tanks] **WHEREAS,** _____ is the owner of the property located at *common address or description of Site location* ("the Site");

WHEREAS, as a result of one or more releases of contaminants [*insert either "from the above referenced underground storage tanks" or "at the above referenced Site"*] ("the Release(s)"), soil and/or groundwater contamination at the Site exceeds the Tier 1 residential remediation objectives of 35 Ill. Adm. Code 742;

WHEREAS, the soil and/or groundwater contamination exceeding Tier 1 residential remediation objectives extends or may extend into the Highway Authority's right-of-way;

WHEREAS, the Owner/Operator or Property Owner is conducting corrective action in response to the Release(s);

WHEREAS, the Parties desire to prevent groundwater beneath the Highway Authority's right-of-way that exceeds Tier 1 remediation objectives from use as a supply of potable or domestic water and to limit access to soil within the right-of-way that exceeds Tier 1 residential remediation objectives so that human health and the environment are protected during and after any access;

NOW, THEREFORE, the Parties agree as follows:

1. The recitals set forth above are incorporated by reference as if fully set forth herein.

2. *[Use this paragraph if IEMA has issued an incident number]* The Illinois Emergency Management Agency has assigned incident number(s) to the Release(s).

3. Attached as Exhibit A is a scaled map(s) prepared by the *[Owner/Operator or Property Owner]* that shows the Site and surrounding area and delineates the

current and estimated future extent of soil and groundwater contamination above the applicable Tier 1 residential remediation objectives as a result of the Release(s). *[Use the following sentence if either soil or groundwater is not contaminated above applicable Tier 1 residential remediation objectives:* [Soil] [Groundwater] is not contaminated above the applicable Tier 1 residential remediation objectives.*]*

4. Attached as Exhibit B is a table(s) prepared by the *[Owner/Operator or Property Owner]* that lists each contaminant of concern that exceeds its Tier 1 residential remediation objective, its Tier 1 residential remediation objective and its concentrations within the zone where Tier 1 residential remediation objectives are exceeded. The locations of the concentrations listed in Exhibit B are identified on the map(s) in Exhibit A.

5. Attached as Exhibit C is a scaled map prepared by the *[Owner/Operator or Property Owner]* showing the area of the Highway Authority's right-of-way that is governed by this agreement ("Right-of-Way"). Because Exhibit C is not a surveyed plat, the Right-of-Way boundary may be an approximation of the actual Right-of-Way lines.

6. *[Use this paragraph if samples have not been collected within the Right-of-Way, sampling within the Right-of-Way is not practical, and contamination does not extend beyond the Right-of-Way].* Because the collection of samples within the Right-of-Way is not practical, the Parties stipulate that, based on modeling, soil and groundwater contamination exceeding Tier 1 residential remediation objectives does not and will not extend beyond the boundaries of the Right-of-Way.

7. The Highway Authority stipulates it has jurisdiction over the Right-of-Way that gives it sole control over the use of the groundwater and access to the soil located within or beneath the Right-of-Way.

8. The Highway Authority agrees to prohibit within the Right-of-Way all potable and domestic uses of groundwater exceeding Tier 1 residential remediation objectives.

9. The Highway Authority further agrees to limit access by itself and others to soil within the Right-of-Way exceeding Tier 1 residential remediation objectives. Access shall be allowed only if human health (including worker safety) and the environment are protected during and after any access. The Highway Authority may construct, reconstruct, improve, repair, maintain and operate a highway upon the Right-of-Way, or allow others to do the same by permit. In addition, the Highway Authority and others using or working in the Right-of-Way under permit have the right to remove soil or groundwater from the Right-of-Way and dispose of the same in accordance with applicable environmental laws and regulations. The Highway Authority agrees to issue all permits for work in the

Right-of-Way, and make all existing permits for work in the Right-of-Way, subject to the following or a substantially similar condition:

As a condition of this permit the permittee shall request the office issuing this permit to identify sites in the Right-of-Way where a Highway Authority Agreement governs access to soil that exceeds the Tier 1 residential remediation objectives of 35 Ill. Adm. Code 742. The permittee shall take all measures necessary to protect human health (including worker safety) and the environment during and after any access to such soil.

10. This agreement shall be referenced in the Agency's no further remediation determination issued for the Release(s).

11. The Agency shall be notified of any transfer of jurisdiction over the Right-of-Way at least 30 days prior to the date the transfer takes effect. This agreement shall be null and void upon the transfer unless the transferee agrees to be bound by this agreement as if the transferee were an original party to this agreement. The transferee's agreement to be bound by the terms of this agreement shall be memorialized at the time of transfer in a writing ("Rider") that references this Highway Authority Agreement and is signed by the Highway Authority, or subsequent transferor, and the transferee.

12. This agreement shall become effective on the date the Agency issues a no further remediation determination for the Release(s). It shall remain effective until the Right-of-Way is demonstrated to be suitable for unrestricted use and the Agency issues a new no further remediation determination to reflect there is no longer a need for this agreement, or until the agreement is otherwise terminated or voided.

13. In addition to any other remedies that may be available, the Agency may bring suit to enforce the terms of this agreement or may, in its sole discretion, declare this agreement null and void if any of the Parties or any transferee violates any term of this agreement. The Parties or transferee shall be notified in writing of any such declaration.

14. This agreement shall be null and void if a court of competent jurisdiction strikes down any part or provision of the agreement.

15. This agreement supersedes any prior written or oral agreements or understandings between the Parties on the subject matter addressed herein. It may be altered, modified or amended only upon the written consent and agreement of the Parties.

16. Any notices or other correspondence regarding this agreement shall be sent to the Parties at following addresses:

Manager, Division of Remediation Management
Bureau of Land
Illinois Environmental Protection Agency

Property Owner or Owner/Operator
[*Address*]

P.O. Box 19276
Springfield, IL 62974-9276

[Contact at Highway Authority]

[Address]

IN WITNESS WHEREOF, the Parties have caused this agreement to be signed by their duly authorized representatives.

[NAME OF LOCAL GOVERNMENT]

Date: _____ By: _____

Its: _____

Property Owner or Owner/Operator

Date: _____ By: _____

Title

(Source: Added at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX E Highway Authority Agreement Memorandum of Agreement

HIGHWAY AUTHORITY AGREEMENT MEMORANDUM OF AGREEMENT

This Memorandum of Agreement is entered by and between the Illinois Environmental Protection Agency ("Agency") and *Name of Local Government* ("Highway Authority"), collectively known as the "Parties."

[Use this paragraph for sites with petroleum leaking underground storage tank(s)]
WHEREAS, the Highway Authority is the owner or operator of one or more leaking underground storage tanks presently or formerly located at *common address or description of Site location* ("the Site");

[Use this paragraph for sites where the highway authority is also the property owner]
WHEREAS, the Highway Authority is the owner of the property located at *common address or description of Site location* ("the Site");

WHEREAS, as a result of one or more releases of contaminants [*insert either "from the above referenced underground storage tanks" or "at the above referenced Site"*] ("the Release(s)"), soil and/or groundwater contamination at the Site exceeds the Tier 1 residential remediation objectives of 35 Ill. Adm. Code 742;

WHEREAS, the soil and/or groundwater contamination exceeding Tier 1 residential remediation objectives extends or may extend into the Highway Authority's right-of-way adjacent to the Site;

WHEREAS, the Highway Authority is conducting corrective action in response to the Release(s);

WHEREAS, the Parties desire to prevent groundwater beneath the Highway Authority's right-of-way that exceeds Tier 1 residential remediation objectives from use as a supply of potable or domestic water and to limit access to soil within the right-of-way that exceeds Tier 1 residential remediation objectives so that human health and the environment are protected during and after any access;

NOW, THEREFORE, the Parties agree as follows:

1. The recitals set forth above are incorporated by reference as if fully set forth herein.

2. [Use this paragraph if IEMA has issued an incident number] The Illinois Emergency Management Agency has assigned incident number(s) to the Release(s).

3. Attached as Exhibit A is a scaled map(s) prepared by the Highway Authority that shows the Site and surrounding area and delineates the current and estimated future extent of soil and groundwater contamination above the applicable Tier 1 residential remediation objectives as a result of the Release(s). [Use the following sentence if either soil or groundwater is not contaminated above applicable Tier 1 residential remediation objectives: [Soil] [Groundwater] is not contaminated above the applicable Tier 1 residential remediation objectives.]

4. Attached as Exhibit B is a table(s) prepared by the Highway Authority that lists each contaminant of concern that exceeds its Tier 1 residential remediation objective, its Tier 1 residential remediation objective and its concentrations within the zone where Tier 1 residential remediation objectives are exceeded. The locations of the concentrations listed in Exhibit B are identified on the map(s) in Exhibit A.

5. Attached as Exhibit C is a scaled map prepared by the Highway Authority showing the area of the Highway Authority's right-of-way that is governed by this agreement ("Right-of-Way"). Because Exhibit C is not a surveyed plat, the Right-of-Way boundary may be an approximation of the actual Right-of-Way lines.

6. *[Use this paragraph if samples have not been collected within the Right-of-Way, sampling within the Right-of-Way is not practical, and contamination does not extend beyond the Right-of-Way].* Because the collection of samples within the Right-of-Way is not practical, the Parties stipulate that, based on modeling, soil and groundwater contamination exceeding Tier 1 residential remediation objectives does not and will not extend beyond the boundaries of the Right-of-Way.

7. The Highway Authority stipulates it has jurisdiction over the Right-of-Way that gives it sole control over the use of the groundwater and access to the soil located within or beneath the Right-of-Way.

8. The Highway Authority agrees to prohibit within the Right-of-Way all potable and domestic uses of groundwater exceeding Tier 1 residential remediation objectives.

9. The Highway Authority further agrees to limit access by itself and others to soil within the Right-of-Way exceeding Tier 1 residential remediation objectives. Access shall be allowed only if human health (including worker safety) and the environment are protected during and after any access. The Highway Authority may construct, reconstruct, improve, repair, maintain and operate a highway upon the Right-of-Way, or allow others to do the same by permit. In addition, the Highway Authority and others using or working in the Right-of-Way under permit have the right to remove soil or groundwater from the Right-of-Way and dispose of the same in accordance with applicable environmental laws and regulations. The Highway Authority agrees to issue all permits for work in the

Right-of-Way, and make all existing permits for work in the Right-of-Way, subject to the following or a substantially similar condition:

As a condition of this permit the permittee shall request the office issuing this permit to identify sites in the Right-of-Way where a Highway Authority Memorandum of Agreement governs access to soil that exceeds the Tier 1 residential remediation objectives of 35 Ill. Adm. Code 742. The permittee shall take all measures necessary to protect human health (including worker safety) and the environment during and after any access to such soil.

10. This agreement shall be referenced in the Agency's no further remediation determination issued for the Release(s).

11. The Agency shall be notified of any transfer of jurisdiction over the Right-of-Way at least 30 days prior to the date the transfer takes effect. This agreement shall be null and void upon the transfer unless the transferee agrees to be bound by this agreement as if the transferee were an original party to this agreement. The transferee's agreement to be bound by the terms of this agreement shall be memorialized at the time of transfer in a writing ("Rider") that references this Highway Authority Memorandum of Agreement and is signed by the Highway Authority, or subsequent transferor, and the transferee.

12. This agreement shall become effective on the date the Agency issues a no further remediation determination for the Release(s). It shall remain effective until the Right-of-Way is demonstrated to be suitable for unrestricted use and the Agency issues a new no further remediation determination to reflect there is no longer a need for this agreement, or until the agreement is otherwise terminated or voided.

13. In addition to any other remedies that may be available, the Agency may bring suit to enforce the terms of this agreement or may, in its sole discretion, declare this agreement null and void if the Highway Authority or a transferee violates any term of this agreement. The Highway Authority or transferee shall be notified in writing of any such declaration.

14. This agreement shall be null and void if a court of competent jurisdiction strikes down any part or provision of the agreement.

15. This agreement supersedes any prior written or oral agreements or understandings between the Parties on the subject matter addressed herein. It may be altered, modified or amended only upon the written consent and agreement of the Parties.

16. Any notices or other correspondence regarding this agreement shall be sent to the Parties at following addresses:

Manager, Division of Remediation Management
Bureau of Land
Illinois Environmental Protection Agency

P.O. Box 19276
Springfield, IL 62974-9276

[Contact at Highway Authority]

[Address]

IN WITNESS WHEREOF, the Parties have caused this agreement to be signed by their duly authorized representatives.

[NAME OF LOCAL GOVERNMENT]

Date: _____ By: _____

Its: _____

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY

Date: _____ By: _____

Director

(Source: Added at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX F: Environmental Land Use Control

PREPARED BY:

Name: _____

_____

Address: _____

RETURN TO:

Name: _____

_____

Address: _____

THE ABOVE SPACE FOR RECORDER'S OFFICE

Model Environmental Land Use Control

THIS ENVIRONMENTAL LAND USE CONTROL ("ELUC"), is made this _____ day of _____, 20__, by _____, ("Property Owner") of the real property located at the common address_____("Property").

WHEREAS, 415 ILCS 5/58.17 and 35 Ill. Adm. Code 742 provide for the use of an ELUC as an institutional control in order to impose land use limitations or requirements related to environmental contamination so that persons conducting remediation can obtain a No Further Remediation determination from the Illinois Environmental Protection Agency ("IEPA"). The reason for an ELUC is to ensure protection of human health and the environment. The limitations and requirements contained herein are necessary in order to protect against exposure to contaminated soil, groundwater, or soil gas that may be present on the property as a result of [**VARIABLE**] activities. Under 35 Ill. Adm. Code 742, the use of risk-based, site-specific remediation objectives may require the use of an ELUC on real property, and the ELUC may apply to certain physical features (e.g., engineered barriers, indoor inhalation building control technologies, monitoring wells, caps, etc.).

WHEREAS, _____ [**the party performing remediation**] intends to request risk-based, site specific soil, groundwater, or soil gas remediation objectives from IEPA under 35 Ill. Adm. Code 742 to obtain risk-based closure of the site, identified by Bureau of Land [**10-digit LPC or Identification number**] _____ , utilizing an ELUC.

NOW, THEREFORE, the recitals set forth above are incorporated by reference as if fully

set forth herein, and the Property Owner agrees as follows:
Date: _____ By: _____

Director

Section One. Property Owner does hereby establish an ELUC on the real estate, situated in the County of _____, State of Illinois and further described in Exhibit A attached hereto and incorporated herein by reference (the "Property").

Attached as Exhibit B are site maps that show the legal boundary of the Property, any physical features to which the ELUC applies, the horizontal and vertical extent of the contaminants of concern above the applicable remediation objectives for soil, groundwater, or soil gas, and the nature, location of the source, and direction of movement of the contaminants of concern, as required under 35 Ill. Adm. Code 742.

Section Two. Property Owner represents and warrants **he/she** is the current owner of the Property and has the authority to record this ELUC on the chain of title for the Property with the Office of the Recorder or Registrar of Titles in _____ County, Illinois.

Section Three. The Property Owner hereby agrees, for **himself/herself**, and **his/her** heirs, grantees, successors, assigns, transferees and any other owner, occupant, lessee, possessor or user of the Property or the holder of any portion thereof or interest therein, that [**INSERT RESTRICTION (e.g. the groundwater under the Property shall not be used as a potable supply of water, and any contaminated groundwater or soil that is removed, excavated, or disturbed from the Property described in Exhibit A herein must be handled in accordance with all applicable laws and regulations**)].

Section Four. This ELUC is binding on the Property Owner, **his/her** heirs, grantees, successors, assigns, transferees and any other owner, occupant, lessee, possessor or user of the Property or the holder of any portion thereof or interest therein. This ELUC shall apply in perpetuity against the Property and shall not be released until the IEPA determines there is no longer a need for this ELUC as an institutional control; until the IEPA, upon written request, issues to the site that received the no further remediation determination a new no further remediation determination approving modification or removal of the limitation(s) or requirement(s); the new no further remediation determination is filed on the chain of title of the site subject to the no further remediation determination; and until a release or modification of the land use limitation or requirement is filed on the chain of title for the Property.

Section Five. Information regarding the remediation performed on the Property may be obtained from the IEPA through a request under the Freedom of Information Act (5 ILCS 140) and rules promulgated thereunder by providing the IEPA with the [10-digit LPC or identification number] listed above.

Section Six. The effective date of this ELUC shall be the date that it is officially recorded in the chain of title for the Property to which the ELUC applies.

WITNESS the following signatures:

Property Owner(s)

By:_____

Its: _____

Date: _____

STATE OF ILLINOIS)
) SS:
COUNTY OF)

I, _____the undersigned, a Notary Public for said County and State, DO HEREBY CERTIFY, that _____ and _____, personally known to me to be the Property Owner(s) of _____, and personally known to me to be the same persons whose names are subscribed to the foregoing instrument, appeared before me this day in person and severally acknowledged that in said capacities they signed and delivered the said instrument as their free and voluntary act for the uses and purposes therein set forth.

Given under my hand and official seal, this _____ day of _____, 20__.

Notary Public

STATE OF _____)
) S.S.
COUNTY OF _____)

I, _____, a notary public, do hereby certify that before me this day in person appeared _____, personally known to me to be the Property Owner(s), of _____, each severally acknowledged that they signed and delivered the foregoing instrument as the Property Owner(s) herein set forth, and as their own free and voluntary act, for the uses and purposes herein set forth.

Given under my hand and seal this _____ day of _____, 20__.

Notary Public

PIN NO. XX-XX-XXX-XXX-XXXX
(Parcel Index Number)

Exhibit A

The subject property is located in the City of _____, _____ County, State of Illinois, commonly known as _____, _____, Illinois and more particularly described as:
LIST THE COMMON ADDRESS;
LEGAL DESCRIPTION; AND
REAL ESTATE TAX INDEX OR PARCEL #
(PURSUANT TO SECTION 742. 1010(d)(2))

PIN NO. XX-XX-XXX-XXX-XXXX

Exhibit B

IN ACCORDANCE WITH SECTION 742.1010(d)(8)(A) through (D), PROVIDE ALL THE FOLLOWING ELEMENTS. ATTACH SEPARATE SHEETS, LABELED AS EXHIBIT B, WHERE NECESSARY.

(A) A scaled map showing the legal boundary of the property to which the ELUC applies.

(B) Scaled maps showing the horizontal and vertical extent of contaminants of concern above the applicable remediation objectives for soil, groundwater, and soil gas to which the ELUC applies.

(C) Scaled maps showing the physical features to which an ELUC applies (e.g., engineered barriers, indoor inhalation building control technologies, monitoring wells, caps, etc.).

(D) Scaled maps showing the nature, location of the source, and direction of movement of the contaminants of concern.

(Source: Amended at 37 Ill. Reg. 7506, effective July 15, 2013)

Section 742.APPENDIX G Model Ordinance

ORDINANCE NUMBER _____

AN ORDINANCE PROHIBITING THE USE OF GROUNDWATER AS A POTABLE WATER SUPPLY BY THE INSTALLATION OR USE OF POTABLE WATER SUPPLY WELLS OR BY ANY OTHER METHOD

WHEREAS, certain properties in the City [Village] of _____, Illinois have been used over a period of time for commercial/industrial purposes; and

WHEREAS, because of said use, concentrations of certain chemical constituents in the groundwater beneath the City [Village] may exceed Class I groundwater quality standards for potable resource groundwater as set forth in 35 Illinois Administrative Code 620 or Tier 1 remediation objectives as set forth in 35 Illinois Administrative Code 742; and

WHEREAS, the City [Village] of _____ desires to limit potential threats to human health from groundwater contamination while facilitating the redevelopment and productive use of properties that are the source of said chemical constituents;

NOW, THEREFORE, BE IT ORDAINED BY THE CITY COUNCIL OF THE CITY [VILLAGE] OF _____, ILLINOIS:

Section One. Use of groundwater as a potable water supply prohibited.

[Except for such uses or methods in existence before the effective date of this ordinance,] The use or attempt to use as a potable water supply groundwater from within the corporate limits of the City [Village] of _____, as a potable water supply, by the installation or drilling of wells or by any other method is hereby prohibited. This prohibition [expressly includes] [does not include] the City [Village] of _____.

Section Two. Penalties.

Any person violating the provisions of this ordinance shall be subject to a fine of up to _____ for each violation.

Section Three. Definitions.

"Person" is any individual, partnership, co-partnership, firm, company, limited liability company, corporation, association, joint stock company, trust, estate, political subdivision, or any other legal entity, or their legal representatives, agents or assigns.

"Potable water" is any water used for human or domestic consumption, including, but not limited to, water used for drinking, bathing, swimming, washing dishes, or preparing foods.

Section Four. Memorandum of Understanding.

'[This Section is only necessary if ordinance does not expressly prohibit installation of potable water supply wells by the city or village--could be separate resolution]

The Mayor of the City [Village] of _____ is hereby authorized and directed to enter into a Memorandum of Understanding with the Illinois Environmental Protection Agency ("Illinois EPA") in which the City [Village] of _____ assumes responsibility for tracking all sites that have received no further remediation determinations from the Illinois EPA, notifying the Illinois EPA of changes to this ordinance, and taking certain precautions when siting public potable water supply wells.

Section Five. Repealer.

All ordinances or parts of ordinances in conflict with this ordinance are hereby repealed insofar as they are in conflict with this ordinance.

Section Six. Severability.

If any provision of this ordinance or its application to any person or under any circumstances is adjudged invalid, such adjudication shall not affect the validity of the ordinance as a whole or of any portion not adjudged invalid.

Section Seven. Effective date.

This ordinance shall be in full force and effect from and after its passage, approval and publication as required by law.

ADOPTED: _____ APPROVED: _____

(Date) (Date)

_____ _____

(City Clerk) (Mayor)

Officially published this _____ day of _____, 20___.

(Source: Added at 31 Ill. Reg. 4063, effective February 23, 2007)

Section 742.APPENDIX H Memorandum of Understanding

MEMORANDUM OF UNDERSTANDING BETWEEN _____ AND THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY REGARDING THE USE OF A LOCAL GROUNDWATER OR WATER WELL ORDINANCE AS AN ENVIRONMENTAL INSTITUTIONAL CONTROL

I. PURPOSE AND INTENT

A. This Memorandum of Understanding ("MOU") between _____ and the Illinois Environmental Protection Agency ("Illinois EPA") is entered into for the purpose of satisfying the requirements of 35 Ill. Adm. Code 742.1015 for the use of groundwater or water well ordinances as environmental institutional controls. The Illinois EPA has reviewed the groundwater or water well ordinance of _____ (Attachment A) and determined that the ordinance prohibits the use of groundwater for potable purposes and/or the installation and use of new potable water supply wells by private entities but does not expressly prohibit those activities by the unit of local government itself. In such cases, 35 Ill. Adm. Code 742.1015(a) provides that the unit of local government may enter into an MOU with the Illinois EPA to allow the use of the ordinance as an institutional control.

B. The intent of this Memorandum of Understanding is to specify the responsibilities that must be assumed by the unit of local government to satisfy the requirements for MOUs as set forth at 35 Ill. Adm. Code 742.1015(i).

II. DECLARATIONS AND ASSUMPTION OF RESPONSIBILITY

In order to ensure the long-term integrity of the groundwater or water well ordinance as an environmental institutional control and that risk to human health and the environment from contamination left in place in reliance on the groundwater or water well ordinance is effectively managed, _____ hereby assumes the following responsibilities pursuant to 35 Ill. Adm. Code 742.1015(d)(2) and (i):

A. _____ will notify the Illinois EPA Bureau of Land of any proposed ordinance changes or requests for variance at least 30 days prior to the date the local government is scheduled to take action on the proposed change or request (35 Ill. Adm. Code 742.1015(i)(4));

B. _____ will maintain a registry of all sites within its corporate limits that have received "No Further Remediation" determinations in reliance on the ordinance from the Illinois EPA (35 Ill. Adm. Code 742.1015(i)(5));

C. _____ will review the registry of sites established under paragraph II. B. prior to siting public potable water supply wells within the area covered by the ordinance (35 Ill. Adm. Code 742.1015(i)(6)(A));

D. _____ will determine whether the potential source of potable water has been or may be affected by contamination left in place at the sites tracked and reviewed under paragraphs II. B. and C. (35 Ill. Adm. Code 742.1015(i)(6)(B)); and

E. _____ will take action as necessary to ensure that the potential source of potable water is protected from contamination or treated before it is used as a potable water supply (35 Ill. Adm. Code 742.1015(i)(6)(C)).

NOTE: Notification under paragraph II. A. above or other communications concerning this MOU should be directed to:

Manager, Division of Remediation Management
Bureau of Land
Illinois Environmental Protection Agency
P.O. Box 19276
Springfield, IL 62794-9276

III. SUPPORTING DOCUMENTATION

The following documentation is required by 35 Ill. Adm. Code 742.1015(i) and is attached to this MOU:

A. Attachment A: A copy of the groundwater or water well ordinance certified by the city clerk or other official as the current, controlling law (35 Ill. Adm. Code 742.1015(i)(3));

B. Attachment B: Identification of the legal boundaries within which the ordinance is applicable (certification by city clerk or other official that the ordinance is applicable everywhere within the corporate limits; if ordinance is not applicable throughout the entire city or village, legal description and map of area showing sufficient detail to determine where ordinance is applicable) (35 Ill. Adm. Code 742.1015(i)(2));

C. Attachment C: A statement of the authority of the unit of local government to enter into the MOU (council resolution, code of ordinances, inherent powers of mayor or other official signing MOU -- attach copies) (35 Ill. Adm. Code 742.1015(i)(1)).

IN WITNESS WHEREOF, the lawful representatives of the parties have caused this MOU to be signed as follows:

FOR: _____
(Name of city or village)

BY: _____ DATE: _____
(Name and title of signatory)

FOR: Illinois Environmental Protection Agency

BY: _____ DATE: _____
Manager, Division of Remediation Management
Bureau of Land

(Source: Added at 31 Ill. Reg. 4063, effective February 23, 2007)

Attachment

1A

2

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	R08-18
PROPOSED AMENDMENTS TO	)	(Rulemaking-Public Water Supplies)
GROUNDWATER QUALITY	)	
STANDARDS	)	
(35 Ill. Adm. Code 620)	)	

NOTICE

Dorothy Gunn, Clerk
Illinois Pollution Control Board
James R. Thompson Center
100 W. Randolph St., Suite 11-500
Chicago, Illinois 60601

Bill Richardson
Chief Legal Counsel
Illinois Dept. of Natural Resources
One Natural Resources Way
Springfield, Illinois 62702-1271

Matt Dunn
Environmental Bureau Chief
Office of the Attorney General
James R. Thompson Center
100 W. Randolph, 12th Floor
Chicago, Illinois 60601

Richard McGill
Illinois Pollution Control Board
James R. Thompson Center
100 W. Randolph St., Suite 11-500
Chicago, Illinois 60601

Service List

PLEASE TAKE NOTICE that I have today filed with the Office of the Clerk of the Illinois Pollution Control Board the Illinois Environmental Protection Agency's Errata Sheet Number 2 and Pre-filed Testimony of Richard P. Cobb and Thomas Hornshaw a copy of which is herewith served upon you.

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: Kimberly A. Geving

Kimberly A. Geving
Assistant Counsel
Division of Legal Counsel

DATED: May 28, 2008

1021 North Grand Ave. East
P.O. Box 19276
Springfield, Illinois 62794-9276
(217) 782-5544

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
PROPOSED AMENDMENTS TO	)	R08-18
GROUNDWATER QUALITY	)	(Rulemaking-Public Water Supplies)
STANDARDS	)	
(35 Ill. Adm. Code 620)	)	
	)	

ERRATA SHEET NUMBER 2

NOW COMES the Illinois Environmental Protection Agency through one of its attorneys, Kimberly Geving, and submits this ERRATA SHEET NUMBER 2 to the Illinois Pollution Control Board and the participants on the Service List. Please note that the errata changes reflect amendments to our proposal as submitted to the Board on February 15, 2008 and not to the existing rule.

Tom Hornshaw or Rick Cobb will provide testimony in support of these changes at the first hearing.

Section		
620.410(b)	Anthracene	0.043 ~~0.0434~~
	Benzo(k)fluoranthene	0.00017 ~~0.0008~~
	Chrysene	0.0063 ~~0.0016~~
	1,1-Dichloroethane	1.4 ~~0.7~~
	Fluoranthene	0.21 ~~0.206~~
	Indeno(1,2,3-cd)pyrene	0.000022 ~~0.00043~~
	2-Methylnaphthalene	0.028 ~~0.0028~~
	p-Dioxane	0.0077 ~~0.005~~
	Pyrene	0.21 ~~0.135~~
	2,4-Dinitrotoluene	0.0001 ~~0.00002~~
620.420(b)	Anthracene	0.043 ~~0.0434~~
	Benzo(a)pyrene	0.0016 ~~0.00162~~
	Chrysene	0.0063 ~~0.0016~~
	1,1-Dichloroethane	7.0 ~~3.5~~
	Fluoranthene	0.21 ~~0.206~~
	Indeno(1,2,3-cd)pyrene	0.000022 ~~0.00043~~
	p-Dioxane	0.0077 ~~0.005~~
	Pyrene	1.05 ~~0.135~~

2,4-Dinitrotoluene	0.0001 ~~0.00002~~

Respectfully submitted,

ILLINOIS ENVIRONMENTAL
PROTECTION AGENCY

By: _____
Kimberly A. Geving
Assistant Counsel
Division of Legal Counsel

DATED: May 28, 2008

1021 North Grand Avenue East
P.O. Box 19276
Springfield, Illinois 62794-9276
(217) 782-5544

BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
GROUNDWATER	)	R08-18
QUALITY STANDARDS	)	
AMENDMENTS:	)	(Rulemaking-Public Water Supplies)
35 ILL. ADM. CODE 620	)	

TESTIMONY OF RICHARD P. COBB, P.G., ON NEW PROPOSED GROUNDWATER QUALITY STANDARDS

My name is Richard P. Cobb. I am a licensed professional geologist and the Deputy Manager of the Division of Public Water Supplies of the Illinois Environmental Protection Agency's ("EPA") Bureau of Water ("BOW"). My primary responsibilities include managing the Groundwater and Source Water Protection, Field Operations, and the Administrative Sections of the Division. Further, I assist with administering the public water supervision program under the federal Safe Drinking Water Act ("SDWA"). Additionally, my responsibility includes the integration of source water protection with traditional water supply engineering and treatment practices, and to further assist with linking Clean Water Act and SDWA programs. I also directly manage the BOW's Groundwater Section. The groundwater section applies Geographic Information System ("GIS") programs, global positioning system technology, hydrogeologic models (including, 3D geologic visualization, vadose zone, groundwater flow, particle tracking, solute transport, and geochemical models), and geostatistical programs for groundwater protection and remediation. I represent the BOW on Illinois EPA's Contaminant Evaluation Group, Strategic Management Planning Team, Environmental Justice Committee, Information Management Steering Committee, and GIS Steering Committee. Since 1985 I have worked on the development of legislation, rules, and regulations. I have also served as a primary Agency witness at Illinois

Pollution Control Board ("Board") proceedings in the matter of groundwater quality standards, technology control regulations, regulated recharge areas, maximum setback zones, clean-up regulations, and water well setback zone exceptions. Furthermore, I have served as a primary Agency witness in enforcement cases under these laws and regulations.

For further detail on my qualifications I have enclosed a copy of my Curriculum Vitae in Attachment I. This testimony, the statement of reasons, and attachments included with this testimony describe the basis for the proposed regulations.

I. PURPOSE OF THE PROPOSAL

The purpose of my testimony today is to provide the background required to propose new groundwater quality standards ("Standards") under Section 8 of the Illinois Groundwater Protection Act ("IGPA") (415 ILCS 55/8(b)(1) (1998)). The term Standards includes the nondegradation requirements for Class I and Class III groundwater as specified by the Board's adopted regulations at:

> SUBPART D: GROUNDWATER QUALITY STANDARDS
>
> Section 620.401 Applicability
>
> Groundwaters must meet the standards appropriate to the groundwater's class as specified in this Subpart and the nondegradation provisions of Subpart C.

Further, the Board's Groundwater Standards Final Opinion and Order (R89-14 (B)), November 1991, on Page 16 had this to say about nondegradation:

> It has sometimes been said that casting the nondegradation provision as it is today is equivalent to allowing pollution up to the standard. The Board believes that this characterization is too simplistic. Among other matters, the whole preventive notification and response program (see following) is directed toward an early alert to, and staving off of, **any increase in contamination in the sensitive groundwater/potential source situations.** (Emphasis added)

Dr. Thomas Hornshaw, Manager of the Illinois EPA's Toxicity Assessment Unit, will provide the supporting toxicological basis for the proposed groundwater standards where appropriate.

II. BACKGROUND

Since the inception of the Illinois Environmental Protection Act ("Act") in 1970, it has been the policy of the State of Illinois to restore, protect, and enhance the groundwater of the State as a natural and public resource. Groundwater has an essential and pervasive role in the social and economic well-being of Illinois, and it is vitally important to general health, safety, and welfare. Groundwater resources should be utilized for beneficial and legitimate purposes; waste and degradation of the resource should be prevented; and the underground water resource should be managed to allow for maximum benefit of the State. Groundwater used as drinking water is one of the highest beneficial uses of the groundwater resource. The IGPA defines "Resource groundwater" as groundwater that is presently being or in the future capable of being put to beneficial use by reason of being of suitable quality (415 ILCS 55/3(j)).

The Act included Sections 11 and 12(a). Section 11 describes part of the purpose of Title III, as follows:

> "...assure that no contaminants are discharged into waters without being given the degree of treatment or control necessary to prevent pollution."

Section 11(b) of the 2005 Act includes the same purpose statement. Water pollution was defined in Section 3(a) of the 1970 version of the Act the same as it is to this day. Moreover, Section 12(a) of the 1970 version of the Act includes the following:

> **" No person shall: (a) cause, threaten or allow the discharge of contaminants into the environment in any State so as to cause or tend to cause water pollution** in Illinois, either alone or in combination with matter from other sources,

> or so as to violate regulations or standards adopted by the Pollution Control Board under this Act." (Emphasis added)

The term "threat" in Section 12(a) of the Act established Illinois' original narrative nondegradation standard. The Board's final order and opinion, for 35 Ill. Adm. Code: Subtitle C, indicated that:

> "...Standards are **applicable to groundwaters that are a present or are a potential source of water for potable use or for food** processing, except where deviation is due to natural causes. It is significant to note that these standards **apply in situ; that is they are ambient water quality standards. They also apply irrespective of whether they are used by a public water supplier, a private water supplier, or have the potential for being so used.** (Emphasis added).

Additionally, the Board's opinion, in regard to *Water Quality Standards Revisions* (#R71-14), and *Water Quality Standards for Intrastate Waters* (SWB-14) (#R71-20) indicated the following:

> "203 General Standards. Today's revision is based upon the principle that **all waters should be protected against nuisances and against health hazards to those near them;** that all waters naturally capable of supporting aquatic life, with the exception of a few highly industrialized streams consisting primarily of effluents in the Chicago area, should be protected to support such life; **and that waters that are used for public water supply should be clean enough that ordinary treatment processes will assure their potability..."**
>
> "...**Since general criteria apply to all waters designated for public water supply**, the present regulation omits separate requirements for those parameters whose general standards are tight enough to protect public water supplies: boron, chromium, copper, fluoride, mercury, silver and zinc. The remaining standards are based largely upon Public Health Service standards, as amplified by the Green Book and by McKee and Wolf. While the PHS explicitly states that its standards are intended to prescribe the quality of finished rather than of raw water, **it is clear from the evidence that many of the metals and other contaminants here listed are not substantially affected by ordinary water supply treatment, and therefore, as the Green Book recommends, the raw water must itself meet the standard to assure satisfactory finished water." (Emphasis added)**

The phrase "ordinary treatment processes", emphasized in the Board's opinion above, is one of the keys to understanding Illinois' nondegradation requirements for groundwater. First, it is

important to note that there is a significant difference between what is considered ordinary treatment processes for surface water versus groundwater sources of drinking water. All community water systems ("CWS") using surface water apply coagulation, sedimentation, filtration, disinfection, and treatment for taste and odor. Private drinking water systems do not use surface water as a source of drinking water, due to the inherent vulnerability of surface water resources to contamination and the associated cost for treating such water. A private drinking water system is defined as a system that serves an owner occupied single family dwelling (415 ILCS 55/9(a)). Secondly, there is a significant difference between what is considered ordinary treatment processes for a small CWS using groundwater versus a private drinking water system well. The small CWS using groundwater has more treatment infrastructure resources available than the owner of a private well. Lastly, a private well owner typically only has to chlorinate his or her well to use the groundwater for potable uses. Thus, this defines the lowest common denominator of what ordinary treatment processes means to the protection of Class I: Potable Resource Groundwater. In other words, the Act and Board regulations prohibit a person from causing, threatening or allowing contamination of potable resource groundwater above what is not removed by ordinary treatment processes in a private drinking water system well. For example, a plume of tritium at a concentration above background or naturally occurring levels, moving toward a private drinking water system well, is considered a threat to diminishing the existing Class I groundwater resource, since tritium cannot be removed by advanced treatment processes let alone ordinary treatment processes. This diminishment of resource groundwater (415 ILCS 55/3(j)) may lead to preclusion of the use of the well if the private well owner chooses not to use it (e.g., suitability) due to the contamination.

The Illinois Supreme Court also determined the following in *Central Illinois Public Service Company v. Pollution Control Board*, 116 Ill.2d 397:

> The Board, at the outset, disagrees with CIPS' interpretation of the definition of water pollution in the Act. **The Board argues that the Act treats water as a resource, and that pollution occurs whenever contamination is likely to render water unusable. Under the Board's interpretation there is not need to show that harm *will* occur, only that harm *would* occur if the contaminated water were to be used.** Since the Board is charged with administering the Environmental Protection Act, its interpretation of the statute is entitled to deference. (*Massa v. Department of Registration & Education* (1987), 116 Ill.2d 376, 107 Ill.Dec, 661, 507 N.E.2d 814; *Illinois Consolidated Telephone Co. v. Illinois Commerce Com.* (1983), 95 Ill.2d 142, 152, 69 Ill.Dec. 78, 447 N.E.2d 295.) **Under the Board's view any contamination which prevents the State's water resources from being usable would constitute pollution, thus allowing the Board to protect those resources from necessary diminishment.** CIPS' interpretation, on the other hand would mean that water rendered unusable would not be polluted so long as use of the water ceased subsequent to contamination. **We find the Board's interpretation preferable to CIPS' interpretation, especially considering the deference we must accord to the Board.** (Emphasis added)

The Illinois EPA framed and the Board adopted the following after the legal cornerstones detailed above:

> Section 620.301 General Prohibition Against Use Impairment of Resource Groundwater
>
> a) **No person shall cause, threaten or allow the release of any contaminant to a resource groundwater such that:**
>
> 1) Treatment or additional treatment is necessary to continue an existing use or to assure a potential use of such groundwater; or
>
> **2) An existing or potential use of such groundwater is precluded.** (Emphasis added)

In summary, the conclusions and facts, provided above, clearly provide the supporting foundation for Standards, as follows:

- The original Act and regulations establish that no person shall discharge contaminants that threaten, cause or allow contamination;
- The intent of this multi-tiered standard is to prevent degradation of the resource up to the numerical standard;
- The Board clearly established that current and potential sources of potable resource groundwater were to be protected;
- Section 12(a) of the Act is integrated with Board regulations to prohibit polluting up to the numerical standards in such regulations;
- The Board's opinions in the matters, quoted above, indicate that resource groundwater should be protected such that a private water supply would be able to obtain clean drinking water through ordinary treatment processes;
- The Board's opinion on Illinois' Groundwater Protection Plan is "...that unreasonable waste and degradation of the resources be prohibited;"
- Public Act 85-863 indicates that waste and degradation of the resources be prevented and includes this as a factor that the Board must consider in adopting comprehensive groundwater quality standard regulations;
- The Illinois Supreme Court has upheld the Board's view that any contamination that prevents the State's water resources from being usable would constitute pollution, thus allowing the Board to protect those resources from unnecessary diminishment; and
- The Board's final opinion and order on groundwater quality standards indicates that the numerical standard is not meant to be a level to pollute up to and included

specific preventive standards prohibiting contamination above detectable levels that threaten a preclusion of use.

III. CONTAMINANTS COMMONLY DETECTED IN ILLINOIS GROUNDWATER

The Illinois EPA evaluated the electronically reported groundwater data for all Illinois Resource Conservation and Recovery Act ("RCRA") and Solid Waste facilities under our purview. The Illinois EPA Bureau of Land ("BOL") Permit Section provides regulatory oversight for these facilities. A data query was conducted for a list of approximately 300 groundwater parameters not included in 35 Ill. Adm. Code 620, Subpart C. The data set evaluated spanned from April 1984 to April 2004. Illinois EPA has also evaluated confirmed groundwater contaminants at various cleanup sites.

The Illinois EPA is proposing a groundwater standard for perchlorate based on the new reference dose published by United States Environmental Protection Agency ("U.S. EPA") and recommended by the National Academy of Science ("NAS"). The Illinois EPA proposes to use the default Relative Source Contribution ("RSC") term of 20 percent. Additionally, Illinois EPA is proposing an amendment to the existing groundwater standard of 0.050 milligrams per liter ("mg/l") for arsenic based on the amendment of the federal drinking water standard or maximum contaminant level ("MCL") for arsenic to 0.010 mg/l. The Board MCL for drinking water has already been amended to 0.010 mg/l at 35 Ill. Adm. Code 611.301.

Arsenic is a naturally occurring element in the earth's crust. Arsenic is found in the deep bedrock materials throughout Illinois, as well as in the shallow glacial materials that cover the northern two-thirds of the State. Arsenic is dissolved naturally from these materials and enters groundwater. For further detail, see *Arsenic in Illinois Groundwater*, developed by Rick Cobb,

Ken Runkle of IDPH and Steve Wilson of the Illinois State Water Survey ("ISWS"), published on November 20 2001, in Attachment II and at http://www.epa.state.il.us/water/groundwater/publications/arsenic/index.html.

There are also a number of incorporations by reference that need to be updated in the Board's groundwater quality standards regulation.

IV. THE AGENCY'S PROPOSAL

A. Section 620.110 Definitions

Illinois EPA proposes that the federal register reference (56 Fed. Reg. 3526-3597) in the Method Detection Limit ("MDL") portion of the "Detection" definition should be replaced by 40 CFR Part 136, Appendix B (2006) citation.

Illinois EPA also proposes to add the "Wellhead protection area" or "WHPA" definition from 35 Ill. Adm. Code 611.101 to 35 Ill. Adm. Code 620.110. This definition is used in conjunction with 2 new proposed incorporations by reference. These new provisions support an amendment to Subsection 620.505(a)(5) for enhancing the hydrogeologic processes in that Section. A WHPA represents a CWS well's recharge area delineated beyond an applicable setback zone, as described in Subsection 17.1(a)(2) of the Act (415 ILCS 5/17.1(a)(2)). Hydrogeologists refer to this as a "contributing recharge area." Groundwater modelers also call this a "capture zone." These terms are used interchangeably by hydrogeologists. In addition, the definition of "Wellhead Protection Program" ("WHPP"), also codified by the Board under 35 Ill. Adm. Code 611.101, is also proposed to be added here since it elaborates on the definition of WHPA, and the two new incorporations by reference that are being proposed. This existing Board definition also provides the nexus between a WHPP and a groundwater protection needs assessment.

There were also amendments made to existing Board definitions due to the revisions of the cross referenced citations in the Act.

B. Section 620.125 Incorporations by Reference

There are new references that need to be added to the Incorporations by Reference in the Board's Standards. The American Society for Testing and Materials ("ASTM") standard has been revised. Further, changes have been proposed because the Federal Register citations have been codified into the Code of Federal Regulations ("CFR"). In addition, many new supplements have been adopted and included in 40 CFR. Additionally, original references have been updated. Further, the sources from which this information is obtained has changed and has been revised. Illinois also proposes a new incorporation by reference to include Illinois' Approved Wellhead Protection Program Document and Guidance Document for Groundwater Protection Needs Assessment because they are used to improve hydrogeologic assessment aspects of Section 620.505 Compliance Determination provisions.

C. The word "or" needs to be deleted from Section 620.201(a)(4).

D. Section 620.310(a)(3)(A)(ii) Preventive Response Activities

Illinois EPA proposes that the Board amend this subsection to include preventive response levels for the following contaminants, which are not classified as carcinogens, with proposed Class I standards based on a statistical increase above background:

> Acenaphthene; acetone; anthracene; benzoic acid; carbon disulfide; 2-butanone (MEK); dicamba, dichlorodifluoromethane; 1,1-dichloroethane; diethylphthalate; di-n-butyl phthalate; di-n-octylphthalate; fluoranthene; fluorine; isopropylbenzene (Cumene); MCPP (Mecoprop); 2-methylnaphthalene; 2-methylphenol; molybdenum; naphthalene; pyrene; trichlorofluoromethane; and vanadium.

E. Section 620.410(a)

The standards proposed below are based on either a U.S.EPA or Board MCL, a reference dose ("RfD") in U.S. EPA's Integrated Risk Information System ("IRIS"), U.S. EPA Health Effects Assessment Summary Table ("HEAST") RfD, Provisional Peer Reviewed Toxicity Values ("PPRTV") RfD, IRIS Slope Factor ("SFo"), or MDL used to derive the 35 Ill. Adm. Code 620, Subpart F, Appendix A: Human Threshold Toxicant Advisory Concentration for Tiered Approach for Correction Objectives ("TACO") (35 Ill. Adm. Code 742) groundwater ("GW") objectives. In addition, we are proposing to amend the existing Subpart F procedures to establish a new procedure that utilizes a 10^{-6} risk level versus the default PQL that was used in the 1980's. Many of the PQLs have dropped significantly and may be well below the respective10-6 risk level. Since 1991, the 10^{-6} risk level has become widely accepted and is used in the Board's TACO regulations. In addition to the 10^{-6} risk level, water solubility is being proposed as a factor. If an organic chemical is present at concentrations in groundwater exceeding its solubility a two-phase system may exist, and the behavior and migration of the chemical in such a system may be difficult to predict. Thus, the water solubility is the proposed basis for several contaminants where the 10^{-6} risk level exceeds water solubility. Dr. Hornshaw's testimony will go into more detail on these proposed amendments.

Carcinogens are denoted in the proposed Class I standards by an asterisk ("*"). Illinois EPA proposes that the Board amend Subsection 620.410(a) to include a Class I: Potable Resource Groundwater Standard for the following inorganic contaminants listed below:

Inorganic Chemicals *Denotes a carcinogen	Proposed Class I Standard (mg/l)	Basis
Arsenic*	0.010	Board and U.S. EPA MCL
Molybdenum	0.035	IRIS RfD
Perchlorate	0.0049	IRIS RfD
Vanadium	0.049	TACO GW Objective

F. Section 620.410(b)

The Illinois EPA proposes to the Board that this subsection be amended to include a Class I: Potable Resource Groundwater Standard for the following organic contaminants:

Volatile Organic Compounds ("VOCs") * Denotes a carcinogen	Proposed Class I Standard (mg/l)	Basis
Acetone	6.3	TACO GW Objective
2-Butanone (MEK)	4.2	IRIS RfD
Carbon disulfide	0.7	TACO GW Objective
Chloroform*	0.0002	TACO GW Objective
Dichlorodifluoromethane	1.4	IRIS RfD
1,1-Dichloroethane	1.4	PPRTV RfD
Isopropylbenzene (Cumene)	0.7	IRIS RfD
Trichlorofluoromethane	2.1	IRIS RfD

Semivolatile Organic Compounds * Denotes a carcinogen	Proposed Class I Standard (mg/l)	Basis
Acenaphthene	0.42	TACO GW Objective
Anthracene	0.043	Water Solubility
Benzo(a)anthracene*	0.00013	TACO GW Objective
Benzo(b)fluoranthene*	0.00018	TACO GW Objective
Benzo(k)fluoranthene*	0.00017	TACO GW Objective
Benzoic acid	28.0	TACO GW Objective
Chrysene*	0.0063	Water Solubility
Dibenzo (a,h,) anthracene*	0.0003	TACO GW Objective
Diethyl phthalate	5.6	TACO GW Objective
Di-n-butyl phthalate	0.7	TACO GW Objective
Di-n-octyl phthalate	0.02	Water Solubility
Fluoranthene	0.21	Water Solubility
Fluorene	0.28	TACO GW Objective
Indeno(1,2,3-cd)pyrene*	0.000022	Water solubility

2-Methylnaphthalene	0.028	IRIS RfD
2-Methylphenol	0.35	TACO GW Objective
Naphthalene	0.14	TACO GW Objective
p-Dioxane*	0.0077	10^{-6} cancer risk
Pyrene	0.21	TACO GW Objective
Pesticides/PCBs * Denotes a carcinogen		
alpha-BHC*	0.00011	TACO GW Objective
Dicamba	0.21	IRIS RfD
MCPP (Mecoprop)	0.007	IRIS RfD

G. Section 620.410(c)

The Illinois EPA proposes that the Board amend the Class I: Potable Resource Groundwater Standards to include a new subsection to for the following explosive contaminants:

Explosives * Denotes a carcinogen	**Proposed Class I Standard (mg/l)**	**Basis**
1,3-Dinitrobenzene	0.0007	IRIS RfD
2,4-Dinitrotoluene*	0.0001	10^{-6} Cancer risk level
2,6-Dinitrotoluene*	0.00031	TACO GW Objective
HMX	1.4	IRIS RfD
Nitrobenzene	0.0035	TACO GW Objective
RDX	0.084	IRIS RfD
1,3,5-Trinitrobenzene	0.84	IRIS RfD
2,4,6-Trinitrotoluene	0.014	IRIS RfD

H. Section 620.420(a)

The final opinion and order of the Board (Docket R89-14(B)), for establishing Class II groundwater quality standards (35 Ill. Adm. Code 620), published November 7, 1991, pages 19 and 20 states that:

> Section 620.420 establishes standards for Class II: General Resource Groundwaters. Because groundwaters are placed in Class II because they are quality-limited, quantity-limited, or both (see Subpart B discussion above), it is necessary that the standards that apply to these waters reflect this range of possible attributes. Among the factors considered in determining the Class II numbers are the capabilities of

treatment technologies to bring Class II waters to qualities suitable for potable use (R3 at 75) ["R3" means the transcript from the Board's May 1991 hearing on this matter, and "at 75" is page 75]. Thus, many Class II standards are based on MCL's as modified to reflect treatment capabilities. For some parameters the Class II standards are based on support of a use other then potability (e.g., livestock water, irrigation, industrial use) where a different use requires a more stringent standard (R3 at 1148)).

The same principle is applied to these proposed Class II standards here today. The standards proposed below are based on irrigation and livestock watering from the National Academy of Sciences, 1972, *Water Quality Criteria*.

Illinois EPA proposes that Subsection 620.410(a) be amended to include a Class II: General Resource Groundwater Standard for the following inorganic chemicals:

Inorganic Chemicals	Reference Proposed Class I Standard (mg/l)	Proposed Class II Standard (mg/l)	Basis for Class II
Arsenic	0.010	0.2	Irrigation
Molybdenum	0.035	0.035	Class I standard (Irrigation criterion is 10)
Perchlorate	0.0049	0.0049	0X
Vanadium	0.049	0.1	Irrigation

I. Section 620.420(b)

Most of the original groundwater standards for organic compounds were based on U.S. EPA MCLs. Best Available Treatment ("BAT") technology removal efficiencies are published in the CFR and 35 Ill. Adm. Code 611, Subpart F. However, none of the contaminants in this proposal, with the exception of arsenic standard, has a codified MCL. Thus, the Illinois EPA used some of the same factors used by U.S. EPA to develop BAT surrogates.

Air Stripping - Due to the high volatility of many of the organic compounds, air stripping is an efficient and cost effective treatment technology (35 Ill. Adm. Code 611, Subpart F). Air

stripping is a proven, effective means to remove VOCs from groundwater as detailed in 35 Ill. Adm. Code 611, Subpart F. Less volatile compounds (e.g., low Henry's Law Constants) are not as easily removed via air stripping (Canter, L.W., and R.C. Knox, 1985, (*Ground water pollution control*, Lewis Publishers). In contrast, compounds with lower Henry's Law Constants are more difficult to remove by air stripping than compounds with high constants. Methylene chloride has one of the lowest Henry's Law Constants at 8.98 X 10^{-2} unit less at 20° Celsius ("C") (35 Ill. Adm. Code 742) in comparison to other VOCs. Where multiple VOCs are present, the compound with the lowest Henry's Law Constant will generally be the limiting compound in the design of the air stripper.

Carbon Adsorption - Carbon adsorption is also is an efficient and cost effective treatment technology (35 Ill. Adm. Code 611, Subpart F) for removing various organic contaminants. Activated carbon is widely used to remove organic compounds (American Water Works Association, 1995, *Water Treatment*). The process of adsorption onto activated carbon requires the contaminated groundwater to come into contact with carbon, which selectively adsorbs organic constituents by a surface attraction phenomenon (due to chemical and physical properties). The organic molecules are attracted to the internal pores of the carbon granules (U.S. EPA, 1989). A coefficient referred to as partition or sorption coefficient (Log K_{oc}) represents the ratio of adsorbed chemical per unit weight of organic carbon to the aqueous concentration (Montgomery, J.H., and L.M. Welkom, 1995, *Groundwater chemicals desk reference*, Volume I, Lewis Publishers.). This value provides an indication of the tendency of a chemical to partition between organic carbon particles and water (Montgomery, 1995). Compounds that bind strongly to organic carbon have characteristically low solubilities, whereas compounds, such as methyl

tertiary butyl ether ("MTBE"), with low tendencies to adsorb to organic particles have high solubilities. Correlations between K_{oc} and the solubility of organic compounds in water have shown a log-log linear relationship (Montgomery, 1995).

Treatment Factor - A five fold treatment factor ("5X") was used to derive a proposed Class II standard for organic compounds with a K_{oc} value greater than (>) ethylbenzene's K_{oc} of 363 liters per kilogram at 20° C or a Henry's Law constant greater than methylene chloride's (8.98 X 10^{-2} unit less at 20° C). A five fold treatment factor equates to a removal efficiency of 80%. This is a very economical approach, since most of the BATs achieve a 99% removal rate. The Class I standard was proposed where either or both were below the factors detailed above.

Illinois EPA proposes that the Board amend Subsection 620.420(b) to include a Class II: General Resource Groundwater Standard for the following organic compounds:

Volatile Organic Compounds	Reference Proposed Class I Standard (mg/l)	Proposed Class II Standard (mg/l)	Basis For Class II
Acetone	6.3	6.3	TACO GW Objective
2-Butanone (MEK)	4.2	4.2	0X
Carbon disulfide	0.7	3.5	TACO GW Objective
Chloroform	0.0002	0.001	TACO GW Objective
Dichlorodifluoromethane	1.4	7.0	5X
1,1-Dichloroethane	1.4	7.0	5X
Isopropylbenzene (Cumene)	0.7	3.5	5X
Trichlorofluoromethane	2.1	10.5	5X
Semivolatile Organic Compounds			
Acenaphthene	0.42	2.1	TACO GW Objective
Anthracene	0.043	0.043	Water Solubility
Benzo(a)anthracene	0.00013	0.00065	TACO GW Objective
Benzo(b)fluoranthene	0.00018	0.0009	TACO GW Objective
Benzo(a)pyrene	0.0002	0.0016	Water Solublity
Benzo(k)fluoranthene	0.00017	0.0008	Water Solubility
Benzoic acid	28.0	28.0	TACO GW Objective
Chrysene	0.0063	0.0063	Water Solubility

Diethyl phthalate	5.6	5.6	TACO GW Objective
Dibenzo (a,h,) anthracene	0.0003	0.0015	TACO GW Objective
Di-n-butyl phthalate	0.7	3.5	TACO GW Objective
Di-n-octyl phthalate	0.02	0.02	Water Solubility
Fluoranthene	0.21	0.21	Water Solubility
Fluorene	0.28	1.4	TACO GW Objective
Indeno(1,2,3-cd)pyrene	0.000022	0.000022	Water Solubility
2-Methylnaphthalene	0.028	0.14	5X
2-Methylphenol	0.35	0.35	TACO GW Objective
Naphthalene	0.14	0.22	TACO GW Objective
p-Dioxane	0.0077	0.0077	10^{-6} Cancer risk
Pyrene	0.21	1.05	TACO GW Objective
Pesticides/PCBs			
alpha-BHC	0.00011	0.00055	TACO GW Objective
Dicamba	0.21	0.21	0X
MCPP (Mecoprop)	0.007	0.035	5X
Methoxychlor	0.04	0.045	Water Solubility

In addition, the existing Class II standard for Benzo(a)pyrene should be amended to 0.0002 mg/l based on its water solubility. Additionally, the Class II standard for Methoxychlor should be amended to 0.045 mg/l based on its water solubility.

J. Section 620.420(c)

This new subsection has been amended to propose a Class II: General Resource Groundwater Standard for the following explosive compounds:

Explosives	Reference Proposed Class I Standard (mg/l)	Proposed Class II Standard (mg/l)	Basis
1,3-Dinitrobenzene	0.0007	0.0007	0X
2,4-Dinitrotoluene	0.0001	0.0001	0X
2,6-Dinitrotoluene	0.00031	0.00031	TACO GW Objective
HMX	1.4	1.4	0X
Nitrobenzene	0.0035	0.0035	0X
RDX	0.084	0.084	0X
1,3,5-Trinitrobenzene	0.84	0. 84	0X
2,4,6-Trinitrotoluene	0.014	0.014	0X

K. Section 620.440 Groundwater Quality Standards for Class IV: Other Groundwater

Explosives are used during various phases of mining. It is not expected that residuals of explosive contaminants will even be detectable after reclamation has been completed for a previously mined area (e.g., excavation left after the coal seam(s) have been removed). However, the Illinois EPA and the Office of Mines and Minerals believe it is important to establish a Class IV standard for explosives within previously mined areas. Designation of a previously mined area is being proposed because it moves the compliance point from the pit of the mine to the boundary of the permitted area. This is necessary because we want to establish a monitoring point that will assess the potential for off-site contamination of resource groundwater beyond the permitted boundary. The alternative would establish a compliance point in the pit of the mine. We already know, expect, and accept that this area would be impacted by TDS, sulfate and other contaminants listed in Class IV previously mined areas. It is simply an environmental cost of doing coal mining. In addition, setting a compliance point at the permitted boundary is certainly consistent with monitoring programs under other Board regulations such as the solid waste landfill monitoring requirements.

L. Section 620.450 Alternative Groundwater Quality Standards

Section 620.450(b) establishes the applicable coal reclamation groundwater standards. A new Subsection 620.450(b)(3)(D) is proposed to include explosive contaminants. Thus, the existing concentration of these contaminants, if present post reclamation, is proposed to be the applicable standard within the permitted area. This establishes an appropriate compliance point for resource groundwater beyond the permitted area.

M. Section 620.505(a)(5)

Section 620.505 contains the provisions for determining compliance with the Board's groundwater quality standards. Appropriate monitoring points are selected based on the ability to represent groundwater quality accurately. When this Section was originally drafted and ultimately adopted by the Board in 1991, the Groundwater Protection Needs Assessment ("GPNA") provisions of the Act (415 ILCS 5/17.1) had not been fully developed or implemented. In addition, Illinois' WHPP had not yet been approved by U.S. EPA. Further, not much progress had been made with delineations during subsequent groundwater standards update proceedings. However, as a result of the source water assessment requirements and grant funding available under the 1996 SDWA amendments, over 300 WHPA's have been delineated beyond the applicable setback zones for CWS wells. These delineations have been conducted based on Illinois' *Guidance Document for Conducting Groundwater Protection Needs Assessments* and Illinois' approved WHPP. Therefore, this proposed amendment is intended to utilize this new hydrogeologic data where it is available.

Before proceeding it is important to provide some historical background. The 1986 amendments to the SDWA required States to develop and implement WHPPs after approval by U.S. EPA. In 1987, U.S. EPA published its *Guidelines for Delineation of Wellhead Protection Areas.* The development of U.S. EPA's WHPA delineation guidance included a *Model Assessment for Delineating Wellhead Protection Areas.* Paul van der Heijde of the International Ground-Water Modeling Center ("IGWMC") conducted this assessment for U.S. EPA. After the SDWA requirements in 1986 to delineate WHPAs and the subsequent requirements to provide recharge area delineation technical assistance under Illinois law in 1987, the ISWS developed new models, such as GWPATH (Shafer, J.M., 1987, *Reverse pathline calculation of time-related capture zones in nonuniformflow*, Ground Water, v. 25, no. 3, pp. 283-289) and conducted

research pursuant to Section 7 of the IGPA (415 ILCS 55/7(b)(4)) that further advanced WHPA modeling techniques (Wehrmann, H.A., and M.D. Varljen, 1990, *A comparison between regulated setback zones and estimated recharge areas around several municipal wells in Rockford*, IL, Proceedings of the NWWA 1990 Conference, February 20-21, 1990, Kansas City, MO. pp. 497-511).

The ISWS, Illinois State Geological Survey ("ISGS"), and the Illinois EPA collaborated to develop a nexus between WHPAs, and the delineation requirements under the Act, so that one approach would meet both requirements. The hydrologic term that describes the recharge area of a pumping well is referred to as a "contributing recharge area" because it is a subset of an overall aquifer recharge area supplying groundwater to a well. Another term used to describe a contributing recharge area is referred to as a capture zone. Capture zone models predict the pathlines of groundwater flow that contribute recharge to a pumping well. The outline of a capture zone, containing pathlines, is a WHPA or contributing recharge area. A time of travel ("TOT") can also be related to a capture zone. Illinois uses the 5-year TOT travel as the minimum threshold for WHPA delineations. This approach was presented to the Interagency Coordinating Committee on Groundwater ("ICCG") (415 ILCS 55/4) and the Groundwater Advisory Council ("GAC") (415 ILCS 55/5). After ICCG and GAC approval, this was presented to U.S. EPA for their approval. U.S. EPA fully approved Illinois' WHPP in1991.

The Illinois EPA and the surveys then collaborated on four pilot GPNA projects, starting in 1991, to further develop the technical basis for WHPA delineation. Pilot GPNA's were conducted for the following CWSs: Pleasant Valley Public Water District ("PWD"); Village of Cary; Village of Woodstock; and City of Pekin. These sites were purposely chosen because of their location within two priority groundwater protection planning regions, established by the ICCG and GAC,

and based on the hydrogeologic data provided by *Illinois Potential for Aquifer Recharge Map,* developed by the ISWS and ISGS pursuant to Subsections 17.2(a) and 17.2(b) of the Act.

Another reason for choosing the City of Pekin for a pilot GPNA was that the ISWS was conducting research specifically on the uncertainty associated with modeling WHPAs at the Pekin wells (Varljean, M.D., and J.M. Shafer, October 1991, *Assessment of Uncertainty in Time-Related Capture Zones Using Conditional Simulation of Hydraulic Conductivity,* Ground Water, v. 29, no. 5, pp. 737-748). This allowed for further collaboration between the ISWS and Illinois EPA on developing and applying appropriate WHPA modeling techniques.

The industry standard United States Geological Survey ("USGS"), numerical groundwater flow model ("MODFLOW"), and groundwater pathline model ("MODPATH") was used on these pilot projects. The IGWMC's WHPA modeling assessment (van der Heijde, P.K.M, 1988, *Model Assessment for Delineating Wellhead Protection Areas)* document, evaluated the usability and the reliability of these USGS models. Evaluation of reliability included if: 1) the model's theory and coding had been "peer reviewed"; 2) if the models had been "verified"; and 3) if these models had been "field validated". MODFLOW and MODPATH met all of these criteria.

MODFLOW and MODPATH were used to delineate the WHPAs for Pekin, Cary, and Pleasant Valley PWD GPNAs. The Illinois EPA and the ISWS worked on the development of the Pekin flow model, Clark Engineers developed the Pleasant Valley PWD model, and Baxter and Woodman developed the model for the Village of Cary. The Woodstock pilot, conducted by the ISGS, focused on geologic mapping techniques that can be used in the GPNA (Berg, R.C. 1994. *Geologic Aspects of a Groundwater Protection Needs Assessment for Woodstock, Illinois*: Illinois State Geological Survey Environmental Geology 146).

Illinois EPA worked with the Central Groundwater Protection Planning Committee, designated by our Director pursuant to Section 17.2 of the Act, and comprised of local stakeholders, to develop a local overlay zoning ordinance based on the Pekin WHPA. The Central Committee facilitated working with the City of Pekin, the Illinois American Water Company, local business, and other interest groups to develop and adopt Pekin's WHPA ordinance. The ordinance was adopted by the Pekin City Council in 1995.

Following the implementation of the pilot GPNA's the Illinois EPA, ISGS and ISWS began work on the development of *A Guidance Document for Conducting Groundwater Protection Needs Assessments* that was published 1995. The WHPA delineation process described in this guidance document was based on: 1) the ISWS's vast experience (For example; ASTM's *Standard Guide for Subsurface Flow and Transport Modeling,* ASTM 5880-95, was based in part on ISWS's ground breaking modeling work done in the late 60's and early 70's); 2) U.S. EPA's *Guidelines for Delineation of Wellhead Protection Areas;* 3) IGWMC's *Model Assessment for Delineating Wellhead Protection Areas;* and 4) the four pilot GPNAs.

The Illinois EPA used the WHPA modeling conducted for the Pleasant Valley PWD to develop a regulated recharge area proposal for submission to the Board. The Illinois EPA prepared this proposal in response to a request from the Central Committee pursuant their authority under Section 17.3 of the Act. Illinois EPA must prepare a proposal in response to a regional committee request. The Board held a series of hearings at which the WHPA modeling techniques were discussed in detail. Illinois' *Guidance Document for Conducting Groundwater Protection Needs Assessments* was included as an attachment to my testimony in that proceeding. The Board adopted a regulated recharge area for Pleasant Valley based on Illinois' WHPA modeling, which became effective September 1, 2001.

The Village of Marquette Height's maximum setback zone proposed by the Illinois EPA and adopted by the Board on May 4, 2007 was based Illinois' WHPA modeling. This proposal was also based on a recommendation from the Central Committee.

In summary, Illinois' *Guidance Document for Conducting Groundwater Protection Needs Assessments* is used to provide technical assistance on how to conduct WHPA modeling pursuant to Subsection 17.1(i) of the Act. Moreover, over 300 WHPA's have been delineated on a state wide basis for CWS wells using these processes.

The amendment to this subsection is intended to utilize the hydrologeologic information where WHPAs have been delineated for CWS wells according to the hydrogeologic procedures in a *Guidance Document for Conducting Groundwater Protection Needs Assessments.*

N. Section 620.510

The proposed amendments to the monitoring and analytical requirements are a result of the updates and revisions to the incorporations by reference in Section 620.125.

O. Section.620.605

The federal register citations have been updated to the relevant Code of Federal Regulation references.

P. Section 620.Appendix A

Section 620.Appendix A(b)(2) has also been amended to replace federal register with 40 CFR citations.

Q. Section 620.Appendix A

Section 620.Appendix A(b)(2) has also been amended to replace federal register with 40 CFR citations.

Dr. Hornshaw will provide testimony on further changes to Subpart F.

V. CONCLUSION

This concludes my testimony. I will be happy to address any questions.

ATTACHMENT I - CURRICULUM VITAE of RICHARD P. COBB, P.G.

Work Experience

Deputy Manager, Division of Public Water Supplies (DPWS), Bureau of Water (BOW), Illinois Environmental Protection Agency (EPA). (5/02- Present) My primary responsibilities include managing the: Groundwater Section, Field Operation Section, and the Administrative Support Unit of the Division. Further, I assist with administering the public water supervision program under the federal Safe Drinking Water Act ("SDWA") and the Wellhead Protection Program ("WHPP") approved by the United States Environmental Protection Agency ('U.S. EPA"). Additionally, my responsibility includes the integration of source water protection with traditional water supply engineering and treatment practices, and to further assist with linking Clean Water Act and SDWA programs. I also directly manage the BOW's Groundwater Section. The Groundwater Section applies Geographic Information System ("GIS") programs, global positioning system ("GPS") technology, hydrogeologic models (3D geologic visualization, vadose zone, groundwater flow, groundwater particle tracking, solute transport, and geochemical models), and geostatistical programs for groundwater protection and remediation projects. The Groundwater Section also continues to operate a statewide ambient groundwater monitoring program for the assessment of groundwater protection and restoration programs. I also do extensive coordination with federal, state and local stakeholders including the Governor appointed Groundwater Advisory Council ("GAC"), the Interagency Coordinating Committee on Groundwater ("ICCG"), four Priority Groundwater Protection Planning Committees, Illinois Source Water Protection Technical and Citizens Advisory Committee, and with the Ground Water Protection Council ("GWPC"), Association of State Drinking Water Administrators ("ASDWA"), and the Association of State and Interstate Water Pollution Control Administrators ("ASWIPCA") to develop and implement groundwater protection policy, plans, and programs. I represent the BOW on Illinois EPA's: Contaminant Evaluation Group ("CEG"); Strategic Management Planning Team; Environmental Justice Committee; Information Management Steering Committee; and the GIS Steering Committee. Since starting with Illinois EPA in 1985, I have worked on the development of legislation, rules and regulations. I have also served as a primary Illinois EPA witness before Senate and House legislative committees, and at Illinois Pollution Control Board ("Board") proceedings in the matter of groundwater quality standards, technology control regulations, cleanup regulations, regulated recharge areas, maximum setback zone, and water well setback zone exceptions. Furthermore, I have served as primary Illinois EPA witness in enforcement matters.

Manager, Groundwater Section, DPWS, BOW, Illinois EPA. (9/92-5/02) My primary responsibilities included development and implementation of Illinois statewide groundwater quality protection, USEPA approved WHPP, and source water protection program. The Groundwater Section worked with the United States Geological Survey ("USGS") to refine Illinois EPA's ambient groundwater monitoring network using a random stratified probability based design. The Groundwater Section continued to operate a statewide ambient groundwater monitoring program for the assessment of groundwater protection and restoration programs based on the new statistically-based design. I co-authored a *Guidance Document for Conducting*

Groundwater Protection Needs Assessments with the Illinois State Water and Illinois State Geological Surveys. I also continued to conduct extensive coordination with federal, state and local stakeholders including the Governor appointed GAC, the ICCG, four Priority Groundwater Protection Planning Committees, Illinois Source Water Protection Technical and Citizens Advisory Committee, and at the national level as Co-chair of the GWPC Ground Water Division to develop and implement groundwater protection policy, plans, and programs. I also served periodically as Acting Manager for the Division of Public Water Supplies. Additionally, the Groundwater Section provided hydrogeologic technical assistance to the BOW Permit Section and Mine Pollution Control Program to implement source water protection, groundwater monitoring and aquifer evaluation and remediation programs. I continued to work on the development of legislation, rules and regulations. I also served as a primary Illinois EPA witness at Board proceedings in the matter of groundwater quality standards, technology control regulations, regulated recharge areas and water well setback zone exceptions. Furthermore, I served as an Agency witness in enforcement matters.

Acting Manager, Groundwater Section, DPWS, BOW, Illinois EPA. (7/91-9/92) My responsibilities included continued development and implementation of Illinois statewide groundwater quality protection, U.S. EPA approved WHPP, and ambient groundwater monitoring program. The Groundwater Section developed the Illinois EPA's WHPP pursuant to Section 1428 of the SDWA and was fully approved by U.S. EPA. Illinois EPA was the first state in U. S. EPA Region V to obtain this approval. I performed extensive coordination with state and local stakeholders including the Governor appointed GAC, the ICCG to develop and implement groundwater protection, plans, policy, and programs. Developed and implemented the establishment of Illinois' Priority Groundwater Protection Planning Committees. Developed and implemented Pilot Groundwater Protection Needs Assessments. The Groundwater Section also provided hydrogeologic technical assistance to the BOW Permit Section and Mine Pollution Control Program staff to develop groundwater monitoring and aquifer evaluation, remediation and/or groundwater management zone programs. I also served as a primary Agency witness at Board proceedings in the matter of groundwater quality standards and technology control regulations. Additionally, I served as an Agency total quality management ("TQM") facilitator, and TQM trainer.

Manager of the Hydrogeology Unit, Groundwater Section, DPWS, Illinois EPA (7/88-7/91) Managed a staff of geologists and geological engineers that applied hydrogeologic and groundwater modeling principals to statewide groundwater protection programs. Developed, and integrated the application of GIS, GPS, geostatistical, optimization, vadose zone, solute transport, groundwater flow and particle tracking computer hardware/software into groundwater protection and remediation projects. Conducted extensive coordination with state and local stakeholders including the Governor appointed GAC and ICCG to develop and implement groundwater protection policy, plans, and programs. Developed and implemented a well site survey program to inventory potential sources of contamination adjacent to community water supply wells. Additionally, I worked on the development of rules to expand setback zones based on the lateral area of influence of community water supply wells. Furthermore, I provided administrative support to the Section manager in coordination, planning, and supervision of the groundwater program. I also assisted with the development of grant applications and subsequent management

of approved projects. In addition, I assisted the section manager with regulatory and legislative development in relation to the statewide groundwater quality protection program. I also served on the Illinois EPA's Cleanup Objectives Team ("COT").

Environmental Protection Specialist I, II, and III, Groundwater Section, DPWS, Illinois EPA. (7/85-7/88) I was the lead worker and senior geologist in the development and implementation of Illinois statewide groundwater quality protection program. I worked on the development of Illinois EPA's ambient groundwater monitoring network, and field sampling methods and procedures with the USGS. I published the first state-wide scientific paper on volatile organic compound occurrence in community water supply wells in Illinois. In addition, I assisted with the development of *A Plan for Protecting Illinois Groundwater*, and the legislation that included the *Illinois Groundwater Protection Act*.

Consulting Well Site Geologist, Geological Exploration (GX) Consultants, Denver Colorado. (3/81-12/83) I worked as a consulting well site geologist in petroleum exploration and development for major and independent oil companies. I was responsible for the geologic oversight of test drilling for the determination and presence of petroleum hydrocarbons. Prepared geologic correlations and performed analysis of geophysical logs, drilling logs and drill cuttings. Supervised and analyzed geophysical logging. Made recommendations for conducting and assisted with the analysis of drill stem tests and coring operations. In addition, I provided daily telephone reports and final written geologic reports to clients.

Undergraduate Teaching Assistant, Geology Department, Illinois State University. (3/79-3/81) I was responsible for teaching and assisting with lecture sessions, lab sessions, assignment preparation and grading for Petrology, Stratigraphy and Geologic Field Technique courses.

Undergraduate Education

B.S Geology, 1981, Illinois State University ("ISU"). Classes included field geology at South Dakota School of Mines and Technology, and Marine Ecology Paleoecology at San Salvador Field Station, Bahamas

Post Graduate Education

Applied Hydrogeology, 1984, ISU Graduate Hydrogeology Program

Engineering Geology, 1984, ISU Graduate Hydrogeology Program

Geochemistry for Groundwater Systems, 1986, USGS National Training Center

Hydrogeology of Waste Disposal Sites, 1987, ISU Hydrogeology Program

Hydrogeology of Glacial Deposits in Illinois, 1995, ISU Graduate Hydrogeology Program

MODFLOW, MODPATH and MT3D groundwater modeling, 1992, USGS National Training Center

24 Hour Occupational Health & Safety Training, 1994

Computer Modeling of Groundwater Systems, 1995, ISU Graduate Hydrogeology Program

Introduction to Quality Systems Requirements and Basic Statistics, 2001, U.S. EPA

Source Water Contamination Prevention Measures, 2001, U.S.EPA, Drinking Water Academy

Fate and Transport Processes and Models, 2006, Risk Assessment and Management Group, Inc.,

National Response Plan (NRP), an Introduction IS-00800.A, 2007, EMI

National Incident Management System (NIMS) an Introduction *IS-00700*, 2006, Emergency Management Institute (EMI),

Intermediate ICS for Expanding Incidents *IS-00300*, 2008, EMI

ICS for Single Resources and Initial Action Incidents *IS-00200*, 2006, EMI,

Introduction to the Incident Command System (ICS) *IS-00100*, 2006, EMI

License

Licensed Professional Geologist 196-000553, State of Illinois, expires 3/31/2009

Certification

Certified Professional Geologist 7455, Certified by the American Institute of Professional Geologists 4/88

Certified Total Quality Management Facilitator, 5/92, Organizational Dynamics Inc.,

Summary of Computer Skills

I am proficient with using the following computer programs ARC VIEW, Aqtesolv, SURFER, WHPA, DREAM, AQUIFEM, MODFLOW, MODPATH, and MT3D.

Professional Representation

Illinois EPA liaison to the ***GAC*** and representative on the ***ICCG*** (1988 – present)

Senate Working Committee on Geologic Mapping.

Illinois EPA representative and subcommittee chairman, ***State Certified Crop Advisory Board***, and ***Ethics and Regulatory Subcommittee*** established in association with the American Society of Agronomy/American Registry of Certified Professionals in Agronomy, Crops and Soils (1995 – 2001)

Illinois groundwater quality standards regulations technical work group (1988 – 1991).

ICCG State Pesticide Management Plan Subcommittee for the protection of groundwater.

Illinois EPA representative, ***State task group involved with developing the siting criteria for a low level radioactive waste site in Illinois.***

Fresh Water Foundation's Groundwater Information System (GWIS) project in the great lakes basin.

Illinois EPA technical advisor, ***four priority regional groundwater protection planning committees*** designated by the Director to advocate groundwater protection programs at the local level (1991 – present)

Groundwater Subcommittee of the National Section 305(b) Report, of the Clean Water Act Consistency Workgroup.

Ground Water Protection Council's Wellhead Protection Subcommittee.

Co-Chair, ***Groundwater Division of the GWPC*** on (September 1997 to 2003)

Chairman, ***Illinois' Source Water Protection Technical and Citizens Advisory Committee.***

United States Environmental Protection Agency National Ground Water Report Work Group. One of 10 state representatives serving on a work group sponsored by U.S. EPA headquarters charged with development of a national report to be submitted to the U.S. Congress on the status and needs for groundwater protection programs across the country. (January 1999 to July 2000)

Illinois EPA representative, ***Northeastern Illinois Planning Commission Water Supply Task Force.*** The purpose of this task force is to assist the Commission in the development of a Strategic Plan for Water Resource Management. (March 1999 to 2001)

GWPC/U.S. EPA Futures Forum Work Group providing input on source water protection for the next 25 years. (January 1999 to 2001)

GWPC/ASDWA work group providing input into the U.S. EPA Office of Ground and Drinking Water Strategic Plan for Source Water Protection. June 2000 to March 2005.

Co-Chair, ***U.S. EPA Headquarters/GWPC/ASDWA/ASWIPCA workgroup to develop the second Ground Water Report to Congress***. March 2002 –present.

Chair, ***ICCG Groundwater Contamination Response Subcommittee*** responsible for developing a new strategy for responding to groundwater contamination and the subsequent notification of private well owners. March 2002 – April 2002.

Illinois EPA representative, ***ICCG Water Quantity Planning Subcommittee*** working on development of a surface and groundwater quantity- planning program for Illinois. June 2002 – January 2003

Chair, ***ICCG Right-to-Know (RTK) Subcommittee,*** *2006*

GWPC, Groundwater Science and Research Advisory Board, 2007

Professional Affiliation

American Institute of Professional Geologists
Illinois Groundwater Association
Ground Water Protection Council
National Groundwater Association -Association of Groundwater Scientists and Engineers
Sigma Xi – The Scientific Research Society

Honors

Sigma Xi - Elected to *Sigma Xi* The Scientific Research Society for undergraduate research conducted and presented to the Illinois Academy of Science. 4/81

Director's Commendation Award - Participation in the development of the City of Pekin, Il. Groundwater Protection Program and commitment to the protection of Illinois groundwater. 7/95

Certificate of Appreciation - Outstanding contribution to the development of the Ground Water Guidelines for the National Water Quality Inventory 1996 Report to Congress from the United States Environmental Protection Agency Office of Ground Water and Drinking Water. 8/96

Groundwater Science Achievement Award - Illinois Groundwater Association for outstanding leadership and service in the application of groundwater science to groundwater protection in Illinois and in the development of the wellhead protection program and pertinent land-use regulations. 11/97

Certificate of Appreciation - GWPC for distinguished service, remarkable dedication, valuable wisdom and outstanding contribution as a GWPC member, division co-chair and special committee member. 9/99

Drinking Water Hero Recognition - United States Environmental Protection Agency Administrator Carol Browner at the 25th Anniversary of the Federal Safe Drinking Water Act Futures Forum in Washington D.C. 12/99.

Certificate of Recognition - United States Environmental Protection Agency Region V Administrator Fred Lyons for outstanding achievements in protecting Illinois' groundwater resources. 12/99

Exemplary Systems in Government (ESIG) Award - Nomination by the Governor's Office of Technology from the Urban and Regional Information Systems Association (URISA) for the Illinois EPA's Source Water Assessment and Protection Internet Geographic Information System. 6/01

Expert Witness Experience

IN THE MATTER OF: GROUNDWATER QUALITY STANDARDS (35 ILL. ADM. CODE 620), R89-14(B) (Rulemaking). Subject: I served as the principal witness recommending adoption of this Illinois EPA Agency proposal. R89-14(B) was adopted by the Board. The standards became effective January 1991.

STATE OIL COMPANY vs. DR. KRONE, McHENRY COUNTY and ILLINOIS EPA, PCB 90-102 (Water Well Exception). Subject: This case involved obtaining an exception from the owner of a non-community water supply well for placing new underground gasoline storage tanks within the 200-foot setback zone of well. I served as the principal witness for Illinois EPA on this case. The Board granted the exception with conditions.

People vs. AMOCO OIL COMPANY and MOBIL CORPORATION, Case no. 90-CH-79, Tenth Judicial Court, Tazewell County, Illinois. Subject: Groundwater contamination resulting from releases at above ground bulk petroleum storage terminals resulting in violation of Illinois' Groundwater Quality Standards Regulations (35 Illinois Administrative Code 620). I served as the principal Illinois EPA witness on this case. The case was settled with a penalty of $125,000 and the requirement of a comprehensive corrective action program.

IN THE MATTER OF: GROUNDWATER PROTECTION: REGULATIONS FOR EXISTING AND NEW ACTIVITIES WITHIN SETBACK ZONES AND REGULATED RECHARGE AREAS (35 ILL. ADM. CODE 601, 615, 616 and 617), R89-5 (Rulemaking). Subject: I served as the principal Illinois EPA witness supporting adoption of this Agency proposal. R89-5 was adopted by the Board and became effective January 1992.

HOUSE BILL 171 METHYL TERTIARY BUTYL ETHER (MTBE) ELIMINATION ACT, House Environmental and Energy Committee. Subject: This law required the phase out MTBE within 3 years of enactment. I served as a principal Illinois EPA witness in support of the proposed legislation. The legislation was adopted as Public Act 92-0132 on July 24 2001. PA 92-132 required the ban of MTBE within three years.

IN THE MATTER OF: GROUNDWATER QUALITY STANDARDS (35 ILL. ADM. CODE 620), R93-27 (Rulemaking). Subject: I served as the principal Illinois EPA witness recommending amendments of new constituent standards in this Agency proposal.

SHELL OIL COMPANY vs. COUNTY of DuPAGE and THE ILLINOIS ENVIRONMENTAL PROTECTION AGENCY, PCB 94-25 (Water Well Setback Exception). Subject: A new underground gasoline storage tank was seeking an exception from the Illinois Pollution Control Board in relation to a private drinking water supply well setback zone. The DuPage County and the Illinois EPA held that the tank would be a significant hazard and opposed the exception. I served as the principal Illinois EPA witness. Shell withdrew the petition from the Board after hearings were held.

People ex rel. Ryan v. STONEHEDGE, INC., 288 Ill.App.3d 318, 223 Ill.Dec. 764, 680 N.E.2d 497 (Ill.App. 2 Dist. May 22, 1997). Subject: The State brought Environmental Protection Act action against company engaged in business of spreading deicing salt, alleging that salt stored on company's industrial property leaked into area's groundwater supply, thereby contaminating it. The Circuit Court, McHenry County, James C. Franz, J., granted company's motion for summary judgment. State appealed. The Appellate Court, Colwell, J., held that: (1) wells existing before Illinois Water Well Construction Code was enacted are not "grandfathered" in as being in compliance with Code, so as to be automatically subject to testing for groundwater contamination, and (2) fact issues precluded summary judgment on claim arising from alleged deposit of at least 50,000 pounds of salt in pile within 200 feet of two existing water supply wells. Affirmed in part and reversed in part; cause remanded.

People vs. STONEHEDGE INC. Case no. 94-CH-46, Circuit Court of the 19th Judicial Circuit, McHenry County. Subject: This case involved a violation of the potable well setback zone provisions of Section 14.2 of the Illinois Environmental Protection Act. Stonehedge Inc. placed a salt pile of greater than 50,000 pounds within the 200 foot setback of multiple private drinking water supply wells. I served as an Agency principal witness. Stonehedge Inc. was found to be guilty of violating the setback prohibition in this case and was assessed a penalty of $1,500 and attorneys fees of $4,500.

SALINE VALLEY CONSERVANCY DISTRICT vs. PEABODY COAL COMPANY, Case No. 99-4074-JLF, United States District Court for the Central District of Illinois. Subject: Groundwater contamination from the disposal of 12.8 million tons of coarse coal refuse, slurry and gob. Witness for the Illinois EPA. This is an on-going case.

IN THE MATTER OF: PROPOSED REGULATED RECHARGE AREAS FOR PLEASANT VALLEY PUBLIC WATER DISTRICT, PROPOSED AMENDMENTS TO (35 ILL. ADM. CODE 617), R00-17 (Rulemaking). Subject: I served as the principal Illinois EPA witness supporting adoption of this Agency proposal. The proposal was adopted on July 26, 2001 and became effective September 1, 2001.

IN THE MATTER OF: PROPOSED AMENDMENTS TO TIERED APPROACH TO CORRECTIVE ACTION OBJECTIVES (35 Ill. Adm. Code 742), (R00-19(A) and R00-19(B)) (Rulemaking). Subject: I served as a supporting Illinois EPA witness recommending inclusion of MTBE in this Agency proposal.

IN THE MATTER OF: NATURAL GAS-FIRED, PEAK-LOAD ELECTRICAL GENERATION FACILITIES (PEAKER PLANTS), R01-10 (Informational Hearing) Subject: I served as a supporting Illinois EPA witness to discuss the impact of peaker plants on groundwater.

IN THE MATTER OF: GROUNDWATER QUALITY STANDARDS AND COMPLIANCE POINT AMENDMENTS (35 ILL. ADM. CODE 620), R01- 14 (Rulemaking). Subject: I served as the principal Illinois EPA witness recommending amendments of a groundwater standard for MTBE and compliance point determinations in this Agency proposal. The Board adopted the proposal unanimously on January 24, 2002.

TERESA LeCLERCQ; AL LeCLERCQ; JAN LeCLERCQ; WALT LeCLERCQ, individually; and on behalf of all persons similarly situated vs. THE LOCKFORMER COMPANY, a division of MET-COIL SYSTEMS CORPORATION, Case no. 00 C 7164, United States District Court, Northern District of Illinois. Subject: I was called as a witness by Lockformer Company to testify about a Well Site Survey prepared and published in 1989 by the Illinois EPA for Downers Grove community water supply.

TERESA LeCLERCQ; AL LeCLERCQ; JAN LeCLERCQ; WALT LeCLERCQ, individually; and on behalf of all persons similarly situated vs. THE LOCKFORMER COMPANY, a division of MET-COIL SYSTEMS CORPORATION, Case no. 00 C 7164, United States District Court, Northern District of Illinois. Subject: I was called as a witness by Lockformer Company to testify about groundwater contamination in the Lisle and Downers Grove area.

HOUSE BILL 4177 PRIVATE WELL TESTING PROPERTY TRANSFER and DISCLOSURE ACT, House Environmental and Energy Committee. Subject: Legislation to require volatile organic chemical contamination testing of private wells at the time of property transfer and reporting to the Illinois Department of Public Health and the Illinois EPA. I served as a principal Illinois EPA witness in support of the proposed legislation. The legislation was not supported due to the opposition from the realtors association.

MATTER OF PEOPLE vs. PEABODY COAL, PCB 99-134 (Enforcement). Subject: the State of Illinois developed an amended complaint against Peabody Coal Company (PCC) for violation of the groundwater quality standard for total dissolved solids, chloride, iron, manganese, and sulfate. I developed testimony to address PCC's affirmative defense of challenging the basis for the groundwater quality standards for these contaminants.

IN THE MATTER OF: PROPOSED AMENDMENTS TO TIERED APPROACH TO CORRECTIVE ACTION OBJECTIVES (35 Ill. Adm. Code 742) (TACO), (Rulemaking). Subject: I served as the Illinois EPA witness supporting amendments TACO to include wellhead protection areas. September 2004.

MAXMIUM SETBACK ZONES FOR MARQUETTE HEIGHTS PUBLIC WATER SUPPLY (35 ILL. ADM. CODE 618), R05-09 (Rulemaking). Subject: Pursuant to request by the Village of Marquette Heights the Illinois EPA developed a maximum setback zone for the Marquette Heights community water supply wells. I served as Illinois EPA's principal witness. The proposal was adopted on May 4, 2006.

IN THE MATTER OF: STANDARDS AND REQUIREMENTS FOR POTABLE WATER WELL SURVEYS AND FOR COMMUNITY RELATIONS ACTIVITIES PERFORMED IN CONJUNCTION WITH AGENCY NOTICES OF THREATS FROM CONTAMINATION UNDER P.A. 94-134 (35 Ill. Adm. Code 1505), R06-023 (Rulemaking), JANUARY 2006. I served as an Agency panel witness to support the adoption of the RTK regulation.

IN THE MATTER OF: PROCEDURES REQUIRED BY P. A. 94-849 FOR REPORTING RELEASES OF RADIONUCLIDES AT NUCLEAR POWER PLANTS: NEW 35 Ill. Adm. Code 1010, R07-20. I served as the Agency primary witness in this proceeding.

Publications

Cobb, R.P., 1980. ***Petrography of the Houx Limestone in Missouri***. Transactions of the Illinois Academy of Science Annual Conference, Illinois Wesleyan, Bloomington, IL.

A Plan for Protecting Illinois Groundwater, 1986, Illinois Environmental Protection Agency, January. 65 p.

Cobb, R.P., and Sinnott, C.L., 1987. ***Organic Contaminants in Illinois Groundwater***. Proceedings of the American Water Resources Association, Illinois Section, Annual Conference, Champaign, IL, April 28-29, p. 33-43.

Clarke, R.P., and Cobb, R.P., 1988. ***Winnebago County Groundwater Study***. Illinois Environmental Protection Agency. 58 pp.

Groundwater in Illinois: A Threatened Resource, A Briefing Paper Regarding the Need for Groundwater Protection Legislation, April 1987, Governors Office and Illinois Environmental Protection Agency, 34 pp.

Clarke, R.P., Cobb, R.P. and C.L. Sinnott, 1988. ***A Primer Regarding Certain Provisions of the Illinois Groundwater Protection Act.*** Illinois Environmental Protection Agency. 48 pp.

Cobb, R.P., etal, 1992. ***Pilot Groundwater Protection Needs Assessment for the City of Pekin.*** Illinois Environmental Protection Agency. 111 pp.

Cobb, R.P., 1994. ***Briefing Paper and Executive Summary on the Illinois Groundwater Protection Act and Groundwater Protection Programs with Recommendations from the Illinois Environmental Protection Agency Regarding the Siting of a Low Level Radioactive Waste Site***. Presented to the Low Level Radioactive Waste Task Force on December 9, 1994 in Champaign-Urbana.

Cobb, R.P., 1994. ***Measuring Groundwater Protection Program Success***. In the proceedings of a national conference on Protecting Ground Water: Promoting Understanding, Accepting

Responsibility, and Taking Action. Sponsored by the Terrene Institute and the United States Environmental Protection Agency in Washington D.C., December 12-13, 1994.

Cobb, R.P., Wehrman, H.A., and R.C. Berg, 1994. ***Groundwater Protection Needs Assessment Guidance Document***. Illinois Environmental Protection Agency. +94 pp.

Cobb, R.P., and Dulka, W.A., 1995. ***Illinois Prevention Efforts: The Illinois Groundwater Protection Act Provides a Unified Prevention-Oriented Process to Protect Groundwater as a Natural and Public Resource***, The AQUIFER, Journal of the Groundwater Foundation, Volume 9, Number 4, March 1995. 3pp.

Cobb, R.P., 1995. ***Integration of Source Water Protection into a Targeted Watershed Program***. In the proceedings of the Ground Water Protection Council'S Annual Ground Water Protection Forum in Kansas City Missouri.

Dulka, W.A., and R.P. Cobb, 1995. ***Grassroots Group Forges Groundwater Protection Law***. American Water Works Association, Opflow, Vol. 21 No. 3. 2pp.

Cobb, R.P., 1996. ***A Three Dimensional Watershed Approach: Illinois Source Water Protection Program***. In the proceedings of the Ground Water Protection Council's Annual Ground Water Protection Forum in Minneappolis Minnesota.

Cobb, R.P., and W.A. Dulka, 1996. ***Discussion Document on the Development of a Regulated Recharge Area for the Pleasant Valley Public Water District***. Illinois Environmental Protection Agency. pp 28.

Cobb, R.P., 1996. ***Illinois Source Water Protection Initiatives-Groundwater Perspective***. In the proceedings of the American Water Works Association's Annual Conference and Exposition in Toronto Canada. pp 585- 594.

Cobb, R.P., and Dulka, W.A., 1996. ***Illinois Community Examines Aquifer Protection Measures.*** American Water Works Association Journal. p10.

Cobb, R.P., etal. October 1999, ***Ground Water Report to Congress***, United States Environmental Protection Agency.

Cobb, R.P., December 2001.***Using An Internet Geographic Information System (GIS) to Provide Public Access to Hydrologic Data,*** Association of Groundwater Scientists and Engineers, National Groundwater Association, National Conference Proceedings, Nashville, Tennessee.

Cobb, R.P., September 2001, ***Regulated Recharge Area Proposal for the Pleasant Valley Public Water District,*** Ground Water Protection Council Annual Forum Proceedings, Reno Nevada, 13 pp.

Wilson, S., Cobb, R.P., and K. Runkle, January 2002. ***Arsenic in Illinois Groundwater***. Illinois State Water Survey, Illinois Environmental Protection Agency, and Illinois Department of Public Health. http://www.epa.state.il.us/water/groundwater/publications/arsenic/index.html, 7 pp.

R.P., Cobb, August 2002, ***Development of Water Quantity Planning and Protection in Illinois – A New Direction***, Proceedings of the Annual Ground Water Protection Council Technical Forum, San Francisco, California, 10pp.

P.C. Mills, K.J. Halford, R.P. Cobb, and D.J. Yeskis, 2002. ***Delineation of the Troy Bedrock Valley and evaluation of ground-water flow by particle tracking, Belvidere, Illinois***, U.S. Geological Survey Water-Resources Investigations Report 02-4062, 46 pp.

Illinois Environmental Protection Agency's ***Homeland Security Strategy***, March 2003, 20pp.

Illinois Environmental Protection Agency' ***Strategic Plan***, *Bureau of Water Section*, September 2003, pp.

Opinions and Conclusions of Richard Cobb for the Matter of People v. Peabody Coal, PCB 99-134 (Enforcement), May 23, 2003. 60 pp.

Cobb, R.P., Fuller, C., Neibergall, K., and M. Carson, February 2004. ***Community Water Supply Well Shooting/Blasting near the Hillcrest Subdivision Lake County, Illinois Fact Sheet***. Illinois Environmental Protection Agency. 4 pp.

Cobb, R.P., and J Konczyk, April 2007. ***Increasing Volatile Organic Compound Detections in Illinois Groundwater***, National Ground Water Association's Groundwater Monitoring and Remediation Journal, [Under review]. 8 pp.

Additional Legislative Publications that I Participated in Developing

A Plan for Protecting Illinois Groundwater, Illinois Environmental Protection Agency, January 1986. 65 p.

Groundwater in Illinois: A Threatened Resource, A Briefing Paper Regarding the Need for Groundwater Protection Legislation, Governors Office and Illinois Environmental Protection Agency, April 1987. 34 pp.

Illinois Groundwater Protection Act, Public Act 85-0863, September 1987. 68 pp.

Public Act 92-0132 (***MTBE Elimination Act***), July 24 2001.

Executive Order #5 - requires the ICCG to designate a subcommittee to develop an integrated groundwater and surface water resources agenda and assessment report. The report shall analyze the burden's on Illinois finite water resources, quantify Illinois' water resources, and prioritize an agenda to plan for the protection of these water resources. The Director of the Department of

Natural Resources chaired this subcommittee. The ICCG and GAC shall use the subcommittee's agenda and report to establish a water-quantity planning procedure for the State. The Governor signed executive order #5 on Earth Day April 22, 2001.

Amendments to Sections 2, 3 and 4 of the Illinois Groundwater Protection Act 415 ILCS 55/2 to establish a Groundwater and Surface Water Quantity Protection Planning Program, January 2002, 3 pp. These amendments were never adopted due to opposition from the Illinois Farm Bureau.

Public Act 92 –652 (Senate Bill 2072)- Amends the Illinois Groundwater Protection Act to require the Environmental Protection Agency to notify the Department of Public Health, unless notification is already provided, of the discovery of any volatile organic compound in excess of the Board's Groundwater Quality Standards or the Safe Drinking Water Act maximum contaminant level. The Governor signed this into law as Public Act 29-652 (effective July 25, 2002).

House Bill 4177 - Amends the Illinois Groundwater Protection Act. Provides that before property that has a well used for drinking water on it can be sold, the owner must have the well water tested for volatile organic chemical groundwater contaminants. Provides that if the well water does not meet the Illinois Pollution Control Board's Groundwater Quality Standards (35 Il Adm Code Part 620), the owner shall notify the Illinois Department of Public Health (IDPH) and the prospective buyer of the property. The realtors association July 2002 opposed House Bill 4177.

House Resolution 1010 - The resolution drafted by in cooperation with Senator Patrick Dunn' staff urge the Illinois Environmental Protection Agency to further strengthen its public outreach efforts by developing, after negotiations with individuals representing areas affected by contamination and other relevant State agencies, a procedure to notify property owners whenever the Agency has confirmed an exceedence of applicable health and safety standards, using scientifically credible data and procedures under Illinois regulations. HR 1010 was adopted by voice vote on June 1, 2004.

Public Act 94-314 (Senate Bill 0214) – This is referred to as Right-to-Know (RTK) law. The law includes providing the Illinois EPA with administrative order authority (AO), information order authority, and established the requirements for providing notices to residents or business exposed or potentially exposed to contamination. The Illinois EPA had been seeking this type of AO authority for the past 35 years. Senate Bill 0214 was unanimously passed by both the Senate and the House May 2005. The legislation was signed into law by the Governor July 27, 2005.

Public Act 94-849 (House Bill 1620) - Amends the Environmental Protection Act. Requires the owner or operator of a nuclear power plant to report to the Environmental Protection Agency any unpermitted release of a contaminant within 24 hours. The bill was sign by the Governor on June 12, 2006.

Arsenic in Illinois Groundwater

Illinois Department of Natural Resources
Illinois Department of Public Health
Illinois Environmental Protection Agency

November 20, 2001

What are the Sources of Arsenic in Illinois Groundwater?

Arsenic is a naturally occurring element in the earth's crust. Arsenic is found in the deep bedrock materials throughout Illinois, as well as in the shallow glacial materials that cover the northern two-thirds of the state. Arsenic is dissolved naturally from these materials and enters groundwater. Many other states have a similar problem.

Why is Arsenic a Concern?

Dissolved arsenic found in Illinois' groundwater is generally in inorganic form and is considered toxic. The Department of Health and Human Services lists arsenic as a known carcinogen. Ingesting inorganic arsenic over many years (chronic exposure) increases the risk of skin cancer and tumors of the bladder, kidney, liver, and lung. It has also been found to cause blood vessel damage, heart problems, darkening of the skin, and nervous system damage. Recently, the National Academy of Sciences (NAS) has reviewed the updated toxicological data base for arsenic and has determined that cancer risks from arsenic exposures are greater than previously estimated. This prompted a call to lower the drinking water standard for arsenic in order to protect human health.

About 35 percent of Illinois residents use groundwater for their water source. In addition, approximately 90 percent of rural citizens utilize groundwater from private wells for their source of water. In total, over four million Illinois residents use groundwater.

Regulation of Arsenic in Drinking Water

The national drinking water standard for arsenic has been 50 micrograms per liter (µg/L), which is 50 parts per billion. This standard was set in 1942. The World Health Organization has set a guideline of 10 µg/L. The United States Environmental Protection Agency (USEPA) recently evaluated the drinking water standard for arsenic and on October 30, 2001, USEPA recommended that the standard be lowered to 10 µg/L.

The new rule will be phased in such that compliance to the national drinking water standard for arsenic will be required by 2006 for all public water supplies. The Illinois Pollution Control Board has final say on Illinois' adoption of the national standard and typically the national regulation is quickly adopted and made law in Illinois. It is anticipated that the adoption of the national standard in Illinois will be finalized before the end of the year. At that point, the federal standard will become the law in Illinois as well. In Illinois, the IEPA and Illinois Department of Public Health (IDPH) are responsible for enforcing the standard for all

community and non-community public water supplies.

An ongoing debate centers around the high costs of removing arsenic, especially for regulated small communities and unregulated individual homes, versus the health benefits of lowering the arsenic standard.

Public Groundwater Supplies

The new national standard for arsenic, 10 µg/L, applies to all community and non-community public water supplies. Figure 1 shows the locations of community public groundwater supplies where, utilizing existing treatment, finished water will likely exceed the new standard unless additional treatment is applied. These data, based on sampling conducted by the Illinois Environmental Protection Agency (IEPA), indicate that arsenic is prevalent in groundwater in many parts of the state.

For the community water supply data included in the Illinois State Water Survey (ISWS) Water Quality Database, which are of well water samples taken prior to treatment, 2.1 percent of the samples had arsenic levels of 50 µg/L or greater, and about 11 percent were above 10 µg/L.

Communities with arsenic levels above the standard in the raw water supplies treat their water so that the finished water provided to their residents is below the standard.

How Much Arsenic Is Found In Illinois' Private Wells?

Private water supplies and wells are not regulated for the amount of arsenic present in their drinking water. Because most private water supplies are not tested for arsenic and few have available treatment to remove arsenic, the health risks to domestic well owners may be higher than for public water supplies.

In Illinois, only a few private well data are available statewide for determining the concentration of arsenic in groundwater. One exception is in Tazewell County, where the Tazewell County Health Department sampled 590 private wells for arsenic in 1985.

Groundwater is the sole source of drinking water in most of Tazewell County. There is wide variability in arsenic concentration over relatively small areas (Figure 2). Little information regarding well depth was available for evaluating these data, but additional research in Tazewell, McLean, and Logan counties by the ISWS indicated that the shallower aquifers had a greater percentage of samples with high arsenic concentrations, compared to the deeper sand and gravel aquifer. These results suggest that shallow glacial materials may contribute more to the arsenic found in groundwater than previously believed.

In Tazewell County, of the 590 samples tested, 202 (34 percent) had arsenic concentrations at or above 50 µg/L. 350 samples (59 percent) had arsenic concentrations at or above 10 µg/L. This is much greater than the percentage of arsenic concentrations in public wells, which are usually much deeper. Only 7 (4 percent) of the more than 180 community groundwater wells in Tazewell County had arsenic concentrations above 50 µg/L and about 70 (39 percent) had arsenic concentrations above 10 µg/L in their ambient water samples

That arsenic concentrations vary dramatically over a relatively short distance indicates

that local conditions dictate arsenic concentration in groundwater, and it is difficult to make regional generalizations.

Effect of a New Arsenic Rule in Illinois

For Illinois residents served by public water supplies, compliance with a new arsenic rule will reduce their exposure to arsenic in groundwater. It will also likely have economic impacts due to higher treatment costs. Illinois residents served by untreated private wells will continue to experience the same exposures and health risks as before, but because the arsenic standard will be lower, the perceived health risks may be greater. Private well water can be treated at a cost.

In Illinois, many communities remove iron and manganese from their raw water to reduce the hardness of the water. Data from the IEPA on the water quality of both raw water and finished water indicate that some communities also are lowering their arsenic levels by using these treatment methods. For example, at Monticello, the change in arsenic concentration from raw water to finished water was 53 µg/L to 10.7 µg/L. At Clinton, arsenic concentrations dropped from 36 µg/L to 18.9 µg/L.

The USEPA recently published a report outlining the available technologies and associated increased costs for removing arsenic. For large communities, the average cost per person will likely be reasonable, but for small communities, the average cost per person will likely be significant. The USEPA estimates that the average cost per person in a community with 200 people will be 10 times the average cost per person in a community with 20,000 people. IEPA has estimated a total cost of $40 million to remove arsenic below 10 µg/L for 50 community water supplies in Illinois. Figure 3 illustrates the distribution of the 50 community water supplies relative to the projected cost of reducing arsenic to less than 10 µg/L.

The rule also, for the first time, requires non-community, non-transient public water supplies (e.g., schools, small businesses, etc.) to comply with the new standard. At this time it is unknown how many of the existing 440 supplies in this category will have to install treatment. The rule requirements for monitoring, reporting, and possible additional treatment could present financial burdens on these facilities.

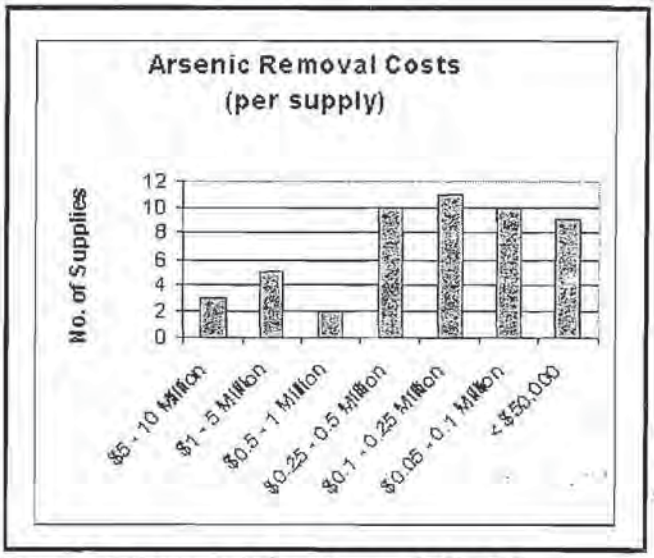


Figure 3. Projected Number and Cost of Removing Arsenic from Community Water Supply Wells in Illinois

There are a number of options for individual well owners who are concerned about the arsenic levels in their drinking water. Reverse osmosis, ultra-filtration, and ion exchange are available technologies for removing arsenic from private well water. These treatments, especially reverse osmosis, are effective in removing arsenic, but there are installation and maintenance costs. According to USEPA, the cost of treating a private system would range

between $400 and $5000 a year, depending on the amount of treated water and the method of treatment. Purchasing bottled water is another option.

What Activities Are Being Undertaken to Address The Arsenic Problem?

The Public Service Laboratory Program at the ISWS provides Illinois residents with water sample analyses for their private drinking water supplies (http://www.sws.uiuc.edu/chem/psl/), but arsenic is not routinely determined. The ISWS is looking into adding arsenic to the constituents evaluated in water samples. The Midwest Technology Assistance Center for Small Public Water Systems (MTAC), housed at the ISWS, is currently supporting research on arsenic treatment technologies that will lead to reduced treatment costs for the removal of arsenic in small public water systems. The ISWS is discussing with other agencies the feasibility of initiating a program to sample public, non-community wells and private wells throughout Illinois. Their goal is to characterize arsenic occurrence throughout Illinois and to identify the characteristics of the aquifers and groundwater chemistry that promote high arsenic concentrations.

The IDPH has a regulatory role for non-community public water supplies and an advisory role for private water supplies. The new rule will require, for the first time, non-community public water supplies test for arsenic. Residents with private well results can contact the IDPH Toxicology Section for a health-based assessment of the data. IDPH has a pamphlet entitled "Arsenic in Groundwater" available on the IDPH home page (http://www.idph.state.il.us) and by mail.

The IEPA is involved with various activities associated with implementing the federal Safe Drinking Water Act, the Illinois Groundwater Protection Act, and the Clean Water Act. IEPA has analyzed applicable treatment technologies and costs associated with the treatment of arsenic in community water supplies that would potentially exceed a maximum contaminant level (MCL) of 10 µg/L. Presentations have been made at seminars and meetings explaining the content of the published federal drinking water standards and possible impacts on water supplies that exceed the new standard. Water supplies that exceed the standard are required to provide Public Notice to customers of adverse health effects associated with arsenic and publish this notice in a newspaper having local circulation. All water supplies that exceed half the arsenic standard are also required to notify customers in the annual Consumer Confidence Report. IEPA has a monitoring program in place to evaluate arsenic in ambient groundwater used by community water supplies, and surface water in rivers and streams. These data from community water supply wells have been further assessed in relation to the supplies that potentially exceed an MCL of 10 µg/L, and to their existing treatment and treatment processes. The IEPA has made the arsenic data collected from community water supply wells available to the public from the Source Water Assessment and Protection Internet Geographic Information System at http://www.epa.state.il.us/water/groundwater/source-water-quality-program.html

Contacts

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	R08-18
PROPOSED AMENDMENTS TO	)	(Rulemaking-Public Water Supplies)
GROUNDWATER QUALITY	)	
STANDARDS	)	
(35 Ill. Adm. Code 620)	)	
	)	

TESTIMONY OF THOMAS C. HORNSHAW

Qualifications

My name is Thomas C. Hornshaw. I am a Senior Public Service Administrator and the Manager of the Toxicity Assessment Unit of the Illinois Environmental Protection Agency (Agency). I have been employed at the Agency since August of 1985, providing expertise to the Agency in the area of environmental toxicology. Major duties of my position include development and use of procedures for toxicity and risk assessments, review of toxicology and hazard information in support of Agency programs and actions, and critical review of risk assessments submitted to the Agency for various cleanup and permitting activities.

I was a member of the Agency's Cleanup Objectives Team until February of 1993, when that Team's responsibilities were assumed mainly by the Toxicity Assessment Unit. I was also a member of the Groundwater Standards Technical Team during the development of the Groundwater Quality Standards. These two teams have looked in depth at the problems involved with determining acceptable residual concentrations of chemicals in soil and/or groundwater.

I received Bachelor of Science (with honors) and Master of Science degrees in Fisheries Biology from Michigan State University, East Lansing, Michigan. I also received a dual Doctor of Philosophy degree from Michigan State University, in Animal Science and Environmental Toxicology. I am a member of the Society of Environmental Toxicology and Chemistry and

Sigma Xi, the Scientific Research Society. I have authored or co-authored six papers published in peer-reviewed scientific journals, one report issued through the U.S. Environmental Protection Agency, and have written or co-written six articles which have appeared in trade journals. I have also presented nineteen posters and/or talks describing facets of my graduate work and my work at the Agency at various regional and national meetings. A more descriptive account of my work and educational background and a list of publications, posters, and talks are included in a Curriculum Vitae presented as Exhibit A to this testimony.

Testimonial Statement

In my testimony today I will describe how chemicals that have been detected and confirmed in groundwater were selected for addition to Part 620. I will also discuss updates needed in Part 620 to make this rule consistent with groundwater remediation objectives listed in Part 742 (TACO) for several chemicals detected in groundwater but not currently included in Part 620. Then I will briefly discuss the development of the new standards that are proposed for this update. But first I will describe changes in the hierarchy for selecting the most appropriate toxicity information for evaluating a chemical, changes that have impacted the development of groundwater standards for certain chemicals.

I. TOXICITY INFORMATION HIERARCHY

On December 5, 2003, USEPA issued a memorandum (OSWER Directive 9285.7-53) from Michael B. Cook, Director of the Office of Superfund Remediation and Technology Information, to the Superfund National Policy Managers, Regions 1-10, on Human Health Toxicity Values in Superfund Risk Assessments. This memo revised the hierarchy for selecting human health toxicity values that had been used since the issuance of the original hierarchy in the 1989 Risk Assessment Guidance for Superfund (RAGS). The RAGS hierarchy, which has

also been used by the Toxicity Assessment Unit in developing human health toxicity values, was to first use values from EPA's Integrated Risk Information System (IRIS) database, if available, or else values from the most recent Health Effects Assessment Summary Tables (HEAST). If no toxicity value was available from these sources, then values could be derived from literature sources or a request could be made to EPA's National Center for Environmental Assessment (NCEA) for provisional toxicity values.

The revised hierarchy still specifies the IRIS database as the first option for toxicity values, but now includes second and third tiers of data sources. The second tier is a recently introduced database, EPA's Provisional Peer Reviewed Toxicity Values (PPRTVs) available from NCEA. The third tier, Other Toxicity Values, includes three named sources but could also include other sources as appropriate. The three named sources are the Agency for Toxic Substances and Disease Registry's (ATSDR) Minimal Risk Levels (MRLs), developed for ATSDR risk assessments, California EPA's toxicity values, developed to support various rules and programs, and EPA's HEAST, which was last updated in 1997.

The Toxicity Assessment Unit has adopted this hierarchy, with some minor revisions, as the basis for determining the toxicity values for its activities. As we began using the new hierarchy, we became aware of some minor issues that ultimately lead to certain revisions of the hierarchy. To keep this portion of my testimony brief, I will illustrate three issues that resulted in a minor revision:

- PPRTVs are retired by EPA after a certain period of time, leading us to question what should be the role of retired values; we ultimately decided to continue using them instead of going to tier three.

- EPA does not provide guidance on which value to use if more than one value is available from the three named sources in tier three; we ultimately decided to use the lowest of the tier three values available in such cases.
- IRIS does not contain values for subchronic exposures, only values for chronic exposures, so there is essentially no first tier for shorter-duration exposures; however, some chronic IRIS values use an Uncertainty Factor to extrapolate to chronic exposures from a study of subchronic duration, and we have used the IRIS value with this Uncertainty Factor removed as the first tier when available.

The Toxicity Assessment Unit has used this new hierarchy to re-evaluate the soil and groundwater objectives for all the chemicals currently included in Part 742 (TACO), other than those groundwater objectives that are based on a Maximum Contaminant Level from the Safe Drinking Water Act (which would require a change at the federal level). Changes needed in TACO because of this new hierarchy will be included when the Agency's next revision to TACO is proposed to the Board, and these changed values for groundwater are also included in the list of chemicals we are proposing to be added to Part 620 to make it consistent with TACO, as discussed below. We have also used this hierarchy to derive the proposed standards for the newly detected chemicals that must be added to Part 620, also discussed below. Finally, as Mr. Cobb has testified, the Agency has determined that no groundwater standard or objective should exceed the water solubility for a chemical, and the one-in-a-million risk concentration for a carcinogen should be considered along with the chemical's analytical detection limit to derive the most appropriate groundwater objective. These factors have been included in the development of the Agency's proposal for updating Part 620.

II. ADDING NEWLY DETECTED CHEMICALS

In preparation for updating Part 620, the Agency's Bureau of Land reviewed analytical results from many remediation activities to determine what chemicals had been detected in groundwater and confirmed by resampling. The master list from this review, consisting of chemicals detected primarily at Resource Conservation and Recovery Act (RCRA), solid waste disposal, and Department of Defense and Superfund sites, was then reviewed by the Toxicity Assessment Unit to determine which of the chemicals had sufficient toxicity values to support the development of a new groundwater standard. After this review, the Toxicity Assessment Unit decided that, in addition to those chemicals already included in TACO, any chemical on the master list for which a toxicity value was available in the IRIS database would be an appropriate candidate for inclusion in the Part 620 update. Thus, in addition to the TACO chemicals discussed below, the following chemicals were added to the proposed update: 2-Butanone (Methyl ethyl ketone, MEK), Dicamba, Dichlorodifluoromethane, p-Dioxane, 1,3-Dinitrobenzene, the explosive HMX, Isopropylbenzene (Cumene), Mecoprop (MCPP), 2-Methylnaphthalene, Molybdenum, Perchlorate, the explosive RDX, Trichlorofluoromethane, 1,3,5-Trinitrobenzene, and 2,4,6-Trinitrotoluene (TNT).

III. ADDING GROUNDWATER OBJECTIVES FROM TACO

In this proposed update to Part 620 the Agency determined that it is now necessary that chemicals that have Groundwater Remediation Objectives in TACO Part 742.Appendix B, Table E, are not included in Sections 620.410 and 620.420, and have been detected and confirmed in groundwater should be added to Sections 620.410 and 620.420, so that these two rules are consistent. Thus, after all the TACO Groundwater Remediation Objectives were updated

according to the new toxicity value hierarchy as described above and compared to solubility limits and one-in-a-million cancer risk concentrations (for carcinogens), the following chemicals not included in Part 620 were added to this proposed update: Acenaphthene, Acetone, Anthracene, Benzo(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzoic acid, alpha-BHC, Carbon disulfide, Chloroform, Chrysene, Dibenzo(a,h)anthracene, Di-n-butyl phthalate, Diethyl phthalate, 2,4- and 2,6-Dinitrotoluene, Di-n-octyl phthalate, Fluoranthene, Fluorene, Indeno(1,2,3-c,d)pyrene, 2-Methylphenol, Naphthalene, Nitrobenzene, Pyrene, and Vanadium.

IV. DERIVING THE NEW STANDARDS

In order to accommodate the changes regarding the one-in-a-million cancer risk concentration for carcinogens and solubility limits for all chemicals, it was necessary to change the wording of Part 620. Subpart F. Specifically, changes were needed in Section 620.605, as follows:

- In Section 620.605(b)(1), insert at the beginning "Except as specified in Section 620.605(c), if..." (no changes to the rest of this section).
- Amend Section 620.605(b)(2) to read "Except as specified in Section 620.605(c), if the chemical substance is a carcinogen, the guidance level for any such chemical substance is the one-in-one-million cancer risk concentration, unless the concentration for such substance is less than the lowest appropriate PQL specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods," EPA Publication No. SW-846, incorporated by reference at Section 620.125 for such substance. If the concentration for such substance is less than the lowest appropriate PQL for the substance specified in SW-

846, the guidance level is the lowest appropriate PQL. The one-in-one-million cancer risk concentration, the Human Nonthreshold Toxicant Advisory Concentration (HNTAC), shall be determined according to the following equation:" The equation then follows the text in the proposed amendment.

- Add a new Section 620.605(c), "If the guidance level determined for a chemical pursuant to Section 620.605(a) or (b) exceeds the water solubility of that chemical, the guidance level shall be the water solubility."

The new hierarchy for toxicity values was used to identify the most appropriate values for the chemicals to be added, and the procedures of Part 620. Subpart F (including the new sections described above), were used to derive the standards that are being proposed. Please note that all proposed values now contain two significant figures, to be consistent with a Toxicity Assessment Unit policy decision that specifies two significant figures be used for all new and updated standards and objectives.

V. TESTIMONY REGARDING ERRATA SHEET 2

In Errata Sheet Number 2 the Agency proposes to make changes to its initial filing regarding certain chemicals listed in Sections 620.410(b) and 620.420(b). Additional corrections are necessary for several reasons. First, selection criteria for groundwater standards have been revised and now require a comparison of each carcinogenic constituent's health-based concentration (the 10^{-6} risk level) with its corresponding analytical method detection limit. The greater of these values is then compared to the constituent's reported water solubility. The lower of these two values then becomes the standard. Second, source information used by the Agency concerning chemical/physical properties was recently updated and has subsequently been incorporated into the Agency's database. Finally, crucial sources for toxicological values that

form the bases for health-based standards have been updated and expanded necessitating subsequent updates to the Agency database.

A brief explanation of the reason for each of the changes follows:

Section 620.410(b):

- Anthracene- The solubility-based standard is corrected to two significant figures.
- Benzo(k)fluoranthene- For this carcinogen, the standard is corrected to the detection limit of 0.00017 mg/L since it does not exceed the water solubility value of 0.0008 mg/L.
- Chrysene- The 10^{-6} risk level of 0.012 mg/L would be the standard because it is greater than the detection limit of 0.0016 mg/L; however, the standard is corrected to the chemical water solubility of 0.0063 mg/L, which should not be exceeded.
- 1,1-Dichloroethane- The standard is corrected to reflect a revised toxicological reference dose value.
- Fluoranthene- The solubility-based standard is corrected to two significant figures.
- Indeno(1,2,3-cd)pyrene- The standard is corrected to the chemical's water solubility, which should not be exceeded.
- 2-Methylnaphthalene- The standard is revised to correct a typographical error.
- P-Dioxane- The standard is corrected to the 10^{-6} cancer risk concentration of 0.0077 mg/L because it is greater than the detection limit of 0.005 mg/L.
- Pyrene- The standard is corrected to the health-based value of 0.21 mg/L following an increase in pyrene's reported water solubility concentration.
- 2,4-Dinitrotoluene- The standard is corrected to the 10^{-6} cancer risk level of 0.0001 mg/L because it is greater than the originally proposed detection limit of 0.00002 mg/L.

Section 620.420(b):

- Anthracene- The solubility-based standard is corrected to two significant figures.
- Benzo(a)pyrene- The solubility-based standard is corrected to two significant figures.
- Chrysene- The 10^{-6} risk level of 0.012 mg/L would be the standard because it is greater than the detection limit of 0.0016 mg/L; however, the standard is corrected to the chemical water solubility of 0.0063 mg/L, which should not be exceeded.

- 1,1-Dichloroethane- The standard is corrected to reflect a revised toxicological reference dose value.
- Fluoranthene- The solubility-based standard is corrected to two significant figures.
- Indeno(1,2,3-cd)pyrene- The standard is corrected to the chemical's water solubility, which should not be exceeded.
- p-Dioxane- The standard is corrected to the 10^{-6} cancer risk concentration of 0.0077 mg/L because it is greater than the detection limit of 0.005 mg/L.
- Pyrene- The standard is corrected to the health-based value of 1.05 mg/L following an increase in pyrene's reported water solubility concentration.
- 2,4-Dinitrotoluene- The standard is corrected to the 10^{-6} cancer risk level of 0.0001 mg/L because it is greater than the originally proposed detection limit of 0.00002 mg/L.

Concluding Statement

This concludes my portion of the Agency's testimony for the proposed amendments to Part 620.

STATE OF ILLINOIS)
)
COUNTY OF SANGAMON)

PROOF OF SERVICE

I, the undersigned, on oath state that I have served the attached Errata Sheet Number 2 and Pre-filed testimony of Richard P. Cobb and Thomas Hornshaw upon the person to whom it is directed, by placing a copy in an envelope addressed to:

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Illinois Pollution Control Board
James R. Thompson Center
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and mailing it from Springfield, Illinois on May 28, 2008 with sufficient postage affixed.

Kimberly A Geving

SUBSCRIBED AND SWORN TO BEFORE ME

This 28th day of May, 2008.

Brenda Boehner
Notary Public

OFFICIAL SEAL
BRENDA BOEHNER
NOTARY PUBLIC, STATE OF ILLINOIS
MY COMMISSION EXPIRES 11-3-2009

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Total number of participants: 8

Attachment

1A

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ILLINOIS POLLUTION CONTROL BOARD
November 7, 1991

IN THE MATTER OF:	)	
	)	
GROUNDWATER QUALITY STANDARDS	)	R89-14(B)
(35 ILL. ADM. CODE 620)	)	(Rulemaking)

ADOPTED RULE. FINAL ORDER.

OPINION AND ORDER OF THE BOARD (by R.C. Flemal):

This matter comes before the Board pursuant to Section 8 of the Illinois Groundwater Protection Act ("IGPA"), Ill. Rev. Stat. 1989, ch. 111½, pars. 7451 et seq. Section 8 mandates inter alia that the Board promulgate "regulations establishing comprehensive water quality standards which are specifically for the protection of groundwater" (IGPA at Section 8(a)). The purpose of today's action is make final adoption of these groundwater quality standards and the associated basic framework.

PROCEDURAL HISTORY

The Agency filed its original proposal on September 21, 1989.

On January 31, 1990 the Department of Energy and Natural Resources ("DENR") filed the Economic Impact Statement ("EcIS"), which pursuant to the IGPA was prepared concurrently with development of the Agency's proposal.

On March 26, 1990 an alternative proposal was filed by the McHenry County Defenders, Citizens for a Better Environment, and the Illinois Chapter of the Sierra Club (collectively as "Defenders").

On June 1, 1990 the Agency filed its second proposal within Public Comment ("PC") #16.

Hearings on the various proposals and the EcIS were held on December 12 and 13, 1989, and February 14, March 29, and May 7, 1990[1].

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[1] Transcripts of the December 1989 to May 1990 hearings, which are numbered consecutively, are herein cited in the form "R1 at ____".

The Board wishes to acknowledge the special contribution made by Michelle C. Dresdow, who has served as Hearing Officer throughout these proceedings.

Based on the cumulative record then available, the Board on September 27, 1990 advanced its own proposed rule, which was published for First Notice on November 2, 1990[2]. Hearings were held on this proposal on December 4 and 5, 1990[3].

On February 19, 1991 the Agency filed its third amended proposal, which the Board on February 28, 1991 proposed for First Notice as Docket B[4]. Hearing was held on the Docket B proposal on May 30, 1991[5]. At hearing the Agency offered further amendments to its proposal based on renewed discussions, conferences, and negotiation sessions with interested persons. The text of these amendments, which was entered as Exhibit T[6], had been distributed to interested persons prior to the May 30, 1991 hearing.

On July 25, 1991 the Board proposed the Docket B regulations, with modifications, for Second Notice[7]. Given the magnitude of the changes, the Board withheld filing of the Second Notice proposal with the Joint Committee on Administrative Rules ("JCAR") to allow for an additional comment period of 15 days. No comments filed during this period persuaded the Board to recede from its July 25 action, and the proposal was accordingly filed with JCAR.

On October 22, 1991 JCAR issued a certificate of no objection to the proposed rules. Prior to the issuance of the certificate, JCAR staff alerted the Board to several non-substantive grammatical and typographical errors. In addition,

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The Board wishes to acknowledge the special contribution made by Michelle C. Dredow, who has served as Hearing Officer throughout these proceedings.

[2] 14 Ill. Reg. 17822, November 2, 1990.

[3] Transcripts of the December 1990 hearings are herein cited in the form "R2 at _____".

[4] Publication occurred at 15 Ill. Reg. 4234, March 22, 1991.

[5] The transcript of the May 1991 hearing is herein cited in the form "R3 at _____".

[6] The text of the proposed amendments entered as Exhibit T are often referred to in the transcript of the May 30, 1991 hearing as the "May 15 proposal", based upon the date contained on that document.

[7] By the same Order the Board Closed Docket A and withdrew its provisions from further consideration, save for the amendment to 35 Ill. Adm. Code.Part 303 which was transferred to Docket B.

JCAR staff recommended seven other changes involving sections 620.110, 620.250(a)(2), 620.302(b)(1), 620.450(b)(3)(A)(ii), 620.510(b)(1), 620.601(b) and 620.Appendix A(c)(1)(iii). These changes are discussed below in appropriate parts of this opinion and are incorporated into today's order.

The Board is pleased with the high quality perspective that has been brought to bear on this matter, both in hearing testimony and public comments. The Board expresses its appreciation to the many persons who have contributed in one form or another.

STATUTORY FRAMEWORK

The IGPA was enacted by the Illinois General Assembly as an outgrowth of long-standing concern by the General Assembly and the citizens of the State that the State's rich and valued groundwater resources be protected. The IGPA is a multi-faceted policy and program statement designed to provide that protection and to assure the continued viability of the State's groundwater resources. The policy statement of the IGPA is found at Section 2(b) (Ill. Rev. Stat. 1989, ch. 111½, ¶7452(b)), and reads:

> ... it is the policy of the State of Illinois to restore, protect, and enhance the groundwaters of the State, as a natural and public resource. The State recognizes the essential and pervasive role of groundwater in the social and economic well-being of the people of Illinois, and its vital importance to the general health, safety, and welfare. It is further recognized as consistent with this policy that the groundwater resources of the State be utilized for beneficial and legitimate purposes; that waste and degradation of the resources be prevented; and that the underground water resources be managed to allow for maximum benefit of the people of the State of Illinois.

The particular mandate of the IGPA pertinent to today's action occurs at Section 8 (Ill. Rev. Stat. 1989, ch. 111½, ¶7458). Section 8 reads:

> a. The Agency, after consultation with the Committee and the Council, shall propose regulations establishing comprehensive water quality standards which are specifically for the protection of groundwater. In preparing such regulations, the Agency shall address, to the extent feasible, those contaminants which have been found in the groundwaters of the State and which are known to cause, or suspected of causing, cancer, birth defects, or any other adverse effect on human health according to nationally accepted

guidelines. Such regulations shall be submitted to the Board by July 1, 1989.

b. Within 2 years after the date upon which the Agency files the proposed regulations, the Board shall promulgate the water quality standards for groundwater. In promulgating these regulations, the Board shall, in addition to the factors set forth in Title VII of the Environmental Protection Act, consider the following:

1. recognition that groundwaters differ in many important respects from surface waters, including water quality, rate of movement, direction of flow, accessibility, susceptibility to pollution, and use;

2. classification of groundwaters on an appropriate basis, such as their utility as a resource or susceptibility to contamination;

3. preference for numerical water quality standards, where possible, over narrative standards, especially where specific contaminants have been commonly detected in groundwaters or where federal drinking water levels or advisories are available;

4. application of nondegradation provisions for appropriate groundwaters, including notification limitations to trigger preventive response activities;

5. relevant experiences from other states where groundwater protection programs have been implemented; and

6. existing methods of detecting and quantifying contaminants with reasonable analytical certainty.

c. To provide a process to expedite promulgation of groundwater quality standards, the provisions of this Section shall be exempt from the requirements of subsection (b) of Section 27 of the "Environmental Protection Act", approved June 29, 1970, as amended; and shall be exempt from the provisions of Sections 4 and 5 of "An Act in relation to natural resources, research, data collection and environmental studies", approved July 1, 1978, as amended.

d. The Department of Energy and Natural Resources, with the cooperation of the Committee and the Agency, shall conduct a study of the economic impact of the regulations developed pursuant to this Section. The study shall include, but need not be limited to, consideration of the criteria established in subsection (a) of Section 4 of "An Act in relation to natural resources, research, data collection and environmental studies", approved July 1, 1978, as amended. This study shall be conducted concurrently with the development of the regulations developed pursuant to this Section. Work on this study shall commence as soon as is administratively practicable after the Agency begins development of the regulations. The study shall be submitted to the Board no later than 60 days after the proposed regulations are filed with the Board.

The Department shall consult with the Economic Technical Advisory Committee during the development of the regulations and the economic impact study required in this Section and shall consider the comments of the Committee in the study.

e. The Board may combine public hearings on the economic impact study conducted by the Department with any hearings required under Board rules.

In the following sections of this Opinion the Board discusses the various provisions that comprise today's adopted rules.

PART 303
CONFORMING AMENDMENT

Although the principal regulations adopted today consist of new Part 620, the promulgation of Part 620 requires a conforming amendment to 35 Ill. Adm. Code: Subtitle C, Part 303. Since the proposed amendments to Part 303 were published in the Illinois Register on November 2, 1990, more than one year ago, the Board is required to return to first notice on the Part 303 amendments only. Therefore, under separate Opinion and Order, the Board opens a docket C in this proceeding and again sends the Part 303 amendments to first notice.

PART 620
SUBPART A: GENERAL PROVISIONS

New 35 Ill. Adm. Code. Part 620 is designed to contain the principal provisions of today's action. It consists of six

Subparts plus two appendices. Subpart A sets out the general provisions applicable to the entire Part 620.

Except for generally non-substantive changes within the definitions and incorporations sections made in response to post-First Notice public comments[8], Subpart A remains essentially as proposed by the Agency in its third amended proposal.

Purpose -- Section 620.105

Section 620.105 sets forth the purpose of the Part. The Defenders had suggested insertion within this Section of the phrase "to assure that the groundwater resources of the State be utilized for beneficial and legitimate purposes, that waste and degradation of the resources be prevented, and that the underground water resource be managed to allow for maximum benefit of the people of the State of Illinois" (Def. Exh. 7 at proposed Section 620.101). This language comes from the policy statement found at Section 2(b) of the IGPA. While the Board fully stands behind this policy statement, the Board believes that today's Section 620.105 language is a better descriptor of the contents of the Part 620 rules, and therefore opts to use this version. The Board believes that this narrow purpose statement more clearly alerts the public to what is being regulated.

Definitions -- Section 620.110

Section 620.110 contains definitions applicable to Part 620. The intent is to present those definitions necessary for a reading of Part 620, including both definitions that are particular to the Part and those that are statutory; statutory definitions are capitalized, pursuant to standard form.

At second notice, JCAR recommended deletion of the phrase "unless otherwise provided" from the introductory statement at the beginning of this definitions section. The Board agrees to make this change.

General Prohibitions -- Section 620.115

Section 620.115 contains a general prohibition against threatening, causing or allowing a violation of the Illinois Environmental Protection Act, IGPA, or Board regulations, including this Part.

Incorporations by Reference -- Section 620.125

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[8] See discussion at p. 5-8 of Second Notice Opinion, July 25, 1991.

Section 620.125 sets forth incorporations by reference as used within Part 620.

Exemptions from Subtitle C Standards -- Section 620.130

Section 620.130 exempts groundwaters from the General Use Standards or Public and Food Processing Standards of Subparts B and C of 35 Ill. Adm. Code 302. This change, in combination with amendment to Section 303.203 (see above), clarifies the relationship between 35 Ill. Adm. Code.Subtitle C and today's rules.

Exclusion for Underground Water in Certain Man-Made Conduits -- Section 620.135

Section 620.135 explicitly excludes any underground waters that occur in certain man-made conduits from the application of today's regulations. The man-made conduits included are subsurface drains, tunnels, reservoirs, storm sewers, tiles, and sewers[9]. Waters in such conduits do not have the conventional characteristics and properties of groundwater, and it is therefore inappropriate to apply to them water quality standards that are based upon groundwater characteristics and properties.

It is perhaps arguable that Section 620.135 is not necessary since the definition of groundwater itself would seemingly exclude water in most, if not all, of the man-made conduits listed. Thus, if these waters are not groundwaters, groundwater standards would not apply to them. However, the record attests that there is sufficient confusion on this matter (see Agency Statement of Reasons, p. 11; PC #9; PC #10; PC #13) to warrant a definitive exclusion for water in man-made conduits.

It should be recognized that water in man-made conduits is not excused from all water quality standards. To the extent that such waters are "Waters of the State", they would be subject to the water quality standards of Subtitle C. As well, if such waters are discharged to the surface, they would be subject to water quality standards applicable to surface waters. The Illinois Department of Agriculture notes this conclusion with respect to drainage from agricultural field tiles:

> It is inappropriate to apply any numbers or standards to water in a drainage tile except surface water standards at the point of discharge to a surface water,

[9] Specifically not included are waters within wells or other structures designed to tap groundwater.

at which point one also must consider the effects of mixing. PC #9 at p. 1.

PART 620
SUBPART B: GROUNDWATER CLASSIFICATION

Subpart B sets out today's general groundwater classification system, criteria for classifying specific groundwaters, the concept of management zones, and procedures for amending the classification of any specific groundwater.

Groundwater classification is a well-recognized tool for the optimizing of groundwater protection efforts. Among its many utilities are the opportunity of recognizing the different values, uses, and vulnerabilities of groundwaters (Defenders Exh. 6). Today's rules specifically accord with the mandate of the IGPA at Section 8(b)(2) that the Board consider "classification of groundwaters on an appropriate basis, such as their utility as a resource or susceptibility to contamination".

List of Groundwater Classes -- Section 620.201

Section 620.201 establishes that there are four classes of groundwater. In addition, it establishes that some groundwaters may fall into groundwater management zones, pursuant to Section 620.201. Every groundwater in the State belongs to one of the four classes or to the waters in a groundwater management zone.

The four classes of groundwater derive from concepts presented over the full history of this proceeding, beginning with the Agency's original proposal and the Defenders' counter proposal, and culminating in the Agency's third amended proposal (Docket B). Perhaps no other facet of this proceeding has focused as much effort as has determining how best to classify the State's groundwaters.

Basic to the groundwater classification effort is the concept that groundwater constitutes a valued resource. This principle is articulated in the opening sentence of the State's Groundwater Protection Policy:

> . . . it is the policy of the State of Illinois to restore, protect, and enhance the groundwaters of the State, as a natural and public resource. IGPA, Section 2(b).

It is recognized, however, that not all groundwaters constitute the same level of resource; some groundwaters have greater resource value by virtue of their higher quality, quantity, accessibility, etc. Moreover, it is generally agreed that the degree of protection required is in some measure a function of the nature of the particular groundwater resource. This concept constitutes one of the bases for groundwater classification, and the application of different water quality standards, monitoring and remedial requirements, etc., to the different classes.

It is to be further recognized that potability[10] generally constitutes the "highest" use to which groundwaters are put. Potability, as a further generality, requires the highest degree of protection, including the most stringent standards, to maintain the use. Potable-use also is by far the largest use to which groundwaters in Illinois are put, and will be put in any foreseeable future. Given these circumstances, it is apparent that any successful program of groundwater management must give special focus to potable groundwaters. Emphasis on potable groundwaters is recognized in the declaration that the first class of Illinois groundwaters consists of the potable resource groundwaters.

Potable Groundwaters Class -- Section 620.210

Section 620.210 establishes the definition of a Class I: Potable Resource Groundwater. Included are all groundwaters that are located 10 feet or more below the land surface and that, by any one of several tests, produce groundwater in quantities sufficient to sustain a potable use. In addition, Section 620.210 specifically identifies that the Board may add groundwaters to Class I via the adjusted standards procedures spelled out at 620.260.

The tests used to determine potable quantities include demonstrated use, thicknesses associated with aquifers found in various rock types, or suitable hydrogeologic parameters. The latter include water in strata capable of a sustained yield of at least 150 gallons per day in a borehole of reasonable size and over a typical collection thickness[11].

Class I groundwaters clearly include a very broad range of groundwaters. This is fully intended. Moreover, it should be noted that Class I groundwaters include groundwaters of potential potable use as well as groundwaters currently experiencing potable use. A recurrent question regarding the resource-protection concept of groundwater protection has been whether potentially usable groundwaters should be afforded like

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[10] "Potable" is defined at in the IGPA as meaning "generally fit for human consumption in accordance with accepted water supply principles and practices" (Ill. Rev. Stat. 1989, ch. 111 1/2, par. 7453(h)).

[11] The 150 gallons per day limit is that limit which the USEPA defines as a yield sufficient for a groundwater to serve as water source for a household unit (Defender's Exh. 6, p. 39, 45; PC #16 at p. 12-16). The qualifications regarding sustainability of yield and size of borehole plus the hydraulic conductivity condition were first proposed and discussed by the Agency at the May 30, 1991 hearing (R3 at 18-23; Exh. T).

protection to groundwaters actually being used (e.g., R1 at 26, 968-9; PC #6 and #8). The Board previously addressed this issue in R86-8[12]. It there noted:

> Resource groundwaters are, at the minimum, those groundwaters which are presently being put to conventional use by reason of being of suitable quality, having local demand, and having been actually developed. Much of the record also shows that resource groundwaters ought also to include those groundwaters which have the potential for being put to conventional use. This perspective is straightforward, in that it suggests that potential resources should be protected against the eventuality that at least some of them will find use in the future. The Board believes that this is a wholly correct perspective, and accordingly concludes that resource groundwaters should include groundwaters of potential use. (Id. at II-3)

The Board believes that this perspective remains correct today, and accordingly it is incorporated into today's rules. Moreover, the Board believes that the General Assembly also endorsed this perspective by defining in the IGPA that a "'resource groundwater' means a groundwater that is presently being or in the future capable of being put to beneficial use" (IGPA at Section 3(j), Ill. Rev. Stat. 1989, ch. 111 1/2, par. 7453(j); emphasis added). That is, the Board believes that among the most necessary facets of the State's groundwater protection program is the need to protect all drinkable water at a drinkable level. Similarly, the Board does not believe that current actual use should be the sole control of whether potable groundwater is afforded the protection necessary to maintain potability; we simply cannot allow the sullying of a resource that future generations may need. For the same reason the term "Potable Resource Groundwater", rather than "Potable Use Groundwater", is employed in the title of this class.

The Board also notes that today's rules do not attempt to limit the definition of potability by qualifiers relating to time of travel to existing wells or stratigraphic position, as have some earlier proposals. This is in keeping with the position that all naturally potable groundwaters should be recognized as such, irrespective of whether they are currently experiencing use as a potable water supply.

Among the concepts not adopted today is the proposition espoused by the Defenders that to Class I groundwaters should be added all groundwaters hydrologically connected to and upgradient of potable resource groundwaters (R2 at 523; R3 at 269-70); under

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[12] In the Matter of: A Plan for Protecting Illinois Groundwater, R86-8, Report of the Board, August 28, 1986.

the instant rules most such groundwaters would be Class II groundwaters. The Defender's concept is not adopted because it offers little additional groundwater protection at a substantial increase in the regulatory burden.

Lastly, the Board notes that the 10-foot rule arises from the need to recognize that many surface activities can impact very shallow underground water without also impacting the great bulk of potable groundwaters. For example, the agricultural community has expressed substantial concern that establishing standards for groundwater would critically impact agriculture by disallowing the chemical alteration of all subsurface waters, including disallowing use of agricultural chemicals that operate through roots. To assure that this erroneous interpretation is not fostered, and to assure that legitimate use of agricultural chemicals or other legitimate activities are not proscribed, it was proposed at the December 4, 1990 hearing that the potable resource (Class I) groundwater standards specifically apply only to groundwaters below a depth of 10 feet, irrespective of whether these waters would otherwise qualify as potable waters; groundwaters shallower than 10 feet would always be Class II, III, or IV, depending upon the local circumstances.

The Board today endorses the "10-foot" rule as a reasonable compromise between the need to protect potable groundwaters and the need to carry on legitimate surface activities, of which agriculture is but one.

As a further observation on the "10-foot" rule, the Board notes that question has been raised whether potable groundwaters found below 10 feet, but located in a geologic unit that meets one of the thickness criteria only because a part of the unit is at a depth less than 10 feet, would still be considered a Class I water (R3 at 300). The Board intends that the answer to this question be "yes". Simply, if the water is below 10 feet and is naturally potable, it should be supported as a potable water resource. Prior to Second Notice a Board Note to this effect was added to Section 620.210 upon the recommendation of the Agency (PC #58 ¶4).

The General Resource (Default) Class -- Section 620.220

Class II: General Resource Groundwater is, by definition at Section 620.220, the default groundwater class. That is, Class II consists of those groundwaters that are not Class I, III, or IV. For example, a groundwater occurring in a thin shale unit that is not actually producing potable groundwater and that has a hydraulic conductivity less than 1×10^{-4} cm/sec would fall into Class II unless one of the special conditions of Class III or IV should apply. In general, a groundwater would fall into Class II if it is not potable by virtue of quantity or quality limitations, if it has not been otherwise specially classified

according to Class III procedures, or if it is not otherwise limited pursuant to Class IV qualifications.

The Board anticipates that groundwaters in "tight" hydrogeologic units will constitute one of the most common occurrences of Class II groundwaters. These are groundwaters that are unavailable in quantities sufficient for most uses. Another common occurrence is likely to be groundwaters that are not so saline as to warrant classification as Class IV: Other Groundwater, but that nevertheless are too saline to be potable without treatment.

Given the several ways that a groundwater may be classified as a Class II groundwater, in the long term it may be advisable to either subdivide Class II or split out additional classes from Class II. However, the Board believes that this endeavor, should it be undertaken, best awaits some experience with the more general classification adopted here.

Special Resource Groundwater -- Section 620.230

Section 620.230, Class III: Special Resource Groundwater, is derived in concept from the Defenders' proposal, which in turn is based on the United States Environmental Protection Agency's ("USEPA") groundwater classification strategies[13]. The Defenders contend that in certain circumstances a groundwater may take on an ecologically vital role, as for example when its discharge supports a vital wetland (R1 at 969-971). Other examples might include caves, lakes, ponds, streams, and perhaps even the more moist varieties of prairies and forests. In general, the Board believes that the concept of special treatment of unique or ecologically vital groundwaters via more stringent standards is a meritorious concept.

In its First-Notice form Section 620.230 provided for the placement of a groundwater in Class III only though the formal action of the Board pursuant to Section 620.250. At the May 30, 1991 hearing, with the support of the Illinois Nature Preserves Commission (PC #50) and the Illinois Department of Conservation (PC #52), the Agency proposed that groundwaters that contribute to a dedicated nature preserve, as listed by the Agency, also be designated as Class III groundwaters via an alternate, more expeditious route found at subsection (b) (R3 at 24-7). As the Agency notes:

> This will provide a more expedited process to list sites that have already been designated by the Nature Preserve Commission, and also will allow for a review

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[13] See Guidelines for Ground-Water Classification under the EPA Ground-Water Protection Strategy, USEPA Office of Ground-Water Protection, November 1986: Defender's Exh. 6.

> of these sites on a case-by-case basis. Sixty sites have been identified by the Commission as nature preserves that may have an important relationship to groundwaters. The review of this information on a case-by-case basis is important to help determine what relation groundwater has to these sites. (R3 at 26)

The Board notes that any person who feels aggrieved by an Agency decision under subsection (b) would still have recourse to bring the action before the Board pursuant to subsection (a). Moreover, as the Defenders correctly observe, the listing process would "only resolve the question of which nature preserves would be designated as containing Class III groundwater; there may still need to be a Board proceeding to determine the appropriate groundwater standards to apply" (PC #57 at p. 6).

Other Groundwater -- Section 620.240

Section 620.240 sets out criteria for classifying Class IV: Other Groundwater. The purpose of the class is to accommodate certain waters that, due to particular practices or natural conditions, are limited in their resource potential. Included are groundwaters that are naturally saline, groundwaters that occur in the zone of attenuation surrounding a landfill, groundwaters in mining-disturbed areas, and affected groundwaters associated with potential primary or secondary sources, as defined in the IGPA. The class also would contain any groundwater designated by the Board as an exempt aquifer.

Several modifications of Section 620.240 were made in response to First Notice comments. The interested person is direction to the Second Notice Opinion of July 25, 1991 at p. 13-15 for a description and discussion of these modifications.

Groundwater Management Zones -- Section 620.250

Section 620.250 provides for establishment of a management zone within each class of groundwater. A management zone is identified by the Agency for groundwaters that have become impaired due to contamination. In any management zone the goal is remediation, if practicable, of the groundwater to the level of the standards applicable to that class of groundwater (R3 at 32).

Unlike most of the other provisions of today's rules, the concept of a management zone was first introduced into this proceeding in the Agency's Docket B proposal. Previously the various proposals had entertained a "Remedial Groundwater" class into which various "substandard" but potentially remediable groundwaters were to reside temporarily or permanently (e.g., see Section 620.230 of the Board's Docket A proposal, September 27, 1990). As the Agency observes, a persistent problem with a remedial class of groundwater concerns the class to which

remedial groundwaters return after remediation (R3 at 32). As an alternative to a separate remedial class, the Agency turned to the groundwater management zone (Id.). Moreover, the management zone concept also provides a better coupling with RCRA and CERCLA regulations (Id. at 33).

At second notice, JCAR recommended that the form required for the confirmation of an adequate corrective action pursuant to 35 Ill. Adm. Code 620.250(a)(2) be made an appendix to the rule. The Board has agreed to do so, and the form is placed at Appendix D in today's order.

Adjusted Standards -- Section 620.260

Section 620.260 specifies that reclassification of any groundwater can occur as a result of an adjusted standard proceeding before the Board, in accord with the adjusted standard provisions of the Illinois Environmental Protection Act. The Section also specifies the level of justification required of a petitioner and other information to allow the Board to determine the adjusted standard, pursuant to Section 28.1 of the Act.

The Board notes that, in addition to an adjusted standard, recourse to reclassification of a particular groundwater also would be available via the site-specific rulemaking process. Since, there are differences in proofs, conduct of hearings, etc., between adjusted standards proceedings and rulemaking proceedings, any interested person would be advised to consider both before choosing a course of action.

PART 620
SUBPART C: NONDEGRADATION AND PREVENTIVE NOTIFICATION/RESPONSE

Subpart C contains nondegradation provisions and general preventive notification and response actions. These, in part, set the framework for the remainder of Part 620.

Nondegradation -- Section 620.301

Section 620.301 states the basic nondegradation provision of today's rules. Its essence is a prohibition against impairment of any existing or potential use of groundwaters.

A principal area of contention in this proceeding has been whether nondegradation ought to encompass some more stringent prohibition. Alternate proposals have included a prohibition against causing or allowing a statistically significant alteration in groundwater chemistry, or of causing or allowing any change in groundwater chemistry.

The Board today declines to generally extend nondegradation beyond the prohibition against loss of use[14]. The Board does this with some reluctance. Perhaps at some time in the future this step can be taken. However, today we simply do not have the information base, or resources necessary to obtain the information base, upon which to found universal judgments of no (statistical) change in groundwater chemistry.

It has sometimes been said that casting the nondegradation provision as it is today is equivalent to allowing pollution up to the standard. The Board believes that this characterization is too simplistic. Among other matters, the whole preventive notification and response program (see following) is directed toward an early alert to, and staving off, of any increase in contamination in the most sensitive groundwater/potential source situations. Moreover, in other regulations, such as the Board's landfill regulations at 35 Ill. Adm. Code 810-815 and the groundwater regulations at 35 Ill. Adm. Code 615 and 616[15], additional proscriptions against allowing of groundwater quality modifications also occur.

The Board believes that the proper way to characterize today's nondegradation provision is that it consists of the baseline, rule-of-general-applicability. In specific circumstances dictated both by today's rule and by other regulations license to alter the State's groundwaters is significantly more proscribed. Moreover, the Board also would anticipate, as we gain better understanding of the many dynamics of groundwater and sources of groundwater pollution, that these proscriptions also will expand. In fact, at subsection (d) the Board emphasizes its intention of providing for such different nondegradation provisions, including more stringent provisions, applicable under specific circumstances.

Applicability of Preventive Notification/Response -- Section 620.302

Section 620.302 sets forth the circumstance under which preventive notification and preventive response is applicable; the section is a prelude to the preventive notification and response provisions found in Section 620.305 and 620.310. In

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[14] There is one area in which the Board has previously determined that no statistical increase in groundwater contaminants is allowable. That is at the bounds of the zone of attenuation associated with landfills. The Board intends that nothing in today's action overturn this prior determination.

[15] In the Matter of: Groundwater Protection: Regulations for Existing and New Activities Within Setback Zones and Regulated Recharge Areas, (35 Ill. Adm. Code 601, 615, 616, and 617), R89-5, currently in Second Notice.

general, preventive notification and response is applicable only to persons who conduct groundwater monitoring pursuant to some other State or Federal program (R3 at 39). In addition, preventive notification and response is associated only with the high-quality, high-use groundwaters, Class I and Class III groundwaters.

At the recommendation of JCAR, the Board has agreed to insert the appropriate citations after the phrase "state or Federal law or regulation" at 620.302(b)(1).

Preventive Notification Procedures -- Section 620.305

Preventive notification consists of (a) confirmation of results and (b) notification of interested persons. If it is the owner or operator who is required to monitor, the appropriate regulatory agency must be notified of the results. If it is a government agency that is required to monitor, it is the owner or operator who must be notified.

The preventive notification procedures for Class I groundwaters are triggered when numerical limits associated with either of two classes of contaminants is exceeded. These are the contaminants found at Section 620.310(a)(3)(A), which consist of the toxic heavy metals and the more common organic and petrochemical contaminants, and the contaminants identified as carcinogens at Section 620.410(b). For Class III groundwaters the preventive notification trigger is the detection of a contaminant for which there is a standard pursuant to Section 620.430.

It is important to note that these preventive notification triggers are generally much lower than the water quality standard for the same constituents. This is in keeping with the philosophy of becoming alert and reacting to potential problems in high-value groundwaters before these problems can grow to an unmanageable scale. It is further in keeping with the principle that in general it is much more expensive, including public expense, to remediate contaminated groundwater than it is to prevent the occurrence of groundwater contamination.

Preventive Response Activities and Levels -- Section 620.310

Section 620.310 describes preventive response activities that are required upon receipt of a preventive notification. The Section also specifies the preventive response levels[16] used to determine if a detected concentration requires a preventive response. In either case, the purpose of this Section to is to

[16] Prior to the submission of the Docket B proposal, these limits were called "corrective action levels" (e.g., R1 at 114-129).

provide a nexus between the body of today's rules and existing and future regulatory programs that need triggers for corrective action. No new corrective action program is today adopted.

The preventive response levels are set with several conditions in mind (R3 at 43). Among these are that all levels are at or above the practical quantitation limit (PQL); carcinogens, which have potable resources standards set at PQLs (see Section 620.410), are not listed because there is no basis for establishing a preventive response level below a PQL (PC #47 at p. 15). Exceedence[17] of background is employed for metals and the non-carcinogenic organic constituents.

PART 620
SUBPART D: GROUNDWATER QUALITY STANDARDS

Subpart D constitutes the focus of the instant regulations. Within it are contained the actual groundwater standards as mandated by the IGPA. Since the groundwater standards are closely tied to the groundwater classification system of Subpart B, the form of Subpart D parallels that of Subpart B.

Introduction -- Sections 620.401 and 620.405

Section 620.401 establishes the connection between the groundwater classification system presented in Subpart B and the groundwater standards of Subpart D, which is that all groundwater must meet the standards specified for the class to which the groundwater belongs. Section 620.405 provides a narrative standard that prohibits violation of the numeric standards of this Subpart.

Standards for Potable Resource Groundwater -- Section 620.410

Section 620.410 contains the groundwater standards applicable to the Potable Resource Groundwater found in Class I (see discussion of Section 620.210, above). In general, the standards found in this Section are equal to the USEPA's Maximum Concentration Levels ("MCLs)" applicable "at-the-tap" pursuant to the Safe Drinking Water Act ("SDWA"). The MCL levels are specified as water quality standards under the principle that groundwaters that are naturally potable should be available for drinking water supply without treatment.

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[17] The Board notes that within Section 620.310 and several subsequent sections of the First Notice proposal, the word *exceedence* was incorrectly spelled as exceedance. *Exceedence* is derived from the verb *exceed*, which in turn is derived from the Latin *excedere* via the Middle French *exceder* and the Middle English *exceden*; Latin infinitives ending in "ere" generate English nouns ending in "ence".

An historical difficulty with incorporation of numeric standards within regulations is the need to constantly revise the numbers as new information is developed. This difficulty has a particular presence in the instant matter because the USEPA is in the process of a major MCL promulgation effort. Even over the short course of this proceeding the Agency has had to several times revise its standards recommendations in keeping with USEPA's action on MCLs (e.g., PC #47 at p. 17-9; R3 at 49-50; Exh. T; PC #52 at p. 25, 27); it is to be expected that the current MCL list will continue to experience changes within the coming years.

At the First Notice of Docket A the Board proposed to address the matter of changing standards/MCLs of Section 620.410 in what it considered a novel and advantageous method. The Board noted:

> Ordinarily [the USEPA promulgation of new standards/MCLs] would imply that Part 620 regulations would have to be regularly reopened and updated to accommodate new MCLs. However, the Board today proposes a stratagem that both forestalls the need to constantly update the MCL list at Section [620.410] and also assures that the MCLs of Section [620.410] remain current. The stratagem consists of identifying the groundwater standards that apply to Potable Resource Groundwaters as being identical with the MCLs found at 35 Ill. Adm. Code 611.Subpart F. 35 Ill. Adm. Code 611.Subpart F contains the "identical in substance" MCLs promulgated pursuant to the SDWA and the Act. As such, 611.Subpart F is subject to updates every six months, pursuant to the Board's SDWA "identical in substance" update program. (Docket A, Opinion p. 17)

At Second Notice of the instant rules the Board receded from this stratagem in the interest of moving this proceeding forward. However, the Board there noted and here continues to note that it expects from the Agency regular[18] updates of the groundwater standards, parallel to those undertaken for the Public Water Supply Standards at 611.Subpart F.

General Resource Groundwater Standards -- Section 620.420

Section 620.420 establishes standards for Class II: General Resource Groundwaters. Because groundwaters are placed in Class II because they are quality-limited, quantity-limited, or both (see Subpart B discussion above), it is necessary that the

[18] The Board notes that the Defenders urge a regular (perhaps every three years) review of both the Class I and Class II standards (e.g., R3 at 257).

standards that apply to these waters reflect this range of possible attributes. Among the factors considered in determining the Class II numbers are the capabilities of treatment technologies to bring Class II waters to qualities suitable for potable use (R3 at 75). Thus, many Class II standards are based on MCLs as modified to reflect treatment capabilities. For some parameters the Class II standards are based on support of a use other than potability (e.g., livestock watering, irrigation, industrial use) where the different use requires a more stringent standard (R3 at 114-8).

Standards Applicable to Special Resource Groundwaters -- Section 620.430

Section 620.430 specifies that the standards applicable to Class III: Special Resource Groundwater are the same standards applicable to Class I groundwater, except as may be provided by the Board in a proceeding pursuant to Section 620.260. Accordingly, the default values of the standards are the Class I standards, with more stringent standards possible if a justification is made for them.

Standards Applicable to Other Groundwater -- Section 620.440

The existing concentration is the basic standard to be applicable to Class IV Groundwater. It is also provided that specific exceptions apply to groundwaters within a zone of attenuation of a landfill, as defined pursuant to 35 Ill. Adm. Code 811 and 814, and within a previously mined area as defined at Section 620.110. Within a zone of attenuation existing concentrations are not to be exceeded except as caused by leachate. Within a previously mined area existing concentrations are not to be exceeded except for pH, total dissolved solids, and those major ions (chloride, iron, manganese, and sulfate), which are typically disturbed as a result of coal mining.

Alternate Groundwater Standards -- Section 620.450

Section 620.450 recognizes that special groundwater standards are necessarily associated with certain activities, as contrasted to native types of groundwater. These activities today are identified to include sites undergoing corrective action or equivalent corrective processes and sites for surface and underground coal mining activities.

At the recommendation of JCAR, the Board agreed to cite the appropriate citation to 62 Ill. Adm. Code 1780.21(f) and (g) at 620.450(b)(3)(A)(ii).

PART 620
SUBPART E: GROUNDWATER MONITORING AND ANALYTICAL PROCEDURES

Subpart E sets out some minimal conditions associated with groundwater monitoring and analytical procedures. These constitute rules-of-general-applicability; in other regulations further conditions and proscriptions may be added to these. It is to be particularly noted that today's rules contain no new required monitoring program.

An important part of Subpart E is found in the Compliance Procedures of Section 620.505. This Section specifies where compliance determinations may be made. As the Agency notes, this Section "recognizes the practical limitations associated with groundwater monitoring and cleanup under a building, landfill, or tank" (PC #47 at p. 23). Also specified in Section 620.505 are the conditions necessary for a water or monitoring well to serve as a compliance point.

For Section 620.510(b)(1), at the recommendation of JCAR, the Board inserted the word "regulatory" after the word "appropriate", to make it clear that what is referred to is the appropriate regulatory agency.

PART 620
SUBPART F: HEALTH ADVISORIES

Subpart F establishes procedures for developing and issuing a Health Advisory. A Health Advisory is a means for the Agency to establish a guidance level for a chemical substance or a mixture of chemical substances for which a standard has not yet been set under Subpart D. This advisory process is intended to mirror the procedure used by USEPA to account for substances detected in groundwater that do not have a promulgated standard. Also, the Agency notes that this Subpart would codify existing practice by the Agency (Statement of Reasons, p. 28-36).

Because the Health Advisory provision and its attendant Appendices have been presented to the Board without apparent controversy, and because the Board has not itself proposed substantive amendment to the Agency's version, the Board will not here discuss these matters further. The interested person is directed to the Agency's Statement of Reasons, p. 28-36, for more discussion and explanation.

Two changes to the Health Advisory material were recommended by JCAR and accepted by the Board. These are to update certain phraseology and citations connected with the change in public water supply regulations cited in Section 620.601(b); and to delete the term "approximately" and add the terms "at least 5%" in Section 620.Appendix A(c)(1)(iii).

ECONOMIC IMPACT

EcIS Document

On January 31, 1990, DENR filed the EcIS in this matter, titled: "Economic Impact Study for Proposed Groundwater Quality Standards, 35 IL. Admin. Code 620" (DENR Exh. 5). The EcIS was prepared by Camp Dresser & McKee, Inc. The study evaluated groundwater remediation costs using historical data on groundwater contamination in the State and also examined benefits consisting of reduced health risks through decreased exposure to contaminants in groundwater. Pursuant to Section 8 of the IGPA and in an effort to expedite the promulgation of the regulations, the EcIS was conducted concurrently with the development of the regulations. Therefore, the EcIS document focused on various options under consideration during the development of the original Agency proposal, over two years prior to today's action.

Cost Analysis

The EcIS investigators determined that the most significant costs of the regulations can be expected to be groundwater remediation costs; i.e., those costs associated with returning contaminated groundwater to compliance with the standards.

To estimate remediation costs, the EcIS investigators used historical data on groundwater contamination in the State. The analysis focused on costs for prototypical remediation of six parameters representing organic, inorganic, and pesticide contaminants. Cleanup cost estimates ranged from $8.83-$8.85 million for the organic contaminants, $12.84-$13.64 million for the pesticides, and $9.10 million for the inorganic contaminants, per incident over a 20 year period. To derive statewide cleanup costs, the estimated per facility costs were multiplied by an estimated number of sites of contamination (24 volatile organic compound incidents and four pesticide incidents). The estimated costs for these remedial actions would range from $263-$267 million.

By using data on existing incidence of groundwater contamination, the EcIS investigators further assumed that costs could be higher for three reasons. These are that although the Agency did not report an incidence for inorganic contamination of public water supply facilities, it is highly probable that the incidence would be greater than zero. Also, since the regulations could include a greater number of VOC's than the Safe Drinking Water Act MCLs, a greater incidence of contamination can be expected. Lastly, the EcIS investigators believe that the actual number of cleanups required would more likely be closer to the number of facilities that exceed the detection limit than the number that exceed an MCL. The statement is based on their belief that once a contaminant is detected, groundwater

contamination is already likely to exceed enforcement or potable use standards somewhere at the site[19].

Given these considerations, and based upon estimates derived from existing contamination incidences, the EcIS investigators reported estimated costs of $1,141 million for VOC remediation, $238 million for pesticide remediation, and $610 million for inorganic remediation, leading to a total estimated state-wide cleanup cost of $1.99 billion. This was calculated only for sites within 3000 feet of community water supply wells, since the proposed Class I standards at the time of EcIS development were proposed to be applied only within the 3000-foot distance.

In its most recent comments, DENR estimates that the costs under today's version of the rules could be higher since Class I has been expanded to include a larger volume of the State's groundwaters. The EcIS investigators estimated costs 50% higher should Class I (as defined sometime before the completion of the EcIS in January 1990) include all groundwaters rather than the 3000-foot zone. Therefore DENR states that costs for the entire State would be $3.1 billion. DENR recognizes that the addition of provisions for groundwater management zones and adjusted standards options could offset the increase (PC #55).

Benefits Analysis

The EcIS investigators report that the primary benefit of groundwater standards is "reduced health risks through decreased exposure to contaminants in groundwater". They explain the benefits thusly:

> These benefits can be expressed as decreased health care expenses, lower health insurance premiums, reduction in pain and suffering, and a better quality of life for Illinois citizens. Reductions in excess cancer risks . . . [and a]lthough not examined quantitatively, a corresponding decrease in non-carcinogenic health risks also can be anticipated as a result of the proposed regulations.
>
> A second major benefit of the proposed regulations is preservation of groundwater as a resource for future generations. By preventing contamination where possible through preventive management practices and by

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[19] For the same reasons, the EcIS investigators believe that the economic impact of trigger limits which would be somewhere between detection levels and potable use standards would not result in cost savings due to early detection of contamination. That is, they believe that once there is detection, there would most likely already be contamination above potable use standards somewhere on the site, which would require remediation.

> addressing existing contamination through groundwater remediation, the value of the resource is preserved and the availability of groundwater for future use is greatly enhanced.
>
> Other non-quantifiable benefits include avoided decreases in property values proximal to sites of groundwater contamination, avoided restrictions in siting for private and community potable wells, and avoided negative impact on wildlife and ecology of areas served by groundwater base flow. Additionally, the aesthetic value of the state's groundwater reserves will be enhanced by the proposed regulations. Finally, . . . a major portion of the costs of cleanups can actually be considered benefits for engineering firms, construction firms, water utilities, and other parties involved in groundwater remediation.
>
> (EcIS at 6-8 to 6-10)

In Appendix D to the EcIS the EcIS investigators listed information on the toxicological effects of substances to be regulated. Section 5.2.4 of the EcIS discusses calculations of carcinogenic risk factors based on USEPA risk levels defined in terms of excess cancer risks.

Discussion and Comments

One of the major points brought out in comments surrounding and at the March 29, 1990 EcIS hearing is that the EcIS authors did not base analysis upon what was then the Agency's proposal (R. 697-702; PC #16, R89-14 Board First Notice Opinion and Order, September 27, 1990). This was mainly because the EcIS was statutorially required to be conducted concurrently with the development of the proposal. This was done in an effort to inject economic analysis into the process at an early stage. However, it did not anticipate that the proposal would undergo a series of major revisions after completion of the EcIS. Thus, even had the EcIS been conducted on the Agency's proposal as it stood in March 1990, the EcIS could not have addressed the changes in subsequent proposals, including the rules today adopted. Therefore, any examination of economic impact that includes the EcIS must consider the context in which the study was developed and the lack of availability to the EcIS investigators of subsequent revisions.

With that preface, the Board recognizes that if remediation to the level of today's standards is subsequently required through other programs, costs of remediation of groundwater could be substantial. It is important to remember, however, that these are groundwater quality standards, not cleanup standards or requirements. As the EcIS authors realized, site specific considerations can and most likely will determine the nature of

required remediation and what actual cost is to be borne by any particular entity, industry, or government[20]. As stated concerning the concurrent R89-5 proceeding, there is difficulty in applying economic analysis to a rule of general applicability. This is especially true where there are as many varied conditions and unknown circumstances as are likely to be encountered here. It also must be borne in mind that exception procedures associated with adjusted standards and features such as the groundwater management zones must temper any attempt to broadly cast cost estimates.

Another factor in consideration of the EcIS' cost estimates is that the instant regulations do not create or require any new corrective action program; all such programs are part of other regulations already in place or proposed (e.g., RCRA, CERCLA, LUST, waterwell setback regulations, etc.). It is accordingly not appropriate to attribute to today's regulations the cost of corrective actions that are not prompted by today's regulations. The EcIS investigators recognized that the remedial costs properly associated with the instant rules should be "incremental costs over and above the costs associated with the currently applicable regulations for water quality standards and cleanup criteria", but further stated that they did not consider the costs of these other programs because of "the limited number of remediations brought under the current regulatory scheme" (EcIS at ii).

The fact that the EcIS investigators attributed to today's groundwater quality standards all the costs of any potential future remedial action is a serious flaw in the EcIS analysis. Cleanup of contaminants to the levels stated in these rules as required by an appropriate agency during remediation does not mean that all the costs of cleanup should be attributed to adoption of today's rules. The remediation programs already require cleanup of most of the parameters listed in the instant regulations[21], in some cases to levels more stringent than in today's rules. That to date there have been few such cleanups in Illinois does not make the cost of all further cleanups attributable to today's rules.

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[20] Much discussion at hearing and in subsequent comments concerned different treatment techniques and their costs (See generally, R1 at 889-97, 760-1; PC #5).

[21] Some of the parameters in the instant regulations, including iron, total dissolved solids, and boron, are not regulated under RCRA and CERCLA. However, the EcIS investigators observe that it would be unlikely that these parameters could be exceeded without a simultaneous exceedence of one or more parameters which are regulated under RCRA and CERCLA (R1 at 759).

A comprehensive list of benefits was included in the EcIS, though the EcIS investigators did not attempt to quantify them, save for the carcinogenic health risks. Additional benefits identified at hearing include reduced expenses to obtain alternate water supplies necessary to replace contaminated current supplies, and reduced expenses for treatment of water at well heads to render it potable or suitable for industrial use (R1 at 820, 830-2). It is important to note that although the benefits currently cannot be quantified, they are thereby no less real or substantial; it is only that they cannot be identified in terms of reliable, specific dollar figures.

ORDER

The Clerk of the Board is directed to submit the text of the following amendments to the Secretary of State for final notice pursuant to Section 6 of the Illinois Administrative Procedures Act.

TITLE 35: ENVIRONMENTAL PROTECTION
SUBTITLE F: PUBLIC WATER SUPPLIES
CHAPTER I: POLLUTION CONTROL BOARD

PART 620
GROUNDWATER QUALITY

SUBPART A: GENERAL

SUBPART B: GROUNDWATER CLASSIFICATION

SUBPART C: NONDEGRADATION PROVISIONS FOR APPROPRIATE GROUNDWATERS

SUBPART D: GROUNDWATER QUALITY STANDARDS

SUBPART E: GROUNDWATER MONITORING AND ANALYTICAL PROCEDURES

SUBPART F: HEALTH ADVISORIES

AUTHORITY: Implementing and authorized by Section 8 of the Illinois Groundwater Protection Act (Ill. Rev. Stat. 1989, ch. 111 1/2, par. 7458).

SOURCE: Adopted in R89-14(B) at ____ Ill. Reg., _____, effective _____.

NOTE: Capitalization denotes statutory language.

SUBPART A: GENERAL

Section 620.105 Purpose

This Part prescribes various aspects of groundwater quality, including method of classification of groundwaters, nondegradation provisions, standards for quality of groundwaters, and various procedures and protocols for the management and protection of groundwaters.

Section 620.110 Definitions

The definitions of the Environmental Protection Act (Ill. Rev. Stat. 1989, ch. 111 1/2, par. 1001 et seq.) and the Groundwater Protection Act (Ill. Rev. Stat. 1989, ch. 111 1/2, pars. 7451 et seq.) apply to this Part. The following definitions also apply to this Part.

"Act" means the Environmental Protection Act (Ill. Rev. Stat. 1989, ch. 111 1/2, pars. 1001 et seq.).

"Agency" means the Illinois Environmental Protection Agency.

"AQUIFER" MEANS SATURATED (WITH GROUNDWATER) SOILS AND GEOLOGIC MATERIALS WHICH ARE SUFFICIENTLY PERMEABLE TO READILY YIELD ECONOMICALLY USEFUL QUANTITIES OF WATER TO WELLS, SPRINGS, OR STREAMS UNDER ORDINARY HYDRAULIC GRADIENTS. (Section 3(b) of the IGPA)

"BETX" means the sum of the concentrations of benzene, ethylbenzene, toluene, and xylenes.

"Board" means the Illinois Pollution Control Board.

"Carcinogen" means a chemical, or complex mixture of closely related chemicals, which has been listed or classified in the Integrated Risk Information System or as specified in a final rule adopted by USEPA in accordance with USEPA Guidelines for Carcinogenic Risk Assessment, incorporated by reference at Section 620.125, to be a group A, B_1, or B_2 carcinogen.

"COMMUNITY WATER SUPPLY" MEANS A PUBLIC SUPPLY WHICH SERVES OR IS INTENDED TO SERVE AT LEAST 15 SERVICE CONNECTIONS USED BY RESIDENTS OR REGULARLY SERVES AT LEAST 25 RESIDENTS. (Section 3.05 of the Act)

"CONTAMINANT" MEANS ANY SOLID, LIQUID, OR GASEOUS MATTER, ANY ODOR, OR ANY FORM OF ENERGY, FROM WHATEVER SOURCE. (Section 3.06 of the Act)

"Corrective action process" means those procedures and practices that may be imposed by a regulatory agency when a determination has been made that contamination of groundwater has taken place, and are necessary to address a potential or existing violation of the standards set forth in Subpart D.

"Cumulative impact area" means the area, including the coal mine area permitted under the Surface Coal Mining Land Conservation Act (Ill. Rev. Stat. 1989, ch. 96 1/2, pars. 7901.01 et seq., as amended) and 62 Ill. Adm. Code 1700 through 1850, within which impacts resulting from the proposed operation may interact with the impacts of all anticipated mining on surface water and groundwater systems.

"Detection" means the identification of a contaminant in a sample at a value equal to or greater than the:

"Method Detection Limit" or "MDL" which means the minimum concentration of a substance that can be measured as reported with 99 percent confidence that the true value is greater than zero, pursuant to 56 Fed. Reg. 3526-3597, incorporated by reference at Section 620.125; or

"Method Quantitation Limit" or "MQL" which means the minimum concentration of a substance that can be measured and reported pursuant to "Test Methods for Evaluating Solid Wastes, Physical/ Chemical Methods", incorporated by reference at Section 620.125.

"Department" means the Illinois Department of Energy and Natural Resources.

"GROUNDWATER" MEANS UNDERGROUND WATER WHICH OCCURS WITHIN THE SATURATED ZONE AND GEOLOGIC MATERIALS WHERE THE FLUID PRESSURE IN THE PORE SPACE IS EQUAL TO OR GREATER THAN ATMOSPHERIC PRESSURE. (Section 3.64 of the Act)

"Hydrologic balance" means the relationship between the quality and quantity of water inflow to, water outflow from, and water storage in a hydrologic unit such as a drainage basin, aquifer, soil zone, lake, or reservoir. It encompasses the dynamic relationships among precipitation, runoff, evaporation, and changes in ground and surface water storage.

"IGPA" means the Illinois Groundwater Protection Act. (Ill. Rev. Stat. 1989, ch. 111 1/2, pars. 7451 et seq.)

"LOAEL" or "Lowest observable adverse effect level" means the lowest tested concentration of a chemical or substance which produces a statistically significant increase in frequency or severity of non-overt adverse effects between the exposed population and its appropriate control. LOAEL may be determined for a human population (LOAEL-H) or an animal population (LOAEL-A).

"NOAEL" or "No observable adverse effect level" means the highest tested concentration of a chemical or substance which does not produce a statistically significant increase in frequency or severity of non-overt adverse effects between the exposed population and its appropriate control. NOAEL may be determined for a human population (NOAEL-H) or an animal population (NOAEL-A)

"NON-COMMUNITY WATER SUPPLY" MEANS A PUBLIC WATER SUPPLY THAT IS NOT A COMMUNITY WATER SUPPLY. (Section 3.05)

"Off-site" means not on-site.

"On-site" means on the same or geographically contiguous property which may be divided by public or private right-of-way, provided the entrance and exit between properties is at a crossroads intersection and access is by crossing as opposed to going along the right-of-way. Noncontiguous properties owned by the same person but connected by a right-of-way which he controls and to which the public does not have access is also considered on-site property.

"Operator" means the person responsible for the operation of a site, facility or unit.

"Owner" means the person who owns a site, facility or unit or part of a site, facility or unit, or who owns the land on which the site, facility or unit is located.

"POTABLE" MEANS GENERALLY FIT FOR HUMAN CONSUMPTION IN ACCORDANCE WITH ACCEPTED WATER SUPPLY PRINCIPLES AND PRACTICES. (Section 3.65 of the Act)

"POTENTIAL PRIMARY SOURCE" MEANS ANY UNIT AT A FACILITY OR SITE NOT CURRENTLY SUBJECT TO A REMOVAL OR REMEDIAL ACTION WHICH:

IS UTILIZED FOR THE TREATMENT, STORAGE, OR DISPOSAL OF ANY HAZARDOUS OR SPECIAL WASTE NOT GENERATED AT THE SITE; OR

IS UTILIZED FOR THE DISPOSAL OF MUNICIPAL WASTE NOT GENERATED AT THE SITE, OTHER THAN LANDSCAPE WASTE AND CONSTRUCTION AND DEMOLITION DEBRIS; OR

IS UTILIZED FOR THE LANDFILLING, LAND TREATING, SURFACE IMPOUNDING OR PILING OF ANY HAZARDOUS OR SPECIAL WASTE THAT IS GENERATED ON THE SITE OR AT OTHER SITES OWNED, CONTROLLED OR OPERATED BY THE SAME PERSON; OR

STORES OR ACCUMULATES AT ANY TIME MORE THAN 75,000 POUNDS ABOVE GROUND, OR MORE THAN 7,500 POUNDS BELOW GROUND, OF ANY HAZARDOUS SUBSTANCES. (Section 3.59 of the Act)

"POTENTIAL ROUTE" MEANS ABANDONED AND IMPROPERLY PLUGGED WELLS OF ALL KINDS, DRAINAGE WELLS, ALL INJECTION WELLS, INCLUDING CLOSED LOOP HEAT PUMP WELLS, AND ANY EXCAVATION FOR THE DISCOVERY, DEVELOPMENT OR PRODUCTION OF STONE, SAND OR GRAVEL. (Section 3.58 of the Act)

"POTENTIAL SECONDARY SOURCE" MEANS ANY UNIT AT A FACILITY OR A SITE NOT CURRENTLY SUBJECT TO A REMOVAL OR REMEDIAL ACTION, OTHER THAN A POTENTIAL PRIMARY SOURCE, WHICH:

IS UTILIZED FOR THE LANDFILLING, LAND TREATING, OR SURFACE IMPOUNDING OF WASTE THAT IS GENERATED ON THE SITE OR AT OTHER SITES OWNED, CONTROLLED OR OPERATED BY THE SAME PERSON, OTHER THAN LIVESTOCK AND LANDSCAPE WASTE, AND CONSTRUCTION AND DEMOLITION DEBRIS; OR

STORES OR ACCUMULATES AT ANY TIME MORE THAN 25,000 BUT NOT MORE THAN 75,000 POUNDS ABOVE GROUND, OR MORE THAN 2,500 BUT NOT MORE THAN 7,500 POUNDS BELOW GROUND, OF ANY HAZARDOUS SUBSTANCES; OR

STORES OR ACCUMULATES AT ANY TIME MORE THAN 25,000 GALLONS ABOVE GROUND, OR MORE THAN 500 GALLONS BELOW GROUND, OF PETROLEUM, INCLUDING CRUDE OIL OR ANY FRACTION THEREOF WHICH IS NOT OTHERWISE SPECIFICALLY LISTED OR DESIGNATED AS A HAZARDOUS SUBSTANCE; OR

STORES OR ACCUMULATES PESTICIDES, FERTILIZERS, OR ROAD OILS FOR PURPOSES OF COMMERCIAL APPLICATION OR FOR DISTRIBUTION TO RETAIL SALES OUTLETS; OR

STORES OR ACCUMULATES AT ANY TIME MORE THAN 50,000 POUNDS OF ANY DE-ICING AGENT; OR

IS UTILIZED FOR HANDLING LIVESTOCK WASTE OR FOR TREATING DOMESTIC WASTEWATERS OTHER THAN PRIVATE SEWAGE DISPOSAL SYSTEMS AS DEFINED IN THE PRIVATE SEWAGE DISPOSAL LICENSING ACT, Ill. Rev. Stat. 1989, ch. 111 1/2, par. 116.301 et seq. (Section 3.60 of the Act)

"Practical Quantitation Limit" or "PQL" means the lowest concentration or level that can be reliably measured within specified limits of precision and accuracy during routine laboratory operating conditions in accordance with "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846, incorporated by reference at Section 620.125.

"Previously mined area" means land disturbed or affected by coal mining operations prior to February 1, 1983.

(Board Note: February 1, 1983, is the effective date of the Illinois permanent program regulations implementing the Surface Coal Mining Land Conservation and Reclamation Act (Ill. Rev. Stat. 1989, ch. 96 1/2, pars. 7901.1 et seq., as amended) as codified in 62 Ill. Adm. Code 1700 through 1850.)

"Property class" means the class assigned by a tax assessor to real property for purposes of real estate taxes.

(Board Note: The property class [rural property, residential vacant land, residential with dwelling, commercial residence, commercial business, commercial office, or industrial] is identified on the property record card maintained by the tax assessor in accordance with the Illinois Real Property Appraisal Manual [February 1987], published by the Illinois Department of Revenue, Property Tax Administration Bureau.)

"PUBLIC WATER SUPPLY" MEANS ALL MAINS, PIPES AND STRUCTURES THROUGH WHICH WATER IS OBTAINED AND DISTRIBUTED TO THE PUBLIC, INCLUDING WELLS AND WELL STRUCTURES, INTAKES AND CRIBS, PUMPING STATIONS, TREATMENT PLANTS, RESERVOIRS, STORAGE TANKS AND APPURTENANCES, COLLECTIVELY OR SEVERALLY, ACTUALLY USED OR INTENDED FOR USE FOR THE PURPOSE OF FURNISHING WATER FOR DRINKING OR GENERAL DOMESTIC USE AND WHICH SERVE AT LEAST 15 SERVICE CONNECTIONS OR WHICH REGULARLY SERVE AT LEAST 25 PERSONS AT LEAST 60 DAYS PER YEAR. A PUBLIC WATER SUPPLY IS EITHER A "COMMUNITY WATER SUPPLY" OR A "NON-COMMUNITY WATER SUPPLY". (Section 3.28 of the Act)

"Regulated entity" means a facility or unit regulated for groundwater protection by any state or federal agency.

"Regulatory agency" means the Illinois Environmental Protection Agency, Department of Public Health, Department of Agriculture, Department of Mines and Minerals, and the Office of State Fire Marshal.

"REGULATED RECHARGE AREA" MEANS A COMPACT GEOGRAPHIC AREA, AS DETERMINED BY THE BOARD pursuant to Section 17.4 of the Act, THE GEOLOGY OF WHICH RENDERS A POTABLE RESOURCE GROUNDWATER PARTICULARLY SUSCEPTIBLE TO CONTAMINATION. (Section 3.67 of the Act)

"RESOURCE GROUNDWATER" MEANS GROUNDWATER THAT IS PRESENTLY BEING, OR IN THE FUTURE IS CAPABLE OF BEING, PUT TO BENEFICIAL USE BY REASON OF BEING OF SUITABLE QUALITY. (Section 3.66 of the Act)

"SETBACK ZONE" MEANS A GEOGRAPHIC AREA, DESIGNATED PURSUANT TO THIS ACT, CONTAINING A POTABLE WATER SUPPLY WELL OR A POTENTIAL SOURCE OR POTENTIAL ROUTE HAVING A CONTINUOUS BOUNDARY, AND WITHIN WHICH CERTAIN PROHIBITIONS OR REGULATIONS ARE APPLICABLE IN ORDER TO PROTECT GROUNDWATERS. (Section 3.61 of the Act)

"Site" MEANS ANY LOCATION, PLACE, TRACT OF LAND, AND FACILITIES, INCLUDING BUT NOT LIMITED TO, BUILDINGS AND IMPROVEMENTS USED FOR PURPOSES SUBJECT TO REGULATION OR CONTROL BY the ACT OR REGULATIONS THEREUNDER. (Section 3.43 of the Act)

"Spring" means a natural surface discharge of an aquifer from rock or soil.

"Threshold dose" means the lowest dose of a chemical at which a specified measurable effect is observed and below which it is not observed.

"Treatment" means the technology, treatment techniques, or other procedures for compliance with 35 Ill. Adm. Code: Subtitle F.

"UNIT" MEANS ANY DEVICE, MECHANISM, EQUIPMENT, OR AREA (EXCLUSIVE OF LAND UTILIZED ONLY FOR AGRICULTURAL PRODUCTION). (Section 3.62) of the Act)

"USEPA" or "U.S. EPA" means the United States Environmental Protection Agency.

Section 620.115 Prohibition

No person shall cause, threaten or allow a violation of the Act, the IGPA or regulations adopted by the Board thereunder, including but not limited to this Part.

Section 620.125 Incorporations by Reference

a) The Board incorporates the following material by reference:

ASTM. American Society for Testing and Materials, 1976 Race Street, Philadelphia, Pa. 19103 (215) 299-5585

"Standard Practice for Description and Identification of Soils (Visual Manual Procedure)" D2488-84

GPO. Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20401, (202) 783-3238):

Maximum Contaminant Level Goals and National Primary Drinking Water Regulations for Lead and Copper; Final Rule, 56 Fed. Reg. 26460-26564 (June 7, 1991).

National Primary Drinking Water Regulations, Final Rule, 56 Fed. Reg. 3526-3597 (January 30, 1991).

USEPA Guidelines for Carcinogenic Risk Assessment, 51 Fed. Reg. 33992-34003 (September 24, 1986).

NCRP. National Council on Radiation Protection, 7910 Woodmont Ave., Bethesda, MD (301) 657-6252

"Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure", NCRP Report Number 22, June 5, 1959.

NTIS. National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 487-4600.

"Methods for Chemical Analysis of Water and Wastes," EPA Publication No. EPA-600/4-79-020, (March 1983), Doc. No. PB 84-128677

"Methods for the Determination of Organic Compounds in Drinking Water", EPA, EMSL, EPA-600/4-88/039 (Dec. 1988), Doc. No. PB 89-220461

"Practical Guide for Ground-Water Sampling", EPA Publication No. EPA/600/2-85/104 (September 1985), Doc. No. PB 86-137304

"Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846 (Third Edition, 1986, as amended by Revision I (December 1987), Doc. No. PB 89-148076

USGS. United States Geological Survey, 1961 Stout St., Denver, CO 80294 (303) 844-4169

"Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected Unstable Constituents", Book I, Chapter D2 (1981).

b) This Section incorporates no later editions or amendments.

Section 620.130 Exemption from General Use Standards and Public and Food Processing Water Supply Standards

Groundwater is not required to meet the general use standards and public and food processing water supply standards of 35 Ill. Adm. Code 302.Subparts B and C.

Section 620.135 Exclusion for Underground Waters in Certain Man-Made Conduits

This Part does not apply to underground waters contained in man-made subsurface drains, tunnels, reservoirs, storm sewers, tiles or sewers.

SUBPART B: GROUNDWATER CLASSIFICATION

Section 620.201 Groundwater Designations

All groundwaters of the State are designated as:

a) One of the following four classes of groundwater in accordance with Sections 620.210 through 620.240:

1) Class I: Potable Resource Groundwater

2) Class II: General Resource Groundwater;

3) Class III: Special Resource Groundwater;

4) Class IV: Other Groundwater; or

b) A groundwater management zone in accordance with Section 620.250.

Section 620.210 Class I: Potable Resource Groundwater

Except as provided in Sections 620.230, 620.240, or 620.250, Potable Resource Groundwater is:

a) Groundwater located 10 feet or more below the land surface and within:

1) The minimum setback zone of a well which serves as a potable water supply and to the bottom of such well;

2) Unconsolidated sand, gravel or sand and gravel which is 5 feet or more in thickness and that contains 12 percent or less of fines (i.e. fines which pass through a No. 200 sieve tested according to ASTM Standard Practice D2488-84, incorporated by reference at Section 620.125);

3) Sandstone which is 10 feet or more in thickness, or fractured carbonate which is 15 feet of more in thickness; or

4) Any geologic material which is capable of a:

A) Sustained groundwater yield, from up to a 12 inch borehole, of 150 gallons per day or more from a thickness of 15 feet or less; or

B) Hydraulic conductivity of 1×10^{-4} cm/sec or greater using one of the following test methods or its equivalent:

i) Permeameter;

ii) Slug test; or

iii) Pump test.

b) Any groundwater which is determined by the Board pursuant to petition procedures set forth in Section 620.260, to be capable of potable use.

(Board Note: Any portion of the thickness associated with the geologic materials as described in subsections 620.210(a)(2), (a)(3) or (a)(4) should be designated as Class I: Potable Resource Groundwater if located 10 feet or more below the land surface.)

Section 620.220 Class II: General Resource Groundwater

Except as provided in Section 620.250, General Resource Groundwater is:

a) Groundwater which does not meet the provisions of Section 620.210 (Class I), Section 620.230 (Class III), or Section 620.240 (Class IV).

b) Groundwater which is found by the Board, pursuant to the petition procedures set forth in Section 620.260, to be capable of agricultural, industrial, recreational or other beneficial uses.

Section 620.230 Class III: Special Resource Groundwater

Except as provided in Section 620.250, Special Resource Groundwater is:

a) Groundwater that is determined by the Board, pursuant to the procedures set forth in Section 620.260, to be:

1) Demonstrably unique (e.g., irreplaceable sources of groundwater) and suitable for application of a water quality standard more stringent than the otherwise applicable water quality standard specified in Subpart D; or

2) Vital for a particularly sensitive ecological system.

b) Groundwater that contributes to a dedicated nature preserve that is listed by the Agency as set forth below:

1) A written request to list a dedicated nature preserve under this subsection must contain, at a minimum, the following information:

   A) A general description of the site and the surrounding land use;

   B) A topographic map or other map of suitable scale denoting the location of the dedicated nature preserve;

   C) A general description of the existing groundwater quality at and surrounding the dedicated nature preserve;

   D) A general geologic profile of the dedicated nature preserve based upon the most reasonably available information, including but not limited to geologic maps and subsurface groundwater flow directions; and

   E) A description of the interrelationship between groundwater and the nature of the site.

2) Upon confirmation by the Agency of the technical adequacy of a written request, the Agency shall publish the proposed listing of the dedicated nature preserve in the Environmental Register for a 45-day public comment period. Within 60 days after the close of the public comment period, the Agency shall either publish a final listing of the dedicated nature preserve in the Environmental Register or provide a written response to the requestor specifying the reasons for not listing the dedicated nature preserve.

3) At least once annually, the Agency shall publish in the Environmental Register a complete listing of all dedicated nature preserves listed under this subsection.

4) For purposes of this Section the term "dedicated nature preserve" means a nature preserve that is dedicated pursuant to the Illinois Natural Areas Preservation Act (Ill. Rev. Stat. 1989, ch. 105, pars. 701 et seq.).

Section 620.240 Class IV: Other Groundwater

Except as provided in Section 620.250, Other Groundwater is:

a) Groundwater within a zone of attenuation as provided in 35 Ill. Adm. Code 811 and 814;

b) Groundwater within a point of compliance as provided in 35 Ill. Adm. Code 724, but not to exceed a distance of 200 feet from a potential primary or secondary source.

c) Groundwater that naturally contains more than 10,000 mg/L of total dissolved solids;

d) Groundwater which has been designated by the Board as an exempt aquifer pursuant to 35 Ill. Adm. Code 730.104; or

e) Groundwater which underlies a potential primary or secondary source, in which contaminants may be present from a release, if the owner or operator of such source notifies the Agency in writing and the following conditions are met:

1) The outermost edge is the closest practicable distance from such source, but does not exceed:

A) A lateral distance of 25 feet from the edge of such potential source or the property boundary, whichever is less; and

B) A depth of 15 feet from the bottom of such potential source or the land surface, whichever is greater;

2) The source of any release of contaminants to groundwater has been controlled;

3) Migration of contaminants within the site resulting from a release to groundwater has been minimized;

4) Any on-site release of contaminants to groundwater has been managed to prevent migration off-site; and

5) No potable water well exists within the outermost edge as provided in subsection (e)(1).

f) Groundwater which underlies a coal mine refuse disposal area not contained within an area from which overburden has been removed, a coal combustion waste disposal area at a surface coal mine authorized under Section 21(s) of the Act, or an impoundment that contains sludge, slurry, or precipitated process material at a coal preparation plant, in which contaminants may be present, if such area or impoundment was placed into

operation after February 1, 1983, if the owner and operator notifies the Agency in writing, and if the following conditions are met:

1) The outermost edge is the closest practicable distance, but does not exceed:

A) A lateral distance of 25 feet from the edge of such area or impoundment, or the property boundary, whichever is less; and

B) A depth of 15 feet from the bottom of such area or impoundment, or the land surface, whichever is greater;

2) The source of any release of contaminants to groundwater has been controlled;

3) Migration of contaminants within the site resulting from a release to groundwater has been minimized;

4) Any on-site release of contaminants to groundwater has been managed to prevent migration off-site; and

5) No potable water well exists within the outermost edge as provided in subsection (e)(1).

g) Groundwater within a previously mined area, unless monitoring demonstrates that the groundwater is capable of consistently meeting the standards of Sections 620.410 or 620.420. If such capability is determined, groundwater within the previously mined area shall not be Class IV.

Section 620.250 Groundwater Management Zone

a) Within any class of groundwater, a groundwater management zone may be established as a three dimensional region containing groundwater being managed to mitigate impairment caused by the release of contaminants from a site:

1) That is subject to a corrective action process approved by the Agency; or

2) For which the owner or operator undertakes an adequate corrective action in a timely and appropriate manner and provides a written confirmation to the Agency. Such confirmation must be provided in a form as prescribed by the Agency.

b) A groundwater management zone is established upon concurrence by the Agency that the conditions as specified in subsection (a) are met and groundwater management continues for a period of time consistent with the action described in that subsection.

c) A groundwater management zone expires upon the Agency's receipt of appropriate documentation which confirms the completion of the action taken pursuant to subsection (a) and which confirms the attainment of applicable standards as set forth in Subpart D. The Agency shall review the on-going adequacy of controls and continued management at the site if concentrations of chemical constituents, as specified in Section 620.450(a)(4)(B), remain in groundwater at the site following completion of such action. The review must take place no less often than every 5 years and the results must be presented to the Agency in a written report.

Section 620.260 Reclassification of Groundwater by Adjusted Standard

Any person may petition the Board to reclassify a groundwater in accordance with the procedures for adjusted standards specified in Section 28.1 of the Act and 35 Ill. Adm. Code 106.Subpart G. In any proceeding to reclassify specific groundwater by adjusted standard, in addition to the requirements of 35 Ill. Adm. Code 106.Subpart G, and Section 28.1(c) of the Act, the petition shall, at a minimum, contain information to allow the Board to determine:

a) The specific groundwater for which reclassification is requested, including but not limited to geographical extent of any aquifers, depth of groundwater, and rate and direction of groundwater flow and that the specific groundwater exhibits the characteristics of the requested class as set forth in Sections 620.210(b), 620.220(b), 620.230, or 620.240(b);

b) Whether the proposed change or use restriction is necessary for economic or social development, by providing information including, but not limited to, the impacts of the standards on the regional economy, social benefits such as loss of jobs or closing of facilities, and economic analysis contrasting the health and environmental benefits with costs likely to be incurred in meeting the standards would be beneficial or necessary;

c) Existing and anticipated uses of the specific groundwater;

d) Existing and anticipated quality of the specific groundwater;

e) Existing and anticipated contamination, if any, of the specific groundwater;

f) Technical feasibility and economic reasonableness of eliminating or reducing contamination of the specific groundwater or of maintaining existing water quality;

g) The anticipated time period over which contaminants will continue to affect the specific groundwater;

h) Existing and anticipated impact on any potable water supplies due to contamination;

i) Availability and cost of alternate water sources or of treatment for those users adversely affected;

j) Negative or positive effect on property values; and

k) For special resource groundwater, negative or positive effect on:

1) The quality of surface waters; and

2) Wetlands, natural areas, and the life contained therein, including endangered or threatened species of plant, fish or wildlife listed pursuant to the Endangered Species Act, 16 U.S.C. 1531 et seq., or the Illinois Endangered Species Protection Act (Ill. Rev. Stat. 1989, ch. 8, par. 331 et seq.).

SUBPART C: NONDEGRADATION PROVISIONS FOR APPROPRIATE GROUNDWATERS

Section 620.301 General Prohibition Against Use Impairment of Resource Groundwater

a) No person shall cause, threaten or allow the release of any contaminant to a resource groundwater such that:

1) Treatment or additional treatment is necessary to continue an existing use or to assure a potential use of such groundwater; or

2) An existing or potential use of such groundwater is precluded.

b) Nothing in this Section shall prevent the establishment of a groundwater management zone pursuant to Section 620.250 or a cumulative impact area within a permitted site.

c) Nothing in this Section shall limit underground injection pursuant to a permit issued by the Agency under the Act or issued by the Department of Mines and Minerals under "An Act in relation to oil, gas, coal and other surface and underground resources and to repeal an Act herein named" (Ill. Rev Stat. 1989, ch. 96 1/2, pars. 5401 et seq., as amended).

d) Nothing in this Section shall limit the Board from promulgating nondegradation provisions applicable to particular types of facilities or activities which impact upon groundwater, including but not limited to landfills regulated pursuant to 35 Ill. Adm. Code.Subtitle G.

Section 620.302 Applicability of Preventive Notification and Preventive Response Activities

a) Preventive notification and preventive response as specified in Sections 620.305 through 620.310 applies to:

1) Class I groundwater under Section 620.210(a)(1), (a)(2), or (a)(3) which is monitored by the persons listed in subsection (b); or

2) Class III groundwater which is monitored by the persons listed in subsection (b).

b) For purposes of subsection (a), the persons that conduct groundwater monitoring are:

1) An owner or operator of a regulated entity for which groundwater quality monitoring must be performed pursuant to State or Federal law or regulation (e.g. Sections 106 and 107 of the Comprehensive Environmental Response, Compensation and Liability Act, (42 U.S.C. 9601, et seq.); Sections 3004 and 3008 of the Resource Conservation and Recovery Act, (42 U.S.C. 6901, et seq.); Sections 4(q), 4(v), 12(g), 21(d), 21(f), 22.2(f), 22.2(m) and 22.18 of the Act; 35 Ill. Adm. Code 724, 725, 730, 731, 750, 811 and 814.)"

2) An owner or operator of a public water supply well who conducts groundwater quality monitoring; or

3) A state agency which is authorized to conduct, or is the recipient of, groundwater quality monitoring data (e.g., Illinois Environmental Protection Agency, Department of Public Health, Department of Conservation, Department of Mines and Minerals, Department of Agriculture, Office of State Fire Marshall or Department of Energy and Natural Resources).

c) If a contaminant exceeds a standard set forth in Section 620.410 or Section 620.430, the appropriate remedy is corrective action and Sections 620.305 and 620.310 do not apply.

Section 620.305 Preventive Notification Procedures

a) Pursuant to groundwater quality monitoring as described in Section 620.302, a preventive notification must occur whenever a contaminant:

1) Listed under Section 620.310(a)(3)(A) is detected (except due to natural causes) in Class I groundwater; or

2) Denoted as a carcinogen under Section 620.410(b) is detected in Class I groundwater; or

3) Subject to a standard under Section 620.430 is detected (except due to natural causes) in Class III groundwater.

b) When a preventive notification is required for groundwater which is monitored by a regulated entity for the subject contaminant, the owner or operator of the site shall confirm the detection by resampling the monitoring well. This resampling shall be made within 30 days of the date on which the first sample analyses are received. The owner or operator shall provide a

preventive notification to the appropriate regulatory agency of the results of the resampling analysis within 30 days of the date on which the sample analyses are received, but no later than 90 days after the results of the first samples were received.

c) When a preventive notification is required for groundwater which is monitored by a regulatory agency, such agency shall notify the owner or operator of the site where the detection has occurred. The owner or operator shall confirm the detection by resampling within 30 days of the date of the notice by the regulatory agency. The owner or operator shall provide preventive notification to the regulatory agency of the results of the resampling analysis within 30 days of the date on which the sample analyses are received, but no later than 90 days after the results of the first samples were received.

d) When a preventive notification of a confirmed detection has been provided by an owner or operator pursuant to this Section, additional detections of the same contaminant do not require further notice, provided that the groundwater quality conditions are substantially unchanged or that preventive response is underway for such contaminant.

Section 620.310 Preventive Response Activities

a) The following preventive assessment must be undertaken:

1) If a preventive notification under Section 620.305(c) is provided by a community water supply:

A) The Agency shall notify the owner or operator of any identified potential primary source, potential secondary source, potential route, or community water supply well that is located within 2,500 feet of the wellhead.

B) The owner or operator notified under subsection (a)(1)(A) shall, within 30 days of the date of issuance of such notice, sample each water well or monitoring well for the contaminant identified in the notice if the contaminant or material containing such contaminant is or has been stored, disposed, or otherwise handled at the site. If a contaminant identified under Section 620.305(a) is detected, then the well must be resampled within 30 days of the date on which the first sample analyses are received. If a

contaminant identified under Section 620.305(a) is detected by the resampling, preventive notification must be given as set forth in Section 620.305.

C) If the Agency receives analytical results under subsection (a)(1)(B) that show a contaminant identified under Section 620.305(a) has been detected, the Agency shall:

i) Conduct a well site survey pursuant to Section 17.1(d) of the Act, if such a survey has not been previously conducted within the last 5 years; and

ii) Identify those sites or activities which represent a hazard to the continued availability of groundwaters for public use unless a groundwater protection needs assessment has been prepared pursuant to Section 17.1 of the Act.

2) If a preventive notification is provided under Section 620.305(c) by a non-community water supply or for multiple private water supply wells, the Department of Public Health shall conduct a sanitary survey within 1,000 feet of the wellhead of a non-community water supply or within 500 feet of the wellheads for multiple private water supply wells.

3) If a preventive notification under Section 620.305(b) is provided by the owner or operator of a regulated entity and the applicable standard in Subpart D has not been exceeded:

A) The appropriate regulatory agency shall determine if any of the following occurs for Class I: Potable Resource Groundwater:

i) The levels set forth below are exceeded or are changed for pH:

Constituent	Criterion (mg/L)
para-Dichlorobenzene	0.005
ortho-Dichlorobenzene	0.01
Ethylbenzene	0.03
Phenols	0.001
Styrene	0.01
Toluene	0.04

Xylenes	0.02

ii) A statistically significant increase occurs above background (as determined pursuant to other regulatory procedures (e.g., 35 Ill. Adm. Code 616, 724, 725 or 811)) for arsenic, cadmium, chromium, cyanide, lead or mercury (except due to natural causes); or for aldicarb, atrazine, carbofuran, endrin, lindane (gamma-hexachlor cyclohexane), 2,4-D, 1,1-dichloroethylene, cis-1,2-dichloroethylene, trans-1,2-dichloroethylene, methoxychlor, monochlorobenzene, 2,4,5-TP (Silvex) and 1,1,1-trichloroethane.

iii) For a chemical constituent of gasoline, diesel fuel, or heating fuel, the constituent exceeds the following:

Constituent	Criterion (mg/L)
BETX	0.095

iv) For pH, a statistically significant change occurs from background.

(Board Note: Constituents that are carcinogens have not been listed in subsection (a)(3)(A) because the standard is set at the PQL and any exceedence thereof is a violation subject to corrective action.)

B) The appropriate agency shall determine if, for Class III: Special Resource Groundwater, the levels as determined by the Board are exceeded.

C) The appropriate regulatory agency shall consider whether the owner or operator reasonably demonstrates that:

i) The contamination is a result of contaminants remaining in groundwater from a prior release for which appropriate action was taken in accordance with laws and regulations in existence at the time of the release;

ii) The source of contamination is not due to the on-site release of contaminants; or

iii) The detection resulted from error in sampling, analysis, or evaluation.

D) The appropriate regulatory agency shall consider actions necessary to minimize the degree and extent of contamination.

b) The appropriate regulatory agency shall determine whether a preventative response must be undertaken based on relevant factors including, but not limited to, the considerations in subsection (a)(3).

c) After completion of preventive response pursuant to authority of an appropriate regulatory agency, the concentration of a contaminant listed in subsection (a)(3)(A) in groundwater may exceed 50 percent of the applicable numerical standard in Subpart D only if the following conditions are met:

1) The exceedence has been minimized to the extent practicable;

2) Beneficial use, as appropriate for the class of groundwater, has been assured; and

3) Any threat to public health or the environment has been minimized.

d) Nothing in this Section shall in any way limit the authority of the State or of the United States to require or perform any corrective action process.

SUBPART D: GROUNDWATER QUALITY STANDARDS

Section 620.401 Applicability

Groundwaters must meet the standards appropriate to the groundwater's class as specified in this Subpart and the nondegradation provisions of Subpart C.

Section 620.405 General Prohibitions Against Violations of Groundwater Quality Standards

No person shall cause, threaten or allow the release of any contaminant to groundwater so as to cause a groundwater quality standard set forth in this Subpart to be exceeded.

Section 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater

a) Inorganic Chemical Constituents

Except due to natural causes or as provided in Section 620.450, concentrations of the following chemical constituents must not be exceeded in Class I groundwater:

Constituent	Units	Standard
Arsenic	mg/L	0.05
Barium	mg/L	2
Boron	mg/L	2
Cadmium	mg/L	0.005
Chloride	mg/L	200
Chromium	mg/L	0.1
Cobalt	mg/L	1
Copper	mg/L	0.65
Cyanide	mg/L	0.2
Fluoride	mg/L	4.0
Iron	mg/L	5
Lead	mg/L	0.0075
Manganese	mg/L	0.15
Mercury	mg/L	0.002
Nickel	mg/L	0.1
Nitrate as N	mg/L	10
Radium-226	pCi/L	20
Radium-228	pCi/L	20
Selenium	mg/L	0.05
Silver	mg/L	0.05
Sulfate	mg/L	400
Total Dissolved Solids (TDS)	mg/L	1,200
Zinc	mg/L	5

b) Organic Chemical Constituents

Except due to natural causes or as provided in Section 620.450 or subsection (c), concentrations of the following organic chemical constituents must not be exceeded in Class I groundwater:

Constituent	Standard (mg/L)
Alachlor*	0.002
Aldicarb	0.003
Atrazine	0.003
Benzene*	0.005
Carbofuran	0.04
Carbon Tetrachloride*	0.005
Chlordane*	0.002
Endrin	0.002
Heptachlor*	0.0004
Heptachlor Epoxide*	0.0002
Lindane (Gamma-Hexachlor cyclohexane)	0.0002
2,4-D	0.07
ortho-Dichlorobenzene	0.6
para-Dichlorobenzene	0.075
1,2-Dichloroethane*	0.005
1,1-Dichloroethylene	0.007
cis-1,2-Dichloroethylene	0.07
trans-1,2-Dichloroethylene	0.1
1,2-Dichloropropane*	0.005
Ethylbenzene	0.7
Methoxychlor	0.04
Monochlorobenzene	0.1
Pentachlorophenol*	0.001
Phenols	0.1
Polychlorinated Biphenyls (PCB's) (as decachloro-bipehnyl)*	0.005
Styrene	0.1
2,4,5-TP (Silvex)	0.05
Tetrachloroethylene*	0.005
Toluene	1
Toxaphene*	0.003
1,1,1-Trichloroethane	0.2
Trichloroethylene*	0.005
Vinyl Chloride*	0.002
Xylenes	10

*Denotes a carcinogen.

c) Complex Organic Chemical Mixtures

Concentrations of the following chemical constituents of gasoline, diesel fuel, or heating fuel must not be exceeded in Class I groundwater:

Constituent	Standard (mg/L)
Benzene*	0.005
BETX	11.705

*Denotes a carcinogen.

d) pH

Except due to natural causes, a pH range of 6.5 - 9.0 units must not be exceeded in Class I groundwater.

e) Beta Particle and Photon Radioactivity

1) Except due to natural causes, the average annual concentration of beta particle and photon radioactivity from man-made radionuclides shall not exceed a dose equivalent to the total body organ greater than 4 mrem/year in Class I groundwater. If two or more radionuclides are present, the sum of their dose equivalent to the total body, or to any internal organ shall not exceed 4 mrem/year in Class I groundwater except due to natural causes.

2) Except for the radionuclides listed in subsection (e)(3), the concentration of man-made radionuclides causing 4 mrem total body or organ dose equivalent must be calculated on the basis of a 2 liter per day drinking water intake using the 168-hour data in accordance with the procedure set forth in NCRP Report Number 22, incorporated by reference at in Section 620.125(a).

3) Except due to natural causes, the average annual concentration assumed to produce a total body or organ dose of 4 mrem/year of the following chemical constituents shall not be exceeded in Class I groundwater:

Constituent	Critical Organ	Standard (pCi/1)
Tritium	Total body	20,000
Strontium-90	Bone marrow	8

Section 620.420 Groundwater Quality Standards for Class II: General Resource Groundwater

a) Inorganic Chemical Constituents

1) Except due to natural causes or as provided in Section 620.450 or subsection (a)(3) or (d), concentrations of the following chemical constituents must not be exceeded in Class II groundwater:

Constituent	Standard (mg/L)
Arsenic	0.2
Barium	2
Cadmium	0.05
Chromium	1
Cobalt	1
Cyanide	0.6
Fluoride	4.0
Lead	0.1
Mercury	0.01
Nitrate as N	100

2) Except as provided in Section 620.450 or subsection (a)(3) or (d), concentrations of the following chemical constituents must not be exceeded in Class II groundwater:

Constituent	Standard (mg/L)
Boron	2.0
Chloride	200
Copper	0.65
Iron	5
Manganese	10
Nickel	2
Selenium	0.05
Total Dissolved Solids (TDS)	1,200
Sulfate	400
Zinc	10

3) The standard for any inorganic chemical constituent listed in subsection (a)(2), for barium, or for pH does not apply to groundwater within fill material or within the upper 10 feet of parent material under such fill material on a site not within the rural property class for which:

A) Prior to the effective date of this Part, surficial characteristics have been altered by the placement of such fill material so as to impact the concentration of the parameters listed in subsection (a)(3), and any on-site groundwater monitoring of such parameters is available for review by the Agency.

B) On the effective date of this Part, surficial characteristics are in the process of being altered by the placement of such fill material, which proceeds in reasonably continuous manner to completion, so as to impact the concentration of the parameters listed in subsection (a)(3), and any on-site groundwater monitoring of such parameters is available for review by the Agency.

4) For purposes of subsection (a)(3), the term "fill material" means clean earthen materials, slag, ash, clean demolition debris, or other similar materials.

b) Organic Chemical Constituents

1) Except due to natural causes or as provided in Section 620.450 or subsection (b)(2) or (d), concentrations of the following organic chemical constituents must not be exceeded in Class II groundwater:

Constituent	Standard (mg/L)
Alachlor*	0.010
Aldicarb	0.015
Atrazine	0.015
Benzene*	0.025
Carbofuran	0.2
Carbon Tetrachloride*	0.025
Chlordane*	0.01
Endrin	0.01
Heptachlor*	0.002
Heptachlor Epoxide*	0.001
Lindane (Gamma-Hexachlor cyclohexane)	0.001
2,4-D	0.35
ortho-Dichlorobenzene	1.5
para-Dichlorobenzene	0.375
1,2-Dichloroethane*	0.025
1,1-Dichloroethylene	0.035
cis-1,2-Dichloroethylene	0.2
trans-1,2-Dichloroethylene	0.5

1,2-Dichloropropane*	0.025
Ethylbenzene	1.0
Methoxychlor	0.2
Monochlorobenzene	0.5
Pentachlorophenol*	0.005
Phenols	0.1
Polychlorinated Biphenyls (PCB's) (as decachloro-biphenyl)*	0.0025
Styrene	0.5
2,4,5-TP	0.25
Tetrachloroethylene*	0.025
Toluene	2.5
Toxaphene*	0.015
1,1,1-Trichloroethane	1.0
Trichloroethylene*	0.025
Vinyl Chloride*	0.01
Xylenes	10

*Denotes a carcinogen.

2) The standards for pesticide chemical constituents listed in subsection (b)(1) do not apply to groundwater within 10 feet of the land surface, provided that the concentrations of such constituents result from the application of pesticides in a manner consistent with the requirements of the Federal Insecticide, Fungicide and Rodenticide Act (7 U. S. C. 136 et seq.) and the Illinois Pesticide Act (Ill. Rev. Stat. 1989, ch. 5, pars. 801 et seq.).

c) Complex Organic Chemical Mixtures

Concentrations of the following organic chemical constituents of gasoline, diesel fuel, or heating fuel must not be exceeded in Class II groundwater:

Constituent	Standard (mg/L)
Benzene*	0.025
BETX	13.525

*Denotes a carcinogen.

d) pH

Except due to natural causes, a pH range of 6.5 - 9.0 units must not be exceeded in Class II groundwater that is within 5 feet of the land surface.

Section 620.430 Groundwater Quality Standards for Class III: Special Resource Groundwater

Concentrations of inorganic and organic chemical constituents must not exceed the standards set forth in Section 620.410, except for those chemical constituents for which the Board has adopted a standard pursuant to Section 620.260.

Section 620.440 Groundwater Quality Standards for Class IV: Other Groundwater

a) Except as provided in subsections (b) or (c), Class IV: Other Groundwater standards are equal to the existing concentrations of constituents in groundwater.

b) For groundwater within a zone of attenuation as provided in 35 Ill. Adm. Code 811 and 814, the standards specified in Section 620.420 must not be exceeded, except for concentrations of contaminants within leachate released from a permitted unit.

c) For groundwater within a previously mined area, the standards set forth in Section 620.420 must not be exceeded, except for concentrations of TDS, chloride, iron, manganese, sulfates, or pH. For concentrations of TDS, chloride, iron, manganese, sulfates, or pH, the standards are the existing concentrations.

Section 620.450 Alternative Groundwater Quality Standards

a) Groundwater Quality Restoration Standards

1) Any chemical constituent in groundwater within a groundwater management zone is subject to this Section.

2) Except as provided in subsections (a)(3) or (a)(4), the standards as specified in Sections 620.410, 620.420, 620.430, and 620.440 apply to any chemical constituent in groundwater within a groundwater management zone.

3) Prior to completion of a corrective action described in Section 620.250(a), the standards as specified in Sections 620.410, 620.420, 620.430, and 620.440 are not applicable to such released chemical constituent, provided that the initiated action proceeds in a timely and appropriate manner.

4) After completion of a corrective action as described in Section 620.250(a), the standard for such released chemical constituent is:

A) The standard as set forth in Section 620.410, 620.420, 620.430, or 620.440, if the concentration as determined by groundwater monitoring of such constituent is less than or equal to the standard for the appropriate class set forth in those sections; or

B) The concentration as determined by groundwater monitoring, if such concentration exceeds the standard for the appropriate class set forth in Section 620.410, 620.420, 620.430, or 620.440 for such constituent, and:

i) To the extent practicable, the exceedence has been minimized and beneficial use, as appropriate for the class of groundwater, has been returned; and

ii) Any threat to public health or the environment has been minimized.

5) The Agency shall develop and maintain a listing of concentrations derived pursuant to subsection (a)(4)(B). This list shall be made available to the public and be updated periodically, but no less frequently than semi-annually. This listing shall be published in the Environmental Register.

b) Coal Reclamation Groundwater Quality Standards

1) Any inorganic chemical constituent or pH in groundwater, within an underground coal mine, or within the cumulative impact area of groundwater for which the hydrologic balance has been disturbed from a permitted coal mine area pursuant to the Surface Coal Mining Land Conservation and Reclamation Act (Ill. Rev. Stat. 1989, ch. 96 1/2, pars. 7901.1 et seq., as amended) and 62 Ill. Adm. Code 1700 through 1850, is subject to this Section.

2) Prior to completion of reclamation at a coal mine, the standards as specified in Sections 620.410(a) and (d), 620.420(a) and (e), 620.430 and 620.440 are not applicable to inorganic constituents and pH.

3) After completion of reclamation at a coal mine, the standards as specified in Sections 620.410(a) and (d), 620.420(a), 620.430, and 620.440 are

applicable to inorganic constituents and pH, except:

A) The concentration of total dissolved solids (TDS) must not exceed:

i) The post-reclamation concentration or 3000 mg/L, whichever is less, for groundwater within the permitted area; or

ii) The post-reclamation concentration of TDS must not exceed the post-reclamation concentration or 5000 mg/L, whichever is less, for groundwater in underground coal mines and in permitted areas reclaimed after surface coal mining if the Illinois Department of Mines and Minerals and the Agency have determined that no significant resource groundwater existed prior to mining (62 Ill. Adm. Code 1780.21(f) and (g)); and

B) For chloride, iron, manganese and sulfate, the post-reclamation concentration within the permitted area must not be exceeded.

C) For pH, the post-reclamation concentration within the permitted area must not be exceeded within Class I: Potable Resource Groundwater as specified in Section 620.210(a)(4).

4) A refuse disposal area (not contained within the area from which overburden has been removed) is subject to the inorganic chemical constituent and pH requirements of:

A) 35 Ill. Adm. Code 303.203 for such area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing;

B) Section 620.440(c) for such area that was placed into operation prior to February 1, 1983, and has remained in continuous operation since that date; or

C) Subpart D for such area that is placed into operation on or after the effective date of this Part.

5) For a refuse disposal area (not contained within the area from which overburden has been removed) that was placed into operation prior to February 1, 1983, and is modified after that date to include additional area, this Section applies to the area that meets the requirements of subsection (b)(4)(C) and the following applies to the additional area:

   A) 35 Ill. Adm. Code 303.203 for such additional refuse disposal area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing; and

   B) Subpart D for such additional area that was placed into operation on or after the effective date of this Part.

6) A coal preparation plant (not located in an area from which overburden has been removed) which contains slurry material, sludge or other precipitated process material, is subject to the inorganic chemical constituent and pH requirements of:

   A) 35 Ill. Adm. Code 303.203 for such plant that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing;

   B) Section 620.440(c) for such plant that was placed into operation prior to February 1, 1983, and has remained in continuous operation since that date; or

   C) Subpart D for such plant that is placed into operation on or after the effective date of this Part.

7) For a coal preparation plant (not located in an area from which overburden has been removed) which contains slurry material, sludge or other precipitated process material, that was placed into operation prior to February 1, 1983, and is modified after that date to include additional area, this Section applies to the area that meets

the requirements of subsection (b)(6)(C) and the following applies to the additional area:

A) 35 Ill. Adm. Code 303.203 for such additional area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing; and

B) Subpart D for such additional area that was placed into operation on or after the effective date of this Part.

SUBPART E: GROUNDWATER MONITORING AND ANALYTICAL PROCEDURES

Section 620.505 Compliance Determination

a) Compliance with standards at a site is to be determined as follows:

1) For a structure (e.g., buildings), at the closest practical distance beyond the outermost edge for the structure.

2) For groundwater that underlies a potential primary or secondary source, the outermost edge as specified in Section 620.240(e)(1).

3) For groundwater that underlies a coal mine refuse disposal area, a coal combustion waste disposal area, or an impoundment that contains sludge, slurry, or precipitated process material at a coal preparation plant, the outermost edge as specified in Section 620.240(f)(1) or location of monitoring wells in existence as of the effective date of this Part on a permitted site.

4) For a groundwater management zone, as specified in a corrective action process.

5) At any point at which groundwater monitoring is conducted using any water well or monitoring well that meets the following conditions:

A) For a potable well other than a community water supply well, a construction report has been filed with the Department of Public Health for such potable well, or such well has been located and constructed (or reconstructed) to meet the Illinois Water Well Construction Code (Ill. Rev. Stat. 1989, ch. 111 1/2, pars. 116.111 et seq., as amended) and 35 Ill. Adm. Code 920.

B) For a community water supply well, such well has been permitted by the Agency, or has been constructed in accordance with 35 Ill. Adm. Code 602.115.

C) For a water well other than a potable water well (e.g., a livestock watering well or an irrigation well), a construction report has been filed with the Department of Public Health or the Department of Mines and Minerals for such well, or such well has been located and constructed (or reconstructed) to

meet the Illinois Water Well Construction Code (Ill. Rev. Stat. 1989, ch. 111 1/2, pars. 116.111 et seq., as amended) and 35 Ill. Adm. Code 920.

D) For a monitoring well, such well meets the following requirements:

i) Construction must be done in a manner that will enable the collection of groundwater samples;

ii) Casings and screens must be made from durable material resistant to expected chemical or physical degradation that do not interfere with the quality of groundwater samples being collected; and

iii) The annular space opposite the screened section of the well (i.e., the space between the bore hole and well screen) must be filled with gravel or sand if necessary to collect groundwater samples. The annular space above and below the well screen must be sealed to prevent migration of water from adjacent formations and the surface to the sampled depth.

b) For a spring, compliance with this Subpart shall be determined at the point of emergence.

Section 620.510 Monitoring and Analytical Requirements

a) Representative Samples

A representative sample must be taken from locations as specified in Section 620.505.

b) Sampling and Analytical Procedures

1) Samples must be collected in accordance with the procedures set forth in the documents pertaining to groundwater monitoring and analysis, "Methods for Chemical Analysis of Water and Wastes, "Methods for the Determination of Organic Compounds in Drinking Water", "Practical Guide for Ground-Water Sampling", "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods" (SW-846), "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected

Unstable Constituents", incorporated by reference at Section 620.125 or other procedures adopted by the appropriate regulatory agency.

2) Groundwater elevation in a groundwater monitoring well must be determined and recorded when necessary to determine the gradient.

3) The analytical methodology used for the analysis of constituents in Subparts C and D must be consistent with both of the following:

A) The methodology must have a PQL at or below the preventive response levels of Subpart C or the groundwater standard set forth in Subpart D, whichever is applicable; and

B) The methodology must be consistent with methodologies contained in "Methods for Chemical Analysis of Water and Wastes", "Methods for the Determination of Organic Compounds in Drinking Water", "Practical Guide for Ground-Water Sampling", "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods" (SW-846), "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected Unstable Constituents", incorporated by reference at Section 620.125.

c) Reporting Requirements

At a minimum, groundwater monitoring analytical results must include information, procedures and techniques for:

1) Sample collection (including but not limited to name of sample collector, time and date of the sample, method of collection, and identification of the monitoring location);

2) Sample preservation and shipment (including but not limited to field quality control);

3) Analytical procedures (including but not limited to the method detection limits and the PQLs); and

4) Chain of custody control.

SUBPART F: HEALTH ADVISORIES

Section 620.601 Purpose of a Health Advisory

This Subpart establishes procedures for the issuance of a Health Advisory that sets forth guidance levels that, in the absence of standards under Section 620.410, must be considered by the Agency in:

a) Establishing groundwater cleanup or action levels whenever there is a release or substantial threat of a release of:

1) A hazardous substance or pesticide; or

2) Other contaminant that represents a significant hazard to public health or the environment.

b) Determining whether the community water supply is taking its raw water from a site or source consistent with the siting and source water requirements of 35 Ill. Adm. Code 611.114 and 611.115.

c) Developing Board rulemaking proposals for new or revised numerical standards.

d) Evaluating mixtures of chemical substances.

Section 620.605 Issuance of a Health Advisory

a) The Agency shall issue a Health Advisory for a chemical substance if all of the following conditions are met:

1) A community water supply well is sampled and a substance is detected and confirmed by resampling;

2) There is no standard under Section 620.410 for such chemical substance; and

3) The chemical substance is toxic or harmful to human health according to the procedures of Appendix A, B, or C.

b) The Health Advisory must contain a general description of the characteristics of the chemical substance, the potential adverse health effects, and a guidance level to be determined as follows:

1) If disease or functional impairment is caused due to a physiological mechanism for which there is a threshold dose below which no damage occurs, the guidance level for any such substance is the Maximum Contaminant Level Goal ("MCLG"), adopted

by USEPA for such substance, 56 Fed. Reg. 26460-26564, and 56 Fed. Reg. 3526-3597, incorporated by reference at Section 620.125. If there is no MCLG for the substance, the guidance level is the Human Threshold Toxicant Advisory Concentration for such substance as determined in accordance with Appendix A, unless the concentration for such substance is less than the lowest appropriate PQL specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846 (SW-846), incorporated by reference at Section 620.125 for the substance. If the concentration for such substance is less than the lowest appropriate PQL for the substance specified in SW-846, incorporated by reference at Section 620.125, the guidance level is the lowest appropriate PQL.

2) If the chemical substance is a carcinogen, the guidance level for any such chemical substance is the lowest appropriate PQL specified in SW-846, incorporated by reference at Section 620.125 for such substance.

Section 620.610 Publishing Health Advisories

a) The Agency shall publish the full text of each Health Advisory upon issuance and make the document available to the public.

b) The Agency shall publish and make available to the public, at intervals of not more than 6 months, a comprehensive and up-to-date summary list of all Health Advisories.

Section 620.615 Additional Health Advice for Mixtures of Similar-Acting Substances

a) The need for additional health advice appropriate to site-specific conditions shall be determined by the Agency when mixtures of chemical substances are detected, where two or more of the chemical substances are similar-acting in their toxic or harmful physiological effect on the same specific organ or organ system.

b) If mixtures of similar-acting chemical substances are present, the procedure for evaluating the mixture of such substances is specified in accordance with Appendices A, B, and C.

Section 620.Appendix A Procedures for Determining Human Threshold Toxicant Advisory Concentration for Class I: Potable Resource Groundwater

a) Calculating the Human Threshold Toxicant Advisory Concentration

For those substances for which USEPA has not adopted a Maximum Contaminant Level Goal ("MCLG"), the Human Threshold Toxicant Advisory Concentration is calculated as follows:

HTTAC = RSC x ADE/W

Where:

HTTAC = Human Threshold Toxicant Advisory Concentration in milligrams per liter (mg/L);

RSC = Relative contribution of the amount of the exposure to a chemical via drinking water when compared to the total exposure to that chemical from all sources. Valid chemical-specific data shall be used if available. If valid chemical-specific data are not available, a value of 20% (=0.20) must be used;

ADE = Acceptable Daily Exposure of substance in milligrams per day (mg/d) as determined pursuant to subsection (b); and

W = Per capita daily water consumption equal to 2 liters per day (L/d).

b) Procedures for Determining Acceptable Daily Exposures for Class I: Potable Resource Groundwater

1) The Acceptable Daily Exposure (ADE) represents the maximum amount of a threshold toxicant in milligrams per day (mg/d) which if ingested daily for a lifetime results in no adverse effects to humans. Subsections (b)(2) through (b)(6) list, in prescribed order, methods for determining the ADE in Class I: Potable Resource Groundwater.

2) For those substances for which the USEPA has derived a Verified Oral Reference Dose for humans, USEPA's Reference Dose given in milligrams per kilogram per day (mg/kg/d), as determined in accordance with methods provided in National Primary and Secondary Drinking Water Regulations;

Final Rule, 56 Fed. Reg. 3526-3597, (January 30, 1991), incorporated by reference at Section 620.125, must be used. The ADE equals the product of multiplying the Reference Dose by 70 kilograms (kg), which is the assumed average weight of an adult human.

3) For those substances for which a no observed adverse effect level for humans (NOAEL-H) exposed to the substance has been derived, the ADE equals the product of multiplying one-tenth of the NOAEL-H given in milligrams of toxicant per kilogram of body weight per day (mg/kg/d) by the average weight of an adult human of 70 kilograms (kg). If two or more studies are available, the lowest NOAEL-H must be used in the calculation of the ADE.

4) For those substances for which only a lowest observed adverse effect level for humans (LOAEL-H) exposed to the substance has been derived, one-tenth the LOAEL-H must be substituted for the NOAEL-H in subsection (b)(3).

5) For those substances for which a no observed adverse effect level has been derived from studies of mammalian test species (NOAEL-A) exposed to the substance, the ADE equals the product of multiplying 1/100 of the NOAEL-A given in milligrams toxicant per kilogram of test species weight per day (mg/kg/d) by the average weight of an adult human of 70 kilograms (kg). Preference will be given to animal studies having High Validity, as defined in subsection (c), in the order listed in that subsection. Studies having a Medium Validity must be considered if no studies having High Validity are available. If studies of Low Validity must be used, the ADE must be calculated using 1/1000 of the NOAEL-A having Low Validity instead of 1/100 of the NOAEL-A of High or Medium Validity, except as described in subsection (b)(6). If two or more studies among different animal species are equally valid, the lowest NOAEL-A among animal species must be used in the calculation of the ADE. Additional considerations in selecting the NOAEL-A include:

A) If the NOAEL-A is given in milligrams of toxicant per liter of water consumed (mg/L), prior to calculating the ADE the NOAEL-A must be multiplied by the average daily volume of water consumed by the mammalian test species in liters per day (l/d) and divided by the

average weight of the mammalian test species in kilograms (kg).

B) If the NOAEL-A is given in milligrams of toxicant per kilogram of food consumed (mg/kg), prior to calculating the ADE, the NOAEL-A must be multiplied by the average amount in kilograms of food consumed daily by the mammalian test species (kg/d) and divided by the average weight of the mammalian test species in kilograms (kg).

C) If the mammalian test species was not exposed to the toxicant each day of the test period, the NOAEL-A must be multiplied by the ratio of days of exposure to the total days of the test period.

D) If more than one equally valid NOAEL-A is available for the same mammalian test species, the best available data must be used.

6) For those substances for which a NOAEL-A is not available but the lowest observed adverse effect level (LOAEL-A) has been derived from studies of mammalian test species exposed to the substance, one-tenth of the LOAEL-A may be substituted for the NOAEL-A in subsection (b)(5). The LOAEL-A must be selected in the same manner as that specified in subsection (b)(5). One-tenth the LOAEL-A from a study determined to have Medium Validity may be substituted for a NOAEL-A in subsection (b)(3) if the NOAEL-A is from a study determined to have Low Validity, or if the toxicity endpoint measured in the study having the LOAEL-A of Medium Validity is determined to be more biologically relevant than the toxicity endpoint measured in the study having the NOAEL-A of Low Validity.

c) Procedures for Establishing Validity of Data from Animal Studies

1) High Validity Studies

A) High validity studies use a route of exposure by ingestion or gavage, and are based upon:

i) Data from animal carcinogenicity studies with a minimum of 2 dose levels and a control group, 2 species, both sexes, with 50 animals per dose per sex, and at least 50 percent survival at 15 months

in mice and 18 months in rats and at least 25 percent survival at 18 months in mice and 24 months in rats;

ii) Data from animal chronic studies with a minimum of 3 dose levels and a control group, 2 species, both sexes, with 40 animals per dose per sex, and at least 50 percent survival at 15 months in mice and 18 months in rats and at least 25 percent survival at 18 months in mice and 24 months in rats, and a well-defined NOAEL; or

iii) Data from animal subchronic studies with a minimum of 3 dose levels and control, 2 species, both sexes, 4 animals per dose per sex for non-rodent species or 10 animals per dose per sex for rodent species, a duration of at least 5% of the test species' lifespan, and a well-defined NOAEL.

B) Supporting studies which reinforce the conclusions of a study of Medium Validity may be considered to raise such a study to High Validity.

2) Medium Validity Studies

Medium validity studies are based upon:

A) Data from animal carcinogenicity, chronic, or subchronic studies in which minor deviations from the study design elements required for a High Validity Study are found, but which otherwise satisfy the standards for a High Validity Study;

B) Data from animal carcinogenicity and chronic studies in which at least 25 percent survival is reported at 15 months in mice and 18 months in rats (a lesser survival is permitted at the conclusion of a longer duration study, but the number of surviving animals should not fall below 20 percent per dose per sex at 18 months for mice and 24 months for rats), but which otherwise satisfy the standards for a High Validity Study;

C) Data from animal subchronic or chronic studies in which a Lowest Observable Adverse Effect Level (LOAEL) is determined, but which

otherwise satisfy the standards for a High Validity Study; or

D) Data from animal subchronic or chronic studies which have an inappropriate route of exposure (for example, intraperitoneal injection or inhalation) but which otherwise satisfy the standards for a High Validity Study, with correction factors for conversion to the oral route.

3) Low Validity Studies

Low validity studies are studies not meeting the standards set forth in subsection (c)(1) or (c)(2).

Section 620.Appendix B Procedures for Determining Hazard Indices for Class I: Potable Resource Groundwater for Mixtures of Similar-Acting Substances

a) This appendix describes procedures for evaluating mixtures of similar-acting substances which may be present in Class I: Potable Resource Groundwaters. Except as provided otherwise in subsection (c), subsections (d) through (h) describe the procedure for determining the Hazard Index for mixtures of similar-acting substances.

b) For the purposes of this appendix, a "mixture" means two or more substances which are present in Class I: Potable Resource Groundwater which may or may not be related either chemically or commercially, but which are not complex mixtures of related isomers and congeners which are produced as commercial products (for example, PCBs or technical grade chlordane).

c) The following substances listed in Section 620.410 are mixtures of similar acting substances:

1) Mixtures of ortho-Dichlorobenzene and para-Dichlorobenzene. The Hazard Index ("HI") for such mixtures is determined as follows:

HI = [ortho-Dichlorobenzene]\0.6 +
[para-Dichlorobenzene]\0.075

2) Mixtures of 1,1-Dichloroethylene and 1,1,1-trichloroethane. The Hazard Index ("HI") for such mixtures is determined as follows:

HI = [1,1-Dichloroethylene]\0.007 +
[1,1,1-trichloroethane]\0.2

d) When two or more substances occur together in a mixture, the additivity of the toxicities of some or all of the substances will be considered when determining health-based standards for Class I: Potable Resource Groundwater. This is done by the use of a dose addition model with the development of a Hazard Index for the mixture of substances with similar-acting toxicities. This method does not address synergism or antagonism. Guidelines for determining when the dose addition of similar-acting substances is appropriate are presented in Appendix C.

The Hazard Index is calculated as follows:

HI = [A]\ALA + [B]\ALB + . . . [I]\ALI

Where:

HI = Hazard Index, unitless.

[A], [B], [I] = Concentration of each similar-acting substance in groundwater in milligrams per liter (mg/L).

ALA, ALB, ALI = The acceptable level of each similar-acting substance in the mixture in milligrams per liter (mg/L).

e) For substances which are considered to have a threshold mechanism of toxicity, the acceptable level is:

1) The standards listed in Section 620.410; or

2) For those substances for which standards have not been established in Section 620.410, the Human Threshold Toxicant Advisory Concentration (HTTAC) as determined in Appendix A.

f) For substances which are carcinogens, the acceptable level is:

1) The standards listed in Section 620.410; or

2) For those substances for which standards have not been established under Section 620.410, the lowest appropriate PQL of USEPA-approved analytical methods specified in SW-846, incorporated by reference at Section 620.125, for each substance.

g) Since the assumption of dose addition is most properly applied to substances that induce the same effect by similar modes of action, a separate HI must be generated for each toxicity endpoint of concern.

h) In addition to meeting the individual substance objectives, a Hazard Index must be less than or equal to 1 for a mixture of similar-acting substances.

Section 620.Appendix C Guidelines for Determining When Dose Addition of Similar-Acting Substances in Class I: Potable Resource Groundwaters is Appropriate

a) Substances must be considered similar-acting if:

1) The substances have the same target in an organism (for example, the same organ, organ system, receptor, or enzyme).

2) The substances have the same mode of toxic action. These actions may include, for example, central nervous system depression, liver toxicity, or cholinesterase inhibition.

b) Substances that have fundamentally different mechanisms of toxicity (threshold toxicants vs. carcinogens) must not be considered similar-acting. However, carcinogens which also cause a threshold toxic effect should be considered in a mixture with other similar-acting substances having the same threshold toxic effect. In such a case, an Acceptable Level for the carcinogen must be derived for its threshold effect, using the procedures described in Appendix A.

c) Substances which are components of a complex mixture of related compounds which are produced as commercial products (for example, PCBs or technical grade chlordane) are not mixtures, as defined in Appendix B. Such complex mixtures are equivalent to a single substance. In such a case, the Human Threshold Toxicant Advisory Concentration may be derived for threshold effects of the complex mixture, using the procedures described in Appendix A, if valid toxicological or epidemiological data are available for the complex mixture. If the complex mixture is a carcinogen, the Health Advisory Concentration is the lowest appropriate PQL of USEPA-approved analytical methods specified in SW-846, incorporated by reference at Section 620.125.

Section 620.Appendix D Confirmation of an Adequate Corrective Action Pursuant to 35 Ill. Adm. Code 620.250 (a)(2).

Pursuant to 35 Ill. Adm. Code 620.250(a) if an owner or operator provides a written confirmation to the Agency that an adequate corrective action, equivalent to a corrective action process approved by the Agency, is being undertaken in a timely and appropriate manner, then a groundwater management zone may be established as a three-dimensional region containing groundwater being managed to mitigate impairment caused by the release of contaminants from a site. This document provides the form in which the written confirmation is to be submitted to the Agency.

Note 1. Parts I and II are to be submitted to IEPA at the time that the facility claims the alternative groundwater standards. Part III is to be submitted at the completion of the site investigation. At the completion of the corrective process, a final report is to be filed which includes the confirmation statement included in Part IV.

Note 2. The issuance of a permit by IEPA's Division of Air Pollution Control or Water Pollution Control for a treatment system does not imply that the Agency has approved the corrective action process.

Note 3. If the facility is conducting a cleanup of a unit which is subject to the requirements of the Resource Conservation and Recovery Act (RCRA) or the 35 Ill. Adm. Code 731 regulations for Underground Storage Tanks, this confirmation process is not applicable and cannot be used.

Note 4. If the answers to any of these questions require explanation or clarification, provide such in an attachment to this document.

Part I. Facility Information

Facility Name _____
Facility Address _____

County _____
Standard Industrial Code (SIC) _____

1. Provide a general description of the type of industry, products manufactured, raw materials used, location and size of the facility.

2. What specific units (operating or closed) are present at the facility which are or were used to manage waste, hazardous waste, hazardous substances or petroleum?

	YES	NO
Landfill	_____	_____
Surface Impoundment	_____	_____
Land Treatment	_____	_____
Spray Irrigation	_____	_____
Waste Pile	_____	_____
Incinerator	_____	_____
Storage Tank (above ground)	_____	_____
Storage Tank (underground)	_____	_____
Container Storage Area	_____	_____
Injection Well	_____	_____
Water Treatment Units	_____	_____
Septic Tanks	_____	_____
French Drains	_____	_____
Transfer Station	_____	_____

Other Units (Please describe)

_____

_____

3. Provide an extract from a USGS topographic or county map showing the location of the site and a more detailed scaled map of the facility with each waste management unit identified in Question 2 or known/suspected source clearly identified. Map scale must be specified and the location of the facility must be provided with respect to Township, Range and Section.

4. Has the facility ever conducted operations which involved the generation, manufacture, processing, transportation, treatment, storage or handling of "hazardous substances" as defined by the Illinois Environmental Protection Act? Yes _____ No _____ If the answer to this question is "yes" generally describe these operations.

5. Has the facility generated, stored or treated hazardous waste as defined by the Resource Conservation and Recovery Act? Yes _____ No _____ If the answer to this question is "yes" generally describe these operations.

6. Has the facility conducted operations which involved the processing, storage or handling of petroleum? Yes _____ No _____ If the answer to this questions is "yes" describe these operations.

7. Has the facility ever held any of the following permits?

   a. Permits for any waste storage, waste treatment or waste disposal operation. Yes _____ No _____ If

the answer to this question is "yes", identify the IEPA permit numbers.

b. Interim Status under the Resources Conservation and Recovery Act (filing of a RCRA Part A application). Yes _____ No _____ If the answer to this question is "yes", attach a copy of the last approved Part A application.

c. RCRA Part B Permits. Yes _____ No _____ If the answer to this question is "yes", identify the permit log number.

8. Has the facility ever conducted the closure of a RCRA hazardous waste management unit? Yes _____ No _____

9. Have any of the following State or federal government actions taken place for a release at the facility?

a. Written notification regarding known, suspected or alleged contamination on or emanating from the property (e.g., a Notice pursuant to Section 4(q) of the Environmental Protection Act)? Yes _____ No _____ If the answer to ths question is "yes", identify the caption and date of issuance.

b. Consent Decree or Order under RCRA, CERCLA, EPAct Section 22.2 (State Superfund), or EPAct Section 21(f) (State RCRA). Yes _____ No _____

c. If either of Items a or b were answered by checking "yes", is the notice, order or decree still in effect? Yes _____ No _____

10. What groundwater classification will the facility be subject to at the completion of the remediation? Class I _____ Class II _____ Class III _____ Class IV _____ If more than one Class applies, please explain.

11. Describe the circumstances which the release to groundwater was identified.

Based on my inquiry of those persons directly responsible for gathering the information, I certify that the information submitted is, to the best of my knowledge and belief, true and accurate.

_____	_____
Facility Name	Signature of Owner/Operator
_____	_____
Location of Facility	Name of Owner/Operator

EPA Identification Number _____ Date _____

PART II: Release Information

1. Identify the chemical constituents released to the groundwater. Attach additional documents as necessary.

Chemical Description	Chemical Abstract No.

2. Describe how the site will be investigated to determine the source or sources of the release.

3. Describe how groundwater will be monitored to determine the rate and extent of the release.

4. Has the release been contained on-site at the facility?

5. Describe the groundwater monitoring network and groundwater and soil sampling protocols in place at the facility.

6. Provide the schedule for investigation and monitoring.

7. Describe the laboratory quality assurance program utilized for the investigation.

8. Provide a summary of the results of available soil testing and groundwater monitoring associated with the release at the facility. The summary of results should provide the following information: dates of sampling; types of samples taken (soil or water); locations and depths of samples; sampling and analytical methods; analytical laboratories used; chemical constituents for which analyses were performed; analytical detection limits; and concentrations of chemical constituents in ppm (levels below detection should be identified as "ND").

Based on my inquiry of those persons directly responsible for gathering the information, I certify that the information submitted is, to the best of knowledge and belief, true and accurate and confirm that the actions identified herein will be undertaken in accordance with the schedule set forth herein.

Facility Name _____ Signature of Owner/Operator _____

_____ _____

Location of Facility | Name of Owner/Operator

EPA Identification Number | Date

Part III: Remedy Selection Information

1. Describe the selected remedy.

2. Describe other remedies which were considered and why they were rejected.

3. Will waste, contaminated soil or contaminated groundwater be removed from the site in the course of this remediation? Yes _____ No _____ If the answer to this question is "yes", where will the contaminated material be taken?

4. Describe how the selected remedy will accomplish the maximum practical restoration of beneficial use of groundwater.

5. Describe how the selected remedy will minimize any threat to public health or the environment.

6. Describe how the selected remedy will result in compliance with the applicable groundwater standards.

7. Provide a schedule for design, construction and operation of the remedy, including dates for the start and completion.

8. Describe how the remedy will be operated and maintained.

9. Have any of the following permits been issued for the remediation?

   a. Construction or Operating permit from the Division of Water Pollution Control. Yes _____ No _____

   b. Land treatment permit from the Division of Water Pollution Control. Yes _____ No _____ If the answer to this question is "yes", identify the permit number.

   c. Construction or Operating permit from the Division of Air Pollution Control. Yes _____ No _____ If the answer to this question is "yes", identify the permit number.

10. How will groundwater at the facility be monitored following completion of the remedy to ensure that the groundwater standards have been attained?

Based on my inquiry of those persons directly responsible for gathering the information, I certify that the information submitted is, to the best of my knowledge and belief, true and accurate and confirm that the actions identified herein will be undertaken in accordance with the schedule set forth herein.

Facility Name	Signature of Owner/Operator
Location of Facility	Name of Owner/Operator
EPA Identification Number	Date

PART IV: Completion Certification

This certification must accompany documentation which includes soil and groundwater monitoring data demonstrating successful completion of the corrective process described in Parts I-III.

Facility Name _____
Facility Address _____
_____
_____
County _____
Standard Industrial Code (SIC) _____
Date _____

Based on my inquiry of those persons directly responsible for gathering the information, I certify that an adequate corrective action, equivalent to a corrective action process approved by the Agency, has been undertaken and that the following restoration concentrations are being met:

Chemical Name	Chemical Abstract No.	Concentration (mg/l)

Facility Name	Signature of Owner/Operator
Location of Facility	Name of Owner/Operator

EPA Identification Number Date

IT IS SO ORDERED.

Board Member J.D. Dumelle concurred.

I, Dorothy M. Gunn, Clerk of the Illinois Pollution Control Board, hereby certify that the above Opinion and Order was adopted on the 14 day of November, 1991, by a vote of 7-0.

Dorothy M. Gunn

Dorothy M. Gunn, Clerk
Illinois Pollution Control Board

Attachment

1B

1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

FEB - 6 2014

OFFICE OF
SOLID WASTE AND
EMERGENCY RESPONSE

OSWER Directive 9200.1-120

MEMORANDUM

SUBJECT: Human Health Evaluation Manual, Supplemental Guidance: Update of Standard Default Exposure Factors

FROM: Dana Stalcup, Acting Director
Assessment and Remediation Division
Office of Superfund Remediation and Technology Innovation

TO: Superfund National Policy Managers, Regions 1 - 10

Purpose

The mission of the Superfund program is to protect human health and the environment consistent with the Comprehensive Environmental Response, Compensation and Liability Act, as amended, (CERCLA) and as implemented by the National Oil and Hazardous Substances Pollution Contingency Plan. The purpose of this directive is to update the Interim Final Standard Exposure Factors Guidance (1991), which is reflected in the attached table and is to be used:

- in the CERCLA remedial investigation and feasibility study process (e.g., assessing baseline health risks, developing preliminary remediation goals, evaluating risks of remedial alternatives),
- to evaluate health risks in the CERCLA removal program, and
- in the process of five-year reviews of selected remedies.

This guidance update supplements the *Risk Assessment Guidance for Superfund: Human Health Evaluation Manual, Part A* (RAGS, Part A) that was issued October 13, 1989. This guidance supersedes and replaces certain portions of OSWER Directive 9285.6-03, issued March 25, 1991 and updates the *Risk Assessment Guidance for Superfund, Part E,* issued July 2004 (RAGS, Part E). Other cleanup programs in the Office of Solid Waste and Emergency Response (OSWER) are welcome and encouraged to adopt the recommended exposure factors, much as they have historically adopted other aspects of the *Risk Assessment Guidance for Superfund* (RAGS).

Background

In September 2011, EPA's National Center for Environmental Assessment, Office of Research and Development (ORD/NCEA) issued a substantive update to its exposure assessment recommendations. *Exposure Factors Handbook – 2011 Edition*, referred to as EFH 2011 herein, provides information and recommendations on various physiological and behavioral factors commonly used in assessing exposure of adults and children to environmental chemicals. ORD/NCEA's recommended values for exposure factors are based on the results of studies deemed to be the most up-to-date and scientifically sound, based upon data available up to July 2011, and incorporates revisions made to the *Child-Specific Exposure Factors Handbook*, which was last updated and published in 2008. EFH 2011 is not a Superfund-specific document; rather, it provides a summary of the latest developments in exposure science and provides recommendations for a broad range of EPA programs.

Following the publication of EFH 2011, regional risk assessors received inquiries from other EPA program offices, states, the regulated community, and other interested parties regarding the applicability of the ORD/NCEA's recommendations for use in human health risk assessments. During the October 2011 to August 2012 period, the OSWER Human Health Regional Risk Assessors Forum (OHHRRAF) reviewed the recommendations in EFH 2011 in the context of the default exposure factors used in the Superfund program and to derive Regional screening levels. As a result of a consensus-driven process, the OHHRRAF identified several Superfund-specific default exposure factors that warranted updating, based upon recommendations from ORD/NCEA in EFH 2011. This guidance incorporates and adopts the updates recommended by the OHHRRAF.

Objective

This guidance has been developed to reduce variability and uncertainty in the exposure assumptions used by Regional Superfund staff to characterize exposures to human populations for human health risk assessments.

Implementation

This guidance supplements the *Risk Assessment Guidance for Superfund: Human Health Evaluation Manual* (RAGS), Part A through E. Where numerical values differ from those presented in Part A or E, the factors presented in this guidance should be considered updates to the older values. As new data become available, this Directive may be modified accordingly.

This report can be found at www.epa.gov/oswer/riskassessment/superfund_hh_exposure.htm
Please contact Richard Kapuscinski at (703) 305-7411 if you have questions or concerns.

Attachment

cc: Mathy Stanislaus, OSWER
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OSRTI Managers
Regional Superfund Branch Chiefs, Regions 1 – 10
Lisa Price, Superfund Lead Region Coordinator, Region 6
OSWER/OSRTI Human Health Regional Risk Assessors Forum

CITATIONS

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Attachment 1. Recommended Default Exposure Factors (2014)

Symbol	Definition (units)	Previous Default Value	Currently Recommended Value	Source of current recommendation	Source of previous recommendation
Ingestion and Dermal Contact Rates					
IRW_c	Resident Drinking Water Ingestion Rate - Child (L/day)	1	0.78	U.S. EPA 2011a, Tables 3-15 and 3-33; weighted average of 90th percentile consumer-only ingestion of drinking water (birth to <6 years)	U.S. EPA 1989 (Exhibit 6-11)
IRW_a	Resident Drinking Water Ingestion Rate - Adult (L/day)	2	2.5	U.S. EPA 2011a, Table 3-33; 90th percentile of consumer-only ingestion of drinking water (≥ 21 years)	U.S. EPA 1989 (Exhibit 6-11)
IRS_c	Resident Soil Ingestion Rate - Child (mg/day)	200	200	U.S. EPA 2011a (Table 5-1); "upper-bound values" accounting for both soil and dust ingestion	U.S. EPA 1991a (pg. 15)
IRS_a	Resident Soil Ingestion Rate - Adult (mg/day)	100	100	U.S. EPA 1991a (pp. 6 and 15); EFH 2011 only provides a central tendency value	U.S. EPA 1991a (pg. 15)
IR_{iw}	Indoor Worker Soil Ingestion Rate (mg/day)	50	50	U.S. EPA 1991a (pp. 9-10, 15); EFH 2011 values not provided	U.S. EPA 1991a (pg. 15)
IR_{ow}	Outdoor Worker Soil Ingestion Rate (mg/day)	100	100	U.S. EPA 1991a (pg. 15), same as adult resident; EFH 2011 value not provided	U.S. EPA 1991a (pg. 15)
SA_{sc}	Resident skin surface area - child (cm^2)	2,800	2,373	U.S. EPA 2011a, Tables 7-2 and 7-8; weighted average of mean values for head, hands, forearms, lower legs, and feet (male and female, birth to < 6 years)(forearm and lower leg-specific data used when available, ratios for nearest available age group used elsewhere (per EPA 2011b))	U.S. EPA 2002 (Exhibit 1-2)
SA_{sa}	Resident skin surface area - adult (cm^2)	5,700	6,032	U.S. EPA 2011a, Tables 7-2 and 7-12; weighted average of mean values for head, hands, forearms, and lower legs (male and female, 21+ years)(forearm and lower leg-specific data used for males and female lower leg; ratio of male forearm to arm applied to female arm data).	U.S. EPA 2002 (Exhibit 1-2)
SA_{sow}	Worker skin surface area - adult (cm^2)	3,300	3,527	US EPA 2011a, Table 7-2; weighted average of mean values for head, hands, and forearms (male and female, 21+years) (similar assumptions for forearms as used in EPA 2011b)	U.S. EPA 2002 (Exhibit 1-2)
SA_{wc}	Resident Water Surface area - child (cm^2)	6,600	6,365	U.S. EPA 2011a, Table 7.9; weighted average of mean values for male and female children <6 years.	U.S. EPA 2004 (Exhibit 3-2)
SA_{wa}	Resident Water Surface area - adult (cm^2)	18,000	19,652	U.S. EPA 2011a, Table 7.9; weighted average of mean values for male and female adults, 21-78.	U.S. EPA 2004 (Exhibit 3-2)
AF_c	Resident soil adherence factor - child (mg/cm^2)	0.2	0.2	U.S. EPA 2004 (Exhibit 3-5), RAGS Part E	U.S. EPA 2002 (Exhibit 1-2)
AF_a	Resident soil adherence factor - adult (mg/cm^2)	0.07	0.07	U.S. EPA 2004 (Exhibit 3-5), RAGS Part E	U.S. EPA 2002 (Exhibit 1-2)
AF_{ow}	Worker soil adherence factor - adult (mg/cm^2)	0.2	0.12	U.S. EPA 2011a, Table 7-20 and Section 7.2.2; arithmetic mean of weighted average of body part-specific (hands, forearms, and face) mean adherence factors for adult commercial/industrial activities	U.S. EPA 2002 (Exhibit 1-2)
BW_c	Resident Body Weight - child (kg)	15	15	U.S. EPA 2011a, Table 8-1; weighted average of mean body weights (birth to <6 years)	U.S. EPA 1991a (pg. 15)
BW_a	Resident Body Weight - adult (kg)	70	80	U.S. EPA 2011a, Table 8-3; weighted mean values for adults 21 – 78	U.S. EPA 1991a (pg. 15)
BW_w	Worker Body Weight (kg)	70	80	U.S. EPA 2011a, Table 8-3; weighted mean values for adults 21 – 78	U.S. EPA 1991a (pg. 15)
Exposure Frequency, Exposure Duration, and Exposure Time Variables					

Attachment 1. Recommended Default Exposure Factors (2014)

Symbol	Definition (units)	Previous Default Value	Currently Recommended Value	Source of current recommendation	Source of previous recommendation
EF_r	Resident Exposure Frequency (days/yr)	350	350	U.S. EPA 1991a (pg. 15); value not provided in EFH 2011	U.S. EPA 1991a (pg. 15)
EF_w	Worker Exposure Frequency (days/yr)	250	250	U.S. EPA 1991a (pg. 15); value not provided in EFH 2011	U.S. EPA 1991a (pg. 15)
EF_{iw}	Indoor Worker Exposure Frequency (days/yr)	250	250	U.S. EPA 1991a (pg. 15); value not provided in EFH 2011	U.S. EPA 1991a (pg. 15)
EF_{ow}	Outdoor Worker Exposure Frequency (days/yr)	225	225	U.S. EPA 2002; value not provided in EFH 2011	U.S. EPA 1991a (pg. 15)
ED_r	Resident Exposure Duration (yr)	30	26	EPA 2011a, Table 16-108; 90th percentile for current residence time.	U.S. EPA 1991a (pg. 15)
ED_c	Resident Exposure Duration - child (yr)	6	6	U.S. EPA 1991a, Pages 6 and 15	U.S. EPA 1991a (pg. 15)
ED_a	Resident Exposure Duration - adult (yr)	24	20	EDr (26 years) - EDc (6 years)	U.S. EPA 1991a (pg. 15)
ED_w	Worker Exposure Duration - (yr)	25	25	U.S. EPA 1991a (pg. 15); EFH 2011 only provides a central tendency value	U.S. EPA 1991a (pg. 15)
ED_{iw}	Indoor Worker Exposure Duration - (yr)	25	25	U.S. EPA 1991a (pg. 15); EFH 2011 only provides a central tendency value	U.S. EPA 1991a (pg. 15)
ED_{ow}	Outdoor Worker Exposure Duration (yr)	25	25	U.S. EPA 1991a (pg. 15); EFH 2011 only provides a central tendency value	U.S. EPA 1991a (pg. 15)
ET_{ra}	Resident Air Exposure Time (hours/day)	24	24	The whole day	The whole day
ET_{rs}	Resident Soil Exposure Time (hours/day)	24	24	The whole day	The whole day
ET_w	Worker Air Exposure Time (hr/hr)	8	8	The work day	The work day
ET_{ws}	Worker Soil Exposure Time (hours/day)	8	8	The work day	The work day
ET_{rw}	Resident Water Exposure Time (hours/day)	24	24	The whole day	The whole day
ET_{rwc}	Resident Water Exposure Time - child (hours/event)	1	0.54	U.S. EPA 2011a, Table 16-28; weighted average of 90th percentile time spent bathing (birth to <6 years)	U.S. EPA 2004
ET_{rwa}	Resident Water Exposure Time - adult (hours/event)	0.58	0.71	U.S. EPA 2011a, Tables 16-30 and 16-31; weighted average of adult (21 to 78) 90th percentile of time spent bathing/ showering in a day, divided by mean number of baths/showers taken in a day.	U.S. EPA 2004
Miscellaneous Variables; values not provided in EFH 2011					
AT_r	Averaging time - resident (days/year)	365	365	U.S. EPA 1989 (pg. 6-23)	U.S. EPA 1989 (pg. 6-23)
AT_w	Averaging time - composite worker (days/year)	365	365	U.S. EPA 1989 (pg. 6-23)	U.S. EPA 1989 (pg. 6-23)
AT_{iw}	Averaging time - indoor worker (days/year)	365	365	U.S. EPA 1989 (pg. 6-23)	U.S. EPA 1989 (pg. 6-23)
AT_{ow}	Averaging time - outdoor worker (days/year)	365	365	U.S. EPA 1989 (pg. 6-23)	U.S. EPA 1989 (pg. 6-23)

Attachment 1. Recommended Default Exposure Factors (2014)

Symbol	Definition (units)	Previous Default Value	Currently Recommended Value	Source of current recommendation	Source of previous recommendation
LT	Lifetime (years)	70	70	U.S. EPA 1989 (pg. 6-22), pending additional input from NCEA	U.S. EPA 1989 (pg. 6-22)
IR_{fish}	Fish Ingestion Rate (mg/day)	5.4×10^4	**	Recommend using site-specific values	U.S. EPA 1991a (pg. 15)
$IR_{produce}$	Consumption of homegrown produce (g/day)	42 (fruit); 80 (veg)	**	Recommend using site-specific values	U.S. EPA 1990

References for Cited Sources:

U.S. EPA 1989. Risk assessment guidance for Superfund. Volume I: Human health evaluation manual (Part A). Interim Final. Office of Emergency and Remedial Response. EPA/540/1-89/002.

U.S. EPA 1990. Exposure Factors Handbook. Office of Health and Environmental Assessment. EPA / 8-89 / 043, March 1990.

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U.S. EPA. 1996a. Soil Screening Guidance: User's Guide. Office of Emergency and Remedial Response. Washington, DC. OSWER No. 9355.4-23http://www.epa.gov/superfund/health/conmedia/soil/index.htm#user

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U.S. EPA 2004. Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment) Final. OSWER 9285.7-02EP.July 2004. Document and website http://www.epa.gov/oswer/riskassessment/rags

U.S. EPA, 2005. Guidance on Selecting Age Groupsfor Monitoring and Assessing Childhood Exposures to Environmental Contaminants. EPA/630/P-03/003F, November, 2005.

U.S. EPA 2009. Risk Assessment Guidance for Superfund Volume I: Human Health Evaluation Manual (Part F, Supplemental Guidance for Inhalation Risk Assessment) Final. OSWER 9285.7-82.2009.

U.S. EPA 2011a. Exposure Factors Handbook: 2011 Edition. EPA/ 600/ R-090/052F, September 2011.

EPA. 2011b. "Regional Screening Levels (Formerly PRGs), User's Guide." November. On-Line Address: http://www.epa.gov/reg3hwmd/risk/human/rb-concentration_table/usersguide.htm

Footnote: Users are directed to the *Exposure Factors Handbook* (2011) as a source for specific age-group exposure factors as described in EPA, 2005.

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
SOLID WASTE AND EMERGENCY
RESPONSE

December 5, 2003

OSWER Directive 9285.7-53

MEMORANDUM

SUBJECT: Human Health Toxicity Values in Superfund Risk Assessments

FROM: Michael B. Cook, Director /s/
Office of Superfund Remediation and Technology Innovation

TO: Superfund National Policy Managers, Regions 1 - 10

Purpose

This memorandum revises the hierarchy of human health toxicity values generally recommended for use in risk assessments, originally presented in Risk Assessment Guidance for Superfund Volume I, Part A, Human Health Evaluation Manual (RAGS) (OSWER 9285.7-02B, EPA/540/1-89/009, December 1989). (http://www.epa.gov/superfund/programs/risk/ragsa/index.htm)

It updates the hierarchy of human health toxicity values and provides guidance for the sources of toxicity information that should generally be used in performing human health risk assessments at Comprehensive Environmental Response Compensation and Liability Act (CERCLA or "Superfund") sites. It does not address the situation where new toxicity information is brought to the attention of the U.S. Environmental Protection Agency (EPA). It also does not provide guidance or address toxicity or reference values for ecological risk.

This memorandum presents current Office of Solid Waste and Emergency Response (OSWER) technical and policy recommendations regarding human health toxicity values in risk assessments. EPA and state personnel may use and accept other technically sound approaches, either on their own initiative, or at the suggestion of potentially responsible parties, or other interested parties. Therefore, interested parties are free to raise questions and objections about the substance of this memorandum and the appropriateness of the application of this document to a particular situation. EPA will, and States should, consider whether the recommendations or interpretations in this memorandum are appropriate in that situation. This memorandum does not impose any requirements or obligations on EPA, States, or other federal agencies, or the regulated community. The sources of authority and requirements in this matter are the relevant

statutes and regulations (e.g., CERCLA, Resource Conservation and Recovery Act). EPA welcomes public comments on this memorandum at any time and may consider such comments in future revisions of this memorandum.

Background

Superfund risk assessments are performed for a number of reasons, including to evaluate whether action is warranted under CERCLA, to establish protective cleanup levels, and to determine the residual risk posed by response actions. Generally, toxicity assessment is an integral part of risk assessment. Volume I, Part A of RAGS provides guidance on how to conduct the human health portion of the risk assessment. Chapter 7.4.1 presents a hierarchy of human health toxicity values for use in risk assessments at Superfund sites. The hierarchy presented in RAGS Part A is being updated to reflect that additional sources of peer reviewed values have become available since 1989. In addition, the EPA Health Effects Assessment Summary Tables (HEAST) document, which was identified as the second tier of data, has not been updated since 1997. As a result, HEAST may not provide the most current source of information on some contaminants.

This revised hierarchy recognizes that EPA should use the best science available on which to base risk assessments. In general, if health assessment information is available in the Integrated Risk Information System ["IRIS," http://www.epa.gov/iris/] for the contaminant under evaluation, risk assessors normally need not search further for additional sources of information. Since EPA's development and use of peer review in toxicity assessments, IRIS assessments have undergone external peer review in accordance with Agency peer review guidance at the time of the assessment. IRIS health assessments contain Agency consensus toxicity values. If such information is not available in IRIS, risk assessors should consider other sources of available data based on the hierarchy presented in this memorandum.

EPA recognizes that there may be other sources of toxicological information. As noted in the December 1993 memorandum entitled "Use of IRIS Values in Superfund Risk Assessment" (OSWER Directive 9285.7-16, December 21, 1993):

> "...IRIS is not the only source of toxicology information, and in some cases more recent, credible and relevant data may come to the Agency's attention. In particular, toxicological information other than that in IRIS may be brought to the Agency by outside parties. Such information should be considered along with the data in IRIS in selecting toxicological values; ultimately, the Agency should evaluate risk based upon its best scientific judgement and consider all credible and relevant information available to it."

This memorandum is intended to help regional risk assessors identify appropriate sources of toxicological information as a means of streamlining decisions. It does not specifically address the situation where additional scientific information is brought to the attention of EPA. In those cases, EPA risk assessors and decision makers should consider the information as appropriate on a case by case basis.

Revised Recommended Human Health Toxicity Value Hierarchy

This memorandum revises the recommended hierarchy of toxicological sources of information which Regional risk assessors and managers should initially consider for site-specific risk assessments. The revised recommended toxicity value hierarchy is as follows:

Tier 1- EPA's IRIS

Tier 2- EPA's Provisional Peer Reviewed Toxicity Values (PPRTVs) – The Office of Research and Development/National Center for Environmental Assessment/Superfund Health Risk Technical Support Center (STSC) develops PPRTVs on a chemical specific basis when requested by EPA's Superfund program.

Tier 3- Other Toxicity Values – Tier 3 includes additional EPA and non-EPA sources of toxicity information. Priority should be given to those sources of information that are the most current, the basis for which is transparent and publicly available, and which have been peer reviewed.

IRIS remains in the first tier of the recommended hierarchy as the generally preferred source of human health toxicity values. IRIS generally contains reference doses (RfDs), reference concentrations (RfCs), cancer slope factors, drinking water unit risk values, and inhalation unit risk values that have gone through a peer review and EPA consensus review process. IRIS normally represents the official Agency scientific position regarding the toxicity of the chemicals based on the data available at the time of the review.

The second tier is EPA's PPRTVs. Generally, PPRTVs are derived for one of two reasons. First, the STSC is conducting a batch wise review of the toxicity values in HEAST (now a Tier 3 source). As such reviews are completed, those toxicity values will be removed from HEAST, and any new toxicity value developed in such a review will be a PPRTV and placed in the PPRTV database. Second, Regional Superfund Offices may request a PPRTV for contaminants lacking a relevant IRIS value. The STSC uses the same methodologies to derive PPRTVs for both.

The third tier includes other sources of information. Priority should be given to sources that provide toxicity information based on similar methods and procedures as those used for Tier I and Tier II, contain values which are peer reviewed, are available to the public, and are transparent about the methods and processes used to develop the values. Consultation with the STSC or headquarters program office is recommended regarding the use of the Tier 3 values for Superfund response decisions when the contaminant appears to be a risk driver for the site. In general, draft toxicity assessments are not appropriate for use until they have been through peer review, the peer review comments have been addressed in a revised draft, and the revised draft is publicly available.

Additional sources may be identified for Tier 3. Toxicity values that fall within the third tier in the hierarchy include, but need not be limited to, the following sources.

- The California Environmental Protection Agency (Cal EPA) toxicity values are peer reviewed and address both cancer and non-cancer effects. Cal EPA toxicity values are available on the Cal EPA internet website at http://www.oehha.ca.gov/risk/chemicalDB//index.asp.
- The Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs) are estimates of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure. The ATSDR MRLs are peer reviewed and are available at http://www.atsdr.cdc.gov/mrls.html on the ATSDR website.

- HEAST toxicity values are Tier 3 values. As noted above, the STSC is conducting a batch wise review of HEAST toxicity values. The toxicity values remaining in HEAST are considered Tier 3 values. The radionuclides HEAST toxicity values are available at http://www.epa.gov/radiation/heast/. The HEAST values on chemical contaminants are not currently available on an EPA internet site. They may be obtained by contacting a Superfund risk assessor.

Neither IRIS nor the PPRTV database contains radionuclide slope factors. Because EPA's Office of Radiation and Indoor Air (ORIA) obtains peer review on the radionuclide slope factors contained in Table 4 of HEAST (which are available on EPA/ORIA's internet website at http://www.epa.gov/radiation/heast/download.htm), routine consultation with STSC is generally not necessary on these values even when they may be a risk driver on a Superfund site. These radionuclide slope factors have been adopted by EPA in its Preliminary Remediation Goals for Radionuclide Calculator and are available on EPA's internet website at: http://epa-prgs.ornl.gov/radionuclides/ and the Soil Screening Guidance for Radionuclide documents, which are available at: http://www.epa.gov/superfund/resources/radiation/radssg.

Implementation

This memorandum provides a revised recommended hierarchy of human health toxicity values for Superfund sites and represents a revision of Chapter 7 of RAGS, Volume I, Part A. Superfund risk assessors should look to this hierarchy when evaluating risk for CERCLA response actions. Additional sources of toxicity values, which are not specifically referenced in this recommended hierarchy, can be considered.

Additional Information

Questions regarding this guidance or its use and implementation on a particular site should be directed to an EPA Regional Superfund risk assessor or toxicologist. Questions of a more general nature relating to this guidance should be directed to Mr. Dave Crawford of my staff at (703) 603- 8891, Crawford.Dave@epa.gov.

cc: Nancy Riveland, Superfund Lead Region Coordinator, USEPA Region 9
NARPM Co-Chairs
Joanna Gibson, OSRTI Documents Coordinator
OSRTI Center Directors and Senior Process Managers
Jim Woolford, FFRRO
Debbie Dietrich, OEPPR
Robert Springer, OSW
Cliff Rothenstein, OUST
Linda Garczynski, OBCR
Sandra Connors, FFEO
Susan Bromm, OSRE
Peter Preuss, NCEA
Charles Openchowski, OGC
John Michaud, OGC
David Kling, FFEO
Stephen Luftig, Senior Advisor to OSWER Assistant Administrator

Attachment

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U.S. ENVIRONMENTAL PROTECTION AGENCY OSWER 9285.7-86

Tier 3 Toxicity Value White Paper

Regional Tier 3 Toxicity Value Workgroup
OSWER Human Health Regional Risk Assessors Forum
5/16/2013

Disclaimer: This U.S. Environmental Protection Agency (EPA) document discusses the process of identifying and selecting Tier 3 toxicity values. This document is not a rule or regulation and it may not apply to a particular situation based upon the circumstances. This document does not change or substitute for any law, regulation, or any other legally binding requirement and is not legally enforceable. As indicated by the use of non-mandatory language such as "guidance," "recommend," "may," "should," and "can," it identifies policies and provides recommendations and does not impose any legally binding requirements.

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Acronyms and Abbreviations

ADP	Action Development Process
AEGL	Acute Exposure Guideline Levels
ATSDR	Agency for Toxic Substances and Disease Registry
BMDL	Below minimal detection limit
CaI/EPA	California Environmental Protection Agency
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act
DoD	U.S. Department of Defense
ECOS	Environment Council of the States
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
GLP	Good Laboratory Practice
HEAST	Health Effects Assessment Summary Tables
HHMSSL	Human Health Medium-Specific Screening Level
HQ	Headquarters
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
ITER	International Toxicity Estimates for Risk
IUR	Inhalation unit risk
LOAEL	Lowest observable adverse effect level
LOEL	Lowest observed effect level
MCL	Maximum contaminant level
mg/kg-day	Milligrams per kilogram per day

MOA	Mechanism of action
MOE	Margin of exposure
MRL	Minimal risk level
NCEA	National Center for Environmental Health
NJDEP	New Jersey Department of Environmental Protection
NOAEL	No observed adverse effect level
NTP	National Toxicology Program
NYSDOH	New York State Department of Health
OECD	Organization for Economic Co-operation and Development
OEHHA	Office of Environmental Health Hazard Assessment
OEM	Office of Emergency Management
OEPI	Office of Policy, Economics & Innovation
OH2R2AF	OSWER Human Health Regional Risk Assessors Forum
OMB	Office of Management and Budget
OPM	Office of Program Management
OPP	Office of Pesticide Programs
ORCR	Office of Resource Conservation and Recovery
ORD	Office of Research and Development
OSRTI	Office of Superfund Remediation & Technology Innovation
OSWER	Office of Solid Waste and Emergency Response
OUST	Office of Underground Storage Tanks
PARMS	Policy Analysis & Regulatory Management Staff
PBPK	Physiologically-based pharmacokinetic
PCE	Perchloroethylene
PFOA	Perfluorooctanic acid

PFOS	Perfluorooctane
PMCAO	Program Management, Communications, and Analysis Office
ppm	Parts per million
PPRTV	Provisional Peer Reviewed Toxicity Values
RAGS	Risk Assessment Guidance for Superfund
RBC	Risk-based concentration
RCRA	Resource Conservation and Recovery Act
RfC	Reference concentration
RfD	Reference dose
RIVM	National Institute of Public Health and the Environment of the Netherlands
RME	Reasonable maximum exposure
RP	Responsible party
RSL	Regional Screening Level
SAB	Science Advisory Board
SPB	Science Policy Branch
STSC	Superfund Health Risk Technical Support Center
TCE	Trichloroethylene
WHO	World Health Organization

Tier 3 Toxicity Value White Paper[1]

1 Introduction

1.1 Purpose

The purpose of this white paper is to articulate the issues pertaining to Tier 3 toxicity values and provide recommendations on processes that will improve the transparency and consistency of identifying, evaluating, selecting, and documenting Tier 3 toxicity values for use in the Superfund and Resource Conservation and Recovery Act (RCRA) programs. This white paper will be used to assist regional risk assessors in selecting Tier 3 toxicity values as well as provide the foundation for future regional and national efforts to improve guidance and policy on Tier 3 toxicity values.

1.1.1 Specific Objectives

The specific objectives of this white paper are to:

- Inform the reader of the differences and similarities between Tier 3 toxicity values,
- Discuss existing criteria and guidance that are relevant to selecting the most scientifically defensible Tier 3 toxicity value,
- Compare the available options for identifying, evaluating, selecting, and documenting Tier 3 toxicity values,
- Provide specific examples of how Tier 3 toxicity values have been identified and selected by the regions, and
- Recommend a process for selecting Tier 3 toxicity values.

1.1.2 Scope

This white paper is limited to Tier 3 toxicity values as defined in Office of Solid Waste and Emergency Response (OSWER) Directive 9285.7-53 (2003 Toxicity Value Hierarchy) and provides recommendations on processes for identifying, evaluating, selecting, documenting, and communicating Tier 3 toxicity values for use in site-specific human health risk assessments.[2] This white paper has been reviewed by the regional risk assessors, and the recommendations are based on the consensus of the regional risk assessors. While not guidance or policy itself, the white paper is also written with the intent to assist

[1] *Disclaimer: This U.S. Environmental Protection Agency (EPA) document discusses the process of identifying and selecting Tier 3 toxicity values. This document is not a rule or regulation and it may not apply to a particular situation based upon the circumstances. This document does not change or substitute for any law, regulation, or any other legally binding requirement and is not legally enforceable. As indicated by the use of non-mandatory language such as "guidance," "recommend," "may," "should," and "can," it identifies policies and provides recommendations and does not impose any legally binding requirements.*

[2] The derivation of new toxicity values falls outside of the scope of this white paper.

others (regional risk assessors, regional risk assessment workgroups, Regional Toxics Integration Coordinators, and headquarters risk assessors) in developing formal or informal EPA regional and national guidance or policy.

1.2 Background

Toxicity values (including reference doses [RfD], reference concentrations [RfC], cancer slope factors, and inhalation unit risks) needed for use in human health risk assessment are generally derived by reviewing available dose-response data in animals or humans, selecting a point of departure in the data that is judged most suitable, and adjusting for associated uncertainties. Often, multiple data sets are available, and there may be a variety of options for deriving the toxicity values. In addition, there are a variety of options for fitting the data and selecting and applying uncertainty factors. For these reasons, there can sometimes be a number of alternative toxicity factors available from different sources for a specified chemical.

OSWER has developed a number of guidance documents which include recommendations for selecting toxicity values. The early guidance established the IRIS database as the preferred source for selecting toxicity values (EPA 1989, 1991, 1993). Subsequent guidance confirmed the preference for the use of IRIS values and made suggestions for appropriate sources of toxicological information that could be used for selecting or deriving toxicity factors in cases where no published IRIS value was available for a given chemical. These developments have led to the concept of applying a more formal or prescribed "hierarchy" for consulting data sources to select or derive toxicity values (EPA 2003, 2005, 2009). This section describes the existing policies used by the Superfund Program for selecting toxicity values, and when necessary, deriving appropriate values for site-specific risk assessment activities.

1.2.1 OSWER's Toxicity Value Hierarchy

1.2.1.1 Risk Assessment Guidance for Superfund (RAGS) Parts A and B

The first guidance on the hierarchy for selecting toxicity factors was provided in Risk Assessment Guidance for Superfund (RAGS) Part A (1989) and Part B (1991). These documents specify that the first preference is for toxicity values that are presented in EPA's Integrated Risk Information System (IRIS). The 1993 OSWER Directive titled "Use of IRIS Values in Superfund Risk Assessment" reconfirmed that IRIS values should be given the highest priority for application in Superfund risk assessments and that alternative toxicological information should only be considered on a case-by-case basis (http://www.epa.gov/oswer/riskassessment/pdf/irismemo.pdf). To this day, IRIS generally supersedes all other sources of toxicity information and is considered the "gold-standard" in terms of toxicological assessments. If no value was available in IRIS, the second preference was identified as the Health Effects Assessment Summary Tables (HEAST). HEAST provided up-to-date toxicity values in a tabular format, first quarterly and then annually for several years through 1997. Unlike IRIS, not all HEAST values went through a formal peer or EPA review process, and interim values were also included in the tables.

If toxicity values were not available on IRIS or in HEAST, then RAGS recommended, in no specified order, other sources such as EPA criteria documents (health advisory summaries), Agency for Toxic Substances and Disease Registry (ATSDR) toxicological profiles, or provisional toxicity assessments prepared by the National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office).

1.2.1.2 2003 Directive Human Health Toxicity Values in Superfund Risk Assessments

In 2003, OSWER Directive 9285.7-53 revised Superfund's hierarchy of human health toxicity values, providing three tiers of toxicity values.[3] There were two important reasons for updating the RAGS toxicity hierarchy. First, additional sources of peer-reviewed values had become available, such as EPA's Provisional Peer Reviewed Toxicity Values (PPRTVs). Second, HEAST, which had been identified in RAGS as the second choice for toxicity information, had not been updated since 1997.

The revised hierarchy provided three tiers of toxicity values: IRIS as the first tier, PPRTVs as the second tier, and "other toxicity values" as the third tier. Example sources of Tier 3 toxicity values included California EPA (Cal/EPA) toxicity values, ATSDR Minimum Risk Levels (MRLs), and HEAST.

1.2.1.3 RAGS Part E and F

RAGS Part E (Dermal Guidance) and RAGS Part F (Inhalation Guidance) were the first supplemental guidance documents to be published after the 2003 OSWER directive. Although RAGS Part E, which was released in 2004, does not reference the 2003 OSWER directive or previous toxicity value hierarchies, this guidance discusses a process for estimating dermal toxicity values by extrapolating from approved oral toxicity values. In 2009, RAGS Part F cited the 2003 OSWER directive as the appropriate hierarchy for selecting toxicity values. RAGS Part F notes that extrapolation of toxicity values from the oral to the inhalation exposure route may not be appropriate in all cases.

1.2.2 Limitations of OSWER Guidance on Tier 3 Toxicity Value Selection

When no Tier 1 or Tier 2 toxicity value is available, but there are several Tier 3 values, it is necessary to decide which Tier 3 value is most appropriate. The merit of these values may vary depending on the scientific quality and rigor of the underlying toxicological studies and analysis and the extent of the peer review. Development of some available values (such as ATSDR MRLs[4] and Cal/EPA toxicity values), includes extensive literature review, rigorous data analysis using up-to-date guidance and methods to derive a toxicity value, and thorough peer review. Development of other toxicity values is not

[3] As an OSWER Directive, the hierarchy is also used by the Office of Brownfields & Land Revitalization (Brownfields), the Office of Emergency Management (OEM), the Office of Resource Conservation and Recovery (ORCR), and the Office of Underground Storage Tanks (OUST).

[4] ATSDR MRLs are limited to non-cancer effects only, but can include chronic, subchronic, and acute values.

necessarily based strictly on risk assessment practices, but may consider other factors. EPA Office of Water maximum contaminant levels (MCLs), for example, may be based on technological limitations in measurement or implementation.

The 2003 OSWER directive provides only limited guidance on selection of Tier 3 toxicity values, recommending that priority should be given to studies that are the most current, transparent in terms of their study or derivation methods, and that have been peer reviewed. Given the wide variety of sources for Tier 3 toxicity values, further guidance is warranted to assist risk assessors to select the most appropriate available Tier 3 value for use at Superfund and RCRA sites.

1.2.3 November 2009 Regional Risk Assessors Meeting

During a session of the November 2009 EPA Region Risk Assessors meeting, the regional risk assessors presented and discussed the approaches, challenges, and limitations for identifying and selecting Tier 3 toxicity values. Specific issues covered during the session included, but were not limited to, existing processes that regional risk assessors were using for identifying and selecting Tier 3 toxicity values, differences between Tier 3 toxicity value sources (for example, derivation methods, transparency, and use of uncertainty factors), and who is responsible for and what could be done to improve the Tier 3 toxicity value selection process. As a result of the presentations and ensuing discussions, the Regional Tier 3 Toxicity Value Workgroup was formed, consisting of a small group of regional risk assessors. The workgroup was given the broad task of developing processes for improving the selection of Tier 3 toxicity values. After the November meeting, the members of the workgroup met and charged themselves with building upon OSWER's toxicity value hierarchy by developing, evaluating, and recommending a processes for identifying and selecting Tier 3 toxicity values. Given that the charge and tasks were broad in scope, additional members and contacts were added to the workgroup, including representatives from headquarters and the regions responsible for the Regional Screening Level Table. Also, consistent with the workgroup's charge and tasks specified during the November 2009 meeting, the workgroup decided that these efforts would be documented in the form of a white paper.

2 Tier 3 Toxicity Values

Currently, there are a myriad of potential sources of ready-made Tier 3 toxicity values and additional sources that provide the data necessary to derive a Tier 3 toxicity value. The purpose of this section is to provide examples from each of these sources, since there are far too many to list. This section will also introduce the similarities and differences between the sources of potential ready-made Tier 3 toxicity values.

2.1 Sources

Tier 3 toxicity values and toxicity data can be derived from state, federal (U.S.), and international sources. The following sections provide examples of some of the most commonly used state, federal and international sources of Tier 3 toxicity values and toxicity data used by risk assessors.

2.1.1 Federal (Internal and External to EPA)

Both EPA and its individual program offices can be useful sources of Tier 3 toxicity values and data. Before a chemical file is posted on the IRIS database in its final form, it must undergo a series of drafts, internal and external peer reviews, and revisions. A major part of this process is development of the draft toxicological review document for the individual chemical. This document details all of the available human and animal toxicity data evaluated and the recommendation for a quantitative cancer or noncancer toxicity value. Although the use of draft IRIS toxicity values as Tier 3 values is generally not appropriate except as indicated in USEPA, 2003, the toxicity values and supporting data in the draft IRIS toxicological reviews can be useful when evaluating a potential Tier 3 toxicity value from another source. These draft documents are useful because the literature searches have been completed and documented, the toxicity values derived using EPA-recommended methodologies, and to a greater or lesser extent have undergone peer review. These draft toxicological reviews can be obtained from the Region's IRIS consensus reviewer and are posted on the web during the public review and comment period.

Individual program offices often develop sources of toxicity values, which are not researched and peer reviewed to the same extent as IRIS files, but are useful for specific chemicals and routes of exposure. One example is the HEAST (http://epa-heast.ornl.gov/) developed for EPA's Superfund and RCRA hazardous waste programs. The Office of Pollution Prevention and Toxics Substances maintains the Acute Exposure Guidelines Levels (AEGLs) database, which provides acceptable concentrations for once in a lifetime, short-term exposures to airborne concentrations of acutely toxic, high priority chemicals (http://www.epa.gov/oppt/aegl/index.htm). These acute values are based on the recommendations of a federal advisory committee consisting of scientists from the public and private sectors. The Office of Pesticide Programs and the National Center for Environmental Assessment in the Office of Research and Development (ORD) are other potential sources of toxicity values.

Outside of EPA, perhaps the best known source of federal toxicity values is ATSDR. This agency develops toxicological profiles for individual chemicals (available at http://www.atsdr.cdc.gov/toxprofiles/index.asp), which are similar to the IRIS Toxicological Reviews. In addition to a review of the available human and animal toxicity studies, the profiles recommend quantitative values for risk management decision-making.

2.1.2 State Toxicity Values

A number of state environmental regulatory programs develop and maintain databases of quantitative toxicity values. Perhaps the best known of these is the Cal/EPA toxicity values available on its Internet website at http://www.oehha.ca.gov/risk/chemicalDB/index.asp. Examples of other state databases of toxicity values include New Jersey Department of Environmental Protection (http://www.state.nj.us/dep/dsr), and the Texas Department of Environmental Quality (http://www.tceq.texas.gov/toxicology). States have also derived toxicity values for specific chemicals and routes of exposure. For example, the New York State Department of Health (NYSDOH) developed an air criteria document for trichloroethylene in 2006, which evaluated and derived noncancer and cancer toxicity values (NYSDOH 2006).

2.1.3 International Community

Quantitative toxicity information can be found on the websites for many international regulatory agencies. For example, Health Canada prepares screening assessments of priority chemicals under the Canadian Environmental Protection Act of 1999 (http://www.chemicalsubstanceschimiques.gc.ca/plan/index-eng.php). One database that provides information from a number of international sources is the International Toxicity Estimates for Risk (ITER) database, which can be found at http://iter.ctcnet.net/publicurl/pub_search_list.cfm. In addition to EPA's IRIS and the ATSDR databases, this site includes toxicity values from Health Canada, the International Agency for Research on Cancer (IARC), the International Programme on Chemical Safety (IPCS), the National Institute of Public Health and the Environment of the Netherlands (RIVM), as well as peer-reviewed values by independent parties, such as Toxicological Excellence for Risk Assessment.

2.1.4 Databases for Developing Toxicity Values

In addition to state, federal, and international databases with cancer and noncancer toxicity values, there are also a tremendous number of resources that can be researched to develop toxicity values for specific chemicals.[5] EPA has recently released ToxRefDB (http://actor.epa.gov/toxrefdb/faces/Home.jsp). This database captures detailed study design, dosing, and observed treatment-related effects on thousands of *in vivo* animal toxicity studies on hundreds of chemicals. This database was developed by the National Center for Computational Toxicology in

[5] The derivation of new toxicity values falls outside of the scope of this white paper. However, state, federal, and international databases can be useful resources for evaluating existing Tier 3 toxicity values.

partnership with the Office of Pesticide Programs. Examples of other databases include the National Library of Medicine Toxnet (http://toxnet.nlm.nih.gov/) and Micromedex (http://www.micromedex.com/products/hcs/), and the National Toxicology Program (NTP; http://ntp.niehs.nih.gov/). NTP provides toxicological information on over 500 chemicals through the publication of general Technical Reports on chemicals and chemical mixtures and the Scientific Review documents for chemicals and chemical agents which are listed in the Report on Carcinogens documents.

2.2 Similarities and Differences In How Toxicity Values Are Derived

As shown above, there are a large number of state, federal, and international resources for either obtaining or developing Tier 3 toxicity values. When obtaining toxicity values and data from these sources it is important to recognize that there are similarities and differences in how they develop toxicity values. This is important when comparing methodologies from external agencies and organizations to EPA's methodologies, as well as when comparing competing toxicity values. Similarities and differences may arise from the following elements:

- The quality and usability of the animal and human studies used to derive the toxicity values
- How adverse and critical effects are defined, and
- The methodologies used to derive the cancer or noncancer toxicity value

The first two elements are common to most of the databases and toxicity values discussed above. The methodologies used to calculate quantitative values are typically specific to the regulatory agency involved. These elements or guiding principles, which will be further discussed in Section 5.3.2, will serve as the basis for critical reviews of potential Tier 3 toxicity values.

In the case of competing toxicity values, differences between values may also be simply a result of the age of the toxicity values. Newer values will likely have more studies underlying their derivation. In addition, newer values may incorporate more current methods for evaluating dose/response relationships, such as physiologically-based pharmacokinetic (PBPK) modeling.

Although not discussed further in this white paper, a basic understanding of how to evaluate and assess the data usability of toxicity studies, identify the adverse and critical effect levels in a study, and evaluate the regulatory-specific methodologies used to derive cancer and noncancer toxicity values is useful for comparing, selecting, and developing chemical-specific toxicity values from multiple databases (Ibid).

3 Existing Publications Relevant to Tier 3 Toxicity Value Evaluation, Selection, and Use

This section summarizes existing publications that are relevant to the evaluation, selection and use of Tier 3 toxicity values. These publications include documents internal and external to EPA and include policy directives, guidance documents, handbooks, guidelines, and issue papers. In addition to summarizing these documents, the purpose of this section is to draw attention to elements of these documents that are critical in the evaluation of potential Tier 3 toxicity values.

3.1 Internal EPA Documents

3.1.1 2003 Hierarchy (OSWER Directive 9285.7-53)

As discussed in Section 1.1.1.2, EPA's Superfund program revised its hierarchy of human health toxicity values to incorporate EPA's PPRTVs and address the aging HEAST toxicity values. Although the 2003 guidance established an overall hierarchy for selecting toxicity values, it did not attempt to rank Tier 3 sources. Instead, it provides examples of Tier 3 sources and general recommendations regarding the prioritization of Tier 3 toxicity values. Specifically, in reference to Tier 3 toxicity values, the directive states:

> *Priority should be given to sources that provide toxicity information based on similar methods and procedures as those used for Tier I and Tier II, contain values which are peer reviewed, are available to the public, and are transparent about the methods and processes used to develop the values. Consultation with the Superfund Health Risk Technical Support Center (STSC) or headquarters program office is recommended regarding the use of the Tier 3 values for Superfund response decisions when the contaminant appears to be a risk driver for the site. In general, draft toxicity assessments are not appropriate for use until they have been through peer review, the peer review comments have been addressed in a revised draft, and the revised draft is publicly available.*

Although the directive does not go into great detail on selection of Tier 3 toxicity values, it is clear that it recommends that risk assessors select values that are derived using toxicological and risk assessment methods that are:

(1) Consistent with the Agency's methodologies;
(2) Transparent;
(3) Publicly available; and
(4) Have undergone peer review.

In addition, the directive recommends the involvement of ORD (Superfund Technical Support Center [STSC]) and headquarters and cautions against the use of draft toxicity values to ensure the scientific defensibility of Tier 3 toxicity values, especially risk-driving chemicals.

3.1.2 Peer Review Handbook

As indicated in the 2003 hierarchy memorandum and other publications specific to toxicity value selection and use (see for example, EPA 2009; ECOS 2007), peer review is one of several critical elements in selecting or giving preference to one toxicity value over another. Although not necessarily specific to toxicity value selection, EPA's Peer Review Handbook (EPA 2006) provides important information that is applicable to the evaluation and selection of Tier 3 toxicity values. The 3rd edition of the peer review handbook defines peer review as the following:

> *Peer review is a documented critical review of a specific Agency scientific and/or technical work product. Peer review is conducted by qualified individuals (or organizations) who are independent of those who performed the work, and who are collectively equivalent in technical expertise (i.e., peers) to those who performed the original work. Peer review is conducted to ensure that activities are technically supportable, competently performed, properly documented, and consistent with established quality criteria. Peer review is an in-depth assessment of the assumptions, calculations, extrapolations, alternate interpretations, methodology, acceptance criteria, and conclusions pertaining to the specific major scientific and/or technical work product and of the documentation that supports them. Peer review may provide an evaluation of a subject where quantitative methods of analysis or measures of success are unavailable or undefined such as research and development. Peer review is usually characterized by a one-time interaction or a limited number of interactions by independent peer reviewers. Peer review is encouraged during the early stages of the project or methods selection, and/or as part of the culmination of the work product, as appropriate. Regardless of the timing of peer review, the goal is ensuring that the final product is technically sound. (USEPA, 2006a)*

The importance of peer-review is re-affirmed in EPA's 2006 peer review policy, which states:

> *Peer review of all scientific and technical information that is intended to inform or support Agency decisions is encouraged and expected. Influential scientific information, including highly influential scientific assessments, should be peer reviewed in accordance with the Agency's Peer Review Handbook. All Agency managers are accountable for ensuring that Agency policy and guidance are appropriately applied in determining if their work products are influential or highly influential, and for deciding the nature, scope, and timing of their peer review. For highly influential scientific assessments, external peer review is the expected procedure. For influential scientific information intended to support important decisions, or for work products that have special*

importance in their own right, external peer review is the approach of choice (USEPA, 2006b).[6]

3.1.3 RAGS Part F

RAGS Part F also provides guidance on evaluation and selection of a Tier 3 toxicity value. In reference to EPA's toxicity value hierarchy, RAGS Part F states, "Priority in Tier 3 should be given to sources that are the most current and those that are peer reviewed. Consultation with the Superfund Headquarters office is recommended regarding the use of Tier 3 values for Superfund response decisions when the contaminant appears to be a risk driver for the site." In addition, this guidance provides a list of circumstances when route-to-route extrapolations from oral toxicity values might not be appropriate. This information could be useful in evaluating Tier 3 toxicity values that are based on route-to-route extrapolations.

3.1.4 Risk Assessment Guidelines

Multiple risk assessment guidelines have been published by EPA ranging from the Guidelines for Mutagenicity Assessment (1986) to the 2005 Guidelines for Carcinogen Risk Assessment. These guidelines , as well as other guidance documents pertaining to development of toxicity values (1994 *Methods for Derivation of Inhalation Reference Concentrations [RfCs] and Application of Inhalation Dosimetry*) provide specific guidance (including criteria to be met) on how the Agency derives toxicity values. These documents have and will continue to serve as the benchmark for evaluating toxicity values external to EPA.

3.1.5 Harmonized Test Guidelines

EPA's harmonized test guidelines (http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm) are documents that specify methods for use in testing pesticides and toxic substances and developing test data for submittal to the Agency. The guidelines typically specify the species to be tested, routes of administration, doses to be administered, and duration of study and endpoints to be assessed. These guidelines serve as the "gold standard" for performing toxicity testing and studies and, similar to the risk assessment guidelines discussed in Section 3.1.4, serve as a benchmark for evaluating the adequacy of a toxicity value's underlying study or studies.

3.2 Environmental Council of the States

[6] Influential scientific and highly influential scientific assessments involve precedential, novel, "cutting edge," or controversial issues, or the Agency has a legal or statutory obligation to conduct a peer review. Highly influential scientific assessments have a higher degree of influence, substance, interagency interest, and economic impact (EPA 2006a).

In April 2007, the Environmental Council of the States-U.S. Department of Defense Sustainability Work Group (ECOS-DoD Sustainability Work Group) released the issue paper (ECOS paper) titled "Identification and Selection of Toxicity Values/Criteria for Comprehensive Environmental Response, Compensation, and Recovery Act (CERCLA) and Hazardous Waste Site Risk Assessments in the Absence of IRIS Values." The ECOS paper, which was written in collaboration with EPA, Cal/EPA, and Department of Defense (DoD) scientists and risk assessors, is intended to provide guidance and a suggested framework for identifying and selecting toxicity values in the absence of IRIS values. The ECOS paper provides this guidance and framework in the form of seven preferences for identifying and ranking toxicity values. These preferences are provided below.

(1) *There should be a preference for transparent assessments (in which toxicity values are derived), that clearly identify the information used and how it was used.*

(2) *There should be a preference for assessments which have been externally and independently peer reviewed, where reviewers and affiliations are identified. Other things being equal, there should also be a preference for assessments with more extensive peer review. Panel peer reviews are considered preferable to letter peer reviews.*

(3) *There should be a preference for assessments that were completed with a previously established and publicly available methodology. Methodologies that themselves were externally peer reviewed are preferred over those that were not externally peer reviewed.*

(4) *While there should be a preference for assessments using established methodologies to derive toxicity values, these methodologies should also be informed by the current best scientific information and practices. New assessment methodologies should provide reproducible results and meet quality assurance and quality control requirements.*

(5) *There should be a preference for assessments that consider the quality of studies used, including the statistical power or lack thereof to detect effects; that corroborate data amongst pertinent studies; and that make best use of all available science.*

(6) *There should be a preference for assessments and values which are publicly available or accessible. There may be a further preference for toxicity assessments that invited and considered public comment (as well as, but not in lieu of, external peer review).*

(7) *Other things being equal, there should be a preference for toxicity values that are consistent with the duration of human exposure being assessed. For example, an externally peer reviewed subchronic reference dose (RfD) should be preferred to an externally peer reviewed chronic RfD when assessing an exposure of 2 years for non-cancer toxicity.* (ECOS 2007)

In conjunction with these seven preferences, the ECOS paper provides additional recommendations relevant to the selection of toxicity values. They include the overarching principle that risk assessors should continue to identify the most scientifically defensible toxicity value and that the selecting individuals have an understanding of the available sources of toxicity data and their strengths and weaknesses so that the most appropriate toxicity value is selected. Furthermore, although the seven preferences are generally intended for existing toxicity values, the ECOS paper specifically states that the preferences may be "used if an agency or party would like to propose an alternative to a toxicity value" (ECOS 2007).

4 Current and Past Regional Practices in Identifying and Selecting Tier 3 Toxicity Values

The purpose of this section is to summarize past and current practices used by regional and headquarters risks assessors to evaluate and select Tier 3 toxicity values. Specifically, this section discusses the evaluation and selection processes employed by the regional risk assessors to derive the regional screening levels. Also, this section provides detailed summaries of Tier 3 toxicity value consultations provided by regional and headquarters risk assessors.

4.1 Regional Screening Levels Table (Selection Process)

Risk-based screening levels for soil, air, and water have been in existence for nearly 20 years in EPA's Superfund Program. Similar to human health risk assessments, screening levels are derived using chemical-specific toxicity values combined with standard exposure factors that reflect Superfund's concept of a reasonable maximum exposure (RME). They have traditionally represented the point of departure of an excess lifetime cancer risk level of 1E-06 or a Hazard Quotient of 1 for noncancer effects.

In the past, risk-based screening levels were compiled in individual regional tables such as the Risk-Based Concentrations (RBC) table published by Region 3, the Human Health Medium-Specific Screening Levels (HHMSSL) table published by Region 6, and the Preliminary Remediation Goals (PRG) table published by Region 9. In general, if a substance had been assigned an EPA toxicity value, it was listed in the individual regional screening tables. In the case where a substance had more than one possible toxicity value, a toxicity hierarchy first described in RAGS Part A was applied. In some cases, each Region developed its own unique values (e.g., Region 3 RBCs for Fish Consumption).

One consequence of the 2003 toxicity values hierarchy memorandum (Human Health Toxicity Values in Superfund Risk Assessments, OSWER Directive 9285.7-53, December 5, 2003) was that the risk screening tables needed to be revised to reflect the new Agency preference for toxicity values. The guidance was clear with respect to the first two tiers in the hierarchy, and these tiers were used as "defaults" in the regional tables. However, it was less clear what was to be used as a Tier 3 source when there are competing sources. This lack of clarity could have led to inconsistencies in the regional screening tables if, for example, Region 3 used a different Tier 3 source than Region 9 or Region 6.

The regional offices that created screening tables have had a long history of communication and coordination to reduce (if not avoid) inconsistencies among the individual tables. Nonetheless, inconsistencies still existed. An important milestone was reached in 2008, when the various regional tables were harmonized into a single majority-consensus table known as the Regional Screening Levels (RSL) table. This table updated and superseded previous regional tables. Individual Regions are still able to develop independent (or non-consensus) screening values, however, they are not published as part of the RSL table. Individual Regions may also choose Tier 3 values different from the RSL table. It is not the responsibility of the RSL table workgroup to choose for, or dictate to the Regions. The RSL table workgroup merely makes recommendations. Representatives from all EPA regions and HQ are

encouraged to participate in the RSL table workgroup so that their valuable input is incorporated in the periodic updates and revisions to the screening table.

Establishing which toxicity values to use when there are no applicable Tier 1 or Tier 2 values is a challenge because the 2003 guidance did not provide a ranking or hierarchy for Tier 3 sources The RSL workgroup has proposed and implemented a tentative ranking of Tier 3 sources to include in the screening table. The RSL workgroup readily acknowledges that other toxicity values (e.g., State values) could be used to develop the screening values. It is NOT the mission or goal of the RSL workgroup to independently develop Tier 3 toxicity reference values in the absence of other sources, nor is it a practice of the workgroup to review values from all potential sources.

At present, the Tier 3 toxicity values from the following sources in the order in which they are presented below are used as the defaults in the RSL tables.

(1) The ATSDR Minimal Risk Levels (MRLs)
(2) Cal/EPA, Office of Environmental Health Hazard Assessment (OEHHA), toxicity values
(3) PPRTV Appendix "Screening Toxicity Values"
(4) HEAST

These sources are credible (rely on best available science, have undergone a high degree of scrutiny and peer review, are often considered by other Agencies).

An RSL calculator is also provided, which allows the user to use a different toxicity value or exposure assumptions other than the defaults. The RSL group anticipates that RSL's provisional hierarchy may change in the future to reflect recommendations in this white paper.

4.2 Tier 3 Toxicity Value Consultations

When there is no established Tier 3 value for high-priority chemicals that are likely to be risk drivers at a site, the regions have often performed their own evaluations of the science and/or sought headquarters guidance. With respect to headquarters consultations, key offices that have been involved include, but are not necessarily limited to, OSWER/OSRTI/SPB, OSWER/OEM, OSWER/OPM/PARMS, OSWER/ORCR/PMCAO, and ORD/NCEA. Below are several examples of how Tier 3 values have been evaluated and selected in the past at the regional and headquarters level.

4.2.1 Chromium (VI)

The 1998 IRIS file for chromium (VI) identified it as an inhalation carcinogen and provided an inhalation unit risk (IUR), but oral carcinogenicity could not be determined because no data were located in the available literature that suggested it was carcinogenic by the oral route of exposure (EPA 1998). However, several years later, a study by the National Toxicology Program (NTP) stated that oral exposure to chromium (VI) "provided *clear evidence of carcinogenic activity* in male and female rats and

mice based on the presence of benign and malignant tumors in rat oral mucosa and mouse small intestine" (NTP 2008) and suggested that the compound may be carcinogenic by mutagenic mode of action. In response to this study, some states (New Jersey and California) began the process of revising their water and soil standards based on the NTP study. EPA's Office of Pesticide Programs (OPP) also developed an oral slope factor and published a journal article on the chemical's mutagenic mode of action to support its risk assessment of chromated copper arsenate (McCarroll et al. 2010). In November 2008, the IRIS program began the reassessment of chromium VI for the oral route of exposure.

Region 2 appealed to headquarters in 2009 for guidance while working on a removal site because the state of the science had evolved faster than IRIS could be updated and several potential Tier 3 toxicity values were available. Specifically, Region 2 requested consultation on the use of New Jersey's oral slope factor (NJDEP 2009). In this request, Region 2 noted that although several potential Tier 3 sources are available, only New Jersey's oral slope factor met all the criteria in the 2003 hierarchy directive. The request was submitted to the Senior Science Advisor for OSWER on August 17, 2009, who consulted with representatives of OSRTI and OEM and concurred with this conclusion in an e-mail on September 28, 2009 (see Appendix B).

4.2.2 Perfluorooctanic Acid and Perfluorooctane Sulfonate

Perfluorooctanic acid (PFOA) and perfluorooctane sulfonate (PFOS) are emerging contaminants that have been found at sites in Region 4 and other regions. Because no toxicity values for these compounds are currently available in the IRIS or PPRTV databases, Region 4 requested that OSWER recommend what toxicity values would be appropriate to use. In response, OSRTI and OEM consulted scientists from EPA's Office of Water, Office of Pollution and Toxic Substances, and the Office of Research and Development regarding the use of the Office of Water's 2009 Provisional Health Advisories for PFOA and PFOS.

In an October 28, 2009, memorandum (see Appendix B), OSRTI and OEM recommended use of the provisional drinking water advisories for PFOA and PFOS and interim subchronic RfDs based on the advisory levels. Because the drinking water advisories address only water, OSWER's consultation included derivation of subchronic RfDs so that they could be used to derive removal action levels or screening levels for water and other media. The memorandum also outlines the ways the Provisional Health Advisories meet the criteria for a Tier 3 toxicity value as established in the hierarchy directive. Specifically, the consultation memorandum notes that the provisional advisories underwent internal and external review and draws attention to similarities between the Office of Water's methodology for deriving provisional advisory levels (and the subsequent subchronic RfDs) and IRIS assessments (deriving toxicity values using Benchmark Dose Level (BMDL), no observed adverse effects level [NOAEL], or lowest observed adverse effects level [LOAEL]).

4.2.3 Perchloroethylene

At about the time the 2003 toxicity value hierarchy was being finalized and released to the regional risk assessors, regions sent inquiries to OSWER regarding the use of Cal/EPA's cancer toxicity values for perchloroethyelene (PCE). Found at nearly half of all Superfund sites (ATSDR 1997), including numerous vapor intrusion sites, having toxicity values for this chemical was key to moving risk assessments and remedy decisions forward. Moving these activities forward was of special concern given that health organizations, such as IARC, had classified PCE as a probable human carcinogen (IARC 1995).[7]

In response, the Deputy Director of the Office of Emergency and Remedial Response (currently OSRTI), in consultation with the STSC, sent a letter to Region 10 on June 12, 2003, supporting the use of Cal/EPA's IUR and oral slope factor (see Appendix B), noting that there are similarities between how Cal/EPA and the IRIS program develop toxicity values and that Cal/EPA's presentation on how the toxicity values were developed is full, complete, and transparent. In regards to transparency and the use of the values in Superfund Program decision-making, the letter recommended that the appropriate documentation or link to the Cal/EPA website be provided. In addition, the letter included an excerpt from a Cal/EPA technical support document pertaining to PCE's inhalation unit risk value.

4.2.4 Trichloroethylene

Trichloroethylene (TCE), which is found at more than 1,500 sites, has a long and complicated history at EPA, especially within the IRIS and Superfund Programs. The IRIS cancer assessment and cancer toxicity values for TCE, which were released in 1987, were withdrawn in 1989.[8] Between 1989 and 2001, regions generally relied on the withdrawn values. In 2001, NCEA completed a preliminary draft assessment of the health risks posed by TCE. The new toxicity values, especially the cancer toxicity values, dramatically increased the calculated risks at the same exposure. Although these values were not loaded into the IRIS database, some regions continued to use them since they were briefly endorsed by STSC. After review by EPA's Science Advisory Board (SAB) in 2002, STSC no longer supported the use of the 2001 draft values. However, several regions continued to use the 2001 draft toxicity values. After the 2003 toxicity hierarchy memorandum was released, some regions began using the Cal/EPA toxicity values for TCE or a combination of Cal/EPA toxicity values and the 2001 draft toxicity values, while others continued to use only the 2001 draft toxicity values. The Region 9 PRG, Region 3 RBC, and Region 6 MSSLs used the 2001 draft noncancer and cancer toxicity values up until approximately the time the tables were consolidated into the RSLs in 2008. In 2008, the RSL tables began using the Cal/EPA cancer toxicity values.

[7] Prior to PCE's final Toxicological Review, which was posted on IRIS on February 10, 2012, IRIS only provided an RfD.

[8] TCE's final Toxicological Review was posted on IRIS on September 28, 2011.

In 2006, the NYSDOH released the *Trichloroethene Air Criteria Document*. That document, which underwent peer review, provided a noncancer inhalation toxicity value comparable to an EPA RfC. Because the NYSDOH toxicity value was final, had undergone peer-review, and its derivation was transparent, some regions began considering use of the value to assess noncancer health risks. Its use in risk assessments was significant, especially with respect to the vapor intrusion into indoor air pathway, because the NYSDOH value results in residential indoor air noncancer screening levels corresponding to a cancer risk of approximately 1E-05. In comparison, Cal/EPA provides a noncancer chronic REL that is 60 times greater than the NYSDOH value.

In 2008, Region 10 advised its states about Region 10's evaluation of TCE and provided two options for evaluating cancer risk: (1) use the geometric midpoint of the slope factor range from the 2001 NCEA assessment, or (2) use the Cal/EPA oral slope factor and inhalation unit risk, but adjust them upward by a factor of 10. When noncancer health hazards are evaluated, Region 10 recommended using the NYSDOH criterion.

In January 2009, OSWER released guidance on the recommended cancer and noncancer toxicity values (Cal/EPA cancer toxicity values and the NYSDOH noncancer inhalation toxicity value) (see Appendix B). The memorandum provided an extensive summary and evaluation of the available toxicity values from Cal/EPA, NYSDOH, and the Indiana Department of Environmental Management. It included a discussion on the toxicity values' underlying studies and methods used to derive the toxicity values and a detailed comparison of the competing noncancer inhalation toxicity values. However, the memo was withdrawn by OSWER in April 2009 to further evaluate the recommendations regarding the noncancer toxicity values for use in inhalation risk assessments (see Appendix B).

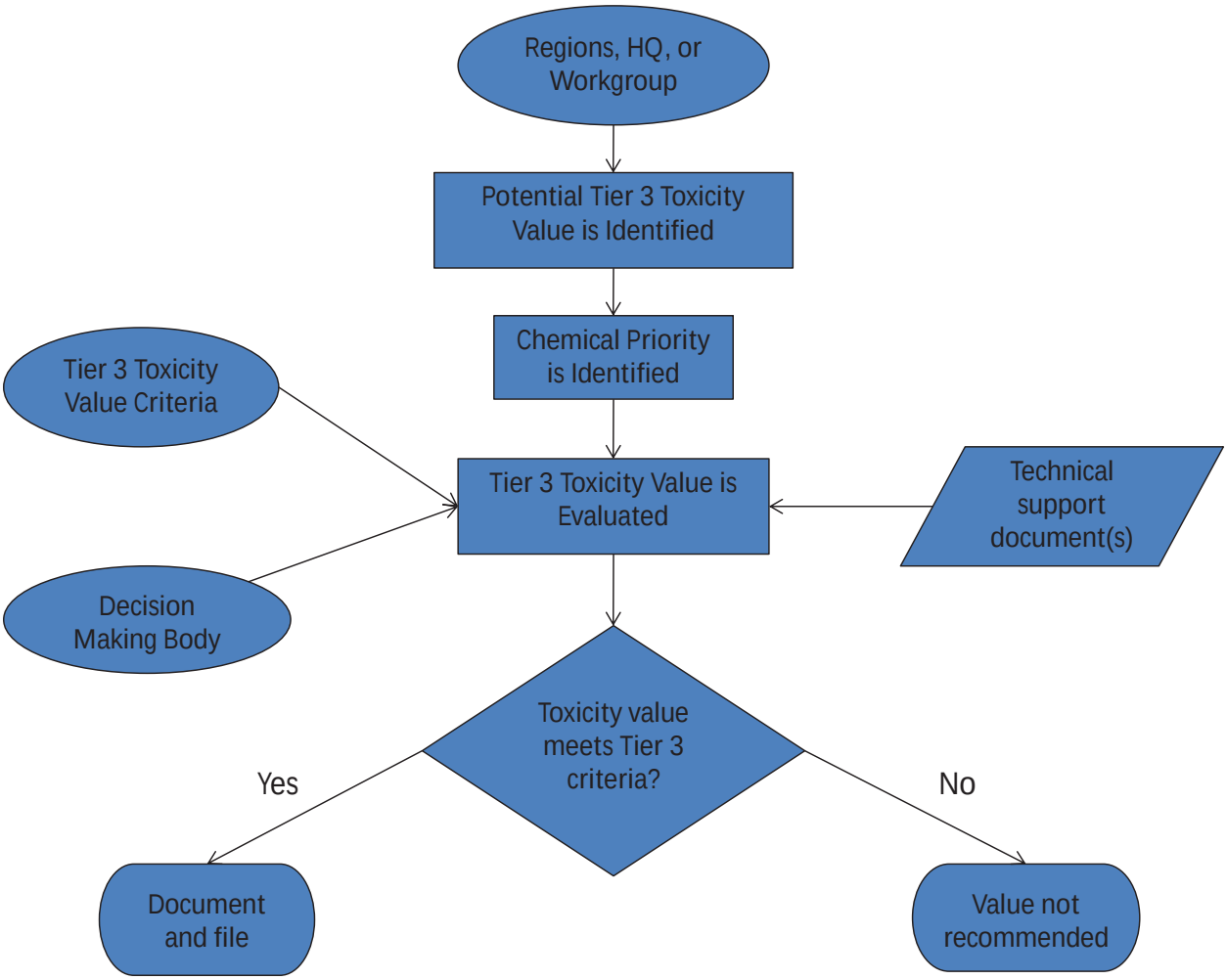
In April 2009, Region 7 provided guidance to the regional RCRA and Superfund programs on TCE toxicity values (see Appendix B). Specifically, the regional risk assessors recommended the use of the Cal/EPA cancer toxicity values and the NYSDOH non-cancer inhalation toxicity value, citing that they met the requirements of Tier 3 toxicity values (for example, had been peer-reviewed). With regards to the competing inhalation toxicity values, Region 7 provided rationale for selecting the NYSDOH value over the Cal/EPA REL.

During the spring 2011 RSL table update, the RSL workgroup provided a noncancer RfC for TCE based on the value derived by the NYSDOH (NYSDOH 2006).

5 Alternatives for Identifying, Evaluating, and Selecting, and Documenting Tier 3 Toxicity Values

As discussed in Section 1, the overall goal of the Regional Tier 3 Toxicity Value Workgroup is to establish a process that enhances the transparency and consistency of Tier 3 toxicity value identification, evaluation, selection, documentation, and communication. The steps in the overall process for selecting Tier 3 toxicity value are shown in Figure 1 below and described in the following sections. For this white paper, communication refers to the flow of information and overall coordination leading to selection and documentation of a Tier 3 toxicity value. Therefore, communication is part of the other steps and is not shown as a separate step. In addition, the priority of a chemical (regional or national interest) may play a significant role in determining the overall selection process and is therefore listed as a step in the selection process.

Figure 1. Tier 3 Toxicity Value Selection



5.1 Toxicity Value Identification

The regional risk assessors and RSL workgroup[9], through their routine work (site risk assessments and table updates), regularly encounter chemicals without Tier 1 and Tier 2 toxicity values. Thus, the identification of potential Tier 3 toxicity values has been largely their responsibility. This approach continues to be an option, however, this white paper also presents other potential avenues for identifying Tier 3 toxicity values. As an alternative to the regional risk assessors and RSL workgroup, a formal toxicity workgroup could be charged with identifying Tier 3 toxicity values, as well as other responsibilities (see Section 5.4.3.1). Although this responsibility is similar to the RSL workgroup, which looks at a broad range of chemicals, it is envisioned that the formal workgroup would look for potential Tier 3 toxicity values beyond the sources consulted by the RSL workgroup (for example, international sources). Furthermore, the workgroup's identification of potential Tier 3 toxicity could outpace the RSL workgroup because the former's sole focus would be to identify, evaluate, select, document, and communicate Tier 3 toxicity values.

If the responsibility for identifying potential Tier 3 toxicity values were assigned to a formal workgroup, several issues would need to be considered. First, the establishment of a new workgroup (assuming responsibilities are not subsumed within an existing workgroup) would require time and resources. In addition, it is uncertain whether the workload (new values being made available) would be sufficient to keep the group active. Furthermore, regional risk assessors and others will likely continue to search for Tier 3 toxicity values in their routine work (conducting risk assessments), leading to a duplication of effort. Thus, the value added of a formal workgroup is uncertain and would likely require the group to have multiple responsibilities to maintain member interest.

5.2 High vs. Low Priority

As a result of resource constraints, time, and other limitations and difficulties (such as potential controversy surrounding some chemicals), it is likely that no one alternative will be suitable as the sole means of evaluating and selecting all Tier 3 toxicity values. Thus, the priority of the chemical will likely dictate the entity that will evaluate and select a Tier 3 toxicity value. For instance, the examples provided in Section 4 clearly indicate that high-priority chemicals are elevated to headquarters.

The process for elevating Tier 3 toxicity values to headquarters and other entities (such as the RSL workgroup) has been rather informal in the past. If a more formalized and structured system of selecting Tier 3 toxicity values is implemented, a formal process for determining a chemical's priority may be needed, including criteria for distinguishing between those chemicals of low, medium, and high priority. This determination can be subjective and vary among the regions. Factors to consider in evaluating priority are described below. Of course, decision-making in regard to these criteria, especially a

[9] During the development of site-specific risk assessments, potentially responsible parties may identify Tier 3 toxicity values.

chemical's prevalence, may require coordination among the regions and headquarters, and the formal process may reinforce this requirement. Continued coordination and communication among potential decision-makers is also important so that elevation of a Tier 3 value to a headquarters or regional workgroup is efficient (chemicals are not elevated and then demoted).[10]

Below are a set of prioritization criteria that could be used to assist risk assessors, risk managers and others in assigning priority to a contaminant. Answering one of the questions below in the affirmative may not be sufficient to designate a contaminant as high priority. However, a preponderance of evidence should be adequate to support a high-priority designation. A contaminant with a high-priority designation would likely require a Tier 3 consultation by headquarters or a regional workgroup to ensure consistency across the Regions. Tier 3 contaminants that are not expected to drive health risks or remediation at a site, may be associated with mild health effects, are not encountered across multiple regions, or are not being considered for national rule making may be considered low priority. In this case, the decision to develop a Tier 3 toxicity value may be best left up to the individual region.[11]

Prioritization Criteria

- Does the contaminant have the potential to drive risks estimates and remediation at a site?

  Answering this question requires a minimum of toxicity information, such as a single subchronic or developmental study administered by the route of exposure expected to occur at the site. This information may be available from the database sources described in Section 2.1 or via an open literature search. If the answer to the question is yes, then the contaminant may be a candidate for a high-priority designation.

[10] Because regional risk assessors that submit potential Tier 3 toxicity values may have significant knowledge of the chemical, they may remain involved in the evaluation and selection process.

[11] In cases where the priority of a chemical falls somewhere in between high and low, best professional judgment should be used in deciding whether that chemical should be evaluated by headquarters or a regional workgroup (chemical evaluated as high priority) versus individual region (chemical evaluated as low priority). In cases of uncertainty, it is recommended a request be sent to the "Tier 3 Toxicity Value Steering Committee" (further discussed in Section 6.2), which would decide the priority designation and ultimately the proper action to be taken on a chemical-specific basis.

- Based on the available toxicity information and the concentration measured at the site, would the estimated human health effects be expected to be severe (irreversible damage affecting the function or viability of a receptor or target organ), moderate, or mild (transient, reversible effects)?

  Similar to the first question, answering this question requires a minimum of toxicity information. If the information suggests that the health effects to an individual would be severe or moderate, then the contaminant may be considered a high priority.

- Is the contaminant associated with a source or industry that is common across the region or multiple regions?

  The more prevalent a contaminant, especially across multiple regions, the more likely it is to receive a high-priority designation.

- Based on the chemical and physical properties of the contaminant, how likely is it that the remediation techniques used for the known risk drivers at the site would also remediate the contaminant in question?

  This question is not necessarily toxicological, but instead is a risk management question. If the remediation techniques being used at a site for the known risk drivers will also be successful in cleaning up the contaminant in question (based on what is known about the chemical and physical properties), it may not be efficient or necessary to delay a project while a Tier 3 toxicity value is being evaluated.

- Is the contaminant under consideration for rulemaking nationally?

  If EPA is considering the contaminant for rulemaking purposes, it should automatically be considered as a high-priority candidate. The best approach would be to ensure a consistent toxicity value across all regions and program offices because of the public visibility of the contaminant.

5.3 Toxicity Value Evaluation (Criteria for Selecting a Tier 3 Toxicity Value)

Per EPA risk assessment guidance and other relevant risk assessment publications, the ultimate goal of selecting a toxicity value for use in risk assessment is to select the most current and scientifically defensible value. With regard to the selection of Tier 3 toxicity values, this value is selected by applying a combination of the general guidance principles discussed in Section 2.2 and the recommendations and preferences discussed in EPA and non-EPA risk assessment guidance (see Section 3). The following sections outline a proposed process that could be used to evaluate and select Tier 3 toxicity values.

5.3.1 Basic Requirements for Consideration as a Tier 3 Values

After a potential Tier 3 toxicity value has been identified, the first step is to determine whether that value meets the basic requirements of a Tier 3 value. As discussed in OSWER's 2003 Toxicity Value Hierarchy, three key factors for a toxicity value to be considered in the selection of a scientifically defensible Tier 3 value are that the value is peer-reviewed, publically available, and that the source is transparent about the methods and procedures used to develop the value. These same factors are also discussed in several of the seven preferences provided in the ECOS paper and echoed in other EPA guidance (such as RAGS Part F). Despite the requirements implied in the aforementioned documents, the level of peer-review is not specified. Thus, per EPA's peer review policy, decision-makers (the entity evaluating a potential Tier 3 toxicity value) have to consider whether the level of peer review matches the significance of the chemical. Availability and transparency are more straightforward. However, decision-makers have to determine, for example, whether an Internet posting of a summary file of a toxicological assessment (instead of the entire toxicological file) meets the availability and transparency criteria unless an internet link to the entire file is provided.

It is also important to evaluate the quality and usability of the underlying data supporting the potential Tier 3 value. Although a precise level of data quality and usability has not been defined, some toxicity values may not be of suitable quality or usability even though they have been peer-reviewed and are publically available. For example, some toxicity values may be based on route-to-route extrapolations of peer-reviewed values. Therefore, this step may focus on major deficiencies that would preclude use of a potential Tier 3 toxicity value. When competing Tier 3 values are available, this step may also indicate the preferred value.

5.3.2 Tier 3 Toxicity Value Critical Review

Section 2.2 introduced the general guiding principles for evaluating the quality and usability of Tier 3 toxicity values. Specifically:

(1) The quality and usability of the animal and human studies used to derive the toxicity values,
(2) How adverse and critical effects are defined, and
(3) The methodologies used to derive the cancer or noncancer toxicity value.

This white paper proposes the use of the guiding principles to conduct a more critical evaluation of the potential Tier 3 toxicity value.

5.3.2.1 Quality and Usability of Toxicity Testing Studies

There are a number of factors to consider in evaluating whether an animal or human toxicity testing study should be used in developing a toxicity value. The first is whether the study was conducted per the appropriate testing guidelines for the regulatory agency. For EPA, these guidelines are the harmonized test guidelines discussed in Section 3.1.5. Other guidelines include the Good Laboratory Practice (GLP) and the Food and Drug Administration (FDA) and Organization for Economic Co-Operation and Development (OECD) guidelines. Per these guidelines and other relevant documents (see for example EPA 1994, 2002, 2005, and 2008), factors to consider in the critical evaluation of the quality and usability of toxicity testing studies include:

- What is the route of administration of test material?
- What is the animal species tested?
- What is the dose duration (acute, sub-chronic, or chronic)?
- Is the apparent difference treatment-related?
- Is the effect dose-dependent?
- Is the effect biologically significant (as opposed to statistically significant)?
- Are the effects seen in multiple species, strains, or both sexes?
- Are the results relevant to humans?
- Were the study results interpreted properly?
- Is supporting evidence such as physiologically based pharmacokinetic modeling, metabolism studies, or structure activity relationship studies available?

Note that both the individual studies and the database of human and animal toxicity testing studies can be ranked as having low, medium, or high confidence based on an evaluation of these factors (see Section 5.3.3).

5.3.2.2 Defining Adverse and Critical Effects

Another critical element in the evaluation of a toxicity value is how adverse and critical effects are defined. An adverse effect is defined by EPA as the biochemical change, functional impairment, or pathological lesion that impairs performance and reduces the ability of an organism to respond to additional challenge (http://www.epa.gov/iris/help_gloss.htm). The lowest dose level at which an adverse effect occurs is defined as the critical effect level and is typically expressed as the LOAEL or lowest observable effect level (LOEL). A dose level at which there are no statistically or biologically significant increases in the frequency or severity of any effect between the exposed population and its appropriate control is the NOAEL. The critical effect level can also be determined using a benchmark dose approach or categorical regression. Thus, it is useful to consider the following in checking a study:

- Were the study results interpreted properly?
- Was the effect identified as adverse truly a biologically significant adverse effect?
- Is the adverse effect consistent with what is known about the chemical and the other studies in the database?

It is also important that the critical effect level be adjusted to the dose metric of interest (for example, parts per million [ppm] in food to milligrams per kilogram per day [mg/kg-day] for the oral route), for duration of exposure (such as from periodic to daily or continuous exposure), and scaled from an animal to a human equivalent body weight or concentration. Without these adjustments, it is not possible to compare effect levels on an equivalent basis. A study that might appear to have the lowest point of departure on first glance may not when the correct dosimetric adjustments are made. The critical effect (NOAEL or LOAEL, point of departure if using a benchmark dose approach, and categorical regression) is used as the starting point for calculating toxicity reference values for threshold toxicants.

5.3.2.3 Derivation of Noncancer and Cancer Toxicity Values

As mentioned in Section 2.2.3, the methodologies used to calculate toxicity values are typically specific to the regulatory agency involved. Understanding their differences and similarities are also useful when potential Tier 3 toxicity values and competing values are evaluated. EPA, for example, uses an RfD approach to calculate toxicity values for threshold toxicants administered by the oral route of exposure. An RfC is estimated for the inhalation route. This approach determines the critical effect level in the principal study or studies and applies uncertainty factors to account for:

(1) Variation in susceptibility among the members of the human population (inter-individual or intraspecies variability);
(2) Uncertainty in extrapolating animal data to humans (interspecies uncertainty);
(3) Uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (extrapolating from subchronic to chronic exposure);
(4) Uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and
(5) Uncertainty associated with extrapolation when the database is incomplete.

The default for each of these uncertainty factors is a value of 10. The exact value (10, 3, or 1) of the uncertainty factor selected may depend on the quality of the studies available, the extent of the database, and scientific judgment. Some factors to consider when the default factor of 10 is replaced with a lesser value are chemical-specific toxicokinetic or toxicodynamic data, the severity of the effect, the slope of the dose-response curve, and the presence of developmental and reproductive studies. For a more in-depth discussion, please see EPA's report titled *A Review of the Reference Dose and Reference Concentration Process* (EPA 2002). When a toxicity value is evaluated from the ATSDR database (or any other state, federal, or international regulatory program), the application and interpretation of uncertainty factors will differ from EPA's approach. Understanding these differences is important because the application of uncertainty factors may alter the final toxicity value by 1 to 5 orders of magnitude.

Some regulatory agencies, such as Health Canada, may use a margin of exposure (MOE) approach. Instead of reducing the critical effect level by a number of uncertainty factors, the MOE approach compares site-specific exposures directly with the critical effect level. The resulting ratio is then evaluated to determine if there is an adequate margin of safety.

For carcinogenic substances, qualitative descriptors are often provided on the likelihood of a chemical agent to cause cancer in humans. EPA currently uses five recommended standard hazard descriptors: "Carcinogenic to Humans," "Likely to Be Carcinogenic to Humans," "Suggestive Evidence of Carcinogenic Potential," "Inadequate Information to Assess Carcinogenic Potential," and "Not Likely to Be Carcinogenic to Humans" (EPA 2005). Different regulatory agencies and health organizations will use different qualitative descriptors. For example, IARC classifies carcinogens as Group 1 (carcinogenic to humans), Group 2A (probably carcinogenic to humans), Group 2B (possibly carcinogenic to humans), Group 3 (not classifiable as to its carcinogenicity to humans) and Group 4 (probably not carcinogenic to humans).

Some regulatory agencies and health organizations will quantify the dose-response assessment of carcinogens, while some may simply regulate a toxicant if it is deemed to be a possible carcinogen. EPA provides a quantitative estimate of the dose-response relationship by fitting the cancer bioassay data within the range of observation and deriving a point of departure (the lowest data point adequately supported by the data). If the mode of action data supports nonlinearity, an RfD or RfC is calculated from the point of departure. If the mode of action data indicate the dose response curve is expected to have a linear component below the point of departure, a linear extrapolation below the point of departure is used. The slope of this line is the slope factor. Agencies may differ on their interpretation of whether the dose response curve is linear or non-linear below the point of departure, resulting in different calculations of a cancer toxicity values.

Other regulatory agencies and health organizations, particularly in Europe and Asia (World Health Organization [WHO], International Programme on Chemical Safety [IPCS], and International Life Science Institute Europe) support a MOE approach for assessing carcinogens, regardless of the mode of action. The MOE approach compares the margin between a dose or an exposure causing cancer in animals or humans (for example, the point of departure) with the estimated human exposure to that substance. The resulting ratio is then evaluated to determine if there is an adequate margin of safety.

5.3.3 Tier 3 Toxicity Value Confidence

This white paper proposes that the confidence in a particular Tier 3 toxicity value could be ranked as low, medium, or high as part of a critical review. Ranking the level of confidence could be useful for determining the relative appropriateness of using Tier 3 toxicity value in various steps of the human health risk assessment process, as well as assisting with the selection of a value when competing values are available. A value that receives a "low" confidence ranking may be helpful during the initial screening process (for example, when determining if an analyte is a chemical of concern and should be carried forward into the baseline risk assessment process); however, a toxicity value with a "low" confidence ranking may be not be suitable for use in the baseline risk assessment or development of preliminary remediation goals because of limitations in this value. For CERCLA and RCRA processes that undergo more critical examination, a toxicity value with a "medium" or "high" confidence ranking would be more appropriate.

Below are some examples using the guiding principles mentioned above and discussed in Section 2.2 in applying confidence rankings to toxicity values.

The first element is the quality and usability of the animal and human studies used to derive the toxicity values. If only one animal species is tested for a subchronic period of exposure using only one dose level by a route of administration not consistent with the exposure route being evaluated at a CERCLA or RCRA site, the confidence in the toxicity value would likely be considered to be "low." The value could be used during the screening process, but would likely be inappropriate for a baseline risk assessment. If the contamination levels at a CERCLA or RCRA site exceed screening levels based on a Tier 3 value with low confidence, then the risk assessor has several choices. One choice would be to move to a qualitative assessment of the contaminant during the baseline risk assessment. Another choice would be to submit the contaminant to the STSC for a more thorough evaluation and a second opinion on the usability of the database and toxicity value. A third option would be to retain the Tier 3 value in the baseline risk assessment and be prepared to defend the scientific credibility of the value as part of the uncertainty assessment.

The second element is how the adverse and critical effects are defined. If the adverse effect is consistent with the definition provided in EPA's IRIS database (http://www.epa.gov/iris/help_gloss.htm) and is both biologically and statistically significant, then a ranking of "medium" or "high" may be assigned.

The third element is an examination of the methodology used to derive the quantitative toxicity value from the defined adverse effect. If the methodology is consistent with the cancer or noncancer methodology described in EPA's IRIS database (http://www.epa.gov/iris/) or adequately accounts for uncertainty and variability within susceptible populations, then a confidence of "medium" or "high" can be assigned. The overall ranking from these elements will be useful in determining where in the CERCLA or RCRA process the toxicity value would be most appropriate to use.

5.4 Options for Tier 3 Toxicity Value Consultations

There are several possible options for the types of decision-making bodies that could provide Tier 3 toxicity value consultations. Some of the possible options, which are discussed in the following sections, include forming or consulting an Action Development Process Workgroup; forming or consulting a headquarters or regional workgroup, or having individual regions evaluate and select values. In addition, the range of potential options is further expanded when considering the scope of consultation. For example, the requestor could be responsible for performing the evaluation and the consultation workgroup provides only a brief review and approval. Alternatively, the consultation workgroup could be charged with conducting the full evaluation of the potential Tier 3 toxicity value. Section 4.1.1.2 provides some examples of how this has been done previously.

One factor that should be considered in making the decision is the potential impact of the Tier 3 toxicity value under consideration and whether it should be considered influential scientific information. Consistent with EPA's Information Quality Guidelines (http://www.epa.gov/QUALITY/informationguidelines/) and the Office Management and Budgets Peer Review Bulletin (http://www.whitehouse.gov/sites/default/files/omb/memoranda/fy2005/m05-03.pdf), influential scientific information is that which the agency reasonably can determine will have or does have a clear and substantial impact on important public policies or private sector decisions. Influential scientific information is expected to maximize quality, objectivity, utility, and integrity.

In addition to the visibility and priority of the chemical, there are several other key issues that will need to be considered in establishing processes for developing Tier 3 toxicity values. These issues include, but are not limited to, the overall coordination and process for requesting consultations, contract support, and documentation. Additional discussion on these issues is provided in the following "options" sections and in Section 5.5

5.4.1 Action Development Process Workgroup

The Action Development Process (ADP) is the Agency's accepted method for producing high-quality actions, such as regulations, policies, and risk assessments. It ensures that EPA uses the best available information to support its actions and that scientific, economic, and policy issues are adequately coordinated with the various stages of action development. More information is available on the Office of Policy, Economics & Innovation's (OPEI) Intranet site http://intranet.epa.gov/adplibrary. Tier 3 toxicity values that would be considered influential scientific information should generally be developed through the ADP. Typically, this process would be initiated by OSWER. Briefly, the process begins with a tiering by the Regulatory Steering Committee. There are three possible tiers related to the level of senior level management involvement and the extent of cross-agency influence: Tier 1 actions are signed by the Administrator and typically have broad cross-agency influence, Tier 2 actions are signed by Assistant Administrators and typically have some cross-agency influence, and Tier 3 actions are typically signed by Office Directors and generally have limited cross-agency influence. Development of Tier 3 toxicity values using the ADP would typically be considered a Tier 3 action. The ADP has a number of prescribed steps that are required for all Tier 1 and Tier 2 actions; Tier 3 actions can be less formal, but typically include Office of Management and Budget (OMB)-led interagency review.

5.4.2 Headquarters Consultation

Headquarters, including offices within OSWER and ORD, have advised regions in the past on the use of Tier 3 toxicity values. Typically, regions have submitted requests to OSWER, which has responded with its recommendations. These requests have included consultations on chromium (VI), PCE, PFOA, and PFOS. Generally, these consultations were led by OSWER, but also included input from ORD. In addition, consultations were often coordinated among various offices within OSWER, including the

science advisor, OSRTI, and OEM. The scope of these consultations also varied. Whereas much of the toxicity value evaluation for chromium (VI) was performed by Region 2, most of the toxicity value evaluation for PFOS and PFOAs was performed by OSWER and consulting programs.

This approach remains a viable method for evaluating and selecting Tier 3 toxicity values, especially for high-priority chemicals where consistency and headquarters support are paramount. The headquarters consultation could continue to be performed on an "informal" basis, or a more formalized consultation process could be adopted in the future. Under the informal process, regions would continue to send requests to any of the multiple risk assessment and toxicology program contacts in OSWER including, but not limited, to OSRTI, OEM, or PARMS. Those offices would be responsible for establishing the consultation workgroup. Under the formal consultation process, it is envisioned that all consultations would be led and authored by a designated office within OSWER (such as OSRTI) and include a small group of technical experts and representatives from various programs, regions, and laboratories (such as ORD).

Regardless of whether an informal or formal approach is taken, several key factors will need to be considered for headquarters consultations. First, headquarters may need to establish a point of contact for consultations to coordinate reviews. In other words, headquarters may need to designate an individual or group of individuals who could receive Tier 3 consultation requests. Likewise, to eliminate redundancy (same requests from multiple regions) and improve the communication of toxicological information, the regional risk assessors may need to establish a process for submitting requests. The OSWER Human Health Regional Risk Assessors Forum (OH2R2AF) and OH2R2AF toxicity workgroup could fulfill this role. Furthermore, depending on the scope of the consult and the resource and time constraints, contract support may be necessary to assist headquarters with the collection, evaluation, coordination, and documentation of information pertaining to the consult.

There are several benefits to using headquarters consultations. Because of its role in providing guidance and policy to the regions, and centralized location within the organization, headquarters-based consultations, which may be provided by a designated office in headquarters, are more likely to maintain a consistent approach in the application of review criteria compared with other alternatives that may rely on multiple entities to provide consults. Furthermore, as a result of its position of authority, headquarters consultations also add "greater weight and credibility" to a Tier 3 value. Headquarters consultations are also more likely to include involvement from other program offices at the national level (e.g.,OPP), which may add greater credibility to and support for a particular Tier 3 toxicity value.

Despite the benefits associated with headquarters consultations, there are some potential challenges. The biggest challenge pertains to the perception that headquarters is setting policy. There are specific requirements for headquarters for the development of guidance and policy (such as interagency and OMB review). Although consultations are not equivalent to agency guidance or policy, the perception that headquarters is setting policy, especially among high-priority chemicals, could stall efforts. Consultations could be delayed if the program office has to defend perceptions of setting policy to

management and others. Subject matter experts from other program offices may also be reluctant to provide input if it appears they are setting policy for their particular program.

Another potential challenge with this alternative is that it may not be well suited to handle low-priority chemicals. Headquarters will tend to have the greatest interest in chemicals that have significant affects on risk management decisions or that are found in numerous regions. Thus, headquarters could exhaust its resources and time in high-priority chemicals and have little time to complete consults on low-priority chemicals. Consults would also have to compete with other headquarters projects and priorities. Therefore, headquarters may have difficulties in getting adequate technical support from subject matter experts for the consult.

5.4.3 Regional Workgroup

Another method for evaluating and selecting a Tier 3 toxicity value is through the use of a regional workgroup. The regional workgroup could be established as a formal regional workgroup or as an *ad hoc* work group consisting of subject matter experts with expertise relevant to the chemicals being evaluated. These workgroups would be led by and generally consist of regional risk assessors and toxicologists.[12] Headquarters risk assessors and toxicologists could be involved, but serve more or less as advisors. It is anticipated that the regional workgroup would primarily focus on low- to medium-priority chemicals, but may provide guidance on the high-priority chemicals that would not be considered influential scientific information.

There are two existing regional workgroups that could evaluate and select Tier 3 toxicity values. They include the RSL workgroup and the newly formed OH2R2AF toxicity workgroup. Because these workgroups' primary roles are to maintain the RSL Table and to address overall toxicity value needs and issues within the regions, a separate workgroup focused on Tier 3 toxicity values may be a viable alternative. However, under this alternative, such a workgroup may require coordination and direction from an overarching workgroup, such as the RSL workgroup and OH2R2AF toxicity workgroup (see below).

The role of these workgroups could vary significantly. The regional workgroup's role could be limited to advising regions that have identified a potential Tier 3 toxicity value, which may include evaluating the toxicity value and providing recommendations regarding the candidate value. In addition to providing consultations, the regional workgroup's role could be expanded to identifying, reviewing, and providing recommendations on Tier 3 toxicity values independent of requests from regions. This latter role would likely require formation of a formal workgroup.

[12] Because regional risk assessors that submit potential Tier 3 toxicity values may have significant knowledge of the chemical, they may remain involved in the evaluation and selection process as a regional workgroup member or advisor.

Although the level of effort of these workgroups will depend on their scope and the amount of toxicological information available for a given compound, contract support may be necessary. Under the consultation role, contract support would likely be limited and vary according to the chemical. The requestor of the consult may perform the bulk of the evaluation. However, contract support may be necessary for a workgroup that is routinely involved in identifying, reviewing, and providing recommendations on Tier 3 toxicity values independent of requests.

5.4.3.1 Formal Regional Workgroup

A formal regional workgroup, presumably under the auspices of the OH2R2AF, could play a dual role as a consulting workgroup and workgroup that actively identifies, reviews, and makes recommendations on Tier 3 toxicity values. This workgroup would generally be composed of and led by regional staff. Its membership could be fixed or consist of a small group of permanent members whereby subject matter experts fill temporary membership positions on a chemical-specific basis. Likely roles for this workgroup, in addition to the those listed above, may include evaluating existing Tier 3 toxicity values provided in the RSL table and periodically reviewing Tier 3 sources for new or updated toxicity values. Additionally, this group could derive new toxicity values. However, the roles involving periodic review of existing Tier 3 toxicity values in the RSL table and the derivation of toxicity values fall outside the scope of this white paper.

There are several strengths and limitations of establishing a formal regional workgroup. It is envisioned that a formal regional workgroup would select a core membership, structure it's organization (perhaps by developing a charter), and schedule regular meetings. Such a group could be more easily tracked in terms of agenda and progress, and a formal structure would make the workgroup easier to manage and have clearer expectations. In addition, both the workgroup and its members would be more visible to headquarters and the regions and provide greater credibility to the selection of a toxicity value. In addition, it is likely that a formal workgroup would more likely maintain a consistent process (for example, in application of review criteria) for evaluating and recommending new Tier 3 toxicity values. However, if the workgroup is formalized and core membership is fixed, the workgroup may lack expertise and/or fail to reach out to others with expertise in a particular chemical or toxicity value development (Ibid). Lack of subject matter expertise would limit the scientific credibility and usability of the toxicity value, which is the end product. Furthermore, the workload may not require regularly scheduled meetings, potential resulting in loss of focus and interest among the workgroup members and less than satisfactory work products.

5.4.3.2 Ad Hoc Regional Workgroups

Regional workgroups, under the direction of a coordinating committee (such as the OH2R2AF toxicity workgroup), could also be formed on an as needed basis to provide consultation on the use of Tier 3 toxicity values. The coordinating committee would receive Tier 3 consultation requests and be charged with staffing an *ad hoc* regional workgroup with regional risk assessors and toxicologists with subject matter expertise relevant to the chemical in question. The group's charge would also include

establishing a workgroup chair (a regional risk assessor or toxicologist) who would be responsible for leading the consultation and documenting the consult (drafting the memorandum). ORD and headquarters could also participate on these workgroups, especially if the regions are lacking subject matter expertise. Given that this workgroup would be formed on an as-needed basis, it is not likely that it will be evaluating and providing recommendations on existing Tier 3 toxicity values or periodically reviewing Tier 3 sources for new or updated toxicity values. Those roles would likely be retained by existing workgroups, such as the RSL table workgroup and the regional risk assessors.

Regardless of whether the coordinating responsibilities fall within a new or existing workgroup, the coordinating committee will have to put itself into position to receive Tier 3 toxicity value consultation requests and assign workgroups in a timely and efficient manner. Thus, the coordinating committee will have to maintain visibility among the regional risk assessors so that it is known to whom requests should be sent. The coordinating committee will also have to maintain a list of subject matter experts to staff the workgroups. Maintaining this list would likely require the coordinating committee to reach out to the regional toxicologists and risk assessors and possibly others in headquarters to determine whether they can and would participate on the workgroup should their expertise be needed.

There are several strengths and benefits with the use of *ad hoc* regional workgroups. Unlike the formal regional workgroup, which is limited to the expertise of its members, an *ad hoc* regional workgroup could be staffed with members who already have expertise on a particular chemical or chemical group. This approach to staffing could decrease the amount of time it takes to provide a consult and provide greater credibility/weight to the consult. In addition, *ad hoc* regional workgroups may also better champion the needs and priority for a Tier 3 toxicity value on a chemical that has a region-specific or limited geographic distribution in the environment. Unlike a formal workgroup or headquarters consult, an *ad hoc* workgroup could be composed of members who all have an interest in the chemical in question and completing a consult. However, this composition also could bias the consult. An *ad hoc* regional workgroup would also be focused on one particular task and less likely to be distracted from competing priorities, thereby decreasing the amount of time for a consultation and potentially improving the quality of the review. Furthermore, assuming the *ad hoc* workgroups are well-coordinated, this option would likely maximize available resource by spreading the responsibilities among many versus a few.

Along with the strengths and benefits of an ad hoc workgroup, this option has its limitations and challenges. Several of these limitations and challenges could stem from the coordinating committee. As indicated above, coordination is a critical component of this option. Thus, this option would lack effectiveness if the coordinating committee is poorly organized and managed. In addition, the formation and staffing of an *ad hoc* work group for each new chemical under consideration may be cumbersome and time consuming for the coordinating committee. Because the *ad hoc* regional workgroup will likely be coordinated by a regional workgroup, it may also suffer from lack of membership or input from EPA in headquarters and ORD (such as OSWER risk assessor or NCEA scientist). From a planning perspective, an *ad hoc* workgroup may make it difficult to staff workgroups

with subject matter experts from ORD or headquarters on an as-needed basis, let alone regional subject matter experts.

Although this option allows for the tailoring of a workgroup around a particular expertise, multiple *ad hoc* workgroups can pose some additional challenges. The use of the *ad hoc* approach could reduce the likelihood that a consistent process would be maintained for evaluating and recommending new Tier 3 toxicity values. One workgroup may apply evaluation criteria differently than another group. Thus, additional guidance and direction on the use of criteria may be needed to improve consistency. Furthermore, an *ad hoc* workgroup needs a mandate or direction that will not ultimately put it at odds with another Tier 3 workgroup (clarity of relationship between *ad hoc* workgroups and the RSL Workgroup).

5.4.4 Joint Headquarters/Regional Workgroup

Risk assessors and toxicologists in the regions and headquarters (OSWER and ORD) have had a long history in working together in developing and implementing risk assessment guidance and toxicological assessments pertaining to Superfund and RCRA. In recent years, additional efforts (such as OH2R2AF) have been undertaken to enhance communication between headquarters and regional Superfund and RCRA risk assessors. A joint workgroup consisting of regional and headquarters risk assessors and toxicologists could be established to provide consults on Tier 3 toxicity values because many of these efforts involve workgroups consisting of a mixture of regional headquarters representatives. This option is nearly identical to the regional workgroup option discussed in Section 5.4.3, except that this workgroup could be led by either a headquarters or a regional risk assessor and would have to include members from both regions and headquarters. Note that the regional workgroups do not necessarily have to include headquarters representatives. Based on headquarters' greater role in such a workgroup, it is likely that this workgroup could work on medium- to high-priority chemicals.

The joint regional and headquarters workgroup also shares many of the same strengths and limitations that the regional workgroup option may offer. In addition, this option allows for more coordination between headquarters and the regions, which could provide greater transparency and credibility to Tier 3 toxicity value consultations over a regional workgroup. A greater role for headquarters may also increase the likelihood that subject matter experts from headquarters will be involved in providing the consult. However, the share of power between the regional risk assessors and headquarters could limit the joint workgroup's effectiveness. Competing interests (completing a site risk assessment versus setting policy) could slow the workgroup activity.

5.4.5 Individual Regions

Under this approach, individual regions would continue to use their current methods for identifying and selecting Tier 3 toxicity values. With the exception of the RSL table (and its predecessors), which have provided recommendations on Tier 3 values, regions have already been largely responsible for identifying Tier 3 toxicity values and providing guidance to responsible parties, states, and other entities.

However, regions have consulted headquarters and other regions for high-priority chemicals (such as chromium VI) or chemicals commonly found at sites. Therefore, this approach is anticipated for use with low- to medium-priority chemicals. The development of Tier 3 toxicity values for high-priority chemicals will likely need the input from a regional workgroup or headquarters, especially risk-driving chemicals. As stated in the 2003 OSWER toxicity value hierarchy, "Consultation with the STSC or headquarters program office is recommended regarding the use of the Tier 3 values for Superfund response decisions when the contaminant appears to be a risk driver for the site" (EPA 2003).

There are several strengths with the individual regions approach. To begin with, a relatively quick turnaround time is associated with the approach. Rather than waiting for a response from headquarters or a workgroup, decisions can be made within the region which assists in a quick turnaround time. Following the individual regions approach allows regions to retain control of the selection of Tier 3 values. Furthermore, it allows for development of a more complete and thorough risk assessment, which limits the possibility of underestimating risks.

As with the previous approaches, there are several limitations to individual regions evaluating and selecting Tier 3 values. For instance, there is potential for lack of transparency and consistency with regard to decision making. At times, information is not shared outside of the region, or even within the region (between the programs). The lack of transparency (or information sharing) creates a problem when different Tier 3 values are recommended by different regions. Because the criteria for selecting a Tier 3 value do not specify the level of peer review, it is possible that several values could be chosen for a chemical by different regions. The credibility of such a toxicity value is more likely to be questioned by a responsible party (RP), resulting in a greater chance of challenge, especially for risk-driving chemicals, which draw an additional level of scrutiny. Since the credibility of regionally selected Tier 3 values may vary greatly, it is important to consult experts who can identify limitations of published values. However, by definition, the regional approach discourages seeking expert advice across regions in decision making. This lack of a cross-regional approach contributes to the limitations since the toxicological expertise of the decision-maker within each region may vary extensively. Finally, this approach does not address high-priority chemicals, which may need to be sent to headquarters for a decision. It should also be noted that although it is possible for individual regions to identify available Tier 3 toxicity values for certain chemicals of use and interest, regions often lack appropriate resources and expertise to adequately evaluate and select a Tier 3 value. In such instances, assistance from headquarters and other groups are often necessary.

5.5 Documentation

As noted in previous sections, transparency is a necessary component of a Tier 3 toxicity value. Therefore, identification and selection of a Tier 3 toxicity value by EPA risk assessors must continue to be transparent. Transparency includes documenting the decisions and recommendations regarding the selection of a Tier 3 toxicity value and its supporting toxicological assessments and making these documents available to the public. The following sections discuss potential methods for documenting

and distributing decision documents and alternatives (repositories) for warehousing decision documents and supporting documentation (for example, toxicological assessments).

5.5.1 Decision Documents and Distribution

As shown in Section 4.2 and Appendix B, consults and recommendations on Tier 3 toxicity values have taken the form of e-mails, formal memoranda, or listings in a table (the RSL table) and the level of detail regarding the support of these values has differed. Although future consults and recommendations may take several forms, development of a process for selecting a Tier 3 toxicity value may need to consider the level of formality needed in consults and recommendations and the type of information to be included in the consult or recommendation. For example, formal signed memoranda may offer more of an authoritative voice than informal e-mails. With regards to the types of information to be provided in consults or recommendations, it may include, but may not be limited to, the following:

- Transparency, peer-review, and availability criteria met,
- Summary of the underlying studies,
- Methods for toxicity value derivation,
- Uncertainty Factors (RfCs and RfDs),
- Carcinogenic mechanism of action (MOA) (if available),
- Target organ and critical effect, and
- Confidence in toxicity value.

Also, before decision documents and toxicological assessments are warehoused (see Section 5.5.2), timely notification of such decisions may be of interest to regional risk assessors. Regional risk assessors have expressed interest in what other regions are doing to avoid re-inventing the wheel or being inconsistent. However, notification does not necessarily mean that all regional decisions have to be distributed outside of the region. There is the potential for inconsistency or that the value is not used in a risk assessment because it may take some time between a decision on a Tier 3 toxicity value and its use, for example, upload into a database. Thus, a process for selecting a Tier 3 toxicity value, should consider a method for notifying regional risk assessors of any decisions regarding a Tier 3 toxicity value. Typically, e-mails have been an effective tool for distributing this type of information and have been the case with most headquarters consults. However, these e-mails have often been distributed from the requesting region and may not have been distributed to all regional risk assessors. In addition, e-mails may not always be read by all recipients. Other potential methods that could expand the risk assessor audience may include broadcasts in the OH2R2AF newsletter or during the OH2R2AF calls. Finally, some consideration should be given to how this information will be shared with other audiences (such as state risk assessors before they are sent to a repository.

5.5.2 Repositories

Decision documents and supporting documentation (in this case, toxicological assessments) behind a Tier 3 toxicity value must be stored and available for retrieval by risk assessors, risk managers, and the public. The following sections discuss potential alternatives for warehousing this information. In addition, the potential repositories discussed below may not apply to all situations because individual regions may continue to develop their own Tier 3 values internally. However, it is expected that the regions that develop their own values would be responsible for storing their decision documents and supporting documentation, unless they plan to distribute the values beyond their region.

In addition, on-line repositories will require storage space, routine maintenance, and a point of contact (for adding or revising a Tier 3 toxicity value). Although it is not the intent of this document to discuss these issues in depth, costs and resources associated with storage and maintenance of decision documents and supporting documentation will have to be considered and evaluated. Given these potential constraints and other considerations (duplication of effort), links to non-EPA websites that contain the toxicological assessments may be a viable alternative to storing the toxicological assessments on EPA's website.

5.5.2.1 PPRTV Assessments Electronic Library

The PPRTV Assessments Electronic Library is a potential repository for Tier 3 toxicity values. The PPRTV electronic library, which has recently become publically available, is administered by OSRTI and maintained by Oak Ridge National Laboratory under an interagency agreement. Notwithstanding contractual arrangements, an additional menu could be added to the PPRTV electronic library to house Tier 3 toxicity values. Similar to the PPRTVs, the menu could contain a list of all chemicals with Tier 3 toxicity values. When a given chemical is selected, the user would be sent to a page that contains the Tier 3 toxicity values, decision documents, and the toxicological assessments.

5.5.2.2 Superfund Health Risk Technical Support Center (STSC)

The STSC provides technical support to EPA program and regional offices in the area of human health risk assessment, such as the development of PPRTV assessments and scientific consultations. In years past, the STSC has served as a repository for health risk assessment documents, such as hard copies of HEAST derivation support documents. For these reasons, the STSC could serve as a repository for Tier 3 consults, recommendations, and supporting documentation. However, , STSC may not be a viable alternative for storing recommendations on non-EPA toxicity values and their technical support documents because the STSC develops PPRTVs and provides support for interpreting EPA publications and guidance.

5.5.2.3 RSL Table Website

The RSL table website, which is posted by Regions 3, 6, and 9, is another potential repository for Tier 3 toxicity values. The RSL table website appears to be a logical choice as a potential repository for supporting documentation because the RSL table is typically the first EPA document to post Tier 3 toxicity values. The user's guide and supporting tables could be expanded to include a page that contains the decision documents. This page could also provide the toxicological assessments or links to the toxicological assessment on non-EPA websites. Because the RSL summary table already contains fields for toxicity values, a separate location listing Tier 3 toxicity values would not be necessary. Furthermore, although the RSL table is not an original source of toxicity values, it often serves as the initial destination for Superfund and RCRA risk assessors seeking the most current toxicity values used by EPA. Thus, use of the RSL table as a repository location for Tier 3 toxicity values could decrease the number of locations risk assessors would have to search for toxicity values. However, as noted above, the RSL table and its supporting documentation (such as the User's Guide) are posted on the Region 3, 6, and 9 websites. While only one Region (Region 3) stores the files (the other two provide links only), this option would require approval and coordination with the Regions' IT and risk assessment staff and management. Note that it is unknown whether the regions currently storing the RSL tables are capable of and willing to take on this additional duty as doing so requires additional storage and resources. Furthermore, the layout of a Tier 3 toxicity value repository would be subject to the individual region's formatting preferences.

5.5.2.4 Tier 3 Toxicity Value Database

Although no such database exists at present, an on-line database strictly for Tier 3 values could be developed. This database would be strictly for Tier 3 toxicity values and, like the IRIS and PPRTV databases, its location will be readily identifiable as a source for recommended Tier 3 toxicity values. It is envisioned that it would be formatted similar to the PPRTV library with drop-down menus. Although such a site would provide a centralized and distinct location for Tier 3 toxicity values, it may require a significant amount of additional money and resources to design and maintain compared with the use of an existing on-line repository.

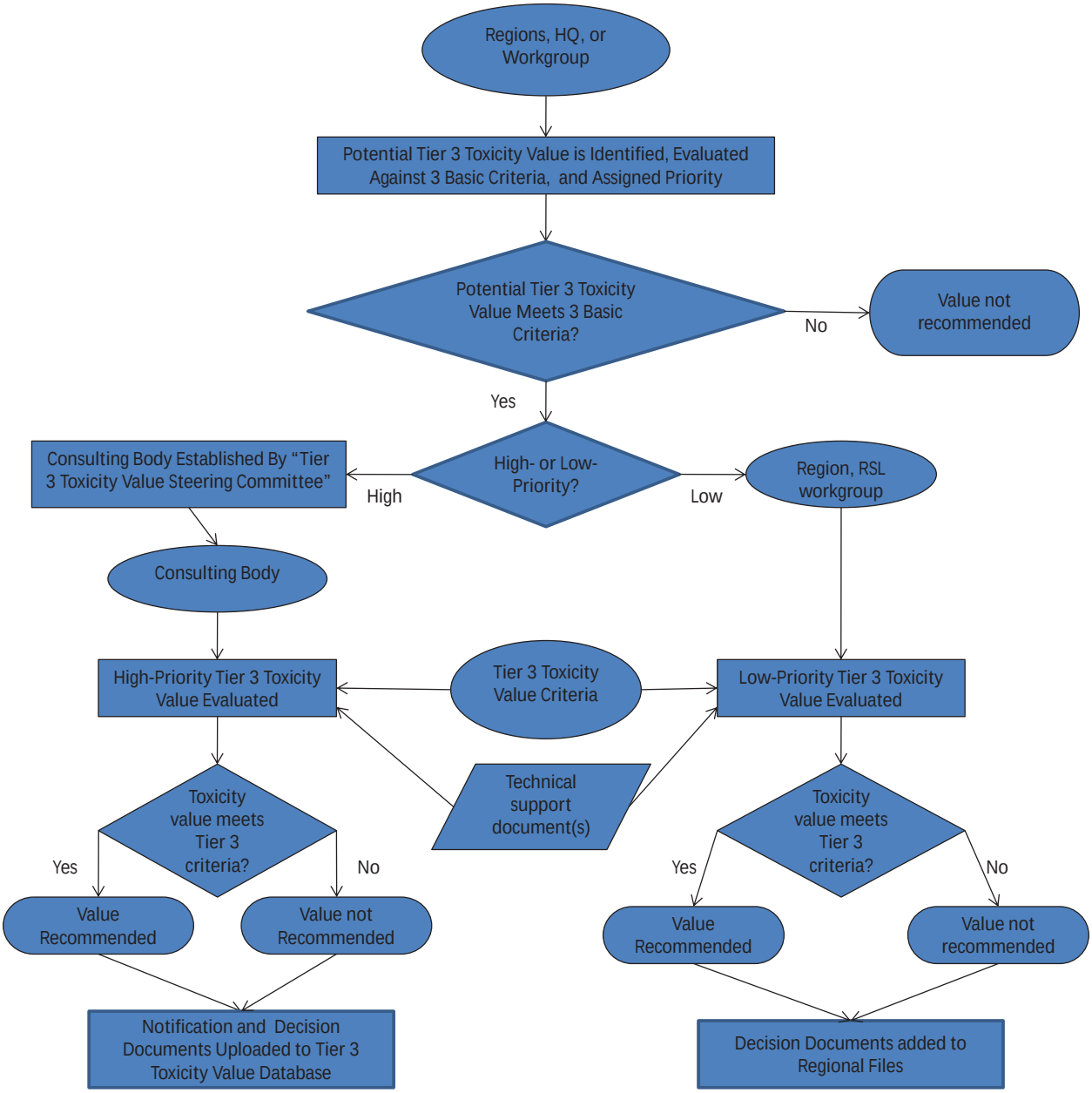
6 Recommended Option/Process and Path Forward

Overall, the Regional Tier 3 Toxicity Value Workgroup recommends a process that is flexible, consistent, efficient, and results in the evaluation and selection of Tier 3 toxicity values that are scientifically defensible. As discussed above, there is no "one size fits all," especially with respect to the decision-making body, for the evaluation and selection of Tier 3 toxicity values, and there are numerous combinations of potential processes for identifying, evaluating, selecting, and documenting Tier 3 toxicity values. Therefore, the following recommendations are provided as a path a candidate Tier 3 toxicity value may take from its initial identification to final selection and documentation. Figure 2 below illustrates this proposed path. Note that the recommendations apply to future Tier 3 toxicity values not already recommended by regional and headquarters risk assessors and the RSL table. However, those involved in the implementation of all or certain aspects of this white paper should consider existing Tier 3 toxicity values.

6.1 Toxicity Value Identification

The Tier 3 toxicity value workgroup recommends that the responsibility of identifying Tier 3 toxicity values remains with the regional and headquarters risk assessors and existing regional risk assessor workgroups (such as the RSL table team) to maintain flexibility and conserve time and resources. As discussed previously, these groups are most likely to encounter a potential Tier 3 toxicity value during development of a human health risk assessment and or a revision to the RSL table. Development of a formal workgroup, as discussed in Section 5.4.3.1, will require time and resources. Furthermore, as indicated in previous sections, the identification of potential Tier 3 values is not a frequent occurrence. Thus, the value of a formal workgroup is unclear, especially when regional risk assessors and others will likely continue to search for Tier 3 toxicity values in their routine work (risk assessments).

Figure 2. Proposed Tier 3 Toxicity Value Selection Process



6.2 Initial Evaluation and Chemical Prioritization

Beyond a more thorough and complete evaluation of a potential Tier 3 toxicity value, some steps must be taken to maintain a flexible and efficient process. We recommend that those who identify a potential Tier 3 toxicity value ensure that the toxicity value meets the three basic criteria outlined in Section 5.3.1, which include transparency, peer-reviewed, and public availability. Of course, these criteria are general in scope and a potential Tier 3 value meeting all three criteria at some level does not guarantee that it is scientifically defensible for use in human health risk assessments. At this time, other factors may also be considered and used to eliminate a potential Tier 3 value (for example, extrapolation of a toxicity value from an occupational standard, such as an Occupational Safety and Health Administration permissible exposure limit).

During the initial evaluation, this white paper recommends that the chemical be designated a low or high priority according to the prioritization criteria in Section 5.2. This designation is essential because it provides the basis for the recommendations in Section 6.3 on the type of consulting body to become involved. Note that additional prioritization of "high" priority chemicals will occur by the "Tier 3 Toxicity Value Steering Committee" (see Section 6.3.2.1). Because two of the prioritization criteria include the chemical's prevalence across the regions and level of interest at the national level (whether it would become the subject of a rule-making, for example), not to mention the potential subjective nature of those determinations, this white paper recommends that these efforts be coordinated with risk assessors and program representatives from other regions and headquarters via the "Tier 3 Toxicity Value Steering Committee."

6.3 Consulting Body

It is of the opinion of the Tier 3 toxicity value workgroup that no single process for evaluating and selecting a Tier 3 toxicity value will be the most efficient and timely for all potential scenarios where a potential Tier 3 toxicity value becomes available. Yet, the Tier 3 toxicity value workgroup also recognizes that a more formal process needs to be established to promote greater consistency and transparency among the regions. To meet these needs, this white paper recommends two separate approaches for evaluating and selecting a Tier 3 toxicity value. Because a chemical's significance and priority have previously defined the level of involvement by regional and headquarters risk assessors and toxicologists, it also serves as the critical determinant in selecting the appropriate approach. Specific details on the two approaches are provided in the following sections.

6.3.1 Low-Priority Chemicals

This white paper recommends that the Tier 3 toxicity values be evaluated and selected by the individual regions for chemicals that are designated as "low priority." However, this alternative does not necessarily preclude a region from consulting with others outside the region (such as STSC) regarding the use of a particular Tier 3 toxicity value. The "individual region" option appears to be the most practical for the "low-priority" chemicals, especially because it may allow for quicker decision making.

Chemicals with regional significance only, for example, may not draw enough interest from risk assessors from other regions or headquarters to staff workgroups, which could stall efforts to evaluate and select a value. A quick turnaround time is beneficial for non-risk driving chemicals so that it does not hold up decisions on risk-driving chemicals. Concerns with transparency and credibility are likely minimal for "low-priority" chemicals, especially non-risk driving chemicals. In addition, the RSL workgroup (under this approach) would continue to be responsible for evaluating and selecting Tier 3 toxicity values for "low-priority" chemicals because the RSL workgroup handles a wide array of chemicals ranging from "low priority" to "high priority."

6.3.2 High-Priority Chemicals

Even among high-priority chemicals, there may be varying expectations on the type of consult to be performed. Thus, it does not appear practical to recommend a specific consulting body. Instead, this white paper recommends a flexible and adaptive approach whereby potential Tier 3 toxicity value consultations be elevated to a "Tier 3 Toxicity Value Steering Committee." This committee (see Section 6.3.2.1) will be responsible for establishing the consulting body (such as an *ad hoc* workgroup, headquarters, or ADP) that best fits the situation and expectations of the risk assessors.

6.3.2.1 Tier 3 Toxicity Value Steering Committee

Although this white paper has not presented or evaluated potential workgroups that could fulfill the role as the "Tier 3 Toxicity Value Steering Committee," this white paper recommends that this role be subsumed by the OH2R2AF toxicity workgroup. This role falls within the scope of the OH2R2AF toxicity workgroup, which is to provide a forum to discuss and provide direction for OSWER human health risk assessors with regard to the use of toxicity values in removal and remedial actions. Furthermore, the OH2R2AF toxicity workgroup consists of members representing several regions and offices within headquarters. This broad range of representation enables the workgroup to more easily reach out to subject matter experts among the regions and headquarters, as well as to stay abreast of regional and national risk assessment issues that may affect the level of review that a potential Tier 3 toxicity value may receive.

Assuming the OH2R2AF toxicity workgroup takes on this responsibility, it may need to establish some guidelines or processes for elevating these chemicals and selecting the appropriate decision-making body. These guidelines and processes may include some of the following elements.

- Points of contact for elevating the chemical to the OH2R2AF toxicity workgroup.
- Criteria for determining which consulting entity will be used.
- Listing of subject matter experts (including regional and headquarters scientists and program representative) interested in participating in consultation workgroups.
- Who will be responsible for performing the review and evaluating the potential Tier 3 toxicity value's health risk assessment (will it be performed by the requestor, consultant, *ad hoc* workgroup members, or headquarters).

- Information requirements (health risk assessments and other documents pertaining to the derivation of a potential Tier 3 toxicity value).
- Who will be responsible for submitting consultation requests (for example, regional risk assessors, RTICs, managers, or division directors).

6.3.2.2 Other Considerations

This white paper generally recommends that the complete evaluation of potential high-priority Tier 3 toxicity values be the responsibility of the consulting body. This responsibility will ensure that subject matter experts are critically reviewing the underlying data behind a toxicity value. However, there is the potential that the consulting body may not perform the full review and evaluation. Previous examples include consultations on chromium VI and PCE. Consulting bodies may have time and resource constraints that prevent them (and individual members) from completing the full review and evaluation. In addition, duplication of effort may be of concern if the requestors perform this activity after a potential Tier 3 toxicity value has been initially identified as a matter of interest or routine. In these instances, consulting bodies may require that others (the requestor) perform the full review and evaluation of the toxicological support documentation and provide a summary of relevant information to the consulting body for additional evaluation and decision-making. The scope of the consulting body's review and evaluation of the underlying toxicological information may vary. As a result, decisions regarding the responsibility and extent of the review will likely require some degree of coordination with the original consultation requestor. These activities could be facilitated by a "Tier 3 Toxicity Value Steering Committee."

6.4 Toxicity Value Evaluation

Regardless of who is responsible for evaluating a potential Tier 3 toxicity value, the same set of criteria should be applied to all Tier 3 toxicity value evaluations. This white paper recommends that the ECOS criteria, guiding principles, and other relevant criteria and guidance outlined in the white paper be adopted as criteria for evaluating potential Tier 3 toxicity values. In addition to adopting the aforementioned criteria, this white paper also recommends that the confidence in the toxicity value be described in the evaluation. Evaluating and assigning confidence to toxicity values including the underlying study and overall database are standard practice and potentially critical elements in risk management decision-making. Confidence in a Tier 3 toxicity value would also be significant (a deciding factor) in instances where there are competing Tier 3 values.

Also, per Section 5.3.2, it is critical that those involved in the evaluation and selection process have, at a minimum, a basic understanding of how to evaluate and assess the data usability of toxicity studies, the adverse and critical effect levels in a study, and the methodologies used to derive toxicity values. Although these skills are likely to be present among the members of regional and headquarters workgroups, it is less certain at the "individual region" level. Thus, training and educational opportunities pertaining to the aforementioned skills should continue to be a priority among the risk assessors.

6.5 Documentation

The following recommendations on documentation are generally intended to address high-priority chemicals. In keeping with the theme of "low-priority" chemicals, decisions on how regions document and store "low-priority" Tier 3 toxicity values will be left to the regions and RSL workgroup. However, the Tier 3 toxicity value workgroup recommends that the regional risk assessors are notified of the selection of Tier 3 toxicity values in case these chemicals ever come up in other regions. To make this process efficient and less of a burden on the risk assessors who select a value, it is recommended that notification and storage of decision documents be coordinated through the "Tier 3 toxicity value steering committee."

6.5.1 Decision Documents

This white paper recommends that a formal system be put into place that documents selection of a Tier 3 toxicity value. This white paper further recommends that all decision documents for high-priority chemicals be provided in a formal memorandum from the selecting entity to the original requestor(s), "Tier 3 toxicity value steering committee" and other relevant workgroups, such as the RSL workgroup and the OH2R2AF toxicity workgroup (if different from the steering committee). The memorandum should provide the rationale for selecting a value (how it meets the evaluation criteria) and contain the following information (where applicable):

- Summary of underlying studies,
- Methods for toxicity value derivation,
- Uncertainty factors (RfDs and RfCs),
- Carcinogenic MOA and cancer classification (if available),
- Target organ/critical effect, and
- Confidence in toxicity value (critical for competing values).

The recommendation above also applies to situations where the consulting body does not recommend the use of a value or selects one value over another in the case of competing values. When a value is not selected, the response will focus on the particular criteria that are not met or other technical reasons for not recommending a value. If the rationale for rejecting a value is not documented, there is the potential that the same requests could be made in the future.

6.5.2 Repository

This white paper recommends that Tier 3 toxicity value decision documents and related documents (such as health risk assessments) be housed electronically at one of the existing EPA toxicity value websites or electronic libraries. To avoid duplication of effort, this white paper also recommends that

decision documents for toxicity values not selected by the consulting body be housed in the repository. Notwithstanding contractual and resource arrangements with EPA websites that contain toxicity value information, use of an existing EPA on-line location would not add to the number of EPA websites to search for a toxicity value and would make use of existing infrastructure and resources. In addition, it is recommended that the electronic library be publicly available and follow a format similar to the PPRTV electronic library (with drop-down menus)

Those involved in posting Tier 3 toxicity value consults, such as the "Tier 3 toxicity value steering committee," will have to consider whether the health risk assessment in support of a particular toxicity value needs to be posted on the website and if so, how this information will be housed. Health risk assessments can be lengthy documents, and posting them on EPA websites may not be feasible. However, health risk assessments in support of toxicity values are often provided electronically by the authors, which are typically federal and state health agencies (as is the case with ATSDR toxicological profiles). Therefore, links to websites containing those assessments may suffice.

7 Summary

While EPA has multiple policies, guidance, and guidelines to assist and/or direct risk assessors in the development and selection of toxicity values, specific guidance on selecting tier 3 toxicity values for use in Superfund and RCRA cleanup programs is limited. As a result, regional risk assessors have shared concerns over transparency and consistency of selecting Tier 3 toxicity values. In response, the Tier 3

Toxicity Workgroup developed this white paper to explore and recommend processes for enhancing the selection of Tier 3 toxicity values.

The process of selecting Tier 3 toxicity values consists of several steps including the identification, prioritization, evaluation, selection, documentation, and communication of Tier 3 toxicity values. Chapters 1 and 2 provide background on guidance and policies regional risk assessors follow to identify toxicity values and examples of some of the most commonly used federal, state and international sources of Tier 3 toxicity values and toxicity data. Chapter 2 also introduced the similarities and differences in how toxicity values are developed within each of those sources and recommended that a basic understanding on how to evaluate and assess the data usability of toxicity studies, identify the adverse and critical effect levels in a study and evaluate the regulatory-specific methodologies used to derive toxicity values is useful for comparing, selecting, and developing chemical-specific toxicity values. A number of publications, both internal and external to EPA, are summarized in Chapter 3, which provide guidance on how to evaluate the underlying basis of a toxicity value and provide a suggested framework for identifying and selecting toxicity values. Chapter 4 summarizes current and past practices of how regional risk assessors have identified, evaluated, and selected Tier 3 toxicity values.

Chapter 5 explores various options for identifying, evaluating, selecting, and documenting Tier 3 toxicity values. The chapter discusses alternatives for who would be responsible for identifying potential Tier 3 toxicity values and proposes a set of criteria for assigning priority to a chemical because a chemical's priority will likely dictate the entity that will provide a Tier 3 consultation. Chapter 5 also proposes a process for evaluating and selecting Tier 3 toxicity values, which includes two steps consisting of a basic evaluation and a critical review. The remainder and bulk of the chapter explores the options for Tier 3 toxicity value consultations and options for documenting and communicating the evaluation and selection of Tier 3 toxicity values. The options for documenting and communicating the selection of Tier 3 toxicity values include methods on how to document and distribute decision documents to regional risk assessors and alternatives for warehousing decision documents. The options for the Tier 3 toxicity value consultation process are summarized in the table below.

After consideration of the strengths and limitations of each of the alternatives and previous and current methods of selecting Tier 3 toxicity values, this white paper recommends a general process that retains flexibility, but also enhances consistency and transparency. Rather than recommend a “one size fits all” approach that could hinder efficiency and lengthen decision-making, this white paper recommends two approaches, one addressing low priority chemicals and the other addressing high priority chemicals. Proposed criteria for assigning priority are presented in Section 5.2.

For low priority chemicals, this white paper recommends that Tier 3 toxicity value decision-making be retained within the regions. While responsibility for selecting Tier 3 toxicity values remains within the regions, this white paper encourages regions to consult others outside of their own region, such as the OH2R2AF, RSL workgroup, and STSC. Regions may lack information, resources, and technical expertise to conduct chemical prioritizations and to evaluate and select Tier 3 toxicity values.

In regard to high priority chemicals, this white paper recommends the establishment of a "Tier 3 Toxicity Value Committee" that will be responsible for the overall coordination of the Tier 3 toxicity value selection process. The "Tier 3 Toxicity Value Committee," a role that can be subsumed by the OH2R2AF toxicity workgroup, would be mainly responsible for establishing the consulting body, i.e., the group responsible for evaluating and selecting a Tier 3 toxicity value, that best fits the needs and expectations of the risk assessors for the specific chemical. In addition, while decisions on how regions document and store "low priority" Tier 3 toxicity values will be left to the regions and RSL workgroup, a more formal and structured process for documenting, storing, and communicating "high priority" Tier 3 toxicity value selections is recommended. Specifically, this white paper recommends that all decision documents be provided in a formal memo from the reviewers to the requestor and would apply to situations where a toxicity value is recommended, not recommended, or one value is recommended over another, i.e., competing toxicity values. Furthermore, this whitepaper recommends that decisions be communicated to the regional risk assessors, via the "Tier 3 Toxicity Value Committee," and that the decision documents and other relevant information (e.g., health risk assessments) be stored within existing EPA toxicity value websites or electronic libraries.

Although this white paper recommends two approaches, it is important to point out that they both share some common recommendations including elements of the identification, prioritization, and evaluation steps. These common recommendations include, but are not limited to, prioritization criteria (discussed above) and the criteria and guiding principles used to evaluate candidate Tier 3 toxicity values. Regardless of the vehicle used to perform the evaluations, the same set of criteria and principles should be used to evaluate all potential Tier 3 toxicity values. Furthermore, to ensure consistent and proper application of review criteria, training will continue to be a critical for those individuals that may be involved in the evaluation and selection process.

Table 1. Options for Tier 3 Consultations

Option	Factors for Consultation	Strengths	Limitations
Action Development Process (ADP) Workgroup	ADP is the Agency's method for producing high quality actions such as regulations, policies, and risk assessments. Tier 3 toxicity values considered influential scientific information should be developed through ADP. Process typically initiated by Office of Solid Waste and Emergency Response (OSWER). Tiering process begun by Regulatory Steering Committee based on level of senior management involvement and extent of cross-agency influence: • Tier 1—actions signed by Administrator and have broad cross-agency influence • Tier 2 – actions signed by assistant Administrator and have some cross-agency influence • Tier 3 – actions signed by Office directors and have limited cross-agency influence	**	**
Headquarters Consultation	Regions submit requests to Headquarters. Typically, consultations are led by OSWER with input from the Office of Research and Development (ORD). Consultations are often coordinated among offices of OSWER including the Science Advisor, OSTRI, and OEM. Currently primarily performed on an "informal" basis. Could be formalized in future with consistent designated lead office within OSWER. Key factors include: • Headquarters establish contact to receive Tier 3 requests • Regional risk assessors establish a consistent process for submitting requests • Contract support may be necessary to assist within consultation	• Promote consistency among regions • More likely to maintain consistent process for providing consultations • Add "greater weight and credibility to Tier 3 values	• Perception that Headquarters is setting policy • Not well-suited to handle low-priority chemicals
Regional Workgroup	Formal or ad hoc group of subject matter experts with expertise relevant to chemicals being evaluated. Led and generally consisting of regional risk assessors and toxicologists. Primary focus would be on low- to medium-priority chemicals. Two existing regional workgroups: • Regional screening level (RSL) workgroup • Regional human health risk assessment forum (OH2R2AF) toxicity workgroup	Formal workgroup • Easier establishment and tracking of expectations and results • Visible to headquarters and regions resulting in greater credibility of Tier 3 values • Maintenance of consistent process Ad hoc workgroup • Formed with selected experts as necessary • May better champion needs and priority for regional-specific Tier 3 values • If well coordinated, will maximize results by spreading duties to many, rather than few	Formal workgroup • Fixed membership may fail to reach out to individuals/groups with particular expertise • Workload may not require regular meetings, resulting in loss of focus and interests among members Ad hoc workgroup • May lack effectiveness if not well-coordinated • Formation and staffing of multiple ad hoc workgroups may be cumbersome • May suffer from lack of headquarters input • Reduced likelihood of consistent process
Joint Headquarters/Regional Workgroup	Joint workgroup consisting of regional and headquarters risk assessors and toxicologists Similar to regional workgroup except the group could be led by either headquarters or regional individual and have members from both groups.	Similar to regional workgroup, as well as • Allows more coordination between headquarters and regions resulting in greater transparency and credibility of Tier 3 values • More likely to include subject matter experts from headquarters (as compared to regional workgroup)	Similar to regional workgroup, as well as • Sharing of power between headquarters and regions could limit effectiveness • Competing interests could slow workgroup activity
Individual regions	Individual regions would continue to use current methods for identifying and selecting Tier 3 values. Anticipated for use primarily with low- to medium-priority chemicals; high priority chemicals expected to include headquarters input.	• Relatively quick turn-around • Allows regions to maintain control of Tier 3 values • Allows development of more complete and thorough risk assessment	• Potential lack of transparency and reduced credibility of Tier 3 values • Lack of cross-regional approach limits access to and use of varied regional expertise • Approach does not address high priority chemicals which require headquarters input • Potential lack of regional resources and expertise in evaluating and selecting a Tier 3 value

**Unlike the other options, the ADP generally applies to specific circumstance as indicated in Section 5.4.1, i.e., Tier 3 toxicity values that are considered highly influential scientific information. Thus, the strengths and limitations of the ADP were not evaluated in this white paper.

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Appendix A – OSWER and ORD Organizational Charts

Figure A-1. Office of Solid Waste and Emergency Response

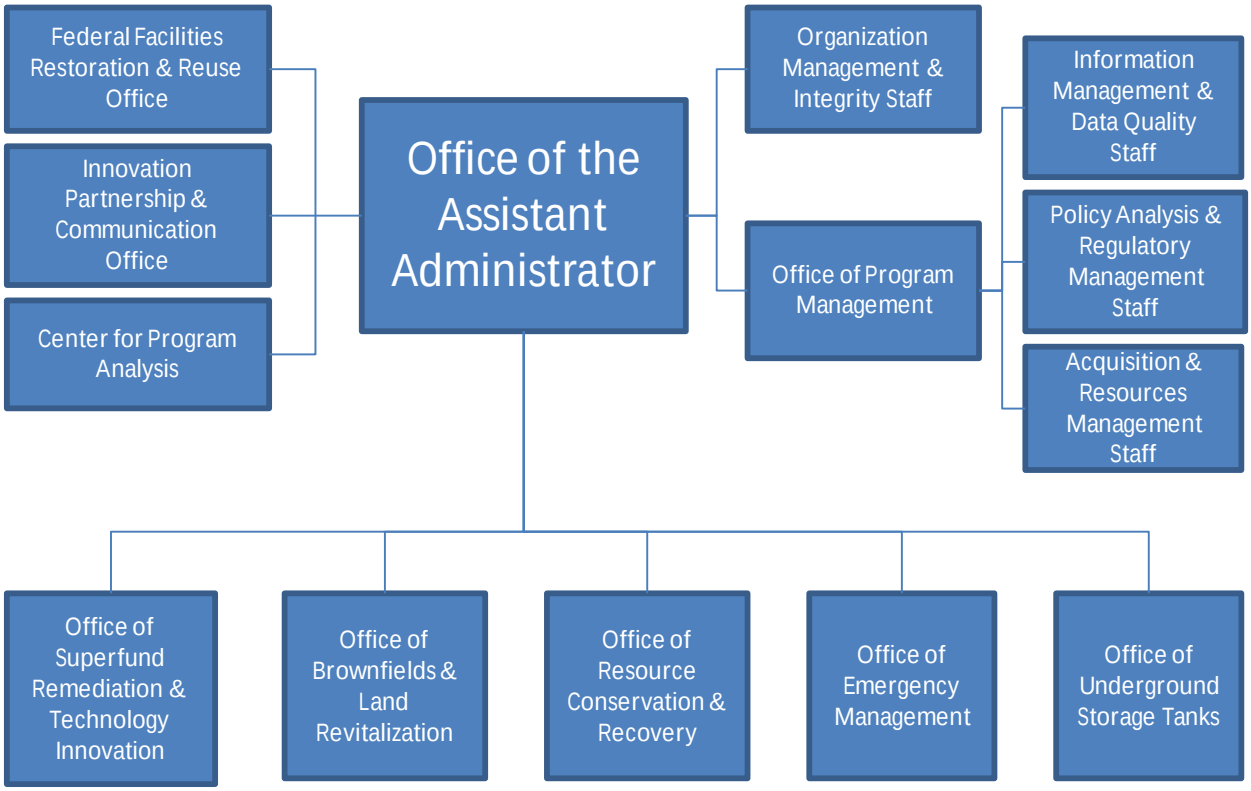
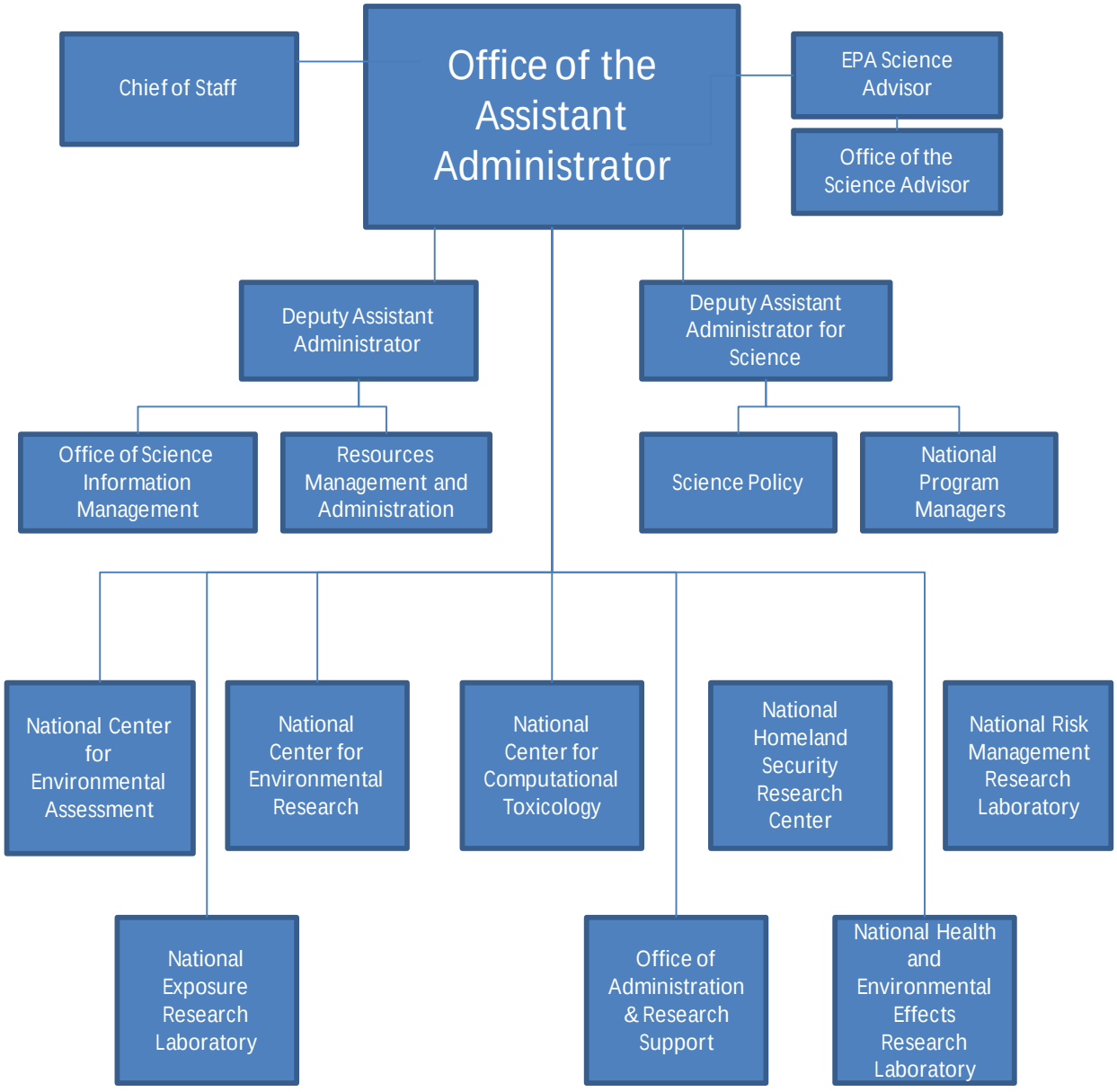


Figure A-2. Office of Research and Development



Appendix B – Consultations

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR – 9 2009

MEMORANDUM

SUBJECT: Withdrawal of the January 15, 2009, OSWER Guidance Entitled "Interim Recommended Trichloroethylene (TCE) Toxicity Values to Assess Human Health Risk and Recommendations for the Vapor Intrusion Pathway Analysis"

FROM: Barry N. Breen, Acting Assistant Administrator
Office of Solid Waste and Emergency Response

TO: Acting Regional Administrators, Regions 1 - 10

On January 15, 2009, the Office of Solid Waste and Emergency Response (OSWER) issued a guidance memorandum entitled "Interim Recommended Trichloroethylene (TCE) Toxicity Values to Assess Human Health Risk and Recommendations for the Vapor Intrusion Pathway Analysis." That guidance was subsequently distributed to EPA regional staff and managers. The Agency is withdrawing this guidance to further evaluate the recommendations regarding the non-cancer TCE toxicity value for use in risk assessments of inhalation exposures. Once this re-evaluation is complete, we will update you.

In the interim, toxicity values for TCE should be determined consistent with the National Contingency Plan (e.g., 40 CFR 300.430(e)) and the 2003 Toxicity Hierarchy (OSWER Directive 9285.7-53, December 5, 2003). The Directive provides guidance on a hierarchy of approaches regarding human health toxicity values in risk assessments, and provides guidance for regional risk assessors to help them identify appropriate sources of toxicological information that should generally be used in performing human health risk assessments at Comprehensive Environmental Response, Compensation and Liability Act (CERCLA or "Superfund") sites. This hierarchy of approaches is also appropriate for human health risk assessments at Resource Conservation and Recovery Act (RCRA) corrective action sites.

The guidance memorandum also addressed the vapor intrusion pathway and recommended a multiple lines of evidence approach in assessing sites for vapor intrusion. EPA expects to issue a separate document that will address the multiple lines of evidence approach as it relates to the vapor intrusion pathway.

If you have any questions, please contact Jayne Michaud in the Office of Superfund Remediation and Technology Innovation at 703-603-8847 or Mary Cooke in the Federal Facilities Restoration and Reuse Office at 703-603-8712.

cc: Regional Superfund Division Directors
Regional RCRA Division Directors
Mary Cooke
Gail A. Cooper
Deborah Dietrich
Matt Hale
Carolyn Hoskinson
Barbara Hostage
Ann Johnson
David Lloyd
Peter Ludzia
Mary Kay Lynch
Ellen Manges
Jayne Michaud
John Michaud
John Reeder
William Sette
Elizabeth Southerland
James Woolford
Renee Wynn

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REGION 7
901 NORTH 5TH STREET
KANSAS CITY, KANSAS 66101

APR 2 9 2009

MEMORANDUM

SUBJECT: Recommended Risk-Based Human Health Screening Levels and Interim Trichloroethylene Toxicity Values Update

FROM: Jeffery Robichaud
Chief
ENSV/EAMB

TO: AWMD – RCRA Branch Chiefs
SUPR – All Division Branch Chiefs

The purpose of this memo is to update the Region 7 RCRA and Superfund programs on the recommended risk-based human health screening levels and to provide recommendations on trichloroethylene (TCE) chronic toxicity values. As a reminder, the recommendations provided in this memo apply to risk assessment-related documents developed by or on behalf of EPA Region 7, as well as any relevant documents submitted to the Region for review and approval.

In a memo, dated December 14, 2007, the Region 7 risk assessors recommended the use of the Region 6 Human Health Medium-Specific Screening Levels (MSSLs) as the primary source of screening levels. At that time, the Region 6 MSSLs were recommended because they were regularly updated and were consistent with current toxicity values and EPA risk assessment guidance and policy. Also, as indicated in that memo, a regional effort was underway to consolidate the existing regional screening tables into a single set of screening values in order to improve consistency and incorporate updated guidance. In the fall of 2008, that effort was completed and the Regional Screening Table was posted on the Region 3 website, followed later by Regions 9 and 6.

Given that the Regional Screening Table is now available on the internet, the Regional risk assessors recommend the use of the Regional Screening Table and its supporting documents (e.g., User's Guide). The links to the table and supporting documentation are provided below.

- Region 3, http://www.epa.gov/reg3hwmd/risk/human/index.htm.
- Region 6, http://www.epa.gov/Region6/6pd/rcra_c/pd-n/screen.htm.
- Region 9, http://www.epa.gov/region09/superfund/prg/index.html.

Also, we have provided additional information to consider when using the tables. That information is provided below.

- Although Regions 3, 6, and 9 continue to use the Risk-Based Concentration (RBC), Preliminary Remediation Goal (PRG), and Medium-Specific Screening Level (MSSL) terminology on their respective websites, they all provide the same Regional Screening Table and supporting documents. Risk assessments and related documents should cite the Regional Screening Table.
- The inhalation exposure pathway screening level equations are consistent with EPA's inhalation dosimetry methodology (USEPA, 1994). Inhalation unit risk (IUR) and reference concentration (RfC) toxicity values are used in place of inhalation cancer slope factors and inhalation reference doses, respectively. Therefore, body weight and inhalation rate are no longer used when evaluating the inhalation pathway. This slightly impacts all screening levels and risk estimates that are based solely, or in part, on the inhalation exposure pathway.
- The Regional Screening Table provides a screening level for industrial air.
- The dermal contact pathway is not accounted for in the tap water screening levels.

With regards to TCE, it is currently undergoing reassessment by the Integrated Risk Information System (IRIS) program and interagency review and external peer review of the draft assessment are projected to begin in the fourth quarter of fiscal year (FY) 2009 and first quarter FY 2010, respectively. Until IRIS provides final toxicity values, specific guidance is provided by EPA headquarters, or new toxicity values become available that fall within EPA's toxicity value hierarchy (e.g., PPRTV database), we recommend the use of the following chronic toxicity values for TCE. When evaluating cancer risks, we recommend the use of California Environmental Protection Agency's (CalEPA) oral slope factor (SFo) of 0.013 $(mg/kg\text{-}day)^{-1}$ and IUR of 2.0E-06 $(\mu g/m^3)^{-1}$. When evaluating chronic non-cancer health hazards, we recommend the use of New York State Department of Health's (NYSDOH) air criterion of 10 $\mu g/m^3$. An oral reference dose (RfDo) is not available at this time and until one becomes available, we recommend that the uncertainties regarding the lack of the value be discussed in site-specific human health risk assessments. The use of these toxicity values is consistent with OSWER Directive 9285.7-53, which is OSWER's current policy on the selection of toxicity values in human health risk assessments. All three values have undergone peer review and are Tier 3 toxicity values.

Also, please note that CalEPA provides a chronic inhalation non-cancer toxicity value for TCE which is 60-fold greater than NYSDOH's air criterion. However, it is our professional judgment that CalEPA's Recommended Exposure Limit (REL) does not afford an adequate level of protection for long-term exposures to TCE and therefore, it should not be used in Superfund or RCRA Corrective Action risk assessments (and related documents) submitted to or conducted on behalf of EPA Region 7. Our reasons for supporting the use of the NYSDOH's non-cancer air criterion include, but are not limited to, the following:

- The NYSDOH value is based on more extensive presentation of health endpoints.
- The NYSDOH value is based on a more recent evaluation of the available health effects

literature, such as developmental and reproductive effects.

- The NYSDOH's critical study has clear strengths over CalEPA's REL critical study. First, the Rasmussen et al. (1993) study, which was used to derive NYSDOH's air criterion, had 99 subjects compared to CalEPA's critical study, the Vandervort and Polankoff (1973) study, which included 19 subjects. Second, the Rasmussen study evaluated clinical neurological endpoints whereas the Vandervort and Polankoff study looked at self-reported health endpoints via a questionnaire. Also, the Rasmussen study included concurrent biological monitoring that was used to estimate TCE air concentrations via pharmacokinetic modeling. The Vandervort and Polankoff study derived an exposure concentration from one day measurements.
- The lowest-observed-adverse-effect-level (LOAEL) used to derive the NYSDOH air criterion is $1/6^{th}$ the LOAEL used to derive the CalEPA REL.
- CalEPA's chronic REL is greater than the Agency for Toxic Substances and Disease Registry's (ATSDR) intermediate Minimal Risk Level (MRL), which covers exposures lasting from 14 days to 1 year. Although the ATSDR intermediate inhalation MRL is based on the subchronic rat study by Arito et al. (1994), the human pharmacokinetic adjusted LOAEL is similar to that of the human equivalent LOAELs observed in several human studies including the studies used by CalEPA and NYSDOH to derive chronic non-cancer inhalation values (NRC, 2006). Note that the ATSDR intermediate MRL is a peer-reviewed value that is recommended for use when evaluating subchronic exposures.

If you or your staff have any questions or need assistance regarding the Regional Screening Table or TCE's toxicity values, please contact Mike Beringer at x7351, Jeremy Johnson at x7510, Greg McCabe at x7709, or Kelly Schumacher at x7963. Specific questions on TCE's reassessment should be direct to Jeremy Johnson, the Region 7 IRIS Consensus Reviewer.

References

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Attachment

U.S. ENVIRONMENTAL PROTECTION AGENCY, REGION II
Emergency and Remedial Response Division
290 Broadway
New York, New York 10007-1866

MEMORANDUM

TO: William Sette, Senior Science Advisor, Office of Solid Waste and Emergency Response

FROM: Chloe Metz, Risk Assessor, Emergency and Remedial Response Division, Region 2

DATE: August 17, 2009

RE: Classification of the Oral Slope Factor for Hexavalent Chromium (Cr VI) Developed by New Jersey as a Tier 3 Toxicity Value

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As defined in OSWER directive 9285.7-53, a Tier 3 value, "Includes additional EPA and non-EPA sources of toxicity information. Priority should be given to those sources of information that are the most current, the basis for which is transparent and publicly available, and which have been peer reviewed." Region 2 believes that the oral slope factor for Cr VI of 0.5 $(mg/kg\text{-}day)^{-1}$ developed by Alan Stern of the New Jersey Department of Environmental Protection meets the above definition. The assessment is current (released in July, 2009) and was subject to an external peer review which is available online (http://www.state.nj.us/dep/dsr/chromium/peer-review-comments.pdf). Added support for the use of this OSF as a Tier 3 value is the fact that EPA-NCEA reviewed the draft risk assessment and concluded that it was, "Clearly written, understandable, and well organized, and it was, for the most part, consistent with EPA's risk assessment methodologies."

The hierarchy directive goes on to say that, "Consultation with the STSC or headquarters program office is recommended regarding the use of the Tier 3 values for Superfund response decisions when the contaminant appears to be a risk driver for the site." As such, Region 2 respectfully requests that OSWER provide written support for the use of the New Jersey OSF for Cr VI to determine action levels for the Garfield site where Cr VI is the only contaminant of concern.

References

New Jersey Department of Environmental Protection (April, 2009). Derivation of Ingestion-Based Soil Remediation Criterion for Cr^{+6} Based on the NTP Chronic Bioassay Data for Sodium Dichromate Dihydrate. http://www.state.nj.us/dep/dsr/chromium/soil-cleanup-derivation.pdf

United States Environmental Protection Agency, Office of Solid Waste and Emergency Response (2003). Human Health Toxicity Value in Superfund Risk Assessments. Directive No. 9285.7-53. http://www.epa.gov/oswer/riskassessment/pdf/hhmemo.pdf

cc: Michael Sivak
Helen Dawson
Stiven Foster
Janine Dinan
Dave Crawford

Re: Classification of the Oral Slope Factor for Hexavalent Chromium (Cr VI) Developed by New Jersey as a Tier 3 Toxicity Value

William Sette to: Chloe Metz 09/28/2009 02:39 PM

Cc: Dave Crawford, Helen Dawson, Janine Dinan, Michael Sivak, Stiven Foster, Barbara Hostage

History: This message has been replied to and forwarded.

TO: Chloe Metz, Risk Assessor, Emergency and Remedial Response Division, Region 2

FROM: William F. Sette, Senior Science Advisor, Office of Solid Waste and Emergency Response (5103T)

RE: Classification of the Oral Slope Factor for Hexavalent Chromium (Cr VI) developed by New Jersey as a Tier 3 Toxicity Value

The purpose of this email is to provide written confirmation of OSWER's concurrence with Region 2 using the oral cancer slope factor for Cr VI recently finalized by the state of New Jersey. As noted in your memo, attached below, this toxicity value is based on the most recent science, has been peer reviewed, is publicly available, and, in the opinion of EPA's ORD, is "clearly written, understandable,and well organized", i.e. transparent. Thus, it fulfills all of OSWER's criteria for a Tier 3 Toxicity Value. Janine Dinan of the Office of Emergency Management, which is the lead OSWER office for this emergency cleanup activity, as well as Dave Crawford, in the Office of Superfund Remediation and Technology Innovation, and I, concur with this conclusion. If you have any further questions, please feel free to contact me.

William F. Sette, Ph.D.
Senior Science Advisor
Office of Solid Waste and Emergency Response (5103T)
US EPA
1200 Penn Ave NW
Wash DC 20004
202 566 1928
202 566 1934 fax
sette.william@epa.gov

Chloe Metz Bill, Attached is the memo we discussed. Plea... 08/17/2009 05:43:02 PM

From: Chloe Metz/R2/USEPA/US
To: William Sette/DC/USEPA/US@EPA
Cc: Stiven Foster/DC/USEPA/US@EPA, Michael Sivak/R2/USEPA/US@EPA, Janine Dinan/DC/USEPA/US@EPA, Helen Dawson/DC/USEPA/US@EPA, Dave Crawford/DC/USEPA/US@EPA
Date: 08/17/2009 05:43 PM
Subject: Classification of the Oral Slope Factor for Hexavalent Chromium (Cr VI) Developed by New Jersey as a Tier 3 Toxicity Value

Bill,

Attached is the memo we discussed. Please let me know if you have any questions. Thanks very much for your assistance throughout this process.

Best,

Chloe

Cr VI Tier 3 Memo.doc

Chloe Metz
Special Assistant
Emergency and Remedial Response Division
US EPA, Region 2
290 Broadway, 19th Floor
New York, NY 10007

212.637.3955 (voice)
212.637.4439 (fax)

Cr+6 Muta MOA for Carcinongenicity paper is published

William Sette to: Chloe Metz, Dave Crawford, Helen Dawson, Janine Dinan, Michael Sivak, Stiven Foster, Barbara Hostage 09/29/2009 10:02 AM

Cc: Michael Beringer, Nancy McCarroll

History: This message has been replied to and forwarded.

hi;

Attached please find this paper reflecting OPP's analysis that finds that this chemical has a mutagenic mode of action for carcinogenicity and that recommends that ADAFs be applied, consistent with EPA's Cancer Guidelines.

So it's recent, publically available, peer reviewed, and I leave the transparent open until we read it. That's all the criteria for Tier 3 use.

Bill

[attachment "McCarroll et al 2009.pdf" deleted by Chloe Metz/R2/USEPA/US]

William F. Sette, Ph.D.
Senior Science Advisor
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
SOLID WASTE AND EMERGENCY
RESPONSE

June 12, 2003

OSWER No. 9285.7-75

Marcia L. Bailey, D. Env.
Environmental Toxicologist
U.S. Environmental Protection Agency, Region 10
Office of Environmental Assessment, Risk Evaluation Unit
1200 Sixth Avenue, OEA-095
Seattle, Washington 98101

Dear Dr. Bailey:

I am responding to recent inquiries concerning cancer toxicity values to evaluate inhalation and ingestion risks from exposure to tetrachloroethylene, also commonly known as perchloroethylene or "PCE," and specifically whether it would be appropriate to use a California Environmental Protection Agency (Cal EPA) inhalation unit risk value and oral slope factor. This letter supercedes an earlier version of this letter, which identified an incorrect source of the oral slope factor. This letter is consistent with the earlier letter regarding the inhalation unit risk value and its source.

In the absence of relevant values in the U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) or a value from EPA's National Center for Environmental Assessment/Superfund Technical Health Risk Support Center (STSC), which are the first two tiers of human health toxicity values in the EPA Superfund hierarchy, we would support consideration of the Cal EPA inhalation unit risk value from the Air Toxics Hot Spots Program and the oral slope factor from the Cal EPA Public Health Goal in Drinking Water.

In general, Cal EPA develops its toxicity values in a manner which is quite similar to the EPA IRIS program, in that many of the same databases and considerations are used. Cal EPA's assessments used information from some of the same sources or studies that EPA typically considers in the IRIS program, including the most recent relevant studies known to exist, and also considered this information in a manner similar to the EPA IRIS program.

In summary, having consulted on this matter with the STSC, the Office of Emergency and Remedial Response (OERR) supports use of the Cal EPA Air Toxics Hot Spots Program inhalation unit risk of 5.9 E-6 $(\mu g/m^3)^{-1}$ for Superfund sites as the best value available at this time until a U.S. EPA value becomes available. Having consulted with the STSC about the Cal EPA Public Health Goal in Drinking Water oral slope factor of 5.4E-1 $(mg/kg\text{-}day)^{-1}$ for PCE, we also support the use of this value until a U.S. EPA value becomes available.

The Cal EPA presents a full, complete and transparent presentation of the relevant information on their development of these values on their internet website. Documentation on the Air Toxics Hot Spots Program inhalation unit risk value can be found at this internet website: http://www.oehha.ca.gov/air/hot_spots/pdf/TSDNov2002.pdf . Since this website does not take you directly to the PCE discussion, and this can be difficult to find on the internet website, we have downloaded the eight pages pertaining to PCE and include them as an enclosure to this letter. Documentation on the Public Health Goal in Drinking Water oral slope factor can be found at this Cal EPA internet website: http://www.oehha.ca.gov/water/phg/pdf/PCEAug2001.pdf Because of the size of this document (75 pages) and because this website does take you directly to this document, we have not included this document as an enclosure to this letter. With respect to the transparency of any Superfund Program decisions which may use these values in selecting a response action, we recommend that the appropriate documentation from the Cal EPA website be provided, or the link to the relevant Cal EPA internet website be identified.

Thank you for your inquiry. If you have any questions, please contact Mr. Dave Crawford of my staff at (703) 603-8891.

Sincerely,

/s/

Elizabeth Southerland, Deputy Director
Office of Emergency and Remedial Response

cc: Harlal Choudhury ORD/NCEA/STSC
Sarah Levinson, Region 1
Matthew Hale, OSWER/OSW
Barnes Johnson, OSWER/OSW
Renee Wynn, OSWER/FFRO
James Woolford, OSWER/FFRO
Regional Risk Leads, Regions 1-10
Nancy Riveland, Superfund Lead Region Coordinator, USEPA Region 9
Paul Sieminski, RCRA Lead Region Coordinator, USEPA Region 6
OERR NARPM Co-Chairs
Joanna Gibson, OERR Document Coordinator

Enclosure: California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Air Toxics Hot Spots Program Risk Assessment Guidelines, Part II, Technical Support Document for Describing Available Cancer Potency Factors, December 2002 (excerpt pertaining to tetrachloroethylene)

PERCHLOROETHYLENE

CAS No: 127-18-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	165.83
Boiling point	121°C
Melting point	-19 °C
Vapor pressure	18.47 mm Hg @ 25°C
Air concentration conversion	1 ppm = 6.78 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 5.9 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 2.1 E-2 $(mg/kg\text{-}day)^{-1}$
[Male mouse hepatocellular adenoma and carcinoma incidence data (NTP, 1986), cancer risk estimate calculated using a linearized multistage procedure and PBPK model dose adjustment (CDHS, 1991).

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiological studies of perchloroethylene (PCE) exposure have been reviewed by Reichert (1983) and by the U.S. EPA (1985). Blair *et al.* (1979) analyzed the death certificates of 330 union laundry and dry-cleaning workers (out of a cohort of 10,000). Of 330 decedents, 279 had worked solely in dry-cleaning establishments. Increased mortality from cancers of the respiratory tract, cervix, and skin was documented, and when all malignancies were evaluated together, the number of observed deaths was significantly greater than expected ($p < 0.05$). Although an excess of liver cancer and leukemia was also observed, these increases were not statistically significant.

In an expanded study, Blair *et al.* (1990) reported on mortality among 5,365 dry cleaning union members. Statistically significant excesses of cancer of the esophagus and cervix and non-significant excesses for cancer of the larynx, lung, bladder, and thyroid were reported. Lack of PCE exposure data and lack of accounting for potential confounding factors, such as economic status, tobacco, or alcohol use, prevents any firm conclusion as to the association of PCE exposure and excess cancer.

Katz and Jowett (1981) analyzed the mortality patterns of 671 white female laundry and dry-cleaning workers. Occupational codes listed on the certificates did not distinguish between the two types of work. Data on the duration of employment were not available, nor were the investigators able to determine to which solvent(s) the individuals were exposed. Smoking history was not known. A significant increase in risk of death from cancer of the kidneys ($p < 0.05$) and genitals ($p < 0.01$) was

documented. An excess risk from skin and bladder cancer was also found; however, neither increase was statistically significant.

Other studies of laundry and dry-cleaning workers have also reported an increased risk of death from cervical cancer (Blair *et al.*, 1979; Kaplan, 1980); however, these investigators have not compared mortality data by low-wage occupation. Although not definitive, the findings of Katz and Jowett (1981) suggest that factor(s) other than (or in addition to) solvent exposure are important contributors to cervical cancer.

Kaplan (1980) completed a retrospective mortality study of 1,597 dry-cleaning workers exposed to PCE for at least one year (prior to 1960). The solvent history of approximately half of the dry-cleaning establishments was known. The inability of Kaplan to quantify solvent exposure adds an important confounding variable to the study (Kaplan, 1980). The mean exposure concentration of individuals to PCE was calculated to be 22 ppm for dry-cleaning machine operators and 3.3 ppm for all other jobs. Kaplan found an elevated SMR (182) for malignant neoplasms of the colon (11 observed deaths, 6.77 to 6.98 expected deaths). In addition to colon cancer, malignant neoplasms of the rectum, pancreas, respiratory system, urinary organs, and "other and unspecified sites (major)" were observed (Kaplan, 1980). Although the relatively small cohort in this study limits conclusions about the carcinogenic potential of PCE, the study (Kaplan, 1980) results suggest a relationship between colon cancer and solvent exposure.

A group of Danish laundry and dry-cleaning workers was identified from the Danish Occupational Cancer Register (Lynge *et al.*, 1990). From cancer incidence data for a 10-year period, a significant excess risk was found for primary liver cancer among 8,567 women (standardized incidence ratio 3.4, 95% confidence interval 1.4-7.0). No case of primary liver cancer was observed among 2,033 men, for whom the expected value was 1.1. Excess alcohol consumption did not appear to account for the excess primary liver cancer risk for women. However, no data was available on actual exposures of the study group to PCE or other chemicals.

Duh and Asal (1984) studied the cause(s) of mortality among 440 laundry and dry-cleaning workers from Oklahoma who died during 1975 to 1981. Smoking histories were not available and separation of the two groups by occupation was not possible. NIOSH reported that, although 75% of dry-cleaning establishments in the U.S. use PCE, Oklahoma may be unique in that petroleum solvents account for more that 50% of total solvents used during this period (NIOSH, 1980). Analysis of deaths due to cancer showed an increase for cancers of the respiratory system, lung, and kidney.

Brown and Kaplan (1987) conducted a retrospective, cohort-mortality study of workers employed in the dry-cleaning industry to evaluate the carcinogenic potential from occupational exposure to PCE. The study cohort consisted of 1,690 members of four labor unions (located in Oakland, Detroit, Chicago, and New York City). Individuals selected for the study had been employed for at least one year prior to 1960 in dry-cleaning shops using PCE as the primary solvent. Complete solvent-use histories were not known for about half of the shops included in the study. Because petroleum solvents were widely used by dry cleaners prior to 1960, most of the cohort had known or potential exposure to

solvents other than PCE (primary, various types of Stoddard solvents). The investigators also identified a subcohort of 615 workers who had been employed only in establishments where PCE was the primary solvent. The PCE exposure in shops included in the study was evaluated independently (Ludwig *et al.*, 1983). The geometric mean of time-weighted-average exposures was 22 ppm PCE for machine operators and approximately 3 ppm for other workers.

In summary, a statistically significant excess of deaths from urinary tract cancer was observed in those workers that were potentially exposed to both PCE and petroleum solvents. Individuals employed in shops where PCE was the primary solvent did not have an increased risk of mortality from kidney or bladder cancer. Although these findings do not rule out PCE as the causative agent of urinary tract cancer, the data suggest that other factors or agents may have contributed to the development of neoplastic disease. CDHS stated in the Toxic Air Contaminant document "Health Effects of Tetrachloroethylene" that until studies are completed that include a thorough analysis and quantification of PCE exposures, epidemiological studies will not be useful for the assessment of the human health risks of PCE (CDHS, 1991).

Animal Studies

Two lifetime bioassays have been completed on PCE (NCI, 1977; NTP, 1986). Additionally, three other studies have addressed the question of PCE carcinogenicity (Rampy *et al.*, 1978; Theiss *et al.*, 1977).

The National Cancer Institute (NCI) conducted a study in which B6C3F$_1$ mice and Osborne Mendel rats were administered PCE in corn oil by gavage, 5 days/week for 78 weeks (NCI, 1977). The time-weighted average daily doses of PCE were 536 and 1072 mg/kg for male mice, 386 and 722 mg/kg for female mice, 471 and 941 mg/kg for male rats, and 474 and 949 mg/kg for female rats. PCE caused a statistically significant increase in the incidence of hepatocellular carcinomas in mice of both sexes and both dosage groups ($p < 0.001$). The time to tumor development was considerably shorter in treated than in control mice. In untreated and vehicle control mice, hepatocellular carcinoma were first detected at about 90 weeks. In comparison, hepatocellular carcinomas in male mice were detected after 27 weeks (low dose) and 40 weeks (high dose) and in female mice after 41 weeks (low dose) and 50 weeks (high dose) (Table 1). The median survival times of mice were inversely related to dose. For control, low dose and high dose male mice, their median survival times were 90 weeks, 78 weeks and 43 weeks, respectively; for female mice, their median survival times were 90 weeks, 62 and 50 weeks, respectively. Early mortality occurred in all groups of rats dosed with PCE. NCI (1977) determined that the early mortality observed in rats in this bioassay were inappropriately high and because the optimum dosage was not used, the rat results preclude any conclusions regarding the carcinogenicity of PCE in rats. In addition, the PCE used in the NCI mouse and rat bioassays had a purity of 99%, with epichlorohydrin (ECH) used as a stabilizer. It has been suggested that the presence of this contaminant may have directly contributed to tumor induction.

The most definitive study of the carcinogenic potential of PCE was conducted by Battelle Pacific Northwest Laboratories for the National Toxicology Program (NTP, 1986). In this experiment,

$B6C3F_1$ mice and F344/N rats were exposed to 99.9% pure PCE by inhalation, 6 hours/day, 5 days/week for 103 weeks. Mice were exposed to concentrations of 0, 100, or 200 ppm; rats were exposed to concentrations of 0, 200, or 400 ppm. Both exposure concentrations produced significant increases in mononuclear cell leukemia in female rats (incidence in control, 18/50 animals; in rats receiving 200 ppm, 30/50; and in rats receiving 400 ppm, 29/50). Treated male rats also developed mononuclear cell leukemia in greater numbers than controls (controls, 28/50 animals; 200 ppm, 37/50; 400 ppm, 37/50) [Table 1]. Male rats (at the 200 ppm and 400 ppm PCE exposure levels) exhibited an increased incidence of both renal tubular-cell adenomas and adenocarcinomas. Although the increases were not statistically significant, they appeared to be dose-related.

Brain glioma (a rare tumor of neuroglial cells) was observed in one male control rat and in four male rats that were exposed to 400 ppm PCE (NTP, 1986). This increase was not statistically significant. However, because the historical incidence of these tumors is quite low (0.2% at Battelle Laboratories), the increased incidence in treated animals in this study is noteworthy. Both concentrations of PCE produced a statistically significant increase of hepatocellular carcinomas in treated mice of both sexes ($p < 0.001$). The incidence of these carcinomas in male mice was as follows: controls, 7/49 animals; low-dose, 25/49; and high-dose, 26/50. The incidence of hepatocellular carcinomas in treated female mice was: controls; 1/48 animals; low-dose, 13/50; high-dose, 36/50. Hepatocellular adenomas occurred in both sexes of mice and at both concentrations of PCE (Table 1). The incidence of adenomas was not statistically significant. However, the combined incidence of hepatocellular adenomas and hepatocellular carcinomas was significant. In males, the combined incidence was: controls, 16/49 animals; low-dose 31/49; ($p = 0.002$); adenomas and carcinomas was: controls, 4/48 animals; low-dose, 17/50 ($p = 0.001$); and high-dose, 38/50 ($p < 0.001$).

Table 1: PCE-induced tumor incidence in mice and rats

Study	Species	Sex	Concentration or dose	Tumor response	
				Type[a]	Incidence
NCI, 1977	Mice	Males	0 mg/kg-d	HC	2/17
			536 mg/kg-d	HC	32/49*
			1072 mg/kg-d	HC	27/48*
		Females	0 mg/kg-d	HC	2/20
			386 mg/kg-d	HC	19/48*
			772 mg/kg-d	HC	19/48*
NTP, 1986	Mice	Males	0 ppm	HC; HAC	7/49 ; 16/49
			100 ppm	HC; HAC	25/49*; 8/49(NS)
			200 ppm	HC: HAC	26/50*; 18/50(NS)
		Females	0 ppm	HC; HAC	1/48 ; 3/48
			100 ppm	HC; HAC	13/50*; 6/50(NS)
			200 ppm	HC; HAC	36/50*; 2/50(NS)

[a] HC = hepatocellular carcinomas; HAC = hepatocellular adenoma; ML = mononuclear cell leukemia.
*$p < 0.001$, Fisher Exact Test; **Probability level, Life Table Analysis. NS = not statistically significant

The NTP (1986) determined that, under the conditions of the study, there was "clear evidence of carcinogenicity" of PCE for male F344/N rats, "some evidence of carcinogenicity" of PCE for female

F344/N rats, and "clear evidence of carcinogenicity" of PCE for both sexes of $B6C3F_1$ mice. IARC reevaluated the evidence of carcinogenicity of PCE in 1987 using data from the NTP study and concluded that there was sufficient evidence that PCE is carcinogenic to animals (IARC, 1987). Other studies on PCE included those by Rampy *et al.* (1978) and Theiss *et al.* (1977). Rampy *et al.* (1978) exposed male and female Sprague-Dawley rats to PCE by inhalation (300 or 600 ppm) 6 hours/day, 5 days/week for 12 months. Animals were subsequently observed for 18 months. Pathological changes in the liver or kidney were not observed. Theiss and coworkers studied the ability of PCE to induce lung adenomas in A/St male mice (Theiss *et al.*, 1977). Animals 6 to 8 weeks old were given 80, 200, or 400 mg/kg of PCE in tricaprylin (intraperitoneally) three times a week. Each group received 14, 24, or 48 injections. Treated animals did not exhibit a significant increase in the average number of lung tumors when compared to controls.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Perchloroethylene has been observed to induce mononuclear cell leukemia in male and female rats and liver tumors in male and female mice (NTP, 1986). CDHS (1992) decided that the tumor incidence data from this study were suitable for use in developing a quantitative risk assessment.

Methodology

Results from the 1986 NTP inhalation study were used as the basis for estimating the carcinogenic risk of PCE to humans. In this bioassay, PCE was 99.9% pure, and animals were exposed 6 hours/day, 5 days/week for 103 weeks. The mice in the 100 and 200 ppm dose groups were exposed to a time-weighted-average (TWA) of 16 and 32 ppm, respectively (e.g., 100 ppm × 6 hours/24 hours × 5 days/7 days). Similarly, rats in the 200 and 400 ppm dose groups were exposed to a TWA of 33 and 66 ppm, respectively.

The CDHS staff used the metabolized dose, adjusted to continuous lifetime exposure, to calculate the carcinogenic potency of PCE (CDHS, 1992). There are several uncertainties using this approach: 1) It was assumed that oxidative metabolism leads to the production of carcinogenic metabolites but the ultimate carcinogen(s) has not been well characterized. The metabolism of PCE is not well quantified in humans, and 20-40% of the absorbed PCE has not been accounted for. 2) The pharmacokinetic models used do not account for individual differences in metabolism and storage. The body burden depended on factors such as age, sex, exercise or workload, body mass, adipose tissue mass, pulmonary dysfunctional states, and individual differences in the intrinsic capacity to metabolize PCE.

Two pharmacokinetic models, the steady-state and the PB-PK approaches were used. They incorporated an 18.5% estimated applied dose as the fraction of the dose that is metabolized in humans. For the low-dose PCE risk assessment, the Crump multistage polynomial (Crump, 1984) was chosen.

This model, rather than a time dependent form of the multistage model, was chosen because most tumors were discovered only at the time of sacrifice, and survival in this study was relatively good. The cancer potency values derived using the two different pharmacokinetic approaches using the 1986 NTP rat and mouse studies ranged from 0.12 - 0.95 $(mg/kg\text{-}d)^{-1}$. When expressed as a function of human applied dose the values obtained ranged from 0.0025 to 0.093 $(mg/kg\text{-}d)^{-1}$. Using an estimated human weight of 70 kg, estimated breathing rate of 20 m^3/day and the PCE conversion factor of 1 ppb = 6.78 $\mu g/m^3$, the cancer unit risk values for PCE ranged from 0.2 - 7.2 $\times 10^{-5}$ $(ppb)^{-1}$. After considering the quality of the cancer bioassays and the uncertainty of human metabolism, CDHS (1992) decided that the best value for the PCE cancer unit risk was 4.0 $\times 10^{-5}$ $(ppb)^{-1}$ [5.9 $\times 10^{-6}$ $(\mu g/m^3)^{-1}$]. This value is derived from the tumor incidence data for the most sensitive species, sex, and tumor site, male mouse hepatocellular adenomas or carcinomas (NTP, 1986).

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OCT 2 8 2009

MEMORANDUM

SUBJECT: The Toxicity of Perfluorooctanic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

FROM: Jamine Dinan, Environmental Health Scientist
Office of Emergency Management (OEM)
Office of Solid Waste and Emergency Response (OSWER)

Dave Crawford, Environmental Scientist
Office of Superfund Remediation and Technology Innovation (OSRTI)
Office of Solid Waste and Emergency Response (OSWER)

TO: Glenn Adams, Chief
Technical Services Section
Superfund Division
US EPA Region 4

Background

PFOA and PFOS have been found at sites in EPA Region 4 and in other regions. As a result, Region 4 has asked the Headquarter's Office of Superfund Remediation and Technology Innovation (OSRTI) and the Office of Emergency Management (OEM) to recommend toxicity values.

On December 5, 2003, OSRTI released guidance (OSWER Directive 9285.7-53) establishing a three-tiered hierarchy of human health toxicity values. Tier 1 is EPA's Integrated Risk Information System (IRIS). Tier 2 is the provisional peer reviewed toxicity values (PPRTVs) completed for the EPA Superfund Program by the EPA Superfund Health Risk Technical Health Risk Support Center. Tier 3 are toxicity values from other credible sources such as other federal or State agencies. Three sources of Tier 3 toxicity values were identified in

2003, but OSRTI also stated that additional Tier 3 sources may exist, and that additional Tier 3 sources may be identified in the future. As there are no toxicity values for PFOA or PFOS available in IRIS or as PPRTVs, this memorandum constitutes a Tier 3 consultation and recommends Tier 3 toxicity values for PFOA and PFOS.

Process

OSRTI and OEM consulted with several EPA program offices to discuss the use of the EPA Office of Water (OW) provisional health advisories as Tier 3 toxicity values. After weighing input from these offices, we make the following recommendations regarding the OW advisory and the interim oral non-cancer toxicity values for PFOA and PFOS.

Recommendations

On January 8, 2009 OW completed and released Provisional Health Advisories for PFOA and PFOS (See Attachment 1). Prior to the release of this assessment, OW invited, received and considered internal and external peer review comments on the then draft assessment. Although derived using methods that differ from the Superfund program's risk-based approaches, OSRTI and OEM find the OW provisional drinking water advisories of 0.4 µg/l for PFOA and 0.2 µg/l for PFOS credible as protective health-based concentrations for these contaminants in drinking water.

Because the OW provisional health advisories address only water consumption, oral reference dose values (RfDs), which can be used to address oral exposure to other media such as soil, were not developed. However, the methodology used by OW in deriving its provisional health advisories can also be used to derive subchronic RfD values for PFOA and PFOS, as shown below:

- **Perflurooctanoic Acid (PFOA)**

  For PFOA, the OW provisional health advisory relies on data from a sub-chronic study in mice (Lau, et al 2006) to derive a Benchmark Dose Level ($BMDL_{10}$) of 0.46 mg/kg-day[1]. When calculating toxicity values such as an RfD, a BMDL or a No Observed Adverse Effect Level (NOAEL) can be used to derive an RfD. In deriving an RfD for PFOA, certain numerical factors are applied to the BMDL to account for differences in the metabolism and sensitivity among test animals and humans to the effects of PFOA. Using the numerical factors presented in OW's provisional health advisory, a subchronic RfD can be developed, as follows:

[1] EPA toxicity assessments, including Integrated Risk Informaiton System (IRIS) assessments, using BML modeling in the derivation of an RfD typically use the 10% response level from the BML modeling ($BMDL_{10}$) to derive an RfD.

Subchronic RfD = $(BMDL_{10}) / UF_H * (UF_A = UF_{pharmacodynamic} * UF_{pharmacokinetic})$
= (0.46 mg/kg-day) / 10 * (3 * 81)
= **2E-4 mg/kg-day**

UF_H = a factor of 10 to account for variations in the dose-response (i.e., sensitivity) among humans to the effects of PFOS

UF_A = a factor to account for differences in the metabolism of PFOA in mice vs. humans
- $UF_{pharmacodynamic}$ = a factor of 3 to account for variations in the dose-response among mice to the effects of PFOA
- $UF_{pharmacokinetic}$ = a factor of 81[2] to account for differences in the rate of clearance of PFOA in mice vs. humans

- **Perfluorooctane Sulfonate (PFOS)**

For PFOS, the OW provisional health advisory relies on data from a sub-chronic study in monkeys (Seacat, et al. 2002) to derive a NOAEL of 0.03 mg/kg-day. As with PFOA, certain numerical factors are applied to the NOAEL to account for differences in the metabolism and sensitivity among test animals and humans to the effects of PFOS. Using the numerical factors presented in OW's provisional health advisory, a subchronic RfD can be developed, as follows:

Subchronic RfD = $(NOAEL) / UF_H * (UF_A = UF_{pharmacodynamic} * UF_{pharmacokinetic})$
= 0.03 mg/kg-day / 10 * (3 * 13)
= **8E-5 mg/kg-day**

UF_H = a factor of 10 to account for variations in the dose-response (i.e., sensitivity) among humans to the effects of PFOS
UF_A = a factor to account for differences in the metabolism of PFOS in monkeys vs. humans
- $UF_{pharmacodynamic}$ = a factor of 3 to account for variations in the dose-response among monkeys to the effects of PFOS
- $UF_{pharmacokinetic}$ = a factor of 13[3] to account for differences in the rate of clearance of PFOS in monkeys vs. humans

Currently, OEM has not established removal action levels for PFOA or PFOS as the basis for considering alternate water supplies, nor have these contaminants been addressed in the Regional Screening Levels for Chemical Contaminants at Superfund Sites. However, the Tier 3 sub-chronic RfDs presented in this memorandum may be used in the Superfund program's risk-based equations to derive Removal Action Levels and/or Screening Levels for water and other media, as appropriate.

[2] See Attachment 1, page 4 for additional details about this UF.
[3] See Attachment 1, pages 4 and 5 for additional details about this UF.

3

Please be aware that the recommendations made in this memorandum may be modified by OSRTI and OEM as the state of the science evolves with respect to deriving toxicity values and determining protective concentrations of PFOA and PFOS. Such changes may include the availability of an IRIS or a PPRTV assessment and/or the promulgation of a Safe Drinking Water Act Maximum Contaminant Level by OW.

Questions related to the use of this memorandum and its recommendations may be directed to Dave Crawford (703-603-8891) and to Janine Dinan (202-564-8737) in OEM.

Attachment 1

Attachment

1C

3

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

May 26, 2021

OFFICE OF
LAND AND EMERGENCY
MANAGEMENT

MEMORANDUM

SUBJECT: Recommendations on the Use of Chronic or Subchronic Noncancer Values for Superfund Human Health Risk Assessments

FROM: Brigid Lowery, Director Brigid Lowery
Assessment and Remediation Division
Office of Superfund Remediation and Technology Innovation

TO: Superfund Emergency Management Divisions Directors, Regions 1 - 10

PURPOSE

The purpose of this memorandum is to provide recommendations from the Office of Land and Emergency Management (OLEM) regarding the use of the subchronic toxicity value rather than the chronic value for 19 specific chemicals as noted in the attachment.

This recommendation is based on OLEM's Human Health Regional Risk Assessment Forum's (OHHRRAF) Toxicity Workgroup evaluation of the toxicity of 32 chemicals. The OHHRRAF recommended using subchronic values in place of chronic values for 19 of the 32 chemicals OLEM concurs with the OHHRRAF recommendation. The Forum's recommendations may be applicable to EPA regional offices' activities to evaluate and address hazardous waste releases under the Comprehensive Environmental Response, Compensation and Liability Act, as amended (e.g., Hazard Ranking System scoring, remedial investigation and feasibility study process, and five-year reviews), and other OLEM risk evaluation efforts.

BACKGROUND

The Office of Solid Waste and Emergency Response[1] (OSWER) Directive 9285.7-53 **(***Human Health Toxicity Values in Superfund Risk Assessments*; December 5, 2003; commonly referred to as "the 2003 hierarchy guidance"), identifies an updated source hierarchy for human health toxicity values to consider when carrying out Superfund site risk assessments. It also states that "[t]his revised hierarchy recognizes that EPA should use the best science available on which to base risk assessments." Furthermore, the 2003 hierarchy guidance states that, "EPA and state personnel may use and accept other technically sound approaches," acknowledging "that there may be other sources of toxicological information," referring specifically to OSWER Directive

[1] The former name of what is now EPA's Office of Land and Emergency Management.

9285.7-16 (*Use of IRIS Values in Superfund Risk Assessment*; December 21, 1993), which offers similar guidance.[2]

The OHHRRAF Toxicity Workgroup identified 21 oral and 11 inhalation toxicity values where a subchronic toxicity value was lower than its corresponding chronic toxicity value. After review of relevant information, the Forum recommends use of the subchronic toxicity value rather than the chronic value for 19 of the 32. For the remaining 13 chemical toxicity values, the Forum recommends the chronic toxicity values be used.

The recommendations in the memorandum will be re-evaluated in the future as toxicity values are updated.

Please contact Michele Burgess (703-603-9003) or Laurence Libelo (703-603-8815) if you have any questions or require additional information.

Attachment
CC: Barry Breen, OLEM
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[2] See OSWER Directive 9285.7-53, page 2, quoting OSWER Directive 9285.7-16: "...IRIS is not the only source of toxicology information, and in some cases more recent, credible and relevant data may come to the Agency's attention. In particular, toxicological information other than that in IRIS may be brought to the Agency by outside parties. Such information should be considered along with the data in IRIS in selecting toxicological values; ultimately, the Agency should evaluate risk based upon its best scientific judgement and consider all credible and relevant information available to it."

Selected Chronic Toxicity Values[a]						
Chemical (CASRN)	**Chronic Value**	**Source (Year)**	**Subchronic Value**	**Source (Year)**	**Selected Value**	**Date**
Inhalation (mg/m^3)						
*Acrylic acid (79-10-7)	0.001	IRIS (1994)	0.0002	PPRTV (2010)	0.0002	04/18/19
Ammonia (7664-41-7)	0.5	IRIS (2016)	0.1	PPRTV (2005)	0.5	04/18/19
Chlordane (12789-03-6)	0.0007	IRIS (1998)	0.0002	ATSDR (1994)	0.0007	04/18/19
1,1-Dichloroethylene (75-35-4)	0.2	IRIS (2002)	0.08[b]	ATSDR (1994)	0.2	04/18/19
*2-Ethoxyethanol (110-80-5)	0.2	IRIS (1991)	0.04	PPRTV (2013)	0.04	12/12/2019
*Ethyl chloride (75-00-3)	10	IRIS (1991)	4	PPRTV (2007)	4	04/18/19
*2-Methoxyethanol (109-86-4)	0.2	IRIS (1991)	0.007	PPRTV (2011)	0.007	04/18/19
Methyl tert-butyl ether (1634-04-4)	3	IRIS (1993)	2.5[c]	ATSDR (1996)	3	06/25/2020
Nitromethane (75-52-5)	0.005	PPRTV (2013)	0.004	PPRTV (2013)	0.005	04/18/19
Vinyl acetate (108-05-4)	0.2	IRIS (1990)	0.05[d]	ATSDR (2001)	0.2	06/25/2020
*Vinyl chloride (75-01-4)	0.1	IRIS (2000)	0.08[e]	ATSDR (2006)	0.08	08/27/2020
Oral (mg/kg-day)						
Acrylamide (79-06-1)	0.002	IRIS (2010)	0.001	ATSDR (2012)	0.002	06/27/2019
Acrylic acid (79-10-7)	0.5	IRIS (1994)	0.2	PPRTV (2010)	0.5	03/05/2020
*Acrylonitrile (107-13-1)	0.04	ATSDR (1990)	0.01	ATSDR (1990)	0.01	06/27/2019
*Allyl alcohol (107-18-6)	0.005	IRIS (1987)	0.004	PPRTV (2009)	0.004	04/18/19
*Atrazine (1912-24-9)	0.035	IRIS (1993)	0.003	ATSDR (2003)	0.003	06/27/2019
1,1-Biphenyl (92-52-4)	0.5	IRIS (2013)	0.1	PPRTV (2011)	0.5	04/18/19
*Bromodichloromethane (75-27-4)	0.02	IRIS (1987)	0.008	PPRTV (2009)	0.008	04/18/19
*Cadmium (7440-43-9)	0.0005/0.001f	IRIS (1989)	0.0001[f]	ATSDR (2012)	0.0001	04/18/19
*p-Chloroaniline (106-47-8)	0.004	IRIS (1988)	0.0005	PPRTV (2008)	0.0005	04/18/19
*p-Cresol (106-44-5)	0.1	ATSDR (2008)	0.02	PPRTV (2010)	0.02	04/18/19
Cyclohexanone (108-94-1)	5	IRIS (1987)	2	PPRTV (2010)	5	08/27/2020
Endosulfan (115-29-7)	0.006	IRIS (1994)	0.005	ATSDR (2015)	0.006	06/27/2019
*Ethyl acetate (141-78-6)	0.9	IRIS (1987)	0.7	PPRTV (2013)	0.7	04/18/19
*Ethylbenzene (100-41-4)	0.1	IRIS (1987)	0.05	(PPRTV (2009)	0.05	04/18/19
*Ethylene glycol (107-21-1)	2	IRIS (1987)	0.8	ATSDR (2010)	0.8	04/18/19
Ethylene glycol monobutyl ether (111-76-2)	0.1	IRIS (2010)	0.07	ATSDR (2010)	0.1	04/18/19
*Hepatchlor (76-44-8)	0.0005	IRIS (1987)	0.0001	ATSDR (2007)	0.0001	06/27/2019
*Hexachlorobenzene (118-74-1)	0.0008	IRIS (1988)	0.00001	PPRTV (2010)	0.00001	04/18/19
*Hexachlorocyclohexane, gamma (58-89-9)	0.0003	IRIS (1987)	0.00001	ATSDR (2005)	0.00001	08/27/2020
Pentachlorophenol (87-86-5)	0.005	IRIS (2010)	0.001	ATSDR (2001)	0.005	08/27/2020
*1,2,4,5-Tetrachlorobenzene (95-94-3)	0.0003	IRIS (1987)	0.00003	PPRTV (2013)	0.00003	04/18/19

[a]Decisions regarding the most appropriate toxicity value when the subchronic value was more conservative than the chronic value in the Regional Screening Levels (RSLs).
[b]Intermediate-duration MRL = 0.02 ppm
[c]Intermediate-duration MRL = 0.07 ppm
[d]Intermediate-duration MRL = 0.01 ppm
[e]Intermediate-duration MRL = 0.03 ppm
[f]Values for food/water.
[g]Chronic-duration MRL; an intermediate-duration MRL of 0.0005 mg/kg-day was also available (ATSDR 2012).
*Indicates that selection of the subchronic value.

1. **Decisions that Require a Change in the RSLs (Inhalation).**

Acrylic Acid (CASRN 79-10-7). The IRIS chronic RfC (1994) and the PPRTV subchronic p-RfC (2010) are based on the same study (Miller et al. 1981). However, the PPRTV used BMD modeling and dosimetric conversion factors to account for pharmacokinetics differences between mice and people. The PPRTV value is selected based on the use of updated methodology.

Summary Table for Acrylic Acid (CASRN 79-10-7)		
Source (Year)	**IRIS (1994)**	**PPRTV (2010)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	Miller et al. 1981	
Species/Strain/Sex	B6C3F1 mice (15/sex/group)	
Study Duration	6 hours/day, 5 days/week, for 13 weeks	
Critical Effect(s)	Focal degeneration of the nasal olfactory epithelium	
POD	$LOAEL_{[HEC]}$ = 0.33 mg/m^3	$BMDL10_{[HEC]}$ = 0.02 mg/m^3
Composite UF	300[a]	100[b]
Toxicity Value (mg/m^3)	0.001	0.0002
Selected Value (mg/m^3)	0.0002	
Rationale	Updated methodology	

[a]The composite UF of 300 is based on 10 for UF_H, 3 for UF_S, 3 for UF_A, and 3 for UF_L.
[b]The composite UF of 100 if based on 3 for UF_A, 10 for UF_H, and 3 for UF_D

References:

- Miller, RR; Ayres, JA; Jersey GC; et al. (1981) Inhalation toxicity of acrylic acid. Fund. Appl. Toxicol. 1:271–277.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0002_summary.pdf#nameddest=rfc
- U.S. EPA. (2010) Provisional peer-reviewed toxicity values for acrylic acid (CASRN 79-10-7). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/AcrylicAcid.pdf

2-Ethoxyethanol (2-EE) (CASRN 110-80-5). The subchronic p-RfC (PPRTV 2013) is based on a developmental toxicity study. The PPRTV determined that, based on duration-adjusted concentrations, minor and major skeletal defects in the offspring of Dutch rabbits (Doe 1984b) is a more sensitive endpoint than testicular (and hematological) effects in adult New Zealand White rabbits (Barbee et al. 1984a), which was the critical effect identified by IRIS (1991). In support, fetal effects (minor skeletal defects) were also observed in a developmental toxicity study using Wistar rats (Doe 1984b). BMD modeling (i.e., updated methodology) was used in the PPRTV assessment to determine the POD (compared to the IRIS $NOAEL_{HEC}$). A benchmark response (BMR) of 5% extra risk was used; it is standard EPA practice to use a BMR of 5% for developmental endpoints. Although the data from Doe (1984b) were provided on a per pup basis (rather than a per-litter basis), the sample size of each exposure group was calculated from the data provided. The PPRTV also noted that BMD modeling could not be applied to the less sensitive endpoints from the Barbee et al. (1984a) study because an abnormally large standard deviation was reported for one of the testis weights values, and no quantitative data for seminiferous tubule degeneration were provided. The PPRTV value is selected based on the evaluation of sensitive (developmental) endpoints and the use of updated methodology.

Summary Table for 2-Ethoxyethanol (CASRN 110-80-5)		
Source (Year)	**IRIS (1991)**	**PPRTV (2013)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	Barbee et al. 1984a	Doe 1984b
Species/Strain/Sex	New Zealand White rabbits (10/sex/group)	Dutch rabbits (24 females/group)
Study Duration	6 hours/day, 5 days/week for 13 weeks	6 hours/day on GDs 6-18
Critical Effect(s)	Decreased hemoglobin, decreased testis weight, and seminiferous tubule degeneration	Fetal skeletal effects
POD	$NOAEL_{[HEC]}$ of 68 mg/m^3	$BMDL_{[5\%HEC]}$ of 4.23 mg/m^3
Composite UF	300[a]	100[b]
Toxicity Value (mg/m^3)	0.2	0.04
Selected Value (mg/m^3)	0.04	

Rationale	Different study; updated methodology

[a]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 100 if based on 3 for UF_A, 3 for UF_D, and 10 for UF_H

References:

- Barbee, S.J., J.B. Terrill, D.J. DeSousa and C.C. Conaway. 1984a. Subchronic inhalation toxicology of ethylene glycol monoethyl ether in the rat and rabbit. Environ. Health Perspect. 57: 157-163.
- Doe, JE. (1984b) Ethylene glycol monoethyl ether and ethylene glycol monoethyl ether acetate teratology studies. Environ Health Perspect 57:33−41.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0513_summary.pdf#nameddest=rfc
- U.S. EPA. (2013) Provisional peer-reviewed toxicity values for 2-ethoxyethanol (CASRN 110-80-5). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Ethoxyethanol2.pdf.

Ethyl Chloride (CASRN 75-00-3). The IRIS chronic RfC (1991) and the PPRTV subchronic p-RfC (2007) are based on the same study (Scortichini et al. 1986). However, the PPRTV Assessment used BMD modeling to determine the POD (i.e., updated methodology). The PPRTV value is selected based on the use of updated methodology.

Summary Table for 2-Ethyl Chloride (CASRN 75-00-3)		
Source (Year)	**IRIS (1991)**	**PPRTV (2007)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	Scortichini et al. 1986	
Species/Strain/Sex	CF-1 mice (30 females/group)	
Study Duration	6 hours/day on GDs 6-15	
Critical Effect(s)	Delayed ossification of the skull bones	
POD	$NOAEL_{[HEC]}$ = 4000 mg/m^3	$LEC_{10[ADJ]}$ = 1078 mg/m^3
Composite UF	300[a]	300[b]
Toxicity Value (mg/m^3)	10	4
Selected Value (mg/m^3)	4	
Rationale	Updated methodology	

[a]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_D.
[b]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_D.

References:

- Scortichini, B.H., K.A. Johnson, J.J. Momany-Pfruender, and T.R. Hanley, Jr. 1986. Ethyl chloride: Inhalation teratology study in CF-1 mice. Dow Chemical Co. EPA Document #86- 870002248.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0523_summary.pdf#nameddest=rfc
- U.S. EPA. (2007) Provisional peer-reviewed toxicity values for chloroethane (CASRN 75-00-3). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Chloroethane.pdf

2-Methoxyethanol (CASRN 109-86-4). The IRIS chronic RfC (1991) and the PPRTV subchronic p-RfC (2011) are based on the same study (Miller et al. 1983). However, the PPRTV Assessment used BMD modeling to determine the POD (i.e., updated methodology). The PPRTV value is selected based on the use of updated methodology.

Summary Table for 2-Methoxyethanol (CASRN 109-86-4)		
Source (Year)	**IRIS (1991)**	**PPRTV (2011)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	Miller et al. 1983	
Species/Strain/Sex	New Zealand White rabbits (5/sex/group)	
Study Duration	6 hours/day, 5 days/week, for 13 weeks	
Critical Effect(s)	Reduction in testis size	
POD	$NOAEL_{[HEC]}$ = 17 mg/m^3	$BMDL_{[10HEC]}$ = 0.73 mg/m^3
Composite UF	1000[a]	100[b]
Toxicity Value (mg/m^3)	0.02	0.007
Selected Value (mg/m^3)	0.007	
Rationale	Updated methodology	

[a]The composite UF of 1000 is based on 3 for UF_A, 3 for UF_D, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 3 for UF_D.

References:

- Miller, R.R., J.A. Ayres, J.T. Young and M.J. McKenna. 1983. Ethylene glycol monomethyl ether. I. Subchronic vapor inhalation study with rats and rabbits. Fund. Appl. Toxicol. 3(1): 49- 54.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0525_summary.pdf#nameddest=rfc
- U.S. EPA. (2011) Provisional peer-reviewed toxicity values for 2-methoxyethanol (CASRN 109-86-4) and 2-methoxyethanol acetate (CASRN 110-49-6). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/MethoxyethanolAcetate2.pdf

Vinyl Chloride (CASRN 75-01-4). The IRIS assessment is based on a dietary study that used PBPK modeling for route-to-route (R2R) extrapolation. The ATSDR assessment is based on a study (Thornton et al., 2002) that was not available when the IRIS assessment was completed (2000). Other differences between the ATSDR intermediate-duration MRL (2006) and the IRIS chronic RFC (2000) were due to rounding. IRIS divided the POD of 2.5 mg/m^3 by the composite uncertainty factor (UF_C) of 30 to arrive at 0.08 mg/m^3, which was rounded to 0.1 mg/m^3. ATSDR identified a POD of 1.25 ppm, which was rounded to 1 ppm prior to the application of uncertainty factors. The POD divided by the UF_C of 30 generated an intermediate-duration MRL of 0.03 ppm; using the conversion factor 1 ppm = 2.56 mg/m^3 resulted in a toxicity value of 0.08 mg/m^3. Differences in the toxicity values is an artifact of the derivation process used by each agency. The ATSDR value is selected based on new information.

Summary Table for Vinyl Chloride (CASRN 75-01-4)		
Source (Year)	**IRIS (2000)**	**ATSDR (2006)**
Toxicity Value	Chronic RfC	Intermediate-duration MRL
Critical Study	Til et al. 1983, 1991	Thornton et al. 2002
Species/Strain/Sex	Wistar rats (50 to 100/sex/group)	Sprague-Dawley rats (30/sex/group)
Study Duration	Lifetime dietary	4 hours/day for two generations
Critical Effect(s)	Liver cell polymorphism	Centrilobular hypertrophy (F1 females)
POD	$NOAEL_{[HEC]}$ = 2.5 mg/m^3 (based on PBPK model R2R extrapolation)	$LEC_{[10HEC]}$ = 1 ppm
Composite UF	30[a]	30[b]
Toxicity Value	0.1 mg/m^3	0.03 ppm (0.08 mg/m^3)
Selected Value (mg/m^3)	0.08	
Rationale	New study	

[a]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.
[b]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2006). Toxicological profile vinyl chloride. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp20.pdf
- Thornton SR, Schroeder RE, Robison RL, et al. 2002. Embryo-fetal developmental and reproductive toxicology of vinyl chloride in rats. Toxicol Sci 68:207-219.
- Til, HP; Feron, VJ; Immel, HR. (1991) Lifetime (149-week) oral carcinogenicity study of vinyl chloride in rats. Food Chem Toxicol 29:713-718.
- Til, HP; Immel, HR; Feron, VJ. (1983) Lifespan oral carcinogenicity study of vinyl chloride in rats. Final report. Civo Institutes. TNO Report No. V 83.285/291099, TSCATS Document FYI-AX-0184-0353, Fiche No. 0353.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/1001_summary.pdf#nameddest=rfc

2. Decisions that Require a Change in the RSLs (Oral).

Acrylonitrile (CASRN 107-13-1). The study used to derive the intermediate-duration MRL (Tandon, 1988) identified a serious LOAEL for decreased sperm count and testicular tubule degeneration at 10 mg/kg-day and used a UF_C of 1000 to derive a chronic-duration MRL of 0.01 mg/kg-day (see footnote f of Table 2-2 in the ATSDR Toxicological Profile). According to the same table, the chronic-duration MRL was derived from Biodynamics (1980) based on a NOAEL of 4.2 mg/kg-day for decreased red blood cells (footnote h). Figure 2-2 erroneously shows that the chronic-duration MRL was derived from Biodynamics (1980) using a NOAEL based on decreased red blood cells of 0.14 mg/kg-day. The intermediate-duration ATSDR value is selected because it is more protective than the chronic-duration MRL.

Summary Table for Acrylonitrile (CASRN 107-13-1)		
Source (Year)	**ATSDR (1990)**	**ATSDR (1990)**
Toxicity Value	Chronic-duration MRL	Intermediate-duration MRL
Critical Study	Biodynamics 1980	Tandon 1988
Species/Strain/Sex	F344 rats	Mice
Study Duration	24 months	60 days
Critical Effect(s)	Decreased red cells	Decreased sperm count and testicular tubule degeneration
POD	NOAEL = 4.2 mg/kg-day	LOAEL = 10 mg/kg-day
Composite UF	100[a]	1000[b]
Toxicity Value (mg/kg-day)	0.04	0.01
Selected Value (mg/kg-day)	0.01	
Rationale	Different methodology	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1996). Toxicological profile for acrylamide. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp125.pdf
- Bio/dynamics. 1980b. A twenty-four month oral toxicity/carcinogenicity study of acrylonitrile administered in the drinking water to Fischer 344 rats. Biodynamics, Inc., Division of Biology and Safety Evaluation, East Millstone, NJ. Project No. BDN-77-27.
- Tandon R, Saxena DK, Chandra SV, et al. 1988. Testicular effects of acrylonitrile in mice. Toxicol Lett 42:55-63.

Allyl Alcohol (CASRN 107-18-6). The PPRTV (2009) assessment used a new study (NTP 2006) that was not available when the IRIS assessment was completed (1987) and used the BMD modeling to determine the POD (i.e., updated methodology). The PPRTV is selected based on new information and the use of updated methodology.

Summary Table for Allyl Alcohol (CASRN 107-18-6)		
Source (Year)	**IRIS (1987)**	**PPRTV (2009)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Carpanini et al. 1978	NTP 2006
Species/Strain/Sex	Wistar rats (15/sex/group)	F344/N rats (10/sex/group)
Study Duration	15 weeks	5 days/week for 14 weeks
Critical Effect(s)	Impaired renal function and kidney weights	Squamous hyperplasia of the forestomach epithelium (females)
POD	NOEL = 4.8 mg/kg-day	BMDL = 1.3 mg/kg-day
Composite UF	1000[a]	300[b]
Toxicity Value (mg/kg-day)	0.005	0.004
Selected Value (mg/kg-day)	0.004	
Rationale	New study; updated methodology	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_D.

References:

- Carpanini, F.M.B., I.F. Gaunt, J. Hardy, S.D. Gangalli, K.R. Butterworth and H.G. Lloyd. 1978. Short-term toxicity of allyl alcohol in rats. Toxicology. 9: 29-45.
- NTP (National Toxicology Program). 2006. NTP technical report on the comparative toxicity studies of allyl acetate, allyl alcohol and acrolein administered by gavage to F344/N rats and B6C3F1 mice. National Toxicology Program Toxicity Report Series Number 48. July 2006. Online. http://ntp.niehs.nih.gov/files/TS48_Web.pdf.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0004_summary.pdf#nameddest=rfd.U.S. EPA. (2009) Provisional peer-reviewed toxicity values for allyl alcohol (CASRN107-18-6). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/AllylAlcohol.pdf

Atrazine (CASRN 1912-24-9). The intermediate-duration MRL from ATSDR (2003) is based on a study (Gojmerac et al. 1999) that was not available at the time of the IRIS assessment (1993). The critical effect identified by ATSDR is indicative of the potential for endocrine disruption, and results in a lower toxicity value than the chronic RfD. The ATSDR value is selected based on new information.

Summary Table for Atrazine (CASRN 1912-24-9)		
Source (Year)	**IRIS (1993)**	**ATSDR (2003)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Ciba-Geigy Corp. 1986	Gojmerac et al. 1999
Species/Strain/Sex	Sprague-Dawley rats (20/sex/group)	Swedish Landance/Large Yorkshire pigs (9 young females/group)
Study Duration	2 years	19 days
Critical Effect(s)	Decreased body weight gain	Delayed estrus
POD	NOAEL = 3.5 mg/kg-day	LOAEL = 1 mg/kg-day
Composite UF	100[a]	300[b]
Toxicity Value (mg/kg-day)	0.035	0.003
Selected Value (mg/kg-day)	0.003	
Rationale	New study	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 300 is based on 10 for UF_A, 3 for UF_H, and 10 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2003). Toxicological profile for atrazine. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp153.pdf
- Ciba-Geigy Corporation. 1986. MRID No. 00141874, 00157875, 00158930, 40629302. HED Doc. No. 005940, 006937. Available from EPA. Write to FOI, EPA, Washington, DC 20460.
- Gojmerac T, Uremovic M, Uremovic Z, et al. 1999. Reproductive disturbance caused by an s-triazine herbicide in pigs. Acta Vet Hung 47(1):129-135.
- U.S. EPA. (1993). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0209_summary.pdf

Bromodichloromethane (CASRN 75-27-4). The subchronic p-RfD (PPRTV 2009) is based on a study (Bielmeier et al. 2001) that was not available at the time of the IRIS assessment (1987). The PPRTV value is selected based on new information.

Summary Table for Bromodichhloromethane (CASRN 75-27-4)		
Source (Year)	**IRIS (1987)**	**PPRTV (2009)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	NTP 1986	Bielmeier et al. 2001
Species/Strain/Sex	B6C3F1 mice (50/sex/group)	F344 rats (8-11 females/group)

Study Duration	102 weeks	GD 9
Critical Effect(s)	Renal cytomegaly	Full litter resorption
POD	LOAEL = 17.9 mg/kg-day	$BMDL_{05}$ = 0.76 mg/kg-day
Composite UF	1000[a]	100[b]
Toxicity Value (mg/kg-day)	0.02	0.008
Selected Value (mg/kg-day)	0.008	
Rationale	New study	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, 3 for UF_L, and 3 for UF_D.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Bielmeier, S.R., D.S. Best, D.L. Guidici et al. 2001. Pregnancy loss in the rat caused by bromodichloromethane. Toxicol. Sci. 59:309−315.
- NTP (National Toxicology Program). 1986. Toxicology and Carcinogenesis Studies of Bromodichloromethane in F344/N Rats and B6C3F1 Mice (gavage studies). NTP Technical Report, Ser. No. 321, NIH Publ. No. 87-2537.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0213_summary.pdf
- U.S. EPA. (2009) Provisional peer-reviewed toxicity values for bromodichloromethane (CASRN 75-27-4). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Bromodichloromethane.pdf

Cadmium (CASRN 7440-43-9). The chronic-duration MRL was derived from a meta-analysis of several studies assessing the effect of dietary cadmium on renal function in humans. The chronic-duration ATSDR value is selected based on new information (i.e., meta-analysis data).

Summary Table for Cadmium (CASRN 7440-43-9)			
Source (Year)	**IRIS (1989)**	**ATSDR (2012)**	**ATSDR (2012)**
Toxicity Value	Chronic RfD	Chronic-duration MRL	Intermediate-duration MRL
Critical Study	U.S. EPA 1985	Buchet et al. 1990; Järup et al. 2000; Suwazono et al. 2006	Brzóska et al. 2005a, 2005b; Brzóska and Moniuszko-Jakoniuk 2005
Species/Strain/Sex	Human studies involving chronic exposures	General population and residents of cadmium-polluted and non-polluted areas	Wistar rats (40 females/group)
Study Duration	Various	Various	12 months
Critical Effect(s)	Significant proteinuria	Renal dysfunction (proteinuria)	Decreased bone mineral density
POD	NOAEL (water) = 0.005 mg/kg-day NOAEL (food = 0.01 mg/kg-day	$UCDL_{10}$ = 0.00033 mg/kg-day (females)	$BMDL_{1SD}$ = 0.05 mg/kg-day
Composite UF	10[a]	3[b]	100[c]
Toxicity Value (mg/kg-day)	0.0005 (water) 0.001 (food)	0.0001	0.0005
Selected Value (mg/kg-day)	0.0001		
Rationale	New study		

[a]The composite UF of 10 is on 10 for UF_H.
[b]The composite UF of 3 is based on 3 for UF_H.
[c]The composite UF of 100 is based on 10 for UF_A and 10 for UF_H.
UCDL = lower limit on urinary cadmium dose

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2003). Toxicological profile for cadmium. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf
- Brzóska MM, Majewska K, Moniuszko-Jakoniuk J. 2005a. Bone mineral density, chemical composition and biomechanical properties of the tibia of female rats exposed to cadmium since weaning up to skeletal maturity. Food Chem Toxicol 43(10):1507-1519.

- Brzóska MM, Majewska K, Moniuszko-Jakoniuk J. 2005b. Weakness in the mechanical properties of the femur of growing female rats exposed to cadmium. Arch Toxicol 79(5):277-288.
- Brzóska MM, Moniuszko-Jakoniuk J. 2005. Disorders in bone metabolism of female rats chronically exposed to cadmium. Toxicol Appl Pharmacol 202(1):68-83.
- Buchet JP, Lauwerys R, Roels H, et al. 1990. Renal effects of cadmium body burden of the general population. Lancet 336:699-702.
- Järup L, Hellstrom L, Alfven T, et al. 2000. Low level exposure to cadmium and early kidney damage: The OSCAR study. Occup Environ Med 57(10):668-672.

p-Chloroaniline (CASRN 106-47-8). The subchronic p-RfD (PPRTV 2008) is based on a study (NTP 1989) that was not available when the IRIS assessment was completed (1988). The PPRTV value is selected based on new information.

Summary Table for p-Chloroaniline (CASRN 106-47-8)		
Source (Year)	**IRIS (1988)**	**PPRTV (2008)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	NCI 1979	NTP 1989
Species/Strain/Sex	F344 rats (20 to 50/sex/group)	F344 rats (15/sex/group)
Study Duration	78 weeks (24 weeks observation)	6 months (interim)
Critical Effect(s)	Non-neoplastic lesions of splenic capsule	Methemoglobin formation
POD	LOAEL = 12.5 mg/kg-day	$LOAEL_{[ADJ]}$ = 1.4 mg/kg-day
Composite UF	3000[a]	3000[b]
Toxicity Value (mg/kg-day)	0.004	0.0005
Selected Value (mg/kg-day)	0.0005	
Rationale	New study	

[a]The composite UF of 3000 is based on 10 for UF_A, 10 for UF_H, 10 for UF_L, and 3 for UF_D.
[b]The composite UF of 3000 is based on 10 for UF_A, 10 for UF_H, 3 for UF_L, and 10 for UF_D.

References:

- NCI (National Cancer Institute). 1979. Bioassay of p-chloroaniline for possible carcinogenicity. NCI Carcinogenesis Tech. Rep. Ser. No. 189. NTIS PB 295896.
- NTP (National Toxicology Program). 1989. Toxicology and carcinogenesis studies of parachloroaniline hydrochloride (CAS No. 20265-96-7) in F344/N rats and B6C3F1 mice (gavage studies). NTP-TR-351. NIH Pub. No. 89-2806.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0320_summary.pdf#nameddest=rfd
- U.S. EPA. (2008) Provisional peer-reviewed toxicity values for p-chloroaniline (CASRN 106-47-8). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Chloroanilinep.pdf

p-Cresol (CASRN 106-44-5). The chronic-duration MRL (ATSDR 2008) was based on a 2-year study that used a mixture of p- and m-cresols (CASRNs 95-48-7, 108-39-4, 1319-77-3, and 106-44-5); ATSDR considers the BRRC (1998) study, used in the PPRTV assessment to derive a subchronic p-RfD, an acute toxicity study (as per ATSDR policy). The PPRTV value is selected based on new information (i.e., data from a study not considered relevant to the ATSDR chronic-duration MRL).

Summary Table for p-Cresol (CASRN 106-44-5)		
Source (Year)	**ATSDR (2008)**	**PPRTV (2010)**
Toxicity Value	Chronic-duration MRL	Subchronic p-RfD
Critical Study	NTP 2008	BRRC 1988
Species/Strain/Sex	B6C3F1 mice (50/sex/group)	New Zealand White rabbits (14 females/group)
Study Duration	2 years	GDs 6-18
Critical Effect(s)	Bronchiolar hyperplasia of the lung and thyroid follicular degeneration	Mortality and clinical signs

POD	LOAEL = 100 mg/kg-day	NOAEL = 5 mg/kg-day
Composite UF	1000[a]	300[b]
Toxicity Value (mg/kg-day)	0.1	0.02
Selected Value (mg/kg-day)	0.02	
Rationale	Different study	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L
[b]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_D.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2003). Toxicological profile for cresols. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp34.pdf
- BRRC (Bushy Run Research Center). (1988) Developmental toxicity evaluation of o-, m- or p-cresol administered by gavage to rabbits and rats with cover letter dated 07/06/88. Final Project Report 51-508. TSCA Section 4 Submission. U.S. EPA Doc. No. 40-8860253. Fiche No. OTS0517695.
- NTP. 2008. Toxicology and carcinogenesis studies of cresols (CAS No. 1319-77-3) in male F344/N rats and female B6C3F1 mice (feed studies). Research Triangle Park, NC: National Toxicology Program. TR-550. Draft technical report.
- U.S. EPA. (2010) Provisional peer-reviewed toxicity values for 4-methylphenol (p-cresol) (CASRN 106-44-5). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Methylphenol4.pdf

Ethyl Acetate (CASRN 141-78-6). The IRIS assessment and the PPRTV are based on the same study (American Biogenics 1986), but the PPRTV assessment (2013) used body weight$^{3/4}$ to calculate a human equivalent dose and reduced the interspecies UF to 3. The PPRTV value is selected based on updated methodology.

Summary Table for Ethyl Acetate (CASRN 141-78-6)		
Source (Year)	**IRIS (1987)**	**PPRTV (2013)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	American Biogenics 1986	
Species/Strain/Sex	Sprague-Dawley rats (30/sex/group)	
Study Duration	90 days	
Critical Effect(s)	Mortality and body weight loss	Clinical signs
POD	NOAEL = 900 mg/kg-day	$NOAEL_{[HED]}$ = 216 mg/kg-day
Composite UF	1000[a]	300[b]
Toxicity Value (mg/m^3)	0.9	0.7
Selected Value (mg/m^3)	0.7	
Rationale	Updated methodology	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_D.

References:

- American Biogenics Corporation. (1986) Rat oral subchronic study with ethyl acetate. Office of Solid Waste, U.S. Environmental Protection Agency, Washington, DC. 699273 (Cited in IRIS as: U.S. EPA. 1986. Rat oral subchronic study with ethyl acetate. Office of Solid Waste, Washington, DC.)
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0157_summary.pdf
- U.S. EPA. (2013) Provisional peer-reviewed toxicity values for ethyl acetate (CASRN 141-78-6) a. Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/EthylAcetate.pdf

Ethylbenzene (CASRN 100-41-4). The PPRTV assessment (2009) used BMD methodology and a toxicity study that was not available at the time of the IRIS assessment (1987). The PPRTV value is selected based on new information and the use of updated methodology.
*Note: The IRIS program is updating this assessment.

Summary Table Ethylbenzene (CASRN 100-41-4)

Source (Year)	IRIS (1987)	PPRTV (2009)
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Wolf et al. 1956	Mellert et al. 2007
Species/Strain/Sex	Albino rats (10 females/group and 20 female controls)	Wistar rats (10/sex/group)
Study Duration	5 days/week for 182 days	7 days/week for 13 weeks
Critical Effect(s)	Liver and kidney toxicity	Centrilobular hepatocyte hypertrophy (males)
POD	NOAEL = 97.1 mg/kg-day	$BMDL_{10}$ = 48 mg/kg-day
Composite UF	1000[a]	1000[b]
Toxicity Value (mg/kg-day)	0.1	0.05
Selected Value (mg/kg-day)	0.05	
Rationale	New study; updated methodology	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_D.

References:

- Mellert, W., K. Deckardt, W. Kaufmann et al. 2007. Ethylbenzene: 4- and 13-week rat oral toxicity. Arch. Toxicol. 81:361–370.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0051_summary.pdf
- U.S. EPA. (2009) Provisional peer-reviewed toxicity values for ethylbenzene (CASRN 100-41-4). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Ethylbenzene.pdf
- Wolf, M.A., V.K. Rowe, D.D. McCollister, R.L. Hollingsworth and F. Oyen. 1956. Toxicological studies of certain alkylated benzenes and benzene. Arch. Ind. Health. 14: 387-398.

Ethylene Glycol (CASRN 107-21-1). ATSDR (2010) used a new study (Neeper-Bradley et al. 1995) that was not available when the IRIS assessment was completed (1987). In addition, the ATSDR assessment used BMD modeling to determine the POD (i.e., updated methodology). The ATSDR value is selected based on new information and the use of updated methodology.

Summary Table Ethylene Glycol (CASRN 107-21-1)		
Source (Year)	IRIS (1987)	ATSDR (2010)
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	DePass et al. 1986	Neeper-Bradley et al. 1995; Tyl 1989
Species/Strain/Sex	F344 rats (30/sex/group)	CD-1 mice (30 females/group)
Study Duration	2 years	GDs 6-15
Critical Effect(s)	Kidney toxicity	Bilateral extra lumbar ribs (offspring)
POD	NOEL = 200 mg/kg-day	$BMDL_{10}$ = 75.59 mg/kg-day
Composite UF	100[a]	100[b]
Toxicity Value (mg/kg-day)	2	0.8
Selected Value (mg/kg-day)	0.8	
Rationale	New study; updated methodology	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2010). Toxicological profile for ethylene glycol. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp96.pdf
- DePass, L.R., R.H. Garman, M.D. Woodside, et al. 1986a. Chronic toxicity and oncogenicity studies of ethylene glycol in rats and mice. Fund. Appl. Toxicol. 7: 547-565.
- Neeper-Bradley TL, Tyl RW, Fisher LC, et al. 1995. Determination of a no-observed-effect level for developmental toxicity of ethylene glycol administered by gavage to CD rats and CD-1 mice. Fundam Appl Toxicol 27:121-130.
- Tyl RW. 1989. Developmental toxicity evaluation of ethylene glycol administrated by gavage to CD-1 mice:

Determination of a "no-observed-effect-level" (NOEL). Bushy Run Research Center, CMA Project Report 51-591.

- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0238_summary.pdf

Heptachlor (CASRN 76-44-8). The ATSDR assessment derived a toxicity value based on developmental toxicity studies (Smialowicz et al. 2000; Moser et al. 2001) that evaluated sensitive endpoints and were not available when the IRIS assessment was completed (1987). The ATSDR value is selected based on the evaluation of sensitive (developmental) endpoints and new information.

Summary Table Heptachlor (CASRN 76-44-8)		
Source (Year)	**IRIS (1987)**	**ATSDR (2007)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Velsicol Chemical 1955	Smialowicz et al. 2001†; Moser et al. 2001*
Species/Strain/Sex	CF white rats (20/sex/group)	Sprague-Dawley rats (15 to 20 females/group)
Study Duration	2 years	†GD 12-PND 71; pups exposed to day 42 *GD 12-PND 7 (dams); *PND 7-PND 21 or 42 (pups)
Critical Effect(s)	Increased liver weight (males)	†Immunological and *neurological effects
POD	NOEL = 0.15 mg/kg-day	LOAEL = 0.03 mg/kg-day
Composite UF	300[a]	300[b]
Toxicity Value (mg/kg-day)	0.0005	0.0001
Selected Value (mg/kg-day)	0.0001	
Rationale	New study	

[a]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_D.
[b]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2007). Toxicological profile for heptachlor and heptachlor epoxide. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp12.pdf
- Moser VC, Shafer TJ, Ward TR, et al. 2001. Neurotoxicological outcomes of perinatal heptachlor exposure in the rat. Toxicol Sci 60(2):315-326.
- Smialowicz RJ, Williams WC, Copeland CB, et al. 2001. The effects of perinatal/juvenile heptachlor exposure on adult immune and reproductive system function in rats. Toxicol Sci 61(1):164-175.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0243_summary.pdf
- Velsicol Chemical Corporation. 1955a. MRID No. 00062599. Available from EPA. Write to FOI, EPA, Washington, DC 20460.

Hexachlorobenzene (CASRN 118-74-1). The PPRTV (2010) derived a toxicity value from a study (Bourque et al. 1995) that was not available when the IRIS assessment was completed (1988). The PPRTV value is selected based on new information.

Summary Table Hexachlorobenzene (CASRN 118-74-1)		
Source (Year)	**IRIS (1988)**	**PPRTV (2010)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Arnold et al. 1985	Bourque et al. 1995
Species/Strain/Sex	Sprague-Dawley rats (50/sex/group)	Cynomolgus monkeys (4 females/group)
Study Duration	130 weeks	13 weeks
Critical Effect(s)	Liver effects	Degenerative changes in primary and growing ovarian follicles

POD	NOAEL = 0.08 mg/kg-day	LOAEL = 0.01 mg/kg-day
Composite UF	100[a]	1000[b]
Toxicity Value (mg/kg-day)	0.0008	0.00001
Selected Value (mg/kg-day)	0.00001	
Rationale	New study	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Arnold, D.L., C.A. Moodie, S.M. Charbonneau, et al. 1985. Long-term toxicity of hexachlorobenzene in the rat and the effect of dietary Vitamin A. Fd. Chem. Toxic. 23(9): 779- 793.
- Bourque, AC; Singh, A; Lakhanpal, N; et al. (1995) Ultrastructural changes in ovarian follicles of monkeys administered hexachlorobenzene. Am J Vet Res 56:1673–1677.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0374_summary.pdf
- U.S. EPA. (2010) Provisional peer-reviewed toxicity values for hexachlorobenzene (CASRN 118-74-1). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Hexachlorobenzene.pdf

Hexachlorocyclohexane, gamma (CASRN 58-89-9). The ATSDR assessment (2005) derived a toxicity value based on a study (Meera et al. 1992) that was not available when the IRIS assessment was completed (1987). The ATSDR value is based on the use of a newer and longer study (24-week study). Despite the use of fewer animals (6 females/group), the study is of higher quality than the principal study used for the IRIS assessment (Zoecon Corp. 1983) and identified a lower POD. The ATSDR value is selected based on new information.

Summary Table Hexachlorocyclohexane (CASRN 58-89-9)		
Source (Year)	**IRIS (1987)**	**ATSDR (2005)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Zoecon Corp. 1983	Meera et al. 1992
Species/Strain/Sex	Wistar KFM-Han rats (20/sex/group)	Swiss mice (6 females/group)
Study Duration	12 weeks; 5/sex/group maintained on control diet for an additional 6 weeks	Up to 24 weeks
Critical Effect(s)	Liver and kidney toxicity (females)	Reduced activity of lymphoid follicles with prominent megakaryocytes and delayed hypersensitivity to immune challenge
POD	NOAEL = 0.33 mg/kg-day	LOAEL = 0.012 mg/kg-day
Composite UF	1000[a]	1000[b]
Toxicity Value (mg/kg-day)	0.0003	0.00001
Selected Value (mg/kg-day)	0.00001	
Rationale	New study	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2005). Toxicological profile for alpha-, beta-, gamma-, and delta-hexachlorocyclohexane. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp43.pdf
- Meera P, Rao PR, Shanker R, et al. 1992. Immunomodulatory effects of γ-HCH (lindane) in mice. Immunopharmacol Immunotoxicol 14:261-282.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0065_summary.pdf
- Zoecon Corporation. 1983. MRID No. 00128356. Available from EPA. Write to FOI, EPA, Washington D.C. 20460.

1,2,4,5-Tetrachlorobenzene (CASRN 95-94-3). The PPRTV (2013) derived a toxicity value from a 28-day study that identified a LOAEL (0.041 mg/kg-day for thyroid effects) lower than the NOAEL (0.34 mg/kg-day for liver effects) in a chronic study used in the IRIS assessment (1987). The Chu et al. (1983) study, while mentioned in the IRIS assessment summary, was not considered for the derivation of a chronic RfD (duration < 90 days). In addition, the PPRTV assessment used body weight$^{3/4}$ to derive the POD. The PPRTV value is selected based on the use of updated methodology.

Summary Table 1,2,4,5-Tetrachlorobenzene (CASRN 95-94-3)		
Source (Year)	**IRIS (1987)**	**PPRTV (2013)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Chu et al. 1984	Chu et al. 1983
Species/Strain/Sex	Weanling Sprague-Dawley rats (15/sex/group)	Sprague-Dawley rats (10/sex/group)
Study Duration	13 weeks	28 days
Critical Effect(s)	Kidney lesions	Thyroid toxicity (males)
POD	NOAEL = 0.34 mg/kg-day	$LOAEL_{[HED]}$ = 0.0098 mg/kg-day
Composite UF	1000[a]	300[b]
Toxicity Value (mg/kg-day)	0.0003	0.00003
Selected Value (mg/kg-day)	0.00003	
Rationale	Different study; updated methodology	

[a]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Chu, I; Villeneuve, D; Secours, V; Valli, VE. (1983) Comparative toxicity of 1,2,3,4-, 1,2,4,5-, and 1,2,3,5-tetrachlorobenzene in the rat: results of acute and subacute studies. J Toxicol Environ Health 11(4−6):663−677. 677338.

- Chu, I., D.C. Villeneuve, V.E. Valli and V.E. Secours. 1984. Toxicity of 1,2,3,4-, 1,2,3,5- and 1,2,4,5-tetrachlorobenzene in the rat: Results of a 90- day feeding study. Drug Chem. Toxicol. 7: 113-127.

- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0107_summary.pdf

3. Decisions that Require No Change in the RSLs (Inhalation).

Ammonia (CASRN 7664-41-7). The IRIS chronic RfC (2016) and the PPRTV subchronic p-RfC (2005) are based on the same study (Holness et al. 1989). However, the IRIS assessment included more details with respect to the occupational cohort and used a different approach to select the POD. The IRIS assessment also indicated that, although there are no developmental toxicity studies and studies of reproductive and other systemic endpoints are limited, the likelihood of effects at the RfC is small because: 1) ammonia is endogenously produced in humans and animals, and changes in blood ammonia levels at the POD would be small relative to normal blood ammonia levels; and 2) EPA is not aware of any mechanisms by which ammonia can exert effects at the point of contact (the respiratory system) that could directly or indirectly affect tissues distal to the point of contact. The more recent and highly-peer reviewed IRIS value (2016) is chosen as a Tier 1 value using standard EPA methods (e.g., application of UFs). The IRIS value is retained based on updated methodology.

Summary Table for Ammonia (CASRN 7664-41-7)		
Source (Year)	**IRIS (2016)**	**PPRTV (2005)**
Toxicity Value	Chronic RfC	Subchronic p-RfC
Critical Study	Holness et al. 1989	
Species/Strain/Sex	52 humans; occupationally exposed	
Study Duration	Average = 12.2 years	
Critical Effect(s)	Decreased lung function and respiratory symptoms	
POD	$NOAEL_{[HEC]}$ = 4.9 mg/m^3	$NOAEL_{[HEC]}$ = 2.3 mg/m^3
Composite UF	10[a]	30[b]
Toxicity Value (mg/m^3)	0.5	0.1
Selected Value (mg/m^3)	0.5	
Rationale	Updated IRIS assessment (including methodology and application of UFs)	

[a]The composite UF of 10 is based on 10 for UF_H.
[b]The composite UF of 30 if based on 10 for UF_H, and 3 for UF_D.

References:

- Holness, D.L., J.T. Purdham and J.R. Nethercott. 1989. Acute and chronic respiratory effects of occupational exposure to ammonia. Am. Ind. Hyg. Assoc. J. 50: 646-650.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0422_summary.pdf#nameddest=rfc
- U.S. EPA. (2005) Provisional peer-reviewed toxicity values for ammonia (CASRN 7664-41-7). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Ammonia.pdf

Chlordane (CASRN 12789-03-6). Minor hepatic effects (hepatocellular vacuolization and hypertrophy) identified as adverse by ATSDR (1994) were not considered biologically significant in the IRIS assessment (1998). The IRIS assessment considered the NOAEL to be 1.0 mg/m^3 (rather than 0.1 mg/m^3), adjusted for continuous exposure, applied the regional deposited dose ratio (RDDR) to extrapolate from rats to humans, lowered the UF for interspecies extrapolation (UF_A) from 10 to 3, and accounted for the lack of a reproduction study (UF_D). The IRIS value is retained based on the use of updated methodology.

Summary Table for Chlordane (CASRN 12789-03-6)		
Source (Year)	**IRIS (1998)**	**ATSDR (1994)**
Toxicity Value	Chronic RfC	Intermediate-duration MRL
Critical Study	Khasawinah et al. 1989	
Species/Strain/Sex	Wistar rats (35 to 47/sex/group)	
Study Duration	8 hours/day, 5 days/week, for 13 weeks	
Critical Effect(s)	Increased liver weight and changes in blood chemistry	Mild liver lesions and changes in blood chemistry
POD	$NOAEL_{[HEC]}$ = 0.65 mg/m^3	NOAEL[ADJ] = 0.024 mg/m^3
Composite UF	1000[a]	100[b]

Toxicity Value (mg/m^3)	0.0007	0.0002
Selected Value (mg/m^3)	0.0007	
Rationale	Updated methodology	

[a]The composite UF of 1000 is based on 3 for UF_A, 3 for UF_D, 10 for UF_H, and 10 for UF_S.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1994). Toxicological profile for chlordane. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp31.pdf
- Khasawinah, A., C. Hardy, and G. Clark. 1989. Comparative inhalation toxicity of technical chlordane in rats and monkeys. J. Toxicol. Environ. Health 28(3): 327-347. (The 90-day rat study.)
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0142_summary.pdf#nameddest=rfc

1,1-Dichloroethylene (CASRN 75-35-4). Compared to the ATSDR intermediate-duration MRL (1994), the IRIS chronic RfC (2002) was based on a later study that evaluated a more comprehensive set of endpoints, used the 1994 Inhalation Dosimetry approach to calculate the HEC, and used BMD methodology. Chronic-duration studies (e.g., Quast et al. 1986) were not considered in the derivation of an intermediate-duration MRL. ATSDR did not derive a chronic-duration MRL based on the Quast et al. (1986) study because a serious LOAEL was identified for developmental effects following acute-duration exposure at a lower exposure concentration, precluding the derivation of a chronic-duration MRL. The IRIS value is retained based on new information (i.e., a study not considered for the derivation of the intermediate-duration MRL) and the use of updated methodology.

Summary Table for 1,1-Dichloroethylene (CASRN 75-35-4)		
Source (Year)	**IRIS (2002)**	**ATSDR (1994)**
Toxicity Value	Chronic RfC	Intermediate-duration MRL
Critical Study	Quast et al. 1986	Prendergast et al. 1967
Species/Strain/Sex	Sprague-Dawley rats (86 animals/group)	Hartley guinea pigs (15/group)
Study Duration	6 hours/day, 5 days/week, for up to 18 months	24 hours/day for 90 days
Critical Effect(s)	Liver toxicity (fatty change)	Liver effects (increased ALT and AP; decreased lipid content)
POD	$BMDL_{[10HEC]}$ = 6.9 mg/m^3	NOAEL = 5 ppm
Composite UF	30[a]	300[b]
Toxicity Value	0.2 mg/m^3	0.02 ppm (0.08 mg/m^3)
Selected Value (mg/m^3)	0.2	
Rationale	Different study and updated methodology	

[a]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.
[b]The composite UF of 300 if based on 10 for UF_H, 10 for UF_A, and 3 as a modifying factor to account for the close proximity of serious effects observed at the range of 10-25 ppm.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1994). Toxicological profile for 1,1-dichloroethene. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp39.pdf
- Prendergast JA, Jones RA, Jenkins LJ, et al. 1967. Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene. Toxicol Appl Pharmacol 10:270-289.
- Quast, JF; Mckenna, MJ; Rampy, LW; et al. (1986) Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats. Fundam Appl Toxicol 6:105-144.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0039_summary.pdf#nameddest=rfc

Methyl *tert*-Butyl Ether (MTBE) (CASRN 1634-04-4). The IRIS chronic RfC (1993) is based on a chronic inhalation study (Chun et al. 1992); the ATSDR intermediate-duration MRL (1996) is based on a reproductive study (Neeper-Bradley 1991). Each study was evaluated in both assessments; dosimetric conversion factors were used in the IRIS assessment. The other difference between the two toxicity values is the conversion of the ATSDR MRL from ppm to mg/m^3. Rounded to one significant figure, the two reference values are identical. The RSL tables round to two digits and values; however, values are added as shown in the source document. The IRIS value is retained based on a different study (that results in a similar toxicity value as the intermediate-duration MRL) and the use of updated methodology.

Summary Table for MTBE (CASRN 1634-04-4)		
Source (Year)	**IRIS (1993)**	**ATSDR (1996)**
Toxicity Value	Chronic RfC	Intermediate-duration MRL
Critical Study	Chun et al. 1992	Neeper-Bradley 1991
Species/Strain/Sex	F344 rats (50/sex/group)	CD Sprague-Dawley rats (25/sex/group)
Study Duration	6 hours/day, 5 days/week, for 24 months	6 hours/day, 5 days/week, for up to 19 weeks
Critical Effect(s)	Increased liver and kidney weights; increased severity of renal lesions; clinical signs	Hypoactivity, lack of startle response, blepharospasm
POD	$NOAEL_{[HEC]}$ = 259 mg/m^3	$NOAEL_{[ADJ]}$ = 71 ppm
Composite UF	100[a]	100[b]
Toxicity Value	3 mg/m^3	2.5 mg/m^3 (0.7 ppm)
Selected Value (mg/m^3)	3	
Rationale	Different study; updated methodology	

[a]The composite UF of 100 is based on 3 for UF_A, 3 for UF_D, and 10 for UF_H.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1996). Toxicological profile for methyl *tert*-butyl ether. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp91.pdf
- Chun, J.S., H.D. Burleigh-Flayer, and W.J. Kintigh. 1992. Methyl tertiary butyl ether: vapor inhalation oncogenicity study in Fischer 344 rats (unpublished material). Prepared for the MTBE Committee by Bushy Run Research Center, Union Carbide Chemicals and Plastics Company Inc. Docket No. OPTS- 42098.
- Neeper-Bradley TL. 1991. Two-generation reproduction study of inhaled methyl tert-butyl ether in CD Sprague-Dawley rats. Project ID 53-594. Bushy Run Research Center, Export, PA.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0545_summary.pdf#nameddest=rfc

Nitromethane (CASRN 75-52-5). The PPRTV subchronic and chronic p-RfC (2013) are based on the same study (NTP 1997). However, to derive the chronic p-RFC, the PPRTV used the phase of the study that was longer and used more animals (50/group rather than 10/group). The larger number of animals reduces the confidence limits when the BMD methodology is used. The chronic p-RfC value is retained based on new information (i.e., consideration of the longer/more robust phase of the same principal study).

Summary Table for Nitromethane (CASRN 75-52-5)		
Source (Year)	**PPRTV (2013)**	**PPRTV (2013)**
Toxicity Value	Chronic p-RfC	Subchronic p-RfC
Critical Study	NTP 1997	
Species/Strain/Sex	B6C3F1 mice (50/sex/group)	B6C3F1 mice (10/sex/group)
Study Duration	6.2 hours/day, 5 days/week, for 103 weeks	6.2 hours/day, 5 days/week, for 13 weeks
Critical Effect(s)	Hyaline degeneration of the respiratory epithelium	Hyaline droplets of the respiratory epithelium
POD	$BMDL_{[10HEC]}$ = 1.60 mg/m^3	$BMDL_{[10HEC]}$ = 1.31 mg/m^3
Composite UF	300[a]	300[b]

Toxicity Value (mg/m^3)	0.005	0.004
Selected Value (mg/m^3)	0.005	
Rationale	Same study	

[a]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_D.
[b]The composite UF of 300 is based on 3 for UF_A, 10 for UF_H, and 10 for UF_D.

References:

- NTP (National Toxicology Program). (1997) Toxicology and carcinogenesis studies of nitromethane in F344/N rats and B6C3F1 mice (inhalation studies). U.S. Department of Health and Human Services, Public Health Service, Research Triangle Park, NC; Technical Report Series No 461. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr461.pdf.
- U.S. EPA. (2013) Provisional peer-reviewed toxicity values for nitromethane (CASRN 75-52-5). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://hhpprtv.ornl.gov/issue_papers/Nitromethane.pdf

Vinyl Acetate (CASRN 108-05-4). IRIS (1990) and ATSDR (1992) used 104-week and 90-day studies, respectively. The NOAEL/LOAEL and the toxicological endpoint are the same in both studies. IRIS applied the regional gas dose to respiratory region (RGDR) to extrapolate from rats to humans and lowered the UF for interspecies extrapolation (UF_A) from 10 to 3. ATSDR also applied the RGDR conversion but retained the UF_A at 10. The difference between the toxicity values is the selection of UFs. The decision about the most appropriate value to use does not consider the UFs used (which is subjective) but rather evaluates whether new information was available and/or updated methodology was used. The IRIS value is retained because the intermediate-duration MRL is not based on new information or updated methodology.

Summary Table for Vinyl Acetate (CASRN 108-05-4)		
Source (Year)	**IRIS (1990)**	**ATSDR (1992)**
Toxicity Value	Chronic RfC	Intermediate-duration MRL
Critical Study	Owen 1988; Beems 1988, Dreef-van der Meulen 1988	Hazleton 1980
Species/Strain/Sex	(Crl:CD-1[ICR]BR) mice (90/sex/group)	CD-1 mice (10/sex/group)
Study Duration	6 hours/day, 5 days/week, for 104 weeks	6 hours/day, 5 days/week, for 90 days
Critical Effect(s)	Nasal epithelial lesions	Respiratory effects (inflammation of nasal turbinate epithelium; mild multifocal bronchitis)
POD	$NOAEL_{[HEC]}$ = 5 mg/m^3	$NOAEL_{[HEC]}$ = 5 mg/m^3
Composite UF	30[a]	100[b]
Toxicity Value	0.2 mg/m^3	0.05 mg/m^3 (0.01 ppm)
Selected Value (mg/m^3)	0.2	
Rationale	Different methodology (selection of UFs)	

[a]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1992). Toxicological profile vinyl acetate. Available online at https://www.atsdr.cdc.gov/ToxProfiles/tp59.pdf
- Beems, R.B. 1988. Report No. V 88.133: Histopathology of the respiratory tract of mice used in a 104-week inhalation study (Owen, 1988) with vinyl acetate. (TNO-CIVO Institutes, April 1988).
- Dreef-van der Meulen, H.C. 1988. Report No. V 88.033/270836: Histopathology of the respiratory tract of rats used in a 104 week inhalation study (Owen, 1988) with vinyl acetate: Revised version. (TNO-CIVO Institutes, October 1988).
- Hazleton. 1980. Vinyl acetate: 3 month inhalation toxicity study in the mouse. U.S. EPA/OTS public files. Hazleton Labs Europe Ltd. Document no. FYI-OTS-0184-0278.
- Owen, P.E. 1988. Vinyl acetate: 104 week inhalation combined chronic toxicity and carcinogenicity study in the rat and mouse. Report prepared by Hazleton Laboratories Europe Ltd., Harrogate, England for the Society of the Plastics Industry, Inc., New York. Report No.: 5547-51/15. November 1988.

- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0512_summary.pdf#nameddest=rfc

4. Decisions that Require No Change in the RSLs (Oral).

Acrylamide (CASRN 79-06-1). The ATSDR (2012) assessment identified a study with a lower HED than the study used by IRIS (2010). The ATSDR assessment calculated the HED using a PBPK rat model (Sweeney et al. 2010). The rat PBPK model was used to estimate rat dose metrics to predict the rat blood time-weighted average (TWA) acrylamide dose associated with the rat NOAEL of 0.2 mg/kg/day, resulting in an HED of 0.038 mg acrylamide/kg/day The IRIS assessment used BMD modeling to characterize the dose-response relationship and determine the POD, resulting in a $BMDL_{05HED}$ of 0.27 mg/kg/day. A different method (using ADME data) was used to calculate the HED in the IRIS assessment. An internal dose in the rat (area under a time-concentration curve, AUC) was derived from the external exposure to acrylamide based on methods and data that characterize the relationship between hemoglobin adducts, serum levels, and administered dose as reported in several rat studies. The studies were used to estimate the internal dose in rats, to extrapolate to an internal dose in humans, and to estimate the daily human intake of acrylamide needed to produce that internal human dose. Advantages to the IRIS value include 1) effects observed at the LOAEL in Burek et al. (1980) were slight and reversible, 2) the identification of a NOAEL for the Burek et al. (1980) study was limited by the selection of dose levels, 3) the IRIS assessment considered Burek et al. (1980) and other chronic-duration studies and used updated methodology when the toxicity value was updated in 2010, and 4) the selection of the IRIS value adheres to the toxicity hierarchy (and there is no overwhelming evidence to switch to a similar toxicity value based on subchronic exposure). The IRIS value was retained based on a different study and updated methodology.

Summary Table for Acrylamide (CASRN 79-06-1)		
Source (Year)	**IRIS (2010)**	**ATSDR (2012)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Johnson et al. 1986	Burek et al. 1980
Species/Strain/Sex	F344 rats (90/sex/group)	F344 rats (10/sex/group)
Study Duration	Up to 2 years	Up to 93 days
Critical Effect(s)	Degenerative nerve changes	Neurological effects (ultrastructural degeneration in sciatic nerve fibers)
POD	$BMDL_{[05HED]}$ = 0.053 mg/kg-day	$NOAEL_{[HED]}$ = 0.038 mg/kg-day
Composite UF	30[a]	30[b]
Toxicity Value (mg/kg-day)	0.002	0.001
Selected Value (mg/kg-day)	0.002	
Rationale	Updated methodology	

[a]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.
[b]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (1996). Toxicological profile for acrylamide. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp203.pdf
- Burek JD, Albee RR, Beyer JE, et al. 1980. Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. J Environ Pathol Toxicol 4(5- 6):157-182.
- Johnson KA; Gorzinski SJ; Bodner KM; Campbell RA; Wolf CH; Friedman MA; Mast RW (1986). Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol, 85: 154-168.
- Sweeney LM, Kirman CR, Gargas ML, et al. 2010. Development of a physiologically-based toxicokinetic model of acrylamide and glycidamide in rats and humans. Food Chem Toxicol 48(2):668- 685.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0286_summary.pdf#nameddest=rfd

Acrylic Acid (CASRN 79-10-7). The PPRTV (2010) and IRIS (1994) assessments used the same study. However, the PPRTV included a UF_D of 3 to account for the lack of a developmental study by oral exposure. The PPRTV assessment noted that BMD modeling could not be performed because of the lack of measured variation for the critical endpoint. The assessment relied heavily on the published version of the study (Hellwig et al. 1997); additional data are available in the unpublished version (BASF 1993). The IRIS assessment indicated that an uncertainty factor for database inadequacy was not considered necessary owing to evidence from bioavailability studies (oral and intravenous routes) that there is no difference in the rate of elimination of acrylic acid in rats and mice. The IRIS value was retained because the PPRTV value is not based on new information or updated methods.

Summary Table for Acrylic Acid (CASRN 79-10-7)		
Source (Year)	**IRIS (1994)**	**PPRTV (2010)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	BASF 1993, Hellwig 1997	
Species/Strain/Sex	Wistar rats (25/sex/group)	
Study Duration	Two generations	
Critical Effect(s)	Reduced pup weight	
POD	NOAEL = 53 mg/kg-day	
Composite UF	100[a]	300[b]
Toxicity Value (mg/kg-day)	0.5	0.2
Selected Value (mg/kg-day)	0.5	
Rationale	Retain IRIS based upon application of UFs	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_D.

References:

- BASF (Badische Anilin- und Sodafabrik). 1993. Reproduction toxicity study with acrylic acid in rats: Continuous administration in the drinking water over 2 generations (1 litter in the first and 1 litter in the second generation). Project No. 71R0114/92011. BASF Aktiengesellschaft, Dept. of Toxicology, Rhein, FRG.
- Hellwig, J; Gembardt, C; Murphy, SR. (1997) Acrylic acid: Two-generation reproduction toxicity study in Wistar rats with continuous administration in the drinking water. Food Chem. Toxicol. 35(9):859–868.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0002_summary.pdf#nameddest=rfd
- U.S. EPA. (2010) Provisional peer-reviewed toxicity values for acrylic acid (CASRN 79-10-7). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/AcrylicAcid.pdf

1-1'-Biphenyl (CASRN 92-52-4). IRIS (2013) selected a different study to derive the RfD. In the IRIS assessment, the $BMDL_{10HEC}$ for kidney effects (Umeda et al. 2002) was lower than the $BMDL_{05}$ for developmental effects from Khera et al. (1979). The BMDL value selected by IRIS (2013) for Khera et al. (1979) was different than the BMDL value selected for the PPRTV due to differences in the critical endpoints selected. IRIS modeled the litter incidence of missing or unossified sternabrae (as the only anomaly that exhibited a dose-related increase when considered individually), whereas the PPRTV (2009) modeled the litter incidence of skeletal anomalies (combined). The PPRTV did not derive a chronic p-RfD; the PPRTV indicated that, "IRIS has derived a chronic value of 0.05 mg/kg-day based on a chronic-duration toxicity study of albino rats by Ambrose et al. (1960) with kidney damage as the critical effect. The IRIS database (U.S. EPA, 2010) should be checked to determine if any changes have been made" (the assessment has since been updated). The IRIS value was retained because the PPRTV value is not based on new information or updated methods.

Summary Table for 1,1'-Biphenyl (CASRN 92-52-4)		
Source (Year)	**IRIS (2013)**	**PPRTV (2011)**

Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Umeda et al. 2002	Khera et al. 1979
Species/Strain/Sex	F344 rats (50/sex/group)	Wistar rats (18-20 females/group)
Study Duration	2 years	GDs 6-15
Critical Effect(s)	Renal papillary mineralization (males)	Increased incidence of litters with fetal skeletal anomalies
POD	$BMDL_{10HED}$ = 13.9 mg/kg-day	$BMDL_{05}$ = 9.59 mg/kg-day
Composite UF	30[a]	100[b]
Toxicity Value (mg/kg-day)	0.5	0.1
Selected Value (mg/kg-day)	0.5	
Rationale	Different study	

[a]The composite UF of 30 is based on 3 for UF_A, and 10 for UF_H.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Khera, KS; Whalen, C; Angers, G; et al. (1979) Assessment of the teratogenic potential of piperonyl butoxide, biphenyl, and phosalone in the rat. Toxicol Appl Pharmacol 47(2):353−358.
- Umeda, Y; Arito, H; Kano, H; Ohnishi, M; Matsumoto, M; Nagano, K; Yamamoto, S; Matsushima, T. (2002). Two-year study of carcinogenicity and chronic toxicity of biphenyl in rats. J Occup Health 44: 176-183.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0013_summary.pdf
- U.S. EPA. (2009) Provisional peer-reviewed toxicity values for 1,1'-biphenyl (CASRN 92-52-4). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Biphenyl11.pdf

Cyclohexanone (CASRN 108-94-1). The PPRTV included a UF_D of 3 whereas the IRIS assessment did not because it was not EPA practice at that time. BMD modeling could not be conducted because the body weight data were not provided in the principal study. The PPRTV value is based on a pilot study for the longer study (by the same authors) that forms the basis for the IRIS assessment. The PPRTV study was based on smaller group numbers (5/sex/group compared to 52/sex/group) and over a shorter duration (25 weeks compared to 2 years). The IRIS value was retained because the PPRTV value is not based on new information or updated methods.

Summary Table for Cyclohexanone (CASRN 108-94-1)		
Source (Year)	**IRIS (1987)**	**PPRTV (2010)**
Toxicity Value	Chronic RfD	Subchronic p-RfD
Critical Study	Lijinsky and Kovatch 1986	
Species/Strain/Sex	F344 rats (52/sex/group)	F344 rats (5/sex/group)
Study Duration	2 years	25 weeks
Critical Effect(s)	Depression in body-weight gain (both sexes)	Decreased weight gain (males)
POD	NOAEL = 462 mg/kg-day	NOAEL = 731 mg/kg-day
Composite UF	100[a]	300[b]
Toxicity Value (mg/kg-day)	5	2
Selected Value (mg/kg-day)	5	
Rationale	Different (phase of) study	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_D.

References:

- Lijinsky, W. and M. Kovatch. 1986. A chronic toxicity study of cyclohexanone in rats and mice (NCI study). J. Natl. Cancer Inst. 77(4): 941-949.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0219_summary.pdf#nameddest=rfd

- U.S. EPA. (2010) Provisional peer-reviewed toxicity values for cyclohexanone (CASRN 108-94-1). Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. Available online at https://cfpub.epa.gov/ncea/pprtv/documents/Cyclohexanone.pdf

Endosulfan (CASRN 115-29-7). ATSDR (2015) used a lower NOAEL from a study (Banerjee and Hussain 1986) showing depressed immune response that was presumably not considered in the IRIS assessment (i.e., not mentioned in the IRIS summary documentation). Advantages to the IRIS value include 1) it is not known why the principal study used for derivation of the ATSDR MRL was not evaluated by the IRIS program, 2) data for depressed immune response in rats are not amenable to benchmark dose modeling (i.e., updated methodology was not used); and 3) selection of the IRIS value adheres to the toxicity hierarchy (and there is no overwhelming evidence to switch to a similar toxicity value based on subchronic exposure). The IRIS value was retained because the ATSDR value is not based on new information or updated methods.

Summary Table for Endosulfan (CASRN 115-29-7)		
Source (Year)	**IRIS (1994)**	**ATSDR (2015)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Hoechst Celanese Corp 1989	Banerjee and Hussain 1986
Species/Strain/Sex	Sprague-Dawley rats (50/sex/group)	Wistar rats (10 to 12 males/group)
Study Duration	2 years	Up to 22 weeks
Critical Effect(s)	Decreased body weight gain; increased incidence of marked progressive glomerulonephrosis and blood vessel aneurysms (males)	Depressed immune response
POD	NOAEL = 0.6 mg/kg-day	NOAEL = 0.45 mg/kg-day
Composite UF	100[a]	100[b]
Toxicity Value (mg/kg-day)	0.006	0.005
Selected Value (mg/kg-day)	0.006	
Rationale	Retain IRIS value	

[a]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.
[b]The composite UF of 100 is based on 10 for UF_A, and 10 for UF_H.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2015). Toxicological profile for endosulfan. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp41.pdf
- Banerjee BD, Hussain QZ. 1986. Effect of sub-chronic endosulfan exposure on humoral and cell-mediated immune responses in albino rats. Arch Toxicol 59:279-284.
- Hoechst Celanese Corporation. 1989. MRID No. 40256502, 41099502. HED Doc. No. 007937. Available from EPA. Write to FOI, EPA, Washington, DC 20460.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0235_summary.pdf#nameddest=rfd

Ethylene Glycol Monobutyl Ether (CASRN 111-76-2). IRIS (2010) derived a toxicity value from a chronic inhalation study (NTP 2000) using a PBPK model and BMD methodology to calculate the HED. The ATSDR assessment derived a toxicity value from a 13-week study (NTP 1993) with no NOAEL. The IRIS value was retained based on updated methodology.

Summary Table Ethylene Glycol Monobutyl Ether (CASRN 111-76-2)		
Source (Year)	**IRIS (2010)**	**ATSDR (2010)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	NTP 2000	NTP 1993
Species/Strain/Sex	F344/N rats and B6C3F1 mice (50/sex/group)	F344/N rats (10/sex/group)
Study Duration	6 hours/day, 5 days/week, for 2 years (inhalation)	13 weeks

Critical Effect(s)	Hemosiderin deposition in the liver	Hepatic effects
POD	BMDL[HED] = 1.4 mg/kg-day	LOAEL = 69 mg/kg-day
Composite UF	10[a]	1000[b]
Toxicity Value (mg/kg-day)	0.1	0.07
Selected Value (mg/kg-day)	0.1	
Rationale	Different study; updated methodology	

[a]The composite UF of 10 is based on 10 for UF_H.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2010). Toxicological profile for 2-butoxyethanol and 2-butoxyethanol acetate. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp118.pdf
- NTP (National Toxicology Program) (2000) NTP technical report on the toxicology and carcinogenesis studies of 2 butoxyethanol (CAS No. 111 76 2) in F344/N rats and B6C3F1 mice (inhalation studies).
- NTP. 1993. Ethylene glycol ethers, 2-ethoxyethanoI, 2-butoxyethanol administered in drinking water to F344/N rats and B6C3Fl mice. NTP toxicity report series no. 26. National Toxicology Program, National Institutes of Health, Public Health Services, U.S. Department of Health and Human Services. NIH publication 93-3349.
- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0500_summary.pdf

Pentachlorophenol (CASRN 87-86-5). ATSDR (2001) derived chronic and intermediate-duration toxicity values based on a two-generation and one-generation reproduction studies in mink that only used one dose. The studies in mink were not well-conducted and observations in the one-generation study could not be replicated in the two-generation study (as mentioned in the IRIS assessment). The IRIS value was retained because the ATSDR value is not based on new information or updated methodology; the principal study is not considered reliable.

Summary Table Pentachlorophenol (CASRN 87-86-5)		
Source (Year)	**IRIS (2010)**	**ATSDR (2001)**
Toxicity Value	Chronic RfD	Intermediate-duration MRL
Critical Study	Mecler 1996	Beard et al. 1997
Species/Strain/Sex	Beagle dogs (4/sex/dose)	Mink (10 females/group)
Study Duration	52 weeks	3 weeks prior to mating and throughout pregnancy and lactation
Critical Effect(s)	Hepatotoxicity	Reproductive effects
POD	LOAEL = 1.5 mg/kg-day	LOAEL = 1 mg/kg-day
Composite UF	300[a]	1000[b]
Toxicity Value (mg/kg-day)	0.005	0.001
Selected Value (mg/kg-day)	0.005	
Rationale	Different study and methodology	

[a]The composite UF of 300 is based on 10 for UF_A, 10 for UF_H, and 3 for UF_L.
[b]The composite UF of 1000 is based on 10 for UF_A, 10 for UF_H, and 10 for UF_L.

References:

- Agency for Toxic Substances and Disease Registry (ATSDR) (2001). Toxicological profile pentachlorophenol. Available online at https://www.atsdr.cdc.gov/toxprofiles/tp51.pdf
- Beard AP, McRae AC, Rawlings NC. 1997. Reproductive efficiency in mink (Mustela vison) treated with the pesticides lindane, carbofuran, and pentachlorophenol. J Reprod Fertil 111:21-28.
- Mecler, F. (1996) Fifty-two week repeated dose chronic oral study of pentachlorophenol administered via capsule to dogs. Study conducted by TSI Mason Laboratories, Worcester, MA; TSI Report #ML-PTF-J31-95-94. Submitted to the Pentachlorophenol Task Force, c/o SRA International, Inc., Washington, DC. U.S. Environmental Protection Agency, Washington, DC; MRID 439827-01. Unpublished report.

- U.S. EPA. (2018). Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0086_summary.pdf

Attachment

1D

1

Vanadium pentoxide; CASRN 1314-62-1

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Vanadium pentoxide

File First On-Line 01/31/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	06/30/1988
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	not evaluated	

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1
Last Revised — 06/30/1988

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased hair cystine **Rat Chronic Oral Study** **Stokinger et al., 1953**	NOAEL: 17.85 ppm converted to 0.89 mg/kg/day LOAEL: none	100	1	9E-3 mg/kg/day

*Conversion Factors: Adult rat food consumption assumed to be 5% bw/day.

I.A.2. Principal and Supporting Studies (Oral RfD)

Stokinger, H.E., W.D. Wagner, J.T. Mountain, F.R. Stacksill, O.J. Dobrogorski and R.G. Keenan. 1953. Unpublished results. Division of Occupational Health, Cincinnati, OH. (Cited in Patty's Industrial Hygiene and Toxicology, 3rd ed., 1981)

In this chronic study, an unspecified number of rats were exposed to dietary levels of 10 or 100 ppm vanadium (about 17.9 or 179 ppm vanadium pentoxide) for 2.5 years. The results of this unpublished study were summarized by Stokinger et al. (1981). The criteria used to evaluate vanadium toxicity were growth rate, survival, and hair cystine content. The only significant change reported was a decrease in the amount of cystine in the hair of animals ingesting vanadium.

Of the subchronic and chronic animal studies available, the lower dose level (17.9 ppm vanadium pentoxide) reported in the Stokinger et al. (1953) study is the highest oral NOAEL upon which an RfD can be derived. An oral RfD of 0.009 mg/kg/day (0.62 mg/day for a 70-kg person) can be calculated by assuming that rats eat food equivalent to 5% of their body weight and by applying an uncertainty factor of 100.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 100 was applied, 10 for interspecies extrapolation and a factor of 10 to provide added protection for unusually sensitive individuals.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

In a subchronic feeding study (Mountain et al., 1953), groups of five male Wistar rats were fed vanadium pentoxide at levels of 0, 25, or 50 ppm for 35 days, after which dietary levels of vanadium were increased to 100 and 150 ppm and continued for 68 days. There was a decrease in the amount of cystine in the hair of the high-dosed (50-150 ppm or 2.5-7.5 mg/kg/day, based on food consumption of 5% bw) rats. A significant decrease was also reported in erythrocyte and hemoglobin levels of the high-dosed rats. In an abstract of a subchronic inhalation study (Suguira, 1978), mice and rats exposed to 1 to 3 mg/cu.m vanadium pentoxide for 3 months, 6 hours/day developed histopathologic changes in their lungs and had a decrease in growth rate. Adverse effects were not detected in either species similarly exposed at 0.1 to 0.4 mg/cu.m.

Although several epidemiologic studies have been conducted on factory workers exposed to vanadium pentoxide for several years, the air concentration levels of vanadium pentoxide were measured only at scattered intervals, making it impossible to determine a minimum effective dose. Also, in cases of humans exposed to relatively high atmospheric concentrations of vanadium pentoxide for short periods of time, all individuals developed respiratory symptoms that usually subsided within 7-14 days.

I.A.5. Confidence in the Oral RfD

Study — Low
Database — Low
RfD — Low

Because of the lack of details in the reference study and the scarcity of data available on vanadium pentoxide, low confidence is assigned to both the study and the database. Low confidence in the RfD follows.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — This assessment is not presented in any existing U.S. EPA document.

Other EPA Documentation — None

Agency Work Group Review — 02/26/1986

Verification Date — 02/26/1986

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Vanadium pentoxide conducted in September 2002 identified one or more significant new studies. IRIS users may request the references for those studies from the IRIS Hotline at hotline.iris@epa.gov or (202)566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1

The NTP (1985) has approved vanadium pentoxide for carcinogenicity testing; however, the route of administration has not been determined (i.e., oral, inhalation).

III. [reserved]

IV. [reserved]

V. [reserved]

VI. Bibliography

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1

VI.A. Oral RfD References

Mountain, J.T., L.L. Delker and H.E. Stokinger. 1953. Studies in vanadium toxicology. Arch. Ind. Hyg. Occup. Med. 8: 406-411.

Stokinger, H.E. 1981. The metals: Vanadium. In: Patty's Industrial Hygiene and Toxicology, 3rd revised ed., Vol. 2A, G.D. Clayton and F.E. Clayton, Eds. John Wiley and Sons, Inc., New York. p. 2013-2033.

Stokinger, H.E., W.D. Wagner, J.T. Mountain, F.R. Stocksill, O.J. Dobrogorski and R.G. Keenan. 1953. No title given. Unpublished results. Division of Occupational Health, Cincinnati, OH. (Cited in: Patty's Industrial Hygiene and Toxicology, 3rd ed., 1981).

Sugiura, S. 1978. Inhalation toxicity of vanadium pentoxide dust in rats and mice. Shikoku Igaku Zasschi. 34(5): 209-219.

VI.B. Inhalation RfC References

None

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1

Date	Section	Description
06/30/1988	I.A.1., II.	NOAEL and RfD corrected, message added to cancer assessment
12/03/2002	I.A.6.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Vanadium pentoxide
CASRN — 1314-62-1
Last Revised — 01/31/1987

- 1314-62-1
- CI 77938
- Divanadium Pentaoxide
- Divanadium Pentoxide
- Vanadic Anhydride
- Vanadium Oxide
- Vanadium Pentaoxide
- Vanadium Pentoxide

Attachment

1D

2

United States Environmental Protection Agency

EPA/690/R-09/070F
Final
9-30-2009

Provisional Peer-Reviewed Toxicity Values for

Vanadium and Its Soluble Inorganic Compounds Other Than Vanadium Pentoxide

(CASRN 7440-62-2 and Others)

Derivation of Subchronic and Chronic Oral RfDs

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Commonly Used Abbreviations

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor
UF_A	animal to human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete to complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR VANADIUM AND ITS SOLUBLE INORGANIC COMPOUNDS OTHER THAN VANADIUM PENTOXIDE (CASRN 7440-62-2 and others)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) U.S. EPA's Integrated Risk Information System (IRIS).
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The U.S. Environmental Protection Agency's (U.S. EPA) Integrated Risk Information System (IRIS) (U.S. EPA, 2008) contains a file for vanadium pentoxide describing a chronic RfD and containing a message about assessing its carcinogenicity—but no chronic RfC. IRIS currently contains no files for elemental vanadium or other vanadium compounds. The Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) does not include an RfD for any vanadium compound. The Health Effects Assessment Summary Table (HEAST; U.S. EPA, 1997) lists subchronic and chronic oral RfDs of 7×10^{-3} mg/kg-day for vanadium and 2×10^{2} mg/kg-day for vanadium sulfate. RfDs for both vanadium and vanadium sulfate were based on a chronic study in which rats were exposed to 5 mg/L vanadium as vanadyl sulfate for their lifetimes (Schroeder et al., 1970), as derived in U.S. EPA (1987). A total UF of 100 was used to derive the RfDs. The Agency for Toxic Substance and Disease Registry (ATSDR, 1992) derived an intermediate-duration oral minimal risk level (MRL) for vanadium of 3×10^{-3} mg/kg-day based on a NOAEL of 0.3 mg/kg-day in a 3-month drinking water study in rats by Domingo et al. (1985); renal and respiratory effects (renal hemorrhagic foci and pulmonary vascular infiltration) were seen at higher doses (0.6 mg/kg-day). A total UF of 100, reflecting UFs of 10 each for interspecies extrapolation and intraspecies variability, was applied to the NOAEL. ATSDR (1992) does not derive a chronic oral MRL.

Neither IRIS (U.S. EPA, 2008) nor the HEAST (U.S. EPA, 1997) reports an RfC for vanadium. ATSDR (1992) derived an acute-duration inhalation MRL of 0.0002 mg/m^3 for vanadium based on a study of human exposure to vanadium pentoxide, but it does not provide inhalation MRLs for other vanadium compounds. The American Conference of Governmental Industrial Hygienists (ACGIH, 2007) lists a time weighted average-threshold limit value (TWA-TLV) of 0.05 mg V_2O_5/m^3 for vanadium pentoxide dust or fume (respirable fraction), with a "notice of intended change" to 0.02 mg V/m^3 (inhalable fraction) based on upper and lower respiratory tract irritation. The National Institute for Occupational Safety and Health (NIOSH, 2008) lists a recommended exposure limit (REL) of 0.05 mg V/m^3 for vanadium

pentoxide dust or fume as a 15-minute ceiling value. NIOSH includes a note that this REL applies to all vanadium compounds except vanadium metal and vanadium carbide—for which a REL of 1 mg/m^3 TWA and 3 mg/m^3 short-term exposure limit (STEL) applies (by analogy to ferrovanadium dust). The Occupational Safety and Health Administration (OSHA, 2008) permissible exposure limit (PEL) applicable to vanadium pentoxide is a ceiling of 0.1 mg V_2O_5/m^3 for fume and 0.5 mg V_2O_5/m^3 for dust.

An assessment of the carcinogenicity of vanadium is not available on IRIS (U.S. EPA, 2008), in the HEAST (U.S. EPA, 1997), or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994) includes a Health Effects Assessment (HEA) for vanadium and compounds (U.S. EPA, 1987) that assigned vanadium to cancer weight-of-evidence Group D (Not Classifiable as to Human Carcinogenicity) based on inconclusive animal data (under U.S. EPA 1986 Guidelines for Carcinogen Risk Assessment). Vanadium has not been evaluated under the U.S. EPA (2005) Guidelines for Cancer Risk Assessment. Vanadium is not included in the 11th Report on Carcinogens available from the National Toxicology Program (NTP, 2005). The International Agency for Research on Cancer (IARC, 2008) has not evaluated vanadium for potential carcinogenicity. For vanadium pentoxide, ACGIH (2007) has posted notice of intended change in cancer notation from A4 (Not Classifiable) to A3 (Confirmed Animal Carcinogen).

To identify toxicological information pertinent to the derivation of provisional toxicity values, literature searches were conducted from 1960s through December 2007 using the following databases: MEDLINE, TOXLINE, BIOSIS, TSCATS1/2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents (prior 6 months). Vanadium pentoxide (CASRN 1314-62-1) was excluded from the search because it has both an IRIS record and a separate PPRTV document. In addition to searching for vanadium and its subheadings in these databases, the following vanadium compounds were specifically included as search terms: vanadyl sulfate (CASRN 27774-13-6), sodium metavanadate (CASRN 13718-26-8), sodium orthovanadate (CASRN 13721-39-6), ammonium vanadate (CASRN 7803-55-6), vanadium sulfate (CASRN 16785-81-2), sodium hexavanadate (CASRN 12436-28-1), sodium tetravanadate (CASRN 1258-74-1), vanadious (4+) acid, disodium salt (CASRN 64082-34-4), vanadium dichloride (CASRN 10580-52-6), vanadium trioxide (CASRN 1314-34-7), and vanadium tetrachloride (CASRN 7632-51-1). Review documents by U.S. EPA (1987), ATSDR (1992), the World Health Organization (WHO, 1988, 2001), and Rydzynski (2001) were also consulted for relevant information. An updated literature search on PubMed was performed on August 17, 2009.

REVIEW OF PERTINENT DATA

Vanadium Compounds Assessed

As noted above, vanadium pentoxide is the subject of both an IRIS record and a separate PPRTV document, which should be used in the toxicity assessment of this particular vanadium compound.

Although vanadium has six oxidation states (-1, 0, +2, +3, +4, and +5), the most stable oxidation state is +4 (Rydzynski, 2001). In the environment, vanadium is bound to a variety of elements including oxygen, sodium, sulfur, and chloride; in commerce, vanadium is often used in an iron alloy (ferrovanadium) (Rydzynski, 2001). The literature searches identified toxicity data for the following inorganic compounds: vanadyl sulfate (+4), sodium metavanadate (+5), sodium orthovanadate (+5), and ammonium metavanadate (+5). Table 1 shows the CASRNs, molecular formulas, molecular weights, and vanadium mass fractions for these compounds. These compounds all exhibit some solubility in water (Rydzynski, 2001; ATSDR, 1992) and, thus, can be considered representative of soluble tetravalent and pentavalent vanadium compounds.

Table 1. Soluble Inorganic Vanadium Compounds Considered in this PPRTV

Compound	Chemical Formula	Vanadium Valence	Molecular Weight (g/mol)	Vanadium Mass Fraction[a]
Vanadium	V	various	50.94	1.0
Vanadyl sulfate trihydrate	$VOSO_4 \cdot (H_2O)_3$	+4	217.06	0.235
Vanadyl sulfate pentahydrate	$VOSO_4 \cdot (H_2O)_5$	+4	253.10	0.201
Ammonium metavanadate or ammonium vanadate	NH_4VO_3	+5	116.99	0.435
Sodium metavanadate or sodium vanadate	$NaVO_3$	+5	121.93	0.418
Sodium orthovanadate or sodium vanadium oxide	Na_3VO_4	+5	183.91	0.277

[a]Molecular weight of vanadium divided by molecular weight of compound.

In recent years, organic vanadium compounds have been synthesized in an effort to enhance the lipophilicity and biological uptake of vanadium for use in treating diabetes and/or cancer. Toxicity data for three organic vanadium compounds were located: bis(maltolato)oxyvanadium(IV) (BMOV), bis(ethylmaltolato)oxyvanadium(IV) (BEOV), and vanadyl acetyl acetonate. Because these compounds have been developed as pharmaceutical agents and are believed to have different absorption and/or toxicokinetic properties than soluble inorganic vanadium salts, they are not considered in this review.

There are three early studies of human exposure to vanadium (Curran et al., 1959; Dimond et al., 1963; and Sommerville and Davies, 1962) that employed compounds reported as "ammonium vanadyl tartrate" and "diammonium oxy-tartrato vanadate" or "diammonium vanado-tartrate." Information provided on the chemical form in the studies is limited to the names and the valence state (+4) for the latter compound (reported by Sommerville and Davies, 1962). Reliable chemical structures and valence states for these compounds have not been located; however, the tartrate component is an organic moiety. Given that the compounds administered in these studies are unknown, it is difficult to estimate vanadium doses from the reported doses of the compounds. Further, because the compounds used in these studies were likely organic in nature and may have exhibited different bioavailability than inorganic vanadium salts, these studies have been excluded from consideration in this review.

Oral exposures to either vanadyl or vanadate result in internal exposures to a mixture of vanadyl and vanadate complexes as a result of reduction/oxidation (redox) reactions that occur in the gastrointestinal tract as well as in the blood and tissues (Rydzynski, 2001; Etcheverry and Cortizo, 1998). Available information suggests that conditions in extracellular fluid favor the formation of vanadate, while intracellular (cytosolic) conditions favor the vanadyl redox state (Rydzynski, 2001). As a result of these physiological interconversions, there is no firm toxicological basis for distinguishing dose-response relationships for these two forms given the currently available data: while toxicology studies can be categorized based on whether humans or animals were exposed to vanadyl or vanadate compounds, target organs and tissues are likely exposed to a mix of these ions. For the purpose of this review, exposure to either the vanadyl or vanadate form is treated as biologically equivalent. Therefore, exposure estimates in all of the toxicity studies have been converted to equivalent vanadium doses for the purpose of dose-response assessment.

In summary, this PPRTV document applies to soluble inorganic vanadyl (+4) and vanadate (+5) compounds other than vanadium pentoxide, which is the subject of an IRIS review and separate PPRTV document. Data are not available to assess the toxicity of insoluble compounds or compounds in which vanadium exists in higher or lower valence states. Organic vanadium compounds are expected to exhibit different toxicokinetic properties than inorganic compounds and should be assessed independently if necessary. Finally, vanadyl and vanadate exposures are considered biologically equivalent (on the basis of equivalent vanadium dose) for the purpose of this review.

Human Studies

The possibility that vanadium may be an essential element for humans remains an unanswered question. Etcheverry and Cortizo (1998) reported that deficiencies in vanadium intake could be associated with alterations in bone structure and development, changes in plasma cholesterol, and changes in reproductive performance. However, WHO (2001) considered the issue unresolved and noted that, if vanadium is essential, required levels are very low (in the range of nanograms per day).

Oral Exposure

Fawcett et al. (1997) administered tablets of vanadyl sulfate trihydrate at a dose of 0.5 mg/kg-day (0.1 mg V/kg-day) for 12 weeks to weight trainers. The treatment and control groups each included 15 males and 5 females. The control group received a daily placebo. Subjects in the control and treatment groups were matched with respect to gender, age, height, weight, and weight-training program (e.g., intensity, schedule). Of those starting the study, 11 males and 4 females in the treatment group and 12 males and 4 females in the control group completed the study. There were two males that withdrew from the study because of self-reported side effects (tiredness and/or aggressiveness while weight training); these two subjects were unremarkable with respect to endpoints assessed in this study. There were four subjects that withdrew because of training-related injuries and three subjects withdrew for other reasons not related to health. Blood pressure was measured and blood samples collected periodically during the exposure period for evaluation of hematology (differential cell counts and blood viscosity tests) and serum chemistry (plasma alanine aminotransferase [ALT] and alkaline phosphatase [ALP], albumin, bilirubin, cholesterol, creatinine, high-density lipoprotein, total protein, triglyceride, and urea). No differences were observed between the treatment and control

groups for the following endpoints: body weight, systolic and diastolic blood pressure, hematology or serum chemistry (all data shown). Without corroborating information, the toxicological relevance of the self-reported symptoms of (tiredness and aggressiveness) is uncertain. The administered dose (0.1 mg V/kg-day) is considered a freestanding NOAEL with respect to the endpoints assessed in the study.

In a study designed to evaluate the safety of vanadyl sulfate as a diabetes treatment, Boden et al. (1996) administered 50 mg capsules of vanadyl sulfate twice daily (100 mg/day) for 4–8 weeks to four men and four women with noninsulin-dependent diabetes mellitus. The specific form of vanadyl sulfate was not reported; assuming vanadyl trihydrate, the corresponding dose of vanadium would be 0.34 mg V/kg-day in men and 0.39 mg V/kg-day in females of average body weight (70 kg and 60 kg, respectively). Of the eight patients, four men and two women were treated with placebo for 4 weeks after the end of vanadium treatment to provide reference data. Patients self-monitored their glucose using a glucometer and were examined weekly at a hospital, where blood was drawn for complete blood count, serum chemistry (glucose, insulin, blood urea nitrogen [BUN], fatty acids, vanadium content), liver and kidney function tests, and urinalysis (urinary nitrogen). Self-reported symptoms were recorded at that time. Glycemic control was assessed during and after the exposure period. Of the eight patients, four reported diarrhea with abdominal cramps and/or flatulence, one reported flatulence alone, and one reported slight nausea. Diarrhea lasted for 11 days in one patient but had abated after the first week in the others. Vanadyl sulfate treatment resulted in statistically significant ($p < 0.05$) decreases in fasting glucose concentration and hepatic glucose output during hyperinsulinemia. There were no effects on total body glucose uptake, glycogen synthesis, glycolysis, carbohydrate oxidation, or lipolysis during the euglycemic-hyperinsulenemic clamps. The study authors reported that weekly blood counts, urinalysis, and liver function tests were not affected by treatment (data not shown). A LOAEL of 0.34–0.39 mg V/kg-day is identified from these data based on gastrointestinal symptoms; no NOAEL is identified.

Goldfine et al. (2000) also investigated the use of vanadyl sulfate to treat noninsulin-dependent diabetes mellitus. Participants in the study were 16 diabetes patients (11 males and 5 females) between the ages of 18 and 65 who did not have active cardiovascular, pulmonary, renal, or hepatic disease. After 12 weeks of monitoring to derive baseline information, the subjects were given vanadyl sulfate by tablet at doses of 75, 150, or 300 mg/day for 6 weeks. Based on individual body weights reported in the study and assuming that the trihydrate form of vanadyl sulfate was used, doses are 0.12–0.23, 0.28–0.45, and 0.43–1.14 mg V/kg-day in the 75, 150, and 300 mg/day groups. Blood glucose was monitored throughout the study (other tests of glycemic control were also administered) and the patients were given physical examinations, blood tests (electrolytes, BUN, creatinine, complete blood count), liver and thyroid function tests and urine tests biweekly. To assess lipid peroxidation, levels of thiobarbituric acid-reactive substances in the serum were measured. Ambulatory blood pressure was measured 4 weeks after exposure was terminated. The patients were monitored for 2 additional weeks. Although patients exposed to the lowest dose range did not experience any gastrointestinal symptoms, several patients at the next dose reported complaints and all patients at the high dose reported cramping, abdominal discomfort, and/or diarrhea. The study authors reported that no other signs of toxicity were observed and blood tests and urinalysis did not indicate toxicity (data not shown). Systolic, diastolic, and mean arterial pressure were not changed by exposure nor was heart rate. Insulin sensitivity and glycemic control were not

dramatically improved in this study. A LOAEL of 0.28–0.45 mg V/kg-day is identified from this study based on gastrointestinal symptoms; the NOAEL is in the range of 0.12–0.23 mg V/kg-day.

Cusi et al. (2001) gave a group of 11 patients (four men and seven women, mean age 59 years) with type 2 diabetes doses of 150 mg vanadyl sulfate each day for 6 weeks after a 2-week period of exposure to increasing doses up to 150 mg/day (exposure regimen during run-up not reported). Assuming that vanadyl sulfate was in the trihydrate form, the estimated doses (during the 6-week period) are 0.5 mg V/kg-day in males and 0.6 mg V/kg-day in females (based on default body weights of 70 and 60 kg, respectively). Measures of glycemic control were assessed throughout the exposure period. Effects reported in the subjects included diarrhea (4/11) and abdominal discomfort (2/11). According to the authors, blood chemistry, complete blood count, and urinalysis were not affected by treatment (data not shown), nor was bone mineral density (measured in three subjects) or body weight. Measures of 24-hour ambulatory blood pressure and mean heart rate were not affected by treatment (data shown). Glycemic control was significantly improved. This study suggests a LOAEL of 0.5–0.6 mg V/kg-day based on gastrointestinal symptoms; a NOAEL could not be identified.

Inhalation Exposure

The few studies examining human exposure to vanadium compounds (other than vanadium pentoxide) via inhalation (Woodin et al., 2000; Sorensen et al., 2005; Zhou et al., 2007) do not specify the form of vanadium exposure; in these studies, coexposure to other compounds could not be ruled out. Woodin et al. (2000) found increases in self-reported upper and lower airway respiratory symptoms in 18 boilermaker workers exposed to vanadium compared with 11 utility worker control subjects. The study authors correlated these symptoms with estimated vanadium doses to the lung and upper airway in all but the highest exposure quartile; the authors attributed the high-dose reversal to a possible healthy worker effect. Sorensen et al. (2005) observed a positive association between levels of 7-hydro-8-oxo-2'-deoxyguanosine (a measure of DNA damage) in lymphocytes of 49 students in Copenhagen and concentrations of both vanadium and chromium in PM2.5 samples. Concentrations of platinum, nickel, copper, and iron were not related to the measures of DNA damage. Based on the English abstract of a paper published in Chinese, 106 workers with exposure to vanadium were reported to exhibit more negative moods as well as poorer performance on neurobehavioral tests (Santa Ana dexterity, Benton visual retention and pursuit aiming) than unexposed workers (Zhou et al., 2007). The average concentration of vanadium in the air of the exposed workers ranged from 0.034 to 0.805 mg/m^3; however, the form of vanadium is not specified. No further information is presented in the abstract.

A case report documented symptoms of metal-fume fever in a worker exposed to a vanadium catalyst, vanadyl pyrophosphate (Vandenplas et al., 2002). After exhibiting symptoms in the work environment, the individual was assessed by a physician under controlled conditions of exposure to the vanadium catalyst. Forced vital capacity and forced expiratory volume were decreased and fever and peripheral blood neutrophilia were observed. Concentrations of vanadium to which the individual was exposed in the workplace or under the challenge conditions were not reported.

Animal Studies

Only a few of the available laboratory animal studies provide information on the levels of vanadium in the basal diet and none of the studies considered dietary input to total vanadium dose. Kanisawa and Schroeder (1967), along with Schroeder et al. (1970), reported the concentration of vanadium in their basal diet as 3.2 mg V/kg food. Elfant and Keen (1987) reported a concentration of 1 mg V/kg in a "purified" diet. Finally, Scibior et al. (2006) measured the concentration of vanadium in their standard chow to be 0.45 mg V/kg. For a dietary concentration of 1 mg V/kg, the vanadium dose to rats and mice would be in the range of 0.1 to 0.2 mg V/kg-day (assuming default values for subchronic exposure in female Sprague-Dawley and B6C3F1 mice; U.S. EPA, 1988). This estimate may not be representative of all commercial laboratory animal feeds used in the studies included in this review. Because exposure to vanadium in the basal diet was not taken into account in any of the studies, doses reported in this review may be underestimated to some degree. Further, low-level exposure to vanadium among controls increases the uncertainty in findings of effect at doses near the estimated control dose.

Oral Exposure

Subchronic Studies—Domingo et al. (1985) exposed male Sprague-Dawley rats to sodium metavanadate for 12 weeks. A control group consisted of 10 rats given free access to drinking water without added vanadate. There were three treatment groups that consisted of 10 rats/group exposed to drinking water to which 5, 10, or 50 mg/L sodium metavanadate (2, 4, or 21 mg V/L) had been added. Vanadium doses estimated for this review based on reported water consumption and body weight (of the high-concentration group only) were 0.3, 0.6, and 3.0 mg V/kg-day. Body weight was measured weekly, while food consumption, water intake, and urine volume were assessed daily. At sacrifice, blood was collected from five rats for serum chemistry determinations (AST, ALT, total protein, bilirubin, creatinine, urea, uric acid, glucose, and cholesterol). Selected organs (liver, kidneys, heart, spleen, and lung) from all animals were weighed. Microscopic examination of the heart, kidney, liver, lung, spleen, and stomach was performed on 3 rats/group. Body-weight gain was significantly ($p < 0.05$) increased (42%) over controls in the high-dose group (3.0 mg V/kg-day) during the first 2 weeks of exposure, but not thereafter; actual body weights are not reported. Food and water intake were not affected in the high-dose group. The authors indicated that body weight, food consumption, and water intake were not affected in other treatment groups (data not shown). Urine volume was greater than controls in the high-dose group during the first month (58% to 2-fold higher; $p < 0.05$), but not during the remainder of the study. Compared to the control values, plasma protein, urea, and uric acid concentrations were significantly higher (31%, 28%, and 2-fold, respectively; $p < 0.05$) in the 3.0 mg V/kg-day treatment group but not in other treatment groups. Organ weights were not affected by treatment (data shown). The histopathology findings are summarized qualitatively as mild changes in the kidney (hemorrhagic foci in the corticomedullary region), spleen (hypertrophy and hyperplasia), and lungs (perivascular mononuclear cell infiltration). The authors reported that these changes occurred in all treatment groups, but they are described as "more evident" in the 3.0 mg V/kg-day treatment group. Incidences of these effects are not reported. Given the authors' report of histopathology and clinical chemistry findings in the low-dose group, even though only three animals were examined, 3.0 mg V/kg-day is considered to be a LOAEL.

A number of studies examined the beneficial effects of vanadium exposure on diabetic rats[1]. Most of the studies examined few or no toxicological endpoints and used doses of 10 mg V/kg-day or greater. Those studies that did examine a few toxicological endpoints, included a nondiabetic treatment group, and exposed the animals for at least 28 days are summarized in Table 2. The studies shown in the table indicate that doses of 12 mg V/kg-day and higher result in body weight reductions of at least 10%, often accompanied by marked reductions in fluid intake. The reduced fluid intake may reflect an organoleptic effect of vanadium compounds administered in drinking water. Although body-weight reductions can be related to reduced fluid intake, studies that have observed reduced body weight or body weight gain with dietary or gavage administration of vanadium (e.g., Sanchez et al., 1991, 1998, 1999; Paternain et al., 1990; Elfant and Keen, 1987) suggest that this may be a toxic effect of the element rather than resulting from reduced fluid intake. Thus, the body-weight decrement of at least 10% observed at a dose of 12 mg V/kg-day (Cam et al., 1993) indicates that this dose is a LOAEL.

Most of the studies that examined only effects in diabetic animals are not summarized here—primarily because the studies demonstrated improvements in diabetes-related effects, rather than any toxic effects of vanadium exposure. However, one study examining effects of vanadium exposure in diabetic animals bears special consideration because it identifies enhanced toxicity in the vanadium-treated animals when compared with both nondiabetic and diabetic controls. Domingo et al. (1991) exposed groups of 10 streptozotocin-induced diabetic male Sprague-Dawley rats to three different forms of vanadium: sodium metavanadate (150 mg/L), sodium orthovanadate (230 mg/L), and vanadyl sulfate pentahydrate (310 mg/L) in the drinking water for 28 days. Based on body weights and fluid intake measurements, the authors estimated vanadium doses of 22.7, 15.6, and 6.1 mg V/kg-day for vanadyl sulfate, sodium orthovanadate, and sodium metavanadate, respectively. Sodium chloride (80 mM) was added to the water to inhibit gastrointestinal effects of vanadium. Both diabetic and nondiabetic control groups (10/group) were included for comparison. Mortality, body weight, food and fluid intake and blood glucose were monitored throughout the exposure period. After exposure ended, blood samples were collected for analysis of hematocrit, glucose, urea, creatinine, AST and ALT.

[1]A chronic study that included a broader range of toxicological endpoints is discussed under Chronic Studies (published in three papers: Dai et al., 1994a,b; Dai and McNeill, 1994).

Table 2. Studies of Effects in Streptozotocin-induced Diabetic and Nondiabetic Rats Exposed to Vanadium

Reference	Number and Sex of Rats	Vanadium Compound Administered	Duration	Dose[a] Vanadium (mg V/kg-day)	Significant Adverse Effects
Cam et al., 1993	11–16 males per group	Vanadyl sulfate in drinking water	5 months	12[b] (nondiabetic) 18–20 (diabetic)	Decreased body weight (14%), fluid intake (40%) and food intake (up to 10%) in treated nondiabetic rats relative to control nondiabetic rats.
Thompson et al., 1993	10–16 males per group	Vanadyl sulfate in drinking water	Up to 12 weeks	36 (nondiabetic) 102 (diabetic)	Decreased body weight (30%), decreased fluid intake (54%) in treated nondiabetic rats relative to control nondiabetic rats. Decreased body weight (11%) in treated diabetic rats relative to diabetic controls.
Yao et al., 1997	5–6 males per group	Vanadyl sulfate in drinking water	7 weeks	13 (nondiabetic) 24 or 29 (diabetic)	Decreased body-weight gain (14%), decreased fluid intake (34%) in treated nondiabetic rats relative to control nondiabetic rats.
Tunali and Yanardag, 2006; Akgün-Dar et al., 2007	5–13 males per group	Vanadyl sulfate via daily gavage	60 days	24 [b]	Lower body weight (11%), increased serum glucose and phospholipids, increased aortic lipid peroxidation, decreased stomach and aortic glutathione, decreased aortic diameter and aortic *tunica intima* thickness in treated nondiabetic rats relative to control nondiabetic rats. Decreased tunica muscularis thickness (in aorta) in treated diabetic rats relative to both diabetic and nondiabetic controls.

[a]Doses estimated by authors except where indicated.
[b]Doses estimated for this review based on default body weight and fluid intake (U.S. EPA, 1988). Cam et al. (1993) reported using the trihydrate form of vanadyl sulfate. Tunali and Yanardag (2006) and Akgün-Dar et al. (2007) did not report the form administered; it was assumed to be the trihydrate for the purpose of dose estimation.

In each of the groups exposed to sodium metavanadate and vanadyl sulfate, 3/10 rats died, while 2/10 diabetic rats treated with sodium orthovanadate died (Domingo et al., 1991). By comparison, no control nondiabetic rats died and 1/10 control diabetic rats died. Food and fluid intake in the groups exposed to sodium metavanadate and vanadyl sulfate were increased relative to the nondiabetic controls, but were lower than those of diabetic controls. Relative weight gain was significantly lower in diabetic controls than in nondiabetic controls (8.2% vs. 24% over study duration). However, the vanadium-treated rats lost weight over the exposure period (3.2%, 4.8%, and 7.2% losses in the groups exposed to vanadyl sulfate, sodium orthovanadate and sodium metavanadate, respectively; $p < 0.05$ relative to both diabetic and nondiabetic control groups). Thus, in this study, vanadium treatment enhanced the adverse effect of diabetes on body-weight gain. In addition, vanadium treatment (all forms) resulted in significantly ($p < 0.05$) higher serum urea concentrations relative to both diabetic and nondiabetic control groups. Treatment with vanadyl sulfate also increased the serum creatinine level relative to both control groups. This study suggests a LOAEL of 6.1 mg V/kg-day for body-weight losses in diabetic rats. Although mortality was observed in diabetic rats treated with vanadium (3/10 in the group exposed to 6.1 mg V/kg-day), it is not clear whether the deaths were attributable to the disease or the treatment; one death also occurred in the untreated diabetic group. A NOAEL cannot be determined.

A follow-up study assessing whether Tiron (sodium 4,5-dihydroxybenzene-1,3-disulfonate, a chelating agent) would mitigate the toxicity of vanadium in diabetic rats, provided some confirmation of these findings (Domingo et al., 1992). A group of 10 streptozotocin-induced diabetic rats was given sodium metavanadate at a concentration of 200 mg/L in the drinking water for 5 weeks, with or without Tiron; nondiabetic and diabetic control groups were included. The same parameters as in the earlier study were monitored. The authors estimated a vanadium dose of 23.2 mg V/kg-day in the group without Tiron exposure. As with the previous study, exposure to sodium metavanadate in diabetic rats resulted in body-weight loss (5%) while weight gains of 28% and 8.1% were seen in untreated nondiabetic and diabetic groups (respectively). The decrement was significantly different from both untreated groups at $p < 0.01$. In addition, serum urea was increased relative to both control groups (10.9 mmol/L vs. 6.3 and 8.2 mmol/L in nondiabetic and diabetic controls), while serum creatinine was not. Tiron administration did not ameliorate the effect of vanadium on body-weight gain, but did reduce serum urea concentrations. A LOAEL of 23.2 mg V/kg-day is identified from this study based on body-weight losses in treated diabetic rats.

A series of papers reported hematological effects of exposure to ammonium metavanadate (Gorski and Zaporowska, 1982; Zaporowska and Wasilewski 1989, 1990, 1991, 1992a,b; Zaporowska and Scibior, 1999). With few exceptions, the study protocols are largely the same. In most studies, 2-month old Wistar rats (either male or male and female) were exposed to ammonium metavanadate in drinking water provided ad libitum, typically for 4 weeks. Some studies examined the interaction of vanadium with another toxicant (ethanol or zinc), but some also provided data on exposure to the vanadium compound alone; in all cases, a single concentration of ammonium vanadate was used. Vanadium concentrations in the drinking water ranged from 50–300 mg/L, resulting in doses ranging from 7–29 mg V/kg-day in the various studies. Body weight, fluid intake, and food consumption were monitored during the exposure period. At sacrifice (at the end of exposure), the following hematological parameters were assessed: erythrocyte, reticulocyte, and total and differential leukocyte counts, hematocrit

[Hct], hemoglobin [Hgb], leukocyte composition in bone marrow and frequency of polychromatophilic erythrocytes in peripheral blood and bone marrow. A few other evaluations were conducted in individual studies. Zaporowska and Wasilewski (1992a) also examined the osmotic resistance of erythrocytes and the activities of glucose-6-phosphate dehydrogenase and lactate dehydrogenase in erythrocytes. Zaporowska and Scibior (1999) assessed the phagocytic activity of neutrophils and the activities of myeloperoxidase and alkaline phosphatase in the neutrophils. Based on the abstracts of papers published in Polish, Gorski and Zaporowska (1982) also examined the histopathology of liver and kidneys, and Zaporowska (1987) evaluated kidney histopathology.

Table 3 provides an overview of the study designs and results. Mortality occurred at doses of 13 mg/kg-day and higher in this series of papers. In general, the studies consistently demonstrated significantly depressed body-weight gain, food intake and fluid intake, decreased erythrocyte counts and hemoglobin concentrations and increased reticulocytes and polychromatophilic erythrocytes in exposed animals. Sporadic effects were observed on leukocytes or leukocyte composition and no effects on erythrocyte enzyme activities were reported. Abstracts from two studies (Gorski and Zaporowska, 1982; Zaporowska, 1987, both published in Polish) reported renal histopathology (parenchymatous degeneration with vacuolar degeneration and tubular casts) at doses of 9–29 mg V/kg-day, but the incidences of the renal effects are not given. Gorski and Zaporowska (1982) also reported parenchymatous degeneration of the liver. Neither study has been translated for this review. Taken together, these studies identify a FEL of 13 mg/kg-day based on mortality (Zaporowska and Wasilewski, 1992a).

In contrast to the other publications in this series, Zaporowska et al. (1993) used more than one concentration of ammonium metavanadate and also used lower doses that were not associated with mortality. Groups of 15–16 Wistar rats of each sex were given concentrations of 0, 10, or 50 mg V/L as ammonium metavanadate in drinking water for 4 weeks. Fluid intake was measured daily and body weight recorded weekly; based on these measures, the authors estimated doses of 1.2 or 5 mg V/kg-day in males and 1.5 or 7 mg V/kg-day in females. Food intake was also monitored daily during exposure. Blood was drawn (presumably at sacrifice at the end of exposure, although this is not specified) for hematology (erythrocyte count [RBC], leukocyte count [WBC], Hgb, Hct, leukocyte composition, polychromatophilic erythrocytes, and reticulocytes in peripheral blood) and erythrocyte enzyme activity determinations (catalase,glucose-6-phosphate dehydrogenase, lactate dehydrogenase and δ-aminolevulinic acid dehydratase). Malondialdehyde (MDA), glutathione (GSH) and L-ascorbic acid content of erythrocytes were also measured. At these doses, there was no mortality. Although body-weight gain was lower in exposed groups than in controls (as much as 9% lower at the high dose), the differences are not statistically significant. Food intake was not affected by treatment and fluid intake was decreased only in high-dose males (14% lower than controls, $p < 0.001$). Statistically significant—but modest—changes in erythrocyte count, hemoglobin concentration, and hematocrit are shown in Table 4. In addition to these changes, the percentage of reticulocytes was significantly increased at the high dose in both sexes (data presented graphically, $p < 0.05$). There was no effect on leukocyte composition or enzyme activity in erythrocytes. While MDA tended to be increased and GSH decreased in exposed animals, the changes are not statistically significant. However, the concentration of L-ascorbic acid in the plasma of male rats was reduced at both doses (24% and 37% below controls; $p < 0.05$). The high dose in this study is

Table 3. Studies of Hematologic Effects in Rats Exposed to Ammonium Metavanadate in Drinking Water

Reference	Number and Sex of Rats	Conc. Vanadium (mg V/L)	Duration	Dose[a] (mg V/kg-day)	Significant Effects
Gorski and Zaporowska, 1982 Published in Polish.	5–13 males per group	0, 200	1, 2, or 3 months	29[b]	Based on English abstract and tables only: decreased body-weight gain, decreased erythrocyte count, hemoglobin, and hematocrit; in "single cases," parenchymatous degeneration of liver and kidney, with vacuolar degeneration of kidney and tubular casts.
Zaporowska, 1987 Published in Polish.	15 (sex not given) per group	0, 50, 100, 200	4 weeks	9, 12, 23[b]	Based on English abstract and tables only: decreased body-weight gain at high dose; "renal tubule cylinders" at mid- and high doses.
Zaporowska and Wasilewski, 1989	10–18 per sex per group	0, 300	2, 4, or 8 weeks	21–29	Mortality[c] (6/16 and 2/14 males after 4 and 8 weeks; 2/13, 4/16, and 2/13 females after 2, 4, and 8 weeks), transient diarrhea in "some" rats, decreased body-weight gain, decreased food and water intake, decreased erythrocyte count and hemoglobin concentration, increased number polychromophilic erythroblasts.
Zaporowska and Wasilewski, 1990	10–21 per sex per group	0, 300	4 weeks	22–27	Mortality[c] (6/21 males and 6/21 females), diarrhea, decreased body-weight gain, decreased food and water intake, decreased erythrocyte count, increased reticulocyte count, increased number polychromatophilic erythrocytes, decreased lymphocytes and plasma cells in bone marrow.
Zaporowska and Wasilewski, 1991	10–11 males per group	0, 300	4 weeks	20	Decreased body-weight gain, fluid intake, food intake, erythrocyte count, and hemoglobin concentration. Increased reticulocytes and polychromatophilic erythrocytes in peripheral blood.
Zaporowska and Wasilewski, 1992a	12–13 per sex per group	0, 150	4 weeks	13	Mortality[c] (1/12 males); transient diarrhea (2 rats); decreased body-weight gain, food intake, fluid intake, erythrocytes, hemoglobin count; increased leukocyte count; decreased osmotic resistance of erythrocytes; increased reticulocytes, polychromatophilic erythrocytes, neutrophils and lymphocytes in peripheral blood.
Zaporowska and Wasilewski, 1992b	12–14 per sex per group	0, 300	4 weeks	20–26	Mortality[c] (2/13 males and 3/14 females); frequent diarrhea; decreased body-weight gain, food intake and fluid intake; decreased erythrocyte count and hemoglobin concentration; increased reticulocytes and polychromatophilic erythrocytes in peripheral blood and/or bone marrow.
Zaporowska and Scibior, 1999	10–13 males per group	0, 150	4 weeks	12	Decreased body-weight gain, food intake and fluid intake; decreased phagocytic activity of neutrophils.

[a]Doses estimated by authors based on fluid intake and body weight except where indicated
[b]Doses estimated for this review based on default body weight and fluid intake (U.S. EPA, 1988)
[c]No control animals died in any study

considered a LOAEL (5 mg V/kg-day in males and 7 mg V/kg-day in females) based on a 9% decrease in body-weight gain (albeit not significantly decreased from controls, and possibly related to reduced fluid intake) and modest hematology changes. The low dose (1.2 mg V/kg-day in males and 1.5 mg V/kg-day in females) is considered a NOAEL; the statistically significant hematology changes observed at this dose are not considered toxicologically significant.

Table 4. Hematologic Effects in Rats Exposed to Ammonium Metavanadate for 4 Weeks[a]

Parameter	Control	10 mg V/L	50 mg V/L
Males		**1.2 mg V/kg-day**	**5 mg V/kg-day**
Erythrocytes ($\times 10^{12}$/dm^3)	8.32 ± 0.17	7.38 ± 0.20 [b]	7.47 ± 0.27 [c]
Hemoglobin (mmol/L)	9.37 ± 0.19	8.94 ± 0.28	8.65 ± 0.26 [c]
Hematocrit (%)	0.48 ± 0.001	0.47 ± 0.004 [c]	0.47 ± 0.003 [b]
Females		**1.5 mg V/kg-day**	**7 mg V/kg-day**
Erythrocytes ($\times 10^{12}$/dm^3)	8.24 ± 0.10	7.38 ± 0.14 [d]	7.12 ± 0.17 [d]
Hemoglobin (mmol/L)	9.41 ± 0.12	8.76 ± 0.30	8.72 ± 0.20 [c]

[a]Zaporowska et al., 1993
[b]$p < 0.01$
[c]Significantly different from control, $p < 0.05$
[d]$p < 0.001$

In recent papers by the same group of investigators, sodium metavanadate was used as the test material in studies comparing the effects of vanadium alone or in combination with chromium or magnesium. Scibior (2005) administered sodium metavanadate in the drinking water to a group of 11 male Wistar rats at a concentration of 100 mg V/L; a group of 16 untreated rats served as controls. Food and fluid intake were measured daily and body weight recorded weekly during the 6-week exposure period. After exposure ended, blood was collected for hematology (RBC, Hct, Hgb, mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], and WBC) and assessment of the total antioxidant status of erythrocytes. Based on measured body weight and fluid intake, the authors estimated the vanadium intake to be 8 mg V/kg-day. While total body-weight gain was reduced in the vanadium-exposed group (about 9% less than controls), the difference is not statistically significant. In treated rats, both food and fluid intake were reduced compared to controls (13% and 32% less than controls, respectively; $p < 0.05$). Modest—but statistically significant ($p < 0.05$)—effects observed with exposure include the following: increased erythrocyte count (in contrast to earlier studies that showed a decrease; 10% higher than controls) and decreased MCH (12% lower) and MCHC (4% lower). No other statistically significant effects were observed in the parameters evaluated. A LOAEL of 8 mg V/kg-day is identified for these data based on a 9% decrease in body-weight gain (albeit not significantly decreased from controls, and possibly related to reduced food and fluid intake) and hematology changes; no NOAEL can be determined.

Scibior et al. (2006) exposed male Wistar rats (12/group) to sodium metavanadate at a concentration of 0 or 125 mg V/L in the drinking water for 6 weeks. Based on fluid intake and body weight measurements, the authors estimated the average vanadium intake to be 11 mg V/kg-day in the exposed group. Evaluations were similar to those of previous studies and included body-weight gain, food and fluid intake, hematology (RBC, WBC, Hgb, Hct, MCV,

MCH, MCHC, and red-cell distribution width), leukocyte composition of peripheral blood smears, phagocytic activity of neutrophils in whole blood, erythrocyte concentrations of L-ascorbic acid and malondialdehyde, and the total antioxidant status of the plasma. In this study, significant ($p < 0.05$) effects of treatment included a 15% decline in body-weight gain, along with 6% and 30% decreases in food and fluid intakes (respectively). Erythrocyte count was decreased by 6%, while hemoglobin concentration was depressed by 10.6% compared to controls ($p < 0.05$). MCV and MCH were reduced by 4% and 6%, respectively ($p < 0.05$). Leukocyte count and leukocyte composition of peripheral blood were not affected by treatment. The plasma concentration of L-ascorbic acid was decreased (26%, $p < 0.05$), while malondialdehyde content of erythrocytes was increased (78%, $p < 0.05$). Based on data presented in tables, there are no statistically significant changes in Hct, MCHC, red-cell distribution width, or phagocytic activity of neutrophils with exposure. A LOAEL of 11 mg V/kg-day is identified for these data based on a 15% decrease in body-weight gain (possibly related to reduced food and fluid intake) and hematology changes; no NOAEL can be determined.

In contrast to the studies published by Zaporowska and collaborators, Dai et al. (1995) observed no effects on hematology parameters in groups of eight male Wistar rats exposed to ammonium metavanadate (140 mg/L) and vanadyl sulfate (260 mg/L) in the drinking water for 12 weeks. An additional group of eight rats received untreated water. Body weight, food intake, and fluid intake were measured before exposure and on Weeks 1, 2, 4, 8, and 12 of treatment. These data were used by the authors to estimate vanadium doses of 0.19 and 0.15 mmol V/kg-day for ammonium metavanadate and vanadyl sulfate, respectively; these values correspond to dose estimates of 9.7 and 7.6 mg V/kg-day, respectively. Blood samples were collected on the same schedule as body weight measurements for evaluation of Hct, Hgb, RBC, WBC, platelet count, differential leukocyte count, reticulocyte percentage and erythrocyte osmotic fragility tests. No other evaluations were performed. Vanadium in the drinking water led to significantly ($p < 0.05$) reduced fluid intake, regardless of the compound administered (data presented graphically). However, food intake and body weight were not affected by exposure and there was no statistically significant effect on any hematology parameter at any time (data shown graphically). This study identifies freestanding NOAELs of 9.7 and 7.6 mg V/kg-day (for ammonium metavanadate and vanadyl sulfate, respectively) for hematologic effects in male rats.

Adachi et al. (2000) exposed groups of seven female Wistar rats to sodium metavanadate in the diet for 10 weeks. Concentrations of 0, 50, or 100 ppm (0, 21, or 42 ppm V) were incorporated into the diet. Food intake and body weight were measured weekly; vanadium doses calculated for this review based on food intake (14 g/day) and body weight (0.260 kg) roughly estimated from graphical presentation of these data are 1.1 and 2.3 mg V/kg-day. After exposure was terminated, the animals were sacrificed and blood was collected for hematology (RBC, WBC, platelet count, reticulocyte count, Hgb, cell number, immunoglobulin levels) and serum chemistry (AST, ALT, cholinesterase [ChE], ALP). Thiobarbituric acid levels (a measure of lipid peroxidation) were determined in the liver, kidney, and spleen, while vanadium and metallothionein (a metal-binding protein) contents of the liver and kidney were also assayed. Histopathology was not assessed. Statistically significant decreases ($p < 0.05$) in body weight were observed at both doses after 3 weeks of exposure; however, the body-weight decrements at termination were less than 10% (approximately 5% and 7% lower than controls) at both doses.

Food intake was not affected by exposure. Hematology and serum chemistry data were presented graphically with statistical analysis of differences from control. ALT, ChE, and ALP levels were significantly ($p < 0.05$) decreased at both doses. Although AST levels were reduced by more than half at both doses, the difference was significantly different from control only at the high dose. A decrease in serum liver enzymes is not considered to be of toxicological significance. Hemoglobin content and hematocrit were slightly reduced at both doses ($p < 0.05$), but erythrocyte count was not affected. Based on visual inspection of the graphs, the Hgb decrease was about 4% at both doses, and Hct was decreased from about 51% to about 49%. Platelet and reticulocyte counts were increased, while leukocyte counts were decreased at the high dose only. The decrease in leukocytes was primarily a result of reduced lymphocyte counts, specifically B cells. Plasma levels of immunoglobulin G (IgG) and IgM were also reduced at the high dose. Lipid peroxidation, as measured by thiobarbituric acid content, was increased in the kidney at the high dose only. Metallothionein content of the kidney is very slightly statistically significantly ($p < 0.05$) higher in the exposed groups relative to controls; there was no difference in the liver. Given the minimal changes in hematology and small body-weight decrease (~7%), the high dose (2.3 mg V/kg-day) is considered a NOAEL.

Kasibhatla and Rai (1993) administered vanadium in drinking water to rabbits (strain and sex not given) in a study evaluating limited hematology parameters. Rabbits (4/dose) were exposed to concentrations of 0, 20, 40, or 80 ppm vanadium for 171, 171, 129, or 24 days, respectively. The test material was characterized as "metavanadate." These exposure levels correspond to doses of about 3.3, 6.7, and 13.8 mg V/kg-day based on measured body weights and default values for water intake (U.S. EPA, 1988). An untreated control group received tap water. Body weights were recorded at irregular intervals. Blood samples were collected periodically for evaluation of erythrocyte and leukocyte counts, hemoglobin concentration and packed cell volume. The authors reported clinical signs including diarrhea, conjunctivitis, weakness, white nasal secretions, and loss of appetite in exposed rabbits, but no information on incidences or doses is provided. Body weights were generally lower in the treated groups, but the authors' statistical analysis indicated significantly reduced body weights only in the low-dose group; thus, this finding appears to be spurious. The authors also reported statistically significant ($p < 0.05$) reductions in erythrocyte count, hemoglobin concentration, and packed cell volume in the treated animals. However, the hematology data show decreasing numbers of treated rabbits over time, without explanation. It is not clear whether the missing animals died or were otherwise removed from the study. The poor reporting in this study precludes determination of effect levels.

Steffen et al. (1981) observed increased blood pressure in renally compromised rats exposed to vanadium. Groups of 20 adult male uninephrectomized Sprague-Dawley rats were given rat chow containing 100-ppm vanadium and either tap water or a 1% solution of sodium chloride to drink for 9 weeks. Based on default values for food intake and body weight (U.S. EPA, 1988), the dose for this experiment was estimated to be 9 mg V/kg-day. Control groups of the same size were given untreated rat chow (which contained 0.3-ppm vanadium) with one of the two fluid options. Fluid intake, urine volume, and urinary sodium concentration were measured daily. Body weights and systolic blood pressure (measured by tail cuff) were measured weekly. Upon sacrifice at the end of exposure, heart weights were recorded. There was one rat exposed to vanadium and sodium chloride that died at Week 3 of exposure; cause of death was not noted. Body-weight gain was lower in the vanadium-treated groups than in the

corresponding control groups, with statistically significant ($p < 0.05$) reductions after the 4th week of treatment. Based on visual examination of the data presented graphically, body weights of the treated groups at termination were about 12% lower than corresponding control body weights. The authors reported that vanadium treatment did not alter water consumption, urine volume, or urinary excretion of sodium (data on sodium excretion shown) compared with corresponding control groups. In the vanadium-exposed group consuming tap water, blood pressure was increased over the tap-water control group after the 3rd week of exposure ($p < 0.05$). Blood pressure data are presented graphically; based on visual examination of the data, systolic blood pressure approached 150 mm Hg in the vanadium-tap water group, compared with a value of <130 mm Hg in the tap-water controls. Though blood pressure measures were higher in vanadium-treated rats consuming sodium chloride, the difference from the sodium chloride control group is not statistically significant. Heart weight was not affected by vanadium treatment (data not shown). These data suggest a LOAEL of 9 mg V/kg-day based on decreased body weight. Although one rat died in the first experiment, there are no other indications of severe toxicity that would suggest that the death was related to treatment.

Susic and Kentera (1986) assessed the effects of vanadium administration on pulmonary circulation in adult male Long-Evans rats. After 2 months of exposure to ammonium vanadate in the diet (300 ppm or about 130-ppm vanadium assuming that the administered form was ammonium metavanadate), pulmonary and systemic blood pressure and cardiac output were measured and pulmonary and systemic vascular resistances were calculated from these measurements. Blood pressure was measured directly using a femoral artery cannula in anaesthetized animals. Using default values for food consumption and body weight (U.S. EPA, 1988), this dietary concentration is estimated to result in a dose of about 12 mg V/kg-day. Arterial blood was collected for assessment of hematocrit (timing not reported), but the results are not reported. After the exposure period, the rats were sacrificed and hearts removed for determination of left and right ventricular weights. Body weight, heart rate, mean femoral artery pressure, cardiac output, and total peripheral resistance were not affected by exposure (data shown). Significant ($p < 0.05$) increases in right ventricular systolic and mean pressures, as well as the calculated pulmonary vascular resistance, were observed with exposure (data presented graphically). The right ventricles of exposed rats were slightly enlarged, as shown by increased relative weight compared to controls (5%, $p < 0.05$). These data suggest a LOAEL of 12 mg V/kg-day based on pulmonary hypertension.

Susic and Kentera (1988) compared the hypertensive effects of sodium metavanadate in normal and partially nephrectomized Long-Evans rats. Groups of 18–24 male rats were fed diets containing 0-, 300-, or 3000-ppm sodium metavanadate for 24 weeks. The authors estimated doses of 5 and 47 mg sodium metavanadate per rat per day, corresponding to doses of approximately 4.4 and 42 mg V/kg-day (assuming a body weight of 0.472 kg for male Long-Evans rats [U.S. EPA, 1988a]). A separate group of 38 rats was subjected to partial nephrectomy followed by exposure to either the control diet or a diet with 300-ppm sodium vanadate (calculated to deliver a dose of 4.5-mg sodium metavanadate per rat per day, or 4.0 mg V/kg-day). Measurements of systolic blood pressure, heart rate, and body weight were recorded biweekly and renal function (plasma creatinine concentration, 24-hour creatinine clearance, urinary sodium excretion, and urinary output) was assessed in eight randomly chosen rats per group during Weeks 5 and 6. After exposure was terminated, groups of six randomly selected rats per group were selected for determination of hematocrit as well as plasma and extracellular fluid volumes. The remaining animals were used for measurement of blood

pressure, cardiac output, and total peripheral resistance. The animals were then sacrificed for removal of hearts and measurement of left and right ventricular weights. Body weights were significantly lower at both doses in the nonnephrectomized rats ($p < 0.001$ by t-test performed for this review), but they did not exceed a 7% decrease from control body weight in either group. The authors indicated that food intake was not affected by exposure (data not shown). Graphical and tabular presentation of data indicated that systolic blood pressure, heart rate, and mean arterial pressure were unchanged by vanadium treatment in nonnephrectomized rats. Statistically significant ($p < 0.05$) changes observed in nonnephrectomized rats at the end of exposure included decreased cardiac output and increased total peripheral resistance at both doses and increased hematocrit and decreased extracellular fluid volume at the high dose (see Table 5). In partially nephrectomized rats, systolic blood pressure, mean arterial pressure, and total peripheral resistance were significantly increased by exposure; other parameters were not affected by exposure. The authors indicated that the increase in resistance resulted from a vasoconstrictive effect of vanadium. In rats with intact kidneys, the increased peripheral resistance was offset by a reduction in cardiac output and blood pressure remained stable. In partially nephrectomized rats, there was no compensatory reduction in cardiac output; thus, an increase in blood pressure was observed. Renal function was not modified by vanadium exposure in any of the groups of rats, based on the parameters measured (data shown). These data indicate a LOAEL of 4 mg V/kg-day based on increased blood pressure in partially nephrectomized rats. A NOAEL cannot be determined.

Table 5. Significant Changes in Cardiovascular Parameters in Rats Exposed to Sodium Metavanadate for 24 Weeks[a]

Parameter	Control	4 mg V/kg-day (300 ppm)	47 mg V/kg-day (3000 ppm)
Nonnephrectomized rats			
Cardiac output (mL/min per 100 g)	25.6 ± 1.2	22.2 ± 0.6[b]	21.2 ± 0.9[b]
Total peripheral resistance (mm Hg/mL per min per 100g)	4.44 ± 0.12	5.41 ± 0.23 [c]	5.82 ± 0.31 [c]
Hematocrit	41.9 ± 0.7	43.5 ± 0.5	45.5 ± 1.3[b]
Extracellular fluid volume (mL/100g)	17.0 ± 0.3	15.9 ± 0.4	12.9 ± 0.3[d]
Partially nephrectomized rats			
Mean arterial pressure (mm Hg)	112 ± 4	134 ± 3[d]	NA
Total peripheral resistance (mm Hg/mL per min per 100g)	4.11 ± 0.29	5.15 ± 0.25[b]	NA

[a]Susic and Kentera, 1988
[b]Significantly different from control, $p < 0.05$
[c]$p < 0.01$
[d]$p < 0.001$

Van Vleet et al. (1981; Van Vleet and Boon, 1980) exposed groups of six male pigs to ammonium metavanadate in feed (0 or 200 mg V/kg) for 10 weeks. The dose estimated for this review was 10 mg V/kg-day based on the average body weight reported in the study (12 kg) and assuming a feed consumption rate of 0.6 kg feed/day (Brooks et al., 1984; U.S. EPA, 1988). The authors indicated that food consumption was decreased in the treatment group relative to controls (data not reported); therefore, the calculated dose may overestimate the actual dose in the treatment group. Endpoints assessed include clinical signs, weekly body weight measurements, blood glutathione peroxidase activity, gross necropsy, and microscopic histopathology assessment of heart, kidney, liver, lung, skeletal muscle, stomach, and "other organs with lesions." There were two deaths in the treatment group (33% mortality): one death on Day 60 of

exposure and one on Day 65. Clinical signs observed in treated pigs (and not in controls) were emaciation, rough hair coats, diarrhea, and blood in feces (incidences not reported). Body weights were markedly lower in the treatment group compared to the control group (one-third to one-half of control values; significantly lower at $p < 0.05$) throughout the exposure period; this decrease may have been associated with the reduction in food consumption. Blood glutathione peroxidase concentrations were not different from controls. The histopathology assessment revealed no abnormalities in the control group and the following findings in the treatment group: ulceration of the large intestine (4/4 surviving pigs), bladder cystitis (2/4), periportal infiltration of mononuclear leukocytes in liver (3/4) and necrosis of the heart atria (2/4). The dose used in this study (10 mg V/kg-day) is a FEL based on mortality and emaciation.

Chronic Studies—There were three multiyear bioassays of vanadium published in the 1960s and 1970s that have been identified in the literature searches; none of the studies met current standards for assessment of chronic toxicity and/or carcinogenicity.

Kanisawa and Schroeder (1967) exposed white Swiss mice (53 treated, 198 controls; sex not specified) to vanadyl sulfate in the drinking water at a concentration of 5 mg V/L from birth until natural death. The dose estimated for this review is 1 mg V/kg-day based on default values for body weight and water intake (U.S. EPA, 1988). The group sizes are not specified. Survival and body weight were monitored. Upon death, the animals were examined for gross lesions and the heart, lung, kidney, liver, spleen, and abnormal organs were examined microscopically. The authors emphasized that the tumor data reflected only tumors visible under a magnifying lens since serial sections for histopathology evaluation were not performed. The authors reported that neither survival nor body weight were affected by vanadium treatment (data not shown). Tumor incidences were grouped across sex for reporting. Based on the tabulated results, exposure to vanadium did not increase the incidence of any individual tumor type or the total incidences of "pre-tumorous lesions," benign, or malignant tumors (grouped across target organ). However, statistical analysis of the individual tumor data is precluded by the absence of group size information. These data are not adequate to define effect levels for chronic exposure.

Schroeder et al. (1970) exposed Long-Evans rats to vanadyl sulfate in drinking water from weaning through natural death (up to 45 months in this study). The treatment group consisted of 61 female and 52 male rats that had free access to drinking water to which 5 mg/L vanadium was added. Controls (54 female, 52 males) were exposed to water without added vanadium. Doses estimated for this review based on reported body weights and default fluid intakes (U.S. EPA, 1988) are 0.7 and 0.9 mg V/kg-day in males and females, respectively. Body weight was measured weekly until 6 weeks of age and then monthly thereafter; at the same times, blood pressure was recorded and blood collected for assessment of serum glucose levels. Upon death, animals were necropsied, hearts were removed and weighed, and grossly visible tumors and other lesions were described. An outbreak of pneumonia during this study led to the deaths of 17 treated males, 17 treated females, 19 control males, and 12 control females; the timing of the outbreak was not reported. No differences were observed in the following endpoints: life span and longevity, body weight, blood pressure (measured with arterial cannula in anesthetized animals), urine protein and glucose, and gross tumor incidence (all data other than urine protein were shown). This study found significant ($p < 0.05$) differences between the treatment and control groups in the following endpoints: increased fasting plasma glucose concentrations (21%) in treated females, increased fasting plasma cholesterol concentrations

(18%) in treated males and decreased fasting cholesterol concentrations (41%) in treated females. Absolute and relative heart weights were 18 and 15% lower (respectively) in treated males relative to controls, while female heart weights were higher (4 and 5% higher for absolute and relative weights, respectively). No treatment-related increases in tumor formation were found. Microscopy was performed on "some" tissues; however, a comprehensive microscopy evaluation apparently was not performed or not reported. No microscopic lesions were reported for any animals, although the histological evaluations performed in this study were not adequate to detect any but the most severe lesions. However, a LOAEL of 0.7 mg V/kg-day can be established for increased fasting plasma glucose and cholesterol levels and decreased heart weights.

Using a study design similar to that above, Schroeder and Michener (1975) exposed groups of Swiss mice (54/sex) to vanadyl sulfate in drinking water (5 mg V/L) for their lifetimes; controls (54/sex) were given untreated water. The dose estimated for this review based on reported body weights and default estimates of fluid intake (U.S. EPA, 1988) was 1 mg V/kg-day in both sexes. The toxicological evaluations are the same as reported by Schroeder et al. (1970). Significant differences between the treatment and control groups included increased body weight in treated males and increased life span and longevity in treated males and females. A gross assessment of tumors and microscopy of "some" tissues revealed no treatment-related increases in tumor incidence. The limitations in the histological evaluations performed in this study preclude the identification of effect levels from these data.

Steffen et al. (1981) exposed groups of uninephrectomized rats (group sizes not reported) to dietary concentrations of 100- or 200-ppm vanadium (as sodium orthovanadate) for 56 weeks. Based on default values for food intake and body weight (U.S. EPA, 1988), the doses for this experiment are estimated to be 7 and 14 mg V/kg-day. Body weights and systolic blood pressure (measured by tail cuff) were measured weekly. Upon sacrifice at the end of exposure, heart weights were recorded, and tail artery norepinephrine content was measured. There were two rats given 14 mg V/kg-day that died "early in the experiment"; neither timing nor cause of death was reported (Steffen et al., 1981). In the 14 mg V/kg-day group, body weights were significantly ($p < 0.05$) below controls beginning at Week 20 of treatment; based on graphical presentation of the data, terminal body weight in this group was about 13% below that of controls. Body weight was not significantly different from controls in the low-dose group. In both groups of vanadium-treated rats, systolic blood pressure was significantly ($p < 0.05$) increased over controls, in a dose-dependent fashion, after the first 1–2 months of treatment. Increases of up to 10 and 25 mm Hg were seen in the low- and high-dose groups, respectively. Plasma vanadium concentration measured at sacrifice correlated strongly with the last measure of systolic blood pressure ($r = 0.71$, $p < 0.001$), bolstering evidence for the apparent relationship with exposure. The low dose (7 mg V/kg-day) is a freestanding LOAEL for increased blood pressure in uninephrectomized rats.

Dai et al. (1994a,b; Dai and McNeill, 1994) exposed groups of nondiabetic and diabetic (streptozocin-induced) male Wistar rats to vanadyl sulfate in drinking water for 1 year. The three publications each reported findings of different endpoints. A control group consisted of eight rats given free access to water without added vanadate. There were three treatment groups that consisted of 8 rats/group exposed to water to which vanadyl sulfate was added; the exposures (mg vanadyl sulfate/L) were as follows: treatment group 1500 mg/L for 52 weeks; treatment group 2500 mg/L for 1 week followed by 750 mg/L for 51 weeks; treatment group

3500 mg/L for 1 week followed by 750 mg/L for 1 week, followed by 1250 mg/L for 50 weeks. Food intake, fluid intake, and body weight were recorded every 3–5 weeks throughout the treatment period. On the basis of these measures, the authors estimated the doses of vanadyl sulfate to be 34, 54, and 90 mg/kg-day (8, 13, and 21 mg V/kg-day, using the molecular weight for the trihydrate form) in nondiabetic rats. In diabetic rats, vanadyl sulfate treatment was adjusted up or down in order to control blood glucose or prevent diarrhea and weight loss. The authors estimated vanadyl sulfate doses of 73 to 165 mg/kg-day (17 to 39 mg V/kg-day) at different time points in the diabetic rats. The general condition of the animals—especially the occurrence of diarrhea or cataracts—was assessed during treatment. Nonfasting blood glucose was measured weekly for the first month and then every 2–4 weeks thereafter. Fasting blood glucose, insulin, triglycerides and cholesterol were measured every 3 months during treatment. The following measurements were made after 3, 6, 9, and 12 months of exposure: blood pressure (measured with a tail cuff sensor in conscious animals), pulse rate, hematocrit and plasma concentrations of AST, ALT, and urea. Most animals were sacrificed after the exposure period; however, three control nondiabetic rats, eight treated nondiabetic rats, and five treated diabetic rats were monitored for 16 untreated weeks prior to sacrifice. At sacrifice, a hematology assessment (Hgb, RBC, total and differential WBC, platelet count, reticulocyte count) was conducted and the following organs were weighed and examined microscopically: adrenal, brain, heart, kidney, liver, lung, pancreas, spleen, testis, and thymus.

In nondiabetic rats, 1/8 animals treated at the highest dose died of unknown causes after 18 weeks of exposure (Dai et al., 1994a). Neither food nor fluid intake was significantly affected by exposure to vanadyl sulfate (data shown graphically). However, body weight gain was reduced in a dose-related manner in treated nondiabetic animals relative to control nondiabetic animals. Based on visual inspection of data presented graphically, the body weight decrements at termination were approximately 10% in the low- and mid-dose groups and 20% in the high-dose group; statistical analysis of the data was not presented. Other than body weight data, most information on the nondiabetic treated rats was pooled across the three treatment groups (the authors indicated that there were no differences among the three groups). Vanadyl sulfate treatment did not affect blood or plasma glucose levels, plasma triglycerides, or cholesterol levels, but significantly lowered plasma insulin levels compared with controls at Weeks 12 and 25 (data presented graphically; *p*-value not reported). No significant changes were observed in the treatment group relative to the control group for the following endpoints: systolic blood pressure, pulse rate, hematology endpoints and relative organ weights (Dai et al., 1994a; Dai and McNeill, 1994). Plasma ALT and urea concentrations are significantly ($p < 0.05$) higher (<2-fold higher based on data presented graphically) in the nondiabetic treatment group relative to the corresponding control group after 3 months of exposure but not after 6, 9, 12, or 16 months of exposure (data presented graphically).

Histopathology findings included a high incidence of glomerular and tubular degeneration and interstitial cell infiltration and fibrosis of the kidney in the nondiabetic control group: 3/5 (60%) at the end of exposure and 2/3 (66%) at 16 weeks postexposure, for a combined incidence of 5/8 (63%) for the two assessment times (Dai et al., 1994b). Despite this high incidence in controls, which was probably age- and/or husbandry-related, the treated animals (all three treatment groups pooled) had a higher incidence: 15/15 (100%, $p = 0.053$) at the end of the exposure, 7/8 (88%, $p = 0.049$) 16 weeks postexposure and a combined incidence of 22/23 (96%; $p = 0.043$) (based on Fisher exact test performed for this review). These results are consistent

with the higher plasma urea concentrations in the treatment group. No other histopathology findings are significantly increased with exposure to vanadyl sulfate. Based on the reduced body weight in the low-dose group (~10% lower than controls at termination), and possibly renal pathology, a LOAEL of 8 mg V/kg-day is identified for nondiabetic rats; no NOAEL can be determined.

Vanadyl sulfate treatment of diabetic rats improved or prevented a number of adverse effects seen in untreated diabetic rats, including: mortality; increased food and fluid intake; hypoinsulinemia; polydipsia; cataract formation; elevations of serum glucose, ALT, urea, triglycerides and cholesterol; bradycardia; decreased leukocyte count; increased relative organ weights and occurrence of megacolon (Dai et al., 1994a,b; Dai and McNeill, 1994). No improvement was seen in body-weight gain, which was markedly lower in both untreated and vanadyl sulfate-treated rats than in both control and treated nondiabetic rats. At the end of exposure, body weights were about 30% lower in both groups of diabetic rats when compared with nondiabetic controls (based on graphical presentation of data). Likewise, renal effects that were significantly increased in diabetic controls (compared with nondiabetic controls), including vacuolation of tubular epithelial cells and renal cell tumors, occurred at similar frequency in vanadyl sulfate-treated diabetic rats. As vanadium treatment was not associated with adverse effects in diabetic rats, the dose to this group (17 to 39 mg V/kg-day) is considered a NOAEL in diabetic rats.

Carmignani et al. (1991) exposed male Sprague-Dawley rats to sodium metavanadate in drinking water for 7 months beginning at weaning. Groups of 10 rats were exposed to water to which 0 or 100 mg V/L was added. The calculated dose was 12 mg V/kg-day, based on default values for fluid intake and body weight (U.S. EPA, 1988). At the end of exposure, blood pressure was measured (with an arterial cannula in anesthetized animals), urinalysis was performed on a 24-hour urine collection and both light and electron microscopic evaluation of the heart and kidney were performed. Systolic and diastolic blood pressure were significantly ($p < 0.05$) elevated in the treatment group compared to the control group (systolic: control 122 mmHg, treatment group 144 mmHg; diastolic: control 95 mmHg, treatment 115 mmHg), as was heart rate (control 239 beats per minute, treatment 288 beats per minute). According to the authors, the urinalysis revealed no difference in urine osmolarity, nitrogen, protein, or ionized calcium between the treatment and control group (data not shown). Urinary sodium and potassium excretion were significantly elevated (83% and >3-fold higher, respectively; $p < 0.05$) in the treatment group compared to the control group. The histopathology assessment revealed narrowing of the renal proximal tubules, which contained amorphous protein material and swollen mitochondria, in the treatment group. The incidences of these changes in treated and control animals are not reported. No changes were noted in the hearts of the treatment group relative to the control group. A LOAEL of 12 mg V/kg-day is identified based on the increased blood pressure and kidney histopathology. A NOAEL cannot be identified.

Investigators from the same laboratory (Boscolo et al., 1994) conducted further experiments with male Sprague-Dawley rats exposed to sodium metavanadate in drinking water. Groups of six rats were exposed to water containing 1, 10, or 40 mg V/L for 180, 210, and 210 days, respectively, in two experiments. Each experiment had a separate control group receiving untreated water for the same duration. Doses estimated for this review based on default estimates of fluid intake and body weight (U.S. EPA, 1988) were 0.12, 1.2, or 4.7 mg V/kg-day. The following endpoints were assessed: blood pressure (measured with an

arterial cannula in anesthetized animals); heart rate; plasma renin activity, plasma aldosterone, urinary kallikrein activity and urinary Kininase I and II activities (indicators of status of the renin-angiotensin-aldosterone system); urinalysis (creatinine, total nitrogen, proteins, sodium, potassium, and calcium); and microscopic examination of blood vessels, brain, heart, kidney, liver, and lung. Histochemical analysis of the Na+, K+-ATPase activity was assessed in the kidneys of high dose and control rats. Statistically significant changes in the measured parameters are shown in Table 6. Significantly higher ($p < 0.05$) systolic and diastolic blood pressures were observed in all treatment groups relative to the control group. The magnitude of the increase did not appear to be dependent on dose level. Plasma renin activity, plasma aldosterone concentration, and urinary kallikrein, Kininase I, and Kininase II were significantly elevated in the 1.2 and 4.7 mg V/kg-day treatment groups relative to controls, suggesting stimulation of the renin-angiotensin-aldosterone system at these exposure levels. In addition, Kininase I activity was doubled at 0.12 mg V/kg-day, although not statistically significant. In contrast, Kininase II activity and plasma aldosterone were significantly reduced at the low dose. The histological assessment revealed narrowing of the lumen and amorphous casts in renal proximal tubules and a decrease in histochemically detected Na^+, K^+-ATPase activity in injured tubules in the 4.7 mg V/kg-day treatment group. The authors also reported hydropic degeneration (swelling of the cells) in proximal, distal, and straight tubules. The incidences of the latter effect were not reported; however, the authors indicated that these changes were "less evident" at 1.2 mg V/kg-day and absent at 0.12 mg V/kg-day. These data suggest a LOAEL of 0.12 mg V/kg-day based on increased blood pressure (>20 mm Hg increase in both systolic and diastolic measures) and stimulation of the renin-angiotensin-aldosterone system. A NOAEL for increased blood pressure cannot be determined. However, a NOAEL for kidney effects (histopathology) is established at 0.12 mg V/kg-day, with a LOAEL at 1.2 mg V/kg-day.

Table 6. Significant Effects on Cardiovascular Parameters in Male Rats Exposed to Sodium Metavanadate[a]

Parameter	Control[b]	0.12 mg V/kg-day (180 days)	1.2 mg V/kg-day (210 days)	4.7 mg V/kg-day (210 days)
Systolic blood pressure (mm Hg)	108 ± 5[c] 106 ± 7	130 ± 4[d]	137 ± 5[d]	132 ± 4[d]
Diastolic blood pressure (mm Hg)	84 ± 4 85 ± 5	106 ± 3[d]	112 ± 5[d]	114 ± 7[d]
Plasma renin activity (ng/mL/h)	13.4 ± 3.4 10.3 ± 2.7	10.6 ± 2.4	47.5 ± 14.9[d]	40.6 ± 12.4[d]
Plasma aldosterone (pg/mL)	264 ± 22 188 ± 57	158 ± 11[d]	554 ± 160[d]	265 ± 61
Kallikrein (nM/mg creatinine)	8.02 ± 1.90 8.43 ± 0.96	4.36 ± 0.60[d]	13.67 ± 2.54[d]	11.72 ± 0.80[d]
Kininase I (nM × 10^{-3} of hydrolyzed substrate/mg creatinine)	27.6 ± 5.4 32.0 ± 4.2	56.8 ± 25.3	129.9 ± 14.9[d]	156.8 ± 9.1[d]
Kininase II (nM x 10^{-3} of hydrolyzed substrate/mg creatinine)	2.23 ± 0.33 1.83 ± 0.26	2.30 ± 0.31	2.63 ± 0.13[d]	3.92 ± 4.08[d]
Urinary potassium excretion 11(mEq/g creatinine)	113 ± 25 118 ± 14	106 ± 6	169 ± 18[d]	221 ± 28[d]

[a]Boscolo et al., 1994
[b]First result is for 180-day control group; second is for 210-day control group.
[c]Mean ± standard error of the mean
[d]Significantly different from corresponding control, $p < 0.05$

Reproductive Studies—Effects on reproductive success have been reported with preconception exposure to vanadium compounds. Domingo et al. (1986) administered daily gavage doses of 0, 5, 10, or 20 mg/kg-day sodium metavanadate (2.1, 4.2, or 8.4 mg V/kg-day) to male and female Sprague-Dawley rats (20/sex/dose). Male rats received daily doses for 60 days after which they were mated to female rats that had received the same doses 14 days prior to mating. Dosing of females continued through gestation. Half of the females were sacrificed on gestation day (GD) 14 for assessment of the number of corpora lutea, total implantations, resorptions, and living and dead fetuses. The remaining dams were continued on the exposure regimen through weaning of their pups (postnatal day [PND] 21). Evaluations of offspring included viability, body-weight gain, body and tail lengths and clinical signs on PND 1, 4, and 21. Results for pups were pooled across litters. Upon sacrifice of pups at weaning, the weights of heart, lungs, spleen, liver, kidneys, and testicles were recorded. The authors reported that maternal toxicity was not evident in the treated dams, but did not specify the endpoints measured to assess maternal effects. No significant differences between the treatment and control groups were observed in the various indicators of reproductive success assessed at sacrifice on GD 14 (data shown). A significant decrease ($p < 0.05$) in pup growth occurred in all treatment groups compared to the control group, as indicated by deficits in whole litter weight and pup body weight, head-to-rump length, tail length, and relative kidney and liver weights (organ-/body-weight ratios). Body weight per litter was significantly decreased in the high-dose group on PND 4 and the mid- and high-dose groups on PND 21. Table 7 shows the changes in pup growth parameters (pooled across litters) observed on PND 1, 4, and 21. At the

high dose, significant ($p < 0.05$) decreases in relative heart (males only) and spleen weights (both sexes) were also observed. These data suggest a developmental toxicity LOAEL of 2.1 mg V/kg-day based on growth retardation in pups; a developmental NOAEL was not identified. Due to the lack of information on maternal endpoints evaluated, effect levels for systemic toxicity cannot be determined

Table 7. Significant Effects on Growth Parameters (Pooled Across Litters) in Pups of Dams Exposed to Sodium Metavanadate[a]

Parameter	Control	2.1 mg V/kg-day	4.2 mg V/kg-day	8.4 mg V/kg-day
Males				
Body weight PND 1 (g)	7.9 ± 0.9(63)[b]	7.0 ± 1.1[c] (57)	6.5±0.9[c] (77)	6.7±0.6[c] (48)
Body weight PND 4 (g)	11.7 ± 1.3 (63)	9.6 ± 1.8[c] (57)	9.7 ± 1.2[c] (63)	8.9 ± 0.8[c] (40)
Body weight PND 21 (g)	42.0 ± 8.3(57)	34.3 ± 7.9[c] (56)	33.7 ± 10.8[c] (35)	33.6 ± 7.6[c] (38)
Body length PND 1 (mm)	56.8 ± 3.5	54.2 ± 3.6[d]	53.4 ± 3.4[e]	53.1 ± 3.0[e]
Body length PND 4 (mm)	67.1 ± 3.4	62.0 ± 4.4[c]	64.7 ± 3.6[c]	62.2 ± 2.5[c]
Body length PND 21 (mm)	119.1 ± 6.1	108.0 ± 10.0[c]	102.8 ± 16.2[c]	104.8 ± 10.8[c]
Tail length PND 4 (mm)	30.4 ± 2.4	23.9 ± 3.4[c]	25.8 ± 3.8[c]	23.6 ± 2.3[c]
Relative liver weight (g/100g BW)	5.12 ± 0.58	4.72 ± 0.56[d]	4.63 ± 0.40[d]	4.57 ± 0.54[d]
Females				
Body weight PND 1 (g)	7.6 ± 0.9 (54)	6.8 ± 1.0[c] (58)	6.4 ± 0.9[c] (62)	6.5 ± 0.6[c] (43)
Body weight PND 4 (g)	11.2 ± 1.9 (53)	9.5 ± 1.6[c] (58)	9.3 ± 1.4[c] (48)	8.8 ± 1.1[c] (39)
Body weight PND 21 (g)	41.0 ± 6.7 (51)	32.5 ± 6.3[c] (53)	29.7 ± 7.2[c] (20)	32.1 ± 8.8[c] (38)
Body length PND 1 (mm)	55.5 ± 3.4	53.6 ± 3.7[e]	52.4 ± 3.9[c]	52.0 ± 2.6[c]
Body length PND 4 (mm)	65.5 ± 3.0	61.4 ± 3.8[c]	63.0 ± 3.7[c]	61.5 ± 3.3[c]
Body length PND 21 (mm)	119.7 ± 6.9	105.5 ± 11.3[c]	100.9 ± 11.7[c]	104.4 ± 11.3[c]
Tail length PND 4 (mm)	30.7 ± 2.4	25.1 ± 3.1[c]	26.2 ± 3.8[c]	24.3 ± 2.5[c]
Tail length PND 21 (mm)	70.4 ± 8.0	66.3 ± 7.0[d]	68.9 ± 9.5	61.0 ± 6.0[c]
Relative liver weight (g/100g BW)	5.53 ± 0.45	5.04 ± 0.80[d]	5.01 ± 0.75[d]	4.72 ± 0.63[e]
Relative kidney weight (g/100g BW)	1.56 ± 0.17	1.38 ± 0.22[d]	1.45 ± 0.20[d]	1.32 ± 0.16[e]

[a]Domingo et al., 1986
[b]Mean ± SD (number of animals)
[c]$p < 0.001$
[d]Significantly different from control, $p < 0.05$
[e]$p < 0.01$

Llobet et al. (1993) exposed male Swiss mice to sodium metavanadate in drinking water for 64 days prior to mating for 4 days with unexposed females. There were four treatment groups that consisted of 24 mice per group given water to which 100, 200, 300, or 400 mg/L sodium metavanadate was added. The authors reported the doses as 20, 40, 60, or 80 mg/kg-day sodium metavanadate, which correspond to calculated vanadium doses of 8.4, 17, 25, or 33 mg V/kg-day. The control group consisted of 24 mice given water without added vanadate. Dams were killed 10 days after mating (GD 10–14) and their uteri were examined to evaluate pregnancy outcomes. Endpoints assessed included body weights; reproductive success, including the number of implantations, early or late resorptions and dead or live fetuses; testis and epididymis weights; and sperm counts, motility, and morphology. Body weights in the 33 mg V/kg-day group were significantly lower than in the control group (13%, $p < 0.05$). The absolute (but not relative) epididymis weight was reduced by treatment (12%, $p < 0.01$). There was no difference in the absolute or relative testis weight between the control and treatment groups. A lower number of successful impregnations occurred in the 25 and 33 mg V/kg-day dose groups compared to the control group (43.8% and 62.5%, respectively, compared with

81.3% in controls; $p < 0.01$). There were no differences in the number of resorptions or fetal mortality. Outcomes related to sperm included: lower spermatozoa counts in the 25 and 33 mg V/kg-day groups relative to the control group (44% and 31% lower, respectively); a lower spermatid count in the 33 mg V/kg-day group (30%, $p < 0.01$) and no significant difference in sperm motility or morphology between control and treatment groups. These data indicate a LOAEL of 25.1 mg V/kg-day based on reproductive effects in treated male mice (decreased spermatozoa counts and reduced fecundity); the NOAEL is 17 mg V/kg-day.

In a study comparing reproductive effects of vanadium in diabetic and nondiabetic rats, Ganguli et al. (1994a) administered concentrations of 0, 250, or 500 mg/L sodium orthovandate (~69 or 138 mg V/L) with 0.45% normal saline in the drinking water of female Sprague-Dawley rats. There were six groups of 15 rats/dose that were used (three groups each of nondiabetic and streptozocin-induced diabetic rats). The authors reported that the animals were mated to untreated males at the commencement of treatment (Day 1); however, the balance of the treatment regimen was not described, so the duration of treatment is not known. Body weight, fluid intake, and urine glucose were measured daily; however, data on body weight and fluid intake are not reported or described. In the absence of information on the treatment schedule, it is not possible to estimate doses with any degree of confidence. Pregnant dams were sacrificed one day after giving birth; those treated females that did not become pregnant or failed to deliver were sacrificed for examination of uteri and ovaries. At birth, the total number of pups and total body weight were recorded. In contrast to the findings discussed previously (Dai et al., 1994a,b; Dai and McNeill, 1994), vanadium was severely toxic to diabetic rats; 7/15 females exposed to 500 mg/L died before Day 15 of treatment and the remainder had severe diarrhea and lack of appetite; these animals were sacrificed humanely. Mortality also occurred at the low dose in diabetic rats (3/15). No deaths occurred in controls. In high-dose nondiabetic rats, moderate-o-severe diarrhea was observed in 12/15 rats; this effect was not reported in low-dose nondiabetic rats. The rate of conception was significantly ($p < 0.05$) reduced by vanadium exposure in both diabetic and nondiabetic rats. When compared with nondiabetic controls, the rate of conception is reduced by 13% and 20% at 250 and 500 mg/L (respectively) in nondiabetic rats and by 7%, 33%, and 47% in 0, 250, and 500 mg/L (respectively) diabetic groups. Ability to carry a pregnancy to term was also compromised by vanadium exposure, significantly so in the diabetic animals. Compared with nondiabetic controls, nondiabetic treated animals exposed to 250 and 500 mg/L were 30% and 84% (respectively) less likely to carry pregnancy to term. In diabetic animals, fewer than 10% of animals at the low dose carried pregnancy to term; as the high-dose group was sacrificed early, there are no data on this endpoint. Effect levels cannot be determined from these data since duration of treatment is unknown and doses could not be estimated.

Faria de Rodriguez et al. (1998a) conducted three experiments to evaluate the effects of exposure to vanadium on the development of the central nervous system in albino rats. This study was published in Spanish and translated for this review. Groups of four female rats were used in all experiments. In the first experiment, three groups were exposed to 0, 100, or 200 ppm ammonium metavanadate (43.5 or 87 ppm V) in the drinking water from weaning until mating; treatment was discontinued during mating and gestation. Doses estimated for this review based on default values of water intake and body weight (U.S. EPA, 1988) were 7 and 15 mg V/kg-day. From each group, two dams were sacrificed at GD 20, while the other two were allowed to deliver. Litters were sacrificed at birth for gross examination of external and

internal malformations and the CNS was removed for microscopic examination and histochemical assessment of glycosaminoglycans. In another experiment, two groups of neonates whose mothers had been exposed to 100-ppm ammonium metavanadate from 37 days of age until mating were exposed to concentrations of 0 or 100 ppm via lactation until weaning and then via drinking water until mating. As with the first experiment, half of each group was sacrificed at GD 20 and half after delivery; evaluation of litters was also the same. In the final experiment, newborn rats of untreated mothers were exposed via lactation and then via drinking water to 0 or 200 ppm ammonium metavanadate. All rats of the final experiment were permitted to deliver. Females in all of the control groups delivered litters averaging from 5–11 offspring each. All four rats exposed to 7 mg V/kg-day in the first experiment became pregnant, delivering an average of 11 offspring per litter. At 15 mg V/kg-day, one rat died, one delivered a litter of 11 offspring, and the other 2 did not become pregnant. In the second experiment, of four rats exposed to 7 mg V/kg-day from birth to mating, only two became pregnant and delivered litters, averaging six offspring each. Similarly, in the third experiment, exposure to 15 mg V/kg-day resulted in only 2/4 females delivering litters (4 and 10 offspring each). No gross external malformations were observed in any of the groups. Data on the microscopic examination of brains were grouped across the experiments, so a dose-response relationship could not be discerned. Of the 81 brains obtained from the offspring of treated animals, 13 exhibited unilateral hypoplasia of the olfactory bulb and one exhibited unilateral hypoplasia of the cerebral hemisphere; the remaining brains were characterized as normal. Microscopic effects on the olfactory bulbs (for example, thinning or disorganization of the glomerular layer) were also seen in the brains of animals with grossly observable effects; the incidences of specific effects were not reported. All brains of control offspring were normal both macroscopically and microscopically. Histochemical studies indicate that exposure to 15 mg V/kg-day increased the glucosaminoglycan content—specifically those of a low grade of sulfation. Effect levels cannot be determined from these data due to the lack of incidence data, the grouping of effect information across treatment groups, and incomplete reporting.

Faria de Rodriguez et al. (1998b) exposed male and female Swiss albino mice to ammonium metavanadate from birth until the animals were mated. This study was published in Spanish and translated for this review. The test compound was administered in drinking water to mothers so that the offspring were exposed via lactation until weaning, when they were continued on the same exposure via drinking water (0, 100, or 200 ppm) until mating. These concentrations correspond to 43.5 and 87 ppm vanadium or dose estimates of 15 and 30 mg V/kg-day (males) and 14 and 28 mg V/kg-day (females) based on default values[2] for body weight and water intake (U.S. EPA, 1988). The animals' body weight, body length, and tail length were measured weekly. At maturity, the males and females of each exposure group were mated with same-treated mice or cross-mated with untreated mice to evaluate separately the effects on each gender. Numbers of offspring, as well as weight and length of offspring, were assessed after successful mating. The authors reported the results of statistical analysis of the parameters, but did not report the data for any endpoints. In addition, results for the same exposure level were grouped across sex. The authors reported that there were no treatment-related differences in body weight of the parents. Body length of treated mice was significantly ($p < 0.05$) reduced with exposure to 28–30 mg V/kg-day, and tail length was significantly lower at both exposure levels when compared with control animals. In contrast, neither weight nor length of offspring was affected in any of the matings. Further, the number of

[2]Assuming body weight and water intake at weaning.

offspring was higher in the exposure groups than in the control group. Effect levels cannot be determined from these data due to inconsistent outcomes and poor reporting.

The same group of investigators conducted additional experiments on female Swiss albino mice (Nava de Leal et al., 1998). This study was also published in Spanish and translated for this review. Ammonium metavanadate was administered in drinking water at concentrations of 0, 100, or 200 ppm (0, 43.5, or 87 ppm vanadium) at various times as shown in Table 8. Dose estimates calculated for this review are 11 or 23 mg V/kg-day based on default values[3] for body weight and water intake (U.S. EPA, 1988). In each experiment, exposure was suspended during 8 days of mating with untreated males (ratio of 1 female to 2 males) and during gestation. After mating, the mice were housed individually and weighed twice weekly. Pregnant mice were allowed to deliver; pregnancy rates and number of offspring were recorded. Those mice that failed to become pregnant were sacrificed 21 days after mating for evaluation of the following parameters: uterine and ovarian weights; corpora lutea counts and histopathology examination of the ovaries. The reporting of results was limited by some inconsistencies and apparent typographical errors. As Table 8 shows, the pregnancy rate was significantly reduced from controls in 2/3 groups (C1 and F1C, but not A2) exposed to 23 mg V/kg-day but not in any group exposed to 11 mg V/kg-day. The absence of an effect on pregnancy rate in Group A2 (exposed to 23 mg V/kg-day from weaning until mating) contrasts with the findings in Group C1 (exposed to 11 mg V/kg-day until first mating and then to 23 mg V/kg-day from parturition until second mating) and suggests that cumulative exposure may be an important factor in the effects of vanadium on mating success. In the statistical analysis of litter sizes, groups exposed for different time periods to the same concentration were combined (details unclear). The results shown in Table 8 indicate that litter size is significantly smaller in mice exposed to 23 mg V/kg-day compared with controls (*p*-value not given). A similar approach was used to compare the numbers of corpora lutea; this analysis also showed a reduced average number of corpora lutea in mice exposed to 23 mg V/kg-day compared with untreated controls. Corpora lutea were counted only in mice that failed to become pregnant, which may have biased the findings.

[3]Assuming body weight and water intake for subchronic exposure.

Table 8. Exposures, Group Sizes, and Pregnancy Outcomes Among Mice Exposed to Ammonium Metavanadate[a]

Group	Exposure Period	Dose (mg V/kg-day)	No. Mice	Pregnancy Rate (%)	Average Litter Size
A1	Weaning to adulthood (mating)	0 11	8 12	NR[b] 50	13 13
A2	Weaning to adulthood	0 23	8 12	50 66.6	9 8
B	Weaning to adulthood	0 11	8 12	75 83.3	11 9
B1	Second mating of Group B; no exposure between matings	0 11	6 10	100 100	10 10
F1B	Offspring of Group B	0 11	9 26	66.6 73	NR NR
C	Weaning to adulthood	0 11	8 12	75 83.3	9 10
C1	Mice Group C that successfully became pregnant; exposed from parturition until second mating	0 23	6 10	100 20[c]	9 3
F1C	Offspring of Group C; exposed via lactation until weaning and drinking water until adulthood	0 23	8 24	65 0[c]	12 0

[a]Nava de Leal et al., 1998
[b]Not reported
[c]Significantly different from control, $p < 0.0001$

Microscopic examination of the ovaries from mice that failed to become pregnant showed histopathology associated with exposure to vanadium (Nava de Leal et al., 1998). Most (94%) samples of ovaries from control mice that failed to become pregnant were reportedly normal. In contrast, ovaries of mice exposed to 11 mg V/kg-day (Groups A1 and B) exhibited fewer follicles and/or follicular atresia (absence of follicles due to degeneration); the follicles that were seen were enlarged and conferred a "polycystic aspect" on the ovaries. Histopathology findings in the ovaries of mice exposed to 23 mg V/kg-day (Groups A2, C1, and F1C) were more pronounced, including absence of mature follicles and corpora lutea, marked follicular atresia, thickening of the external theca, loss of ovarian parenchymal architecture, cellular disaggregation, and cytoplasmic vacuolation in granulosa lutein cells. The authors reported the incidences of these findings in the ovaries of mice that did not become pregnant; however, the overall incidences of these effects in treated mice were not available, as histopathology was not assessed in mice that became pregnant. Ovarian histopathology changes in mice exposed to 11 mg V/kg-day suggest that this dose may be a LOAEL, despite the lack of effect on pregnancy success; however, the absence of data on overall incidences in the treated and control groups (including those that became pregnant), in addition to reporting problems, precludes definition of reliable effect levels for this study.

Morgan and El-Tawil (2003) also assessed the effects of vanadium exposure on reproductive success. Groups of 10 male and 20 female Sprague-Dawley rats were given ammonium metavanadate at concentrations of 0 or 200 mg/L (87 mg V/L) in the drinking water. Based on default values of water intake and body weight (U.S. EPA, 1988), the dose is estimated to be 28 and 30 mg V/kg-day in males and females, respectively. Exposed male rats were treated

for 70 days prior to mating with untreated females; exposed females were treated for 14 days premating and during mating, gestation, and lactation (total of 61 days). During premating, the estrous cycles of females were monitored. Maternal body weights were recorded at the end of gestation. Half of each group of females was sacrificed on GD 20, while the other half, along with their pups, was sacrificed after weaning on PND 21. Gravid uterine and placental weights were recorded. Males were sacrificed after mating for assessment of body, testes, epididymis, prostate, and seminal vesicle weights. Reproductive parameters assessed in the study included: gestation duration; signs of dystocia; numbers of corpora lutea, implantation sites, resorptions, pre- and postimplantation losses; live and dead fetuses; fetal body weight at birth and on PND 4, 7, 14, and 21 and fetal survival during lactation. During lactation, pups were examined for learning and memory responses; however, the specific methods and endpoints were not described. All pups were examined for gross malformations at sacrifice; two-thirds were examined for skeletal abnormalities and the remainder for visceral abnormalities. Exposure to ammonium metavanadate resulted in profound effects on reproductive success and offspring development, regardless of whether males or females were treated. Statistically significant adverse effects are reported for nearly every reproductive parameter assessed, including maternal body, uterine and placental weights; litter parameters; viability of offspring at birth; pup body weight during lactation and incidences of gross, visceral and skeletal malformations. In addition, fewer treated females exhibited normal estrous cycles; treatment of females also resulted in reduced survival and viability indices of offspring. Body weight of treated males was not affected, but testes, epididymis, prostate gland and seminal vesicle weights were significantly ($p < 0.05$) reduced by exposure. Few offspring were produced in the treated groups (20 and 35 in the offspring of treated males and females, respectively, compared with 216 controls). Those that were produced had a high frequency of gross, visceral, and skeletal anomalies. Data were reported using the fetus, rather than the litter, as the unit of statistical analysis, so it is not possible to assess the litter distribution of effects. These data suggest a freestanding LOAEL of 28 mg V/kg-day for reproductive toxicity in rats.

Developmental Studies—Elfant and Keen (1987) exposed groups of at least 14 pregnant Sprague-Dawley rats to diets containing 0- or 75-ppm vanadium (as sodium metavanadate) throughout pregnancy and lactation. Based on default values for body weight and food intake[4] (U.S. EPA, 1988), the dose of vanadium was around 7 mg/kg-day. Maternal weight and food intake were recorded daily. When the dams gave birth, live and dead pups were counted. Pup weights were recorded at birth and every second day thereafter until PND 21. Sacrifice of both dams and pups was performed at PND 21, whereupon brain, kidney, spleen, pancreas, heart, thymus, and testes were weighed. Liver samples were collected for analysis of lipid peroxidation products (reduced glutathione, thiobarbituric acid reactivity, and superoxide dismutase activity).

The authors reported that both food intake and body-weight gain were lower in the exposed dams (statistical analysis not reported); at parturition, the cumulative weight gain appeared to be about 25% lower in exposed animals relative to controls based on visual examination of body-weight gain data presented graphically (Elfant and Keen, 1987). Data on food intake are not reported. The percentage of pups born alive was smaller in exposed dams (about 80%) than in controls (about 90%) and survival to weaning was also lower (about 40% vs. about 70% in controls based on visual examination of data presented graphically and without

[4]Default values for body weight and food intake are uncertain estimates of weight and intake for pregnant animals, but they do provide an approximate estimate of dose in the absence of study-specific data on these parameters.

statistical analysis). The cumulative weight gain of the surviving pups was lower in exposed offspring; at weaning, mean body weights of exposed pups were about 34% lower than controls (data shown graphically and without statistical analysis). Pups of exposed dams were reported to exhibit diarrhea, seborrhea, lethargy, staggered gaits, and ocular exudate (incidences not reported). The relative weights of the liver, brain, and testes were higher in exposed vs. control pups (18%, 36%, and 15%, $p < 0.05$). Reductions in body-weight gain among exposed pups complicate the interpretation of these organ weight changes. Thiobarbituric acid reactivity was elevated in whole cell homogenates from the livers of both dams and pups exposed to vanadium; reduced glutathione was lower in exposed pups than in control pups but was not affected in dams. The latter findings suggest increases in lipid peroxidation with vanadium exposure that may contribute to developmental toxicity. These data indicate a maternal and developmental LOAEL of about 7 mg/kg-day based on reduced maternal food intake and weight gain, as well as reduced pup survival, body weight, growth and clinical signs in pups. A NOAEL cannot be identified.

The effects of sodium metavanadate on development were further studied by Paternain et al. (1987). Groups of 20 pregnant Sprague-Dawley rats were treated with sodium metavanadate via gavage at doses of 0, 5, 10, or 20 mg/kg-day during GD 6–15. Equivalent doses of vanadium were 2, 4, and 8 mg V/kg-day. On GD 20, the uteri were opened by Caesarean section for examination of corpora lutea, implantations, live and dead fetuses, and resorptions. Placental weights were recorded and fetal body weight, body length, and tail length were measured. Gross abnormalities were assessed in all fetuses; half were examined for skeletal abnormalities and half were examined for visceral anomalies. The paper does not report any evaluation of maternal toxicity parameters. At the high dose, fewer litters were produced than in controls or in other dose groups (14, 14, 12, and 8 in control, 2, 4, and 8 mg V/kg-day groups, respectively), but the decrease is not statistically significant. The numbers of resorptions were increased and numbers of live fetuses decreased at both 4 and 8 mg V/kg-day; however, these differences were also not statistically significant. A slight—but statistically significant—decrease in tail length was observed at 2 and 8 mg V/kg-day (4–5%, $p < 0.01$), but not at 4 mg V/kg-day; there was no apparent dose-response relationship. The authors reported that the incidences of skeletal and visceral abnormalities were not affected by treatment (data not shown). A higher percentage of fetuses in the high-dose group exhibited facial (18%) and dorsal (10%) hemorrhages when compared with controls (2% for facial and 2% for dorsal); however, a litter-based comparison between the groups is not presented. The authors characterized the 4 mg V/kg-day dose as a NOAEL for developmental effects on the basis of the hemorrhages observed at the high dose. However, the lack of information on the litter distribution of fetuses with hemorrhages precludes a reliable determination of effect levels from these data. Further, as maternal parameters were not evaluated, no determination of maternal effect levels can be made.

Paternain et al. (1990) administered gavage doses of 0, 37.5, 75, or 150 mg/kg-day vanadyl sulfate pentahydrate (7.5, 15, or 30 mg V/kg-day) to female Swiss mice on GD 6–15. The control group included 20 mice and the treatment groups consisted of 16 or 20 mice per group. Body weight and food consumption were recorded daily and observations for morbidity and mortality were also made daily. Dams were killed on GD 18 and fetuses harvested by Caesarean section; dams were then examined for gross pathology. The following litter parameters were evaluated: number of implants, number of resorptions, and number of live and dead fetuses. Fetal sex, weight, and length were noted. Pups were examined for external,

visceral, and skeletal abnormalities. Treatment did not result in mortality or clinical signs, and food consumption was not different between the treatment and control groups. A significant ($p < 0.05$) decrease in body-weight gain of the dams occurred in all treatment groups during the treatment period (46%, 53%, and 59% below controls at low, mid-, and high doses, respectively). At termination, body weights corrected for gravid uterine weights were reduced at 15 and 30 mg V/kg-day (16% below controls at both doses; $p < 0.05$). At these doses, absolute liver and kidney weights were also reduced proportionate to the body weight decrements. A significant ($p < 0.05$) increase in early resorptions occurred in all treatment groups relative to the control group (2–6 fold higher, without a clear dose-response relationship). Fetal body weights were significantly lower (13–21%, $p < 0.001$) in all treatment groups compared to the control group. The following external and internal soft-tissue abnormalities were observed at significantly elevated incidences (with litter as unit of statistical measure, $p < 0.05$) in fetuses of treated dams: hematomas of the dorsal area (all dose levels), hematomas of the facial area and neck (15 and 30 mg V/kg-day only), anophthalmia/microphthalmia (15 mg V/kg-day), cleft palate, and micrognathia (30 mg V/kg-day). The incidences of litters with external defects (grouped across type) were 2/20, 8/20, 11/20, and 17/20 in control, low, mid-, and high doses, respectively; these were significantly ($p < 0.05$) elevated above control at all dose levels. While the incidences of soft tissue abnormalities (exclusively hydrocephaly) were increased at the mid- and high-dose, the increases were not statistically significant. However, the incidence of skeletal defects were increased at all doses (4/20, 9/16, 15/20, 20/20 affected litters in control through high dose; $p < 0.05$ for all treatment groups). The skeletal abnormalities consisted of poorly ossified supraoccipital bone, carpus, tarsus and sternebrae, as well as bipartite sternebrae and irregular ribs. These data indicate a freestanding LOAEL of 7.5 mg V/kg-day for both maternal toxicity (reduced body-weight gain during treatment) and developmental toxicity (increased resorptions, skeletal malformations, and growth delays).

In a later study by the same laboratory, Sanchez et al. (1991) administered daily gavage doses of 0, 7.5, 15, 30, or 60 mg/kg-day sodium orthovanadate (equivalent to 0, 2.1, 4.2, 8.3, or 17 mg V/kg-day) to groups of 14–20 pregnant Swiss mice on GD 6–15. Maternal appearance, body weight and food consumption were recorded daily. The dams were sacrificed on GD 18 for evaluation of body weight, liver and kidney weights, gravid uterine weight, and uterine parameters (numbers of implants, early and late resorptions, live and dead fetuses). Live fetuses were weighed, sexed, and examined grossly for abnormalities; two-thirds were then prepared for skeletal examination and one-third for visceral examination. Exposure to doses of 8.3 or 17 mg V/kg-day proved to be lethal; 4/18 dams dosed at 8.3 mg V/kg-day died, while 17/19 given the high dose died. Body-weight gain during treatment was reduced at 8.3 mg V/kg-day (30% less than controls, $p < 0.01$) and not at lower doses. Food consumption was significantly ($p < 0.05$) reduced at the beginning of treatment at both 4.2 and 8.3 mg V/kg-day. Body weight at termination, corrected for gravid uterine weight, was unaffected at any dose. Relative kidney weight was slightly—but statistically significantly—increased at 8.3 mg V/kg-day; however, the body-weight reduction at this dose may have contributed to the increased relative kidney weight. Litter parameters were not affected by exposure; at 8.3 mg V/kg-day, one litter contained no viable implants, but the incidence of litters with resorptions was not significantly increased. External and visceral malformations were not increased in exposed groups relative to controls; however, the numbers of litters containing fetuses with incompletely ossified sacrococcygeal vertebrae, forelimb and hindlimb proximal phalanges were increased at 8.3 mg V/kg-day. The authors identified the low dose (2.1 mg V/kg-day) as a NOAEL for maternal toxicity,

presumably considering the decreased food consumption at 4.2 mg V/kg-day. Given the evidence for frank effects at the next higher dose (mortality at 8.3 mg V/kg-day), the decrease in food consumption is considered potentially indicative of toxicity and is used to define the 4.2 mg V/kg-day dose as a LOAEL. The authors considered the 4.2 mg V/kg-day dose to be a NOAEL for developmental effects. For the purpose of this review, the LOAEL for developmental toxicity is 8.3 mg V/kg-day based on increases in the incidence of litters with incomplete skeletal ossification.

Ganguli et al. (1994b) exposed female Sprague-Dawley rats to 250 mg/L sodium orthovanadate (~69 mg V/L) added to drinking water on GD 10–20. The study compared the effects of treatment in diabetic (streptozocin-induced) and nondiabetic rats. The doses (calculated for this review based on reported fluid intakes and estimated body weight[5] of 250 g) were 7.5 and 17 mg V/kg-day in the nondiabetic and diabetic treatment groups, respectively. The treatment groups consisted of 11 diabetic and 7 nondiabetic pregnant females; the control groups consisted of 6 diabetic and 5 nondiabetic pregnant females given water without the addition of vanadate. Endpoints examined in dams included blood and urine glucose concentrations and fluid intake. On GD 20, the animals were sacrificed; the number of live pups and the pups' weights were recorded. Maternal uteri, ovaries, and placentas were examined grossly. Vanadium treatment was lethal in diabetic pregnant rats; only 6/11 dams survived until termination. No deaths occurred in other groups. Intake of drinking water was significantly decreased by vanadium treatment in both nondiabetic and diabetic rats. Fluid intake in the nondiabetic treatment group was approximately half that of the corresponding control group; in diabetic treated rats, fluid intake was about one-third that of the diabetic controls, who had significantly higher water intake than nondiabetic controls. Blood glucose was significantly decreased in vanadium-treated diabetic rats ($p = 0.006$), but the levels were still above those of nondiabetic rats. Urine glucose was not affected by vanadium exposure. Statistical analysis is not reported, and only data on pooled litters are reported; thus, a statistical group comparison cannot be made. Nevertheless, the outcomes included a lower average number of live fetuses on GD 20 (6.71 vs. 9.6 in treated vs. control nondiabetic rats and 5.5 vs. 11.3 in treated vs. control diabetic rats) and lower average pup mass in nondiabetic rats (3.60 vs. 4.02 g in treated vs. control) but not in diabetic rats (statistical analysis not reported). These data suggest that 17 mg V/kg-day is an FEL based on maternal mortality in the treated diabetic rats. Other effect levels cannot be determined due to poor reporting of data and limited endpoints evaluated.

Poggioli et al. (2001) assessed the effects of prenatal and postnatal exposure to vanadyl sulfate on the growth and behavior of Wistar rats. Concentrations of 0 or 300 mg/L of vanadyl sulfate (corresponding to 70 mg V/L according to the authors) were administered in the drinking water along with 5 g/L NaCl to reduce gastrointestinal effects of vanadium. An untreated control group received water without vanadyl sulfate or NaCl. Dams were exposed beginning three days before the last day of pregnancy and continued until weaning; thus, the pups were exposed during 3 days of gestation and via lactation until weaning. Litters were culled to 8–10 pups 1 day after birth and at weaning the groups were again reduced to 10/sex/dose. After weaning, the pups were given the same drinking water as their mothers until they were 100 days of age. Body weight was recorded at regular intervals and food and water intake were measured at 2 months of age. Based on recorded water intake, the vanadium dose was estimated by the authors to be about 10 mg V/kg-day. Neurobehavioral assessments were performed at 1 month

[5]The starting weights were 210–230 g; however, ending body weights were not reported.

of age (locomotor activity and open field evaluation of ambulation, rearing, grooming and defecation) and 100 days of age (memory test assessing time spent exploring new and familiar objects). Survival to weaning was significantly reduced by vanadyl sulfate treatment when compared with either the NaCl or untreated controls (61% in treated vs. 100% and 94% in NaCl and untreated controls, respectively; $p < 0.0001$). Neither food nor water intake was affected by exposure. Body weights were significantly lower than untreated controls beginning at weaning (PND 25) in the vanadyl sulfate group. However, body weights were also reduced in the NaCl group, so the effect of vanadium exposure cannot be distinguished. Locomotor activity was not different among the groups (data shown). In contrast, the open field evaluation revealed significantly ($p < 0.05$) fewer outer ambulation (ambulation in the outer area of the cage), rearing and grooming events and increased defecation in treated male rats when compared with the NaCl group; the treated males also exhibited reduced rearing events compared with untreated controls. The memory test revealed similar impairment in both the NaCl and vanadium exposure groups, which the authors attributed to NaCl exposure rather than vanadium (as the effect was similar in both). A LOAEL of 10 mg V/kg-day is identified based on reduced survival to weaning; a NOAEL cannot be identified.

Neurotoxicity

Sanchez et al. (1998) exposed male Sprague-Dawley rats to daily gavage doses of sodium metavanadate at dose levels of 0, 1.7, 3.4, or 6.8 mg V/kg-day for 8 weeks (12 animals per group). Endpoints assessed include body-weight gain and two neurobehavioral assessments: open-field activity and active avoidance (electric shock with auditory and light stimulus as the conditioned stimulus). Body-weight gains were significantly lower (10% below controls at the end of the exposure period, $p < 0.05$) in the 6.8 mg V/kg-day group relative to the control group. Open-field activity was lower in the 3.4 and 6.8 mg V/kg-day groups relative to the control group—but only during the first of three testing sessions ($p < 0.05$, data presented graphically). Similarly, acquisition of the avoidance response to the conditioned stimulus was significantly lower in all treatment groups ($p < 0.05$; about one-half as many avoidance responses and latencies about twice that of the control group based on graphical presentation of the data)—but only during the last of three sessions. Neither parameter exhibited a clear dose-response relationship; the magnitude of change from control was similar at all doses.

These investigators also conducted a follow-up study designed to evaluate whether the chelating agent Tiron would ameliorate the effects of vanadium exposure on behavior (Sanchez et al., 1999). Groups of 10 male Sprague-Dawley rats were given daily gavage doses of water or aqueous sodium metavanadate at a dose of 6.84 mg V/kg-day for 8 weeks. There were two groups that were also given Tiron via i.p. injection at two different doses. Body weight was measured daily. After the end of exposure, open-field activity and active avoidance were assessed as in the previous study. The authors indicated that body weight was not affected by treatment (data not shown). Graphical presentation of the data indicated no effect of exposure on open field motor activity but significant ($p < 0.05$) inhibition of active avoidance. Administration of Tiron mitigated the effects of sodium metavanadate on both of these endpoints.

Immunotoxicity

The limited data on immunotoxicity of vanadium suggest little or no adverse effect on this endpoint. Alexandrova et al. (2002) assessed humoral and cellular immune responses in

BALB/c mice and Wistar rats (both sexes) exposed to ammonium vanadate. Exposure to ammonium vanadate in the drinking water (0.5 mg/L or 0.2 mg V/L) for 40 or 200 days (about 6 or 28 weeks) stimulated both humoral and immune responses, as measured by increases (above control values) in the number of antibody-synthesizing cells in the spleen after challenge with sheep erythrocytes, the titers of serum agglutinins and haemolysins (humoral response) and the migration of spleen cells and peritoneal macrophages in vitro (cellular response). In contrast to the results of Alexandrova et al. (2002), Sharma et al. (1981) observed a decrease (albeit not statistically significant) in antibody-producing cells in the spleen of male Swiss-Webster mice exposed to concentrations of 0, 1, 10, or 50 mg/L vanadium (as sodium orthovanadate) in the drinking water for up to 13 weeks. No treatment-related effects were observed on delayed hypersensitivity reaction and immunoglobulin levels (IgG, IgA, and IgM) were not affected by exposure. Both Alexandrova et al. (2002) and Sharma et al. (1981) observed increased DNA synthesis in splenic lymphocytes treated with vanadium and cultured in the presence of some mitogens (phytohemagglutinin and pokeweed) but not others (bacterial lipopolysaccharide), when compared with cells not treated with vanadium.

Inhalation Exposure

No subchronic or chronic animal studies of inhalation exposure to vanadium compounds (other than vanadium pentoxide) have been identified in the literature search.

Other Studies

Toxicokinetics

In the United States, exposure to vanadium primarily occurs through dietary sources. Estimates of the daily intake of vanadium in the diet are in the range of 10–30 μg V/day or 0.0001 to 0.0004 mg V/kg-day for an adult man (WHO, 2001). Few studies are available on the absorption of vanadium from the gastrointestinal tract in humans or experimental animals; however, existing data suggest a relatively low fractional absorption. WHO (2001) estimated the gastrointestinal absorption of vanadium to be about 3% of the administered dose based on animal studies. Therefore, a relatively small absolute difference in gastrointestinal absorption between rodents and humans could result in a large error in the equivalent dose extrapolation. There are no studies that allow a direct comparison of the absorption of vanadium when administered as vanadyl or vanadate compounds.

Once absorbed, vanadium is distributed primarily to the bone, with smaller amounts distributing to the kidney, liver, spleen, muscle, and testes (ATSDR, 1992; WHO, 2001; Rydzynski, 2001). Vanadium stored in bone is retained much longer than in other tissues, from which vanadium is rapidly excreted (ATSDR, 1992; Rydzynski, 2001). Urine appears to be the major excretory route for absorbed vanadium, while unabsorbed vanadium is excreted in the feces (ATSDR, 1992; WHO, 1988, 2001).

In blood, vanadyl and vanadate ions are interconverted through redox reactions that may involve glutathione, cysteine, ascorbate, and possibly other components of plasma and cytosol (Rehder and Jantzen, 1998). Vanadium in blood partitions between plasma and erythrocytes. In beagle dogs administered single intravenous injections of vanadyl sulfate or ammonium vanadate, approximately 30–45% of the vanadium in blood was associated with erythrocytes and approximately 80% of vanadium in serum was associated with transferrin (Harris et al., 1984). Albumin also participates as a protein ligand for vanadyl and vanadate in plasma

(Chasteen et al., 1986a,b). Vanadyl and vanadate form complexes with a variety of intracellular proteins including ATPases, calmodulin, kinases and phosphatases, ribonucleases and nucleic acids (Rehder and Jantzen, 1998). The redox state of the cytosol favors the intracellular reduction of vanadate to vanadyl, whereas the oxidation of vanadyl to vanadate is favored in plasma; the interconversion occurs in minutes (Etcheverry and Cortizo, 1998).

Antineoplastic Studies

Vanadium has been tested as an antineoplastic agent in animal models of colon, liver, and mammary carcinogenesis. All of the studies of this effect that were identified in the literature searches were conducted by a single laboratory. In all of the studies, vanadium was administered as ammonium monovanadate to rats at a concentration of 0.5 ppm in drinking water. Vanadium coadministration reduced the number of aberrant crypt foci (a preneoplastic lesion in colon cancer) in rats treated with 1,2-dimethylhydrazine and resulted in fewer colon tumors (Kanna et al., 2003, 2004, 2005). Mechanistic data collected in these studies showed that vanadium treatment reduced the number of DNA-protein cross-links and evidence of DNA damage in colon cells, reduced the PCNA index, decreased the frequency of chromosomal aberrations and increased glutathione S-transferase and cytochrome p450 levels when compared with rats treated with carcinogen alone (Kanna et al., 2003, 2004, 2005). Similar findings were observed in rat models of hepatocarcinogenesis. In rats treated with 2-acetylaminofluorene (2-AAF) or diethyl nitrosamine (DEN) and subsequently given vanadium, relative liver weight, incidence of gamma glutamyl transpeptidase (GGT)-positive foci, nodular incidence, number of liver nodules and multiplicity of nodules were reduced compared with treatment with the carcinogen alone (Chakraborty et al., 2005; 2006a,b,c; 2007a,b). Vanadium treatment reduced the frequency of modified DNA bases, DNA damage, and chromosomal aberrations; reduced the expression of metallothionein (a metalloprotein associated with neoplastic cell growth) and Ki-67 nuclear antigen; and increased the expression of p53 tumor suppressor (Chakraborty et al., 2005; 2006a,b,c; 2007a,b). Further evidence of a potential antineoplastic effect of vanadium was provided in studies of rat mammary carcinogenesis. Vanadium treatment reduced the incidence, total number, multiplicity and size of mammary tumors in rats pretreated with 7,12-dimethylbenz(a)anthracene (Ray et al., 2004; 2005a,b; 2006). Ray et al. (2006) used immunohistochemical analysis to show that vanadium exposure increased apoptosis in mammary tissues; p53 and Bax genes were upregulated, while the antiapoptotic protein Bcl2 was downregulated by vanadium. In studies performed in another laboratory, a vanadium-cysteine complex was effective in prolonging survival, reducing the rate of benzo(a)pyrene-induced leiomyosarcoma growth, and inducing some tumor remission when given to male rats beginning on the day a palpable tumor was observed (Evangelou et al., 1997; Liasko et al., 1998).

Mechanistic

Etcheverry and Cortizo (1998) reviewed the action of vanadium on cells in culture. Their review indicated that vanadate acts as an analogue of phosphate, resulting in the modification (stimulation or inhibition) of several enzymes involved in phosphate metabolism. In in vitro systems, vanadium compounds have been shown to inhibit Na+K+ ATPase, Ca2+ ATPase, H+K+ ATPase, H+-ATPase, K+ATPase, Ca+Mg+ATPase, dynein ATPase, actomysosin ATPase, protein tyrosine phosphatase, glutamine dehydrogenase, acid and alkaline phosphatases, glucose-6-phosphatase, phosphofructokinase, alanine aminotransferase, aspargine aminotransferase, ribonuclease, phosphodiesterase, phosphotyrosyl-phosphatase, while stimulating phospholipase C, adenyl cyclase, mitogen-activated protein kinases,

phosphatidylinositol 3-kinase, NADPH oxidase, glycogen synthase, lipoprotein lipase, and tyrosine kinase phosphorylase (Etcheverry and Cortizo, 1998; Rydzynski, 2001). In addition, vanadium is a strong mitogen, inducing cell proliferation in a number of different systems (including fibroblasts, Leydig cells, and bone cells); the mechanism for this effect may be related to the inhibition of protein tyrosine phosphatases (Etcheverry and Cortizo, 1998). The effects of vanadium on various enzymes, which, in turn, affect many systems, may be responsible for the diverse effects seen in vivo—including modulation of diabetes, renal effects, reproductive and developmental toxicity and cardiovascular effects. In a recent review, Coderre and Srivastava (2004) proposed a potential mechanism of action for the cardiovascular effects of vanadium. In the proposed scheme, vanadium inhibition of protein tyrosine phosphatases results in the intracellular release of calcium and activation of phosphatidylinositol 3-kinase (PI3K) and p38-mitogen activated protein kinase (p38 MAPK) signaling pathways; these effects, in turn, stimulate smooth muscle contraction and glucose uptake (Coderre and Srivastava, 2004). Vanadium causes contraction of several types of smooth muscles, including gastric and vascular smooth muscle (Coderre and Srivastava, 2004). The effects of vanadium on smooth muscle contraction and glucose uptake may help to explain the in vivo modulation of blood pressure by vanadium. The authors noted that vanadium has exerted both vasodilation and vasoconstriction effects in different systems; thus, the action of vanadium on blood pressure may vary with dose, duration, and model system (Coderre and Srivastava, 2004).

Genotoxicity

Genotoxicity testing of soluble inorganic vanadium salts have primarily given positive results for mutagenicity and clastogenicity (especially numerical chromosomal aberrations). In the *Bacillis subtilis* Rec^- mutagenicity screening assay, ammonium metavanadate gave a positive result (greater inhibition of the Rec^- strain than the wild type Rec^+ strain) at a concentration of 0.3 M (Kanematsu et al., 1980). However, spot mutation tests with *Escherichia coli* (B/r WP2 and WP2) and *Salmonella typhimurium* (TA1535, TA100, TA98, TA1537, and TA1538) were negative for this compound (Kanematsu et al., 1980). Ammonium metavanadate induced mitotic gene conversion and reverse point mutations in *Saccharomyces cerevisia* (strain D7) when tested at concentrations from 80–210 nM with and without S9 (Bronzetti et al., 1990). Greater numbers of conversions and mutations were observed in the absence of S9, suggesting that the metabolism of ammonium metavanadate may detoxify the compound. In a study of cultured Chinese hamster V79 and V79-derived $hprt^-/gpt^+$ transgenic G12 cells, ammonium metavanadate exposure resulted in weak, but concentration-related increases in *hprt* mutations in V79 cells and in *gpt* mutations in G12 variants when the cells were exposed for 24 hours at concentrations from 5–50 µM (Cohen et al., 1992; Klein et al., 1994). Owusu-Yaw et al. (1990) reported that vanadyl sulfate and ammonium metavanadate both induced significant ($p < 0.01$) increases in the frequency of sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells treated with and without S9. Concentrations resulting in increases in SCE were about 6 and 2 µg V/mL for vanadyl sulfate and ammonium metavanadate, respectively. These compounds also induced dose-related increases in the frequency of chromosomal aberrations at concentrations near those causing cytotoxicity. Cytotoxic concentrations (TC_{50}s) were 23 and 16 µg V/mL for vanadyl sulfate and ammonium metavanadate, respectively (Owusu-Yaw et al., 1990). In human lymphocytes cultured in vitro, sodium metavanadate, sodium orthovanadate and ammonium metavanadate and vanadyl sulfate resulted in increased frequencies of micronuclei and numerical chromosomal aberrations (primarily hypoploidy) at doses as low as 5 uM (Migliore et al., 1993). SCEs were induced at higher doses (Migliore et al., 1993).

In vivo studies in mice indicated that vanadyl sulfate (100 mg/kg body weight), sodium orthovanadate (75 mg/kg) and ammonium metavanadate (50 mg/kg) administered by gavage all increased the frequency of micronucleated polychromatic erythrocytes (2- to 3-fold increase over controls) (Ciranni et al., 1995). The frequencies of hypoploid (missing chromosomes) and hyperploid (having an excess of chromosomes) cells were also increased by both compounds. Only vanadyl sulfate exposure resulted in a statistically significant ($p < 0.05$) increase (up to 7-fold above control values) in structural chromosomal aberrations (Ciranni et al., 1995). Mice exposed for 5 months to sodium orthovanadate in drinking water were observed to exhibit statistically significant increases in bone marrow micronuclei (at exposure concentrations of 750 or 1500 mg/L) as well as evidence of DNA damage in splenocytes (measured by comet assay, at a concentration of 1500 mg/L)—but not in bone marrow cells, testis cells or epididymal sperm (Leopardi et al., 2005). In another study, oral exposure to drinking water containing vanadyl sulfate (2–1000 mg/L) did not increase the frequency of micronuclei in bone marrow polychromatic erythrocytes in male CD-1 mice exposed for 5 weeks (Villani et al., 2007). In reticulocytes from these same mice, the frequency of micronuclei was slightly increased at some exposure levels, but there was no dose-response relationship (Villani et al., 2007).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR VANADIUM AND COMPOUNDS

Equal intakes of vanadium in any of the forms considered (vanadyl sulfate, sodium metavanadate, sodium orthovanadate, and ammonium metavanadate) were treated as toxicologically equivalent for the purpose of deriving provisional oral toxicity values on the following basis: (1) there is very little quantitative information about the gastrointestinal absorption of vanadium and no evidence that the absorption of vanadium will be substantially affected by the form of vanadium ingested for this set of compounds and (2) although there is evidence for pharmacologic specificity of the actions of vanadate and vanadyl ions in various biochemical systems, these forms are rapidly (within minutes) interconverted in the body in oxidation-reduction reactions that take place in the intracellular and extracellular compartments (Etcheverry and Cortizo, 1998; Mendz, 1998; Rydzynksi, 2001).

A total of four studies of humans exposed to vanadium compounds for brief durations (up to 12 weeks) are available; Table 9 provides an overview of the findings in these studies. Of these, three studies were of patients with diabetes. All of the studies used vanadyl sulfate in tablet form. Endpoints assessed in the studies included body weight, gastrointestinal symptoms, hematology, glycemic control, serum chemistry parameters, urinalysis, liver, kidney or thyroid function tests, and blood pressure. None of the studies reported significant effects on any endpoint other than gastrointestinal symptoms. Of particular note is the apparently normal kidney function and the absence of a blood pressure effect at daily doses as high as 0.5 to 1.1 mg V/kg-day. However, the exposure groups were small, no histopathology was possible, and often no referent population is included. While the individual studies are limited, the human studies collectively provide a short-term human LOAEL of approximately 0.3 mg V/kg-day in humans based on symptoms of gastrointestinal distress, including diarrhea, cramping, and discomfort. The NOAEL for these effects is approximately 0.1 mg V/kg-day based on the available studies. Gastrointestinal effects (severe diarrhea) have also been observed in rats

exposed to vanadium in drinking water (Zaporowska and Wasilewski, 1989, 1990, 1992a,b; Ganguli et al., 1994b); in rabbits exposed via drinking water (Khasibhatla and Rai, 1993) and in pigs exposed via the diet (Van Vleet et al., 1981; Van Vleet and Boon, 1980), providing support for the observed relationship between vanadium exposure and diarrhea in humans. The doses resulting in diarrhea in laboratory animals were in the 5–20 mg V/kg-day range. Studies in rats and mice indicate that vanadium exposure may be associated with effects on body weight, hematology, kidney function, blood pressure and reproduction. Animal studies that meet minimum criteria for possible use in deriving subchronic or chronic provisional RfDs (e.g., effect levels could clearly be identified) are summarized in Table 10. It should be noted that some of the LOAELs shown in Table 10 were identified for effects in partially nephrectomized rats (Steffen et al., 1981; Susic and Kentera, 1988) or in diabetic rats (Domingo et al., 1991, 1992).

Table 9. Human Studies of Oral Exposure to Vanadium Compounds

Study Description	Dose (mg V/ kg-day)	Vanadium Form Administered	NOAEL (mg V/ kg-day)	LOAEL (mg V/ kg-day)	Responses at the LOAEL	Comments	Reference
Human, 4 M and 7 F Tablet, daily for 6 weeks after 2-week run-up	0.5 (M) 0.6 (F)	Vanadyl sulfate (assumed trihydrate)	NA	0.5 (M) 0.6 (F)	Gastrointestinal symptoms	Patients with type 2 diabetes	Cusi et al., 2001
Human, 11 M and 5 F Tablet, daily for 6 weeks	0.12–0.23 0.28–0.45 0.43–1.14	Vanadyl sulfate (assumed trihydrate)	0.12–0.23	0.28–0.45	Gastrointestinal symptoms	Patients with type 2 diabetes	Goldfine et al., 2000
Human, 12–13 M and 4 F Tablet, daily for 12 weeks	0, 0.1	Vanadyl sulfate trihydrate	0.1	NA	None	Weight trainers	Fawcett et al., 1997
Human, 4 M and 4 F Tablet, daily for 4–8 weeks	0.34 (M) 0.39 (F)	Vanadyl sulfate (assumed trihydrate)	NA	0.34 (M) 0.39 (F)	Gastrointestinal symptoms	Patients with type 2 diabetes	Boden et al., 1996

Effects on blood pressure have been associated with vanadium exposure, although the available studies provide conflicting results. Boscolo et al. (1994) found a significant increase in systolic and diastolic blood pressure in rats exposed to 0.12, 1.2, or 4.7 mg V/kg-day as sodium metavanadate in the drinking water for 6 months. Carmignani et al. (1991) reported similar findings at a dose of 12 mg V/kg-day (as sodium metavanadate in drinking water). The increases in blood pressure are not corroborated by the Schroeder et al. (1970) chronic rat study or the Dai et al. (1994b) 52-week study in rats. Steffen et al. (1981) and Susic and Kentera (1988) reported increases in blood pressure in partially nephrectomized rats exposed to sodium orthovanadate and sodium metavanadate (respectively). In addition to these subchronic and chronic studies, a shorter-term study reported increased blood pressure in lean Zucker rats exposed to vanadium in the drinking water (about 10 mg V/kg-day) for 25 days (Hopfner et al., 1999). Several differences in the studies need to be taken into consideration in cross-study comparisons; Table 11 shows the major differences, which include the form of vanadium

Table 10. Animal Studies of Oral Exposure to Vanadium Compounds

Study Description	Dose (mg V/kg-day)	Vanadium Form Administered	NOAEL (mg V/kg-day)	LOAEL (mg V/kg-day)	Responses at the LOAEL	Comments	Reference
Shorter-term							
Male Sprague-Dawley rats (10/group) were exposed via drinking water for 28 days	0, 6.1, 15.6, 22.7	Sodium metavanadate, sodium orthovanadate, vanadyl sulfate	NA	6.1	Body-weight loss in diabetic rats	No nondiabetic treatment group	Domingo et al., 1991
Male Sprague-Dawley rats (10/group) were exposed via drinking water for 5 weeks	0, 23.2	Sodium metavanadate	NA	23.2	Body-weight loss in diabetic rats	No nondiabetic treatment group	Domingo et al., 1992
Male and female Wistar rats (15–16/sex/group) were exposed via drinking water for 4 weeks	0, 1.2, 5 (males) 0, 1.5, 7 (females)	Ammonium metavanadate	1.2 (males); 1.5 (females)	5 (males); 7 (females)	Reduced body weight (with reduced fluid intake) and hematology changes		Zaporowska et al., 1993
Subchronic							
Male Sprague-Dawley rats (20/group) were exposed via the diet for 9 weeks	0, 9	Sodium orthovanadate	NA	9	Decreased weight gain and increased blood pressure in uninephrectomized rats		Steffen et al., 1981
Male weanling pigs (6) were exposed via drinking water for 12 weeks	0, 10	Ammonium metavanadate	NA	10 (FEL)	Emaciation and mortality		Van Vleet, 1981
Male Sprague-Dawley rats (10/group) were exposed via drinking water for 12 weeks	0, 0.3, 0.6, 3.0	Sodium metavanadate	NA (0.6, ATSDR, 1992)	0.3 – 3.0 (indeterminate)	Mild changes in the kidney (hemorrhagic foci in the corticomedullary region), spleen (hypertrophy and hyperplasia) and lungs (perivascular mononuclear cell infiltration)	Occurring in all treatment groups, but "more evident" in the high-dose group. Clear AEL at 3 mg/kg-day.	Domingo et al., 1985
Male Long-Evans rats (15/group) were exposed via the diet for 2 months	0, 12	Ammonium metavanadate	NA	12	Pulmonary hypertension		Susic and Kentera, 1986
Male Wistar rats (8/group) were exposed via drinking water for 12 weeks	0, 7.7, 9.7	Ammonium metavanadate, vanadyl sulfate	7.7, 9.7	NA	No effects on food intake, body weight, hematology	Fluid intake was reduced at this dose	Dai et al., 1995

Table 10. Animal Studies of Oral Exposure to Vanadium Compounds

Study Description	Dose (mg V/kg-day)	Vanadium Form Administered	NOAEL (mg V/kg-day)	LOAEL (mg V/kg-day)	Responses at the LOAEL	Comments	Reference
Female Wistar rats (7/group) were exposed via the diet for 10 weeks	0, 1.1, or 2.3	Sodium metavanadate	2.3	NA	Small changes in hematology and body weight were not considered toxicologically significant		Adachi et al., 2000
Male Wistar rats (11–16/group) were exposed via drinking water for 6 weeks	0, 8	Sodium metavanadate	NA	8	Reduced body weight gain (possibly related to reduced food and fluid intake); hematologic effects		Scibior, 2005
Male Wistar rats (12/group) were exposed via drinking water for 6 weeks	0, 11	Sodium metavanadate	NA	11	Reduced body weight gain (possibly related to reduced food and fluid intake); hematologic effects		Scibior et al., 2006
Intermediate							
Male Long-Evans rats (12–24/group) were exposed via the diet for 24 weeks	0, 4.4, 42	Sodium metavanadate	NA	4.4	Increased blood pressure in partially nephrectomized rats	Blood pressure not affected in rats with intact kidneys	Susic and Kentera, 1988
Male Sprague-Dawley rats (10/group) were exposed via drinking water for 7 months	0, 12	Sodium metavanadate	NA	12	Increased blood pressure, kidney histopathology		Carmignani et al., 1991
Male Wistar rats (12/group) were exposed via drinking water for 5 months	0, 12	Vanadyl sulfate	NA	12	Decreased body weight in treated nondiabetic rats relative to nondiabetic controls		Cam et al., 1993
Male Sprague-Dawley rats (6/group) were exposed via drinking water for 180 or 210 days	0, 0.12, 1.2, 4.7	Sodium metavanadate	NA	0.12	Increased blood pressure, stimulation of the renin-angiotensin-aldosterone system, and kidney histopathology		Boscolo et al., 1994

Table 10. Animal Studies of Oral Exposure to Vanadium Compounds

Study Description	Dose (mg V/kg-day)	Vanadium Form Administered	NOAEL (mg V/kg-day)	LOAEL (mg V/kg-day)	Responses at the LOAEL	Comments	Reference
Chronic							
Long-Evans rats were exposed via drinking water from weaning through natural death (up to 45 months)	0, 0.7 (males), d 0.9 (females)	Vanadyl sulfate	NA	NA	No effects observed	Histological evaluations inadequate to detect any but the most severe lesions; effect levels cannot be determined	Schroeder et al., 1970
Male Sprague-Dawley rats (>20/group) were exposed via diet for 56 weeks	0, 7, 14	Sodium orthovanadate	NA	7	Increased blood pressure in uninephrectomized rats		Steffen et al., 1981
Male Wistar rats (8/group) were exposed via drinking water for 52 weeks	0, 8, 13 or 21	Vanadyl sulfate	NA	8	Reduced body-weight gain	Diabetic and nondiabetic rats	Dai et al., 1994a,b; Dai and McNeill, 1994
Reproductive							
Male and female Sprague-Dawley rats (20/sex/group) were exposed via drinking water for 60 (M) or 14 (F) days premating and during gestation and lactation (F)	0, 2.1, 4.2, 8.4	Sodium metavanadate	NA	2.1 (offspring)	Growth retardation in pups	Maternal effect levels could not be identified due to lack of information on endpoints assessed	Domingo et al., 1986
Male and female Sprague-Dawley rats (10 M and 20 F/group) were exposed via drinking water for 70 days (M) or through premating, mating, gestation and lactation (61 days, F)	0 or 28 (M) or 30 (F)	Ammonium metavanadate	NA	28	Effects on reproductive success, litter parameters, postnatal growth, male reproductive organ weights and skeletal malformations		Morgan and El-Tawil, 2003
Male Swiss mice (24/group) were exposed via drinking water for 64 days prior to mating	8.4, 17, 25.1 or 33.4	Sodium metavanadate	17	25.1	Decreased spermatozoa counts and reduced fecundity		Llobet et al., 1993

Table 10. Animal Studies of Oral Exposure to Vanadium Compounds

Study Description	Dose (mg V/kg-day)	Vanadium Form Administered	NOAEL (mg V/kg-day)	LOAEL (mg V/kg-day)	Responses at the LOAEL	Comments	Reference
Developmental							
Pregnant Sprague-Dawley rats (14/group) were exposed via the diet throughout pregnancy and lactation	0, 7	Sodium metavanadate	NA	7 (maternal and develop-mental)	Reduced food intake and weight gain (maternal); reduced pup survival, body weight, growth and clinical signs (developmental)		Elfant and Keen, 1987
Pregnant Swiss mice (16–20/group) were exposed via daily gavage on GD 6–15	0, 7.5, 15.1 or 30.2	Vanadyl sulfate pentahydrate	NA	7.5 (maternal and develop-mental)	Reduced body-weight gain (maternal) Increased resorptions, growth deficits, external and skeletal abnormalities (developmental)		Paternain et al., 1990
Pregnant Swiss mice (14–18/group) were exposed via daily gavage on GD 6–15	0, 2.1, 4.2, 8.3 or 16.6	Sodium orthovanadate	2.1 (maternal) 4.2 (develop-mental)	4.2 (maternal) 8.3 (develop-mental)	Reduced food consumption (maternal) Delayed skeletal ossification (developmental)	Maternal deaths occurred at 8.3 mg V/kg-day	Sanchez et al., 1991
Male and female Wistar rats, (8–10/group) were exposed via drinking water from 3 days before birth until 100 days of age	0, 10	Vanadyl sulfate	NA	10	Reduced survival to weaning		Poggioli et al., 2001

administered, the method of administration, the renal status of the affected animals, the strain of the affected animals and the method by which blood pressure was measured. All the blood pressure increases were from exposure to the vanadate; there were no blood pressure increases in the only two studies that used the vanadyl salt. Given the rapid interconversion of the two forms in plasma and cytosol, this discrepancy cannot be explained. Blood pressure was generally increased by 20–25 mm Hg over a 100-fold dose range within and among studies; this is particularly noted for the companion studies of Carmignani et al. (1991) and Boscolo et al. (1994) in which an interaction with thiopentane cannot be ruled out. Except for the shortest study of 25 days (Hopfner et al., 1999), there is no apparent exposure-duration effect on the magnitude of the blood pressure increase from exposure to vanadium for 9 to 56 weeks. No effects on blood pressure were observed in the human studies at doses as high as 0.5–1 mg V/kg-day (Boden et al., 1996; Fawcett et al., 1997; Goldfine et al., 2000; Cusi et al., 2001). Overall, these studies establish a NOAEL of at least 0.3 mg V/kg-day for blood pressure effects in humans for short-term exposure (6 weeks).

Table 11. Comparison Among Studies in which Blood Pressure was Measured

Study	Observed Effect on Blood Pressure[a]	Magnitude of Effect (mm Hg)	Form and Method of Vanadium Administration	Renal Status and Strain of Affected Animals	Method of Blood Pressure Measurement
Boscolo et al., 1994	Increase at ≥0.12 mg V/kg-day	25 (not dose-related)	Na metavanadate in drinking water for 6 months	Intact Sprague-Dawley rats	Arterial cannula under thiopentane anesthesia
Carmignani et al., 1991	Increase at 12 mg V/kg-day	22	Na metavanadate in drinking water for 7 months	Intact Sprague-Dawley rats	Arterial cannula under thiopentane anesthesia
Steffen et al., 1981	Increase at 9 mg V/kg-day	20	Na metavanadate in diet for 9 weeks	Uninephrectomized Sprague-Dawley rats	Tail cuff in conscious animals
Steffen et al., 1981	Increase at 7, 14 mg V/kg-day	10, 25	Na metavanadate in diet for 56 weeks	Uninephrectomized Sprague-Dawley rats	Tail cuff in conscious animals
Hopfner et al., 1999	Increase at 10 mg V/kg-day	15	Na orthovanadate in drinking water for 25 days	Intact lean Zucker rats	Tail cuff in conscious animals
Susic and Kentera, 1988	Increase at 4.4 mg V/kg-day	22	Na orthovanadate in diet for 24 weeks	Partially nephrectomized Long-Evans rats	Arterial cannula under nembutal anesthesia
Susic and Kentera, 1988	None at 42 mg V/kg-day	0	Na orthovanadate in diet for 24 weeks	Intact Long-Evans rats	Arterial cannula under nembutal
Dai et al., 1994b	None at 21 mg V/kg-day	0	Vanadyl sulfate in drinking water for 1 year	Intact Wistar rats	Tail cuff in conscious animals
Schroeder et al., 1970	None at 0.7 mg V/kg-day	0	Vanadyl sulfate in drinking water for 45 months	Intact Long-Evans rats	Anaesthetized animals; method not specified

[a]Effect observed at lowest dose tested in all positive studies

Studies conducted by Susic and Kentera (1988) in which several cardiovascular endpoints were assessed provide some information as to why vanadium exposure may increase blood pressure in some animals and not in others. In rats with intact kidneys exposed to doses up to 42 mg V/kg-day, a vanadium-related increase in peripheral resistance was offset by a reduction in cardiac output and blood pressure remained stable (no effect on blood pressure was observed). In partially nephrectomized rats, there was no compensatory reduction in cardiac output; thus, an increase in blood pressure was observed (Susic and Kentera, 1988). Thus, one potential explanation as to why blood pressure was not increased in every study is that compensatory mechanisms may serve to modulate the effect on blood pressure. If so, then individuals with health conditions that compromise these compensatory mechanisms (e.g., impaired renal function) may be at greater risk from vanadium exposure, although this hypothesis must be considered somewhat speculative.

Limited mechanistic information also supports a potential relationship between vanadium exposure and blood pressure changes. Boscolo et al. (1994) showed that vanadium exposure can modify plasma levels of proteins involved in blood pressure homeostasis. In this study, exposure to sodium metavanadate at doses of 1.2 or 4.7 mg V/kg-day resulted in increases in plasma renin activity (an enzyme that converts angiotensin to angiotensin I, a precursor to the vasoconstrictor angiotensin II) and aldosterone (a hormone involved in salt:water balance), as well as increases in urinary excretion of kallikrein (an enzyme that releases vasodilating kinins from plasma proteins) and kininases I and II (enzymes that break down kinins). The effects on the renin-angiotensin-aldosterone system are consistent with the observed increases in blood pressure.

Several studies (Domingo et al., 1985; Gorski and Zaporowska, 1982; Zaporowska, 1987; Dai et al., 1994a,b; Dai and McNeill, 1994) have indicated that the kidney is a primary target organ of vanadium toxicity in male rats. Among these, the study identifying effects at the lowest dose was Domingo et al. (1985). This study reported histopathologic changes in kidneys of rats exposed to sodium metavanadate in drinking water at dosages of 0.3 mg V/kg-day and higher. However, as previously noted, this study is limited in that only three animals per exposure group were actually subjected to a histopathological assessment and the results are summarized without a qualitative or quantitative reporting of incidence and severity. As a result, it is difficult to verify that the observed effects were clearly increased by exposure. Boscolo et al. (1994) reported hydropic degeneration in the kidneys of rats exposed to sodium metavanadate at a dose of 1.2 mg V/kg-day (in drinking water) for 1 year, with additional histopathologic changes (narrowing of the lumen and appearance of amorphous casts in the renal proximal tubules) at the next higher dose (4.7 mg V/kg-day). The latter changes were also observed by Carmignini et al. (1991) at a drinking water dose of 12 mg V/kg-day for 1 year. Dai et al. (1994a,b; Dai and McNeill, 1994) reported an increased incidence of glomerular and tubular degeneration, with interstitial cell infiltration and fibrosis in the kidneys of rats exposed to vanadyl sulfate in the drinking water at doses of 8–21 mg V/kg-day for a year. Limited information provided in English abstracts of two Polish studies (Gorski and Zaporowska, 1982; Zaporowska, 1987) suggested renal histopathology in rats exposed to 12–29 mg V/kg-day as ammonium metavanadate. Of all of these studies, only Boscolo et al. (1994) identified an unequivocal NOAEL for kidney effects.

Clinical chemistry changes indicative of renal effects have also been reported in a number of animal studies, although similar changes have not been observed in human studies (Fawcett et al., 1997; Boden et al., 1996). Increases in plasma urea concentrations have been observed at a dose of 3.0 mg V/kg-day in rats treated with sodium metavanadate (Domingo et al., 1985) and at higher doses in a number of studies (Domingo et al., 1991, 1992; Dai et al., 1994a,b; Dai and McNeill, 1994). Serum creatinine was higher in diabetic rats exposed to 6.1–22.7 mg V/kg-day as sodium metavanadate (Domingo et al., 1991) but not in a follow-up study in which diabetic rats were exposed to 23.2 mg V/kg-day as sodium metavanadate (Domingo et al., 1992). Susic and Kentera (1988) observed no changes in indicators of renal function (plasma creatinine, 24-hour creatinine clearance, urinary sodium excretion, and urine output) in normal and partially nephrectomized Long-Evans rats exposed to 4.4 or 42 mg V/kg-day as sodium metavanadate. Boscolo et al. (1994) observed increased potassium excretion after rats were exposed to sodium metavanadate at 1.2 and 4.7 mg V/kg-day, but no changes in urinary creatinine, nitrogen, proteins, sodium, or calcium. It should be noted vanadium was administered in drinking water in all of the studies that indicated clinical chemistry changes related to renal function. A number of studies have shown reductions in fluid intake, including marked reductions at doses of ≥10 mg V/kg-day, when vanadium is incorporated into the drinking water of rats. Thus, the changes in renal function parameters may have been influenced to an unknown degree by decreases in fluid intake, particularly at the higher exposure levels. No changes in renal function were observed in the human studies at doses as high as 0.5–1 mg V/kg-day (Boden et al., 1996; Fawcett et al., 1997; Goldfine et al., 2000; Cusi et al., 2001). Overall, these studies establish a NOAEL of at least 0.3 mg V/kg-day for overt kidney effects in humans for short-term exposure (6 weeks).

Limited mechanistic information in animals also provides some support for potential renal toxicity after vanadium exposure. Adachi et al. (2000) measured higher levels of lipid peroxidation products in the kidneys of rats exposed to 2.3 mg V/kg-day. Boscolo et al. (1994) reported reductions in Na+ K+ ATPase in the kidneys of rats exposed to 4.7 mg V/kg-day as sodium metavanadate; vanadium is known to inhibit the sodium-potassium ATPase (Etcheverry and Cortizo, 1998; Rydzynski, 2001). In addition, studies of vanadium distribution after oral exposure indicate that higher levels of vanadium are observed in the kidneys than in other organs, providing support for this organ as a potential target of vanadium toxicity.

Available data also supports a finding of reproductive and developmental toxicity associated with vanadium exposure. Effects observed in the available studies (see Table 10), conducted in both rats and mice, include diminished fertility, reduced offspring viability, growth retardation of offspring and skeletal malformations (Morgan and El-Tawil, 2003; Poggioli et al., 2001; Llobet et al., 1993; Sanchez et al., 1991; Paternain et al., 1990; Elfant and Keen, 1987; and Domingo et al., 1986). In addition to the studies shown in the table, several other studies are not suitable for derivation of provisional toxicity values, but they do contribute to the overall database for reproductive and developmental toxicity. There were three studies published in Spanish that provide suggestive evidence that vanadium exposure (as ammonium metavanadate) may result in histopathologic changes in the ovaries (Nava de Leal et al., 1998), effects on the developing central nervous system (especially the olfactory bulbs; Faria de Rodriguez et al., 1998a) and growth delays (Faria de Rodriguez et al., 1998b). In a study with poorly-reported information on the treatment regimen,

Ganguli et al. (1994b) reported reduced rate of conception and reduced ability to carry pregnancy to term in rats exposed to sodium orthovanadate.

Subchronic p-RfD

Data pertinent to the derivation of a subchronic p-RfD for vanadium include short-term human studies, short-term (4–5 weeks) and subchronic animal studies, and reproductive and developmental toxicity studies. In addition, several studies of slightly longer duration (5–7 months) have some bearing on the subchronic p-RfD because of the kidney and blood pressure endpoints. Blood pressure and kidney effects were fairly common among the rat studies. Kidney toxicity was implied in the Domingo et al. (1985) 3-month study at doses as low as 0.3 mg/kg-day, although it was not clear as to whether this was a LOAEL; ATSDR (1992) determined that the 0.6 mg/kg-day exposure level was a NOAEL. Subsequent to that determination, Boscolo et al. (1994) found mild kidney lesions in rats after a 6-month exposure to 1.2 mg/kg-day, but none at 0.12 mg/kg-day. The most sensitive effect found by Boscolo et al. (1994) was increased blood pressure at 0.12 mg/kg-day; although a 6-month study is somewhat longer than subchronic, the findings are relevant to the subchronic p-RfD assessment because they establish a much lower LOAEL for this effect. All the kidney and blood-pressure effects occurred in male rats, but there was no direct indication that the kidney toxicity in any of these studies was a result of α_{2u}-globulin accumulation. The relevant studies apparently did not test for the presence of α_{2u}-globulin. However, as vanadium binds readily to proteins and is a protease inhibitor, accumulation of α_{2u}-globulin in the proximal tubule cells, leading to tissue necrosis, is plausible. The hemorrhagic foci in the corticomedullary region described by Domingo et al. (1985) could indicate proximal tubule necrosis. However, the criteria for establishing an α_{2u}-globulin mode of action have not been met. The human studies collectively identify a NOAEL of at least 0.3 mg V/kg-day for increased blood pressure and overt kidney toxicity, the most sensitive effects in rats; neither of these effects was observed for some subjects at dose levels of 0.5 to 1.1 mg V/kg-day. The human studies, however, were not considered for use in deriving the subchronic RfD. These studies are of short duration, used small numbers of subjects, and are not capable of detecting sub-clinical kidney damage—identifying a portal-of-entry effect (gastrointestinal distress) as the only adverse effect. Given no evidence of systemic effects in the human subjects, many of which were diabetic, the male rat may be particularly susceptible to kidney and blood pressure effects from vanadium exposure. Accordingly the increased blood pressure reported by Boscolo et al. (1994) at the lowest dose level (0.12 mg V/kg-day) is discounted as a basis for the pRfD, but the kidney effects remain relevant for consideration as the basis for the subchronic p-RfD.

The lowest reproductive/developmental toxicity LOAEL is 2.1 mg V/kg-day for growth retardation in the offspring of rats exposed prior to mating (Domingo et al., 1986); a NOAEL is not established. A clear dose-response relationship is reported in both sexes of offspring for a number of growth-related endpoints measured at several postnatal times. Benchmark dose modeling is rejected because the data were pooled across litters, BMD models could not be fit to most of the data, and it is not clear whether those endpoints that were fit successfully were the most sensitive. Therefore, only the LOAEL of 2.1 mg V/kg-day is considered as a potential POD for the subchronic p-RfD.

The NOAEL of 0.12 mg V/kg-day based on kidney histopathology at 1.2 mg V/kg-day in the 6-month rat study of Boscolo et al. (1994) provides the most appropriate basis for the

subchronic p-RfD. However, given exposure to vanadium in the diet, the NOAEL is adjusted upward by 0.1 mg/kg-day, which is the lower end of the range of likely dietary exposure discussed previously in this document. The subchronic p-RfD is derived as follows:

Subchronic p-RfD = NOAEL ÷ UF
= 0.22 mg V/kg-day ÷ 300
= **0.0007 mg V/kg-day or 7×10^{-4} mg/kg-day**

The composite UF of 3000 is composed of the following:

- A full UF of 10 is used to account for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A full UF of 10 is used to account for potentially susceptible individuals in the population in the absence of information on the variability of response to vanadium developmental toxicity in humans.
- A partial UF of 3 ($10^{0.5}$) is used to account for database deficiencies—in particular the lack of a reproductive toxicity study.

Confidence in the key study (Boscolo et al., 1994) is low. The study does not examine the factors that would determine whether the kidney effects in male rats were a result of α_{2u}-globulin accumulation. Confidence in the database is medium. The toxicological database for oral exposure to vanadium includes human studies, several subchronic studies, several reproductive and developmental toxicity studies and limited studies of immunotoxicity and neurotoxicity. However, the majority of the subchronic studies evaluate limited endpoints; there are no comprehensive bioassays of subchronic duration. Although several studies reported kidney and blood pressure effects in male rats, none of them examined the factors that would determine whether the kidney effects were a result of α_{2u}-globulin accumulation. The reproductive toxicity database does not include any adequate standard multigeneration studies. The available 2-generation studies (Faria de Rodriguez et al., 1998a; Nava de Leal et al., 1998) were limited by poor reporting or pooling of data across treatment groups; however, the results provided suggestive evidence for reproductive toxicity. Likewise, two short-term studies of neurotoxicity (Sanchez et al., 1998, 1999) provide suggestive evidence for an effect of vanadium exposure on avoidance response, but neither study conducted comprehensive tests of neurobehavioral endpoints. Low confidence in the subchronic p-RfD follows.

Chronic p-RfD

Kanisawa and Schroeder (1967) and Schroeder et al. (1970; Schroeder and Michener, 1975) conducted chronic mouse and rat studies; however, the histopathologic assessment in these studies included only gross morphologic evaluations after natural deaths of the animals and would not have detected more subtle histopathologic lesions, particularly kidney lesions. Thus, these studies are inappropriate as critical studies for the chronic p-RfD. The lowest LOAEL of the remaining relevant endpoints is 1.2 mg V/kg-day for kidney pathology in male rats after a 6-month exposure to sodium metavanadate in drinking water (Boscolo et al., 1994), the basis for the subchronic p-RfD. As the human studies would not have revealed subclinical tissue damage and were of short duration, chronic kidney damage would be of concern. Therefore, kidney toxicity is selected as the critical effect, with a LOAEL of

1.2 mg V/kg-day and NOAEL of 0.12 mg V/kg-day established in the Boscolo et al. (1994) rat study. As for the subchronic p-RfD, the NOAEL is adjusted to 0.22 mg/kg-day to account for dietary exposure. The chronic p-RfD is derived as follows:

Chronic p-RfD = NOAEL ÷ UF
= 0.22 mg V/kg-day ÷ 3000
= **0.00007 mg V/kg-day or 7×10^{-5} mg/kg-day**

The composite UF of 3000 is composed of the following:

- A full UF of 10 is used to account for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- A full UF of 10 is used to account for potentially susceptible individuals in the population in the absence of information on the variability of human response to vanadium.
- A partial UF of 3 ($10^{0.5}$) is used to account for database deficiencies as per the subchronic p-RfD.
- A full UF of 10 is used to account for extrapolation to chronic exposure duration from a subchronic study.

Confidence in the key study (Boscolo et al., 1994) is low. The study focused on the blood pressure and kidney effects of vanadium; it did not address a comprehensive suite of endpoints. In addition, the issue of α_{2u}-globulin accumulation was not addressed. Confidence in the database is medium as for the subchronic p-RfD. The chronic studies are of limited utility. Low confidence in the chronic p-RfD follows.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR VANADIUM AND COMPOUNDS

There are no inhalation data with which to derive subchronic or chronic p-RfCs for vanadium compounds.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR VANADIUM AND COMPOUNDS

There are no human data on the potential carcinogenicity of soluble inorganic vanadium compounds, nor are there adequate animal carcinogenicity bioassays; thus, under the U.S. EPA (2005) *Guidelines for Carcinogen Risk Assessment*, there is "*Inadequate Information to Assess* [the] *Carcinogenic Potential"* of vanadium. In early carcinogenicity bioassays of vanadium, no increases in tumor incidence were observed in rats or mice exposed chronically (Kanisawa and Schroeder, 1967; Schroeder et al., 1970; Schroeder and Michener, 1975). However, these studies are limited in several ways: there is limited histopathology evaluation,

and tumor findings are not reported by target organ. In addition, the study in rats (Schroeder et al., 1970) is hampered by significant animal loss due to a pneumonia outbreak. A number of studies in rats have indicated that vanadium may exert an antineoplastic effect in chemical carcinogenesis, reducing the number and/or incidence of leiomyosarcomas and tumors of the liver, colon, and mammary glands in rats (Evangelou et al., 1997; Liasko et al., 1998; Ray et al., 2004, 2005a,b, 2006; Chakraborty et al., 2005, 2006a,b,c, 2007a,b; Kanna et al., 2003, 2004, 2005). Mechanistic information supporting the potential antineoplastic effect includes evidence that vanadium can induce apoptosis in mammary tumor cells both in vitro and in vivo (Ray et al., 2006). Limited genotoxicity data have shown that vanadium can induce mutations in yeast and mammalian cells (Bronzetti et al., 1990; Cohen et al., 1992; Klein et al., 1994). In mammalian cells cultured in vitro, vanadium increased the SCE frequency at noncytotoxic concentrations (Owusu-Yaw et al., 1990). Vanadium has induced micronuclei and/or numerical chromosomal aberrations (hypoploidy or hyperploidy) in mice treated in vivo (Ciranni et al., 1995; Leopardi et al., 2005; Villani et al., 2007).

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Attachment

1D

3

United States Environmental Protection Agency | **Office of Water Mail Code 4304T** | **EPA 822-R-16-003 May 2016**

Health Effects Support Document for Perfluorooctanoic Acid (PFOA)

Health Effects Support Document for Perfluorooctanoic Acid (PFOA)

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

EPA Document Number: 822-R-16-003
May 2016

BACKGROUND

The Safe Drinking Water Act (SDWA), as amended in 1996, requires the Administrator of the U.S. Environmental Protection Agency (EPA) to periodically publish a list of unregulated chemical contaminants known or anticipated to occur in public water systems and that may require regulation under SDWA. The SDWA also requires the Agency to make regulatory determinations on at least five contaminants on the Contaminant Candidate List (CCL) every 5 years. For each contaminant on the CCL, before EPA makes a regulatory determination, the Agency needs to obtain sufficient data to conduct analyses on the extent to which the contaminant occurs and the risk it poses to populations via drinking water. Ultimately, this information will assist the Agency in determining the most appropriate course of action in relation to the contaminant (e.g., developing a regulation to control it in drinking water, developing guidance, or deciding not to regulate it).

The PFOA health assessment was initiated by the Office of Water, Office of Science and Technology in 2009. The draft *Health Effects Support Document for Perfluoroctanoic Acid (PFOA)* was completed in 2013 and released for public comment in February 2014. An external peer-review panel meeting was held on August 21 and 22, 2014. The final document reflects input from the panel as well as public comments received on the draft document. Both the peer-reviewed draft and this document include only the sections of a health effects support document (HESD) that cover the toxicokinetics and health effects of PFOA. If a decision is made to regulate the contaminant, this document will be expanded.

One of the challenges inherent in conducting this assessment was the wealth of experimental data published before and during its development. This section provides a synopsis of the approach used in identifying and selecting the publications reflected in the final assessment.

Data were identified through the following:

- Monthly/bimonthly literature searches conducted by EPA library staff (2009–2015) and New Jersey Department of Environmental Protection library staff (2012–2015).
- Papers identified by EPA internal and external peer reviewers.
- Papers identified through public comments on the draft assessments.
- Papers submitted to EPA by the public.

In mid-2013, the EPA library searches were expanded to cover all members of the perfluoroalkane carboxylate family (C4 through C12). Appendix A describes the literature search strategy used by the libraries. Through the literature search, documents were identified for retrieval, review, and inclusion in the HESD using the following criteria:

- The study examines a toxicity endpoint or population not examined by studies already included in the draft document.
- Aspects of the study design such as the size of the population exposed or quantification approach make it superior to key studies already included in the draft document.
- The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
- Elements of the study design merit its inclusion in the draft document based on its contribution to the mode of action (MoA) or the quantification approach.

- The study elucidates the MoA for any toxicity endpoint or toxicokinetic property associated with PFOA exposure.
- The effects observed differ from those in other studies with comparable protocols.

In addition to each publication being evaluated against the criteria above, the relevance of the study to drinking water exposures and to the U.S. population also were considered.

The studies included in the final draft were determined to provide the most current and comprehensive description of the toxicological properties of PFOA and the risk it poses to humans exposed to it in their drinking water. Appendix B summarizes the studies evaluated for inclusion in the HESD following the August 2014 peer review and identifies those selected for inclusion in the final assessment. Appendix B includes epidemiology data that provide a high-level summary of the outcomes across the studies evaluated.

Development of the hazard identification and dose-response assessment for PFOA has followed the general guidelines for risk assessment forth by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (USEPA 2014a). Other EPA guidelines used in the development of this assessment include the following:

- *Guidelines for the Health Risk Assessment of Chemical Mixtures* (USEPA 1986a)
- *Guidelines for Mutagenicity Risk Assessment* (USEPA 1986b)
- *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (USEPA 1988)
- *Guidelines for Developmental Toxicity Risk Assessment* (USEPA 1991)
- *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies* (USEPA 1994a)
- *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (USEPA 1994b)
- *Use of the Benchmark Dose Approach in Health Risk Assessment* (USEPA, 1995)
- *Guidelines for Reproductive Toxicity Risk Assessment* (USEPA 1996)
- *Guidelines for Neurotoxicity Risk Assessment* (USEPA 1998)
- *Science Policy Council Handbook: Peer Review (2nd edition)* (USEPA 2000a)
- *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA 2000b)
- *A Review of the Reference Dose and Reference Concentration Processes* (USEPA 2002a)
- *Guidelines for Carcinogen Risk Assessment* (USEPA 2005a)
- *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (USEPA 2005b)
- *Science Policy Council Handbook: Peer Review (3rd edition)* (USEPA 2006a)
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (USEPA 2006b)
- *Exposure Factors Handbook* (USEPA 2011)
- *Benchmark Dose Technical Guidance Document* (USEPA 2012)
- *Child-Specific Exposure Scenarios Examples* (USEPA 2014b)

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CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

8-OH-dG	8-hydroxydeoxyguanosine
Acot1	acyl-CoA thioesterase (human)
Acox	acyl-CoA oxidase
ADHD	attention deficit hyperactivity disorder
ADX	adrenalectomized
AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
APFO	ammonium perfluorooctanoate
Areg	amphiregulin
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the plasma concentration time curve
AUC_{INF}	area under the plasma concentration time curve, extrapolated to infinity
AUC_{INF}/D	area under the plasma concentration time curve, extrapolated to infinity, normalized to dose
BAX	BCL2-associated X protein
BMD	benchmark dose
BMDL	lower 95^{th} percentile confidence bound on benchmark dose
BMDS	Benchmark Dose Software
BMI	body mass index
BrdU	Bromodeoxyuridine (5-bromo-2-deoxyuridine)
BSA	bovine serum albumin
BSEP	bile salt export pump
BSP	sulfobromophthalein
BUN	blood urea nitrogen
bw	body weight
C	Celsius
C_{max}	peak plasma concentration at the first intestinal absorption loci
CaMKII	calcium/calmodulin-dependent protein kinase II
CAR	constitutive androstane receptor
CAT	carnitine acyltransferase
CCK	cholecystokinin
CCL	Contaminant Candidate List
CCL 3	Contaminant Candidate List 3
CFSE	6-carboxyfluorescein succinimidyl ester
ChAT	choline acetyltransferase
CHO	Chinese hamster ovary
CI	confidence interval
CL	clearance
Cl_p	plasma clearance
CL_R	renal clearance
CoA	coenzyme A
ConA	concanavalinA
COPD	chronic obstructive pulmonary disease
CORT	corticosterone

Cox II	cytochrome c oxidase subunit II
Cox IV	cytochrome c oxidase subunit IV
CPT	carnitine palmitoyltransferase
Crl	Charles River Laboratory
CSF	cancer slope factor
Cte	acyl-CoA thioesterase (rat)
CYP4A10	cytochrome P450 4a10
d	day
DCDQ	Developmental Coordination Disorder Questionnaire
Ddit3	DNA damage inducible transcript
DEHP	di(2-ethylhexyl) phthalate
DHT	5α-dihydroxy-testosterone
dL	deciliter
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DR	dose rate
DTH	delayed-type hypersensitivity
DWI	drinking water intake
E2	17-β estradiol
E3S	estrone-3-sulfate
EC_{50}	half maximal effective concentration
ECF	electrochemical fluorination
eGFR	estimated glomerular filtration rate
EGFR	epidermal growth factor receptor
EPA	U.S. Environmental Protection Agency
ER	endoplasmic reticulum
ERα	estrogen receptor α
Errα	estrogen-related receptor α
FID	flame ionization detector
FSH	follicle-stimulating hormone
FT4	free thyroxine
FXR	farnesoid receptor
g	gram
GAP-43	growth-associated protein-43
GD	gestation day
GEE	generalized estimating equation
GFR	glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
GJIC	gap junction intercellular communication
GlyT	glycogen trophoblast cell
GnRH	gonadotropin releasing hormone
GSD	geometric standard deviation
GST	glutathione-S-transferase
hCG	human chorionic gonadotropin
HDL	high-density lipoprotein
HED	human equivalent dose
HEK	human embryonic kidney
HESD	health effects support document
HET	heterozygous

HFD	high-fat diet
HGFα	hepatocyte growth factor
HL-60	human promyelocytic leukemia cell line
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HPLC/MS	High-performance liquid chromatography mass spectrometry
HPLC/MS/MS	High-performance liquid chromatography tandem mass spectrometry
HR	hazard ratio
HRBC	horse red blood cells
HSA	human serum albumin
HSD17β1	hydroxysteroid 17β dehydrogenase 1
HSD3β1	hydroxysteroid 3β dehydrogenase 1
IC_{50}	half-maximal inhibiting concentration
ICR	imprinting control region
IDL	intermediate density lipoprotein
IgE	immunoglobulin E
IGF-I	insulin like growth factor I
IgM	immunoglobulin M
IHD	ischemic heart disease
IL-6	interleukin 6
INUENDO	Biopersistent Organochlorines in Diet and Human Fertility study
IQR	interquartile range
IRR	incidence rate ratio
IU	international unit
IV	intravenous
K_a	adsorption rate constant
K_d	dissociation constant
K_e	elimination rate constant
kg	kilogram
K_m	substrate concentration at which the initial reaction rate is half maximal
K_{oc}	organic carbon water partitioning coefficient
K_t	affinity constant
L	liter
L-FABP	liver fatty acid binding protein
LC_{50}	lethal concentration for 50% of animals
LCT	Leydig cell tumor
LD	lactation day
LD_{50}	lethal dose for 50% of animals
LDH	lactic dehydrogenase
LDL	low-density lipoprotein
LH	luteinizing hormone
LHWA	Little Hocking Water Association
LLOQ	lower limit of quantification
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOQ	limit of quantitation
LPS	lipopolysaccharide
m	meter
MCAD	medium chain acyl-CoA dehydrogenase
MDA	malondialdehyde

Mdr2	multidrug resistance protein 2
µg	microgram
mg	milligram
min	minute
mL	milliliter
µm	micrometer
µmol	micromole
MMAD	mass median aerodynamic diameter
MOA	mechanism of action
MoA	mode of action
mol	mole
mPL	mouse placental lactogen
mPLP	mouse prolactin-like protein
mRNA	messenger ribonucleic acid
MRP	multidrug resistance-associated protein
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Nd2	NADH dehydrogenase 2
Ndufs8	NADH dehydrogenase iron-sulfur protein 8
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute for Environmental Health Sciences
NJDEP	New Jersey Department of Environmental Protection
NK	natural killer
NM	not monitored
nmol	nanomolar
NMR	nuclear magnetic resonance
NMRI	Naval Medical Research Institute
NOAEL	no observed adverse effect level
Nrf1	nuclear respiratory factor 1
Nrf2	nuclear respiratory factor 2
NTCP	sodium-taurocholate contransporting polypeptide
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OR	odds ratio
OVA	ovalbumin
OVX	ovariectomized
OW	Office of Water
P	progesterone
PACT	pancreatic acinar cell tumor
PAH	polycyclic aromatic hydrocarbon
PB	phenobarbital
PBMC	peripheral blood mononuclear cells
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PenH	enhanced pause airway respiration
PFAA	perfluoroalkyl acid
PFAS	perfluoroalkyl substance
PFC	plaque-forming cell
PFDA	perfluorodecanoic acid

PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
Pgc-1α	peroxisome proliferator-activated receptor gamma coactivator 1α
PH	peroxisomal bifunctional protein
PHA	phytohemagglutinin
PK	pharmacokinetic
pKa	acid dissociation constant
PND	postnatal day
POD	point of departure
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion
ppm	parts per million
PT	peroxisomal thiolase
PWG	pathology working group
PXR	pregnane X receptor
Q	flow in and out of tissues
Q_{filc}	median fraction of blood flow to the filtrate
RfC	reference concentration
RfD	reference dose
RFD	regular fat diet
ROS	reactive oxygen species
RR	relative risk
RSA	rodent serum albumin
RSC	relative source contribution
RT-PCR	reverse transcription polymerase chain reaction
RXRα	retinoid X receptor alpha
SD	standard deviation
SDH	sorbitol dehydrogenase
SDQ	Strengths and Difficulties Questionnaire
SDWA	Safe Drinking Water Act
SHBG	sex hormone-binding globulin
SIAR	SIDS Initial Assessment Report
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SPI	Society of the Plastics Industry
SRBC	sheep red blood cells
S-TGC	sinusoidal trophoblast giant cells
T3	triiodothyronine
T4	thyroxine
$T_{1/2}$	elimination half-time
T_m	transporter maximum
T_{max}	time of maximum plasma concentration
TC	total cholesterol
TCPOBOP	1,4-bis[2-(3,5-dichloropyridyloxy)] benzene
Tfam	transcription factor A

TG	triglyceride
TH	tyrosine hydroxylase
TNFα	tumor necrosis factor α
TPO	thyroid peroxidise
TRR	total reactive residues
TSH	thyroid stimulating hormone
TTP	time to pregnancy
TTR	thyroid hormone transport protein, transthyretin
UA	uric acid
UCMR	Unregulated Contaminant Monitoring Rule
UCMR 1	Unregulated Contaminant Monitoring Rule 1
UCMR 2	Unregulated Contaminant Monitoring Rule 2
UF	uncertainty factor
URAT	urate transporter
USGS	U.S. Geological Survey
V_d	volume of distribution
V_{max}	maximum initial rate of an enzyme catalyzed reaction
VLCAD	very long chain acyl-CoA dehydrogenase
VLDL	very low-density lipoprotein
VOC	volatile organic compound
WHO	World Health Organization
WRF	Water Research Foundation

EXECUTIVE SUMMARY

Perfluorooctanoic acid (PFOA) is a synthetic, fully fluorinated, organic acid used in a variety of consumer products and in the production of fluoropolymers and generated as a degradation product of other perfluorinated compounds. Because of strong carbon-fluorine bonds, PFOA is stable to metabolic and environmental degradation. PFOA is one of a large group of perfluoroalkyl substances (PFASs) that are used to make products more resistant to stains, grease, and water. These compounds have been widely found in consumer and industrial products as well as in food items. Major U.S. manufacturers voluntarily agreed to phase out production of PFOA by the end of 2015. Exposure to PFOA in the United States remains possible due to its legacy uses, existing and legacy uses on imported goods, degradation of precursors, and extremely high persistence in the environment and the human body.

Extensive data on humans and animals indicate ready absorption of PFOA and distribution of the chemical throughout the body by noncovalent binding to plasma proteins. Studies of postmortem human tissues identify its presence in liver, lung, kidney, and bone. PFOA is not readily eliminated from the human body as evidenced by the half-life of 2.3 years among members of the general population. In contrast, half-life values for the monkey, rat, and mouse are 20.8 days, 11.5 days, and 15.6 days, respectively.

Human epidemiology data report associations between PFOA exposure and high cholesterol, increased liver enzymes, decreased vaccination response, thyroid disorders, pregnancy-induced hypertension and preeclampsia, and cancer (testicular and kidney). Epidemiology studies examined workers at PFOA production plants, a high-exposure community population near a production plant in the United States (i.e., the C8 cohort), and members of the general population in the United States, Europe, and Asia. These studies examined the relationship between serum PFOA concentration (or other measures of PFOA exposure) and various health outcomes. Exposures in the highly exposed C8 community are based on the concentrations in contaminated drinking water and serum measures. Exposures among the general population typically included multiple PFASs as indicated by serum measurements. The correlation among eight carbon PFASs is often moderately strong (e.g., Spearman $r > 0.6$ for PFOA and perfluorooctane sulfonate (PFOS) in the general population). Mean serum levels among the occupational cohorts ranged from approximately 1 to 4 micrograms per milliliter (μg/mL) and in the C8 cohort ranged from 0.01 to 0.10 μg/mL. Geometric mean serum values for the National Health and Nutrition Examination Survey (NHANES) general population (≥ age 12; 2003–2008) were 0.0045 μg/mL for males and 0.0036 μg/mL for females.

These epidemiology studies have generally found positive associations between serum PFOA concentration and total cholesterol (TC) in the PFOA-exposed workers and the high-exposure community (i.e., increasing lipid level with increasing PFOA); similar patterns are seen with low-density lipoproteins (LDLs) but not with high-density lipoproteins (HDLs). These associations were seen in most of the general population studies, but similar results also were seen with PFOS, and the studies did not always adjust for these correlations. Associations between serum PFOA concentrations and elevations in serum levels of alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) were consistently observed in occupational cohorts, the high-exposure community, and the U.S. general population. The associations are not large in magnitude, but indicate the potential for PFOA to affect liver function.

Diagnosed thyroid disease in females and female children was increased both in the high-exposure C8 study population and in females with background exposure; thyroid hormones are not consistently associated with PFOA concentration. Associations between PFOA exposure and risk of infectious diseases (as a marker of immune suppression) were not identified, but a decreased response to vaccines in relation to PFOA exposure was reported in studies in adults in the high-exposure community population and in studies of children in the general population; in the latter studies, it is difficult to distinguish associations with PFOA from those of other correlated PFASs. Studies in the high-exposure community reported an association between serum PFOA and risk of pregnancy-related hypertension or preeclampsia, conditions related to renal function during pregnancy; this outcome has not been examined in other populations. An inverse association between maternal PFOA (measured during the second or third trimester) or cord blood PFOA concentrations and birth weight was seen in several studies. It has been suggested that low glomerular filtration rate (GFR) can impact fetal birth weight (Morken et al. 2014). Pharmacokinetic (PK) analyses have shown, however, that in individuals with low GFR, there are increased levels of serum PFOA and lower birth weights. Thus, the impact on body weight is likely due to a combination of the low GFR and the serum PFOA.

The epidemiology studies did not find associations between PFOA and diabetes, neurodevelopmental effects, or preterm birth and other complications of pregnancy. Developmental outcomes including delayed puberty onset in girls also have been reported; however, in the two studies examining PFOA exposure in relation to menarche, conflicting results were observed: either no association or a possible indication of an earlier menarche seen with higher maternal PFOA levels in one study and a later menarche seen with higher maternal PFOA levels in the other study. Increased risk of ulcerative colitis was reported in the high-exposure community study as well as in a study limited to workers in that population.

For PFOA, oral animal studies of short-term subchronic and chronic duration are available in multiple species including monkeys, rats, and mice. These studies report developmental effects, liver and kidney toxicity, immune effects, and cancer (liver, testicular, and pancreatic). Developmental effects observed in animals include decreased survival, delayed eye opening and reduced ossification, skeletal defects, altered puberty (delayed vaginal opening in females and accelerated puberty in males), and altered mammary gland development.

In most animal studies, changes in relative and/or absolute liver weight appear to be the most common effect observed with or without other hepatic indicators of adversity, identifying increased liver weight as a common indicator of PFOA exposure. The liver also contains the highest levels of PFOA when analyzed after test animal sacrifice. The increases in liver weight and hypertrophy, however, also can be associated with activation of cellular peroxisome proliferator-activated receptor α (PPARα) receptors, making it difficult to determine if this change is a reflection of PPARα activation or an indication of PFOA toxicity. The PPARα response is greater in rodents than it is in humans. The U.S. Environmental Protection Agency (EPA) evaluated liver disease and liver function resulting from PFOA exposure in studies where liver weight changes and other indicators of adversity such as necrosis, inflammation, fibrosis, and/or steatosis (fat accumulation in the liver) or increases in liver or serum enzymes indicative of liver damage were observed.

In repeat PFOA dosing studies, rats given 0.64 milligrams per kilogram per day (mg/kg/day) for 13 weeks and monkeys given 3 mg/kg/day for 26 weeks had increased liver weight accompanied by hepatocellular hypertrophy. As part of a two-generation study, male rats had increased liver and kidney weights as well as decreased body weight at 1 mg/kg/day. In shorter term studies, slightly higher or lower doses to rats resulted in increased liver weight, liver necrosis, and developmental delays. In mice, developmental toxicity and increased spleen weight was observed at a dose of 1 mg/kg/day accompanied by increased liver weight. Other doses of similar magnitudes in mice were associated with developmental delays and liver necrosis. Slightly higher doses resulted in decreased immunoglobulin levels. As supported by the epidemiology data, suppression of the immune system in response to PFOA exposure is an area of concern for humans as well as animals.

PFOA is known to activate PPAR pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Based on PFOA-induced transcriptional activation of many other genes in PPARα-null mice, however, other receptors such as the constitutive androstane receptor (CAR), farnesoid receptor (FXR), and pregnane X receptor (PXR) could be involved in PFOA-induced toxicity.

EPA used a peer-reviewed PK model to calculate the average serum concentrations associated with candidate no observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) from six studies for multiple effects to calculate corresponding human equivalent doses (HEDs) for the derivation of candidate reference doses (RfDs). Overall, the toxicity studies available for PFOA demonstrate that the developing fetus is particularly sensitive to PFOA-induced toxicity. In addition to the critical developmental effects described above, other adverse effects include decreased survival, delays in eye opening and ossification, skeletal defects, delayed vaginal opening in females, and altered mammary gland development.

The EPA Office of Water (OW) selected an RfD of 0.00002 mg/kg/day based on effects observed in a developmental toxicity study in mice for PFOA (Lau et al. 2006). The RfD is based on reduced ossification and accelerated puberty (in males). The total uncertainty factor (UF) applied to the HED LOAEL from Lau et al. (2006) is 300 and includes a UF of 10 for intrahuman variability, a UF of 3 to account for toxicodynamic differences between animals and humans, and a UF of 10 to account for use of a LOAEL as the point of departure (POD).

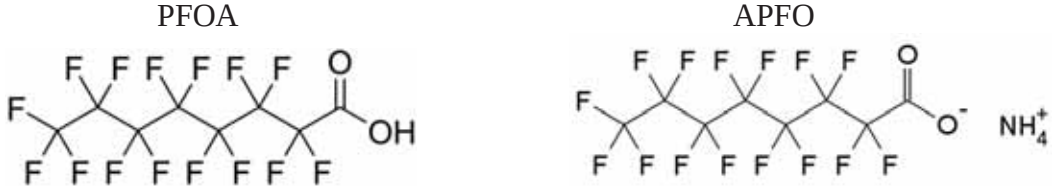
Decreased pup body weights also were observed in studies conducted in mice receiving external doses within the same order of magnitude (1, 3, and 5 mg/kg/day, respectively) as those chosen for the RfD. These studies, however, lacked serum levels and were not amenable to modeling. Overall, the developmental and reproductive toxicity studies available for PFOA demonstrate that the developing fetus is particularly sensitive to PFOA-induced toxicity. The selected RfD is supported by the other candidate RfDs (also 0.00002 mg/kg/day) based on effects on the immune system in a 15-day short-term study by DeWitt et al. (2008) and on the kidneys of F0 and F1 males in a two-generation study of developmental and reproductive toxicity.

Under EPA's *Guidelines for Carcinogen Risk Assessment* (USEPA 2005a), there is "suggestive evidence of carcinogenic potential" for PFOA. Epidemiology studies demonstrate an association of serum PFOA with kidney and testicular tumors among highly exposed members of the general population. Two chronic bioassays of PFOA support a positive finding for its ability to be tumorigenic in one or more organs of rats, including the liver, testes, and pancreas. EPA estimated a cancer slope factor (CSF) of 0.07 $(mg/kg/day)^{-1}$ based on testicular tumors. As a

comparative analysis, the concentration of PFOA in drinking water that would have a one-in-a-million increased cancer risk was calculated using the oral slope factor for testicular tumors, assuming a default adult body weight of 80 kg and a default drinking water intake (DWI) rate of 2.5 liter per day (L/day) (USEPA 2011). This concentration is lower than the concentration for cancer (also derived with adult exposure values), indicating that a guideline derived from the developmental endpoint will be protective for the cancer endpoint.

1 IDENTITY: CHEMICAL AND PHYSICAL PROPERTIES

Perfluorooctanoic acid (PFOA) is a completely fluorinated organic synthetic acid used to produce fluoropolymers. It is manufactured by the Simons electrochemical fluorination (ECF) process or by telomerization. In the ECF process, the carbon-hydrogen bonds on molecules of organic feedstock are replaced with carbon-fluorine bonds when an electric current is passed through a solution of hydrogen fluoride and the organic feedstock. In the telomerization process, fluorine-bearing chemicals and tetrafluoroethylene react to produce fluorinated intermediates that are converted into PFOA (HSDB 2006). The telomerization process produces linear chains (Beesoon et al. 2011). Ammonium perfluorooctanoate (APFO) is the salt of PFOA and is a processing aid in the manufacture of certain fluoropolymers, especially as an emulsifier in aqueous solution during the emulsion polymerization of tetrafluoroethylene (see Figure 1-1). APFO is not consumed during the polymerization process (SPI 2005). Some sources of PFOA in the atmosphere result from the atmospheric degradation or transformation or surface deposition of precursors, including related fluorinated chemicals (e.g., fluorotelomer alcohols, olefins, and perfluoroalkyl sulfonamide substances) (Wallington et al. 2006).



Source: SIAR 2006

Figure 1-1. Chemical Structures of PFOA and APFO

Although PFOA is not a polar molecule, each of the carbon fluoride bonds is a dipole as a result of the electronegativity difference between fluoride (4.1) and carbon (2.5), placing a partial negative charge on each of the covalently bound fluorines and a partial positive charge on each of the fluorinated carbons. Charge repulsion of the partially negative fluorines and steric factors favor a PFOA conformation in which carbons 2 through 7 adopt an *anti* arrangement of substituents resulting in a fairly linear molecular shape as the lowest energy conformer (see Figure 1-2 and Figure 1-3).

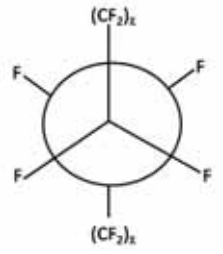


Figure 1-2. PFOA Anti-Conformer

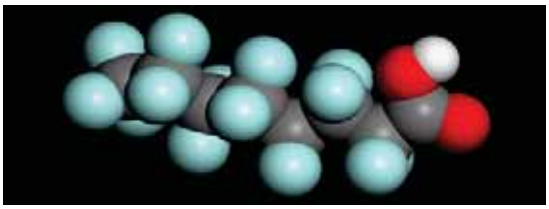


Figure 1-3. PFOA Lowest Energy Conformer

The favored PFOA conformer is very similar to the preferred conformation of the eight-carbon fatty acid, octanoic acid (also known as caprylic acid) except for the sphere of partial negative charge on the fluorines of the exterior surface. The ionized carboxylate grouping and the fluorine's partial negative charges favor electrostatic interactions between PFOA and positively charged surfaces on proteins and other macromolecules.

The ECF process produces branched chain isomers, about 80% linear and 20% branched (Loveless et al. 2008). The samples studied by Loveless et al. (2008) had the following mole (mol) percents of branched chain isomers: 12.6% internal monomethyl (nonalpha), 9% isopropyl, 0.2%, *tert*-butyl, 0.1 gem-dimethyl, and 0.1 alpha monomethyl. A study by Yoshikane et al. (2010) reported finding perfluoro-6-methylheptanoic acid (the isopropyl isomer) using mass spectroscopy analysis of environmental fluorosurfactants in Japan. Branched chain samples evaluated by Beesoon and Martin (2015) had a 7 carbon linear chain with methyl groups on carbons 3, 4, 5, or 6, designated as 3m, 4m, 5m, or 6m (iso), respectively. The composition of a PFOA product is important because the toxicokinetic and physiological properties of the linear and branched chain isomers are different. The nomenclature for the branched chain isomers varied between authors and indicates that differences exist in the composition of the commercial products that were evaluated.

The physical and chemical properties and other reference information for PFOA and its salt APFO are provided in Table 1-1. These properties help to define the behavior of PFOA in living systems and the environment. PFOA and its salt are highly stable compounds. They are solids at room temperature with low vapor pressures. The melting point for PFOA is identified as 54.3 degrees Celsius, and vapor pressures increase at temperatures near the melting point.

PFOA is moderately soluble in water and APFO is even more soluble. Both compounds are considered insoluble in nonpolar solvents, which results in their being described as olephobic. Water solubility is increased by the presence of other ions and is an important factor governing solubility in body fluids. As the concentration of PFOA in aqueous solution increases, it forms colloidal micelles with the carboxyl functional groups on the exterior and the fluorocarbon chain on the interior. The critical micelle concentration has been identified as 3.6–3.7 g/L. Once the critical concentration has been reached, micelles will form and the PFOA molecules will colloidally distribute in the aqueous environment. At levels below the critical micelle concentration, the individual molecules are individually distributed in the solvent.

The acid dissociation constant (pKa) for PFOA has been reported as 2.8. As a result, it will be present in most biological fluids (gastric secretions excluded) primarily as the perfluorooctanoate anion. This is an important feature in governing absorption and membrane transport.

Table 1-1. Chemical and Physical Properties of PFOA

Property	Perfluorooctanoic Acid	Source
Chemical Abstracts Service Registry No. (CASRN) [a]	335-67-1	
CA Index Name	2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanoic acid	
Synonyms	PFOA; Pentadecafluoro-1-octanoic acid; Pentadecafluoro-n-octanoic acid; Octanoic acid, pentadecafluoro-; Perfluorocaprylic acid; Pentadecafluorooctanoic acid; Perfluoroheptanecarboxylic acid	
Chemical Formula	$C_8HF_{15}O_2$	
Molecular Weight (g/mol)	414.09	(HSDB 2012); (Lide 2007); (SRC 2016)
Color/Physical State	White powder (ammonia salt)	(HSDB 2012); (Lewis 2004)
Boiling Point	192.4 ºC; Stable when bound	(HSDB 2012); (Lide 2007); (SRC 2016)
Melting Point	54.3 ºC	(HSDB 2012); (Lide 2007); (SRC 2016)
Vapor Pressure	0.525 mm Hg at 25 ºC (measured) 0.962 mm Hg at 59.25 °C (measured)	(Hekster et al. 2003); (HSDB 2012); (SRC 2016) (ATSDR 2015); (Kaiser et al. 2005)
Henry's Law constant	Not measureable	(ATSDR 2015)
pKa	2.80	(SRC 2016)
K_{oc}	2.06	(Higgins and Luthy 2006)
K_{ow}	Not measurable	(ATSDR 2015); (EFSA 2008)
Solubility in water	9.50×10^3 mg/L at 25 ºC (estimated)	(ATSDR 2015); (Hekster et al. 2003); (HSDB 2012); (Kauck and Diesslin 1951); (SRC 2016)
Half-life in water (25ºC)	Stable	(UNEP 2015)
Half-life in air	Stable when bound	(UNEP 2015)

Note: [a]This CASRN is for linear PFOA, but the toxicity studies are based on a mixture of linear and branched and the RfD applies to both.

2 TOXICOKINETICS

PFOA is stable to metabolic and environmental degradation because of strong carbon-fluorine bonds. It also is resistant to metabolic biotransformation. Thus, the toxicity of the parent compound is the concern. Because of its impact on cellular receptors and proteins, it possesses the ability to impact the biotransformation of dietary constituents, intermediate metabolites, and other xenobiotic chemicals by altering enzyme activities and transport kinetics. PFOA is known to activate PPAR pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, sterol, and bile acid biosynthesis and retinol metabolism genes. Based on transcriptional activation of many genes in PPARα-null mice, however, the effects of PFOA involve far more than activation of PPAR and consequent peroxisome proliferation. The data indicate that it also can activate the CAR, FXR, and PXR and metabolic activities linked to these nuclear receptors.

PFOA is not readily eliminated from humans and other primates. Toxicokinetic profiles and the underlying mechanism for half-life differences are not completely understood, although many of the differences appear to be related to elimination kinetics and factors that control membrane transport. Thus far, three transport families appear to play a role in PFOA absorption, distribution, and excretion: organic anion transporters (OATs), organic anion transporting polypeptides (OATPs), and multidrug resistance-associated proteins (MRPs) (Klaassen and Aleksunes 2010; Launay-Vacher et al. 2006). The transporters are critical for gastrointestinal absorption, uptake by the tissues, and excretion via bile and the kidney. These transport systems are located at the membrane surfaces of the intestines, liver, lungs, heart, blood brain barrier, blood placental barrier, blood testes barrier, and mammary glands where they function to protect the organs, tissues, and fetus from foreign compounds (Ito and Alcorn 2003; Klaassen and Aleksunes 2010, Zaïr et al. 2008).

There are differences in transporters across species, genders, and individuals. For example, more PFOA-specific information is available about the OAT and OATP families than about the MRPs. These limitations have hindered the development of PK models for use in predicting effects in humans based on the data from animal studies. Abbreviations for the various transporters are not totally standardized, and there are inconsistencies across individual publications. The current convention for distinguishing between the transporters in humans and those in animals is to use uppercase letters for humans and lowercase letters for animals. In this document, uppercase letters are used uniformly, thus, the abbreviations indicate the transporter family and not the species studied.

2.1 Absorption

Absorption data are available for oral, inhalation, and dermal exposure in laboratory animals, and extensive data are available from humans demonstrating the presence of PFOA in serum. These data demonstrate absorption by one or more routes but do not quantify the amounts absorbed relative to dose.

The absorption process requires transport across the interface of the gut, lung, and skin with the external environment. Since PFOA is moderately soluble in aqueous solutions and oleophobic (i.e., minimally soluble in body lipids), movement across the apical and basal membrane surfaces of the lung, gastrointestinal tract, and skin involves transporters or mechanisms other than simple diffusion across the lipid bilayer. As discussed above, there are

data that identify involvement of OATs, OATPs, and MRPs in enterocytes in uptake of PFOA (Klaassen and Aleksunes 2010; Zaïr et al. 2008). OAT2, OAT3, OATP2b1, and MRP2 are located in the apical membrane of the microvilli, and MRP1, 3, and 4 are located along the basolateral membrane. Together they function in the uptake of organic anions from gastrointestinal contents and transport of those anions into the portal blood supply (Zaïr et al. 2008). Few studies have been conducted of the intestinal transporters for PFOA in humans or laboratory animals. Most of the research has focused on the kidney and has been carried out using cultured carrier cells transfected with the transporter proteins.

2.1.1 Oral Exposure

Based on animal data, PFOA is well absorbed following oral exposure. Gibson and Johnson (1979) administered a single dose of ^{14}C-PFOA averaging 11.4 mg/kg by gavage to groups of three male 10-week-old CD rats. Twenty-four hours after administration, at least 93% of total carbon-14 was absorbed. In another study, Cui et al. (2010) exposed male Sprague-Dawley rats (10 per group) to PFOA (96% active ingredient) at 0, 5, and 20 mg/kg/day once daily by gavage for 28 days. The percent of the dose absorbed was 92.8% and 92.3% for the low and high dose, respectively, under the assumption that fecal excretion over the first 24 hours after dosing was estimated to be unabsorbed material and did not include biliary loss.

The data from studies of adverse effects on monkeys, rats, and mice receiving PFOA in capsules, food, or drinking water demonstrate gastrointestinal absorption. In cynomolgus monkeys, steady-state serum PFOA levels were reached within 4–6 weeks after dosing with capsules containing 3, 10, and 20 mg/kg PFOA for 6 months (Butenhoff et al. 2004b). Urine steady-state levels were reached after 4 weeks. Serum PFOA concentration in male rats fed diets containing 0.06, 0.64, 1.94, and 6.5 mg PFOA/kg for 90 days was 7.1, 41, 70, and 138 μg/mL, respectively (Perkins et al. 2004). Peak blood levels of PFOA were attained 1–2 hours following a 25-mg/kg dose to male and female rats (Kennedy et al. 2004). Blood levels of PFOA over time were similar in female rats given a single dose of 25 mg PFOA/kg to a female rat given 10 daily doses of 25 mg PFOA/kg (Kennedy et al. 2004). Plasma PFOA concentrations in male Sprague-Dawley rats fed a diet containing 300 parts per million (ppm) PFOA for 1, 7, and 28 days were 259, 234, and 252 μg/mL, respectively (Elcombe et al. 2010).

In rats, a marked gender difference in serum and tissue levels exists following PFOA administration. Males consistently have much higher levels than females with the difference maintained and becoming more pronounced over time. Female rats show much greater urinary excretion of PFOA than do male rats with serum half-life values in hours for females compared with days for males. These differences account for variability in postexposure serum concentrations between males and females.

2.1.2 Inhalation Exposure

Hinderliter (2003) measured the serum concentrations of PFOA following single and repeated inhalation exposures in Sprague-Dawley rats. For the single-exposure study, male and female rats (3/gender/group) were exposed to a single nose-only exposure of an aerosol of 0, 1, 10, and 25 mg/m^3 PFOA. Preliminary range-finding studies demonstrated that aerosol particle sizes were 1.8–2.0 μm mass median aerodynamic diameter (MMAD) with geometric standard deviations (GSDs) ranging from 1.9 to 2.1 μm. Blood samples were collected before exposure; at 0.5, 1, 3, and 6 hours during exposure; and at 1, 3, 6, 12, 18, and 24 hours after exposure. Plasma

was analyzed by liquid chromatography-mass spectrometry (LC-MS). PFOA plasma concentrations increased proportional to aerosol exposure concentrations.

The male plasma C_{max} values were approximately 2–3 times higher than the female C_{max}. The female C_{max} occurred approximately 1 hour after the exposure period with plasma concentrations then declining. In males, Cm_{ax} was observed immediately after the exposure period ended and persisted for up to 6 hours. The data are illustrative of absorption of PFOA via inhalation and are consistent with the gender differences in rate of excretion.

2.1.3 Dermal Exposure

There is evidence that PFOA is absorbed following dermal exposure. Kennedy (1985) treated rabbits and rats dermally with a total of 10 applications of PFOA at doses of 0, 20, 200, and 2,000 mg/kg. Treatment resulted in elevated blood organofluorine levels that increased in a dose-related manner. Organofluorine was measured because, at the time of the study, reliable analytical techniques for measuring serum or plasma PFOA were still under development. O'Malley and Ebbens (1981) treated groups of two male and two female New Zealand White rabbits dermally with doses of 100, 1,000, and 2,000 mg/kg PFOA for 14 days. Mortality among the exposed animals demonstrated dermal uptake. All of the animals died at the highest dose, three of four died in the mid-dose group, and none in the low-dose group. Although these data demonstrate dermal absorption, they do not provide quantitative dose-response data for effects other than mortality.

The results of *in vitro* percutaneous absorption studies of PFOA through rat and human skin have been reported (Fasano et al. 2005). The permeability coefficient for PFOA was calculated to be $3.25 \pm 1.51 \times 10^{-5}$ centimeters per hour (cm/h) and $9.49 \pm 2.86 \times 10^{-7}$ cm/h in rat and human skin, respectively.

2.2 Distribution

Distribution of absorbed material requires vascular transport from the portal of entry to receiving tissues. It has been suggested that PFOA circulates in the body by noncovalently binding to plasma proteins. Several studies have investigated the binding of PFOA to plasma proteins in rats, humans, or monkeys to gain an understanding of its absorption, distribution, and elimination as well as information on species and gender differences.

Protein Binding. Protein binding in plasma from cynomolgus monkeys, rats, and humans was tested with PFOA via *in vitro* methods (Kerstner-Wood et al. 2003). The results are summarized in Table 2-1. Rat, human, and monkey plasma proteins were able to bind 97–100% of the PFOA added at concentrations ranging from 1 to 500 ppm. Human serum albumin (HSA) carried the largest portion of the PFOA among the protein components of human plasma. Serum albumin is a common carrier of hydrophobic materials in the blood, including short- and medium-chain fatty acids, thyroxine (T4), heme, inorganic ions, and some pharmaceuticals (Fasano et al. 2005). Approximately 60% of the serum protein in humans and rats is albumin (Harkness and Wagner 1983; Saladin 2004). At 68%, the percentage bound to albumin in mice is slightly higher than in humans and rats (Harkness and Wagner 1983).

Table 2-1. Protein Binding in Rat, Human, and Monkey Plasma

PFOA Concentration (ppm)	Rat (%)	Monkey (%)	Human (%)
1	~100	~100	~100
10	99.5	99.8	99.9
100	98.6	99.8	99.9
250	97.6	99.8	99.6
500	97.3	99.5	99.4

Source: Kerstner-Wood et al. 2003
Note: % binding values reported as "~100" reflect a nonquantifiable amount of test article in the plasma water below the quantifiable limit <6.25 ng/mL.

Han et al. (2003) investigated the binding of PFOA to rat and human plasma proteins *in vitro*. The authors concluded that there was no correlation between the PFOA persistence and binding of the PFOA to rat serum. The primary PFOA binding protein in plasma was serum albumin. However, the method used (ligand blotting) would not theoretically allow the identification of low-abundance proteins with high affinity for PFOA. Further investigation of purified rodent and HSA binding using labeled ^{19}F nuclear magnetic resonance (NMR) allowed the calculation of disassociation constants for PFOA binding to rodent and HSA. No significant difference in binding to the serum albumin of rat versus human was detected (Table 2-2).

Male and female rats treated *in vivo* showed no gender difference in the binding of PFOA to serum proteins though the persistence of PFOA *in vivo* is much greater in male than female rats.

Table 2-2. Dissociation Constants (K_d) of Binding Between PFOA and Albumin

Parameter	Method	RSA	HSA
K_d (mM)	NMR[a]	0.29 ± 0.10[c]	
K_d (mM)	micro-SEC[b]	0.36 ± 0.08[c]	0.38 ± 0.04
Number of Binding Sites	micro-SEC[b]	7.8 ± 1.5	7.2 ± 1.3

Source: Han et al. 2003
Notes:
RSA = rodent serum albumin; HSA= human serum albumin
a = Average of the two K_d values (0.31 ± 0.15 and 0.27 ± 0.05 mM) obtained by NMR.
b = Values were obtained from three independent experiments and their SDs are shown.
c = On the basis of the result of unpaired t-test at 95% confidence interval, the difference of K_d values determined by NMR and micro-SEC is statistically insignificant.

Wu et al. (2009) examined the interaction of PFOA and HSA. The authors tested their hypothesis that PFOA, after absorption, was transported bound to albumin by dialyzing PFOA solutions in the presence and absence of HSA. In the absence of HSA, 98% of the dissolved PFOA crossed the dialysis membrane into the dialysate within 4 hours. In the presence of HSA, the amount of PFOA found in the dialysate after 4 hours decreased in direct proportion to the albumin concentration, demonstrating binding to the protein. No albumin was identified in the dialysate.

Using the dialysis data and thermodynamic considerations, the authors concluded that albumin could bind up to 12 PFOA molecules on its surface via chemical monolayer absorption with a 13th molecule bound noncovalently in the more hydrophobic interior of the protein. The surface nature of the binding could well indicate potential binding to other serum proteins as well. Circular dichroism measurements of the albumin/PFOA complex suggested a conformational change in the protein as a result of the PFOA binding. The beta-pleated sheet

content of the albumin decreased, and the alpha-helix content increased by 15%. These conformational changes could interfere with the functional properties of serum albumin or other serum proteins impacted by surface monolayers of PFOA. For example, albumin's ability to transport its natural ligands could be decreased by the presence of PFOA on the protein surface. The interaction of albumin with target cellular receptors also could be altered.

MacManus-Spencer et al. (2010) used a variety of approaches to quantify the binding of PFOA to serum albumin (e.g., surface tension measurements, ^{19}FNMR spectroscopy, and fluorescence spectroscopy). When taken together, the results from these analyses suggest the presence of primary and secondary binding sites on albumin. The PFOA-albumin association constants for the primary site (K_1^a) was about 1.5 ± 0.2 x 10^5/mol bovine serum albumin (BSA) while the association constant for the secondary site (K_2^a) was 0.8 ± 0.1 x 10^2/mol BSA at a concentration of 1µmol. When the concentration of BSA increased to 10 µmol, the binding per mol of BSA decreased K_1^a = 0.33 ± 0.004 and K_2^a = 0.53 ± 0.1. Qin et al. (2010) also used fluorescence spectroscopy quenching analysis to study PFOA binding to BSA and concluded that van der Waals forces and hydrogen bonds were the dominant intermolecular binding forces.

The results of the fluorescence spectroscopy suggested a conformational change in BSA following binding of PFOA that moved a tryptophan residue (#214) from a slightly polar region of the protein to a less polar region. The shift in a tryptophan position is consistent with the observations of Wu et al. (2009) and Qin et al. (2010), who reported that BSA underwent a conformational change following the binding of PFOA. The authors considered the results from the fluorescence spectroscopy to be relevant to the potential physiological impact of PFOA at levels found in the environment. Because serum albumin is a carrier for a variety of endogenous and exogenous substrates, a change in conformation can alter the bonding constants between albumin and other serum constituents.

A modeling study by Salvalaglio et al. (2010) was conducted to determine the binding sites of PFOA on HSA and classify them by their interaction energy using molecular modeling; this study builds on the binding studies of Wu et al. (2009) and MacManus-Spencer et al. (2010). It was estimated that the maximum number of PFOA binding sites on HSA was nine. The site locations were common to the natural binding sites for fatty acids, T4, Warfarin, indol, and benzodiazepine (see Figure 2-1) (Salvalaglio et al. 2010). The binding site closest to tryptophan residue #214 had the highest binding affinity (-8.0 kilocalorie/mol).

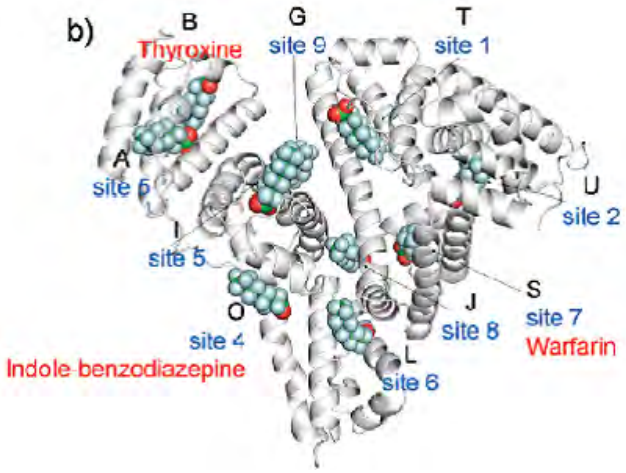


Figure 2-1. PFOA Binding Sites on HSA

Weiss et al. (2009) screened several perfluorinated compounds (n = 30), differing by carbon chain length C4–18, fluorination degree, and functional groups for potential binding to the serum thyroid hormone transport protein, transthyretin (TTR), using a radioligand-binding assay. The natural ligand of TTR is T4. PFOA was one of the chemicals evaluated. Human TTR was incubated overnight with ^{125}I-labeled T4, unlabeled T4, and 10-10,000 nanomolar (nmol) PFOA as a competitor for the T4 binding sites. The unlabeled T4 was used as a reference compound. The levels of T4 in the assay were close to the lower range for total T4 measured in healthy adults. The authors concluded that binding affinity for TTR was highest for the fully fluorinated compounds tested and those having at least an eight carbon length chain, characteristics that apply to PFOA. PFOA demonstrated a high binding affinity for TTR with 949 nmol, causing a 50% inhibition of T4 binding to the TTR.

Beesoon and Martin (2015) examined differences in the binding of the linear and branched chain isomers to serum albumin and human serum proteins. The linear PFOA molecule was found to bind more strongly to calf serum albumin than the branched chain isomers. When arranged in order of increasing binding, the order was 4m < 3m <5m < 6m (iso) <linear. In the isomer-specific binding to spiked total human serum protein, the linear molecule clearly had the strongest binding potential with about 7–10% free. The relationship for the other isomers was 5m > 6m >4m> 3m (15–30% free). Binding was estimated based on the concentrations in the ultrafiltrate after spiking with 5–60 mg/L of technical PFOA. The human serum was diluted tenfold before spiking.

When incubated with separate human-derived plasma protein fractions (Kerstner-Wood et al. 2003), PFOA was highly bound (99.7%) to albumin and showed some affinity for LDLs, formerly beta-lipoproteins (9.6%) with limited binding to alpha-globulins (11.0%) and gamma-globulins (3.0%). Low levels of binding to alpha-2-macroglobulin and transferrin were measured when the protein concentrations were approximately 10% of physiological concentration (see Table 2-3).

Table 2-3. Percent (%) Binding of PFOA to Human Plasma Protein Fractions

Fraction	~10% Physiological Conc.	100% Physiological Conc.
Albumin	96.4	99.7
Gamma-globulin	3.5	3.0
Alpha-globulin	28.5	11.0
Fibrinogen	5.4	<0.1
Alpha-2-macroglobulin	7.9	<0.1
Transferrin	1.0	2.1
LDLs	19.6	39.6

Source: Kerstner-Wood et al. 2003

It also is possible that PFOA will display nonspecific binding to proteins within the cellular matrix as well as in the serum but little work has been done to investigate that probability.

Luebker et al. (2002) conducted *in vitro* studies of the ability of a variety of perfluorinated chemicals to displace a fluorescent substrate (11-(5-dimethylamino-napthalenesulphonyl)-undecanoic acid) from liver fatty acid binding protein (L-FABP). L-FABP is an intracellular lipid carrier protein that reversibly binds long-chain fatty acids, phospholipids, and an assortment of peroxisome proliferators (Erol et al. 2003). It constitutes 2–5% of the cytosolic protein in hepatocytes. Luebker et al. (2002) reported that PFOA (IC_{50}>10µmol) exhibited some binding to

L-FABP, but the binding potential was only about 50% of that for PFOS (IC_{50} = 4.9 μmol) and far less than that of oleic acid (IC_{50} = 0.01 μmol).

L. Zhang et al. (2013) cloned the human L-FABP gene and used it to produce purified protein for evaluation of the binding of PFOA and other PFASs. Nitrobenzoxadiazole-labeled lauric acid was the fluorescent substrate used in the displacement assays. IC_{50} values and dissociation constants were generated for the PFASs studied. Oleic and palmitic acids served as the normal substrates for L-FABP binding. The nitrobenzoxadiazole-labeled lauric acids indicated that there were two distinct binding sites for fatty acids in human FABP with the primary site having a twentyfold higher affinity than the secondary site. The IC_{50} value for PFOA was 9.0 ± 0.7 μmol, suggesting that it has a lower binding affinity than PFOS (IC_{50}=3.3 ± 0.1 μmol). A similar approach was used to compare perfluorohexanoic acid (PFHxA, perfluorohexanesulfonic acid (PFHxS), PFOA, and perfluorononanoic acid (PFNA). The affinity of PFNA for human L-FABP was found to be greater than that for PFOA. The affinities of PFHxA and PFHxS for the protein were much lower. Both PFOA and PFNA bound to the carrier protein in a 1:1 ratio and the interaction was mediated by electrostatic interactions and hydrogen binding of the PFAS with the fatty acid binding site.

2.2.1 Oral Exposure

Tissue Distribution

Human. No clinical studies are available that examined tissue distribution in humans following administration of a controlled dose of PFOA. However, samples collected in biomonitoring and epidemiology studies provide data showing distribution of PFOA. Olsen et al. (2001a) analyzed human sera and postmortem liver samples and found that more than 90% of the liver samples (n = 30) were < limit of quantitation (LOQ). Serum levels ranged from <LOQ–7.0 nanograms per milliliter (ng/mL). PFOA concentrations above the LOQ were detected in 5/6 postmortem liver samples from males in Catalonia, Spain. In females, only 1/6 liver samples was above LOQ (Kärrman et al. 2009). Pirali et al. (2009) measured intrathyroidal PFOA levels (0.4–6.0 ng/g) in thyroid surgical patients and found no correlation between serum and thyroid PFOA concentration. PFOA has been detected in breast milk samples (Tao et al. 2008; Völkel et al. 2008) and cord blood samples (Apelberg et al. 2007; Monroy et al. 2008) at concentrations above the LOQ. These studies indicate that PFOA is distributed within the body and that maternal transfer to offspring can occur.

Pérez et al. (2013) collected tissue samples from 20 adult subjects (aged 28–83) who had been living in Catalonia, Spain, for 10 years and died of a variety of causes. Autopsies and tissue collection (liver, kidney, brain, lung, and bone) were carried out in the first 24 hours after death. The tissues were analyzed for 21 perfluorinated compounds. PFOA was present in 45% of the samples but could be quantified in only 20% (median 1.9 ng/g). PFOA accumulated primarily in the bone (60.2 ng/g), lung (29.2 ng/g), liver (13.6 ng/g), and kidney (2.0 ng/g), with levels below detection in brain based on the mean wet weight tissue concentrations. Detection levels varied with the tissue evaluated.

Animal. Studies of tissue distributions are available for several species including monkeys, rats, and mice. The data are categorized by species in the sections that follow. The distribution data derived from studies during pregnancy and lactation follow the data on nonpregnant animals.

Monkey. Butenhoff et al. (2002, 2004b) studied the fate of PFOA in cynomolgus monkeys in a 6-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at dose rates (DRs) of 0, 3, 10, or 20 mg/kg. The highest dose was initially 30 mg/kg, but due to its toxicity, it was suspended after 12 days. Dosing was resumed on test day 22 using the 20 mg/kg/day dose for the remainder of the 6-month period, resulting in a normalized dose of 20 mg/kg/day for the study. Serum, urine, and fecal samples were collected at 2-week intervals and were analyzed for PFOA concentrations. Liver samples were collected at time of sacrifice.

Serum concentrations reached steady-state levels within 4–6 weeks in all dose groups. Steady-state concentrations of PFOA in serum were 77 ± 39, 86 ± 33, and 158 ± 100 μg/ml after 6 weeks and 81 ± 40, 99 ± 50, and 156 ± 103 μg/ml after 6 months for the 3-, 10-, and 20-mg/kg dose groups, respectively (Butenhoff et al. 2002, 2004b). The mean serum concentration of PFOA in control monkeys was 0.134–0.203 μg/ml. Urine PFOA concentrations reached steady state after 4 weeks and were 53 ± 25, 166 ± 83, and 181 ± 100 μg/ml in the 3, 10, and 20-mg/kg dose groups, respectively, for the duration of the study. Liver PFOA concentrations at terminal sacrifice in the 3-mg/kg and 10-mg/kg dose groups were similar and ranged from 6.29 to 21.9μg/g. Liver PFOA concentrations in two monkeys exposed to 20 mg/kg were 16.0 and 83.3 μg/g. Liver PFOA concentrations in two monkeys dosed with 10 mg/kg/day at the end of a 13-week recovery period were 0.08 and 0.15 μg/g (Butenhoff et al. 2004b).

Rat. Ylinen et al. (1990) administered newly weaned Wistar rats (18/gender/group) doses of 3, 10, and 30 mg/kg/day PFOA by gavage for 28 days. At necropsy, serum was collected as well as brain, liver, kidney, lung, spleen, ovary, testis, and adipose tissue. The concentration of PFOA in the serum and tissues was determined with capillary gas chromatography equipped with a flame ionization detector (FID). A mass spectrometer was used in the selected ion monitoring mode when the PFOA concentration was below the LOQ of the FID (1 μg/ml).

The concentration of PFOA in the serum and tissues following 28 days of administration is presented in Table 2-4. PFOA was not detected in the adipose tissue. The concentrations of PFOA in the serum and tissues were much higher in males than in females. In the males, the levels of PFOA in the serum were generally lower in the 30 mg/kg/day dose group than in the 10 mg/kg/day dose group due to increased urinary elimination in the 30 mg/kg/day group. The tissue levels were similar for the 10 and 30 mg/kg/day doses. In females, there was a dose-related increase in tissue levels while the serum levels were comparable for the 10 and 30 mg/kg/day dose groups. Among solid tissues, the liver had the highest tissue concentration followed by the testis, spleen, lung, kidney, and brain, respectively. In females, the concentration in the kidneys exceeded that in the liver for the 10 and 30 mg/kg/day doses but not at the lowest dose. Ovary and spleen tissue had similar concentrations followed by lower levels in the lung and brain.

Table 2-4. Tissue Distribution of PFOA in Wistar Rats After 28 Days of Treatment

Tissue	Dose (Males[a]) mg/kg/day			Dose (Females[a]) mg/kg/day		
	3	10	30	3	10	30
Serum	48.6 ± 10.3	87.27 ± 20.09	51.65 ± 1.47	2.4[b]	12.47 ± 4.07	13.92 ± 6.06
Liver	39.9 ± 7.25	51.71 ± 11.18	49.77 ± 10.76	1.81 ± 0.49	3.45 ± 1.36	6.64 ± 2.64
Kidney	1.55 ± 0.71	40.56 ± 14.94	39.81 ± 17.67	0.06 ± 0.02	7.36 ± 3.19	12.54 ± 8.24
Spleen	4.75 ± 1.66	7.59 ± 3.5	4.1 ± 1.57	0.15 ± 0.04	0.38 ± 0.17	1.59 ± 0.49
Lung	2.95 ± 0.54	22.58 ± 4.59	23.71 ± 5.42	0.24[b]	0.22 ± 0.15	0.75 ± 0.26
Brain	0.398 ± 0.144	1.464 ± 0.211	0.71 ± 0.32	< LOQ[c]	0.029 ± 0.019	0.044 ± 0.018
Ovary				< LOQ	0.41 ± 0.27	1.16 ± 0.58
Testis	6.24 ± 2.04	9.35 ± 4.02	7.22 ± 3.17			

Source: Ylinen et al. 1990
Notes:
[a] n = 6, mean ± SD, µg/ml tissue.
[b] n = 3, no SD.
[c] Below LOQ of 1µg/mL

Kemper (2003) examined the distribution of PFOA in tissues of male and female Sprague-Dawley rats following administration by gavage. Rats were administered 1, 5, and 25 mg/kg ^{14}C-PFOA by oral gavage. Tissue concentrations were determined at the time of maximum plasma concentration (T_{max}) and at the time that plasma concentration had fallen to one half the maximum ($T_{max/2}$). Values for T_{max} and $T_{max/2}$ for male and female rats were determined from PK experiments. In those experiments, plasma was collected over the course of several days and PFOA concentration was analyzed. Noncompartmental PK models were applied to identify T_{max} and elimination half-time ($T_{1/2}$) from the data. The $T_{max/2}$ was calculated as the time (hr) for the maximum plasma concentration plus the elimination half-time (hr) ($T_{max} + T_{1/2}$). In some cases, elimination could occur in a rapid phase followed by a slower elimination phase. For cases in which biphasic elimination was evident, the rapid phase $T_{1/2}$ was used for calculation of $T_{max/2}$.

Tissues from male rats were collected at 10.5 hours (T_{max}) and 171 hours ($T_{max/2}$) after dosing. Tissues from female rats were collected at 1.25 hours (T_{max}) and 4 hours ($T_{max/2}$) after dosing. The results are summarized in Table 2-5 for males and Table 2-6 for females. Liver, blood, skin, muscle, bone, G.I. tract, and fat were the primary tissues for distribution of ^{14}C-PFOA. In males, the fraction of the dose found in the liver increased between T_{max} and $T_{max/2}$, but remained constant or decreased in other tissues. In females, the fraction of the dose present in all tissues remained constant or decreased between T_{max} and $T_{max/2}$. Liver-to-blood concentration ratios for ^{14}C at T_{max} in males were greater than 1 and increased between T_{max} and $T_{max/2}$. In females distribution levels in blood were between 1 and 2 at all dose levels and remained relatively constant between T_{max} and $T_{max/2}$. In males, the blood distributions levels were tenfold or higher than kidney levels at T_{max} and declined slightly at $T_{max/2}$.

Examination of the residuals from the administered PFOA in the male tissues at $T_{max/2}$ (171 hours) indicate that 40–60% of the dosed PFOA retained was present in the liver, blood, skin, and muscle tissues in decreasing amounts (Table 2-5). In males, about 1% of the label was present in the gastrointestinal tissues and contents at $T_{max/2,}$ while the value for females was about 10%. However, the samples were collected at 1.25 and 4 hours in females and 10.5 and 171 hours in males, providing more time for absorption in the males.

Table 2-5. Distribution of PFOA in Male Sprague-Dawley Rats After Oral Exposure Dose

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
Prostate	0.083 ± 0.039	0.030 ± 0.002	0.071 ± 0.045	0.057 ± 0.020	0.067 ± 0.018	0.028 ± 0.012
Skin[a]	14.77 ± 2.135	6.061 ± 0.274	15.565 ± 0.899	7.233 ± 0.430	13.836 ± 0.969	5.419 ± 0.237
Blood[a]	22.148 ± 0.692	8.232 ± 1.218	24.919 ± 1.942	11.140 ± 0.624	22.905 ± 1.177	7.904 ± 1.032
Brain	0.071 ± 0.018	0.022 ± 0.002	0.051 ± 0.021	0.023 ± 0.008	0.063 ± 0.007	0.019 ± 0.002
Fat[a]	2.281 ± 0.467	0.593 ± 0.136	2.815 ± 0.225	0.916 ± 0.205	2.153 ± 0.430	0.628 ± 0.110
Heart	0.451 ± 0.119	0.195 ± 0.024	0.443 ± 0.037	0.252 ± 0.030	0.461 ± 0.053	0.164 ± 0.032
Lungs	0.74 ± 0.147	0.341 ± 0.043	0.593 ± 0.376	0.344 ± 0.194	0.863 ± 0.103	0.303 ± 0.057
Spleen	0.086 ± 0.011	0.045 ± 0.006	0.096 ± 0.017	0.060 ± 0.007	0.106 ± 0.015	0.042 ± 0.005
Liver	21.708 ± 5.627	32.627 ± 3.601	18.750 ± 2.434	25.231 ± 1.289	17.528 ± 0.900	20.145 ± 3.098
Kidney	1.949 ± 0.402	1.14 ± 0.215	2.170 ± 0.354	1.212 ± 0.115	2.293 ± 0.286	1.003 ± 0.122
G.I. tract	2.930 ± 0.929	0.980 ± 0.300	2.508 ± 0.713	1.052 ± 0.202	2.784 ± 0.608	0.808 ± 0.189
G.I. contents	2.083 ± 0.625	0.239 ± 0.025	2.632 ± 0.934	0.270 ± 0.028	4.186 ± 1.349	0.210 ± 0.084
Thyroid	0.008 ± 0.005	0.004 ± 0.003	0.011 ± 0.006	0.004 ± 0.002	0.009 ± 0.002	0.005 ± 0.001
Thymus	0.085 ± 0.008	0.051 ± 0.018	0.085 ± 0.012	0.053 ± 0.003	0.120 ± 0.025	0.045 ± 0.010
Testes	0.755 ± 0.079	0.356 ± 0.037	0.693 ± 0.180	0.372 ± 0.062	0.623 ± 0.098	0.224 ± 0.031
Adrenals	0.019 ± 0.004	0.010 ± 0.001	0.022 ± 0.004	0.009 ± 0.001	0.026 ± 0.004	0.009 ± 0.003
Muscle[a]	12.025 ± 0.648	4.984 ± 0.745	13.565 ± 0.576	6.429 ± 0.648	12.855 ± 0.841	4.253 ± 0.358
Bone[a]	3.273 ± 0.538	1.120 ± 0.094	3.047 ± 0.544	1.375 ± 0.169	3.062 ± 0.438	0.906 ± 0.100
Total[b]	85.465 ± 6.426	57.026 ± 3.379	88.033 ± 1.420	56.031 ± 1.025	83.937 ± 3.680	42.112 ± 4.740

Source: Kemper 2003

Notes: Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues.

[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.

[b] Totals are calculated from individual animal data.

In the female tissues at $T_{max/2}$ (4 hours), approximately 30% of the dosed PFOA retained was present in the liver, blood, kidney, muscle, and skin tissues in decreasing amounts (Table 2-6). About 14% of the administered dose remained in the gastrointestinal tissues and contents. Based on the timing of the measurements and the results, females appear to absorb and excrete PFOA more rapidly than males.

Lau et al. (2006) studied PFOA's toxicokinetic properties in rats as part of a larger study. The authors gavage-dosed adult male and female Sprague-Dawley rats (n = 8) with 10 mg/kg for 20 days and sacrificed them 24 hours after the last treatment. After 20 days of treatment, male rats had serum PFOA levels of 111 µg/mL compared to 0.69 µg/mL in female rats.

Martin et al. (2007) administered 20 mg PFOA/kg to adult male Sprague-Dawley rats (n = 4 or 5) for 1, 3, and 5 days by oral gavage and determined the liver and serum levels of PFOA. Blood was collected via cardiac puncture and PFOA concentration was determined by high-performance liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS). The mean liver PFOA concentration was 92 ± 6, 250 ± 32, and 243 ± 23 µg/g after 1, 3, and 5 daily doses of 20 mg PFOA/kg/day, respectively. The mean serum concentration was 245 ± 41 µg/mL after 3 daily doses of 20 mg PFOA/kg/day. Serum PFOA concentration was not determined after 1 day and 5 days of dosing due to sample availability.

Table 2-6. Distribution of PFOA in Female Sprague-Dawley Rats after Oral Exposure Dose

Tissue	1 mg/kg		5 mg/kg		25 mg/kg	
	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$	% at T_{max}	% at $T_{max/2}$
Skin[a]	0.434 ± 0.162	0.403 ± 0.096	0.624 ± 0.142	0.307 ± 0.121	0.380 ± 0.166	0.415 ± 0.175
Blood[a]	5.740 ± 1.507	4.438 ± 1.625	8.089 ± 2.080	5.411 ± 1.466	7.158 ± 2.232	6.407 ± 1.406
Brain	0.037 ± 0.009	0.047 ± 0.008	0.066 ± 0.019	0.045 ± 0.010	0.058 ± 0.008	0.058 ± 0.018
Fat[a]	0.134 ± 0.032	0.164 ± 0.079	0.220 ± 0.111	0.110 ± 0.069	0.147 ± 0.053	0.148 ± 0.065
Heart	0.198 ± 0.079	0.253 ± 0.055	0.388 ± 0.057	0.236 ± 0.051	0.317 ± 0.035	0.287 ± 0.069
Lungs	0.454 ± 0.148	0.546 ± 0.082	0.827 ± 0.102	0.570 ± 0.179	0.678 ± 0.067	0.775 ± 0.204
Spleen	0.063 ± 0.027	0.058 ± 0.006	0.101 ± 0.021	0.060 ± 0.012	0.091 ± 0.007	0.070 ± 0.002
Liver	7.060 ± 1.266	6.817 ± 1.537	11.190 ± 2.192	7.176 ± 0.982	10.538 ± 1.723	9.080 ± 0.895
Kidney	3.288 ± 0.948	2.769 ± 0.784	4.293 ± 0.771	2.685 ± 0.736	5.867 ± 0.946	4.749 ± 0.393
G.I. tract	10.699 ± 9.066	8.462 ± 6.519	7.142 ± 2.594	8.255 ± 8.967	6.923 ± 1.846	3.547 ± 1.306
G.I. contents	21.956 ± 13.48	3.891 ± 2.395	2.896 ± 2.305	5.601 ± 6.165	2.491 ± 1.548	1.121 ± 1.010
Thyroid	0.010 ± 0.003	0.016 ± 0.021	0.008 ± 0.002	0.006 ± 0.002	0.009 ± 0.003	0.007 ± 0.002
Thymus	0.052 ± 0.017	0.058 ± 0.024	0.105 ± 0.030	0.068 ± 0.021	0.091 ± 0.032	0.077 ± 0.020
Ovaries	0.047 ± 0.019	0.048 ± 0.006	0.071 ± 0.012	0.041 ± 0.012	0.071 ± 0.012	0.070 ± 0.012
Adrenals	0.014 ± 0.005	0.018 ± 0.004	0.026 ± 0.005	0.015 ± 0.004	0.031 ± 0.005	0.021 ± 0.001
Muscle[a]	0.170 ± 0.051	0.258 ± 0.089	0.325 ± 0.010	0.229 ± 0.031	0.441 ± 0.116	0.304 ± 0.099
Uterus	0.243 ± 0.091	0.374 ± 0.247	0.354 ± 0.046	0.247 ± 0.068	0.358 ± 0.124	0.365 ± 0.029
Bone[a]	0.101 ± 0.017	0.153 ± 0.052	0.174 ± 0.057	0.142 ± 0.078	0.157 ± 0.072	0.181 ± 0.090
Total[b]	50.698 ± 16.48	28.772 ± 10.98	36.897 ± 3.187	31.201 ± 12.63	35.803 ± 2.554	27.680 ± 2.569

Source: Kemper 2003

Notes: Percent of dose recovered at T_{max} and $T_{max/2}$ in tissues.

[a] Percent recovery scaled to whole animal assuming the following: skin=19%, whole blood=7.4%, fat=7%, muscle=40.4%, bone=7.3% of body weight.

[b] Totals are calculated from individual animal data.

Mouse. Lau et al. (2006) gavage-dosed adult male and female CD-1 mice (5–7/group) with 20 mg/kg for 7 and 17 days. The animals were sacrificed 24 hours after the last treatment. After 7 days of treatment, male mice had serum PFOA levels of 181 µg/mL and females had levels of 178 µg/mL. After 17 days of treatment, male mice had serum PFOA levels of 199 µg/mL and females had levels of 171 µg/mL. These data suggest that the gender difference observed by Lau et al (2006) in rats was not seen in the mice under the conditions of this study.

As part of a physiologically based pharmacokinetic (PBPK) modeling exercise, Lou et al. (2009) administered single doses of 1 and 10 mg/kg to groups of three male and three female CD-1 mice. The mice were sacrificed for analysis of plasma, liver, and kidney tissues after 4, 8, and 12 hours and at 1, 3, 6, 9, 13, 20, 27, 34, and 48 days after dosing. This study was repeated for a second analysis that extended the sacrifice times to 55, 62, 70, and 80 days.

Measures of PFOA in serum were presented graphically and indicate that the order of magnitude difference between the doses led to a comparable order of magnitude difference in serum concentrations for both males and females across the 80-day observation period. [The study procedures indicated that *serum* was collected and analyzed, but the graphic presentation described the values as *plasma* values. Contact with one of the authors confirmed that the values should have been listed as *serum* rather than *plasma*.] The peak serum concentrations were

10 and 100 mg/L for the 1 and 10 mg/kg/day doses, respectively. Declines in serum concentrations for females over time were roughly parallel reaching concentrations of about 2 mg/L and <0.2 mg/L for the high and low doses, respectively, at the end of 80 days. Peak serum concentrations were slightly lower in the males (~8 and 80 mg/L) than in the females, and final serum concentrations were higher in the males (~0.5 and 8 mg/L). Liver and kidney concentrations also were higher in males than in females for each of the two doses. These data suggest a longer half-life in males than in females.

Lou et al. (2009) also collected serum data for up to 28 days after administration of a 60-mg/kg dose to groups of three female mice. Based on the graphic presentation of the data, the 60-mg/kg dose was cleared from the serum much more rapidly than the 1- and 10-mg/kg doses. For example, a serum concentration of about 0.4 mg/L was reached in about 28 days for the 60-mg/kg dose, 61 days for the 10-mg/kg dose, and 70 days for the 1-mg/kg dose (values estimated from Figure 3 in Lou et al. [2009]). No measurements were made for liver or kidney in the high-dose animals.

In the final experimental portion of the study, Lou et al. (2009) exposed groups of five female CD-1 mice to 20 mg/kg/day for 17 days. Serum samples were collected 24 hours after the final dose and analyzed for PFOA. The mean serum concentration was 130 ± 23 mg/L, which is comparable to that of 171 µg/mL reported by Lau et al. (2006).

Minata et al. (2010) orally administered 0, 12.5, 25, and 50 micromole per kilogram (µmol/kg) PFOA (~0, 5.4, 10.8, and 21.6 mg/kg PFOA) to groups of male wild-type 129S4/SvlmJ mice (n = 39) and PPARα- null 129S4/SvJae-Pparα^{tm1Gonz}/J mice (n = 40) for 4 weeks. Blood, liver, and bile were collected for determination of PFOA concentration at the end of 4 weeks, as shown in Table 2-7. The PFOA concentration in whole blood and the liver were similar between wild-type and PPARα-null mice at the same dose level and appeared to increase in proportion to dose. In bile, PFOA concentration in wild-type mice increased by a factor of 13.8 from 12.5 to 25 µmol/kg and by a factor of 2.8 from 25 to 50 µmol/kg. In the bile of PPARα-null mice, PFOA concentration increased by a factor of 3.2 from 12.5 to 25 µmol/kg and by a factor of 19.5 from 25 to 50 µmol/kg. The data suggested saturation of PFOA transport from the liver to bile ducts in wild-type mice, but not PPARα-null mice. This may indicate that PPARα plays a role in the clearance of PFOA.

Table 2-7. PFOA Concentrations in Wild-type and PPARα-null Mice (µg/mL)

Dose µmol/kg	Whole Blood		Bile		Liver	
	Wild-type	PPARα-null	Wild-type	PPARα-null	Wild-type	PPARα-null
0	ND	ND	ND	ND	ND	ND
12.5	20.6 ± 2.4	19.3 ± 2.2	56.8 ± 26.9	19.6 ± 2.2	181.2 ± 6.3	172.3 ±8.9
25	46.9 ± 3.2	36.4 ± 2.7*	784 ± 137.6	62.9 ± 16.7**	198.8 ± 15.4	218.3 ± 14.5
50	64.2 ± 6.5	71.2 ± 8.0	2174 ± 322.4	383 ± 109.9**	211.6 ± 13.3	239.7 ± 25.0

Source: Minata et al. 2010
Notes: Mean ± SD; ND= not detected (< 0.001 µg/mL); * $p < 0.05$; ** $p < 0.01$

Tissue Transporters. As identified earlier, protein transporters from a number of families play a role in the tissue uptake of orally ingested PFOA. The transporters are located at the interface between the serum and the liver, kidneys, lungs, heart, brain, testes, ovaries, placenta, and uterus (Klaassen and Aleksunes 2010). The liver is an important uptake site for PFOA. OATPs and MRPs, at least one OAT, and the sodium-taurocholate cotransporting polypeptide (NTCP), a

hepatic bile uptake transporter, have been identified at the interface of the liver with the portal blood and/or the canalicular membranes within the liver (Kim 2003; Kusuhara and Sugiyama 2009; Zaïr et al. 2008).

The impact of PFOA on several membrane transporter systems linked to biliary transport was studied by Maher et al. (2008) as part of a more detailed study of perfluorodecanoic acid (PFDA). A dose of 80 mg/kg by intraperitoneal (i.p.) injection (propylene glycol: water vehicle) was found to significantly increase ($p<0.05$) the expression of MRP3 and MRP4 in the livers of C57BL/6 mice 2 days after treatment as reflected in quantification of their deoxyribonucleic acid (DNA) transcripts. MRP3 and MRP4 are believed to protect the liver from accumulation of bile acids, bilirubin, and potentially toxic exogenous substances by promoting their excretion in bile. There were significant increases in serum bilirubin and bile acids after PFDA exposure, signifying increased export. Conversely, there were significant decreases ($p<0.05$) in the protein levels for OATP1a1, OATP1a4, and OATP1b2 as determined by Western Blot analysis and messenger ribonucleic acid (mRNA) measurements following exposure to 40 mg PFOA/kg (Cheng and Klaassen 2008). There was no significant impact on NTCP protein or the serum levels of bile acids. The OATPs are transporters responsible for the uptake of bile acids and other hydrophobic substances such as steroid conjugates, ecosinoids, and thyroid hormones into the liver.

These studies, all by the same laboratory, were carried out at high, single-dose exposures, which limit their value in extrapolating to low- and repeat-dose scenarios. The results suggest a decrease in the uptake of favored substrates into the liver and an increase in removal of favored substrates from the liver via bile. Upregulation of MRP3 and MRP4, coupled with decreased OATp levels, could be beneficial due to increased biliary excretion of bile acids, bilirubin, and conjugated metabolites of toxic chemicals, including PFOA. Based on the results with the more extensive evaluation of PFDA including mouse strains null for several receptors (PPARα, CAR, PXR, and FXR), the authors concluded that the changes in receptor proteins were primarily linked to activation of PPARα.

Impact of Developmental Age. Han (2003) administered groups of 4–8-week-old Sprague-Dawley rats (10 per gender per age) a single dose of 10 mg/kg/day PFOA by oral gavage. Blood samples were collected 24 hours after dosing and the plasma concentration of PFOA was measured by high-performance liquid chromatography mass spectrometry (HPLC/MS). In the 4-week-old rats, the concentration of plasma PFOA was approximately 2.7 times higher in males than in the females (Table 2-8). In the 5- and 6-week-old female rats, the plasma PFOA concentrations were about twofold lower than in the 4-week-old rats. However, in the 5-week-old males, the concentration of plasma PFOA was about fivefold higher than in the 4-week-old group, suggesting a developmental change in excretion rate. Plasma concentrations did not differ appreciably among 5-, 6-, 7-, and 8-week-old rats within each gender but did differ between genders. In fact, PFOA plasma concentrations were 35–65-fold higher in males than in females at every age except at 4 weeks. Thus, it appears that maturation of the transport features responsible for the gender difference in elimination occurs between the ages of 4 and 5 weeks in the rat.

Table 2-8. Plasma PFOA Concentrations (µg/ml) in Postweaning Sprague-Dawley Rats

Age (weeks)	Males	Females
4	7.32 ± 1.01[a]	2.68 ± 0.64
5	39.24 ± 3.89	1.13 ± 0.46
6	43.19 ± 3.79	1.18 ± 0.52
7	37.12 ± 4.07	0.57 ± 0.29
8	38.55 ± 5.44	0.81 ± 0.27

Source: Han 2003
Notes:
[a] Mean ± SD; samples from 10 animals/gender/group

Hinderliter (2004) and Hinderliter et al. (2006a) continued the investigation of the relationship between age and plasma PFOA in male and female Sprague-Dawley rats. Immature rats at 3, 4, and 5 weeks of age were administered PFOA via oral gavage at a single dose of 10 or 30 mg/kg. Rats were not fasted prior to dosing. Two hours after dosing, five rats per gender per age group and dose group were sacrificed and blood samples were collected. The remaining five rats per gender per age and dose group were placed in metabolism cages for 24-hour urine collection. These rats were sacrificed at 24 hours and blood samples were collected.

In the male rats, plasma PFOA concentrations for either the 10- or 30-mg/kg dosage groups did not differ significantly by sample time (at 2 and 24 hours) or by animal age (3, 4, and 5 weeks), except at 2 hours for the 5-week-old group ($p<0.01$), which showed the lowest PFOA level (Table 2-9). PFOA plasma concentrations following a 30-mg/kg dose were 2–3 times higher than those following a 10-mg/kg dose. These data do not demonstrate a difference between the 5-week-old rats and the younger 3- and 4-week-old groups at 24 hours after dosing, and thus do not support the observations from the Han study (2003).

Table 2-9. Plasma PFOA Concentrations in Male Rats

Age (weeks)	Dose (mg/kg)	Plasma PFOA (µg/ml)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	41.87	4.01	34.22	7.89
4	10	39.92	4.45	42.94	5.33
5	10	26.32*	6.89	40.60	3.69
3	30	120.65	12.78	74.16	18.23
4	30	117.40	18.10	100.81	13.18
5	30	65.66*	15.53	113.86	23.36

Source: Hinderliter 2004
Note: *Statistically significantly different by sample time and animal age ($p<0.01$).

In the female rats, plasma PFOA concentrations were significantly lower in the 5-week-old group than in the 3- or 4-week-old groups at the 24-hour time period for both doses and for the 30-mg/kg dose group at 2 hours (Table 2-10). Plasma PFOA concentrations following a 30-mg/kg dose were approximately one and one half to four times higher than those observed following a 10-mg/kg dose.

At 24 hours post-dose, plasma PFOA levels in the female rats were significantly lower than the plasma PFOA levels in male rats, especially at 5 weeks of age. The data for the 5-week-old female rats compared to the 3- and 4-week-old groups at 24 hours are consistent with the Han (2003) data

in that they demonstrate a decline in plasma levels compared to their earlier measurements. Thus, the developmental change is one that appears to be unique to the female rat.

Table 2-10. Plasma PFOA Concentrations in Female Rats

Age (weeks)	Dose (mg/kg)	Plasma PFOA (μg/ml)			
		2 Hours Post-Dose		24 Hours Post-Dose	
		Mean	SD	Mean	SD
3	10	37.87	5.77	13.55 [b]	3.83
4	10	29.88	12.15	18.98 [b]	7.01
5	10	33.23	7.41	1.36 [a, b]	0.87
3	30	84.86	10.51	51.43 [b]	13.61
4	30	80.67	14.10	28.01 [b]	9.90
5	30	56.90 [a]	29.66	3.42 [a, b]	1.95

Source: Hinderliter 2004
Notes:
[a] Statistically significantly different from the 3- and 4-week values ($p < 0.01$).
[b] Statistically significantly different from 2-hour values ($p < 0.01$).

The data demonstrate that both dose and gender influence plasma levels. Post-dosing clearance (CL) is slow for both doses at 2 and 24 hours in males and females at postnatal weeks 3 and 4. At 5 weeks, however, the plasma levels after 24 hours are greater than those at 2 hours in males. In females, for the high dose at 2 hours, plasma levels are similar to those in males, while at 24 hours they are only 3% of the value for males. This suggests that uptake from the intestines is similar while the rate of excretion at 5 weeks and beyond is considerably greater for female rats than males. They are comparable for postnatal weeks 3 and 4.

In a supplemental study to determine the effect of fasting (Hinderliter [2004] and Hinderliter et al. [2006a]), 4-week-old rats, 4 rats per gender, were administered 10 mg/kg PFOA via oral gavage. Animals (two per gender) were fasted overnight for 12 hours before dosing with PFOA. All the rats were sacrificed at 24 hours post dosing and blood was collected for analysis of PFOA in plasma. Plasma PFOA concentrations in male rats were 64.95 and 30.00 μg/ml for the fasted and nonfasted animals, respectively. Plasma PFOA concentrations in the female rats were 68.16 and 26.54 μg/ml for the fasted and nonfasted animals, respectively. Given the consistency in the 4-week-old rat plasma PFOA concentrations, the authors concluded that age-dependent changes in female PFOA elimination are observable between 3 and 5 weeks of age. PFOA uptake was greater in the fasted animals than the fed animals, suggesting competition for uptake in the presence of food components that share common transporters and/or decreased contact of PFOA with the intestinal epithelium in the presence of dietary materials.

Distribution during Pregnancy and Lactation

Humans. T. Zhang et al. (2013) recruited pregnant females for a study to examine the distribution of PFOA between maternal blood, cord blood, the placenta, and amniotic fluid. Thirty-two females from Tianjin, China, volunteered to take part in the study. Samples were collected at time of delivery. Maternal ages ranged from 21 to 39 years, gestation periods ranged from 35 to 37 weeks. It was the first child for 26 of the females and a second child for 6. The study yielded 31 maternal whole blood samples, 30 cord blood samples, 29 amniotic fluid samples, and 29 placentas. The maternal blood contained variable levels of 10 PFASs, eight acids, and two sulfonates. The mean maternal blood concentration was highest for PFOS

(14.6 ng/mL) followed by PFOA (3.35 ng/mL). In both cases, the mean was greater than the median, indicating a distribution skewed toward the higher concentrations.

PFOA was found in all fluids/tissues sampled. PFOA was transferred to the amniotic fluid to a greater extent than PFOS, based on their relative proportions in the maternal blood and cord blood. Compared to the mean PFOA blood levels in the pregnant females, the mean levels in the cord blood, placenta, and amniotic fluid were 47%, 59%, and 1.3%, respectively, of those in the mother's blood. The correlation coefficients between the maternal PFOA blood levels and placenta, cord blood, and amniotic fluid levels were good (0.7–0.9) and the relationships statistically significant ($p<0.001$).

Rat. An oral two-generation reproductive toxicity study of PFOA in rats was conducted (Butenhoff et al. 2004a). Five groups of rats (30 gender/group) were administered PFOA by gavage at doses of 0, 1, 3, 10, and 30 mg/kg/day. At scheduled sacrifice, after completion of the cohabitation period in F0 male rats and on lactation day (LD) 22 in F0 female rats, blood samples (3/gender/group-control; 10/gender/group-treated) were collected from animals dosed with 0, 10, and 30 mg/kg for analysis of PFOA. Serum analysis for the F0 generation males in the control, 10-, and 30-mg/kg/day groups sampled at the end of cohabitation showed that PFOA was present in all samples tested, including controls. Control males had an average concentration of 0.0344 ± 0.0148 μg/ml PFOA. Levels of PFOA were similar in the two male dose groups; treated males had 51.1 ± 9.30 and 45.3 ± 12.6 μg/ml, respectively, for the 10- and 30-mg/kg/day dose groups. In the F0 female controls, serum PFOA was below LOQ (0.00528 μg/ml). Levels of PFOA found in female sera increased between the two dose groups; treated females had an average concentration of 0.37 ± 0.0805 and 1.02 ± 0.425 μg/ml, respectively, for the 10- and 30-mg/kg/day dose groups.

PFOA levels during gestation and lactation were studied by Hinderliter et al. (2005) and Mylchreest (2003). Groups of 20 pregnant Sprague-Dawley rats were dosed with 0, 3, 10, and 30 mg/kg/day of PFOA during days 4–10, 4–15, and 4–21 of gestation, or from gestation day (GD) 4 to LD 21. Maternal blood samples were collected at 2 hours ± 30 minutes (mins) post-dose on a daily basis. Clinical observations and body weights were recorded daily. Five animals per dose group were sacrificed at specific time periods to harvest the conceptus and/or placenta and amniotic fluid. On GD 10, only embryos were recovered, and on GDs 15 and 21, the placentas, amniotic fluid, and embryos/fetuses were collected.

The remaining five rats per group were allowed to deliver their pups. On LDs 0, 3, 7, 14, and 21, the pups were counted, weighed (genders separate), and examined for abnormal appearance and behavior. Randomly selected pups were sacrificed and blood samples were collected. On LDs 3, 7, 14, and 21, the dams were anesthetized and milk and blood samples were collected; dams were removed from their litters 1–2 hours prior to collection.

Plasma, milk, amniotic fluid extract, and tissue homogenate (placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC/MS. Maternal PFOA plasma levels during gestation and lactation are presented in Table 2-11. Maternal plasma levels at 2 hours post-dosing (approximately the time of peak blood levels following a gavage dose) were fairly similar during the course of the study with a mean level of 11.2, 26.8, and 66.6 μg/ml in the 3-, 10-, and 30-mg/kg/day groups, respectively; PFOA levels in the control group were below the LOQ (0.05 μg/ml).

Table 2-11. Maternal Plasma PFOA Levels (µg/ml) in Rats During Gestation and Lactation

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4 - GD 10	GD 10 plasma	8.53 ± 1.06	23.32 ± 2.15	70.49 ± 8.94
GD 4 - GD 15	GD 15 plasma	15.92 ± 12.96	29.40 ± 14.19	79.55 ± 3.11
GD 4 - GD 21	GD 21 plasma	14.04 ± 2.27	34.20 ± 6.68	76.36 ± 14.76
GD 4 - LD 3	LD 3 plasma	11.01 ± 2.11	22.47 ± 2.74	54.39 ± 17.86
GD 4 - LD 7	LD 7 plasma	10.09 ± 2.90	25.83 ± 2.07	66.91 ± 11.82
GD 4 - LD 14	LD 14 plasma	9.69 ± 0.92	23.79 ± 2,81	54.65 ± 11.63
GD 4 - LD 21	LD 21 plasma	9.04 ± 1.01	28.84 ± 5.15	64.13 ± 1.45
NA	Average plasma	11.19 ± 2.76	26.84 ± 4.21	66.64 ± 9.80

Source: Hinderliter et al. 2005; Mylchreest 2003
Notes: Mean ± SD; samples were from five dams/group/time point and were collected 2 hours post-dosing.

PFOA levels in the placenta, amniotic fluid, and embryo/fetus are presented in Table 2-12. The levels of PFOA in the placenta on GD 21 were approximately twice the levels observed on GD 15, and the levels of PFOA in the amniotic fluid were approximately four times higher on GD 21 than on GD 15. The concentration of PFOA in the embryo/fetus was highest in the GD 10 embryo and lowest in the GD 15 embryo; PFOA levels in the GD 21 fetus were intermediate.

Table 2-12. Placenta, Amniotic Fluid, and Embryo/Fetus PFOA Concentrations in Rats (µg/ml)

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–GD 10	GD 10—embryo	1.40 ± 0.30	3.33 ± 0.81	12.49 ± 3.50
GD 4–GD 15	GD 15—placenta —amniotic fluid —embryo	2.22 ± 1.79 0.60 ± 0.69 0.24 ± 0.19	5.10 ± 1.70 0.70 ± 0.15 0.53 ± 0.18	13.22 ± 1.03 1.70 ± 0.91 1.24 ± 0.22
GD 4–GD 21	GD 21—placenta —amniotic fluid —fetus	3.55 ± 0.57 1.50 ± 0.32 1.27 ± 0.26	9.37 ± 1.76 3.76 ± 0.81 2.61 ± 0.37	24.37 ± 4.13 8.13 ± 0.86 8.77 ± 2.36

Source: Hinderliter et al. 2005; Mylchreest 2003
Note: Mean ± SD; samples were pooled by litter and were collected 2 hours post-dosing.

The concentrations of PFOA in the plasma of the GD 21 fetus were approximately half the levels observed in the maternal plasma (Table 2-11). The values were about twice as high in the dams as in the pups with mean values of 14.04, 34.20, and 76.36 µg/ml, respectively, in the 3-, 10-, and 30-mg/kg/day groups for the dams and 5.88, 14.48, and 33.11 µg/ml, respectively, for the pups. Pup plasma levels decreased between birth and LD 7 (Table 2-13) and were, thereafter, similar to the levels observed in the milk (Table 2-14). The pups were not separated by gender.

The concentration of PFOA in the milk also was fairly similar throughout lactation and was approximately one-tenth of the PFOA levels in the maternal plasma (see Table 2-11); the mean values for maternal milk were 1.1, 2.8, and 6.2 µg/ml in the 3-, 10-, and 30-mg/kg/day groups, respectively (Table 2-14).

Table 2-13. Fetus/Pup PFOA Concentration (µg/ml) in Rats During Gestation and Lactation

Exposure Period	Tissue	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–GD 21	GD21—fetal plasma	5.88 ± 0.69	14.48 ± 1.51	33.11 ± 4.64
GD 4–LD 3	LD 3—pup plasma	2.89 ± 0.70	5.94 ± 1.44	11.96 ± 1.66
GD 4–LD 7	LD 7—pup plasma	0.65 ± 0.20	2.77 ± 0.58	4.92 ± 1.28
GD 4–LD 14	LD 14—pup plasma	0.77 ± 0.10	2.22 ± 0.38	4.91 ± 1.12
GD 4–LD 21	LD 21—pup plasma	1.28 ± 0.72	3.25 ± 0.52	7.36 ± 2.17

Source: Hinderliter et al. 2005; Mylchreest 2003
Note: Mean ± SD; samples were pooled by litter and were collected 2 hours post-dosing.

Table 2-14. PFOA Levels (µg/ml) in Rats Maternal Milk During Lactation

Exposure Period	Sample Time	Dose		
		3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
GD 4–LD 3	LD 3–milk	1.07 ± 0.26	2.03 ± 0.33	4.97 ± 1.20
GD 4–LD 7	LD 7–milk	0.94 ± 0.22	2.74 ±0.91	5.76 ±1.26
GD 4–LD 14	LD 14–milk	1.15 ± 0.06	3.45 ± 1.18	6.45 ± 1.38
GD 4–LD 21	LD 21–milk	1.13 ± 0.08	3.07 ± 0.51	7.48 ± 1.63
NA	Average milk	1.07 ± 0.09	2.82 ± 0.60	6.16 ± 1.06

Source: Hinderliter et al. 2005; Mylchreest 2003
Notes: Mean ± SD; samples were from 5 dams/group/time point and were collected 2 hours post-dosing.

Mouse. Fenton et al. (2009) orally dosed pregnant CD-1 mice (n = 25/group) with 0, 0.1, 1, and 5 mg PFOA/kg on GD 17. On GD 18, five dams/group were sacrificed and trunk blood, urine, amniotic fluid, and the fourth and fifth mammary glands were collected. One fetus/dam was euthanized and retained for whole-pup analysis. The remaining dams were allowed to litter. Biological samples as described above excluding amniotic fluid also were collected on postnatal days (PNDs) 1, 4, 8, and 18. As before, at each time-point, a single pup was euthanized and retained for whole-pup analysis. Blood from the remaining pups was collected and pooled. Milk was collected from dams on PNDs 2, 8, 11, and 18 following a 2-hour separation of the pups from the dam.

The concentration of PFOA in dam serum was approximately twice that detected in amniotic fluid (Table 2-15). Compared to the amniotic fluid, the concentration of PFOA in the fetuses was increased by 2.3-, 3.1-, and 2.7-fold at 0.1, 1, and 5 mg/kg, respectively. The highest concentration of PFOA was detected in the serum of nursing dams. In the dams, the concentration of PFOA in the serum exhibited a U-shaped response curve; the lowest serum concentration was observed at the time of peak lactation. Dam mammary tissue and milk PFOA concentrations showed a U-shaped response that mirrored that found in the dam's serum. The concentration of PFOA in pup's serum was significantly higher than PFOA concentration in dam's serum and appeared to decrease as the time for weaning approached. When pup PFOA concentration was calculated with consideration for pup body weight gain, PFOA body burden increased through the peak of lactation and began to decrease by PND 18, showing an inverse U-shaped response curve.

Table 2-15. PFOA Levels (ng/ml) in Mice During Gestation and Lactation in Selected Fluids and Tissues

Tissue	Day	Dose		
		0.1 mg/kg	1 mg/kg	5 mg/kg
Dam Serum	GD 18	143 ± 19	1697 ± 203	7897± 663
	PND 1	217.5 ± 35	1957.0 ± 84	9845.6 ± 1478
	PND 4	110.0 ± 12	1269.4 ± 235	6776.6 ± 561
	PND 8	46.7 ± 21	360.8 ± 98	1961.8 ± 414
	PND 18	123.3 ± 41	1035.2 ± 305	5156.5 ± 1201
Amniotic Fluid	GD 18	99.0 ± 28	865.3 ± 191	3203.8 ± 492
Dam Urine	GD 18	21.9 ± 8.6	104.9 ± 69.7	666.7 ± 169
	PND 1	7.7 ± 1.7	116.8 ± 64	492.3 ± 119
	PND 4	8.4 ± 6.4	53.5 ± 15	401.5 ± 117
	PND 8	0.8 ± 0.22	11.6 ± 6.2	40.1 ± 17
	PND 18	1.8 ± 1.1	18.7 ± 8.6	91.7 ± 49
Mammary Gland	GD 18	18.9 ± 1.9	307.2 ± 30.4	1429± 186
	PND 1	27.4 ± 6.8	343.8 ± 53	1933.5 ± 194
	PND 4	9.6± 8.4	239.2 ± 53	1461.8 ± 267
	PND 8	2.4 ± 3.8	71.7 ± 22	411.8 ± 78
	PND 18	17.1 ± 10	239.9 ± 76	1372.8 ± 240
Milk	PND 2	32.5 ± 12	716.7 ± 145	1236.6 ± 1370
	PND 8	11.6 ± 8.1	77.4 ± 19	245.1 ± 26
	PND 11	5.4 ± 1.0	42.3 ± 9.1	282.5 ± 162
	PND 18	43.5 ± 19	251.8 ± 147	909.8 ± 308
Whole Pup	GD 18	136.3 ± 15	1665.8 ± 213	6256.5 ± 751
	PND 1	150.9 ± 21	1606.9 ± 288	7134.5 ± 1097
	PND 4	91.8 ± 8.9	1183.2 ±187	5071.4 ± 267
	PND 8	60.9 ± 16	729.0 ± 92	3118.5 ± 424
	PND 18	17.5 ± 11	251.9 ± 112	1391.5 ± 118
Pup Serum	PND 1	324.7 ± 36	3926.8 ± 480	16,286.4 ± 1372
	PND 4	267.6 ± 47	3020.8 ± 223	11,925.2 ± 1077
	PND 8	260.2 ± 56	2548.2 ± 245	9215.8 ± 594
	PND 18	111.8 ± 30	1124.8 ± 236	5894.3 ± 743

Source: Fenton et al. 2009

Pregnant C57BL/6/Bkl mice were fed diets containing 0.3 mg PFOA/kg/day from GD 1 through the end of pregnancy. At birth, the PFOA concentrations in the offspring were 0.7 ± 0.1 μg/g in the brain and 16.3 ± 4.1 μg/g in the liver (Onishchenko et al. 2011).

Macon et al. (2011) gavage-dosed CD-1 mice with 0, 0.3, 1.0, or 3.0 mg PFOA/kg from GD 1 to GD 17 or with 0, 0.01, 0.1, and 1.0 mg PFOA/kg from GD 10 to GD 17. In the full gestation experiment (GD 1–17), offspring were sacrificed on PNDs 7, 14, 21, 28, 42, 63, and 84, and in the half gestation experiment (GDs 10–17), female offspring were sacrificed on PNDs 1, 4, 7, 14, and 21. Serum, liver, and brain from the offspring were analyzed for PFOA by HPLC/MS/MS.

At the lowest dose, PFOA concentration in the serum peaked at or before PND 7, but the two higher doses peaked around PND 14 (Table 2-16). Calculated blood burdens which take into account the increasing blood volumes and body weights for females showed an inverted U-shaped curve peaking at PND 14 for all doses. In the liver, PFOA concentration decreased over time with the highest concentration observed at PND 7. Lower concentrations of PFOA were detected in the brain of the offspring on PND 7 and 14.

Table 2-16. Female Offspring PFOA Levels (ng/ml) in Mice After GD 1-17 Exposure

Tissue	Day	Dose		
		0.3 mg/kg	**1.0 mg/kg**	**3.0 mg/kg**
Serum	PND 7	4980 ± 218	11026 ± 915	20700 ± 3900
	PND 14	4535 ± 920	16950 ± 3606	26525 ± 2446
	PND 21	1194 ± 394	377 ± 607	8343 ± 1078
	PND 28	630 ± 162	1247 ± 208	4883 ± 1378
	PND 42	377± 81	663 ± 185	2058 ± 348
	PND 63	55 ± 17	176 ± 85	–
	PND 84	16 ± 5	71 ± 8	125
Liver	PND 7	2078 ± 90	8134 ± 740	16700 ± 749
	PND 14	972 ± 124	4152 ± 483	10290 ± 1028
	PND 21	1188 ± 182	1939 ± 637	2339 ± 1241
	PND 28	678 ± 130	2007 ± 560	7124 ± 1081
	PND 42	342 ± 87	617 ± 145	1145 ± 274
	PND 63	118 ± 22	320 ± 113	417 ± 160
	PND 84	43 ± 12	55± 12	235 ± 79
Brain	PND 7	150 ± 26	479 ± 41	1594 ± 162
	PND 14	65 ± 12	241 ± 20	650 ± 44
	PND 21	<LOQ	31 ± 5	133 ± 23
	PND 28	<LOQ	<LOQ	62 ± 93
	PND 42	<LOQ	<LOQ	<LOQ
	PND 63	<LOQ	<LOQ	<LOQ
	PND 84	<LOQ	<LOQ	<LOQ

Source: Macon et al. 2011
Notes:
– = not measured
LOQ: serum full gestation = 10-20 ng/g; liver = 35 ng/g; brain = 35 ng/g; late gestation serum = 5 ng/mL

After an exposure to low doses of PFOA from GD 10 to GD 17, serum PFOA concentration in the female offspring declined from PND 1 through the end of the experiment (Table 2-17). Calculated blood burden showed a gradual increase from PND 1 to PND 14, followed by a decline through PND 21.

Table 2-17. Female Offspring Serum PFOA Levels (ng/ml) in Mice After GD 10-17 Exposure

Tissue	Day	Dose		
		0.01 mg/kg	0.1 mg/kg	1.0 mg/kg
Serum	PND 1	284.5 ± 21.0	2303.5 ± 114.4	16305.5 ± 873.5
	PND 4	184.1 ± 12.1	–	–
	PND 7	150.7 ± 20.9	1277.8 ± 122.6	11880.3 ± 1447.6
	PND 14	80.2 ± 13.9	645.4 ± 114.2	6083.7 ± 662.6
	PND 21	16.5 ± 2.1	131.7 ± 24.5	2025.1 ± 281.9
Blood Burden (calculated)	PND 1	15.2 ± 1.7	114.3 ± 5.4	926.0 ± 47.6
	PND 4	20.6 ± 0.1	–	–
	PND 7	27.3 ± 3.8	221.7 ± 24.9	1965.9 ± 256.7
	PND 14	27.0 ± 4.6	218.5 ± 39.8	2033.6 ± 293.5
	PND 21	7.9 ± 1.0	66.4 ± 12.8	984.7 ± 142.8

Source: Macon et al. 2011
Note: – = not measured, blood burden determined by (body weight x (58.5/1000) x serum x 0.55)

White et al. (2011) measured serum PFOA concentrations in three generation of CD-1 mice (Table 2-18). Pregnant mice (F0, n = 10–12 dams/group) were gavage-dosed with 0, 1, and 5 mg PFOA/kg from GD 1–17. A separate group of pregnant mice (n = 7–10 dams/group) were gavage-dosed with either 0 or 1 mg PFOA/kg from GD 1–17 and received drinking water containing 5 parts per billion (ppb) PFOA beginning on GD 7 and continuing until the end of the study for their offspring, except during breeding and early gestation, to simulate a chronic low-dose exposure. An increase in serum PFOA concentration was observed in the control + 5 ppb PFOA groups in the F1 and F2 generations and in the 1-mg/kg + 5-ppb PFOA group of the F2 generation. A decrease was observed for the remaining groups.

Table 2-18. Serum PFOA Levels (ng/ml) in Mice Over Three Generations

	Generation/ Day	Dose			
		0 mg/kg + 5 ppb	1 mg/kg	1 mg/kg + 5 ppb	5 mg/kg
Dams at weaning	F0/ PND 22	74.8 ± 11.3	6658.0 ± 650.5	4772.0 ± 282.4	26980.0 ± 1288.2
	F1/~PND 91	86.9 ± 14.5	9.3 ± 2.6	173.3 ± 36.4	18.7 ± 5.2
Offspring	F1/PND 22	21.3 ± 2.1	2443.8 ± 256.4	2743.8 ± 129.7	10045 ± 1125.6
	F1/PND 42	48.9 ± 4.7	609.5 ± 72.2	558.0 ± 55.8	1581.0 ± 245.1
	F1/PND 63	66.2 ± 4.1	210.7 ± 21.9	187.0 ± 24.1	760.3 ± 188.3
	F2/PND 22	26.6 ± 2.4	4.6 ± 1.2	28.5 ± 3.7	7.8 ± 1.9
	F2/PND 42	57.4 ± 2.9	0.4 ± 0.0	72.8 ± 5.8	0.4 ± 0.0
	F2/PND 63	68.5 ± 9.4	1.1 ± 0.5	69.2 ± 4.3	1.2 ± 0.5

Source: White et al. 2011

Subcellular Distribution. Han et al. (2005) examined the subcellular distribution of PFOA in the liver and kidney of male and female rats. Male and female Sprague-Dawley Crl:CD (SD)IGS BR rats were gavage-dosed with 25 mg/kg [^{14}C] PFOA and sacrificed 2 hours after dosing. Blood was collected and the liver and kidneys were removed. Five subcellular fractions (nuclei

and cell debris, lysosome and mitochondria, microsome, light microsome and ribosome, and membrane-free cytosol) were obtained by differential centrifugation. The radioactivity per gram (g) of each fraction and the total radioactivity were measured.

In the male liver, the highest proportion of total reactive residues (TRR) of PFOA was located in the nuclei and cell debris (40%). The TRR for the other subcellular fractions were as follows: membrane-free cytosol 26 percent% TRR, lysosome and mitochondria ~14% TRR, and microsome ~16% TRR. The level of PFOA in the light microsome and ribosome was ~1% TRR. In the female liver, the highest proportion of PFOA was found in the membrane-free cytosol, 48% TRR. The TRR were nuclei and cell debris ~31% TRR, lysosome and mitochondria ~12% TRR, and microsome ~8% TRR. As observed in the males, the level of PFOA in the light microsome and ribosome was ~1% TRR (Han et al. 2005).

In the male kidney, the level of PFOA was 79% TRR in the membrane-free cytosol, 15% TRR in the nuclei and cell debris, and 4% TRR in the lysosome and mitochondria/microsome/ light microsome and ribosome (combined). In the female kidney, the level of PFOA was 71% TRR in the cytosol, 21% TRR in the nuclei and cell debris, and 8% TRR in the lysosome and mitochondria/ microsome/light microsome and ribosome (combined). Further examination showed that in both genders, 98% of PFOA in the plasma was protein bound. The protein-bound fraction of PFOA in the liver cytosol was 56% TRR. In the kidney, the protein-bound fraction of PFOA in males was 42% TRR and 17% TRR in females (Han et al. 2005).

Based on the results, the authors concluded that subcellular distribution of PFOA in the rat liver was gender-dependent because the proportion of PFOA in the liver cytosol of female rats was almost twice that of the male rats. They hypothesized that the female might have a greater amount than the male of an unknown liver cytosolic binding protein with an affinity for perfluorinated acids. They also hypothesized that the unknown protein or protein complex might normally aid in transport of fatty acids from the liver. In the kidney, the subcellular distribution did not show the gender difference seen with the liver; however, the protein-bound fraction for the males (42%) was about twice that for the females (17%) (Han et al. 2005).

Inhalation Exposure

In a repeated exposure study, Hinderliter (2003) and Hinderliter et al. (2006b) exposed 6–8-week-old male and female rats (5 per gender per group) to 0-, 1-, 10-, and 25-mg/m^3 aerosol concentrations of PFOA for 6 hours/day, 5 days/week for 3 weeks. Blood was collected immediately before and after the daily exposure period 3 days/week. The aerosols had MMADs of 1.3–1.9 μm with GSDs of 1.5–2.1. PFOA plasma concentrations were proportional to the inhalation exposure concentrations, and repeated exposures produced little plasma carryover in females, but significant day-to-day carryover in males. Male rats reached steady-state plasma levels of 8, 21, and 36 μg/ml for the 1-, 10-, and 25-mg/m^3 groups, respectively, by 3 weeks. In females, the post-exposure plasma levels were 1, 2, and 4 μg/ml for the 1-, 10-, and 25-mg/m^3 groups, respectively. When measured immediately before the next daily exposure, plasma levels had returned to baseline in females, demonstrating CL within 24 hours of each daily dose.

Dermal Exposure

No data were identified on tissue distribution following dermal exposures.

2.3 Metabolism

Several studies have examined metabolism of PFOA. However, no studies show clear evidence of metabolism. Ophaug and Singer (1980) found no change in fluoride ion level in the serum or urine following oral administration of PFOA to female Holtzman rats. Ylinen et al. (1989) found no evidence of phase II metabolism of PFOA following a single intraperitoneal PFOA dose (50 mg/kg) in male and female Wistar rats. The free anionic and possible conjugated forms of PFOA in the urine were separated using BondElut tubes. The tubes contain NH_2, which is a weaker anion exchange sorbent and a good choice for retaining strong anions. The samples were aspirated through the tube, washed with water, and eluted with sodium bicarbonate/carbonate-buffer. The aspirate and eluate from the separation method were analyzed by gas chromatography. PFOA was not detected in the aspirate, but was retained with the cationic amino phase found in the eluate. This also occurred in control blanks spiked with PFOA. The authors concluded that because the PFOA anion was completely bound to the weak cationic amino phase in both the spiked controls and urine samples, PFOA in urine is not altered by phase II metabolism (Ylinen et al. 1989).

2.4 Excretion

Excretion data are available for oral exposure in humans and laboratory animals. Several studies have investigated the elimination of PFOA in humans, cynomolgus monkeys, and rats. In human females, elimination pathways include pregnancy (cord blood) and lactation (breast milk) (Apelberg et al. 2007; Tao et al. 2008; Thomsen et al. 2010; Völkel et al. 2008; von Ehrenstein et al. 2009).

Elimination half-lives differ among species. There are also significant gender differences in humans and some laboratory animal species. Information from humans does not, at this time, provide sufficient data to determine the magnitude of interindividual and gender differences in excretory half-lives. The transporters appear to play an important role in renal excretion of PFOA and possibly its biliary elimination as well.

Humans. The urinary excretion of PFOA in humans is impacted by the isomeric composition of the mixture present in blood and the gender/age of the individuals. The half-lives of the branched-chain PFOA isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains.

Y. Zhang et al. (2013) determined half-lives for PFOA isomers based on paired serum samples and early morning urine samples collected from healthy volunteers in two large Chinese cities. Half-lives were determined using a one compartment model and an assumption of first order CL. The V_d applied in the analysis as determined by Thompson et al. (2010) was 170 mL/kg. CL was estimated from the concentration in urine normalized for creatinine and assuming excretion of 1.2 and 1.4 L/day of urine and 0.9 and 1.1 mg creatinine/day for males and females, respectively. The mean half-life for the sum of all PFOA isomers in younger females (n = 12) was 2.1 years (range 0.19–5.2 years) while that for all males and older females (n = 31) was 2.6 (range 0.0059–14 years); the medians were 1.8 and 1.7 years, respectively. The mean values for the four branched-chain isomers of PFOA were lower than the value for the linear chain, suggesting that resorption transporters might favor uptake of the linear chain over the branched-chain isomers. Older females and males have longer half-lives than young females, suggesting the importance of monthly menstruation as a pathway for excretion (Y. Zhang et al. 2013).

T. Zhang et al. (2014) derived estimates for PFOA's urinary excretion rate using paired urine and blood samples from 54 adults (29 males and 25 females) in the general population and 27 pregnant females in Tainjin, China. The age range for the general population was 22–62 years and for the pregnant females was 21–39 years. Urinary excretion was calculated based on the concentration in the urine times volume of urine wherein a urinary volume of 1,200 mL/day was applied to all females and 1,600 mL/day for all males. Urine samples were first-draw morning samples. Total daily intakes for PFOA were calculated from the concentration in blood using first order assumptions, a half-life of 2.3 years (Bartell et al. 2010) and a V_d of 170 mL/kg (Lorber and Egeghy 2011; Thompson et al. 2010). PFOA was detected in the blood samples for all participants but for only 76% of the urine samples from the general population and 30% for the pregnant females. There was a direct correlation between the PFOA concentrations in blood and creatinine adjusted urine ($r = 0.348$ $p = 0.013$) for the general population but not for the pregnant females. When limited to the eight females who had detectable levels in both blood and urine, there was a significant correlation ($r= 0.724$, $p = 0.042$).

Among the general population, the daily urinary excretion rate accounted for 25% of the estimated intake with the excretion higher in males (31%) than in females (19%). The urine: blood ratio was lower for pregnant females than for nonpregnant females (0.0011 versus 0.0029), suggesting other removal pathways such as placenta and cord blood. There was little difference between the younger menstruating females (21–50 years versus 51–61 years), but there is no indication that data were collected from the participants relative to menstruation status on the day of blood and urine collection.

Wong et al. (2014) looked at the role of menstrual blood as an excretory pathway to explain the shorter half-life of PFOS in females than in males. They fit a population-based PK model to six cross-sectional NHANES data sets (1999–2012) for males and females. They concluded that menstruation could account for about 30% of the PFOS elimination half-life difference between females and males. Although Wong et al. (2014) studied PFOS and not PFOA, their findings are relevant to both chemicals.

Elimination of PFOA by way of the gastrointestinal tract was reported in a case history of a single human male with high serum levels of perfluorinated chemicals that appeared to originate from household dust following the installation of new carpeting (Genuis et al. 2010). Treatment with cholestyramine, a bile acid sequestrant for 20 weeks (4g/day, three times a day), lowered his serum PFOA concentration from 5.9 ng/g serum to 4.1 ng/g serum. More dramatic decreases were observed with serum PFOS (23–14.4 ng/g serum) and PFHxS (58–46.8 ng/g serum), which were present at higher levels in the serum. This observation suggests that excretion with bile and possible enterohepatic resorption via intestinal transporters limits the loss of absorbed PFOA via feces in the absence of a binding agent such as cholestyramine.

2.5 Animal Studies

Oral Exposure

Monkey. Butenhoff et al. (2004b) studied the fate of PFOA in cynomolgus monkeys in a 6-month oral exposure study. Groups of four to six male monkeys each were administered PFOA daily via oral capsule at DRs of 0, 3, 10, and 30/20 mg/kg for 6 months. Two monkeys exposed to 10 mg/kg and three monkeys exposed to 20 mg/kg were monitored for 21 weeks (recovery period) following dosing. Urine and fecal samples were collected at 2-week intervals and were analyzed for PFOA concentrations.

Urine PFOA concentrations over the duration of the study were 53 ± 25, 166 ± 83, and 181 ± 100 μg/ml in the 3-, 10-, and 30-/20-mg/kg dose groups, respectively, and reached steady-state after 4 weeks. Within two weeks of recovery, urine PFOA concentrations were <1% of the value measured during treatment and decreased slowly thereafter. Fecal PFOA concentrations were 6.8 ± 5.3, 28 ± 20, and 50 ± 33 μg/g in the 3-, 10-, and 20-mg/kg dose groups, respectively. Within two weeks of recovery, fecal PFOA concentrations dropped to less than 10% of the last value during treatment, and then declined slowly. These results are consistent with both renal and biliary excretion in male monkeys.

Rat. There have been a number of studies of excretion in rats because of the gender differences noted in serum levels. Hinderliter (2004) and Hinderliter et al. (2006a) investigated the relationship between age and urine PFOA concentrations in male and female Sprague-Dawley rats. Immature rats 3, 4, or 5 weeks of age were administered PFOA via oral gavage as a single dose of 10 or 30 mg/kg. Two hours after dosing, five rats per gender per age group and dose group were sacrificed and blood samples were collected (see section 2.2.1). The remaining five rats per gender per age and dose group were placed in metabolism cages for 24-hour urine collection. Urinary output (volume) was not quantified or standardized for creatinine levels.

Urine PFOA concentrations differed significantly with age, dose, and gender ($p<0.01$, Table 2-19). Urinary excretion of PFOA was substantially higher in females than in males, and the female urine PFOA concentrations increased with age. In male rats, 24-hour urine PFOA concentrations decreased with age up to five weeks. In both genders, urine PFOA was higher (2.5 to 6.5 times) at the 30-mg/kg dose as compared to the 10-mg/kg dose.

There was a difference in urinary excretion between the 3-week-old and 4/5-week-old male rats, with the older rats excreting ~50% less PFOA in the urine than the younger rats at 10 mg/kg and 30 mg/kg. If the data from urine are integrated with the plasma data in the same study (Table 2-9), the male plasma levels increased from the 3-week value and were relatively stable for weeks 4 and 5. In the females, urine excretion increased gradually with age (Table 2-19) and plasma concentrations decreased (Table 2-10).

Table 2-19. Urine PFOA Concentrations in Male and Female Rats

Age (weeks)	Dose (mg/kg)	Urine PFOA (μg/ml at 24 hours post-dose)			
		Male		Female	
		Mean	SD	Mean	SD
3	10	9.57	4.86	21.17	8.95
4	10	4.53	2.45	23.26	15.27
5	10	4.03	2.36	49.77	24.64
3	30	51.76	28.86	94.89	26.26
4	30	28.70	18.84	104.12	28.97
5	30	15.65	6.24	123.16	51.56

Source: Hinderliter 2004

Hundley et al. (2006) examined excretion of PFOA in one male and one female CD rat (sexually mature). Each was given a single dose of 10 mg/kg ^{14}C-PFOA and housed in a metabolism cage. Urine and feces were collected at 12, 24, 48, 72, 96, and 120 hours post-dose. The female rat excreted more PFOA over the 120-hour collection period than the male rat. In the male rat, 25.6% and 9.2% ^{14}C-PFOA were excreted in the urine and feces, respectively. In the female rat, 73.9% and 27.8% ^{14}C-PFOA were excreted in the urine and feces, respectively. The

female rat excreted almost all of the PFOA by 48 hours compared with only 19% of the dose excreted by the male rat over the same amount of time. The cumulative percent of the dose excreted is shown in Table 2-20.

Table 2-20. Cumulative Percent ^{14}C-PFOA Excreted in Urine and Feces by Rats

Rat	Hours After Dosing					
	12	24	48	72	96	120
Male	0.6	8.7	19.2	23.4	30.2	34.3
Female	52.5	96.4	99.8	100.0	100.0	100.0

Source: Hundley et al. 2006

Adult male Sprague-Dawley rats (n = 7) were given a single gavage dose of 0.5 mg PFOA/kg and monitored for 38 days (Benskin et al 2009). Over the course of the study, the rats were held in metabolic cages and urine and feces were collected. The mean blood PFOA concentration was 1.1 µg/mL 24 hours post-dose. During the first 24 hours post-dose, 65% of PFOA was excreted in the urine; most of the PFOA that was not absorbed was excreted in the feces. After that time period, 91–95% of the daily excreted PFOA was eliminated in the urine. On day 3, the mean PFOA concentration in urine and feces were 265 ng/g and 28 ng/g. The half-life for elimination from plasma in male rats was 13.4 days.

Cui et al. (2010) exposed 2-month-old male Sprague-Dawley rats (10 per group) to PFOA (96% active ingredient) at 0, 5, and 20 mg/kg/day once daily by gavage for 28 days. Urine and fecal samples were collected through use of metabolism cages at 24-hour intervals immediately following dosing on days 1, 2, 5, 7, 10, 14, 18, 21, 24, and 28 of the study. Daily urine volume and fecal weight were comparable across all groups throughout the study. As measured by excretion 24-hours after the first dose, 17.9% of the applied dose was excreted in the urine of the low-dose group and 22% for the high-dose group. The percent of the absorbed dose was 92.8% and 92.3% for the low and high doses, respectively, when the fecal excretion over the 24 hours following dosing was estimated to be unabsorbed material. During week 1, a sharp increase in urinary and fecal excretion expressed as percent of dose/day was observed in rats of both groups. The excretion rate leveled off at about 50% for the low-dose animals for the remainder of the 28 days. In the case of the high-dose animals, the urinary excretion remained level at about 80% for the second and third weeks and then increased sharply to about 140% at 28 days. The fecal excretion rates were 7.2% and 7.7% for rats in the 5- and 20-mg/kg groups, respectively, during the first 24 hours post-dosing and continued an upward trend throughout the 28 days with the terminal percent/day about 25% for the low-dose group and 40% for the high-dose group.

Dose is an important variable that impacts excretion. Rigden et al. (2015) exposed groups of five male Sprague-Dawley rats to doses of 0, 10, 33, and 100 mg/kg/day for 3 days and maintained them for 3 additional days; overnight urine was collected and body weight was measured daily. Of greatest interest relative to the limitations on renal resorption, is the dose-related increase in urine PFOA concentration and urine PFOA concentration per mg creatinine for the 33- and 100-mg/kg/day groups compared to the 10-mg/kg/day group. The peak in PFOA excretion normalized to creatinine occurred on day 3 after the cessation of dosing. The concentration at 33 mg/kg/day was 500 times greater than that at 10 mg/kg/day. At the 100-mg/kg/day dose, the peak concentration was about 3,200 times greater than for the low dose. The low-dose excretion was only slightly greater than the controls. The urine results support the renal resorption hypothesis concept and suggest that there is a threshold limit on resorption that,

once exceeded, dramatically increases PFOA loss in urine. As a consequence, half-life for continuous low-dose exposures will be longer than for single or short-term high-dose exposures.

Other Species. Hundley et al. (2006) examined excretion of PFOA in CD mice, BIO-15.16 hamsters, and New Zealand White rabbits. One male and one female of each species was given a single dose of 10-mg/kg ^{14}C-PFOA and housed in metabolism cages. Urine and feces were collected at 12, 24, 48, 72, 96, and 120 hours post-dose. Additional samples were collected from rabbits at 144 and 168 hours post-dose.

Over 120 hours, the male mouse excreted 3.4% ^{14}C-PFOA in urine and 8.3% ^{14}C-PFOA in feces, and the female mouse excreted 6.7% ^{14}C-PFOA in urine and 5.7% ^{14}C-PFOA in feces. The mice were similar in the amounts excreted. The male hamster excreted 90.3% and 8.2% ^{14}C-PFOA in urine and feces, respectively, and the female hamster excreted 45.3% and 9.3%^{14}C-PFOA. The male hamster excreted a greater amount of ^{14}C-PFOA than the female hamster. Over 84% of ^{14}C-PFOA was excreted 24 hours after dosing by the male hamster compared to less than 25% of ^{14}C-PFOA excreted by the female hamster at 24 hours after dosing. Over 168 hours, the male rabbit excreted 76.8% and 4.2% ^{14}C-PFOA in urine and feces, respectively, and the female rabbit excreted 87.9% and 4.6% ^{14}C-PFOA. Both rabbits excreted most of the dose by 24 hours. The cumulative percentage of ^{14}C-PFOA excreted is shown in Table 2-21.

Table 2-21. Cumulative Percent ^{14}C-PFOA Excreted in Urine and Feces

		Hours After Dosing						
Species	**Gender**	**12**	**24**	**48**	**72**	**96**	**120**	**168**
Mouse	Male	0.4	4.1	6.7	8.6	9.1	10.8	-
	Female	0.2	4.1	6.5	8.4	9.0	11.0	-
Hamster	Male	67.3	84.5	96.1	97.4	98.2	98.4	-
	Female	11.3	24.6	36.4	43.9	50.1	54.0	-
Rabbit	Male	77.8	80.2	80.4	80.4	80.4	80.4	80.4
	Female	86.7	90.5	92.0	92.2	92.7	92.9	93.0

Source: Hundley et al. 2006

When the data in Table 2-21 are integrated with the data from rats, the gender differences in PFOA excretion rate appear to be species-specific. Female rats, male hamsters, and both genders of rabbits appear to be good excreters based on their response to a radiolabeled dose of 10 mg/kg. Most of the dosed material is excreted within 24 hours after dosing. Female hamsters apparently are moderate excreters. Males and female mice excreted only about 10% of the dose over the 120 hours (5 days) after dosing. Mice do not show a gender difference but retain more of the dose than do hamsters, rabbits, and female rats. The long half-lives in humans suggest that their excretion rates are more like mice or male rats.

Inhalation Exposure

Although no data were identified on urine or fecal excretion of PFOA following inhalation exposures, the Hinderliter study (2003) provides evidence of CL following single and repeated inhalation exposures in Sprague-Dawley rats. Plasma PFOA concentrations following a single exposure to 1, 10, and 25 mg/m^3 PFOA declined 1 hour after exposure in females and 6 hours after exposure in males. In females, the elimination of PFOA was rapid at all exposure levels and, by 12 hours after exposure, their plasma levels had dropped below the analytical LOQ (0.1 µg/ml). In males, the plasma elimination was much slower and, at 24 hours after exposure, the plasma concentrations were approximately 90% of the peak concentrations at all exposure

levels. In the repeated exposure study, male and female rats were exposed to the same concentrations for 6 hours/day, 5 days/week for 3 weeks. Steady-state plasma levels were reached in males by 3 weeks, but plasma PFOA levels in females returned to baseline with 24 hours of each dose. The data are illustrative of distinct toxicokinetic differences between male and female rats in their response to PFOA exposure (Hinderliter 2003).

Dermal Exposure

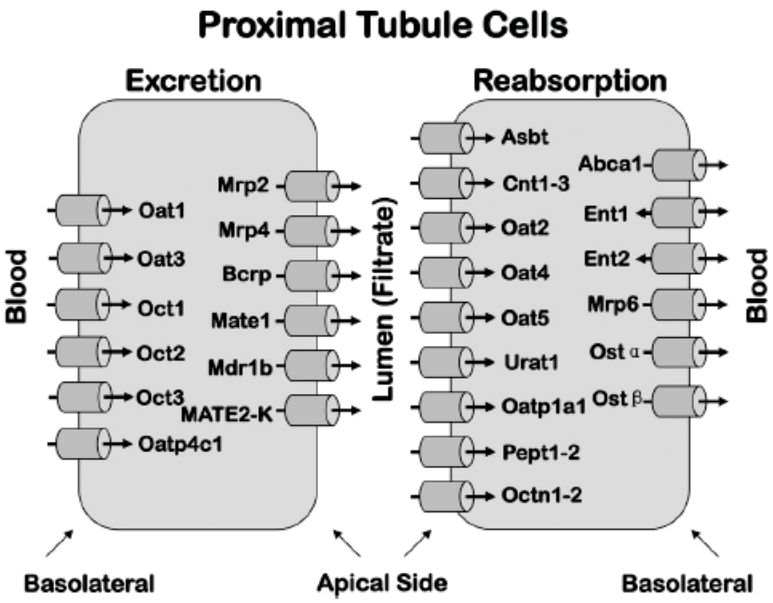
No data were identified on excretion following dermal exposures. Minimal fecal excretion is anticipated for the dermal route of exposure although the biliary pathway can be a route for excretion of material absorbed through the skin, distributed to the liver, and discharged to the gastrointestinal tract.

2.5.1 Mechanistic Studies of Renal Excretion

Several studies have been conducted to elucidate the cause of the gender difference in the elimination of PFOA by rats. Many of the studies have focused on the role of transporters in the kidney tubules. Most studies have examined the OATs located in the proximal portion of the descending tubule. OATs are found in other tissues as well and were discussed earlier for their role in absorption and distribution. In the kidney, they are responsible for delivery of organic anions, including a large number of medications from the serum into the kidney tubule for excretion as well as reabsorption of anions from the glomerular filtrate. The transporters are particularly important in excretion of PFOA because it binds to surfaces of serum proteins (particularly albumin), which makes much of it unavailable for removal during glomerular filtration. Other transporter families believed to be involved in renal excretion are the OATPs and the MRPs. However, they have not been evaluated as extensively as the OATs for their role in renal excretion.

OATs are located on both the basolateral (serum interface) and apical surfaces of the brush boarder of the proximal tubule inner surface. At the basolateral surface, the OATs transport the perfluorooctanoate anion from the serum to the tubular cells (Anzai et al. 2006; Cheng and Klaassen 2008; Klaassen and Aleksunes 2010; Klaassen and Lu 2008; Nakagawa et al. 2007, 2009). OAT1, 2, and 3 are located on the basolateral membrane surface. OAT4 and OAT5 are located on the apical surface of the tubular cells, where they reabsorb the PFOA anions from the glomerular filtrate. Figure 2-2 diagrams the flow of organic anions such as the PFOA anion from serum to the glomerular filtrate for excretion and resorption of organic acids from the glomerular filtrate with transport back to serum. OATs can function for uptake into the cell across both the basolateral and apical surfaces.

Several MRP transporters also appear to function in the kidney and move organic anions in and out of cells at both the basolateral surface (e.g., MRP2/4) and the apical surface (e.g., MRP1) as well as one or more OATPs on each surface (Cheng and Klaassen 2009; Klaassen and Aleksunes 2010; Klaassen and Lu 2008; Kusuhara and Sugiyama 2009; Launay-Vacher et al. 2006; Yang et al. 2009). Bidirectional movement of PFOA across both the basolateral and apical surfaces is driven by concentration gradients and/or active transport. Far more data exist on PFOA and OATs in the kidneys than on OATPs and MRPs. Abbreviations for individual transporters on the basolateral and apical surfaces differ across publications. The accepted convention is to use uppercase letters to refer to human transporters and lowercase letters to refer to animal transporters. For this report, the data are not reported by species but by transporter family and the uppercase letters are used.



Source: Klaassen and Aleksunes 2010

Figure 2-2. Localization of Transport Proteins

Knowledge about specific OAT, OATP, and MRP transporters in the kidneys is rapidly evolving. A low membrane density or blockage of basolateral OATs will decrease PFOA excretion while low membrane densities or blockage of apical OATs will increase excretion because they decrease resorption of anions from the glomerular filtrate.

The earliest studies of the impact of gender on urinary excretion were conducted by Hanhijarvi et al. (1982) using probenecid, an inhibitor of renal excretion of organic acids on PFOA excretion in male and female Holtzman rats. The female rats that had not received the probenecid excreted 76% of the administered dose of PFOA over a 7-hour period, while males excreted only 7.8% of the administered dose over the same period of time. Probenecid administration modified the cumulative excretion curve for males only slightly. In females, however, probenecid markedly reduced PFOA elimination to 11.8%. The authors concluded that the female rat possesses an active secretory mechanism that rapidly eliminates PFOA from the body that male rats do not possess.

Kudo et al. (2002) examined the role of sex hormones and OATs on the renal clearance (CL_R) of PFOA. Renal mRNA levels of specific OATs in castrated male and ovariectomized (OVX) female Wistar rats also were determined. Castration of male rats caused a 14-fold increase in CL_R of PFOA. The elevated PFOA CL_R in castrated males was reduced by treating them with testosterone. Treatment of male rats with estradiol increased the CL_R of PFOA. In female rats, ovariectomy caused a significant increase in CL_R of PFOA (a twofold increase), but the administration of estradiol to OVX female rats returned CL_R of PFOA to normal values. Treatments of female rats with testosterone reduced the CL_R of PFOA.

Treatment with probenecid, a known inhibitor of OAT1–6 and OAT8, markedly reduced the CL_R of PFOA in male rats, castrated male rats, and female rats (Kudo et al. 2002). Accordingly, the male sex hormones appear to decrease the presence of OATs in the renal basolateral membranes while the female sex hormones appear to increase the transporters.

To identify the transporter molecules responsible for PFOA transport in the rat kidney, renal mRNA levels of specific OATs were determined in male and female rats under various hormonal states and compared with the CL_R of PFOA. The level of OAT2 mRNA in male rats was only 13% of the level in female rats. Castration or estradiol treatment increased the level of OAT2 mRNA whereas treatment of castrated male rats with testosterone reduced it. Ovariectomy of female rats significantly increased the level of OAT3 mRNA. Multiple regression analysis of the data suggested that OAT2 and OAT3 are responsible for urinary elimination of PFOA in the rat; however, the possibility of a resorption process mediated by OATP1 was mentioned as a possible factor in male rat retention of PFOA. OAT2 and OAT3 are located on the basolateral cell surface. OATP1 is located on the apical surface of the renal tubule cells (Kudo et al. 2002).

Cheng et al. (2006) examined whether sex hormones influenced gender-specific OATP expression in the kidneys of adult male and female C57BL/6 mice. Gonadectomized mice were used for the studies in conjunction with hormone replacement measures (5α-dihydroxy-testosterone [DHT] or 17-β estradiol [E2]). OATP1a1 and OATP3a1 were evaluated. Treatment with DHT resulted in significant increase in both OATPs in the kidneys of male and female gonadectomized mice. In both cases, the change in males was greater than the change in females. Treatment with E2 almost abolished the expression of OATP1a1 in the kidneys but caused no significant change in OATP3a1. In the intact control animals, almost no expression of OATP1a1 occurred in the kidneys of females and a significantly lower expression of OATP3a1 ($p<0.05$) occurred. In the gonadectomized control animals, little or no expression of OATP1a1 occurred in either gender, and expression of OATP3a1 was equivalent in both genders.

Nakagawa et al. (2007) investigated the role of OATs in the renal excretion of PFOA using *in vitro* methods. HEK293-transformed cells, derived from human embryonic kidney (HEK), were transfected with human or rat OAT1, OAT2, or OAT3 constructs. Cells from the S2 segment of the proximal tubule were transfected with human or rat OAT2 constructs. HEK293 and S2 cells transfected with the vector served only as control cells. The transfected HEK293 cells were incubated for 1 min with or without 0, 10, and 100 µmol [^{14}C]PFOA and/or varying concentrations of favored OAT substrates to determine inhibitory effects of PFOA as follows: 5 µmol [^{14}C]para-aminohippuric acid (OAT1), 20 nmol [^{14}C]estrone sulfate (OAT3), and 10 nmol [^{14}C]prostaglandin $F_{2\alpha}$ (OAT2).

PFOA significantly inhibited para-aminohippuric acid and estrone sulfate uptake mediated by OAT1 and OAT3, respectively. At 10 µmol PFOA, uptake of 5 µmol [^{14}C] para-aminohippuric acid was 75–85% of the control level and, at 100 µmol PFOA, uptake was reduced to 35–45% of control. Estrone sulfate uptake by human OAT3 was 65% of the control level at 10 µmol PFOA and 40% of control at 100 µmol PFOA. Estrone sulfate uptake by rat OAT3 was 15% of the control level in the presence of 10 µmol PFOA and was almost completely inhibited at 100 µmol PFOA. Prostaglandin $F_{2\alpha}$ uptake by OAT2 was inhibited moderately by PFOA, 75–85% of control at 10 µmol PFOA, and 65% of control at 100 µmol PFOA.

In the second part of their study, Nakagawa et al. (2007) incubated HEK293 and S2 transfected cells with 10 µmol [^{14}C]PFOA for 1 min to determine uptake. Time-dependent uptake of 5 µmol [^{14}C]PFOA from 0 to 30 mins was conducted in the HEK293 cells transfected with human or rat OAT1, OAT2, or OAT3. Experiments were conducted in triplicate. Uptake of PFOA was stimulated ($p<0.001$) in cells transfected with human or rat OAT1 or OAT3, while no uptake was stimulated in cells transfected with OAT2 in either cell line. In the time-dependent experiments, uptake by human or rat OAT1 or OAT3 increased linearly up to 2 mins and reached a plateau in about 15 mins. Kinetic evaluations resulted in substrate concentration at which the

initial reaction rate is half maximal (K_m) values of 48.0, 51.0, 49.1, and 80.2 μmol for human OAT1, rat OAT1, human OAT3, and rat OAT3, respectively. The authors showed that both human and rat OAT1 and OAT3 transport PFOA in the kidney while human and rat OAT2 do not (Nakagawa et al. 2007).

Yang et al. (2009) investigated the role of OAT polypeptide 1a1 (OATP1a1) in the renal elimination of PFOA. The polypeptide is located on the apical side of proximal tubule cells and could be the mechanism for renal reabsorption of PFOA in rats. The level of mRNA of OATP1a1 in male rat kidney is 5–20-fold higher than in female rat kidney, OATP1a1 protein expression is higher in male rat kidneys, and it is regulated by sex hormones. One of its known substrates is estrone-3-sulfate (E3S). A substantial presence of OATP1a1 in male rats would favor resorption of PFOA in the glomerular filtrate and reduce excretion.

Chinese hamster ovary (CHO) cells were transfected with rat OATP1a1 complementary DNA. The transfected CHO cells were incubated with 4 μmol [^{14}C]PFOA for up to 10 mins or with 0–1,000 μmol [^{14}C]PFOA for 2 mins to determine uptake. The difference between the uptake velocities of CHO OATP1a1-transfected cells and CHO vector-transfected cells was defined as active PFOA uptake by the tubular epithelium. The transfected CHO cells were incubated with 5 μmol [^{14}C]PFOA for 2 mins in the absence or presence of inhibitors (e.g., BSP, taurocholate, probenecid, *p*-aminohippurate, and naringin [a flavonoid found in grapefruit]) for inhibition studies. The transfected CHO cells were incubated with 2 μmol E3S and 0, 0.1, or 1 mM perfluorocarboxylates with carbon chain lengths ranging from 4 to 12, including PFOA (C8) for 30 seconds for E3S inhibition studies.

In time-dependent uptake experiments, uptake of PFOA by OATP1a1-transfected cells increased proportionally to time during the first 2 mins of incubation. Vector-transfected cells had a significant level of uptake of PFOA attributed to nonspecific passive diffusion. In the concentration-dependent uptake experiments, uptake velocity of PFOA in OATP1a1-transfected cells increased with increasing concentration and saturation levels were not reached. In vector-transfected cells, uptake velocities increased linearly with increasing concentration of PFOA, demonstrating a passive diffusion mechanism. Active PFOA uptake—the difference between the uptake of the OATP1a1 cells and the vector-transfected cells—could be described by the Michaelis-Menton equation and exhibited saturable kinetics.

Inhibition experiments with substrates of OATs and OATPs showed that BSP, taurocholate, and naringin inhibited PFOA uptake to 10–30% of control and *p*-aminohippurate inhibited PFOA uptake to 62% of control. Probenecid, an OAT inhibitor, did not inhibit PFOA uptake at all. In OATP1a1-transfected cells, uptake of E3S was inhibited to less than 10% of control uptake following incubation with 1 mM [^{14}C]PFOA. Inhibition of E3S was less than 50% of control uptake after incubation with 0.1 mM [^{14}C]PFOA. Based on the results of the uptake and inhibition experiments, the authors suggested that passive diffusion could be an important route of PFOA distribution and that renal reabsorption in the male rat could be mediated by OATP1a1.

Nakagawa et al. (2009) investigated the role that the human organic acid transporter (OAT4) plays in transporting PFOA. Human OAT4 is located on the apical side of proximal tubule cells and mediates reabsorption of organic anions. Transformed cells derived from HEK cells, HEK293, were transfected with human OAT1, OAT3, or OAT4 constructs. HEK293 cells transfected with only the vector served as control cells. The transfected HEK293 cells were incubated with 10 μmol [^{14}C]PFOA for 15 mins to determine uptake. Transfected cells also were incubated with 10 μmol [^{14}C]PFOA for 15 mins and then washed with incubation medium

containing 1%, 3%, and 5% BSA to investigate the contribution of nonspecific binding of PFOA on the cell membrane. Experiments were conducted in triplicate.

Uptake of PFOA was significantly stimulated ($p<0.01$) in cells transfected with human OAT1, OAT3, and OAT4. Uptake of PFOA in human OAT1 transfected cells was 1.6-fold higher than in control cells. In human OAT3 transfected cells, PFOA uptake was ~2.4-fold higher than in control cells. In human OAT4 transfected cells, PFOA uptake was 2.7-fold higher than in control cells. Accumulation of PFOA in transfected human OAT4 cells also was significantly greater than in human OAT1 cells ($p<0.01$). Washing the cells with BSA reduced PFOA uptake by 30% at most, suggesting mediation by the transporters into the transfected cells. The experiments showed that human OAT4 transports PFOA and that human OAT4 activity might play a role in reabsorption of PFOA from the tubule, resulting in poor urinary excretion.

Yang et al. (2010) examined cellular uptake of PFOA by OATP1A2, OAT4, and urate transporter 1 (URAT1) to determine their roles in mediating human renal reabsorption. CHO and HEK293 cells were transfected with OATP1A2, OAT4, and URAT1 plasmid DNA or vector DNA (control). In uptake studies, PFOA incubation times were 10 seconds (OAT4) and 30 seconds (URAT1). Cells transfected with OAT4 were incubated with 5 µmol PFOA for up to 1 min in time-dependent uptake experiments. In inhibition studies, cells transfected with OAT4 were incubated with 5 µmol [^{14}C]PFOA for 10 seconds in the presence and absence of 100 µmol sulfobromophthalein (BSP), probenecid, glutarate, or polycyclic aromatic hydrocarbon (PAH). Perfluorinated carboxylates with differing chain lengths (C4–C12) were used in chain length-dependent inhibition experiments. Incubations with ^{3}H-E3S (OAT4 and OATP1A2) or 6 µmol C^{14}-uric acid (URAT1) in the presence and absence of 100 µmol perfluorinated carboxylate lasted 10 seconds (OAT4), 30 seconds (OATP1A2), and 1 min (URAT1).

PFOA uptake in OATP1A2-transfected HEK293 cells was no different than uptake in control cells. At 100 µmol, E3S uptake was inhibited ~30% by PFOA (C8), ~62% by C9, ~70% by C10, ~42% by C11, and ~18% by C12. E3S uptake was not inhibited by C4–C7. In CHO cells transfected with OAT4, time-dependent uptake experiments showed a saturation phase after an incubation time of approximately 10 seconds. A pH-dependent increase in PFOA uptake was observed with approximately 90% uptake at pH 8 and 250% at pH 5.5.

In concentration-dependent uptake experiments, uptake increased with increasing PFOA concentration (0–1000 µmol) in OAT4-transfected CHO cells at pH 7.4 and 6. PFOA uptake was cis-inhibited by BSP and probenecid and trans-stimulated by PAH and glutarate at pH 7.4. A chain length-dependent effect was observed in E3S inhibition on OAT4-expressing cells in the presence of C7 (30%) through C10 (~80%). Inhibition in the presence of C11 and C12 were ~52% and ~30%, respectively. Inhibition of E3S in the presence of C4, C5, and C6 was less than 20% for each.

PFOA uptake in HEK293 cells transfected with URAT1 was not statistically different from control cells in the presence and absence of Cl^-. Under both conditions, PFOA intake was enhanced especially in the absence of Cl^- in which PFOA uptake was greater than fourfold compared to uptake in control cells. Time-dependent PFOA (5 µmol) uptake by URAT1 increased with time during the 5-min incubation period, and a concentration-dependent increase in PFOA uptake was observed (0–700 µmol). Urate uptake was inhibited in a chain length-dependent manner. Inhibition in the presence of C7–C10 was ~70% each, ~60% in the presence of C6 and C11, ~50% in the presence of C5, ~30% in the presence of C12, and ~25% in the presence of C4. Based on the results, Yang et al. (2010) concluded that PFOA was not a

substrate for OATP1A2, but that OAT4 and URAT1 were probably involved in the renal reabsorption of PFOA.

Weaver et al. (2010) published *in vitro* studies on the transport activities of the rat renal transporters OAT1, OAT2, OAT3, OATP1a1, and URAT1. The transporters were transfected into one of several cell lines and exposed to a series of perfluorinated carboxylates having chain lengths ranging from 2 to 18 carbons (C). The activity of the perfluorinated carboxylate on the transporters was quantified on the basis of its ability to inhibit the transport of a favored radiolabeled substrate. The PFAS inhibition of the individual transporters varied with chain length. The perfluorinated carboxylate with 6, 7, and 8 carbon chains caused a significant decrease in OAT1 transport of tritiated p-aminohippurate, with the C7 acid having the strongest effect. The perfluorinated carboxylates with 5 through 10 carbon chains caused a significant decrease in transport of tritiated E3S by OAT3, with C8 and C9 acids having the strongest effect. The transport of tritiated estadiol-17β-glucuronide by OATP1a1 was significantly inhibited by perfluorinated carboxylates with 6 through 11 carbon chains, with C10 acid having the strongest effect. The perfluorinated carboxylate did not inhibit OAT2 or URAT1 transport of favored substrates.

The kinetic response of the OAT1, OAT3, and OATP1a1 transporters to increasing concentrations of selected perfluorinated carboxylates also was evaluated by Weaver et al. (2010). The change in transport velocity (ng/mg protein/min) with increasing concentrations of the perfluorinated carboxylate exhibited a Michaelis-Menton-type response. The kinetic data were analyzed to determine the K_m and V_{max}, and the results are summarized in Table 2-22 below.

Table 2-22. Kinetic Parameters of Perfluorinated Carboxylate Transport by OAT1, OAT3, and OATP1a1

Transporter	PFAS	K_m (µmol)	Vmax (nmol/mg protein/min)
OAT1	C7	50.5 ± 13.9	2.2 ± 0.2
	C8	43.2 ± 15.5	2.6 ± 0.3
OAT3	C8	65.7 ± 12.1	3.8 ± 0.5
	C9	174.5 ± 32.4	8.7 ± 0.7
OATP1a1	C8	126.4 ± 23.9	9.3 ± 1.4
	C9	20.5 ± 6.8	3.6 ± 0.5
	C10	28.5 ± 5.6	3.8 ± 0.3

Source: Weaver et al. 2010

The Michaelis-Menton kinetic data (K_m and V_{max} [maximum initial rate of an enzyme catalyzed reaction]) indicate that there are substantial differences in the affinity of the perfluorinated carboxylate with 8 and 9 carbon chains for OAT3, with the C8 acid favored over the C9 acid. OAT3 is an export transporter located on the basolateral side of the tubular cells; thus, when present in a mixture consisting of comparable concentrations of both, renal tubular excretion of the C8 acid would tend to decrease excretion of the C9 acid. For OATP1a1, a resorption transporter located on the apical side of the renal tubular cells, the C9 and C10 acid have a greater affinity for the transport protein than the C8 acid. The kinetic data suggest that the net impact of these relationships would be to favor excretion of the C8 acid over the C9 acid and possibly the C10 acid when all three fluorocarbons are present in the exposure matrix at approximately equal concentrations. There were minimal kinetic differences between transport

of the C7 and C8 acids by OAT1, an export transporter on the basolateral surface of the renal tubular cells.

Based on the Hinderliter study (2004), a developmental change in renal transport occurs in female rats between 3 and 5 weeks of age that allows for expedited excretion of PFOA. When the transporters become active, there is a decrease in plasma PFOA levels and an increase in urinary excretion (Table 2-23). The developmental change in male rats appears to have the opposite effect. Sexual maturity appears to influence these events because castrated male rats become more like females and OVX females become more like males in their PFOA excretion capabilities. The change in female rats seems to involve the OATs (Kudo et al. 2002) while the change in males seems to involve the OATPs (Cheng et al. 2006).

Table 2-23. Plasma and Urine PFOA Concentration 24-hr After Treatment with 30 mg/kg PFOA

Age (weeks)	Female		Male	
	Plasma (µg/ml)	Urine (µg/ml)	Plasma (µg/ml)	Urine (µg/ml)
3	51.43 ± 13.61	94.89 ± 26.26	74.16 ± 18.23	51.76 ± 28.86
4	28.01 ± 9.90	104.12 ± 28.97	100.81 ± 13.18	28.70 ± 18.84
5	3.42 ± 1.95	123.16 ± 51.56	113.86 ± 23.36	15.65 ± 6.24

Source: Hinderliter 2004

When considered together, the studies of the transporters suggest that female rats are efficient in transporting PFOA across the basolateral and apical membranes of the proximal kidney tubules into the glomerular filtrate, but male rats are not. Males, on the other hand, have a higher rate of resorption than females for the smaller amount they can transport into the glomerular filtrate via OATP1a1 in the apical membrane. This scenario might explain the inverse relationship between the levels of PFOA in female urine and plasma and the plateau of plasma PFOA in male rats compared to their losses via urine.

Unfortunately, much work remains to be done to explain the gender differences between male and female rats and to determine whether it is relevant to humans. Similarities are possible because the long half-life in humans suggests that they might be more like the male rat than the female rat. There is a broad range of half-lives in human epidemiology studies suggesting a variability in the unbound fraction of PFOA in serum or in human transport capabilities resulting from genetic variations in structures and consequently in function. Genetic variations in human OATs and OATPs are described in a review by Zaïr et al. (2008).

2.6 Toxicokinetic Considerations

2.6.1 PK Models

One of the earliest PK models was done using the post-dosing plasma data from the Butenhoff et al. study (2004b) in cynomolgus monkeys (Andersen et al. 2006). In this study, groups of six monkeys (three per gender per group) were dosed for 26 weeks with 0, 3, 10, and 20 mg/kg PFOA (high-dose =30 mg/kg PFOA for the first 12 days), followed for >160 days after dosing. Metabolism cages were used for overnight urine collection. Since urine specimens could account for only overnight PFOA excretion, the total volume and total PFOA were extrapolated to 24-hour values based on the excretion rate (volume/hour) for the volume collected and the hours of collection.

The Andersen et al. model (2006) was based on the hypothesis that saturable resorption capacity in the kidney would possibly account for the unique half-life properties of PFOA across species and genders. The model structure, shown in Figure 2-3, was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells (Andersen et al. 2006).

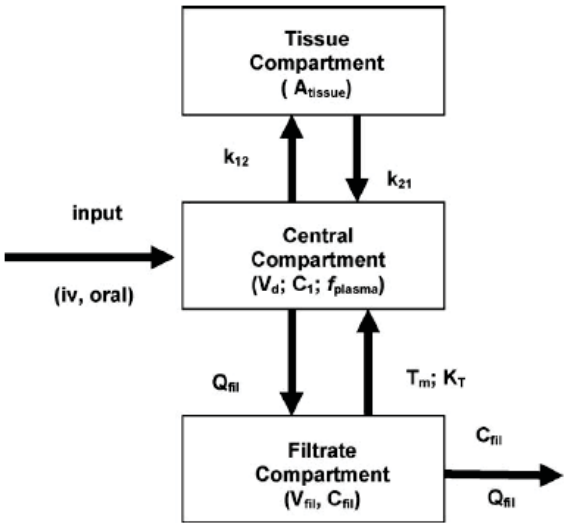


Figure 2-3. Schematic for a Physiologically Motivated Renal Resorptions PK Model

The renal-resorption model includes a central compartment that receives the chemical from the oral dose and a filtrate compartment for the glomerular filtrate from which resorption and transfer to the central compartment can occur. Transfer from the filtrate compartment to the central compartment decreases the rate of excretion. The resorption in the model was saturable, meaning that there was less resorption and greater excretion at high serum PFOA concentrations than at low concentrations.

The model was parameterized using the body weight and urine output of cynomolgus monkeys (Butenhoff et al. 2004b) and a cardiac output of 15 L/h-kg from the literature (Corley et al. 1990). A 20% blood flow rate to the kidney was assumed based on data from humans and dogs. Other parameters were optimized to fit the data for plasma and urine at lower concentrations and then applied for the 20 mg/kg/day dose, which was assumed to represent a concentration at which renal resorption was saturated. Based on the data for the dose of 20 mg/kg/day, the model was able to predict the decline in plasma levels after the cessation of dosing. The predictions were fairly adequate for one of the three modeled monkeys; for the other two monkeys, the model predicted higher levels than were observed. That result could have occurred because the model did not allow for efflux of PFOA into the glomerular filtrate through transporters on the basolateral surface of the tubular cells. The authors observed that three monkeys had faster CL_R of PFOA than the other three monkeys.

Tan et al. (2008) divided the second compartment in the Andersen et al. model (2006) into a liver compartment and a tissue compartment. A storage compartment was added between the filtrate compartment and urinary excretion (Figure 2-4) (Tan et al. 2008).

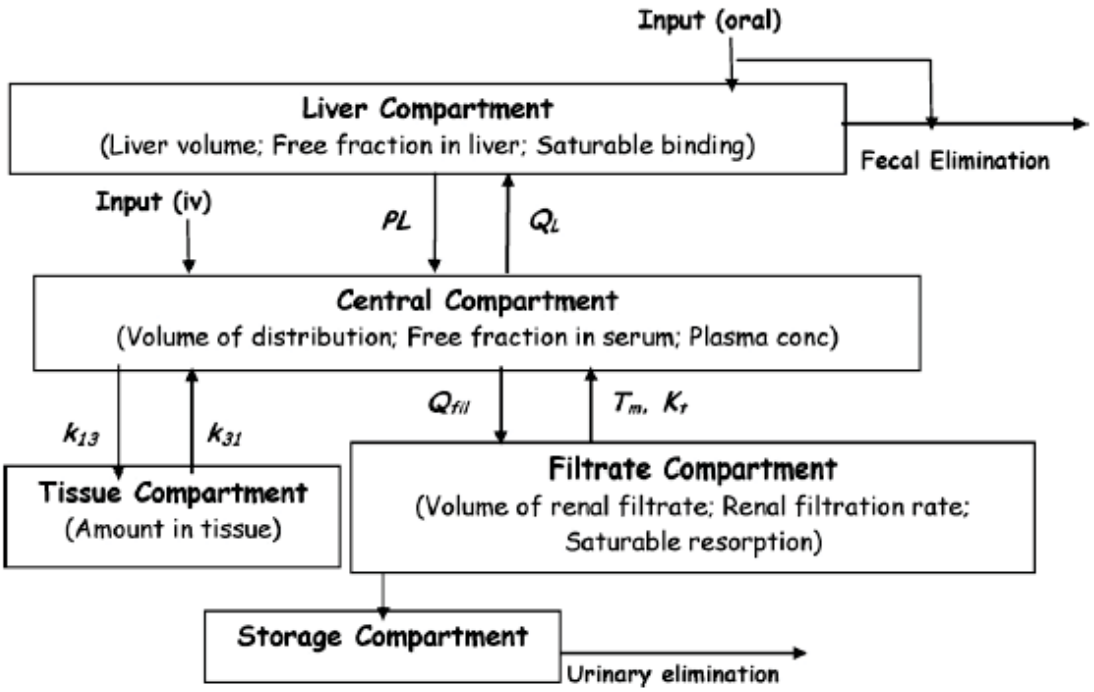


Figure 2-4. Physiologically Motivated Pharmacokinetic Model Schematic for PFOA-Exposed Rats

The models were parameterized and applied to the Kemper data (2003) for male and female CD rats given doses of 1, 5, and 25 mg/kg/day. The model did not provide a satisfactory fit between the predictions of plasma concentration or urine + fecal excretion and experimental data for either gender.

Lou et al. (2009) used the data they collected on the serum, liver, and kidney PFOA concentration (see section 2.2.1) in CD-1 mice to examine if one- or two-compartment PK models would fit the experimental data for 1, 10, and 60 mg/kg/day single gavage doses (see Figure 2-5 for the one-compartment model). Both models assumed first order absorption and elimination. The two-compartment model included a central compartment that received PFOA after absorption and transferred it to a second compartment for excretion. The excretion compartment was coupled with bidirectional flow between the two compartments. The net loss from the central compartment differed during and after distribution. The models were fit using a general nonlinear least squares approach. A likelihood ratio squared approach was applied to determine which model achieved the best fit to the data.

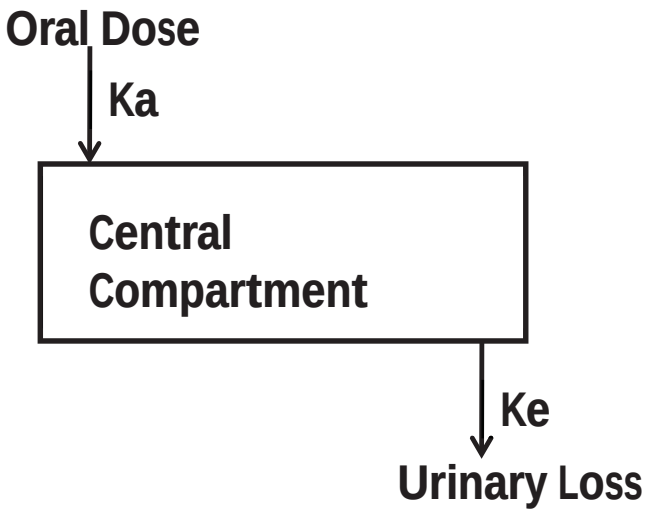


Figure 2-5. Schematic for One-Compartment Model.

The one compartment model performed well for serum, liver, and kidney in this analysis, and output was not significantly improved with use of a two-compartment model. The input parameters for the one-compartment model included V_d, serum half-life, and absorption rate constant (K_a) and elimination rate constant (K_e) for serum, liver, and kidney. There were slight differences in the fitted values between males and females for some parameters. The K_e values were consistently higher in the female mice (Table 2-24). The quantitative measures for liver and kidney were only available for the 1- and 10-mg/kg/day doses.

Table 2-24. Model Parameters for 1 and 10 mg/kg Single Doses of PFOA to CD1 Mice

Tissue	Parameter (abbreviation)	Females	Males
Serum	Volume of distribution (V_d)	0.135 L/kg	0.266 L/kg
	Absorption rate constant (K_a)	0.537 L/hr	
	Elimination rate constant (K_e)	0.00185 L/hr	0.00133 L/hr
	Half-life ($T_{½}$)	15.6 days	21.7 days
Liver	Volume of distribution (V_d)	0.161 L/kg	0.120 L/kg
	Absorption rate constant (K_a)	0.5170 L/hr	
	Elimination rate constant (K_e)	0.00161 L/hr	0.00129 L/hr
Kidney	Volume of distribution (V_d)—1 mg/kg	0.822 L/kg	1.280 L/kg
	Volume of distribution (V_d)—10 mg/kg	1.092 L/kg	1.170 L/kg
	Absorption rate constant (K_a)	0.527 L/hr	
	Elimination rate constant (K_e)	0.00151 L/hr	0.00113 L/hr

Source: Lou et al. 2009

The one-compartment model described above was not able to predict serum concentration in female mice given a single 60-mg/kg dose, suggesting a change in kinetics with the 60-mg/kg dose compared to the 1- and 10-mg/kg doses. This conclusion is supported by comparison of the serum measurements made during the 30-day post-dosing period for all three doses. The serum PFOA concentration at the 60-mg/kg dose declined more rapidly with time than serum PFOA concentrations at the 1- and 10-mg/kg doses. For example, a serum concentration of about 0.4 mg/L was reached in about 28 days at the 60-mg/kg dose, 61 days at the 10-mg/kg dose, and 70 days at the 1-mg/kg dose (values estimated from Figure 3, Lou et al. 2009). The one-

compartment model also produced a poor fit for the serum level measurements taken 24 hours after the cessation of a 17-day exposure to 20 mg/kg/day. The two-compartment model provided a better fit with experimental serum concentration data for the single 60-mg/kg dose and the repeat 20-mg/kg/day dose, but the fit was still unsatisfactory.

Lou et al. (2009) also tried the Andersen et al. renal-resorption model (2006) to determine if it provided an improved fit for the data. The Andersen et al. model (2006) fit to the data was superior to that of the one- and two-compartment models of Lou et al. (2009) for the 60-mg/kg single-dose and the 20-mg/kg/day repeat-dose scenarios.

The Andersen et al. model (2006) includes a second tissue compartment that articulates with the central compartment but not the filtrate compartment. In addition to values for V_d, K_a, and K_e, the model includes values for cardiac output, volume for the renal filtrate, renal blood filtration rate, intercompartmental CL, transport maximum, transport affinity constant (K_t), and the proportion of free PFOA in serum. With the exception of body weight and cardiac output, the input parameters for the model were either assumed (i.e., volume of renal filtrate and proportion of free serum PFOA) or optimized for the model. The wide confidence bounds around the optimized values are indicative of considerable parameter uncertainty.

The Lou et al. parameter estimates (2009) indicate that there may be several biological limitations to the Andersen et al. (2006) PK model for adult mice including the fact that it requires an unreasonably high portion of the cardiac output to pass through the kidneys to optimize fit to the experimental data. It also does not include excretion via export transporters in the renal tubular cells or consider that the bound fraction in the serum could vary with the magnitude of the dose and duration of dosing. Much of the emerging data is consistent with a variety of tubular transporters functioning in both efflux and resorption from the glomerular filtrate. In addition, there are opportunities for protein binding within organs that could function to retard distribution to the cytosol, especially at low doses. The binding of PFOA with L-FABP is an example. Once binding sites are saturated, the concentration in the cytosol will increase.

A model also has been developed that applied to female CD-1 mice during gestation and lactation (Rodriguez et al. 2009). The gestational model includes two compartments, one for the dam and the other for the litter. They are linked by placental blood flow. The biological data used to set the parameters for the two compartments were based on the data from the Lau et al. (2006) and Abbott et al. (2007) studies in CD-1 and 129S1/SvlmJ mice, respectively. Exposure was assumed to be limited by blood flow, and only the experimental doses that did not impact litter size (i.e., 0.1–1.0 mg/kg/day for CD-1 mice and 1–10 mg/kg/day for 129S1/SvlmJ mice) were used in model development.

Lactational exposure was modeled as a dynamic relationship between the dam (n = 10) and the litter, and they were connected by a milk compartment. Milk yield information was obtained from the literature. Milk was assumed to be consumed as it was produced without any circadian impact on consumption patterns. PFOA excreted in pup urine was routed back to the dam.

Both absorption and excretion were assumed to be first order processes as was lactation transfer from the dam to the litter (Figure 2-6) (Rodriguez et al. 2009). Resorption of a portion of the PFOA urinary efflux was included in the model. The renal excretion/resorption was parameterized for cardiac output, kidney blood flow, GFR, urine flow rate, volume of renal plasma (fraction of body weight), and volume of renal filtrate (fraction of body weight). The fraction of free PFOA in serum reaching the glomerulus was assumed to be 0.01 based on

protein binding information. As was the case with the Lou et al. model (2009), Rodriguez et al. (2009) did not include parameters to adjust for transporter-mediated efflux from the renal tubular cells into the glomerular filtrate.

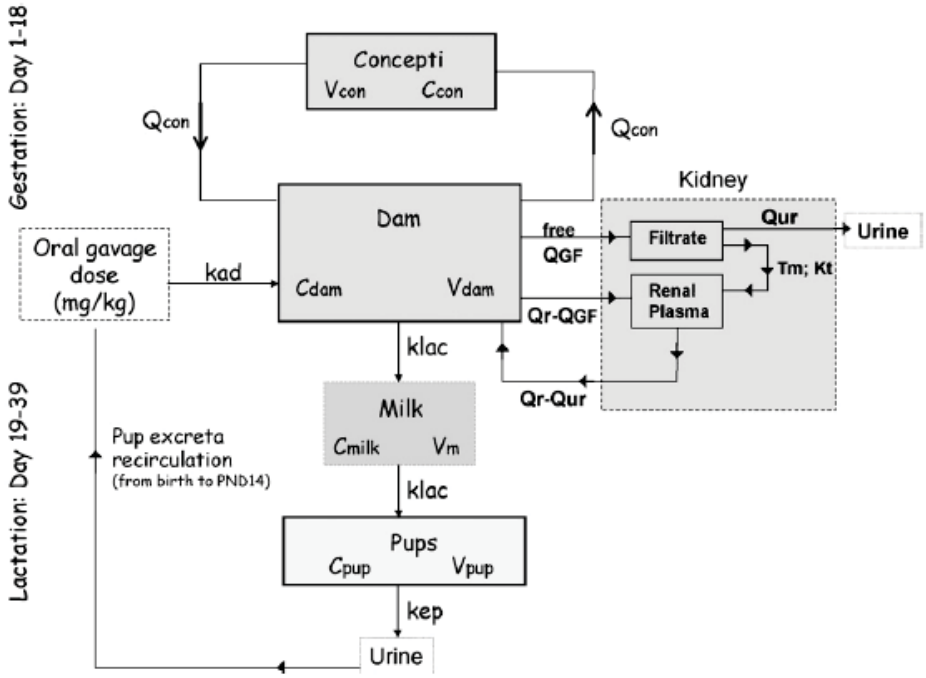


Figure 2-6. PK Model of Gestation and Lactation in Mice

One of the limitations of the Rodriguez et al. modeling effort (2009) was the limited amount of laboratory data against which to evaluate projections. Serum measures from the Lau et al. (2006) and Abbott et al. (2007) studies were available for only a few time points. Nevertheless, the authors reached several conclusions based on the model projections as follows:

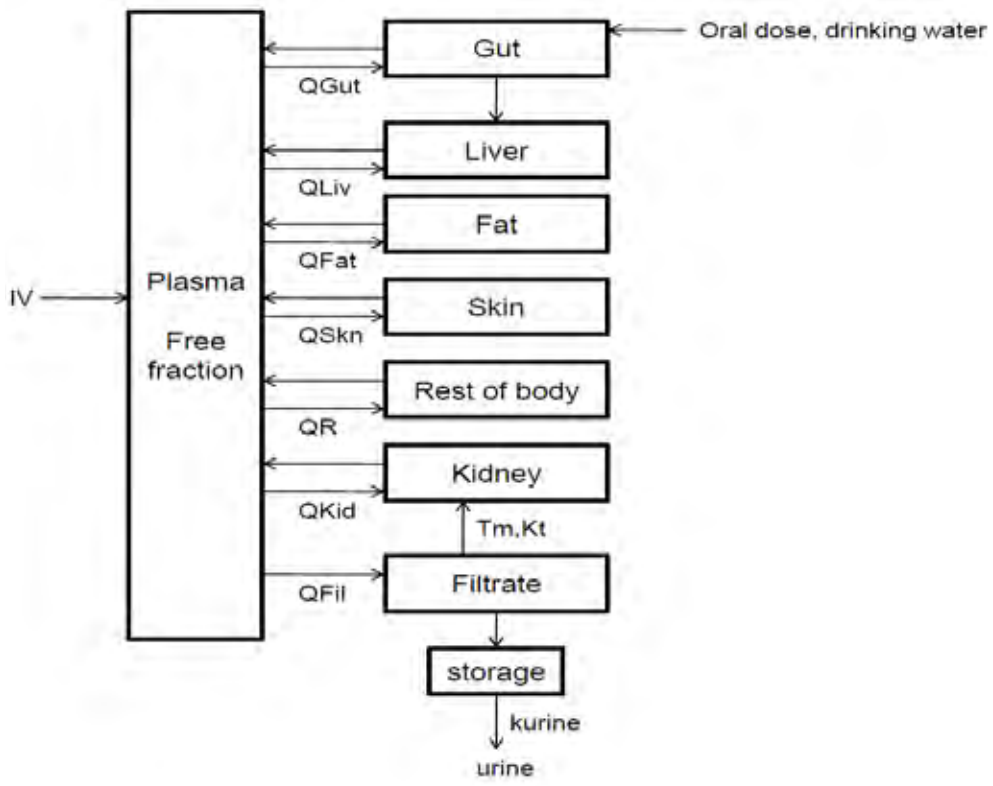
- The model had a tendency to overestimate serum levels, suggesting nonlinearity as doses increased.
- Gestation and lactation as a source of exposure contributed about equally to the pups of 129S1/SvlmJ dams exposed only during gestation.
- The contributions to the pups from gestation exceeded those from lactation in the CD-1 mice.
- Exposure to the pups via lactation increased over time.
- Lactation is a CL pathway for the dam.

A number of uncertainties accompany the model because of the assumptions regarding the flow limitation on transport to the fetus and to maternal milk: the first order K_e for the pups and, for the milk, the maternal serum partition coefficient and the limited knowledge regarding the renal tubular transporters. They caution that the model should not be applied for cross-species or high-to-low dose extrapolation.

Loccisano et al. (2011) developed a PFOA PBPK model for monkeys based on the Andersen et al (2006) and Tan et al. (2008) models, and then extrapolated it for use in humans (Figure 2-7). The model reflects saturable renal absorption of urinary PFOA by the proximal tubule of

the kidney. This is represented in Figure 2-7 by the interactions between the plasma and kidney plus the interaction of the filtrate compartment with both plasma and kidney.

The fraction of PFOA free in plasma and available for glomerular filtration was based on data fit and estimated to be less than 10% because of binding to serum proteins, especially albumin. Lacking the kinetic data on tubular resorption, the rate was based on the best fit to the plasma/urine data. A storage compartment was added to the model between filtrate and urine. Tissue plasma partition coefficients were derived from the data by Kudo et al. (2007) following the disposition pattern of a single intravenous (IV) dose to male Wistar rats.



Notes:
T_m = transporter maximum, K_t = affinity constant, and Q = flow in and out of tissues.

Figure 2-7. Structure of the PFOA PBPK Model in Monkeys and Humans

Existing IV and oral data sets from Butenhoff et al. (2004b) for the cynomolgus monkey were used to develop the monkey model. In the oral study (section 2.2.1), animals were dosed for 6 months and followed for 90 days after dosing. Plasma and urine samples were analyzed periodically during dosing and recovery. The model projections for the oral study were in good agreement with the Butenhoff et al. data (2004b) for the 10-mg/kg dose, showing a rapid rise to plasma steady state and a slow terminal half-life. The model performance for the high dose (30/20 mg/kg/day) did not fit as well, partially as a consequence of the observed toxicity with the initial 30 mg/kg/day dose that necessitated cessation of dosing on study day 12, followed by resumption of dosing at 20 mg/kg/day on study day 22.

The structure of the human model was similar to that used for the monkeys. Human serum data (means with standard deviations [SDs] or medians) for PFOA are available for occupational and general populations (Bartell et al. 2010; Calafat et al. 2007a, 2007b; Emmett et al. 2006;

Hölzer et al. 2008; Olsen et al. 2005; Steenland et al. 2009). The fact that the serum data were the results from measurements made following uncertain routes and uncertain exposure durations presented a challenge in the assessment of model fit. The human half-lives used for the model (3.8 and 2.3 years) came from an occupational study (Olsen et al. 2005) and a study of the Little Hocking, Ohio, population after reduction of the PFOA in drinking water as a result of treatment (Bartell et al. 2010). See section 2.6.2. Both half-life values were used in evaluating the model's ability to predict serum concentration at the time the serum samples were collected.

The model produced results that can be characterized as fair to good when compared to the reported average serum measurements. For the Little Hocking population studied by Emmett et al. (2006), the model indicated the need for a 30-year exposure to reach steady-state concentrations. The model indicated that both half-life values provided reasonable results when compared to the measured serum values. The authors concluded that more data are needed on the kinetics of renal transporters and intrahuman variability, plus more definitive information on exposures to further refine the human model.

Fàbrega et al. (2014) adapted the Loccisano et al. model (2011) to include compartments for the brain and lung, and to remove the skin. They applied the adjusted model to humans by using intake and body burden data from residents in Tarragona County, Spain. Food and drinking water were the major sources of exposure. Body burden information came from blood samples from 48 residents; tissue burdens came from 99 samples of autopsy tissues. The adjusted model overpredicted PFOA serum levels by a factor of about 9, the liver by a factor of 4.5, and the kidney by a factor of about 18. Model predictions for PFOS were far more consistent with the tissue concentration experimental data.

The authors also looked at the impact of using data for partition coefficients from human tissues in place of the Loccisano et al. rat data (2011) for the estimation of steady-state tissue concentrations. The PFOA simulation values were closer to the human experimental data when using the human partition coefficient values for the brain and lung, but not for the liver. In the case of the kidney, the simulated projections were generally equivalent with both the human and rat partition coefficients. The authors suggested that both saturable resorption and variations in protein binding are important parameters for PK models. With the exception of serum albumin, the existing models have not considered protein-binding constants within tissues. Even though the use of human partition coefficients improved the steady-state predictions for tissues, overall there were still considerable differences between the experimental values and the predictions with both models.

Loccisano et al. (2012a) also used the saturable resorption hypothesis when developing a model for adult Sprague-Dawley rats (Figure 2-8). The structure of the model is similar to that for the monkey/human model depicted in Figure 2-7, but lacks the fat and skin compartments and includes a storage compartment to accommodate fecal loss of unabsorbed dietary PFOA as well as loss from biliary secretions. Oral and IV data used in model development came from studies by Kemper (2003), Kudo et al. (2007) and Perkins et al. (2004). Partition coefficients for liver:plasma, kidney:plasma, and rest of the body:plasma were derived from unpublished data on mice by DePierre (2009) through personal communication with the authors (Loccisano et al. 2012a). Most of the other kinetic parameters were based on values providing the best fit to the experimental data. Because a number of the renal transporters involved with PFOA resorption are known, available kinetic information was used where appropriate. Model performance was evaluated primarily based on its ability to predict plasma and liver concentrations from the

studies identified above. Performance was generally good given the limitations in the primary data sources, as was the case for the monkey model.

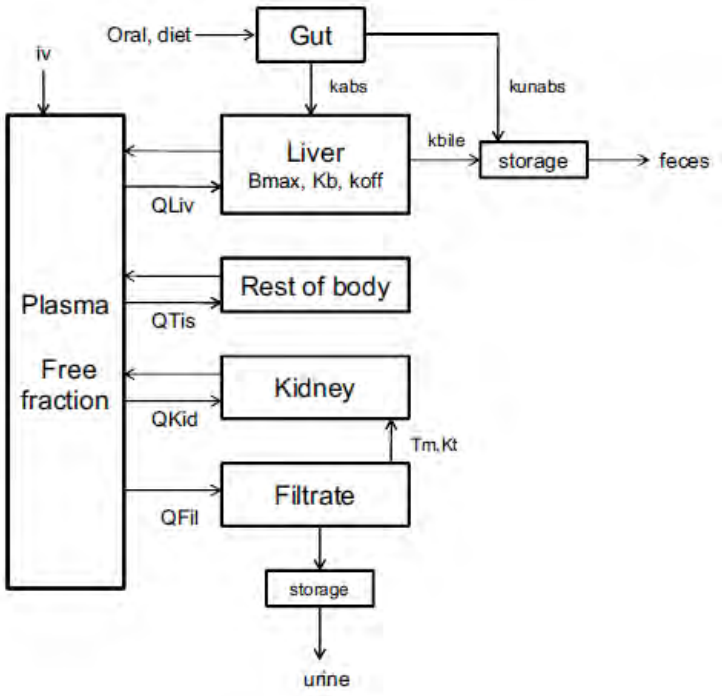


Figure 2-8. Structure of the PBPK Model for PFOA in the Adult Sprague-Dawley Rat

Loccisano et al. (2012b) expanded the adult Sprague-Dawley rat model to cover gestational and lactational exposure to the fetus and pups through their dams. The data from Hinderliter et al. (2005) were used in model development for both the gestation and lactation periods. The gestational model structure for the dams is similar to the model structure shown in Figure 2-8. The model was expanded to include the fetuses linked to the dams by way of the placenta. Uptake from the placenta was described by simple diffusion; the fetal plasma compartment was separate from the dams as was distribution to fetal tissues and amniotic fluid. Based on the transporter data for PFOA, elimination differed for male and female rats and was considered to be developmentally regulated, resulting in faster elimination for female rats than for male rats after sexual maturation. The lactation model linked the pups to their dams through mammary gland secretions. Pup compartments included the gut, liver, kidney, renal filtrate, plasma, and rest of the body.

Model performance was judged by its ability to predict concentrations in maternal and fetal plasma, amniotic fluid, and milk. The predictive capability of the model ranged from fair to good, depending on the medium. The fit of the projections to the data was weakest for the whole embryo during gestation, for which measured levels were greater than projection for two of three data points and for neonate plasma during lactation, for which all data points fell below the predictions.

Loccisano et al. (2013) extended their model development to cover humans during pregnancy and lactation, building on the work done with rodents and recognizing the limitations of the human data available for evaluating the model predictions. Figure 2-9 illustrates the structure of

the model used. The basic structure was derived from the rat model discussed above. Following are some of the key features of the model:

- The fetus is exposed via the placenta through simple bidirectional diffusion.
- Transfer rates to the fetus from the amniotic fluid are governed by bidirectional diffusion.
- Transfer from the fetal plasma to tissues is flow-limited.
- Maternal plasma is directly linked to the milk compartment and considered to be flow-limited; only the free fraction in plasma is transferred to maternal milk.
- The neonate is exposed to PFOA only via maternal milk for the first 6 months postpartum.
- The infant in the model is treated as a single compartment with a V_d.

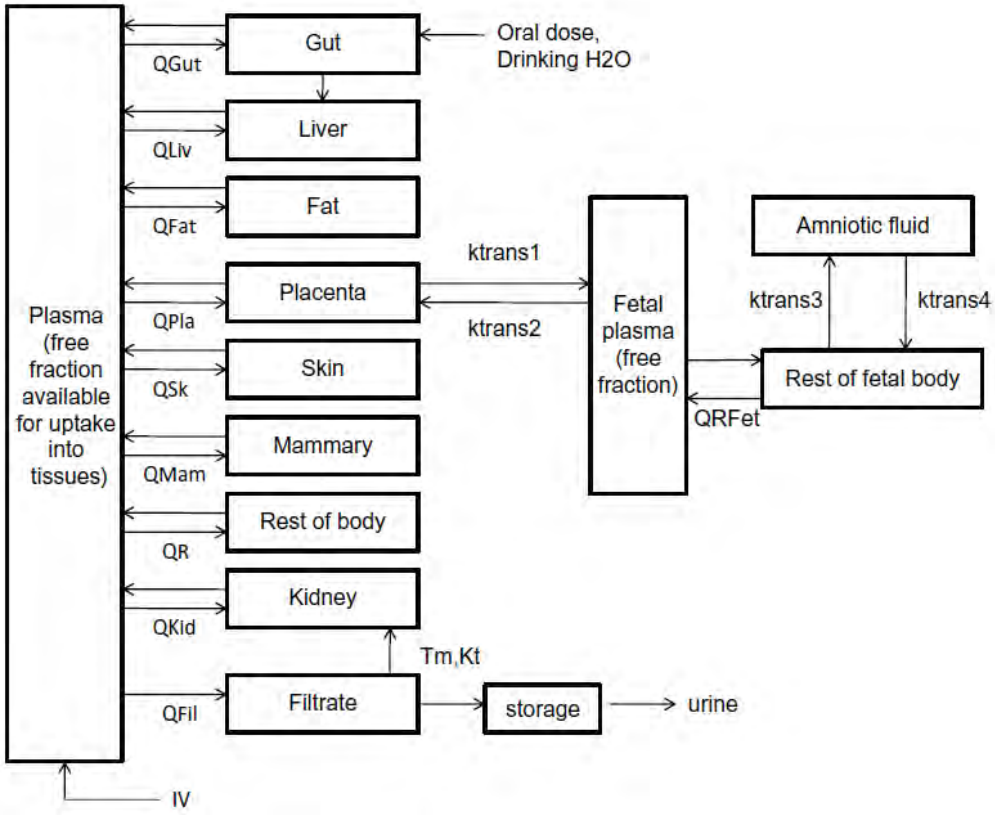


Figure 2-9. PBPK Model Structure for Simulating PFOA and PFOS Exposure During Pregnancy in Humans (Maternal, Left; Fetal, Right)

Limitations to the model are acknowledged and attributed primarily to lack of data to support a more mechanistic approach. Physiological parameters applicable to a pregnant or lactating woman, the fetus, and the nursing infant were obtained from a variety of referenced publications.

To obtain a plasma value at the time of conception, the model was run until it reached a prepregnancy steady-state concentration. The model predicted 30 years as the exposure necessary to reach steady state for the general female population (1 E-4 to 2 E-3 µg/kg body weight [bw] /day). The model performance simulations for PFOA were run using an exposure of 1.5×10^{-4} µg/kg bw/day. Projections were developed for maternal plasma, fetal plasma, infant plasma, and maternal milk. Agreement between the observed concentrations (µg/L) and the predicted values was considered satisfactory if the predicted value was within 1% of the

observed value. Model output was compared to maternal and fetal plasma values at delivery or at specific time points, and for the infant plasma and milk data where available. Predicted maternal:fetal plasma (cord blood) concentration ratios were more consistent for PFOA than for PFOS when compared to the published data. The projections for fetal internal dose were reasonable, and there was good agreement between the model and the available human lactation data. The modeled maternal plasma was 2.4 μg/L at the time of conception; it slowly decreased across the gestation period and increased slightly at delivery. For the most part, the modeled results fell within ± 1 SD of the observed data.

During lactation, there was a decline in maternal plasma across the 6 months of lactation (a change of 1μg/L). Thereafter, plasma values slowly increased and stabilized at about 1.5 μg/L at 6 months postpartum. The fetal plasma concentration was about 2.3 μg/L at the start of gestation and declined to about 1.8 μg/L at the time of delivery. Maternal plasma values are about the same as those for the fetus. During the lactation period, the infant plasma increased in a linear fashion to a terminal value of about 5 μg/L. Milk concentrations declined very slightly across the lactation period with an initial concentration of 0.07 μg/L and a final value of 0.05 μg/L. These concentrations were estimated from the graphic data presentation. Breast milk appears to be an important excretory route for the dam.

The projections for PFOA differed from those for PFOS in several respects. Most importantly, maternal and fetal plasma values were similar for PFOA but for PFOS, maternal levels were approximately twofold higher than fetal levels. Compared with PFOS, there was a much greater decline in maternal PFOA plasma values during lactation accompanied by a comparable decline in the PFOA concentration in milk. The increase in infant plasma across the lactation period was comparable for PFOA and PFOS, with the concentration at 6 months postpartum about 2.5 times higher than at 1 month.

Loccisano et al. (2011, 2012a, 2012b, 2013) determined that the human pregnancy lactation model results, when compared to published data, identified the following important research needs:

- Are there differences in the transporter preferences and transfer rates for the individual PFASs? Do those differences correlate with half-life differences?
- Are there qualitative or quantitative differences between the transporters favored by PFOA and those favored by PFOS?
- What physiological factors influence CL for the mother, the fetus, and the infant during gestation and lactation?
- Are placental transport processes active, facilitated, or passive?

These research needs are more pronounced for PFOS than PFOA because the information supporting renal resorption and tissue uptake via membrane transporters for PFOS is very limited. Most models infer that PFOS and PFOA are similar based on their half-lives rather than on published research on PFOS transporter kinetics.

Building on the work of other researchers, Wambaugh et al. (2013) developed and published a PK model to support the development of an EPA RfD for PFOA. The model was applied to data from studies conducted in monkeys, rats, and mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption PK model was used. This concept has played a fundamental role in the design of all of the published PFOA models summarized in this section. In this case, an oral dosing version of the

original model introduced by Andersen et al. (2006) and summarized early in section 2.6.1 was selected for having the fewest number of parameters that would need to be estimated. A unique feature of the Wambaugh et al. approach was to use a single model for all species in the toxicological studies to examine the consistency in the average serum values associated with effects and with no effects from nine animal studies of PFOA. The model structure is depicted in Figure 2-3, with minor modifications.

Wambaugh et al. (2013) placed bounds on the estimated values for some parameters of the Andersen et al. model (2006) to support the assumption that serum carries a significant portion of the total PFOA body load. The Andersen et al. model is a modified two-compartment model in which a primary compartment describes the serum and a secondary deep tissue compartment acts as a specified tissue reservoir. Wambaugh et al. (2013) constrained the total V_d to a value of not more than 100 times that in the serum. As a result, the ratio of the two volumes (serum versus total) was estimated in place of establishing a rate of transfer from the tissue to serum.

A nonhierarchical model for parameter values was assumed. Under this assumption, a single numeric value represents all individuals of the same species, gender, and strain. The gender assumption was applied to monkeys and mice while male and female rats were treated separately because of the established gender-related toxicokinetic differences. Body weight, number of doses, and magnitude of the doses were the only parameters to vary. In place of external doses, serum concentrations as measured at the time of euthanasia were used as the metric for dose magnitude. Measurement errors were assumed to be log-normally distributed. Table 2-25 provides the estimated and assumed PK parameters applied in the Wambaugh et al. model (2013) for each of the species evaluated.

The PK data that supported the analysis were derived from two PFOA PK *in vivo* studies. The monkey PK data were derived from Butenhoff et al. (2004b), and the data for the rats (M/F) were from Kemper (2003). Two strains of female mice were analyzed separately, with CD1 information derived from Lou et al. (2009) and C57Bl/6 information derived from DeWitt et al. (2008). The data were analyzed within a Bayesian framework using Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and to identify serum levels associated with the NOAEL and LOAEL external doses. The model chose vague, bounded prior distributions on the parameters being estimated, allowing them to be significantly informed by the data. The values were assumed to be log-normally distributed, constraining each parameter to a positive value.

The model predictions were evaluated by comparing each predicted final serum concentration to the serum value in the supporting animal studies. The predictions were generally similar to the experimental values. There were no systematic differences between the experimental data and the model predictions across species, strain, or gender, and median model outputs uniformly appeared to be biologically plausible despite the uncertainty reflected in some of the 95^{th} percentile credible intervals. The application of the model outputs in deriving a human RfD is the focus of section 4.0 of this document.

Table 2-25. Pharmacokinetic Parameters from Wambaugh et al. (2013) Meta-Analysis of Literature Data

Parameter	Units	CD1 Mouse (f)[a]	C57Bl/6 Mouse (f)[a]	Sprague-Dawley Rat (f)[a]	Sprague-Dawley Rat (m)[a]	Cynomolgus Monkey (m/f)[a]
bw[b]	kg	0.02	0.02	0.20 (0.16 – 0.23)	0.24 (0.21 –0.28)	7 (m), 4.5 (f)
Cardiac Output[c]	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
k_a	L/h	290 (0.6 – 73,000)	340 (0.53 – 69,000)	1.7 (1.1 – 3.1)	1.1 (0.83 – 1.3)	230 (0.27 – 73,000)
V_{cc}	L/kg	0.18 (0.16 – 2.0)	0.17 (0.13 – 2.3)	0.14 (0.11 – 0.17)	0.15 (0.13 – 0.16)	0.4 (0.29 – 0.55)
k_{12}	L/h	0.012 (3.1 x e^{-10} – 38,000)	0.35 (0.058 – 52)	0.098 (0.039 – 0.27)	0.028 (0.0096 – 0.08)	0.0011(2.4 x e^{-10} – 35,000)
$R_{V2:V1}$	Unitless	1.07 (0.26 – 5.84)	53 (11 – 97)	9.2 (3.4 – 28)	8.4 (3.1 – 23)	0.98 (0.25 – 3.8)
T_{maxc}	µmol/h	4.91 (1.75 – 2.96)	2.7 (0.95 – 22)	1.1 (0.25 – 9.6)	190 (5.5 – 50,000)	3.9 (0.65 – 9,700)
K_T	µmol	0.037 (0.0057 – 0.17)	0.12 (0.033 – 0.24)	1.1 (0.27 – 4.5)	0.092 (3.4 x e^{-4} – 1.6)	0.043 (4.3 x e^{-5} – 0.29)
Free	Unitless	0.011 (0.0026 – 0.051)	0.034 (0.014 – 0.17)	0.086 (0.031 – 0.23)	0.08 (0.03 – 0.22)	0.01 (0.0026 – 0.038)
Q_{filc}	Unitless	0.077 (0.015 – 0.58)	0.017 (0.01 – 0.081)	0.039 (0.014 – 0.13)	0.22 (0.011 – 58)	0.15 (0.02 – 24)
V_{filc}	L/kg	0.00097 (3.34 x e^{-9} – 7.21)	7.6 x e^{-5} (2.7 x e^{-10} – 6.4)	2.6 x e^{-5} (2.9 x e^{-10} – 28)	0.0082 (1.3 x e^{-8} – 7.6)	0.0021 (3.3 x e^{-9} – 6.9)

Notes:
Means and 95% confidence interval (in parentheses) from Bayesian analysis are reported. For some parameters, the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).
m = male, f = female
[a] Data sets modeled for the CD1 mouse were from Lou et al. (2009), for the C57Bl/6 mouse were from DeWitt et al. (2008), for the rat were from Kemper (2003), and for the monkey from Butenhoff et al. (2004b).
[b] Estimated average body weight for species used except with Kemper study (2003) where individual rat weights were available and assumed to be constant.
[c] Cardiac outputs obtained from Davies and Morris (1993).

2.6.2 Half-Life Data

Human. There have been several studies of half-lives in humans and all support a long residence time for serum PFOA with estimates measured in years rather than months or weeks. Bartell et al. (2010) determined an average half-life of 2.3 years based on a study of the decreases in human serum levels after treatment of drinking water for PFOA removal was instituted by the Lubeck Public Services District in Washington, West Virginia, and the Little Hocking Water Association (LHWA) in Ohio. Source waters for these systems had become contaminated with PFAS from the DuPont Works Plant in Washington, West Virginia, between 1951 and 2000.

The Bartell et al. study (2010) was based on a series of serum measurements (eight over 4 years) from 200 individuals who agreed to participate in the study. Inclusion criteria for the participants included: serum PFOA concentrations ≥ 50 ng/mL, residential water service provided by one of the two treatment plants, never employed at the DuPont plant, not growing their own vegetables, and signed acceptance of the study consent form. The participants were almost equally divided between males and females with an average age of about 50 years (range of 18–89 years). Most of the participants consumed public tap water (172) as their primary source, but a small number (28) consumed bottled water as their source.

The participants were required to report that they primarily used home tap water for cooking, bathing, and showering for the years between 2005 and 2007. The tap water users had to report public water as their primary source of residential water consumption, and bottled water users had to report the use of bottled water as their primary source of residential water consumption. The initial blood draw for serum occurred in June 2007, with subsequent samples at 1, 2, 3, 6, and 12 months after the initial sample. Samples were analyzed by the Centers for Disease Control and Prevention. Nineteen samples from the 2-month blood draw were not analyzed due to mislabeling.

A linear mixed model was used to determine the decline in serum PFOA concentration over time. With these models, the decline from baseline by the participants was essentially first order. The serum PFOA concentration was the only time-varying measurement entered into the model. Serum concentrations were log-normally distributed, as described by the following equation:

$$\ln C = \ln C0 - k t$$

where:

C= serum concentration at time *t*
C0 = baseline serum concentration
k = elimination rate constant
t = time point for the measurement

The results of this assessment showed a 26% decrease in PFOA concentration per year after adjustment for covariates and a half-life of 2.3 years [confidence interval (CI) = 2.1-2.4]. The covariates considered included the water treatment system, the time exposed before and after filtration, public versus bottled water, gender, age, consumption of local or homegrown vegetables, and exposure to the public water supply at work. The only potential confounders determined to be significant were the treatment plant ($p = 0.03$) and homegrown vegetable consumption ($p<0.001$).

Identification of consumption of homegrown vegetables as a significant confounder revealed a weakness in the study design because it had been an exclusion factor, yet was identified as an exposure source at the 12-month interview of the study participants. The researchers concluded that this problem was a result of the way the exclusion question was phrased for the original interview, “Do you grow your own vegetables?” When the question was asked later in the study, it was rephrased, “Do you eat any fruits and vegetables grown at your own home?” Some people who answered “no” to the original questions answered “yes” to the second question.

Changes in the source of drinking water during the study could also have impacted the results. When baseline interview data were compared with the results from the 12-month interview, 39% of the bottled water group reported using public water at home. Some of the public water drinkers (10%) reported using primarily bottled water at the 6-month interview.

In another study, the drinking water supply was contaminated with a mixture of perfluorinated chemicals when a soil-improver mixed with industrial waste was applied upriver to agricultural lands in Arnsberg, Germany (Brede et al. 2010). The PFOA levels in the finished drinking water were measured as 500–640 ng/L in 2006. PFOS and PFHxS also were present. The plasma PFOA levels in the Arnsberg population were 4.5 to 8.3 times higher than those in a reference community at the time the problem was discovered. Charcoal filtration was added to the potable treatment train and succeeded in reducing concentrations in the drinking water.

The authors used the differences in plasma 2008 PFOA measurements from a subset of the participants (children and adults) initially exposed in 2006 to determine the PFOA half-life. The 2008 subjects included 66 males, females, and children from Arnsberg and 73 from the reference community in the evaluation. The drinking water concentration monitoring results (nondetects estimated as one-half of the limit of detection [LOD], 10 ng/L) and DWI estimates obtained by questionnaire and interview were used to estimate PFOA exposures. Plasma PFOA samples were collected during a 2-month period in late 2008. Plasma PFOA had declined in the serum for both the Arnsberg residents (39.2%) and those from the reference community (13.4%). In Arnsberg, the decrease was greater for the exposed females and children than for the males when compared to the reference community, an observation that appeared to reflect the reported lower DWIs of the Arnsberg females and children (0.3 ± 0.2 and 0.8 ± 0.6 L/day compared to 0.7 ± 0.5 and 1.6 ± 0.8 L/day, respectively). The estimate for the human half-life was 3.26 years (geometric mean; range 1.03–14.67 years). Regression analysis of the data also suggested that the elimination rate might have been greater in younger subjects and older subjects.

Seals et al. (2011) determined half-life estimates for 602 residents of Little Hocking, Ohio, and 971 residents of Lubeck, West Virginia, who were part of the C8 study but had relocated to a different area of the country. The half-life estimate was based on the decline in serum PFOA levels after the time of the initial measurement and the years since the change in residential location occurred. A background estimate (5 ng/mL) was subtracted from the serum measurements before analysis. On average, the initial serum PFOA concentrations were higher in Little Hocking (60.6 ng/mL) than in Lubeck (31.0 ng/mL). Due to the nonlinearity in scatter plots of the natural log for adjusted serum PFOA concentrations versus the years elapsed since relocation, the authors used a two-segment linear spline regression approach in their analysis of the data (i.e., Little Hocking—4 years, Lubeck—9 years). The slope of the line decreased for the second time segment compared to the first. In former residents of Little Hocking, a -21.4%-change in serum PFOA was observed in the first 4 years after leaving Little Hocking, and a -7.6%-change was observed beyond 4 years. In former Lubeck residents, the serum PFOA change was -7.8% for the first 9 years and 0.2% (a slight increase) afterwards. The half-life estimates for Little Hocking ranged from 2.5–3.0 years (average 2.9 years) and for Lubeck ranged from 5.9–10.3 years (average 8.5 years).

Based on their analysis, the authors suggested that, if their assumptions were correct, a simple first order elimination model might not be appropriate for PFOA given that the rate of elimination appeared to be influenced by both concentration and time. There was a difference in the CL for the two locations even though the range of years elapsed since relocation was the same for both communities. The authors identified three potential limitations of their analysis: the cross-sectional design, the assumption that exposure was uniform within a water district, and a potential bias introduced by exclusion of individuals with serum values <15 ng/mL.

3M (Burris et al. 2000, 2002) conducted a half-life study on 26 retired fluorochemical production workers from their Decatur, Alabama, (n = 24) and Cottage Grove, Minnesota, (n = 3) plants. Blood was collected from the subjects between 1998 and 2004, a period during which serum samples were drawn every 6 months over a 5-year period, depending on the facility at which the subject had worked. Responses on questionnaires determined whether any of the retirees had occupational exposures after retirement. The average number of years that participants worked was 31 (range 20–36 years) and they had been retired an average of 2.6 years at study initiation (range 0.4–11.5 years). The mean age of the retirees was 61 years (range 55–75) at the beginning of the study.

The initial mean serum PFOA concentration of all of the subjects was 0.691 µg/ml (range 0.072–5.1 µg/mL). At the completion of the study, the mean PFOA concentration was 0.262 µg/mL (range 0.017–2.435 µg/mL). Two of the retirees died during the study period; therefore, they were only followed for 4.2 years. The mean serum elimination half-life of PFOA in these workers was 3.8 years (1378 days, 95% CI, 1131-1624 days) and the median was 3.5 years (Olsen et al. 2005). The range was 1.5–9.1 years (561–3334 days). No association was reported between the serum elimination half-life and with initial PFOA concentrations, age, or gender of the retirees, the number of years retired or working at the production facility, or medication use or health conditions.

Harada et al. (2005) studied the relationship between age, gender, and serum PFOA concentration in residents of Kyoto, Japan. They found that females in the 20–50-year-old age group (all with regular menstrual cycles) had serum PFOA concentrations that were significantly lower than those in females over age 50 (all postmenopausal). Mean serum PFOA concentration in the younger females was 7.89 ± 3.61 ng/ml versus 12.63 ± 2.42 ng/mL in the older females. This age difference in serum PFOA concentrations was not seen in males, and serum PFOA concentrations in males were comparable to those of the older females.

Harada et al. (2005) also estimated the CL_R rate of PFOA in humans and found it to be only about 0.001% of the GFR. There was no significant difference in CL_R of PFOA with respect to gender or age group, and the mean value was 0.03 ± 0.013 ml/day/kg.

Animal. Kemper (2003) examined the plasma concentration profile of PFOA following gavage administration in sexually mature Sprague-Dawley rats. Male and female rats (four per gender per group) were administered single doses of PFOA by gavage at DRs of 0.1, 1, 5, and 25 mg PFOA/kg. After dosing, plasma was collected for 22 days in males and 5 days in females. Plasma concentration versus time data were then analyzed using noncompartmental PK methods (see Table 2-26 and Table 2-27). To further characterize plasma elimination kinetics, animals were given oral PFOA at a rate of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (extended time).

Plasma elimination curves were linear with respect to time in male rats at all dose levels. In males, plasma elimination half-lives were independent of dose level and ranged from approximately 138 hours to 202 hours. To further characterize plasma elimination kinetics, particularly in male rats, animals were given oral PFOA at a dose of 0.1 mg/kg, and plasma samples were collected until PFOA concentrations fell below quantitation limits (2,016 hours in males). The estimated plasma elimination half-life in this experiment was approximately 277 hours (11.5 days) in male rats.

Plasma elimination curves were biphasic in females at the 5-mg/kg and 25-mg/kg dose levels. In females, terminal elimination half-lives ranged from approximately 2.8 hours at the lowest dose to approximately 16 hours at the high dose. The estimated plasma elimination half-life in the extended time experiment was approximately 3.4 hours in females.

Table 2-26. PK Parameters in Male Rats Following Administration of PFOA

Parameter	Dose					
	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg (IV)	0.1 mg/kg extended time
T_{max} (hr)	10.25 (6.45)	9.00 (3.83)	15.0 (10.5)	7.5 (6.2)	NA	5.5 (7.0)
C_{max} (µg/mL)	0.598 (0.127)	8.431 (1.161)	44.75 (6.14)	160.0 (12.0)	NA	1.08 (0.42)
Lambda z (1/hr)	0.004 (0.001)	0.005 (0.001)	0.0041 (0.0007)	0.0046 (0.0012)	0.004 (0.000)	0.0026 (0.0007)
$T_{1/2}$ (hr)	201.774 (37.489)	138.343 (31.972)	174.19 (28.92)	157.47 (38.39)	185.584 (19.558)	277.10 (56.62)
AUC_{INF} (hr·µg/mL)	123.224 (35.476)	1194.463 (215.578)	6733.70 (1392.83)	25,155.61 (7276.96)	1249.817 (113.167)	206.38 (59.03)
AUC_{INF}/D (hr·µg/ml/mg/kg)	1096.811 (310.491)	1176.009 (206.316)	1221.89 (250.28)	942.65 (284.67)	1123.384 (100.488)	2111.28 (586.77)
Cl_p (mL/kg·hr)	0.962 (0.240)	0.871 (0.158)	0.85 (0.21)	1.13 (0.31)	0.896 (0.082)	0.51 (0.17)

Source: Kemper 2003
Notes:
Mean (SD)
AUC_{INF}: area under the plasma concentration time curve, extrapolated to infinity; AUC_{INF}/D: AUC_{INF} normalized to dose; Cl_p: plasma clearance; C_{max}: maximum plasma concentration; Lambda z: terminal elimination constant; T1/2: terminal elimination half-life; T_{max}: time to C_{max}.

Table 2-27. PK Parameters in Female Rats Following Administration of PFOA

Parameter	Dose					
	0.1 mg/kg	1 mg/kg	5 mg/kg	25 mg/kg	1 mg/kg (IV)	0.1 mg/kg extended time
T_{max} (hr)	0.56 (0.31)	1.13 (0.63)	1.50 (0.58)	1.25 (0.87)	NA	1.25 (0.50)
C_{max} (µg/mL)	0.67 (0.07)	4.782 (1.149)	20.36 (1.58)	132.6 (46.0)	NA	0.52 (0.08)
Lambda z (1/hr)	0.231 (0.066)	0.213 (0.053)	0.15 (0.02)	0.059 (0.037)	0.250 (0.047)	0.22 (0.07)
$T_{1/2}$ (hr)	3.206 (0.905)	3.457 (1.111)	4.60 (0.64)	16.22 (9.90)	2.844 (0.514)	3.44 (1.26)
AUC_{INF} (hr·µg/mL)	3.584 (0.666)	39.072 (10.172)	114.90 (11.23)	795.76 (187.51)	33.998 (7.601)	3.34 (0.32)
AUC_{INF}/D (hr·µg/mL/mg/kg)	31.721 (5.880)	38.635 (10.093)	20.78 (2.01)	29.54 (6.92)	30.747 (6.759)	34.39 (3.29)
Cl_p (mL/kg·hr)	32.359 (6.025)	27.286 (7.159)	48.48 (4.86)	35.06 (.88)	34.040 (9.230)	29.30 (3.06)

Source: Kemper 2003
Note: Mean (SD)

Gibson and Johnson (1979) administered a single dose of ^{14}C-PFOA averaging 11.4 mg/kg by gavage to groups of three male 10-week-old CD rats. The elimination half-life of ^{14}C from the plasma was 4.8 days. NRC ([2005], cited in Butenhoff et al. [2004b]) reported half-lives of 4–6 days for male rats and 2–4 hours for female rats; there was no mention of the strains studied.

Kemper (2003) reported half-lives of 6–8 days for male Sprague-Dawley rats (Table 2-26) and 3–16 hours for females (Table 2-27).

Lou et al. (2009) determined values of 21.7 days (95% confidence interval: 19.5–24.1) for male CD1 mice and 15.6 days (95% confidence interval: 14.7–16.5) for females for use in their pharmacokinetic model (see section 2.6.1). NRC ([2005], cited in Butenhoff et al. [2004b]) provided values of 12 days for males and 20 days for females without any information on strains.

Butenhoff et al. (2004b) looked at the elimination half-life in monkeys treated for 6 months with 0, 3, 10, and 20 mg/kg/day via capsules. Elimination of PFOA from serum after cessation of dosing was monitored in recovery monkeys from the 10- and 20-mg/kg dose groups. For the two monkeys exposed to 10 mg/kg, serum PFOA elimination half-life was 19.5 (R^2=0.98) days and indicated first-order elimination kinetics. For three monkeys exposed to 20 mg/kg, serum PFOA elimination half-life was 20.8 days (R^2=0.82) and also indicated first-order elimination kinetics, although dosing was suspended at different time points because of weight loss. The data from NRC (2005), which were provided by Butenhoff et al. (2004b), were about 21 days for females and 30 days for males.

2.6.3 Volume of Distribution Data

Several researchers have attempted to characterize PFOA exposure and intake in humans through PK modeling (Lorber and Egeghy 2011; Thompson et al. 2010). As an integral part of model validation, the parameter for V_d of PFOA within the body was calibrated from the available data. In the models discussed below, V_d was defined as the total amount of PFOA in the body divided by the blood or serum concentration.

Two groups of researchers defined a V_d of 170 ml/kg body weight for humans for use in a simple, single compartment, first-order PK model (Lorber and Egeghy 2011; Thompson et al. 2010). The models developed by these groups were designed to estimate intakes of PFOA by young children and adults (Lorber and Egeghy 2011) and the general population of urban areas on the east coast of Australia (Thompson et al. 2010). In both models, the V_d was calibrated using human serum concentration and exposure data from the NHANES and assumes that most PFOA intake is from contaminated drinking water. Thus, in using the models to derive an intake from contaminated water, the V_d was calibrated so that model prediction of elevated blood levels of PFOA matched those seen in residents.

Butenhoff et al. (2004b) calculated a V_d from noncompartmental PK analysis of data from cynomolgus monkeys. Three males and three females were administered a single IV dose of 10 mg/kg, and serum PFOA concentrations were measured in samples collected up to 123 days post-dosing. The V_d of PFOA at steady state ($V_d$ss) were similar for both genders at 181 ± 12 ml/kg for males and 198 ± 69 ml/kg for females.

2.6.4 Toxicokinetic Summary

Uptake and egress of PFOA from cells is largely regulated by transporters in cell membranes. It is absorbed from the gastrointestinal tract as indicated by serum measurements in humans and treated animals. In serum, PFOA is electrostatically bound to albumin occupying up to nine to twelve sites and sometimes displacing other substances that normally would occupy a site. Linear PFOA chains display stronger binding than branched chains. PFOA binding causes a

change in the conformation of serum albumin, altering its ability to bind with some endogenous and exogenous materials it normally transports.

PFOA is distributed to tissues by a process requiring membrane transporters. Accordingly, the tissue levels vary from organ to organ. The highest tissue concentrations are usually in the liver. Liver accumulation in males is greater than in females. Other tissues with a tendency to accumulate PFOA are the kidneys, lungs, heart, and muscle, plus the testes in males and uterus in females. Post-mortem studies in humans have found PFOA in liver, lungs, bone, and kidneys, but only low levels in brain. PFOA is not metabolized, thus, any effects observed in toxicological studies are the result of parent compound, not metabolites.

Electrostatic interactions with proteins are an important toxicokinetic feature of PFOA. Studies demonstrate binding or interactions with membrane receptors (e.g., PPARα, T3), transport proteins, and enzymes. Saturable renal resorption of PFOA from the glomerular filtrate via transporters in the kidney tubules is a major contributor to the long half-life of this compound. Branched-chain PFOAs are less likely to be resorbed than the linear molecules based on half-life information in humans. All toxicokinetic models for PFOA are built on the concept of saturable renal resorption first proposed by Andersen et al. (2006). Some PFOA is removed from the body with bile, a process that also is transporter-dependent. Accordingly, the levels in fecal matter represent both unabsorbed material and that discharged with bile.

During pregnancy, PFOA is present in the placenta and amniotic fluid in both animals and humans. Post-delivery, PFOA is transferred to offspring through lactation in a dose-related manner. Maternal serum levels decline as those in the pups increase. This also occurs in humans as demonstrated in a study of females breast-feeding their infants in Little Hocking, Ohio.

The half-life in humans for occupationally exposed workers was 3.8 years (95% CI, 1.5-9.1). The average half-life was 2.3 years among people in the Lubeck Public Services District in West Virginia and the LHWA in Ohio, based on changes in serum levels for the general population after treatment of drinking water was implemented. This half-life value reflects humans whose exposure came primarily from their public water system. Half-lives from animals included 21 days (females) and 30 days (males) for monkeys Butenhoff et al. (2004b); 11.5 days (males) and 3.4 hours (females) in Sprague-Dawley rats (Kemper 2003); and 27.1 days (male) and 15.6 days (female) CD1 mice (Lau et al. 2006). The gender difference between male and female rats is not seen in mice. In early life, the half-lives are nearly the same for both genders of rats, but once the animals reach sexual maturity, resorption increases in males, prolonging the half-life (Hinderliter 2004; Hundley et al. 2006). This change appears to be under the control of hormones in both males and females (Cheng et al. 2006; Kudo et al. 2002).

3 HAZARD IDENTIFICATION

This section provides a summary and synthesis of the data from a large number of human epidemiology studies accompanied by studies in laboratory animals designed to identify both the dose response and critical effects that result from exposures to PFOA and to examine the MoA leading to toxicity.

3.1 Human Studies

Epidemiology studies of effects of PFOA have been conducted in three types of populations: workers exposed in chemical plants producing or using PFOA, high-exposure communities (i.e., an area in West Virginia and Ohio that experienced water contamination over a period of more than 20 years), and general population studies with background exposures. These populations differ with respect to exposure levels. The approximate range in serum PFOA concentrations is 0.010–> 2.0 (means around 1–4 μg/mL) in the PFOA-exposed workers, and 0.010–0.100 μg/mL and below LOD to < 0.010 μg/mL in the high-exposure community and general population settings, respectively. Although moderate-to-high correlations between PFOA and PFOS are often seen in general populations ($r > 0.5$), the correlation is lower in the West Virginia and Ohio high-exposure area ($r=0.3$). In evaluating and synthesizing results from these studies, it is important to consider differences in the exposure range within the study population and the exposure level within the referent group, as differences (or inconsistencies) can be expected depending on the shape of the exposure-response curve and the exposure range encompassed by different studies. In addition, the optimal choice of an exposure metric (e.g., cumulative or a time-specific) depends on the specific outcome being examined.

Occupational studies. Large-scale production of PFOA occurred in the United States for several decades. Both 3M (in Alabama and Minnesota) and DuPont (in West Virginia) have been the primary U.S. producers and users of perfluorinated compounds, and both companies have offered voluntary fluorochemical medical surveillance programs to workers at plants that produced or used perfluorinated compounds. The monitoring data collected by 3M and DuPont were used in conjunction with mortality and health effects information in a number of epidemiology studies of cancer and noncancer outcomes in the worker populations. 3M discontinued manufacturing PFOA in 2000, but a subsidiary in Europe (Antwerp, Belgium) continued to manufacture and sell it through 2008.

High-exposure community studies. Members of the general population living in the vicinity of the DuPont Washington Works PFOA production plant in Parkersburg, West Virginia, are the focus of a large-scale, community-based study titled the C8 Health Project. Releases from the Washington Works plant, where PFOA (C8) was used as a processing aid in the manufacture of fluoropolymers, contaminated the ground water of six water districts near the plant resulting in exposures to the general population. The plant began production in the 1950s, with PFOA use and emission from the plant increasing in the 1980s. Study participants from the affected areas ($n = 69,030$; 33,242 males, 35,788 females; <10–70+ years) were identified in 2005–2006, and a series of studies were conducted. The participants all received compensation and provided a blood sample and filled out an extensive questionnaire that included information on drinking water sources, use of home-grown produce, and health information. A variety of approaches to exposure assessment have been used in these studies, with the most detailed incorporating individual residential history and water consumption and source data, emissions data,

environmental characteristics, water pipe installation, PK data, and workplace exposures (Barry et al. 2013; Shin et al. 2011; Vieira et al. 2013). The methods allow the estimation of cumulative and of current exposure at different time periods or ages for individual study participants. Details of the specific analyses undertaken to estimate historical exposures and to ascertain different types of outcomes (retrospective and prospective analyses) are described in detail below. Drinking water concentrations were based on PFOA releases from the DuPont plant and residential address history of the participants (C8 Science Panel 2012).

The C8 Health Project also involved a review of evidence of health effects, considering their own studies and studies conducted by others and in other populations. The conclusions for each health endpoint assessed—"probable link" or "not a probable link"—are available on the C8 Science Panel website in a series of reports completed in 2011–2012 (see http://www.c8sciencepanel.org/index.html).

General population studies. Studies investigating the association between PFOA levels and health effects in the U.S. general population have been conducted using the NHANES data set. NHANES examined representative members of the U.S. population (~5000 adults and children/year) through surveys focusing on different health topics. The study consists of an interview (demographic, socioeconomic, dietary, and medical questions) and examination (medical including blood and urine collection, and dental and physiological parameters). Biomonitoring included a number of PFAS, predominantly PFOA and PFOS.

A study by Jain (2014) examined the influence of diet and other factors on the levels of serum PFOA and other PFAS using NHANES 2003–2004, 2005–2006, and 2007–2008 data. Significantly higher serum PFOA levels were found in males (0.0047 μg/mL) than in females (0.035 μg/mL) and in smokers (0.043 μg/mL) than in nonsmokers (0.040 μg/mL). No significant differences in PFOA serum concentration were seen during the time periods evaluated. There was a positive association of PFOA with increases in serum cholesterol ($p<0.001$), serum albumin ($p<0.001$), and body mass index (BMI) ($p<0.04$) based on the 5,591 records used in the assessment. Intakes of nonalcoholic beverages were positively associated with serum PFOA ($P<0.001$), but no associations were found for other dietary food groupings.

The results of these studies along with other population studies are described in the following sections. In the studies of worker cohorts, the data collected focused on measures of cardiovascular risk, signs of organ damage, standard hematological endpoints, and cancer (primarily cancer-related mortality). Within the general population, data were focused on cardiovascular risk factors and diabetic or prediabetic conditions as well as reproductive and developmental endpoints. The following summary focuses on measures of lipids (e.g., cholesterol, LDL); liver, kidney, and thyroid effects; reproductive effects (e.g., pregnancy-related outcomes, specifically pregnancy-related hypertension and preeclampsia, measures of fetal growth, and pubertal development); and cancer (specifically kidney and testicular cancer). These outcomes were selected either because of the availability of studies in a variety of settings with some indications of effects (e.g., as noted in the C8 Science Panel reports), or to allow comparison with results from studies in animals. Summary tables are included to support evaluation of the weight of evidence and facilitate comparison of the serum concentrations in the epidemiology studies to those in the animals studies summarized in section 3.2.

3.1.1 Noncancer

3.1.1.1 Serum Lipids and Cardiovascular Diseases

Serum Lipids

Occupational studies. Four cross-sectional studies are described in this section and in Table 3-1. Olsen et al. (2000) analyzed data from voluntary medical surveillance examinations of PFOA production workers at a 3M plant in 1993, 1995, and 1997. Cholesterol, LDL, HDL, and triglycerides were measured in male workers (n = 111 in 1993, n = 80 in 1995, and n = 74 in 1997). Multivariable regression analyses, conducted separately by year (cross-sectional), were adjusted for age, BMI, alcohol consumption, and cigarette use. Employees' serum PFOA levels were stratified into three categories—<1, 1- <10, and ≥10 μg/mL. The sample size in the highest category ranged from 11 to 15 in the three examination years. There was little variation by exposure category in mean or median TC, LDL, HDL, or triglycerides across the workers in 1993, 1995, or 1997.

Olsen and Zobel (2007) examined data from the 2000 medical surveillance program at the three 3M plants, which is an expanded and refined analysis of the data reported in Olsen et al. (2003). The fluorochemical workers consisted of males (age 21–67) from the Antwerp, Belgium (n = 196); Cottage Grove, Minnesota (n = 122); and Decatur, Alabama (n = 188) production facilities who volunteered to participate in the medical surveillance program and did not take cholesterol-lowering medication. Blood was collected for fluorochemical concentration determination and serum lipid parameters including cholesterol, LDL, HDL, and triglycerides. Analysis of variance (ANOVA), analysis of covariance, logistic regression, and multiple regression models were used to analyze the data with age, BMI, and alcohol consumption as covariates. Potential associations with PFOS levels were not evaluated because a previous analysis had shown no association between PFOS and the selected outcomes. Serum PFOA concentrations ranged from 0.01 to 92.03 μg/mL for the male workers (all sites combined), with a mean serum PFOA concentration of 2.21, 1.02, 4.63, and 1.89 μg/mL for all sites combined, and the Antwerp, Cottage Grove, and Decatur sites, respectively. Serum PFOA (all sites combined) was not associated with TC or LDL. A negative association was observed between serum PFOA concentration (all sites combined) and HDL. Serum triglyceride was positively associated with serum PFOA at all sites combined and independently at the Antwerp site. Nonadherence to the fasting requirement for blood collection, especially for night-shift workers, and potential binding of PFOA to albumin and LDL, were identified by the authors as possible factors that influenced the triglyceride results.

Sakr et al. (2007a) conducted a cross-sectional analysis of PFOA and lipids among active employees at the DuPont Washington Works fluoropolymer production plant in West Virginia. The employees who volunteered to participate in the study (n = 1025, 782 males, 243 females) each had a physical examination, provided a fasting blood sample, and answered a medical and occupation history questionnaire in 2004. The association between PFOA and lipid levels was evaluated by ANOVA, χ^2 test, student's t-test, and linear regression models. Confounders including age, BMI, gender, alcohol consumption, and parental heart attack were considered in the models. Mean serum PFOA concentration in the workers was 0.428 ± 0.189 μg/mL (interquartile range 0.099–0.381). For those with current occupational exposure to PFOA, the range was 0.0174–9.55 μg/mL and for workers with intermittent occupational exposure, the range was 0.0081–2.07 μg/mL. The range was 0.0086–2.59 μg/mL for workers with past occupational exposure and the 0.0046–0.963 μg/mL for workers with no occupational exposure.

Serum PFOA was positively associated with cholesterol, very low-density lipoprotein (VLDL), and LDL ($p<0.03$) in the participating workers, whether or not they were taking lipid-lowering medication. No association was observed between serum PFOA and HDL or triglycerides. PFOS was not included in the study.

Costa et al. (2009) examined serum lipid data using 30 years of medical surveillance data from workers of a PFOA production plant in Italy. The workers (n = 53 males, 20–63 years of age) participated in the medical surveillance program yearly from 1978 to 2007. The length of work exposure was 0.5–32.5 years. In 2007, 37 males were active workers and 16 males were retired or had transferred to other departments and were no longer being exposed. Unexposed male workers (n = 107, 12 executives and 95 blue collar workers) from different departments also participated in the medical surveillance program and served as controls. Beginning in 2000, serum PFOA was monitored yearly except in 2005. Serum PFOA concentrations in the workers decreased after plant renovations partially automated the PFOA production process and procedures for the use of protective devices were instituted in 2002. In 2007, the geometric mean serum PFOA was 4.02 and 3.76 μg/mL, respectively, in currently exposed and retired workers. Three analyses were conducted: a t-test comparing 34 exposed workers matched to 34 unexposed workers by age, work seniority, day/shift work, and living conditions; linear regression with 34 exposed workers and 107 unexposed workers adjusting for age, work seniority, BMI, smoking, and alcohol consumption; and a repeated measures analysis with a total of 56 individuals with more than one measure, adjusting for age, work seniority, BMI, smoking, alcohol consumption, and year of observation. TC and uric acid were significantly increased ($p<0.05$) in relation to PFOA exposure in each of these analyses. No correlations were observed between serum PFOA concentration and Apo-A (HDL-associated) or Apo-B (LDL-associated) proteins, HDL, or triglycerides in any of the analyses. PFOS was not included in this study.

Three other studies included analyses with multiple measures over time (Table 3-1). Olsen et al. (2003) conducted a longitudinal analysis of the 2000 medical surveillance data from the 3M workers in conjunction with 1995 and 1997 data. This analysis included 175 male employees with data from 2000 and at least one of the other survey dates. Only 41 employees were participants in all three surveillance periods. Mean serum levels for the group sampled in 1995 and 2000 (n = 64) were 1.36 μg/mL and 1.59 μg/mL, respectively. Mean serum levels for the group sampled in 1997 and 2000 (n = 69) were 1.22 μg/mL and 1.49 μg/mL, respectively. Finally, mean serum levels for the group sampled in 1995, 1997, and 2000 (n = 41) were 1.41 μg/mL, 1.90 μg/mL, and 1.77 μg/mL, respectively. When serum levels were analyzed using a repeated measures mixed-model multivariable regression, adjusting for age, BMI, smoking, alcohol consumption, location, year at first entry, years worked (at baseline), and years of follow-up, there was a statistically significant positive association between PFOA and serum cholesterol (Beta[1] = 0.032, 95% CI 0.013, 0.015) and triglycerides (Beta = 0.094, 95% CI 0.045, 0.144) ($p = 0.0002$). PFOS levels were not associated with changes in serum lipids over time.

Sakr et al. (2007b) conducted a longitudinal analysis among the workers at the DuPont Washington Works plant in West Virginia using data from 1979 to 2004. Employee medical records from the medical surveillance program were used to obtain blood lipid (e.g., TC, LDL, HDL, triglycerides), height, and weight data. As part of the medical surveillance program, each employee gave a detailed medical history and had a physical examination at least every 3 years. Serum PFOA concentration was measured every 1–2 years in PFOA-exposed workers and every

[1] The beta coefficient from the regression analysis.

3–5 years in non-PFOA-exposed workers on a volunteer basis. This study included 454 workers who had two or more serum PFOA measurements. The study population included 334 males and 120 females ranging in age from 24 to 66 years who had worked at the plant for at least 1 year since 1979. A linear mixed effects regression model was used to analyze the data and accounted for age (and age-squared), gender, BMI, and decade of hire as potential confounders. Serum PFOA concentrations ranged from 0 to 22.66 μg/mL, with a mean of 1.13 μg/mL over the 23-year monitoring period in the study population. For employees with two or more PFOA measurements, the mean of the first and last sample was 1.04 μg/mL and 1.16 μg/mL, respectively, with an average of 10.8 years between samples. Serum PFOA concentration was positively associated with TC after age, BMI, gender, and decade of hire adjustment in the model (Beta = 1.06, 95% CI 0.24, 1.88) per ppm increase in PFOA. Information on lipid-lowering medications and alcohol intake by the participants was not available. PFOS was not included in this study.

Steenland et al. (2015) conducted an analysis of the incidence of several conditions, including high cholesterol (based on prescription medication use) among 3,713 workers at the Washington Works plant in West Virginia who participated in the C8 Health Project. Yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported incidence of high cholesterol in relation to time-varying cumulative estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. No association was seen when analyzed without a lag (HRs by quartile 1.0, 1.11, 1.06, 1.05; trend p = 0.56 for log cumulative exposure), or when using a 10-year lag (HRs by quartile 1.0, 0.93, 1.01, 0.96; trend p = 0.62).

High-exposure community studies. Several studies examined serum lipids in populations serviced by water districts contaminated by the Washington Works PFOA production plant in Ohio and West Virginia (Table 3-2). Emmett et al. (2006) is a small study (n = 371) with limited analysis (t-tests comparing PFOA levels in people with abnormal versus normal TC); the larger studies were conducted as part of the C8 Health Project. This collection of studies includes analyses of current serum PFOA levels in relation to serum lipids in adults (Steenland et al. 2009) and children (Frisbee et al. 2010), longitudinal analysis of the change in lipids seen in relation to a change in serum PFOA (Fitz-Simon et al. 2013), and analyses of the incidence of hypercholesteremia in relation to modeled exposure (Winquist and Steenland 2014a). With the exception of one set of analyses within the Winquist and Steenland study (2014a), these data provide consistent evidence of positive associations between PFOA exposure (measured directly in blood or modeled based on environmental and drinking water data) and TC.

Emmett et al. (2006) examined the association of serum PFOA concentration with serum TC in residents of the Little Hocking water district in Ohio. The study population (n = 371, 2–>60 years of age) was a random sample of the population served by LHWA. The subjects completed questionnaires (e.g., demographic, occupational, health conditions, and so forth) and provided blood samples. PFOA concentration was determined by HPLC/MS/MS; no other PFASs were measured. Regression models were used to analyze the data. The median serum PFOA concentration was 0.354 μg/mL. No association was observed between serum PFOA and TC.

Steenland et al. (2009) examined the association of PFOA with serum lipids in adult participants of the C8 Health Project (n = 46,294; 18–≥80 years). Serum samples were separated into deciles or quartiles for analysis. TC, HDL, triglycerides, LDL, and non-HDL (TC minus HDL cholesterol) were measured or calculated from blood samples. The data were analyzed by linear regression using the log-transformed values for all variables. Covariates of the model

included age, gender, quartile BMI, education, smoking, regular exercise, and alcohol consumption. A logistic model was used to analyze high cholesterol and serum PFOA concentration (quartiles). The mean serum PFOA concentration was 0.080 μg/mL. All lipid outcomes, except for HDL, showed significant increasing trends with increasing serum PFOA decile. There was a positive association between mean levels of serum PFOA and TC, LDL cholesterol, triglycerides, TC/HDL ratio, and non-HDL. The predicted increase in TC from lowest to highest serum PFOA concentration decile was 11–12 milligrams per deciliter (mg/dL). The odds ratio (OR) for high cholesterol (≥240 mg/dL) increased from the lowest to the highest quartile of serum PFOA concentrations: 1.00, 1.21 (95% CI: 1.12–1.31), 1.33 (95% CI: 1.23–1.43), and 1.38 (95% CI: 1.28–1.51). No association was observed between mean level of serum PFOA and HDL cholesterol. PFOS also was positively associated with TC, LDL cholesterol, and triglycerides. The results of the study were consistent with occupational studies that found a positive association between PFOA exposure and serum lipids.

The study by Frisbee et al. (2010) used a design and analysis strategy similar to that of Steenland et al. (2009), but it was conducted among children (n = 6536; 1–11.9 years) and adolescent (n = 5934; 12.0–17.9 years) participants of the C8 Health Project. The mean serum PFOA concentration was 0.0777 μg/mL and 0.0618 μg/mL, respectively, for children and adolescents. TC, LDL, and triglycerides were positively associated ($p \leq 0.02$) with serum PFOA concentration, adjusting for age, gender, BMI, exercise, and length of fast. Assessment of the quintile trends showed significant differences ($p \leq 0.02$) between the first and fifth quintile for TC and LDL for children and adolescents of both genders combined and separated. A significant difference ($p = 0.04$) was observed for fasting triglycerides in female children only. An increased risk of abnormal TC and LDL were positively associated with serum PFOA. The ORs were 1.0 first (reference), 1.1 (95% CI: 1.0–1.3, second), 1.2 (95% CI: 1.0–1.4, third), and 1.2 (95% CI: 1.1–1.4, fourth and fifth) for TC, and 1.0 (reference, first), 1.2 (95% CI: 1.0–1.5, second), 1.2 (95% CI: 1.0–1.4, third and fourth), and 1.4 (95% CI: 1.2–1.7, fifth) for LDL. An increased risk of abnormal fasting triglyceride and HDL was not associated with serum PFOA. PFOS also was positively associated with TC, LDL cholesterol, and HDL cholesterol.

The C8 Science Panel (2012) used data from the C8 general population cohorts as well as from combined general population and worker cohorts to evaluate the association between PFOA and a medical diagnosis of high cholesterol. Despite inconsistent evidence between studies, they concluded that there is a probable link between PFOA and diagnosed high cholesterol. The worker cohort was not evaluated separately in this analysis.

A cohort of 521 members of the C8 Health Project was evaluated for an association between changes in serum PFOA levels and changes in serum LDL-cholesterol, HDL-cholesterol, TC, and triglycerides over a 4.4-year period (Fitz-Simon et al. 2013). Linear regression models were fit to the logarithm (base 10) of ratio change in each serum lipid measurement in relation to the logarithm of ratio change in PFOA. Mean serum PFOA concentration decreased by approximately one-half between baseline (0.140 ± 0.209 μg/mL) and follow-up (0.068 ± 0.144 μg/mL). No corresponding changes in serum lipids were found. However, those individuals with the greatest declines in serum PFOA had a larger decrease in LDL cholesterol.

Table 3-1. Summary of PFOA Occupational Exposure Studies of PFOA and Serum Lipids

Reference and Study Details	PFOA Level	TC	LDL	HDL	Triglycerides
Cross-sectional					
Olsen et al. 2000 n = 111 in 1993, 80 in 1995, 74 in 1997; 50-70% participation rate Mean age: ~ 40 yrs Mean duration: not reported ANOVA based on three exposure categories, adjusted	(1) 0 to < 1, mean ~ 0.4 µg/mL (2) 1 to < 10, mean ~ 3 µg/mL (3) ≥ 10, mean ~ 30 µg/mL	1993: mean 215, 219, 232 mg/dl (p = 0.45) 1995: mean 207, 212, 221 mg/dl (p = 0.48) 1997: mean 199, 213, 217 mg/dl (p = 0.08)	1993: mean 138, 143, 140 mg/dl (p = 0.84) 1995: mean 131, 133, 130 mg/dl (p = 0.96) 1997: mean 114, 134, 134 mg/dl (p = 0.11)	1993: mean 43, 47, 48 mg/dl (p = 0.32) 1995: mean 42, 43, 41 mg/dl (p = 0.70) 1997: mean 41, 44, 45 mg/dl (p = 0.40)	1993: mean 171, 205, 221 mg/dl (p = 0.77) 1995: mean 152, 123, 183 mg/dl (p = 0.07) 1997: mean 219, 176, 251 mg/dl (p = 0.13)
Olsen and Zobel 2007 3M. Antwerp, Cottage Grove, Decatur combined; 50-65% participation rate n = 506 (men, not taking lipid-lowering medications) Mean age: 40 yrs Mean duration: not reported Linear regression, adjusted [Related reference: Olsen et al. 2003]	Mean 2.21 µg/mL range 0.01–92.03 µg/mL	Beta = 0.0076 (SE 0.0059) (p = 0.20) [log-transformed PFOA and cholesterol]	Beta = 0.0021 (SE 0.0090) (p = 0.81) [log-transformed PFOA and cholesterol]	Beta = -0.0183 (SE 0.0069) (p = 0.01) [log-transformed PFOA and cholesterol]	Beta = 0.0711 (SE 0.0169) (p = 0.0001) [log-transformed PFOA and cholesterol]
Sakr et al. 2007a Washington Works (West Virginia) n = 1025 (782 men, 243 women), 55% participation rate Mean age: 46.5 and 44.4 yrs, respectively for men and women Mean duration: 19.6 and 15.9 yrs, respectively for men and women Linear regression, adjusted	Mean 0.428 µg/mL range 0.005– 9.55 µg/mL	All: Beta = 4.036 (SE 1.284) (p = 0.002) Excluding workers taking lipid-lowering medications: Beta = 5.519 (SE 1.467) p = < 0.001)	All: Beta = 2.834 (SE 1.062) (p = 0.008) Excluding workers taking lipid-lowering medications: Beta = 3.561 (SE 1.213) (p = 0.003)	All: Beta = -0.178 (SE 0.432) (p = 0.68) Excluding workers taking lipid-lowering medications: Beta = 0.023 (SE 0.058) (p = 0.96)	All: Beta = 018 (SE 0.021) (p = 0.38) Excluding workers taking lipid-lowering medications: Beta = 0.030 (SE 0.024 (p = 0.21) [TG log-transformed]
Costa et al. 2009 Italy PFOA production plant n = 37 currently exposed, 16 formerly exposed, 107 controls (different areas in the plant) Mean age: 42 yrs—currently exposed and controls; 52 yrs—formerly exposed Mean duration (in 2007): 14-16 yrs Analysis 1: t-test, 34 currently exposed matched to 34 controls, adjusted Analysis 2: Linear regression, 34 currently exposed and 107 controls, adjusted Analysis 3: linear regression (generalized estimating equation [GEE] modeling), 56 total with concurrent PFOA and lipid measure, adjusted	Currently exposed: mean 12.9, geometric mean 4.02, range 0.2–47 µg/mL Formerly exposed: mean 6.81 geometric mean 3.76, range 0.53–18 µg/mL	Analysis 1: Currently exposed: 237.0 mg/dl Controls: 206.4 mg/dl (p = 0.003) Analysis 2: Beta = 21.7 (95% CI 6.83, 36.6) (p = 0.005) Analysis 3: Beta = 0.028 (95% CI 0.002, 0.055) (p < 0.05)	Not measured	Analysis 1: Currently exposed: 56.68 mg/dl Controls: 57.82 mg/dl (p => 0.05) Analysis 2: Beta = 2.42 (95% CI -2.30, 7.13) (p > 0.05) Analysis 3: Beta = -0.018 (95% CI -0.047, 0.012) (p > 0.05)	Analysis 1: Currently exposed: 150.03 mg/dl Controls: 155.35 mg/dl (p > 0.05) Analysis 2: Beta = -0. 15 (95% CI -34.6, 34.3) (p > 0.005) Analysis 3: Beta = 0.055 (95% CI -0.036, 0.147) (p > 0.05)

Reference and Study Details	PFOA Level	TC	LDL	HDL	Triglycerides
Longitudinal					
Olsen et al. 2003 3M, Antwerp and Decatur combined ~5 yr follow-up period n = 174 (measure in 1995 or 1997, and in 2000) Mean age: not reported Mean duration: not reported Linear mixed effects regression for repeated measures, adjusted	1995 baseline: 1.36-1.41 µg/mL 2000 follow-up: 1.49-1.77 µg/mL	Beta = 0.032 (95% CI 0.013, 0.051) [and statistically significant PFOA-years follow-up interaction, Beta = -0.0004]	Not measured	Reported as "no significant changes"	Beta = 0.094 (95% CI 0.045, 0.144)
Sakr et al. 2007b Washington Works (West Virginia) n = 454 23-yr follow-up (mean 3.7 PFOA measures) Mean age: 27 yrs (at hire) Mean duration: 27 yrs Linear mixed effects regression for repeated measures, adjusted	1.04 µg/mL (first) 1.16 µg/mL (last) Declined since 1980 (mean 4.78 µg/mL in 1980 to 1.00 µg/mL in 2001–2004)	Beta = 1.06 (95% CI 0.24, 1.88)	Beta = 0.46 (95% CI -0.87, 1.79)	Beta = 0.16 (95% CI -0.39, 0.71)	Beta = 0.79 (95% CI -5.99, 7.57)
Steenland et al. 2015 n=3,713 workers Data collected in 2005-2006 and 2008-2011 n=1,298 cases	In 2005-2006: mean 0.325 µg/mL, median 0.113 µg/mL	HR (95% CI), for self-reported use of cholesterol-lowering medications (incidence based on year of diagnosis). Cumulative exposure quartile, no lags: 1.00 (referent) 1.11 (0.94, 1.30) 1.06 (0.89, 1.27) 1.05 (0.87, 1.27) (P_{trend} = 0.56)			

Table 3-2. Summary of High-Exposure Community Studies of PFOA and Serum Lipids

Reference and Study Details	PFOA level	TC	LDL	HDL	Triglycerides
Cross-sectional					
Emmett et al. 2006 n = 371, aged 2–89 yrs, median 50 yrs (317 from stratified random population sample) Linear regression (continuous PFOA) and t-test, PFOA, abnormal versus normal cholesterol	0.354 µg/mL	Regression: Beta = 0.00551 (p = 0.27) p-value of t-test = 0.79 [n = 182, 49% abnormal]	Not measured	Not measured	Not measured
Steenland et al. 2009 n = 46,294, aged 18-80 yrs (not taking cholesterol-lowering medications) Linear regression, quartiles PFOA and continuous PFOA	Mean 0.08 µg/mL Quartiles 0- 0.0131 0.0132-0.0265 0.0266-0.0669 ≥ 0.067	Beta = 0.01112 (SE 0.00076) [log PFOA and lipids] By quartiles (OR): 1.0 (referent) 1.21 (1.12, 1.31) 1.33 (1.23, 1.43) 1.38 (1.28, 1.50)	Beta = 0.01499 (SE 0.00121) [log PFOA and lipids]	Beta = 0.00276 (SE 0.00094) [log PFOA and lipids]	Beta = 0.00169 (SE 0.00219) [log PFOA and lipids]
Frisbee et al. 2010 6,536 children 1-< 12 yrs 5,934 adolescents, 12-18 yrs Linear regression, adjusted	Mean µg/mL Children 0.0777 Adolescents 0.0618	Difference between 1st and 5th quintile PFOA (trend p): Children 5.8 mg/dl (p < 0.0001) Adolescents 4.2 mg/dl (p < 0.0001)	Difference between 1st and 5th quintile PFOA (trend p): Children 4.9 mg/dl (p = 0.001) Adolescents 3.2mg/dl (p = 0.004)	Difference between 1st and 5th quintile PFOA (trend p): Children 5.8 mg/dl (p = 0.88) Adolescents 4.2 mg/dl (p = 0.20)	Difference between 1st and 5th quintile PFOA (trend p): Children 2.0 mg/dl (p = 0.10) Adolescents 3.8 mg/dl (p = 0.10)
Longitudinal (Change in Lipid in Relation to Change in PFOA)					
Fitz-Simon et al. 2013 Longitudinal; 4.4 yrs n = 521 Linear regression of log of ratio change in serum lipid to log of ratio change in PFOA, adjusted for age, gender, interval between measures, fasting status (change in lipid in relation to change in PFOA)	0.140 µg/mL (baseline) 0.068 µg/mL (follow-up)	Percent decrease (95% CI) in lipid per halving PFOA: 1.65 (0.32, 2.97); with additional adjustment for PFOS: 0.63 (-0.88, 2.12)	Percent decrease (95% CI) in lipid per halving PFOA: 3.58 (1.47, 5.66); with additional adjustment for PFOS: 2.92 (0.71, 5.09)	Percent decrease (95% CI) in lipid per halving PFOA: 1.33 (-0.21, 2.85); with additional adjustment for PFOS: 1.24 (-0.34, 2.79)	Percent decrease (95% CI) in lipid per halving PFOA: -0.78 (-5.34, 3.58); with additional adjustment for PFOS: -1.16 (-5.85, 3.33)
Incidence of Hypercholesterolemia					
Winquist and Steenland 2014a n = 32,254 (including 3,713 workers) Data collected in 2005-2006 and 2008-2011 n = 9,653 cases in primary analysis (all diagnoses) n = 1,825 cases in prospective analysis (diagnoses after 2005-2006)	In 2005-2006: mean 0.0866 µg/mL, median 0.0261 µg/mL	HR (95% CI), self-reported use of cholesterol-lowering medications, primary analysis Cumulative exposure quintiles: 1.00 (referent) 1.24 (1.15, 1.33) 1.17 (1.09, 1.26) 1.19 (1.11, 1.27) 1.19 (1.11, 1.28) (P_{trend} = 0.005) Year exposure quintiles: 1.00 (referent) 1.07 (1.01, 1.15) 1.11 (1.04, 1.19) 1.05 (0.99, 1.13) 1.20 (1.12, 1.28) (P_{trend} = 0.001) Diagnoses after 2005: no association with PFOA with either exposure metric			

More recently, participants in the C8 Health Project were examined for an association between PFOA levels and incidence of several conditions, including high cholesterol (based on prescription medication use) (Winquist and Steenland 2014a). The cohort included 28,541 community members and 3,713 workers who had completed study questionnaires during 2008–2011. The median serum PFOA level at enrollment in 2005–2006 was 0.0261 μg/mL for the combined cohort, 0.0242 μg/mL for the community members, and 0.1127 μg/mL for the workers. Retrospective serum levels for the community cohort were estimated from air and water concentrations, residential history, and water consumption rates. For the workers, yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported adult heart disease hazard in relation to time-varying yearly or cumulative (sum of yearly estimates) estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. Using the cumulative exposure metric, the HRs for hypercholesterolemia for quintiles 2–5 versus quintile 1 were 1.24, 1.17, 1.19, and 1.19 (P_{trend} = 0.005). Using the yearly exposure metric, the HRs for high cholesterol for quintiles 2–5 versus quintile 1 were 1.07, 1.11, 1.05, and 1.20 (P_{trend} = 0.001). The strongest association was in males aged 40–59. No associations were found between PFOA level and hypertension or coronary artery disease incidence. (The analysis of these data restricted to the worker population by Steenland et al. [2015] is described in the previous section).

A subset of 290 individuals in the C8 Health Project was evaluated for evidence that PFOA exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al. 2013). RNA was extracted from whole blood samples taken from 144 males and 146 females aged 20–60 years; serum collected at the same time was used to measure PFOA concentration. The association between candidate gene expression levels and PFOA levels was assessed by multivariable linear regression with adjustments for confounders. Inverse associations were found between PFOA levels and expressions of transcripts involved in cholesterol transport (NR1H2, NPC1, and ABCG1; p = 0.002, 0.026, and 0.014, respectively). When genders were analyzed separately, PFOA was negatively associated with expression of genes involved in cholesterol transport in males (NPC1, ABCG1, PPARα) and females (NCEH1). Similar associations were found with PFOS.

General population studies. Several studies examined serum lipids in the general population (Table 3-3). Nelson et al. (2010) examined the relationship between polyfluoroalkyl chemical serum concentration, including PFOA, and lipid and weight outcomes in the general population of the United States by analyzing data from the 2003–2004 NHANES. The population (n = 860) included persons aged 20–80 years with no missing covariate information who were not pregnant, breast-feeding, taking insulin or cholesterol medicine, or undergoing dialysis. Cholesterol (TC, HDL, LDL) was measured from serum samples. Data for covariates predicting cholesterol and body weight including age, gender, race/ethnicity, socioeconomic status, saturated fat intake, exercise, alcohol consumption at ≥ 20 years of age, smoking, and parity were obtained from the questionnaires. Regression analyses were performed for gender and the age groups 12–19 years, 20–59 years, and 60–80 years. The mean PFOA concentration was 0.0046 ± 0.003 μg/mL. A positive association was found between TC and non-HDL (TC-HDL, ~70–80% TC) cholesterol and serum PFOA (effect estimate 9.8; 95% CI, -0.2–19.7). No association was found between serum PFOA concentration and HDL, or LDL. No association was found between serum PFOA concentration and body weight. Similar results were found with PFOS. A similar analysis using 1999–2008 NHANES data for 815 adolescents (aged 12–18 years) by Geiger et al. (2014a) found an association between serum PFOA and TC

(Beta 4.55, 95% CI 0.90, 8.20, per ln-unit increase in PFOA) and LDL (Beta 5.75, 95% CI 2.16, 9.33, per ln-unit increase in PFOA).

Eriksen et al. (2013) examined the association between plasma PFOA (and PFOS) levels and TC levels in a middle-aged Danish population. This cross-sectional study included 663 males and 90 females aged 50–65 years who were enrolled in the Danish Diet, Cancer and Health cohort. Generalized linear models were used to analyze the association between PFOA and TC levels, adjusted for age, gender, education, BMI, smoking, alcohol consumption, egg intake, animal fat intake, and physical activity. The mean plasma PFOA level was 0.0071 μg/mL. A significant, positive association was found between PFOA (and PFOS) and TC such that, in the fully adjusted model, a 4.4-mg/dL (95% CI 0.8, 8.5) higher concentration of TC was found per interquartile range of plasma PFOA (quartile cut-points were not reported).

Fisher et al. (2013) examined the association of plasma PFAS levels, including PFOA, with metabolic function and plasma lipid levels. This population-based sample included 2,700 participants aged 18–74 years (~50% male) in the Canadian Health Measures Survey. The geometric mean PFOA concentration was 0.0025 ± 0.0018 μg/mL. In analyses that included sampling weights, no associations were found between PFOA (or PFOS) and TC, HDL- and LDL-cholesterol, and metabolic syndrome and glucose homeostasis parameters. Covariates considered included age, gender, marital status, income adequacy, race, education, BMI, physical activity, smoking, and alcohol consumption.

Starling et al. (2014) examined the association between PFOA (and six other PFASs) and serum lipids in pregnant females in the Norwegian Mother and Child Cohort Study. Most of the blood samples were drawn during weeks 14–26 of gestation. Weighted multiple linear regression was used to estimate the association between PFOA level and each lipid level. Covariates considered included age, prepregnancy BMI, nulliparous or interpregnancy interval, breast-feeding duration, education, current smoking, gestation week at blood draw, oily fish consumption, and weight gain during pregnancy. The median plasma PFOA level was 0.00225 μg/mL. No association was observed between PFOA and triglycerides, TC, or LDL-cholesterol. PFOA was positively associated with HDL-cholesterol, although the CI was large for the association. With HDL-cholesterol, each interquartile range- (IQR-) unit increase in ln-PFOA was associated with an increase of 1.28 mg/dL (95% CI: -0.15, 2.71). Five of the seven PFASs studied were positively associated with HDL cholesterol and all seven had elevated HDL associated with the highest quartile.

Table 3-3. Summary of General Population Epidemiology Studies of PFOA with Serum Lipids

Reference and Study Details	PFOA level	TC	LDL	HDL	Triglycerides
All Adults					
Nelson et al. 2010 United States, NHANES (2003–2004) n = 860, aged 20-80 yrs (451 men, 409 women) Linear regression, PFOA in quartiles and continuous PFOA, adjusted PFOA-PFOS correlation Spearman r = 0.65	Mean 0.0046 µg/mL, median 0.0038 µg/mL	Beta = 1.22 (95% CI 0.04, 2.40) 9.8 mg/dl increase in top versus bottom quartile (PFOS results similar)	Beta = -0.21 (95% CI -1.91, 1.49) (PFOS results similar)	Beta = -0.12 (95% CI -0.41, 0.16) Different pattern seen with PFOS	Not measured
Eriksen et al. 2013 Denmark n = 753, aged 50-65 yrs (663 men, 90 women) Linear regression, continuous PFOA, adjusted PFOA-PFOS correlation not reported	Mean 0.0071 µg/mL	4.4 mg/dl increase per IQR (PFOS results similar)	Not measured	Not measured	Not measured
Fisher et al. 2013 Canada, Canadian Health Measures Survey n = 2,700, aged 18-74 yrs Linear regression, continuous PFOA (log-transformed PFOA and lipids) PFOS correlation r = 0.36	Mean 0.0025 µg/mL Quartiles: 0.00015-0.00185 0.00186-0.00258 0.00259-0.00355 ≥ 0.0036	Beta = 0.03 (95% CI -0.017, 0.07) [log PFOA and lipids]	Beta = 0.02 (95% CI -0.06, 0.091) [log PFOA and lipids]	Beta = 0.0009 (95% CI -0.04, 0.04) [log PFOA and lipids]	Not measured
Pregnant Women					
Starling et al. 2014 Norway n = 891 pregnant women Plasma PFOA (collected in 2nd trimester) Linear regression, continuous PFOA (log-transformed PFOA), adjusted PFOS correlation Spearman r = 0.64	Median 0.00225 µg/mL	Beta = 2.58 (95% CI -4.32, 9.47) [per ln-unit increase in PFOA]	Beta = 2.25 (95% CI -3.97, 8.48) [per ln-unit increase in PFOA]	Beta = 2.13 (95% CI -0.26, 4.51) [per ln-unit increase in PFOA]	Beta = 0.00 (95% CI -0.07, 0.06) [per ln-unit increase in PFOA]
Adolescents					
Geiger et al. 2014a United States, NHANES (1999-2008) n = 815, aged 12-18 yrs Linear regression, continuous PFOA (log-transformed PFOA), adjusted	Mean 0.0042 µg/mL	Beta = 4.55 (95% CI 0.90, 8.20) [per ln-unit increase in PFOA] (PFOS results similar)	Beta = 5.75 (95% CI 2.16, 9.33) [per ln-unit increase in PFOA] (PFOS results similar)	Beta = -1.52 (95% CI -3.02, -0.03 [per ln-unit increase in PFOA] Attenuated results when adjusted for PFOS	Beta = 1.74 (95% CI -2.88, 6.36) Different pattern seen with PFOS

The association between PFOA and serum lipids has been examined in several studies in different populations. Cross-sectional and longitudinal studies in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b; Steenland et al. 2015) and in the high-exposure community (the C8 Health Project study population) (Fitz-Simon et al. 2013; Frisbee et al 2010; Steenland et al. 2009; Winquist and Steenland 2014a) generally observed positive associations between serum PFOA and TC in adults and children (aged 1–< 18 yrs); most of the effect estimates were statistically significant. Although exceptions to this pattern are present (i.e., some of the analyses examining incidence of self-reported high cholesterol based on medication use in Winquist and Steenland [2014a] and in Steenland et al. [2015]), the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (with means varying between 0.4 and > 12 μg/mL), and the mean serum levels in the C8 population studies were around 0.08 μg/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 0.002–0.007 μg/mL (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014). The interpretation of these general population results is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and PFOA.

3.1.1.2 Cardiovascular Diseases

Occupational exposure studies. Several studies examined cardiovascular-related cause of death among PFOA-exposed workers at the West Virginia Washington Works plant (Leonard et al. 2008; Sakr et al. 2009; Steenland and Woskie 2012) and the 3M Cottage Grove plant in Minnesota (Lundin et al. 2009; Gilliland and Mandel 1993). This type of mortality is of interest because of the relation between lipid profiles (e.g., LDL) and the risk of cardiovascular disease. The most recent West Virginia study included 5,791 individuals who had worked at the plant for at least 1 year between 1948 and 2002, with mortality follow-up through 2008. No associations were found between cumulative PFOA levels and ischemic heart disease (IHD) mortality (standardized mortality ratio [SMR] 1.07, 1.02, 0.87, and 0.93 across four quartiles of cumulative exposure, compared to U.S. referent group). Based on these data from the worker cohorts, the C8 Science Panel (2012) concluded that there is no probable link between PFOA and stroke and coronary artery disease.

The analysis of the Minnesota plant (n = 3,993 workers who began work between 1983 and 1997, with follow-up through 2002) also found no association between cumulative PFOA exposure and IHD risk, but an increased risk of cerebrovascular disease mortality was seen in the highest exposure category (HR 2.1, 95% CI 1.0, 4.6). These studies are limited by the reliance on mortality (rather than incidence) data, which can result in a substantial degree of under ascertainment and misclassification.

3.1.1.3 Liver Enzymes and Liver Disease

Cross-sectional studies and longitudinal studies of PFOA and liver enzymes in various populations are described in this section and summarized in Table 3-4.

Table 3-4. Summary of Epidemiology Studies of PFOA and Liver Enzymes

Reference and Study Details	PFOA level	Results
Cross-sectional: Occupational Exposure Studies		
Olsen et al. 2000 n = 111 in 1993, 80 in 1995, 74 in 1997; 50-70% participation rate Mean age: ~ 40 yrs Mean duration: not reported ANOVA and linear regression adjusted for age, BMI, and alcohol and cigarette use	Mean (range) µg/mL 1993: 5 (0-80) 1995: 6.8 (0-114) 1997: 6.4 (0.1-81)	ALT: Year β (±SE) (p value) 1993: 0.89 (2.88) (p = 0.76) 1995: 0.81 (2.62) (p = 0.75) 1997: 2.77 (1.27) (p = 0.03) Change per 10 µg/mL increase in serum PFOA; stronger association in individuals with BMI< 30. No associations for ALP, GGT, AST, total bilirubin, direct bilirubin.
Olsen and Zobel 2007 3M. Antwerp, Cottage Grove, Decatur combined; 50-65% participation rate n = 506 (men, not taking lipid-lowering medications) Mean age: 40 yrs Mean duration: not reported Linear regression adjusting for ln age, ln BMI, ln alcohol [Related reference: Olsen et al. 2003)	Mean (range) 2.21 (0.01 – 92.03) µg/mL	β change per ln PFOA (±SE) p value ln ALP: All 0.009 (± 0.008) (p = 0.25) Decatur: 0.08 (0.34) (p = 0.02) ln AST: All -0.005 (± 0.009) (p = 0.55) Decatur: 0.011 (0.02) (p = 0.57) ln ALT: All 0.025 (± 0.013) (p = 0.06) Decatur: 0.08 (0.034) (p = 0.02) ln GGT: All 0.033 (± 0.017) (p = 0.05) Decatur: 0.08 (0.034) (p = 0.02) ln total bilirubin: All -0.033 (± 0.01) (p = 0.001) Decatur: -0.054 (± 0.021) (p = 0.01) Replacement of ln BMI with triglycerides in the model resulted in reduced associations for ALT and GGT.
Sakr et al. 2007a Washington Works (West Virginia) n = 1025 (782 men, 243 women), 55% participation rate Mean age: 46.5 and 44.4 yrs, respectively for men and women Mean duration: 19.6 and 15.9 yrs, respectively for men and women Linear regression, adjusting for age, BMI, alcohol consumption, gender, history of heart attack in parent, use of lipid-lowering medications	0.428 µg/ml LOQ 0.0005 µg/ml range 0.005 – 9.55 µg/mL	β (±SE) p value: Full sample / Excluding 178 men on lipid-lowering medications ln AST: 0.012 (± 0.012) (p = 0.317) / ln AST: 0.023 (±0.013) (p = 0.079) ln ALT: 0.023 (± 0.015) (p = 0.124) / ln ALT: 0.031 (±0.017) (p = 0.071) ln GGT: 0.048 (± 0.02) (p = 0.016) / ln GGT: 0.05 (±0.023) (p = 0.03) ln bilirubin: 0.008 (± 0.014) (p = 0.59) / ln bilirubin: 0.1 (±0.017) (p = 0.637)
Costa et al. 2009 Italy Cross-sectional 56 male workers (currently and formerly exposed and unexposed) with concurrent serum PFOA and clinical parameters measured in last 7 yrs GEE models adjusting for age, years of exposure, year of PFOA sampling, BMI, smoking, and alcohol consumption	Currently exposed: mean 12.9, geometric mean 4.02, range 0.2-47 Formerly exposed: mean 6.81 geometric mean 3.76, range 0.53-18 µg/mL	β change per µg PFOA/mL (95% CI) AST: 0.038 (-0.003, 0.080) ALT: 0.116 (0.054, 0.177) GGT: 0.177 (0.076, 0.278) ALP: 0.057 (0.007, 0.107) Total bilirubin: -0.080 (-0.137, -0.024) Conj. bilirubin: -0.034 (-0.09, 0.031)

Reference and Study Details	PFOA level	Results
Longitudinal: Occupational Exposure Studies		
Olsen et al. 2003 3M, Antwerp and Decatur combined ~5 yr follow-up period n = 174 (measure in 1995 or 1997, and in 2000) Mean age: not reported Mean duration: not reported Linear mixed effects regression for repeated measures, adjusted	1995 baseline: 1.36-1.41 µg/mL 2000 follow-up: 1.49-1.77 µg/mL	No associations observed; however, data not provided
Sakr et al. 2007b Washington Works (West Virginia) n = 454 23-yr follow-up (mean 3.7 PFOA measures) Mean age: 27 yrs (at hire) Mean duration: 27 yrs Linear mixed effects regression for repeated measures, adjusted	1.04 µg/mL (first) 1.16 µg/mL (last) Used PFOA measurement from same year as biomarker test or interpolated using two surrounding values	β IU/L change per 1 µg/mL PFOA (95% CI) ALP: (n = 1327) -0.21 (-0.60, 0.18) AST: (n = 1326) 0.35 (0.10, 0.60) ALT: (n = 231) 0.54 (-0.46, 1.54) GGT: (n = 233) 1.24 (-1.09, 3.57) Total bilirubin: (n = 1327) 0.008 (-0.0139, -0.0021)
Cross-sectional: High-Exposure Community Studies		
Emmett et al. 2006 n= 371, aged 2–89 yrs, median 50 yrs (317 from stratified random population sample) Linear regression (continuous PFOA) and t-test, PFOA, abnormal versus normal enzyme levels	Median 0.354 µg/mL (IQR 0.184 – 0.571 µg/mL); nonfasting blood sample	Linear regression, Beta (p-value) n (%) abnormal,(t-test p-value) ALP: -0.00416 (p = 0.65) 6 (2%) (p = 0.63) AST: -0.0007586 (p = 0.76) 9 (2%) (p = 0.03) ALT: -0.00183 (p = 0.65) 28 (8%) (p = 0.30) GGT: 0.00057711 (p = 0.89) 11 (3%) (p = 0.50)
Gallo et al. 2012 West Virginia, United States; C8 Health Project, 46,452 of 56,554 (82.1%) adults Adjusting for age, gender, physical activity, BMI, average household income, educational level, fasting status, month of blood sample collection, race, insulin resistance, alcohol consumption, and cigarette smoking	Median 0.028 µg/mL (IQR 0.135 – 0.71 µg/mL) nonfasting blood sample; LOD 0.0005 µg/mL, n = 32 below LOD	Linear regression, Logistic regression of abnormal values β per 1 unit increase PFOA (95% CI) OR (95% CI) Ln ALT: 0.022 (0.018 – 0.025) ALT: 1.10 (1.07, 1.13) ln GGT: 0.015 (0.01 – 0.019) GGT: 1.01 (0.99, 1.04) n Direct (conjugated) bilirubin: 0.001 (-0.002 – 0.004) Direct bilirubin 0.97 (0.90, 1.05) Analysis of Ln ALT or Ln GGT by decile showed increase from 0.005 to 0.030 µg/mL, then leveling; p value for trend < 0.001; Direct bilirubin showed a U-shaped relation increasing to 0.030 µg/mL, then declining.
Cross-sectional: General Population Studies		
Lin et al. 2010 United States, NHANES (1999-2000; 2003-2004) 1,076 men, 1,140 women of 10,224 enrolled, excluding < 6-hr fast n = 1,802), and missing covariate or no serum PFOA or liver function of metabolic syndrome data Adjusting for age, gender, race/ethnicity, smoking, alcohol consumption, education level, BMI, metabolic syndrome, and iron saturation status	Geometric mean 0.00505 µg/mL 0.00406 µg/mL; 0.4% of samples below LOD (LOD 0.0002 and 0.0001 µg/mL in 1999-2000 and 2003-2004)	β per unit (ng/ml) increase in log serum PFOA (95% CI) ALT: (U/l) 1.86 (1.24, 2.48) Log GGT: (U/l) 0.08 (0.05, 0.11) β per unit (ng/ml) increase in log serum PFOA (95% CI); Same model as above, also controlling for other PFASs ALT: (U/l) 2.19 (1.4, 2.98) Log GGT: (U/l) 0.15 (0.11, 0.19)

Liver Enzymes

Occupational exposure studies. Olsen et al. (2000) analyzed alkaline phosphatase (ALP), GGT, aspartate aminotransferase (AST), ALT, and total- and direct bilirubin data from voluntary medical surveillance examinations of PFOA production workers at a 3M plant in 1993, 1995, and 1997. No association was observed between serum PFOA concentration and the parameters explored in cross-sectional analyses in the workers in 1993 or 1995; although in 1997 increases in AST per unit increase in serum PFOA concentration were observed. When measurements for all years were combined in longitudinal analyses (Olsen et al. 2003), the authors reported that no associations were observed with serum PFOA levels. Other than the analyses of AST, however, quantitative results were not provided.

A subsequent analysis involving these fluorochemical workers and an additional plant (Cottage Grove, Minnesota) that used medical surveillance data collected in 2000 examined the association between serum PFOA concentration and liver enzymes (Olsen and Zobel 2007). Serum samples were analyzed for ALP, GGT, AST, ALT, and bilirubin concentrations. Ln serum PFOA was marginally associated with ln ALT and ln GGT in regression models adjusting for ln age, ln BMI, and ln alcohol consumption, although the association was reduced when ln triglycerides replaced ln BMI in the model. An inverse association between total bilirubin and serum PFOA concentration ($p<0.05$) was observed at all sites combined.

Sakr et al. (2007a) examined the relationship between serum PFOA and several clinical chemistry parameters in workers at the Washington Works plant in West Virginia. A complete blood count, metabolic panel (glucose, blood urea nitrogen [BUN], creatinine, iron, uric acid, electrolytes, creatinine kinase, lactic dehydrogenase [LDH], ALP, protein, albumin, C-reactive protein), liver enzyme panel (AST, ALT, GGT, bilirubin), and serum PFOA concentration were determined from the blood samples. Serum PFOA was associated ($p<0.05$) with increasing GGT in all of the participating workers. It was stated that an association was observed between serum PFOA concentration and iron, LDH, calcium, and potassium, but quantitative results were not included and the direction of association was not specified.

Costa et al. (2009) also examined associations between serum PFOA concentration and liver enzymes in workers at a fluorochemical production plant in Italy. Serum PFOA concentration was associated with increasing ALT, GGT, and ALP levels ($p<0.05$), and inversely associated with total bilirubin ($p<0.01$) in 56 workers with PFOA and liver enzymes measured concurrently over the last 7 years. This subset of 56 workers included currently, formerly, and never exposed.

Sakr et al. (2007b) also conducted a longitudinal study of liver enzymes among workers at the Washington Works plant with two or more PFOA measurements as described previously. Hepatic clinical chemistry (GGT, AST, ALT, ALP, total bilirubin), height, and weight data were analyzed. Serum PFOA concentration was associated in the model with increasing AST levels ($p = 0.009$) and inversely associated with total bilirubin ($p = 0.006$) after adjustment for age, BMI, gender, and decade of hire. No association was observed between serum PFOA concentration and GGT, ALT, and ALP. The regression models did not adjust for alcohol consumption, a potential limitation.

High-exposure community studies. A small study (n = 371) of residents of the Little Hocking water district in Ohio found inconsistent results in different analyses of liver enzymes: An association with AST but not with ALP or ALT was seen when comparing serum PFOA levels between groups with abnormal compared to normal enzyme levels, but no association with any

enzyme was seen in regression analyses with PFOA as a continuous variable (Emmett et al. 2006). A subsequent study, which included a wider set of communities in the contaminated area, investigated the correlation between serum PFOA levels and liver enzymes in a total of 47,092 samples collected from members enrolled in the C8 Health Project (Gallo et al. 2012). The association of ALT, GGT, and direct bilirubin with PFOA was assessed using linear regression models adjusted for various confounders. The median PFOA level was 0.028 μg/mL. The ln-transformed values of ALT were significantly associated with ln-PFOA (and PFOS). There was a steady increase in fitted levels of ALT per decile of PFOA, leveling off after approximately 0.030 μg PFOA/mL. Fitted values of GGT by deciles of PFOA showed a slight positive trend when adjusted for insulin resistance and BMI, but this was not confirmed in the logistic model analysis of elevated enzyme levels. Direct bilirubin levels appeared to increase at lower concentrations and then decline in a U-shaped pattern at 0.030 μg PFOA/mL.

General population studies. Lin et al. (2010) investigated the association between serum PFOA (plus three other PFASs) and liver enzymes in the adult population of the United States by analyzing data from the 1999–2000 and 2003–2004 NHANES. The study population included 2,216 adults (1076 males, 1140 females) older than 20 years who were not pregnant or nursing; had fasted more than 6 hours at the time of examination; were negative for hepatitis B or C virus; had body weight, height, educational attainment, and smoking status data available; and had serum tests for PFAS, liver function, or the five physiological measures associated with metabolic syndrome. Regression models were used to analyze the data and adjust for confounders. Mean PFOA levels were 0.00505 μg/mL and 0.00406 μg/mL for males and females, respectively. Serum PFOA concentration was divided into quartiles (Q1 = ≤ 0.0029; Q2 = ≤ 0.0042; Q3 = ≤ 0.00595; Q4 = > 0.00595 μg/ml). In the univariate regression models, liver enzymes, serum ALT, and log-GGT increased across quartiles of PFOA ($p \leq 0.012$), but total bilirubin showed no trend. The linear regression models were adjusted for (1) age, gender, and race/ethnicity; (2) age, gender, race/ethnicity, and lifestyle (smoking status, drinking status, education level), and (3) additional data for BMI, metabolic syndrome biomarkers, iron saturation status, and insulin resistance. An association was found between serum log-PFOA concentration and increasing serum ALT and log-GGT. One unit increase in serum log-PFOA was associated with an increase of 1.86 units in serum ALT measurements and a 0.08-unit increase in log-GGT measurements. Effect modification was seen: For example, stronger associations between serum PFOA concentration and serum ALT (or GGT) were found among non-Hispanic Caucasians. PFOS also was positively associated with ALT in the fully adjusted model.

The results of the occupational studies provide evidence of an association with increases in serum AST, ALT, and GGT, with the most consistent results seen for ALT. The associations were not large and they might depend on the covariates in the models such as BMI, use of lipid-lowering medications, and triglycerides (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b). Two population-based studies of highly exposed residents in contaminated regions near a fluorochemical industry in West Virginia have evaluated associations with liver enzymes, and the larger of the two studies reported associations of increasing serum ln ALT and ln GGT levels with increasing serum PFOA concentrations (Emmett et al. 2006; Gallo et al. 2012). A cross-sectional analysis of data from NHANES, representative of the U.S. national population, also found associations with ln PFOA concentration with increasing serum ALT and ln GGT levels. Serum bilirubin was inversely associated with serum PFOA in the occupational studies. A U-shaped exposure-response pattern for serum bilirubin was observed among the participants in the C8 Health Project, which might explain the inverse associations reported for occupational cohorts. Overall, an association of

serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational and highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential of PFOA to affect liver function.

Liver Diseases

High-exposure community studies. Few studies of the relationship between PFOA and liver disease are available, but the C8 Health Project did not observe associations with hepatitis, fatty liver disease, or other types of liver disease in their initial studies. The most recent update of disease incidence in the workers identified 35 cases of nonhepatitis liver disease (with medical validation) (Steenland et al. 2015); no association was seen with cumulative exposure when analyzed without a lag (HRs by quartile 1.0, 0.58, 1.43, 1.20; trend p = 0.86 for log cumulative exposure), but there was a possible trend in the analysis using a 10-year lag (HRs by quartile 1.0, 1.46, 2.13, and 2.02; trend p = 0.40).

3.1.1.4 Biomarkers of Kidney Function and Kidney Disease

Kidney Function

PFOA has the potential to affect the kidney's function of tubular resorption because of it uses tubular transporters for excretion and resorption (see section 2.4). Since PFOA is removed from the blood by the kidney, cross-sectional analyses using serum PFOA as the exposure measure are problematic if individuals with compromised kidney function are included: PFOA concentrations could be increased in those individuals and an apparent association with GFR would be observed, even if one did not exist. Studies examining measures of kidney function are described in this section and summarized in Table 3-5.

Table 3-5. Summary of Epidemiology Studies of PFOA and Measures of Kidney Function

Reference and Study Details	PFOA Level	Results
Sakr et al. 2007a Washington Works plant Cross-sectional; all active, nonpregnant employees enrolled over 12 days in 2004 1,025 of 1,863 eligible (55%)	0.428 µg/ml LOQ 0.0005 µg/ml range 0.005–9.55 µg/mL	Reported association with uric acid but quantified results were not provided
Costa et al. 2009 Italy Cross-sectional 56 male workers (currently and formerly exposed and unexposed) with concurrent serum PFOA and clinical parameters measured in last 7 yrs	Currently exposed: mean 12.9, geometric mean 4.02, range 0.2–47 µg/mL Formerly exposed: mean 6.81 geometric mean 3.76, range 0.53–18 µg/mL	β change per µg PFOA/mL (95% CI) Uric acid 0.026 (0.001, 0.053) GEE models adjusting for age, years of exposure, year of PFOA sampling, BMI, smoking, and alcohol consumption

Reference and Study Details	PFOA Level	Results
Steenland et al. 2010 C8 Health Project, West Virginia Cross-section; adult subjects (n = 53,458; 20–≥80 years of age) participating in the C8 Health Project from 2005-2006 Subjects had consumed water for at least 1 year prior to 2004	0.0864 µg/mL, measured in 2005–2006	Increased predicted uric acid of 0.2–0.3 µg/dL with increasing deciles of PFOA or PFOS
Shankar et al. 2011 United States, NHANES Uric acid analysis: 1999–2000, 2003–2004 and 2005–2006 cycles 3,883 out of 3,974 participants ≥ 20 years of age with serum PFOA measurements; excluded subjects with missing data (n = 91); 48.3% male, mean age 46.4 years eGFR analysis: 1999–2000, 2003–2004, 2005–2006, and 2007–2008 cycles 4,587 out of 5,717 (80%) eligible 20 years or older with PFOA measures; excluded self-reported CVD (n = 572), missing data on serum creatinine or covariates (n = 558)	0.0059 µg/mL; LOD 0.1 ng/mL Quartiles, µg/mL, n 1 < 0.0028 µg/mL, 1,176 2 0.0028–0.0041 µg/mL, 1,141 3 0.0042–0.0059 µg/mL, 1,141 4 > 0.0059 µg/mL, 1,129	Mean change in uric acid, mg/dL (95% CI) by quartile 1 referent 2 0.14 (0.04–0.25) 3 0.37 (0.25–0.49) 4 0.44 (0.32–0.56), p trend 0.0001 Mean change in uric acid, mg/dL (95% CI) by ln PFOA: 0.22 (0.15–0.30) Multivariate regression adjusting for age, gender, race/ethnicity, education, smoking, alcohol consumption, BMI, hypertension, diabetes, and serum total cholesterol Hyperuricemia risk by quartile, OR (95% CI) 1 referent 2 1.14 (0.78–1.67) 3 1.90 (1.35–2.69) 4 1.97 (1.44–2.70), p trend 0.0001 Hyperuricemia risk per unit increase in ln PFOA, OR (95% CI): 1.43 (1.16–1.76) Logistic regression adjusting for age, gender, race/ethnicity, education, smoking, alcohol consumption, BMI, hypertension, diabetes, and serum total cholesterol Chronic kidney disease defined as eGFR < 60 mL/min/1.73 m^2 Quartile, OR (95% CI) 1 referent 2 0.83 (0.55–1.24) 3 1.24 (0.75–2.05) 4 1.73 (1.04–2.88) Logistic regression adjusting for age, gender, race/ethnicity, education, smoking, alcohol consumption, BMI, systolic blood pressure, diastolic blood pressure, diabetes, serum TC, and glycohemoglobin Adjustment for PFOS did not alter association with PFOA Multivariate regression of association PFOA with eGFR among subjects with and without chronic kidney disease β (SE) with -1.6 (0.8) and without -2.8 (0.6) chronic kidney disease

Reference and Study Details	PFOA Level	Results
Watkins et al. 2013 West Virginia Cross-sectional population-based survey, residents near the Washington Works plant (C8 Health Project) 9,660 (children < 18 yrs) out of 9,783 eligible with complete data for serum creatinine, height, and serum PFOA	Median measured PFOA 0.0283 µg/mL; range 0.0007–2.071; yearly serum PFOA estimated for each individual from model used to predict serum PFOA at time of enrollment, historical serum PFOA during the first 10 years of life, 3 years before enrollment or at birth	β (95% CI) change in unit eGFR (mL/min/1.73 m^2) per ln serum PFOA, -0.75 (-1.41–-0.010) Linear regression adjusting for age, gender, race, smoking, and household income; additional adjustment for regular exercise, BMI z-score, and TC did not alter association No associations of predicted serum PFOA (modeled) with eGFR

Uric Acid (risk factor for hypertension)

Occupational exposure studies. Costa et al. (2009) examined associations between serum PFOA concentration and uric acid levels in serum in workers at a fluorochemical production plant in Italy. Serum PFOA concentration was associated with uric acid levels ($p<0.05$) in 56 workers assessed concurrently over the previous 7 years. This subset of 56 workers included currently, formerly, and never exposed with relatively high serum PFOA concentrations.

High-exposure community studies. Steenland et al. (2010) examined the association of serum PFOA concentrations with uric acid levels in adult subjects (n = 53,458; 20–≥80 years) participating in the C8 Health Project from 2005–2006. The reference range for uric acid is 2.0–8.5 mg/dL. Serum samples were separated into deciles or quintiles for analysis. The data were analyzed by linear and logistic regression with uric acid as the outcome and PFOA as the exposure variable. Covariates of the model included age, gender, BMI, education, smoking, alcohol consumption, and serum creatinine. The mean serum PFOA concentration was 0.0864 µg/mL. The mean uric acid level was 5.58 mg/dL with an IQR of 4.5–6.6 mg/dL. The increase in uric acid from lowest to highest serum PFOA concentration decile was 0.2–0.3 mg/dL. The OR for high serum uric acid levels increased from the lowest to the highest quintile of PFOA serum concentrations: 1.00, 1.33 (95% CI: 1.24–1.43), 1.35 (95% CI: 1.26–1.45), 1.47 (95% CI: 1.37–1.58), and 1.47 (95% CI: 1.37–1.58). The study showed that higher serum PFOA concentrations were associated with higher incidence of high serum uric acid levels. The serum of C8 study participants included several PFASs; PFOA appeared to have a greater influence on uric acid trends than PFOS in the models employed by Steenland et al. (2010).

The C8 Science Panel (2012) combined the data from the C8 general population cohort with data from worker cohorts and concluded that there is no probable link between PFOA and stroke, hypertension, and coronary artery disease. The general population cohorts were not evaluated separately in these analyses.

General population studies. Shankar et al. (2011) investigated the association between serum PFOA (and PFOS) and uric acid concentration in the adult population of the United States by analyzing data from the 1999–2000, 2003–2004, and 2005–2006 NHANES evaluations. The study population included 3,883 adults (48.3% male) older than 20 years with data available for serum PFOA, plasma uric acid, and important covariates. Regression models were used to analyze associations with serum PFOA as a continuous variable and in quartiles. Logistic regression models analyzed risk for hyperuricemia defined as plasma uric acid > 6.8 mg/dL in males and > 6.0 mg/dL in females. Ln PFOA concentration was associated with increasing uric

acid concentration in multivariate models adjusting for age, gender, race/ethnicity, education, smoking, alcohol consumption, BMI, hypertension, diabetes, and serum TC. Mean uric acid concentration increased by 0.22 (95% CI 0.15–0.30) mg/dL per unit change in ln PFOA. A concentration-response relationship was indicated across all quartiles. In addition, an elevated hyeruricemia risk was observed with increasing serum PFOA concentration (OR 1.43, 95% CI 1.16–1.76).

Glomerular Filtration Rate

High-exposure community studies. Watkins et al. (2013) evaluated the cross-sectional association between PFOA exposure and kidney function among children aged 1<18 years (mean 12.4 ± 3.8 years) enrolled in the C8 Health Project. A total of 9,660 participants had data available on serum PFOA (median 0.0283 μg/mL), as well as serum creatinine and height, which were used to calculate an estimated glomerular filtration rate (eGFR). Linear regression was used to evaluate the association between quartiles of measured serum PFOA concentration and eGFR. A shift from the lowest to the highest quartile of measured, natural log-transformed concentrations of PFOA in serum [IQR ln (PFOA) = 1.63] was associated with a decrease in eGFR of 0.75 mL/min/1.73 m^2 (95% CI: –1.41, –0.1; p = 0.02) adjusting for age, gender, race, smoking status, and household income. With increasing quartile of serum PFOA concentrations, eGFR decreased monotonically, although the change was slight and did not attain statistical significance (p for trend across quartiles = 0.30). PFOS also was associated with a decrease in eGFR and showed a dose-related trend. Modeled predicted serum PFOA and PFOS concentrations were not associated with eGFR.

General population studies. Shankar et al. (2011) also used data from the NHANES to determine whether there was a relationship between serum PFOA levels and chronic kidney disease defined as eGFR (determined from serum creatinine) of less than 60 mL/min/1.73 m^2. Serum PFOA levels were categorized into quartiles: Q1 = <0.0028 μg/mL; Q2 = 0.0028–0.0041 μg/mL; Q3 = 0.0042–0.0059 μg/mL; Q4 = >0.0059 μg/mL. The adjusted OR for chronic kidney disease for individuals in Q4 was 1.73 (95% CI: 1.04, 2.88; p for trend = 0.015) compared with individuals in Q1. The logistic regression model was adjusted for age, gender, race/ethnicity, education, smoking, alcohol consumption, BMI, systolic blood pressure, diastolic blood pressure, diabetes, serum TC, and glycohemoglobin. Although a similar increase in OR was seen for PFOS, additional adjustment for serum PFOS did not alter the association with PFOA. In addition, the inverse association of eGFR with serum PFOA was observed over all quartiles of PFOA, as well as among individuals both with and without chronic kidney disease. Although the possibility of reverse causality could not be excluded, the association between serum PFOA and eGFR among participants without chronic kidney disease suggests a PFOA-related effect on kidney function.

Overall, studies of occupational cohorts (Costa et al. 2009), a highly exposed community (Steenland et al. 2010; Watkins et al. 2013), and the U.S. general population (Shankar et al. 2011) that evaluated uric acid levels or eGFR as a measure of kidney function found associations with decreased function, although reverse causality as an explanation cannot be ruled out. Since the URAT transporter functions in the renal resorption of PFOA, the increase in serum uric acid could be a reflection of systemic transport pharmacodynamics rather than formation biochemistry.

Kidney Disease

The occupational mortality studies have produced generally negative results with respect to the association between PFOA and mortality due to chronic kidney disease (Steenland et al. 2015; Steenland and Woskie 2012; Raleigh et al. 2014). The most recent update of incidence of chronic kidney disease in the workers in the C8 West Virginia community identified 43 cases (with medical validation) (Steenland et al. 2015); no association was seen with cumulative exposure when analyzed without a lag (HRs by quartile 1.0, 0.50, 0.69, 0.67; trend p = 0.92 for log cumulative exposure), or using a 10-year lag (HRs by quartile 1.0, 1.32, 0.50, and 0.67; trend p = 0.99).

In 2012, the C8 Science Panel concluded that there is no probable link between PFOA and chronic kidney disease. Their conclusion was based on findings in combined general population and worker cohorts, data on children enrolled in the C8 Health Project, and published data from NHANES.

3.1.1.5 Immunotoxicity

Immune suppression

Immune function—specifically immune system suppression—can affect numerous health outcomes, including risk of common infectious diseases (e.g., colds, flu, otitis media) and some types of cancer. The World Health Organization (WHO) guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications (WHO 2012).

Associations between prenatal PFOA exposure and risk of infectious diseases (as a marker of immune suppression) were not seen in two studies, although there was some indication of effect modification by gender (i.e., associations seen in females but not in males). Fei et al. (2010a) examined hospitalizations for infectious diseases in early childhood in a Danish birth cohort. Mean maternal PFOA concentration was 0.0056 µg/mL. A slightly higher risk for hospitalizations was observed in females with higher maternal PFOA concentrations (incidence rate ration [IRR] = 1.00, 1.20, 1.63, 1.74 for Q1, Q2, Q3, and Q4, respectively), and the risk for males was below 1.0 for each quartile. Overall, there was no association between hospitalizations due to infectious diseases and maternal PFOA exposure; similar results were found with PFOS.

Okada et al. (2012) examined history of otitis media (and of allergic conditions) in children up to the age of 18 months. Mean maternal PFOA concentration was 0.0014 µg/mL. Cord blood immunoglobulin E (IgE) level decreased significantly with high maternal PFOA concentration among female infants, but not male infants. No significant associations were observed between maternal PFOA levels (and PFOS) with the incidence of otitis media (or specific types of allergies or wheeze). Two other studies, described below, examined reported history of colds and gastroenteritis in children up to age 3 years (Granum et al. 2013) or colds and flu in adults (Looker et al. 2014). Granum et al. (2013) observed associations between prenatal PFOA exposure and frequency of colds or gastroenteritis episodes, but not with a variable based on “ever had” this condition in the past year. Looker et al. (2014) did not observe associations between serum PFOA and “ever had” or frequency of colds or flu in the past year.

In 2012, the C8 Science Panel (2012) concluded that there is no probable link between PFOA and common infections, including influenza, in children or adults. The panel based their

conclusions on a subset of adult members of the cohort, a subset of mother-child pairs, and published data from other researchers.

Three studies have examined response to one or more vaccine (e.g., measured by antibody titer) in relation to higher exposure to PFOA in children (Grandjean et al. 2012; Granum et al. 2013) or adults (Looker et al. 2014); the latter study was conducted in the high-exposure C8 community population (Table 3-6).

Table 3-6. Summary of Epidemiology Studies of PFOA and Immune Suppression (Vaccine Response)

Reference and Study Details	PFOA Level	Results
High-Exposure Community: Adults		
Looker et al. 2014 C8 Health Project, West Virginia 2005-2006 enrollment and baseline blood sample and questionnaires; 2010 follow-up n = 411 with prevaccination blood sample – flu vaccination – 21-day post vaccination blood sample Linear regression: antibody titer rise Logistic regression: seroconversion and seroprotection	Median 0.032 µg/mL Q1: 0.0025-0.0137 Q2: 0.0138 – 0.0315 Q3: 0.0316 – 0.0903 Q4: 0.0904 – 2.14	(Percentage positive) OR (95% CI), by influenza strain: Seroconversion (fourfold increase in antibody titer) / Seroprotection (antibody titer 1:40 following vaccine) Influenza B (62%) (66%) PFOA continuous 0.80 (0.53, 1.21) 1.04 (0.68, 1.60) Q1 1.0 (referent) 1.0 (referent) Q2 1.43 (0.76, 2.70) 0.76 (0.40, 1.45) Q3 1.39 (0.73, 2.66) 1.13 (0.57, 2.23) Q4 0.71 (0.38, 1.36) 0.77 (0.39, 1.50) A/H1N1 (84%) (96%) PFOA continuous (84%) (96%) Q1 1.0 (referent) 1.0 (referent) Q2 0.74 (0.34, 2.70) 0.74 (0.17, 3.28) Q3 1.11 (0.73, 2.66) 1.59 (0.33, 7.70) Q4 2.23 (0.38, 1.36) 6.47 (0.91, 45.9) A/H3N2 (65%) (84%) PFOA continuous 0.76 (0.51, 1.15) 0.66 (0.39, 1.12) Q1 1.0 (referent) 1.0 (referent Q2 0.90 (0.48, 1.68) 0.34 (0.14, 0.83) Q3 1.13 (0.59, 2.17) 0.28 (0.11, 0.70) Q4 0.62 (0.33, 1.66) 0.36 (0.15, 0.99)
General Population: Children		
Grandjean et al. 2012 Faroe Islands Birth cohort, follow-up to age 7 yrs n = 587 Age 5 prebooster (e.g., tetanus, diphtheria) and 4 weeks after booster and age 7 PFOA in 3rd trimester blood sample and in child (age 5) Linear regression, adjusted for gender, age, birth weight, maternal smoking, breast-feeding, and PCBs (and time since booster for post-booster analysis)	Geometric mean Maternal sample 0.0032 µg/mL Child's sample 0.004 µg/mL	Log PFOA and Log antibody Beta (95% CI) [% change in antibody titer per twofold increase in PFOA] Maternal PFOA Tetanus Diphtheria Prebooster -10.5 (-28.2, 11.7) -16.2 (-34.2, 6.7) Postbooster 14.5 (-10.4, 46.4) -6.2 (-22.4, 13.3) Year 7 (adjusted for age 5) 12.3 (-8.6, 38.1) -16.8 (-32.9, 3.3) Child's PFOA Tetanus Diphtheria Prebooster -13.3 (-31.6, 9.9) -6.8 (-28.3, 21.0) Postbooster - 9.7 (-30.7, 17.7) -6.1 (-23.6, 15.5) Year 7 (adjusted for age 5) -28.2 (-42.7, -10.1) -23.4 (-39.3, -3.4) Similar results seen with PFOS
Granum et al. 2013 Norway Birth cohort, Norwegian Mother and Child Cohort Study n = 56 with maternal blood at delivery and child blood samples at 3 yrs Linear regression, considered potential confounders	Mean 0.001 µg/mL	Beta (95% CI) (p-value), PFOA and antibody titer Rubella -0.40 (-0.64, -0.17) (p = 0.001) Measles -0.13 (-0.35, 0.09) (p = 0.24) Tetanus 0.01 (-0.009, 0.10) (p = 0.92) Hib -0.05 (-3.85, 3.74) (p = 0.98) Simlar results for other PFAS

A cohort of 411 adult members of the C8 Health Project was evaluated in 2010 for an association between serum PFOA levels and antibody response following vaccination with an inactivated trivalent influenza vaccine (Looker et al. 2014). A prevaccination serum sample was collected at the time of vaccination and the postvaccination serum sample was collected 21 ± 3 days later. The geometric mean serum PFOA level was 0.0337 μg/mL (95% CI 0.0298, 0.0382) and participants were divided into quintiles for analyses. PFOA was negatively associated with geometric mean A/H3N2 antibody titer rise, but no association was found with antibody titers for A/H1N1 and influenza type B. No association was found between antibody titers and PFOS levels.

Antibody responses to diphtheria and tetanus toxoids following childhood vaccinations were assessed in context of exposure to five perfluorinated compounds (Grandjean et al. 2012). The prospective study included a birth cohort of 587 singleton births during 1999–2001 from the National Hospital in the Faroe Islands. Serum antibody concentrations were measured in children at age 5 years prebooster, approximately 4 weeks after the booster, and at age 7 years. Prenatal exposures to perfluorinated compounds were assessed by analysis of serum collected from the mother during week 32 of pregnancy (PFOA geometric mean 0.0032 μg/mL; IQR 0.00256–0.00401); postnatal exposure was assessed from serum collected from the child at 5 years of age (PFOA geometric mean 0.00406 μg/mL; IQR 0.00333–0.00496). Multiple regression analyses with covariate adjustments were used to estimate the percent difference in specific antibody concentrations per twofold increase in PFOA concentration in both maternal and 5-year serum. Maternal PFOA serum concentration was negatively associated with antidiphtheria antibody concentration (-16.2%) at age 5 before booster. The biggest effect was found in comparison of antibody concentrations at age 7 with serum PFOA concentrations at age 5 where a twofold increase in PFOA was associated with differences of -36% (95% CI, -52%–-14%) and -25% (95% CI, -43%–-2%) for tetanus and diphtheria, respectively. Additionally at age 7, a small percentage of children had antibody concentrations below the clinically protective level of 0.1 international unit (IU) /mL. The ORs of antibody concentrations falling below this level were 4.20 (95% CI, 1.54–11.44) for tetanus and 3.27 (95% CI, 1.43–7.51) for diphtheria when age 7 antibody levels were correlated with age 5 PFOA serum concentrations. Maternal and child PFOS levels also were negatively associated with antibody titers in children.

The effects of prenatal exposure to perfluorinated compounds on vaccination responses and clinical health outcomes in early childhood were investigated in a subcohort of the Norwegian Mother and Child Cohort Study (Granum et al. 2013). A total of 56 mother-child pairs, for whom both maternal blood samples at delivery and blood samples from the children at 3 years of age, were evaluated. Antibody titers specific to measles, rubella, tetanus, and influenza were measured as these vaccines are part of the Norwegian Childhood Vaccination Program. Serum IgE levels also were measured. Clinical health outcomes, including common colds and gastroenteritis, at ages 1, 2, and 3 years were assessed by means of a questionnaire sent to participants. Mean maternal plasma PFOA concentration was 0.0011 μg/mL at delivery; the PFOS level was 0.0056 μg/mL and PFNA and PFHxS were below the LOQ. PFOA levels in the children were not measured. No associations were found with PFOA or any perfluorinated compound and antibody levels to the vaccines with one exception. A slight, but significant, inverse relationship between maternal PFOS level and anti-rubella antibodies in children at 3 years was found (β = -0.8 [95% CI -0.14, -0.02]). Maternal PFOA levels were not associated with adverse childhood health outcomes.

In summary, three studies have reported decreases in response to one or more vaccines (e.g., measured by antibody titer) in relation to higher exposure to PFOA in children (Grandjean

et al. 2012; Granum et al. 2013) and adults (Looker et al. 2014). In the two studies examining exposures in the background range (i.e., general population exposures, < 0.010 μg/ml), the associations with PFOA also were seen with other correlated PFASs. This limitation was not present in the study in adults in the high-exposure C8 community population. Serum PFOA levels in this study population were approximately 0.014–0.090 μg/mL.

Asthma

The association between serum levels of perfluorinated compounds and childhood asthma was investigated by Dong et al. (2013). The cross-sectional study included a total of 231 children aged 10–15 years with physician-diagnosed asthma and 225 age-matched nonasthmatic controls. Between 2009 and 2010, asthmatic children were recruited from two hospitals in Northern Taiwan, while the controls were part of a cohort population in seven public schools in Northern Taiwan. Serum was collected for measurement of 10 perfluorinated compounds, absolute eosinophil counts, total IgE, and eosinophilic cationic protein. A questionnaire was administered to asthmatic children to assess asthma control and to calculate an asthma severity score (e.g., frequency of attacks, use of medicine, and hospitalization) during the previous 4 weeks. Associations of perflourinated compound quartiles with concentrations of immunological markers and asthma outcomes were estimated using multivariable regression models. Nine of 10 perfluorinated compounds were detectable in ≥84.4% of all children with levels generally higher in asthmatic children than in nonasthmatics. Serum concentrations of PFOA in asthmatic and nonasthmatic children were 0.0015 ± 0.0013 μg/mL and 0.0010 ± 0.0011 μg/mL, respectively; four other compounds were measured at higher concentrations with the highest levels for PFOS and perfluorotetradecanoic acid. The adjusted ORs for asthma association with the highest versus lowest quartile levels were significantly elevated for seven of the compounds. For PFOA, the OR was 4.05 (95% CI: 2.21, 7.42). In asthmatic children, absolute eosinophil counts, total IgE, and eosinophilic cationic protein concentration were positively associated with PFOA levels with a significant monotonic trend with increasing serum concentration. None of these biomarkers were significantly associated with PFOA levels in nonasthmatic children. Serum PFOA levels were not significantly associated with asthma severity scores among the children with asthma, although four other compounds did show an association.

Humblet et al. (2014) evaluated a cohort from NHANES to investigate children's PFAS serum levels, including PFOA, and their association with asthma-related outcomes. Sera were analyzed for 12 PFASs with focus on PFOA, PFOS, PFHxS, and PFNA. A total of 1,877 children aged 12–19 years with at least one serum sample available were included. Asthma and related outcomes were self-reported. Median serum PFOA levels were 0.0043 μg/mL for those ever having asthma and 0.0040 μg/mL for children without asthma. In the multivariable adjusted model, a doubling of PFOA level was associated with an increased odds of ever having asthma (OR=1.18, 95% CI 1.01, 1.39). PFOS was inversely associated with asthma and no associations were found between the other PFAS and outcome.

On the basis of epidemiological and other data available, the C8 Science Panel (2012) found no probable link between PFOA and asthma in children and adults and chronic obstructive pulmonary disease (COPD) in adults.

Autoimmune conditions

The most recent report on the worker cohort initially described by Leonard et al. (2008) included 6,026 workers evaluated for disease incidence, not just mortality (Steenland et al.

2015). Lifetime serum cumulative dose was estimated by combining occupational and nonoccupational exposures. Median measured serum level was 0.113 μg/mL based on samples collected in 2005. Statistically significant positive trends were found between log of cumulative exposure and ulcerative colitis and rheumatoid arthritis. Rate ratios for the highest quartile compared to the lowest quartile were 2.74 (95% CI 0.78, 9.65) for ulcerative colitis and 4.45 (95% CI 0.99, 19.9) for rheumatoid arthritis.

The C8 Science Panel (2012) combined these data with findings from the C8 general population cohort and concluded that there is a probable link between PFOA and ulcerative colitis. Using historical estimates for serum PFOA, the C8 Science Panel found a significant positive, dose-response trend with a relative risk (RR) for the highest quartile compared to the lowest of 3.18 (95% CI 1.84, 5.51). The panel concluded that there was no probable link between PFOA and autoimmune diseases, including rheumatoid arthritis, lupus, type1 diabetes, Crohn's disease, or multiple sclerosis. The C8 Science Panel also concluded that there is no probable link between PFOA and osteoarthritis. These analyses by the panel included both worker and general population cohorts.

3.1.1.6 Thyroid Effects

Several epidemiology studies have evaluated thyroid function and/or thyroid disease and its association with serum PFOA concentrations. Thyroid disease is more common in females than in males. Among the PFOA studies, the three most highly powered studies with the largest number of participants are one from the general U.S. population (Melzer et al. 2010) and two from highly exposed individuals within the C8 population (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b). Two of these studies are of adults (Melzer et al. 2010; Winquist and Steenland 2014b) and one is of children/adolescents (Lopez-Espinosa et al. 2012). Hypothyroidism is characterized by elevated thyroid stimulating hormone (TSH) and low T4; elevated TSH in conjunction with normal T4 and triiodothyronine (T3) is defined as subclinical hypothyroidism. Hyperthyroidisim is characterized by elevated T4 and low TSH; low levels of TSH in conjunction with normal T4 and T3 is defined as subclinical hyperthyroidism. Some studies focused on the prevalence of clinically defined disease (or the subclinical state), and others examined variations in TSH, T4, and T3 measurements among people who have not been diagnosed with a thyroid disease. Both hypothyroidism and hyperthyroidism can result from an autoimmune pathogenesis involving destruction of thyroid tissue. A summary of the studies on PFOA's association with thyroid disease or changes in thyroid hormones follows, and is depicted in Table 3-7 (studies in adults) and Table 3-8 (studies in special populations—children and pregnant females).

Occupational exposure studies. Serum PFOA levels were obtained from volunteer workers of the Cottage Grove, Minnesota, PFOA plant in 1993 (n = 111) and 1995 (n = 80) as part of the medical surveillance program and analyzed to determine a relationship between TSH and PFOA concentration (Olsen et al. 1998). Employees were placed into four exposure categories based on their serum PFOA levels: 0–1 μg/mL, 1– < 10 μg/mL, 10– < 30 μg/mL, and >30 μg/mL. Statistical methods used to compare PFOA levels and hormone values included multivariable regression analysis, ANOVA, and Pearson correlation coefficients. TSH was significantly ($p = 0.002$) elevated in 10–<30 μg/mL exposure category for 1995 only (mean serum TSH level was 2.9 ppm). However, mean TSH levels for the other exposure categories, including the ≥30 μg/mL category, were all the same (1.7 ppm). In 1993, TSH was elevated in this same exposure category, but was not statistically significant ($p = 0.09$) when compared to the other exposure categories.

Table 3-7. Summary of Epidemiology Studies of PFOA and Thyroid Effects in Adults

Reference and Study Details	PFOA Level	TSH	T3	T4
Occupational Exposure Studies				
Olsen and Zobel 2007 3M. Antwerp, Cottage Grove, Decatur combined; 50-65% participation rate n= 506 Mean age: 40 yrs Mean duration: not reported Linear regression adjusting for ln age, ln BMI, ln alcohol (Related references: Olsen et al. 1998, 2003)	Mean (range) 2.21 (0.01–92.03) µg/mL	Beta (±SE) (p-value), ln PFOA and ln TSH: 0.0360 (± 0.0207) (p = 0.08)	Beta (±SE) (p-value), ln PFOA and ln T3: 0.0105 (± 0.0053) (p = 0.05)	Beta (±SE) (p-value), ln PFOA and ln T4: -0.0057 (± 0.0054) (p = 0.29) Beta (±SE) (p-value), ln PFOA and ln FT4: -0.0117 (± 0.0043) (p = 0.01)
Steenland et al. 2015 n = 3,713 workers Data collected in 2005-2006 and 2008-2011 n = 82 cases in men, 77 cases in women	In 2005-2006: mean 0.325 µg/mL, median 0.113 µg/mL	HR (95% CI), for self-reported thyroid disease, with medical record validation (incidence based on year of diagnosis). Cumulative exposure quartile, no lag In men: 1.0 (referent) 1.64 (0.82, 3.29) 1.13 (0.50, 2.54) 2.16 (0.98, 4.77) (P_{trend} = 0.98)	 In women: 1.0 (referent) 1.00 (0.54, 1.87) 1.02 (0.48, 2.17) 0.33 (0.08, 1.26) (P_{trend} = 0.97)	
Adults: High-Exposure Community Studies				
Emmett et al. 2006 n = 371, aged 2–89 yrs, median 50 yrs (317 from stratified random population sample) t-test, PFOA in abnormal vs normal TSH levels	0.354 µg/mL	6% abnormal; p-value of t-test comparing PFOA in abnormal and normal = 0.59	Not measured	Not measured
Winquist and Steenland 2014b n = 32,254 (including 3,713 workers) Data collected in 2005-2006 and 2008-2011 n = 2,008 cases in primary analysis n = 454 cases in prospective analysis (diagnoses after 2005-2006) Stratified by gender; also conducted separate analyses for hyperthyroidism and hypothyroidism	In 2005-2006: mean 0.0866 µg/mL, median 0.0261 µg/mL	HR (95% CI), incident thyroid disease (with medical record validation), primary analysis: Cumulative exposure quintiles Full sample: 1.0 (referent); 1.21; 1.17; 1.27; 1.2 (P_{trend} = 0.03) Men: 1.0; 1.12; 0.83; 1.01; 1.05 (P_{trend} = 0.85) Women: 1.0; 1.24; 1.27; 1.36; 1.37 (P_{trend} = 0.03) Year exposure quintiles: Full sample: 1.0 (referent); 1.23; 1.24; 1.10; 1.28 (P_{trend} = 0.04) Men: 1.0; 1.13; 1.11; 1.06; 1.04 (P_{trend} = 0.97) Women: 1.0; 1.26; 1.28; 1.11; 1.38 (P_{trend} = 0.008)		

Reference and Study Details	PFOA Level	TSH	T3	T4
		Diagnoses after 2005: Cumulative exposure quintiles Full sample / Men / Women 1.0 (referent) / 1.0 / 1.0 1.23 / 1.35 / 1.23 1.00 / 1.37 / 0.93 1.06 / 1.44 / 1.00 1.12 / 1.85 / 0.96 (P_{trend} = 0.73) / (P_{trend} = 0.09) / (P_{trend} = 0.55)	Year exposure quintiles: Full sample / Men / Women 1.0 (referent) / 1.0 / 1.0 0.80 / 1.32 / 0.74 0.91 / 2.09 / 0.76 0.93 / 1.83 / 0.82 0.91 / 1.76 / 0.80 (P_{trend} = 0.86) / (P_{trend} = 0.54) / (P_{trend} = 0.53)	
Adults: General Population Studies				
Bloom et al. 2010 United States (New York; New York State Anglers Cohort Study) n = 31 (4 women) Mean age: 39 yrs (31–45 years) Linear regression, adjusted PFOA-PFOS correlation r = 0.35	Geometric mean 0.0013 µg/mL	Log-PFOA and log-TSH: Beta = -0.06 (-0.78, 0.67) (p = 0.87)	Not measured	Log-PFOA and log-T4: Beta = -0.01 (-0.16, 0.14) (p = 0.89)
Shrestha et al. 2015 United States (Upper Hudson River Valley) n = 87 (with serum for analyses); excluded if taking thyroid medicine Aged: 55–74 yrs PFOA-PFOS correlation r = 0.52 Linear regression, adjusted	Geometric mean (IQR) 0.0092 (0.0071–0.0131) µg/mL	Log-PFOA and log-TSH: Beta = 0.102 (-0.047, 0.25) (p = 0.18)	Log-PFOA and log-T3: Beta 3.03 (-1.73, 7.79) (p = 0.21)	Log-PFOA and log-T4: Beta = 0.38 (-0.07, 0.83) (p = 0.97) Log-PFOA and log-FT4: Beta = 0.016 (-0.036, 0.069) (p = 0.54)
Melzer et al. 2010 United States, NHANES 1999–2000, 2003–2004, and 2005–2006 n = 3,974 adults, ages ≥ 20 yrs Linear regression, stratified by gender, adjusted	Men (µg/mL) Q1: 0.0001–0.0036 Q2: 0.0037–0.0052 Q3: 0.0053–0.0072 Q4: 0.0073–0.0459 Similar cut-points in women	Thyroid Disease, self-reported, with medication use: Men / Women 1 (referent) / 1 (referent) 1.17 (0.64–2.15) / 0.98 (0.65–1.50) 0.58 (0.21–1.59) / 1.09 (0.66–1.81) 1.58 (0.79–3.16) / 1.63 (1.07–2.47)		
Wen et al. 2013 United States, NHANES 2007-2008, 2009-2010 n = 1181, adults, aged ≥ 20 yrs Linear regression, adjusted, with sampling weights	Mean 0.004 µg/mL	Beta (95% CI) (p-value) Ln-PFOA and ln-TSH: Men 0004 (-0.081, 0.090) (p = 0.92) Women -0.030 (-0.2157, 0.154) (p = 0.73)	Beta (95% CI) (p-value) Ln-PFOA and ln-T3: Men 0.775 (-3.048, 4.598) (p = 0.67) Women 6.628 (0.545, 12.7) (p = 0.035) Ln-PFOA and ln-FT3: Men 0.016 (0.001, 0.031) (p = 0.04) Women 0.027 (0.009, 0.044) (p = 0.002)	Beta (95% CI) (p-value) Ln-PFOA and ln-T4: Men 0.000 (-0.28, 0.28) (p = 1.0) Women 0.082 (-0.369, 0.532) (p = 0.71) Ln-PFOA and ln-FT4: Men -0.010 (-0.041, 0.022) (p = 0.53) Women -0.004 (-0.047,0.039) (p = 0.83)

Table 3-8. Summary of Epidemiology Studies of PFOA and Thyroid Effects in Special Populations

Reference and Study Details	PFOA Level	TSH	T3	T4
Children: High-Exposure Community				
Lopez-Espinosa et al. 2012 n = 10,725 children, aged 1-17 yrs C8 Health Project Reported thyroid disease based on parent- report of health care provider diagnosis of thyroid disease (and specific types); also included current use of thyroid medications Subclinical disease based on hormone levels excluding people with self-reported thyroid disease or taking thyroid medication (subclinical hypothyroidism = above age-specific reference range for TSH and total T4 within reference range; subclinical hyperthyroidism = below age-specific reference range for TSH and total T4 within or above reference range)	Modeled *in utero* PFOA: median 0.012 µg/mL Measured in children: median 0.0293 µg/mL	Beta (95% CI) for % change in TSH per IQR ln-PFOA: in utero -0.5 (-2.4, 1.5) in child 1.0 (-0.5, 2.7)	Not measured	Beta (95% CI) for % change in total T4 per IQR ln-PFOA: in utero -0.1 (-0.8, 0.6) in child 0.1 (-0.5, 0.6)
		OR (95% CI) for thyroid disease per IQR ln-PFOA: *in utero* PFOA: / Child's PFOA: Any thyroid disease (n = 27) 1.45 (0.95, 2.27) / (n = 61) 1.44 (1.02, 2.03) Hypothyroidism (n = 20) 1.61(0.96, 2.63) / (n = 39) 1.54 (1.00, 2.37) Subclinical hypothyroidism (n = 155) 0.94 (0.76, 1.16) / (n = 365) 0.98 (0.86, 1.15) Subclinical hyperthyroidism (n = 31) 1.10 (0.69, 1.74) / (n = 78) 0.81 (0.58, 1.15) Associations with any thyroid disease and hypothyroidism were not seen with PFOS		
Children: General Population Studies				
de Cock et al. 2014 Netherlands n = 83 newborns PFOA in cord blood samples T4 in heel prick blood Linear regression, stratified by gender; PFOA quartiles, adjusted	0.943 µg/L 0.000943 µg/mL	Not measured	Not measured	Beta T4 (nmol/L) (95% CI) Boys Q1: Referent Q2: 7.9 (-18.04, 33.92) Q3: -2.1 (-20.94, 16.7) Q4: 6.2 (-16.08, 28.50) Girls Q1: Referent Q2: -5.9 (-26.75, 14.94) Q3: 11.8 (-19.08, 42.72) Q4: 38.6 (13.34, 63.83)
Lin et al. 2013 Taiwan, Young Taiwanese Cardiovascular Cohort Study n = 545 (45 with elevated blood pressure); n = 18 hypothyroid- TSH > normal range Aged: 12-30 yrs	Geometric mean 0.00267 µg/mL Q1: <0.00364 Q2: 0.00364 – ≤0.00066 Q3: 0.00666 - ≤0.00971 Q4: >0.009.71	Mean (±SE) Ln TSH by PFOA quartile (adjusted) Q1: 0.48 (± 0.08) Q2: 0.45 (± 0.09) Q3: 0.36 (± 0.11) Q4: 0.41 (± 0.12) No association with risk of hypothyroidism	Not measured	Mean (±SE) free T4 by PFOA quartile (adjusted) Q1: 1.07 (± 0.01) Q2: 1.08 (± 0.02) Q3: 1.10 (± 0.02) Q4: 1.06 (± 0.02)

Reference and Study Details	PFOA Level	TSH	T3	T4
Pregnant Women: General Population Studies				
Chan et al. 2011 Canada n = 96 hypothyroxinemia cases (normal TSH with decreased free T4 – below 10th percentile) and 175 controls (normal TSK and free T4 in 50th–90th percentile; matching based on referring physician and maternal age 2nd trimester blood sample (mean 18 weeks) Conditional logistic regression, adjusted PFOA-PFOS correlation r = 0.5	Geometric mean 0.00135 µg/mL	Ln PFOA OR (95% CI): 0.94 (0.74–1.18) With additional adjustment for PFOS and PFHxS: 0.87 (0.63–1.19)		
Wang et al. 2013 Norway (from case-control study of subfecundity in the Norwegian Mother and Child Cohort Study; cases and controls combined) n = 903 women 2nd trimester blood sample (mean 18 weeks) Linear regression, adjusted	Median 0.00215 µg/mL	PFOA and ln-TSH Beta (95% CI) −0.0001 (−0.045, 0.044)	Not measured	Not measured
Berg et al. 2015 Norway, Northern Norway Mother and Child Contaminant Cohort Study n = 375 2nd trimester blood sample (18 weeks) Thyroid hormones and anti-TPO antibodies measured at 18 weeks gestation and at day 3 and week 6 after delivery Mixed effects linear models Repeated measures of thyroid hormone levels were used in model PFOA-PFOS correlation r = 0.65	Median 0.00153 µg/mL	Highest quartile PFOA associated with higher TSH, but not significant when adjusted for PFOS (quantitative results not reported)	Quantitative results not reported (noted as no association)	Quantitative results not reported (noted as no association)
Webster et al. 2014 Canada (Vancouver Chemicals Health and Pregnancy Study) n = 152, not taking thyroid medicine 2nd trimester blood samples (15 and 18 weeks) Mixed effects linear models, stratified by TPO antibody levels PFOA-PFOS correlation r = 0.71	Median 0.0017 µg/mL	Beta per IQR PFOA and TSH, (95% CI) (p-value) Normal TPO antibody 0.07 (-0.1, 0.2) (p = 0.41) High TPO antibody 0.7 (0.09, 1) (p = 0.02) Similar results for PFOS [IQR PFOA = 0.0014 µg/mL]	not measured	Beta per IQR PFOA and FT4, (95% CI) (p-value) Normal TPO antibody -0.03 (-0.3, 0.2) (p = 0.82) High TPO antibody -0.4 (-1, 0.5) (p = 0.35) [IQR PFOA = 0.0014 µg/mL]

In an expanded and refined analysis of the data reported in Olsen et al. 2003, Olsen and Zobel (2007) looked at the relationship between serum PFOA concentration and TSH, serum and free T4, and T3 levels in workers at the Decatur, Antwerp, and Cottage Grove production plants. The fluorochemical workers consisted of males (aged 21–67) from the Antwerp, Belgium (n = 196); Cottage Grove, Minnesota (n = 122); and Decatur, Alabama (n = 188) production facilities who volunteered to participate in the medical surveillance program in 2000. The mean serum PFOA concentration was 2.21 μg/mL for all sites combined. No association between TSH, serum T4, and PFOA concentration was observed. A negative association ($p<0.01$) between free T4 and serum PFOA concentration was observed in the unadjusted and adjusted (age, BMI, and alcohol consumption) models for all locations combined; no association was observed for the individual locations. A positive association ($p<0.05$) was observed between T3 and serum PFOA concentration in the unadjusted and adjusted models for all locations combined, the Antwerp plant, and the Decatur plant. The authors noted that the results were not considered clinically relevant because the results were within normal reference range. Steenland et al. (2015) did not find an association between self-reported thyroid disease and PFOA levels among 3,713 workers at the Washington Works plant in West Virginia who participated in the C8 Health Project.

Two studies measured thyroid hormones in PFOA-exposed workers, but did not present an analysis of the relation between PFOA exposure and hormone levels. Both studies noted that the thyroid hormone values were in the normal range (Costa et al. 2009; Sakr et al. 2007a).

High-exposure community studies. Emmett et al. (2006) examined the association of serum PFOA with thyroid disease in 371 residents of the Little Hocking, Ohio, water district as described previously. No association was observed between serum PFOA and thyroid disease. Serum PFOA was decreased (not significantly different) in subjects with self-reported disease (e.g., hyperthyroidism, goiter or enlarged thyroid, hypothyroidism) (0.387 μg/mL; n = 40) compared to subjects without thyroid disease (0.451 μg/mL; n = 331). No association was seen between serum PFOA and TSH when analyzed with linear regression or by t-test comparison of PFOA in the abnormal TSH (n = 24, 6%) and normal TSH groups ($p = 0.59$).

Participants in the C8 Health Project were examined for an association between PFOA levels and thyroid disease (Winquist and Steenland 2014b). The cohort included 28,541 community members and 3,713 workers who had completed study questionnaires during 2008–2011. The median serum PFOA level at enrollment in 2005–2006 was 0.0261 μg/mL for the combined cohort, 0.0242 μg/mL for the community members, and 0.1127 μg/mL for the workers. Retrospective serum levels for the community cohort, estimated from air and water concentrations, residential history, and water consumption rates, were used to estimate yearly intakes. For the workers, yearly serum estimates were modeled from work history information and job-specific concentrations. Cox proportional hazard models, stratified by birth year, were used to assess self-reported adult thyroid disease hazard in relation to time-varying yearly or cumulative (sum of yearly estimates) estimated PFOA serum concentration, controlling for gender, race, education, smoking, and alcohol consumption. For the combined cohort, quintiles for yearly exposure were 0.00011–<0.0047, 0.0047–<0.00849, 0.00849–<0.0216, 0.0216–<0.100, and 0.100–3.303 μg/mL; quintiles for cumulative exposure were 0.0001–<0.115, 0.115–<0.202, 0.202–<0.497, 0.497–2.676, and 2.676–97.396 μg/mL·year. As expected, the number of thyroid disease cases was higher among females than among males. Positive associations were seen with the cumulative exposure and the per-year exposure metrics for incidence of all thyroid disease (as well as for specific subtypes), with the observations seen primarily in females

(Table 3-7). When limited to disease occurring after the 2005–2006 serum collection, the number of incident cases was reduced from 2,008 to 454, and the patterns of associations were more variable. No associations between estimated serum PFOA level and thyroid disease were found in the analysis limited to workers in this study population (Steenland et al. 2015).

The C8 Science Panel (2012) used data from the C8 general population cohort and concluded that there is a probable link between PFOA and thyroid disease.

General population studies. Bloom et al. (2010) investigated the associations between serum PFAS, including PFOA, and TSH and free thyroxine (FT4). The serum samples came from 31 participants (27 males, 4 females; mean age 39 years) of the 1995–1997 New York State Angler Cohort Study Dioxin Exposure Substudy. The study subjects each completed a questionnaire and provided a blood sample for serum analysis. The questionnaire contained questions about sport-fish and game consumption, lifestyle, demographic factors, and medical history. The serum samples were analyzed for TSH and FT4 in 2003 by immunometric chemiluminescent sandwich assay and for PFAS in 2006 by ion pair extraction high-performance LC-MS/MS. Regression models were used to analyze the data and adjust for confounders. No subjects reported use of thyroid medication or physician-diagnosed goiter or thyroid conditions. Mean TSH concentration (range 0.43–15.70 μIU/mL) was within normal range (0.40–5.00 μIU/mL) with the exception of one subject. Mean FT4 (0.90–1.55 ng/dL) was within normal range (0.80–1.80 ng/dL) for all subjects. The mean serum PFOA concentration was 0.00133 μg/mL and ranged from 0.00057 to 0.00258 μg/mL. The males had a significantly higher serum PFOA concentration than the females (0.00147 μg/mL versus 0.00105 μg/mL; $p = 0.047$). There was no association between serum PFOA concentration (or PFOS) and TSH or FT4.

The relationship between serum levels of PFOA, PFOS and other persistent organic pollutants and thyroid biomarkers was investigated in older adults (Shrestha et al. 2015). Levels of TSH, FT4, T4, and T3 were measured in 51 males and 36 females with a mean age of 63.6 years. None of the participants had thyroid disease or were taking thyroid medication. Covariates in the analysis included age, gender, education level, the sum of polychlorinated biphenyls (∑PCBs) and polybrominated diphenyl ethers (∑PBDEs), smoking status, and alcohol consumption. The mean PFOA serum level was 0.0104 ± 0.0057 μg/mL for all participants. In both unadjusted and adjusted models, PFOA was significantly ($p<0.05$ or 0.01) and positively associated with T4 and T3; a possible dose-response was not evaluated in this small sample. A statistical interaction was detected between age and PFOA for effects on FT4 and T4 suggesting that the positive associations of PFOA were potentiated by age. PFOS was also positively associated with FT4 and T4.

Melzer et al. (2010) examined the association between serum PFOA concentration and thyroid disease in the general population of the United States by analyzing data from the 1999–2000, 2003–2004, and 2005–2006 NHANES The population included 3,966 adults (2,066 females, 1,900 males) older than 18 years. Each of the participants answered a questionnaire, had a physical examination, and provided blood and urine samples for analysis. Serum samples were analyzed for PFOA concentration by solid-phase extraction coupled to isotope dilution/high-performance LC-MS/MS. Data on diseases diagnosed by a physician and confounding factors, including year of NHANES, age, gender, race/ethnicity, education, smoking status, BMI, and alcohol consumption were obtained from the questionnaire. Individuals were considered to have thyroid disease if they responded on the questionnaire to having a physician-diagnosed disease or if they were taking medication for either hypothyroidism or hyperthyroidism.

Regression models were used to analyze the data and adjust for confounders. Serum PFOA concentration was divided into quartiles for each gender. In females, serum PFOA concentration ranged from 0.0001–0.123 μg/mL (Q1 = 0.0001–0.0026; Q2 = 0.0027–0.004; Q3 = 0.0041–0.0057; Q4 = 0.0057–0.123), and in males, serum PFOA concentration ranged from 0.0001–0.0459 μg/mL (Q1 = 0.0001–0.0036; Q2 = 0.0037–0.0052; Q3 = 0.0053–0.0072; Q4 = 0.0073–0.0459). Females in PFOA Q4 were more likely to report current thyroid disease [OR = 2.24, 95% CI: 1.38–3.65, p = 0.002] compared to females in Q1 and Q2. No association between serum PFOA concentration and thyroid disease was observed in males. With PFOS, the opposite was found, with males in the highest quartile, but not females, more likely to report thyroid disease. Data interpretation was limited by the cross-sectional study design, lack of information on the specific thyroid disorder diagnosis in the questionnaire responses, and single serum samples for PFOA measurements taken at the same time disease status was ascertained through the questionnaire. Thus, the possibility of reverse causality cannot be eliminated.

Another study of 1,181 members of NHANES for survey years 2007–2008 and 2009–2010 examined the association between serum PFOA levels (and 12 other PFASs) and thyroid hormone levels (Wen et al. 2013). Multivariable linear regression models were used with serum thyroid measures as the dependent variable and individual natural log-transformed PFAS concentration as a predictor along with confounders. The geometric mean serum PFOA level was 0.00415 μg/mL. A positive association between PFOA level and free T3 (FT3) was found in females as a 1-unit increase in natural log-serum PFOA increased serum total T3 concentration by 6.628 ng/dL (95% CI 0.545, 12.712, p = .035). However, the association was no longer significant when PFOS, PFNA, and PFHxS levels were included in the model.

A different type of examination was undertaken by Pirali et al. (2009). The study measured intrathyroidal levels of PFOA (and PFOS) in thyroid surgical specimens to determine if a relationship existed between PFOA and the clinical, biochemical, and histological phenotype of thyroid disease patients. Serum PFOA concentration also was measured to determine if a relationship existed between thyroid tissue and serum PFOA levels. Patients (n = 28; 8 males, 20 females; 33–79 years) with benign multinodular goiters (n = 15), Graves' disease (n = 7), malignant papillary carcinoma (n = 5), and malignant follicular carcinoma (n = 1) were included in the study. Informed consent, clinical examination, work history, thyroid hormone and antibody measurements, thyroid ultrasound, fine-needle aspiration of nodules greater than 1 cm, and serum samples (n = 21) were performed or collected prior to surgery. The control group consisted of thyroid tissues collected at autopsy from subjects with no history of thyroid disease (n = 7; 5 males, 3 females; 12–83 years) and serum samples from 10 subjects with no evidence of thyroid disease. The student's t-test, Mann-Whitney U-test, Pearson and Spearman's correlation tests, and chi-square test with Fisher's correction were used to compare group results. Regression analysis was used to test the effect of different variables independently of a covariate.

The median concentration of PFOA in thyroid tissue was 2.0 ng/g (range = 0.4–4.6 ng/g). The patients were divided into three different groups: group I (toxic and nontoxic multinodular goiter, n = 12), group II (differentiated thyroid cancer, n = 6), and group III (Hashimoto's thyroiditis or Graves' disease, n = 10). Thyroid PFOA concentration for the control group, group I, group II, and group III ranged from 1.0–6.0, 0.4–4.4, 1.4–4.0, and 1.0–4.6 ng/g, respectively. Serum PFOA concentration for the control group, group I, group II, and group III ranged from 0.004–0.0137, 0.0012–0.0166, 0.0051–0.0096, and 0.0039–0.0125 μg/ml, respectively. The concentration of PFOA in the thyroid and serum was similar between control and thyroid patients at the time of measurement. Age, gender, residence, working activity, malignant /

nonmalignant conditions, antibodies, thyroid hormone concentrations, and ultrasound parameters were not associated with thyroid or serum PFOA concentration. There also was no correlation between serum and thyroid PFOA concentration. Similar results were obtained with PFOS.

Children. Three studies evaluated thyroid function in children (or children and young adults) (Table 3-8). In the children from the C8 cohort who were highly exposed to PFOA, Lopez-Espinosa et al. (2012) observed positive associations between prenatal PFOA (modeled maternal levels) and any thyroid disease or clinical hypothyroidism; similar results were seen with the child's PFOA level. Associations were not seen with subclinical hypothyroidism or hyperthyroidism, or TSH or total T4 levels among children without thyroid disease. In a study from the Netherlands of 52 males and 31 females, increasing T4 levels in females were associated with increasing prenatal PFOA concentrations (as measured in cord blood samples) (de Cock et al. 2014); no associations were reported in males. A study of adolescents and young adults (aged 12–30 years) from Taiwan did not observe associations between serum PFOA concentrations and TSH or T4 levels (Lin et al. 2013).

Pregnant females. Several studies of thyroid have been conducted in pregnant females (Table 3-8), mostly reporting null associations between maternal PFOA concentration and thyroid status during pregnancy (Berg et al. 2015; Chan et al. 2011; Wang et al. 2013). The exception to these results is the only study that included an analysis stratified by presence of antithyroid peroxidise (anti-TPO) antibodies (Webster et al. 2014), in which associations between PFOA and TSH were seen only among females with high autoantibody levels. This finding supports the importance of further research into the association between PFOA and autoimmunity and autoimmune conditions.

Chan et al. (2011) examined the association between hypothyroxinemia and serum PFOA concentration (and PFOS) in pregnant Canadian females (n = 271; 20.1–45.1 years of age, ≥22 weeks of gestation) in a matched case-control study. Maternal serum from the second trimester was collected between December 15, 2005, and June 22, 2006, as part of an elective prenatal screen for birth defects. Serum samples were analyzed for TSH and FT4 concentrations and PFOA. The cases of hypothyroxinemia (n = 96) had normal TSH concentrations and FT4 concentrations in the lowest 10^{th} percentile (≤8.8 pmol/L). The controls (n = 175) had normal TSH concentrations and FT4 concentrations between the 50^{th} and 90^{th} percentiles (12–14.1 pmol/L). Maternal age, weight, and gestational age at blood draw and dichotomized at 50^{th} percentiles were included as confounders, and race was included as a covariate. Chi-square tests and regression models were used to analyze the data. Overall, the geometric mean PFOA level was 0.00135 µg/mL. Statistical comparisons used the geometric mean serum PFOA concentration in the cases of 3.10 nmol/L and 3.32 nmol/L in the controls. There was no association between serum PFOA concentration (or PFOS) and hypothyroxinemia in pregnant females.

A cross-sectional study of 903 pregnant females evaluated the association between plasma PFOA levels and plasma TSH (Wang et al. 2013). Twelve other PFASs also were quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study and the blood samples were drawn at approximately week 18 of gestation. The median PFOA concentration was 0.0022 µg/mL with an interquartile range of 0.00157–0.00295 µg/mL. No association was found between plasma levels of PFOA and TSH. PFOS was associated with higher TSH levels, but plasma levels of other PFASs were unrelated to TSH.

Expanding on the above study, Berg et al. (2015) investigated the association between a number of PFASs, including PFOA, and TSH, T3, T4, FT3, and FT4. A subset of 375 females in the Norwegian Mother and Child Cohort Study with blood samples at about gestational week 18 and at 3 days and 6 weeks after delivery were included. Seven compounds were detected in more than 80% of the blood samples, with PFOS present in the highest concentration followed by PFOA. The median PFOA level was 0.00153 µg/mL, and the females were assigned to quartiles based on the first blood sample at week 18 of gestation. Females in the highest quartile had significantly higher mean TSH than females in the first quartile; however, when PFOS concentration was included as a covariate, the association was not significant.

A study of Canadian females (n = 152) evaluated maternal serum PFOA levels (and PFHxS, PFNA, and PFOS) for associations with thyroid hormone levels during the early second trimester of pregnancy, weeks 15–18 (Webster et al. 2014). Mixed effects linear models were used to examine associations between PFOA levels and FT4, total T4, and TSH; associations were made for all females and separately for females with high levels of TPO antibody, a marker of autoimmune hypothyroidism. Median serum PFOA was 0.0017 µg/mL. No associations were found between levels of PFOA (or PFOS and PFHxS), and thyroid hormone levels in females with normal antibody levels. PFNA was positively associated with TSH. Clinically elevated TPO antibody levels were found in 14 (9%) of the study population. In the females with high antibody levels, PFOA, as well as PFNA and PFOS, was strongly and positively associated with TSH. An IQR increase in maternal PFOA concentrations was associated with a 54% increase in maternal TSH compared to the median TSH level. PFNA and PFOS concentrations were associated with 46% and 69% increases, respectively, in maternal TSH.

As illustrated above, numerous epidemiology studies have evaluated thyroid function and/or thyroid disease in association with serum PFOA concentrations (Tables 3-7 and 3-8). As noted previously, thyroid disease is more common in females. Several studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. population (Melzer et al. 2010) and the high-exposure C8 community population (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b). Two of these studies are of adults (Melzer et al. 2010; Winquist and Steenland 2014b) and one is of children/adolescents (Lopez-Espinosa et al. 2012). The trend for an association with thyroid disease was seen in females in the C8 population (Winquist and Steenland 2014b) and the general population (Melzer et al. 2010), and in children (Lopez-Espinosa et al. 2012); this was most often hypothyroidism. Association between PFOA and TSH also was seen in pregnant females with anti-TPO antibodies (Webster et al. 2014). In contrast, generally null associations were found between PFOA and TSH or thyroid hormones (T3 or T4) in people who have not been diagnosed with thyroid disease.

3.1.1.7 Diabetes and Related Endpoints

Occupational exposure studies. Leonard et al. (2008) examined cause of death among former workers at the DuPont Washington Works plant in West Virginia. The cohort consisted of 6,027 employees (4,872 males and 1,155 females) who had worked at the plant from 1948 through 2002. The DuPont Epidemiology Registry and U.S. National Death Index were used to obtain causes of death. SMRs were estimated using three reference populations; the populations of the United States and West Virginia and the DuPont regional worker reference population excluding workers at the Washington Works plant. A significant increase in diabetes mortality was observed for Washington Works plant workers compared to the DuPont regional worker

reference population [SMR = 197, 95% CI: 123, 298]. However, no regression analyses were done with PFOA levels.

The Leonard et al. study (2008) was updated in a cohort mortality study conducted by Steenland and Woskie (2012) to include 5,791 individuals who had worked at the DuPont West Virginia plant for at least 1 year between 1948 and 2002. Mean duration of employment was 19 years. Deaths through 2008 were ascertained through either the National Death Index or death certificate data. Exposure quartiles were assessed by estimated cumulative annual serum levels based on blood samples from 1,308 workers taken during 1979–2004 and time spent in various job categories (ppm-years). Referent groups included both nonexposed DuPont workers in the same region and the U.S. population. Overall, the mean cumulative exposure was 7.8 ppm-years and the estimated average annual serum level was 0.35 μg/mL. Compared to the referent rates from other DuPont workers, cause-specific mortality rates were elevated for diabetes (n = 38; SMR=1.90; 95% CI 1.35, 2.61). These data are limited by the small number of cases and the restriction to mortality as an outcome.

The most recent report on the above cohort included 6,026 workers evaluated for disease incidence, not just mortality (Steenland et al. 2015). Lifetime serum cumulative dose was estimated by combining occupational and nonoccupational exposures. Median measured serum level was 0.113 μg/mL based on samples collected in 2005. No association was found between PFOA level and type II diabetes incidence rate.

High-exposure community studies. MacNeil et al. (2009) examined the association of PFOA with type II diabetes in adult participants of the C8 Health Project (n = 54,468; age 20 to >80 years). Serum PFOA concentration was divided into deciles using the population distribution. Other PFAS were not evaluated in this study. Serum PFOA (deciles), BMI, gender, family history of diabetes, race, use of cholesterol-lowering medicine, and use of blood pressure-lowering medicine were used to analyze the data in categorical and logistic regression models for the outcome of type II diabetes. Serum fasting glucose levels were the focus for a linear regression analysis of the study population (n = 21,643) excluding type II diabetics and those who had provided nonfasting blood samples. The mean serum PFOA concentration for the entire study population was 0.0868 μg/mL and 0.0913 μg/mL for subjects with type II diabetes validated by medical review (n = 3,539).

There was no association between serum PFOA concentration and fasting serum glucose level in subjects characterized as nondiabetic. The mean serum PFOA concentration was 0.0929 μg/mL in subjects who self-reported type II diabetes (n = 4,278) and 0.1227 μg/mL in subjects diagnosed in the last 10 years (n = 1,055). No association was observed between type II diabetes and serum PFOA concentration. The OR by decile was 1.00, 0.71, 0.60, 0.72, 0.65, 0.65, 0.87, 0.58, 0.62, and 0.72. The results of the analysis indicated that PFOA exposure is not associated with type II diabetes among the population studied. Data interpretation was limited by the cross-sectional study design, which made it difficult to determine if PFOA exposure preceded disease.

The C8 Science Panel (2012) combined these data from the C8 general population cohort with follow-up data and data from worker cohorts, and concluded that there is no probable link between PFOA and type II diabetes.

General population studies. Preconception serum levels of PFOA (and other PFASs) were evaluated in females attempting pregnancy in relation to risk of developing gestational diabetes

(Zhang et al. 2015). The 258 participants were members of the Longitudinal Investigation of Fertility and the Environment (LIFE) study with blood samples taken during 2005–2009. The ORs and 95% CIs of gestational diabetes associated with each SD increment of preconception serum PFOA concentration (log-transformed) (and six other PFASs) were estimated with the use of logistic regression after adjusting for confounders. Preconception mean serum PFOA levels were 0.0033 μg/mL for the entire cohort, 0.00394 μg/mL in females with gestational diabetes and 0.00307 μg/mL in females without gestational diabetes. A significant positive association was found between PFOA and risk of gestational diabetes in the fully adjusted model (OR=1.86; 95% CI 1.14, 3.02). Associations for six other PFAS were slightly increased (e.g., PFOS OR=1.13), but did not attain statistical significance.

Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease and diabetes. Lin et al. (2009) investigated the association between serum PFOA (plus three other PFASs) and glucose homeostasis and metabolic syndrome in adolescents (aged 12–20 years) and adults (aged >20 years) by analyzing the 1999–2000 and 2003–2004 NHANES data. The National Cholesterol Education Program Adult Treatment Panel III guidelines were used to define adult metabolic syndrome and the modified guidelines were used to define adolescent metabolic syndrome. The study population included 1,443 subjects (474 adolescents, 969 adults) at least 12 years of age who had a morning examination and triglyceride measurement. There were 266 male and 208 female adolescents and 475 male and 493 female adults. Multiple linear regression and logistic regression models were used to analyze the data. Covariates included age, gender, race, smoking status, alcohol intake, and household income. Log-transformed PFOA concentration was 1.51 and 1.48 ng/mL for adolescents and adults, respectively. In adults, serum PFOA concentration was associated with increased β-cell function (β coefficient 0.07, $p<0.05$). Serum PFOA concentration was not associated with metabolic syndrome, metabolic syndrome waist circumference, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents. Both PFOS and PFNA were positively associated with some of the endpoints associated with metabolic syndrome.

Nelson et al. (2010) examined the relationship between polyfluoroalkyl chemical serum concentration, including PFOA, and insulin resistance as previously described for data from NHANES. Fasting insulin and fasting glucose were used to determine the homeostatic model assessment for insulin resistance. No association was found between serum PFOA concentration, or any other PFAS, and insulin resistance.

Overall, these studies show a lack of association of PFOA with diabetes, metabolic syndrome, and related endpoints.

3.1.1.8 Reproductive and Developmental Endpoints

Several studies have examined the relationship between PFOA exposures and reproductive, gestational, and developmental endpoints as well as postnatal growth and maturation in humans. Pregnancy-related endpoints include gestational age (Nolan et al. 2009), measures of fetal growth (Apelberg et al. 2007; Fei et al. 2007, 2008a; Monroy et al. 2008; Nolan et al. 2009; Stein et al. 2009; Washino et al. 2009), miscarriage or preterm birth (Stein et al. 2009), birth defects (Stein et al. 2009), hypertension and preeclampsia (Darrow et al. 2013; Savitz et al. 2012a, 2012b; Stein et al. 2009), and fecundity (Fei et al. 2009; Vélez et al. 2015). Infant growth and development during the first 7 years (Andersen et al. 2010, 2013; Fei et al. 2008b, 2010a, 2010b; Høyer et al. 2015b) and postnatal growth and maturation, including neurodevelopment (Fei and

Olsen 2011; Hoffman et al. 2010; Høyer et al. 2015a; Liew et al. 2014; Stein et al. 2013) and risk of adult obesity (Halldorsson et al. 2012) also have been studied. Male reproductive endpoints evaluated in humans include sperm count and semen quality (Buck Louis et al. 2015; Joensen et al. 2009, 2013; Vested et al. 2013). Female pubertal development was examined in three studies (Christensen et al. 2011; Kristensen et al. 2013; Lopez-Espinosa et al. 2011). As noted previously, the focus of this review is on pregnancy-related outcomes, specifically pregnancy-related hypertension and preeclampsia, measures of fetal growth, and pubertal development. Within each section, the discussion is divided into occupational exposure studies (if applicable), the C8 high-exposure community studies, and general population studies.

Several analyses are based on the Danish National Birth Cohort (Andersen et al. 2010, 2013; Fei et al. 2007, 2008a, 2008b, 2009, 2010a, 2010b; Fei and Olsen 2011). The females (n = 1,400) and their infants were randomly selected, and the study included those who provided their first blood samples between gestational weeks 4 and 14 and gave birth to a single live-born child without congenital malformation. The females participated in telephone interviews—at 12 and 30 weeks gestation, when the children were 6 and 18 months of age, and when the children were 7 years of age—and filled out a food frequency questionnaire. As the children aged, more questionnaires were completed by the mothers with regard to behavioral health and motor coordination. Highly structured questionnaires were used to gather information on possible confounders, including infant gender, maternal age, parity, socio-occupational status, prepregnancy BMI, and smoking during pregnancy. The National Hospital Discharge Register was used to obtain birth weight, gestational age, placental weight, birth length, head and abdominal circumference data, Apgar scores based on heart rate, respiratory effort, reflex, irritability, muscle tone, and skin color. Plasma PFOA concentration was determined from the first blood samples of 1,399 females, from the second blood samples of 200 females, and from cord blood samples of 50 infants by solid-phase extraction high-performance LC-MS/MS. PFOA concentrations were divided into quartiles (Fei et al. 2009, 2010b), with the lowest quartile designated as the reference group, as follows: <lower limit of quantification– (LLOQ–) 0.00390, 0.00391–0.00520, 0.00521–0.00696, and ≥ 0.00697 μg/mL. Regression models were used to analyze the data. Results of these studies are included in the following discussion of results for specific endpoints.

Pregnancy-related hypertension and preeclampsia. There are no occupational exposure and general population studies examining pregnancy-related hypertension and preeclampsia in relation to PFOA exposure. The only data available come from the high-exposure C8 Health Project study population (Table 3-9).

Several studies, using different designs and exposure measures, have examined birth outcomes, including pregnancy-induced hypertension or preeclampsia in infants born to mothers in the high-exposure C8 community population in West Virginia and Ohio (information obtained from questionnaire-based pregnancy histories or from linkage to birth records) (Table 3-9). Stein et al. (2009) used an exposure measure based on individual serum PFOA levels obtained in the 2005–2006 baseline survey to examine birth outcomes (based on self-report) in 1,845 births in the 5 years preceding the PFOA measurement. Savitz et al. (2012a, 2012b) included births from 1990 to 2004, modeling exposure based on the serum measurements in 2005, information obtained in the 2005 baseline questionnaire regarding residential history, information on historical environmental releases, and PKs. In one of the analyses (study II in Savitz et al. 2012b), linkage with birth records was used to verify the preeclampsia outcome.

Table 3-9. Summary of Epidemiology Studies of PFOA and Pregnancy-Induced Hypertension or Preeclampsia

Study	PFOA Level	Results
Stein et al. 2009 United States (C8 Health Project) n = 1,845 pregnancies in the 5 years before enrollment Exposure based on serum collected in 2005 Outcome based on pregnancy history collected in 2005	Median 0.0212 µg/mL	OR (95% CI), preeclampsia per IQR (lnPFOA) increase in PFOA: 1.1 (0.9, 1.3) [IQR(lnPFOA)=0.0395 µg/mL] < 50th percentile 1.0 (referent) ≥ 50th 1.3 (0.9, 1.9) < 50th percentile 1.0 (referent) 50-75th 1.5 (1.0, 2.3) 75-90th 1.2 (0.7, 2.1) > 90th 0.9 (0.5, 1.8)
Savitz et al. 2012a United States (C8 Health Project) n = 11,737 pregnancies in 1990-2006 Modeled exposure based on serum collected in 2005, residential history, and other data Outcome based on pregnancy history collected in 2005		OR (95% CI), preeclampsia per IQR(lnPFOA) increase in PFOA: 1.13 (1.00–1.28) [IQR (lnPFOA)=0.00219 µg/mL] per 0.100 µg/mL increase in PFOA: 1.08 (1.01–1.15) < 40th percentile 1.0 (referent) 40-60th 1.2 (1.0, 1.5) 60-80th 1.1 (0.9, 1.4) ≥ 80th 1.2 (1.0, 1.6) Also noted stronger associations with Bayesian calibration of exposure and among women with highest quality residential history
Savitz et al. 2012b United States (C8 Health Project) n = 4,547 pregnancies in 1990-2004 Modeled exposure based on serum collected in 2005, residential history, and other data Outcome based on linkage to birth records	Median 0.0134 µg/mL	With Bayesian calibration of exposure: OR (95% CI) per IQR (lnPFOA) increase in PFOA: 1.13 (0.92, 1.37) [IQR (lnPFOA)=0.00192 µg/mL] per 0.100 µg/mL increase in PFOA: 0.97 (0.85, 1.11) < 40th percentile 1.0 (referent) 40-60th 1.0 (0.7, 1.4) 60-80th 1.5 (1.1, 2.1) ≥ 80th 1.2 (0.8, 1.7)
Darrow et al. 2013 United States (C8 Health Project) n = 1,330 pregnancies in 2005-2010; 770 first pregnancies after PFOA measures; 947 (pregnancies in 2005-2007) Exposure based on serum collected in 2005	Geometric mean 0.016 µg/mL Mean 0.031	OR (95% CI) per log unit increase in PFOA Full analysis: 1.27 (1.05, 1.55) (adjusted for PFOS) 1.22 (0.99, 1.51) By quintile: Q1 up to 0.0069 µg/mL 1.0 (referent) Q2 0.0069 – < 0.0111 2.39 (1.05, 5.46) Q3 0.0111 – < 0.0189 3.43 (1.50 (7.82) Q4 0.0189 – < 0.0372 3.12 (1.35, 7.18) Q5 ≥ 0.0372 3.16 (1.35, 7.38) First pregnancy after PFOA measure 1.23 (0.92, 1.64) Pregnancies in 2005-2007: 1.35 (1.04, 1.76)

Darrow et al. (2013) examined birth outcomes in births that occurred in the 5 years after the PFOA measurement. In this study, reproductive history in a follow-up interview in 2010 was collected from females who had provided serum for PFOA measurement in 2005–2006. Singleton live births among 1,330 females after January 1, 2005, were linked to birth records to identify outcomes of pregnancy-induced hypertension and other outcomes (e.g., preterm birth, low birth weight, and birth weight among full-term infants). Thus there is a progressively greater refinement and reduction in misclassification (or exposure and outcome) among this set of

studies. Each of these studies provides some evidence of an association between PFOA exposure and risk of pregnancy-induced hypertension or preeclampsia, with the most robust findings from the methodologically strongest study (Darrow et al. 2013). Maternal serum PFOA levels were positively associated with pregnancy-induced hypertension, with an adjusted OR per log unit increase in PFOA of 1.27 (95% CI: 1.15, 1.55). PFOS also was positively associated with pregnancy-induced hypertension.

The C8 Science Panel (2012) considered both hypertension and preeclampsia together in determining a link between PFOA and pregnancy-induced hypertension. Some studies conducted by the panel found no associations while others showed positive associations. Among the studies with positive associations, no clear dose response was indicated. However, the panel decided that the evidence was sufficient to conclude that PFOA has a probable link to pregnancy-induced hypertension.

Fetal growth. Many different measures of fetal growth can be used in epidemiology studies. Birth weight is widely available (as it is routinely collected in medical records and birth certificates). Low birth weight (defined as < 2,500 g) can be a proxy measure for preterm birth (particularly when accurate gestational age dating is not available). Other measures of fetal growth such as small for gestation age might more accurately reflect fetal growth retardation.

Both birth weight and gestational age are characterized as two-part distributions, with a larger Gaussian portion representing term births and a longer tail representing preterm births. Increased risks of complications, including infant mortality, are seen in preterm births (or low birth-weight births). When analyzed as a continuous measure, changes in birth weight might not be clinically significant, as small changes in the distribution among term infants do not result in a shift into the distribution seen in preterm infants (Savitz 2007; Wilcox 2010). This consideration differs from that of some other types of continuous measures, such as neurodevelopment scales, blood pressure, or cholesterol, in which shifts in the distribution are expected to move a greater proportion of the population into an “at risk” or “abnormal” level.

High-exposure community studies. As noted in the previous discussion of preeclampsia, several studies using different designs and exposure measures have examined birth outcomes in infants born to mothers in the high-exposure C8 community population in West Virginia and Ohio (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012a, 2012b; Stein et al. 2009). These studies include analyses of birth weight and of low birth weight, and have not observed associations between PFOA and either birth weight among term births or the risk of low birth weight among all (singleton) births (Table 3-10).

Based on these data, as well as continued follow-up of the community cohort, the C8 Science Panel (2012) concluded that there is no probable link between PFOA and low birth weight.

General population studies. Two studies examined associations between maternal PFOA levels and birth weight among term infants (Fei et al. 2007; Monroy et al. 2008). The larger of these is from the Danish National Birth Cohort by Fei et al. (2007) (Table 3-10). In this study of 1,207 term births, the change in birth weight per log unit increase in PFOA was -9 g (95% CI: -20, 2 g).

Table 3-10. Summary of Epidemiology Studies of PFOA and Birth Weight

Study	PFOA Level	Results
High-Exposure Community		
Darrow et al. 2013 United States (C8 Health Project) n = 1,629 pregnancies in 2005-2010; 770 first pregnancies after PFOA measures; 947 (pregnancies in 2005-2007) Exposure based on serum collected in 2005	Geometric mean 0.0162 µg/mL Mean 0.031 µg/mL	Change in birth weight per log unit increase (95% CI) Full analysis: -8 (-28, 12) g (adjusted for PFOS) -4 (-25, 17) g First pregnancy after PFOA measure 5 (-22, 33) g Pregnancies in 2005-2007: -10 (-34, 14) g OR (95% CI) for low birth weight (< 2500 g) per log unit increase Full analysis: 0.94 (0.75, 1.17) (adjusted for PFOS) 0.91 (0.72, 1.16) First pregnancy after PFOA measure 1.07 (0.78, 1.47) Pregnancies in 2005-2007: 0.91 (0.70, 1.17) [Similar results in Nolan et al. 2009; Savitz et al. 2012a, 2012b; Stein et al. 2009]
General Population: Birth Weight Among Term Births		
Fei et al. 2007 Denmark n = 1,207 (term births) Blood sample at 4-14 weeks	0.0056 µg/mL	Change in birth weight per unit increase (95% CI) -8.7 (-19.5, 2.1)
Monroy et al. 2008 Canada n = 101 Cord blood sample	0.0019 µg/mL (cord blood)	Change in PFOA per g change in birth weight: Beta = 0.000171 (p = 0.65)
General Population: Birth Weight or Low Birth Weight Among All Births (by time of blood collection)		
Fei et al. 2007 Denmark Blood sample at 4-14 weeks n = 1,399 (full sample) 3.8% preterm	0.0056 µg/mL	Change in birth weight per unit increase (95% CI) -10.6 (-20.8, -0.47) g OR (95% CI) for low birth weight (< 2500 g) by quartile Q1 up to 0.00390 µg/mL 1.0 (referent) Q2 0.00391-0.00520 4.3 (0.51, 37) Q3 0.00521-0.00696 3.7 (0.42, 32) Q4 ≥ 0.00697 2.4 (0.27, 22) (Trend p = 0.94)
Hamm et al. 2010 Canada n = 252 Blood sample at 15-16 weeks 8.3% preterm	0.0021 µg/mL	Change in birth weight per ln unit increase (95% CI) -37.4 (-86.0, 11.2) g
Whitworth et al. 2012 Norway n = 849 Blood sample at around 17 weeks 3.9% preterm	0.0021 µg/mL	Change in birth weight z-score per unit increase (95% CI) -0.03 (-0.10, 0.04)
Maisonet et al. 2012 United Kingdom n = 395 Blood sample at 10-28 weeks 3.1% preterm	0.0037 µg/mL	Change in birth weight per log unit increase -34.2 (-54.8, -13) g
Washino et al. 2009 Japan n = 428 Blood sample at 23-35 weeks % preterm not reported	0.0014 µg/mL	Change in birth weight per log unit increase (95% CI) -75 (-191, 42) g

Study	PFOA Level	Results
Apelberg et al. 2007 United States (Baltimore) n = 293 Cord blood sample 13% preterm	0.0016 µg/mL (cord blood)	Change in birth weight per log unit increase (95% CI) -104 (-213, 5) g
Chen et al. 2012 Taiwan n = 429 Cord blood sample 9.3% preterm	0.0018 µg/mL (cord blood)	Change in birth weight per log unit increase (95% CI) -19 (-63, 25) g

Fei et al. (2007, 2008a), and other studies in the general population have examined PFOA in relation to birth weight or risk of low birth weight (or other measures of fetal growth), without restriction to term births (Table 3-10). These studies vary in size from approximately 250 to 1,400 births, and also in terms of timing of exposure measure. Fei et al. (2007, 2008a) used blood samples collected early in pregnancy (4–14 weeks), three studies used samples collected in the second trimester (Hamm et al. 2010; Maisonet et al. 2012; Whitworth et al. 2012), Washino et al. (2009) used samples collected in the third trimester, and two studies used cord blood samples (Apelberg et al. 2007; Chen et al 2012). These studies also differed in the percent of births that were preterm (ranging from approximately 3% to 13%), and presented results using different types of analyses (i.e., the form of the exposure and outcome variables, continuous, ln-transformed, categorical, etc). Each of the analyses indicates a negative association between PFOA levels and birth weight (i.e., a decrease in birth weight with increase in PFOA), although CIs were wide.

In a systematic review based on the Navigation Guide methods (Woodruff and Sutton 2014), Johnson et al. (2014) identified the general population studies shown in Table 3-10 and the high-exposure C8 Health Project studies published through 2012. The results from the meta-analysis showed that a 0.001 µg/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI -29.8, -7.9) difference in birth weight.

Preeclampsia is a condition that causes the pregnant female to be hypertensive because of reduced renal excretion associated with a decrease in GFR. Preeclampsia is often accompanied by low birth weight (Whitney et al. 1987). Morken et al. (2014) used a subset of the Norwegian Mother and Child Cohort to evaluate the relationship between GFR and fetal size. Participants included 470 preeclamptic patients and 483 nonpreeclamptic females; plasma creatinine measured during the second trimester was used to estimate GFR. For the overall cohort, for each mL/min increase in GFR, infant weight at birth increased 0.73–0.83 g, depending on the method used to calculate GFR. The increases were greater and statistically significant in females with preeclampsia. Differences were not statistically significant for the nonpreeclamptic group. Morken et al. (2014) was not a study of perfluorochemicals and there were no serum measurements of any PFASs. However, because PFOA/PFOS serum levels are expected to be higher with a lower GFR, the finding stimulated examination of the GFR as it relates to serum PFAS levels and the low birth weight identified in the epidemiology studies (Verner et al. 2015; Vesterinen et al. 2014).

The evidence for an inverse association between PFOA levels and birth weight raised the question of whether reverse causality linked to maternal GFR played a role in the association of low birth weight with serum PFOA. PFOA excretion by the kidney is dependent, in part, on the

GFR. Conditions that result in impairment of GFR (and thus increased serum PFOA) also could be related to fetal growth restriction, confounding the association between serum PFOA and decreased birth weight. Vesterinen et al. (2014) examined evidence pertaining to the relationship between fetal growth and maternal GFR using Navigation Guide systematic review methods. They identified 35 relevant studies published between 1954 and 2012 that met the Navigation Guide criteria for inclusion in the analysis. All studies were rated as "low" or "very low" quality due to inconsistency of findings among studies, small sample sizes resulting in large CIs around a mean, and high risk of bias in conduct of the study. The quality rating led to the conclusion that data were "inadequate" to determine an association between fetal growth and GFR. However, a more recent publication described below, expanded the database on the relationship between GFR and fetal size.

Verner et al. (2015) modified the human pregnancy/lactation PK model of PFOA by Loccisano et al. (2013) described in section 2.6.1 to evaluate the association between GFR, serum PFOA levels, and birth weight. When GFR was accounted for in the model simulations, the reduction in birth weight associated with increasing serum PFOA was less than that found by the author's meta-analysis of the same data. This finding suggests that a portion of the association between prenatal PFOA and birth weight is confounded by maternal GFR differences within the populations studied. The true association for each 1 ng/mL increase in PFOA could be closer to a 7-g reduction (95% CI -8, -6) compared to the 14.72-g reduction (95% CI: -8.92, -1.09) predicted by meta-analysis of the epidemiology data without a correction for low GFR as observed in individuals with pregnancy-induced hypertension or evidence of preeclampsia.

Other pregnancy outcomes. Gestational age and preterm birth and risk of miscarriage were not associated with PFOA in the studies examining pregnancy outcomes in the high-exposure community (Darrow et al. 2014; Nolan et al. 2009, 2010). In contrast, PFOS was positively associated with miscarriage (Darrow et al. 2014).

Congenital anomalies were diagnosed in 1.8%, 1.9%, and 2.0% of the mothers with water provided completely, partially, or not at all by LHWA, respectively (Nolan et al. 2010). When adjusted for confounders, no statistically significant differences were found. Complications with labor and delivery were observed in 32.5%, 35.9%, and 41.9% of the mothers with water provided completely, partially, or not at all by LHWA, respectively. Mothers with water provided by LHWA did have in increased likelihood of having dysfunctional labor, but the number of reported cases was low. Mothers with one or more maternal risk factors were 37.5%, 34.4%, and 39.3% of the populations with water provided completely, partially, or not at all by LHWA, respectively. Adjusted regression models showed no statistical differences across water service status. An increased likelihood of anemia (crude OR 11, 95% CI: 1.8–64) and dysfunctional labor (crude OR 5.3, 95% CI: 1.2–24) in mothers with water provided by LHWA was found, but the number of reported cases was low. No association was found between PFOA and increased incidence of congenital anomalies, other labor and delivery complications, or maternal risk factors.

The C8 Science Panel (2012) concluded that there is no probable link between PFOA and birth defects, miscarriage, preterm birth, or stillbirth. Their conclusion was based on findings in Nolan et al. (2010), Stein et al. (2009), and other data available to the panel. These other data included historical estimates of serum PFOA generated by the panel based on amounts released from the plant and an individual's residential history.

Fei et al. (2009) examined the association between plasma PFOA concentration and longer time to pregnancy (TTP) as a measure of fecundity in 1,240 females. TTP was categorized as follows: immediate pregnancy (<1 month), 1–2, 3–5, 6–12, and >12 months. Having >12 months TTP or having used fertility treatment to get pregnant were used to define infertility. A total of 620 females had a TTP within the first 2 months of trying to conceive and 379 had a TTP of ≥6 months with 188 of those females having a TTP of >12 months. The mean plasma PFOA concentration was 0.0056 μg/mL for females who planned their pregnancies, and 0.0054, 0.0060, and 0.0063 μg/mL for TTPs <6 months, 6–12 months, and >12 months, respectively. Plasma PFOA concentration was significantly greater ($p<0.001$) in females who had TTPs >6 months than those with TTPs <6 months. The females with TTPs >6 months were more likely to be older, have middle socio-occupational status, and have a history of spontaneous miscarriage or irregular menstrual cycles. The adjusted odds for infertility increased 60–154% among females with >0.00391 μg/mL plasma PFOA concentration compared to females with <0.00391 μg/mL plasma concentration. The fecundity OR was 0.72, 0.73, and 0.60 for the three highest PFOA concentration quartiles. In the likelihood ratio test, the trend was significant ($p<0.001$). Both TTP and infertility also were positively associated with serum PFOS levels in this study. Although the results of the study suggest that plasma PFOA concentration could reduce fecundity, the authors noted that selection bias, the unknown quality of the sperm, unknown frequency and timing of intercourse, and abnormal hormone levels might have an impact on the results and fecundity.

Participants enrolled in the Maternal-Infant Research on Environmental Chemicals Study, a Canadian pregnancy and birth cohort, were evaluated for an association between serum PFOA levels (as well as PFOS and PFHxS) and TTP (Vélez et al. 2015). A total of 1,743 females, enrolled between 2008 and 2011 and having a blood sample collected during the first trimester were included. Infertility was defined as having a TTP of >12 months or requiring infertility treatment for the current pregnancy. The geometric mean plasma PFOA level was 0.00166 μg/mL. The crude fecundity OR per one SD increase in log-transformed serum concentration was significantly lower for PFOA (OR=0.91, 95% CI 0.86, 0.96) (and for PFHxS). In fully adjusted models, PFOA (and PFHxS) was associated with an 11% reduction in fecundability per one SD increase in log-transformed serum concentration (OR=0.89; 95% CI 0.83, 0.94). The adjusted odds of infertility increased by 31% per one SD increase of PFOA (OR=1.31; 95% CI 1.11–1.53) (and of PFHxS). No significant associations were observed for PFOS.

Fei et al. (2010b) reported on the effects of PFOA and PFOS on the length of breast-feeding. Self-reported data on the duration of breast-feeding were collected during the telephone interviews with each mother at 6 and 18 months after birth of the child. Higher levels of PFOA were significantly associated with a shorter duration of breast-feeding. In multiparous females, the adjusted OR for weaning before 6 months was 1.23 (95% CI, 1.13–1.33) for each 1-ng/mL increase in PFOA concentration in the maternal blood and the increase was dose-related. A similar association was observed with PFOS levels. No association was found between length of breast-feeding and PFOA levels in females having their first child. The authors speculate that the observed associations might be noncausally related to previous length of breast-feeding or to reduction of PFOA and PFOS through lactation.

Pubertal development. Two studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples (Christensen et al. 2011; Kristensen et al. 2013), and another study examined PFOA exposure measured concurrently with the assessment of pubertal status in females and in males (Lopez-Espinosa et al. 2011) (Table 3-11).

Table 3-11. Summary of Epidemiology Studies of PFOA and Pubertal Development

Study	PFOA Level	Results
Prenatal Exposure: General Population		
Christensen et al. 2011 United Kingdom Pregnancy cohort, with case-control of early menarche in follow-up n = 218 cases (menarche before age 11.5 yrs) and 230 controls	0.0036-0.0039 µg/mL (maternal)	Median (75th percentile) in cases: 3.9 (5.0) controls: 3.6 (4.7) (p = 0.15) OR (95% CI) above versus below median 1.29 (0.86, 1.93) per ln-unit increase in PFOA 1.01 (0.61, 1.68)
Kristensen et al. 2013 Denmark Pregnancy cohort, with follow-up of 343 (79% of eligible) daughters at age 20 Health questionnaire and exams/hormone measurements (for n = 254)	0.0036 µg/mL (maternal)	Difference in age at menarche (months) by exposure group low (0.001–0.003 µg/mL) 0.0 (referent) medium (0.003–0.0043 µg/mL) 0.9 (-3.0, 4.8) high (0.0044–0.0198 µg/mL) 5.3 (1.3, 9.3) continuous 1.01 (0.22, 1.89)
Peripubertal Exposure: High-Exposure Community		
Lopez-Espinosa et al. 2011 United States (C8 Health Project) n = 2,931 girl and 3,076 boy, aged 8-18 yrs Self-reported menarche (girls) and free or total testosterone (boys)	Median 0.058 µg/mL	Prevalence of menarche in girls OR / 95% CI / days delay Q1: 1.0 (referent) Q2: 1.01 (0.65-1.58) -4 Q3: 1.00 (0.64-1.58) -1 Q4: 0.75 (0.49-1.15) 69 Prevalence of delayed puberty in boys OR / 95% CI / days delay Q1: 1.0 (referent) Q2: 0.54 (0.35-0.84) 142 Q3: 0.50 (0.32-0.87) 163 Q4: 0.57 (0.37-0.89) 130 Results were broadly similar when the analysis was based on estradiol levels to define menarche or when the models included PFOA and PFOS jointly, though significance was reduced in some comparisons.

Christensen et al. (2011) used data from a prospective cohort study in the United Kingdom to perform a nested case-control study examining the association between age at menarche and gestational exposure to perfluorinated chemicals, including PFOA and PFOS. The study population from the Avon Longitudinal Study of Parents and Children included single-birth female subjects who had completed at least two puberty staging questionnaires between the ages of 8 and 13 years and whose mothers provided at least one analyzable prenatal serum sample. If more than one serum sample was available, the earliest sample provided was used for analysis. The study does not provide information as to when samples were collected. The females were divided into two groups: those who experienced menarche prior to age 11.5 years (n = 218) and a random sample of those who experienced menarche after age 11.5 (n = 230). Confounders such as the mother's prepregnancy BMI, age at delivery, age at menarche, educational level, and the child's birth order and ethnic background were included in linear and logistic regression models used to analyze the data. The median maternal serum PFOA concentrations were 0.0039 and 0.0036 µg/mL for the early menarche and nonearly menarche groups, respectively. The authors noted a modest nonsignificant association between the odds of earlier menarche and prenatal serum PFOA concentrations above the median. For all models, the CIs included the null value of 1.0. Similar results were obtained for PFOS.

Effects of prenatal exposure to PFOA (and PFOS) on female and male reproductive function was evaluated in 343 females and 169 males whose mothers participated in a cohort in 1988–1989 (Kristensen et al. 2013; Vested et al. 2013). Maternal blood samples were collected during week 30 of gestation. Follow-up was initiated in 2008 when the offspring were ~20 years old. Median serum PFOA level was 0.0036 μg/mL for the mothers with daughters evaluated. In adjusted regression analysis, daughters from mothers in the highest PFOA tertile had a 5.3-month later age at menarche (95% CI 1.3, 9.3) than those in the lowest tertile. No association was found between prenatal exposure to PFOS and age of menarche. No statistically significant relationships were found between PFOA (or PFOS) exposure and cycle length, reproductive hormone levels, or number of follicles assessed by ultrasound (Kristensen et al. 2013).

Lopez-Espinosa et al. (2011) examined the association of serum PFOA concentration and the age of puberty in exposed children of the Mid-Ohio Valley. Data from the C8 Health project (e.g., sex steroid hormone levels, self-reported menarche status) along with detailed date of birth information were used to determine age of puberty in males (n = 3,076) and females (n = 2,931) aged 8–18 years. Serum PFOA concentrations were divided into quartiles: <0.0114, 0.0114–0.023, >0.023–0.058, and >0.058 μg/mL. Confounders such as age at survey, BMI, BMI *z*-score, height, family income, ethnicity, smoking status, alcohol consumption, and date and time of sample collection were included in the logistic regression models used to analyze the data. The median PFOA concentrations were 0.026 and 0.020 μg/mL in males and females, respectively. No association between PFOA concentration and puberty was observed for males. Reduced odds of having reached puberty was associated with higher PFOA exposure in females (OR=0.57, 95% CI 0.37–0.89). There were 130 days of delay between the highest and lowest quartile. Reduced odds of experiencing menarche at a younger age (10–15 years) also was observed (OR 0.83, 95% CI 0.74–0.93). The results suggested that PFOA was associated with a later age of menarche. PFOS was associated with delayed puberty in both males and females. The authors expressed caution in interpretation of the data because of lack of serum PFOA concentration prior to puberty, PFOA concentration having been measured after the attainment of puberty, and lack of secondary sexual maturation data (i.e., physical, Tanner criteria, and biomarker measurements).

Male reproductive effects. Joensen et al. (2009) examined the association between PFASs, including PFOA, and testicular function in 105 Danish males who provided semen and blood samples as part of reporting for the military draft in 2003. The males chosen for the study had the highest testosterone concentrations (ranging from 30.1 to 34.8 nmol/L; n = 53; 18.2–24.6 years) and lowest testosterone concentrations (ranging from 10.5 to 15.5 nmol/L; n = 52; 18.2–25.2 years). Regression models were used to analyze associations between PFOA and testicular function. Median serum PFOA concentration was 0.0044, 0.0050, and 0.0049 μg/mL in the high testosterone, low testosterone, and combined groups, respectively. A nonsignificant negative association was observed between serum PFOA concentration and semen volume, sperm concentration, sperm count, sperm motility, or sperm morphology. No association was observed between serum PFOA concentration and testosterone, estradiol, sex hormone-binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin B. However, significantly fewer ($p<0.05$) morphologically normal sperm were seen in males with high combined levels of PFOA/PFOS (6.2 million spermatozoa) than in males with low PFOA/PFOS levels (15.5 million spermatozoa).

In a slightly expanded study, Joensen et al. (2013) investigated the associations between PFASs, including serum PFOA concentration, and reproductive hormones and semen quality in

247 healthy young Danish males (mean age 19.6 years). Serum samples were analyzed for PFOA as well as total testosterone (T), estradiol, SHBG, LH, FSH, and inhibin-B. The mean PFOA level was 0.0035 μg/mL. No associations were found between PFOA levels (or 12 other PFAS) and any hormone level or semen quality parameters. PFOS levels were negatively associated with testosterone.

An association between serum levels of seven PFASs and 35 semen quality parameters was evaluated in 462 males enrolled in the LIFE study cohort (Buck Louis et al. 2015). The males were from Michigan and Texas with a mean age of 31.8 years and mean PFOA levels 0.00429–0.00509 μg/mL. PFOA was significantly associated with a lower percentage of sperm with coiled tails, an increased curvilinear velocity, and a slightly larger acrosome area of the head. In total, six PFASs (including PFOA) were associated with changes in 17 semen quality endpoints.

Effects of prenatal exposure to PFOA (and PFOS on male reproductive function was evaluated in 169 males whose mothers participated in a cohort in 1988–1989 (Vested et al. 2013). Maternal blood samples were collected during week 30 of gestation. Follow-up was initiated in 2008 when the offspring were ~20 years old. Median serum PFOA level was 0.0038 μg/mL for mothers with sons evaluated. Multivariable regression models showed significant negative trends for sperm concentration and total sperm count in association with *in utero* exposure to PFOA. A 34% reduction in sperm concentration (95% CI 58, 5%) and a 34% reduction in total count (95% CI 62, 12%) were estimated for the highest exposure tertile compared with the lowest tertile. Maternal PFOA level also was positively associated with higher FSH and LH levels in the sons. No associations were found between PFOA level and percentage of progressive sperm, sperm morphology, semen volume, or testicular volume. PFOS was not associated with any outcome (Vested et al. 2013).

3.1.1.9 Steroid Hormones

Occupational exposure studies. Olsen et al. (1998) examined several hormones, including cortisol, estradiol, FSH, dehydroepiandrosterone sulfate, 17 gamma-hydroxyprogesterone (a testosterone precursor), free testosterone, T, LH, prolactin, and SHBG in male workers at the Cottage Grove, Minnesota, production plant for 1993 and 1995. This was the same population used for the thyroid hormone study described above for 111 workers in 1993 and 80 in 1995. Employees were placed into four exposure categories based on their serum PFOA levels: 0–1 μg/mL, 1– < 10 μg/mL, 10– < 30 μg/mL, and >30 μg/mL. Statistical methods used to compare PFOA levels and hormone values included multivariable regression analysis, ANOVA, and Pearson correlation coefficients. No association between serum PFOA and any hormone was observed, but some trends were observed. When the mean measures of the various hormones were compared by exposure categories, there was a statistically significant elevation in prolactin ($p = 0.01$) in 1993 only for the 10 workers whose serum PFOA levels were between 10 and 30 μg/mL compared to the lower two exposure categories.

Estradiol levels in the >30 μg/mL PFOA group in both years were 10% higher than in the other PFOA groups, but the difference was not statistically significant. These results were confounded by estradiol being correlated with BMI ($r = 0.41$, $p<0.001$ in 1993, and $r = 0.30$, $p<0.01$ in 1995). The authors postulated that the study might not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (e.g., 55 μg/mL PFOA in the CD rat). Only three employees in this study had PFOA serum levels that high. They also

suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

In the Sakr et al. study (2007a) of 1,025 workers at the DuPont Washington Works facility in West Virginia, an association was observed between serum PFOA and serum estradiol ($p = 0.017$) and testosterone ($p = 0.034$) in male workers; however, circadian variations of hormones were not taken into consideration during analysis. The biological significance of the results is unknown.

Costa et al. (2009) found no association between serum PFOA concentration and estradiol or testosterone in 53 male workers at a PFOA production plant in Italy based on medical surveillance data collected between 2000 and 2007.

High-exposure community studies. Knox et al. (2011) examined the endocrine disrupting effects of perfluorocarbons in females from the C8 Health Project by analyzing the relationship between serum PFOA, serum estradiol concentration, and menopause onset. The population included females over age 18 years (n = 25,957). Serum PFOA and estradiol concentrations were determined from blood samples. Females who were pregnant; had had full hysterectomies; and were taking any prescription hormones, selective estrogen receptor modulators, and/or fertility agents were excluded from estradiol analysis. Serum PFOA concentrations were grouped into quintiles (natural log-transformation)—Q1 = 0.00025–0.0112; Q2 = 0.0113–0.0198; Q3 = 0.0199–0.0367; Q4 = 0.0368–0.0849; and Q5 = 0.0850–22.412 μg/mL. Estradiol analysis was calculated by age group—18–42 years, >42 ≤ 51 years, and >51 ≤ 65 years. Menopause was determined by questionnaire. Menopause analysis was calculated by age group—30–42 years, >42 ≤ 51 years, and >51 ≤ 65 years—and excluded those who reported having had hysterectomies. Logistic regression models were adjusted for smoking, age, BMI, alcohol consumption, and regular exercise. PFOA concentration in females who had had hysterectomies was significantly higher than in females who had not had hysterectomies. Serum PFOA and estradiol concentrations were not associated, while PFOS levels were negatively associated with estradiol. The odds of attaining menopause analysis in the oldest group of females, showed that all quintiles were significantly higher for all quintiles than the lowest, and in females between the ages of 42 and 51 years, Q3, Q4, and Q5 were significantly higher than the lowest. PFOS also was associated with increased odds of attaining menopause in women 42–51 years and >51 years. Data interpretation was limited by the cross-sectional study design and survey-reported menopause without age or independent confirmation.

3.1.1.10 Neurodevelopment

High-exposure community studies. A subset of 321 children enrolled in the C8 Health Project was assessed for neurobehavioral development 3–4 years after enrollment (Stein et al. 2013). The children had serum samples collected at enrollment in 2005–2006 with the current follow-up evaluation conducted in 2009–2010, when the children were 6–12 years old. Both the mother and teacher completed surveys to elicit information on each child's executive function, attention deficit hyperactivity disorder- (ADHD-) like behavior, and behavioral problems. Information on family demographics and other health conditions of the child were included as confounders. Linear regression was used to determine the association between PFOA levels and mother and teacher reports.

The median PFOA level was 0.0351 µg/mL with an IQR of 0.0158–0.0941 µg/mL. When comparing the highest to the lowest PFOA quartile, survey results from the mother for both executive function and ADHD showed a favorable association for males, but an adverse association for females. These findings were not replicated when males and females were analyzed together or with results from the teacher surveys. No association was found between PFOA levels and either mother or teacher scores for behavioral problems in females and males.

In 2012, the C8 Science Panel concluded that there is no probable link between PFOA exposure and neurodevelopmental disorders in children, including attention deficit disorders and learning disabilities. Their conclusion was based on epidemiology studies conducted by the panel and other data available.

General population studies. Fei et al. (2008b) examined the association between plasma PFOA concentration in pregnant females and motor and mental developmental milestones of their children. The mothers self-reported the infant's fine and gross motor skills and mental development at 6 and 18 months of age. There was no association between maternal plasma PFOA concentration and Apgar score or between maternal plasma PFOA concentration and fine motor skills, gross motor skills, or cognitive skills at 6 and 18 months of age. The children born to females having higher plasma PFOA concentrations reached developmental milestones at the same times as children born to females having lower plasma PFOA concentrations. The authors concluded that there was no association between maternal early pregnancy levels of PFOA and motor or mental developmental milestones in offspring. However, in children at 18 months, mothers with higher PFOS levels were slightly more likely to report that their babies started sitting without support at a later age.

A subset of the Danish National Birth Cohort was evaluated for an association between prenatal PFAS exposure and the risk of cerebral palsy (Liew et al. 2014). A total of 156 cases of cerebral palsy were identified and matched to 550 randomly selected controls. Stored maternal plasma samples were analyzed for 16 PFAS and six compounds were quantifiable in >90% of the samples. For the cerebral palsy cases and matched controls, median maternal PFOA levels were 0.00456 and 0.00400 µg/mL, respectively, for males and 0.00390 and 0.00404 µg/mL, respectively, for females. Per natural-log unit increase in maternal PFOA level, the risk of developing cerebral palsy in males was significantly increased (RR=2.1; 95% CI 1.2, 3.6). Positive associations were also found with PFOS and perfluoroheptane sulfonate. No association was found between any PFAS level and risk of cerebral palsy in females.

Fei and Olsen (2011) examined the association between prenatal PFOA (and PFOS) exposure and behavior or coordination problems in children at age 7. The children and their mothers were part of the Danish National Birth Cohort. Behavioral problems were assessed using the Strengths and Difficulties Questionnaire (SDQ), and coordination problems were assessed using the Developmental Coordination Disorder Questionnaire (DCDQ) completed by the mothers. A total of 787 mothers completed the SDQ and 537 completed the DCDQ for children aged 7.01–8.47 years (mean age 7.15 years). The mean maternal PFOA concentration was 0.0057 µg/mL, and PFOA levels were divided into quartiles: <LLOQ-0.00395, 0.00396–0.00532, 0.00535–0.00711, and 0.00714–0.02190 µg/mL. A child having higher scores in total difficulties, emotional symptoms, and hyperactivity was negatively associated with the second or third PFOA quartiles (OR=0.56, 95% CI 0.27–1.19; $p<0.05$ and OR=0.36, 95% CI 0.15–0.82; $p<0.05$, respectively) when compared with females in the lowest quartile. ORs adjusted for parity, maternal age, prepregnancy BMI, pregnancy smoking and alcohol consumption, socio-occupational status, child gender, breast-feeding, birth year, home density, gestational age at blood draw, and

parental behavior problem as children did not show a positive association between prenatal PFOA exposure and behavior or coordination problems. Overall, no significant association between behavioral or coordination problems in children 7 years of age and prenatal PFOA (and PFOS) exposure was found.

Similar to the above study, the association between maternal PFOA (and PFOS) levels and offspring behavior and motor development was investigated in a subset of the Biopersistent Organochlorines in Diet and Human Fertility study (INUENDO) birth cohort (Høyer et al. 2015a). Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,106 mother-child pairs at follow-up between January 2010 and May 2012, when the children were 7–9 years old. The study population consisted of 526 pairs from Greenland, 89 pairs from Poland, and 491 pairs from Ukraine. Maternal blood samples for measurement of plasma PFOA levels were taken any time during pregnancy. Behavior of children was assessed with SDQ score, and logistic regression models were used in the analyses of PFOA tertile levels and behavioral problems. Motor development was assessed with DCDQ score, and linear regression was used for analyses. All analyses were performed on the entire cohort as well as by country, except that not all analyses could be performed on the Polish subset because of the small number of cases. The median maternal plasma PFOA level was 0.0014 μg/mL for the combined population and 0.0018, 0.001, and 0.0027 μg/mL for the pregnant females from Greenland, Ukraine, and Poland, respectively.

No associations were found between PFOA (and PFOS) levels and motor development score. Total SDQ score was not associated with PFOA levels; however, the OR of having an abnormal total SDQ score was 2.7 (95% CI 1.2, 6.3) for all groups combined. PFOS levels were associated with higher total SDQ score only in Greenland. The highest PFOA tertile was associated with a 0.5-point higher hyperactivity score in both the combined analysis and in Greenland, but no associations were found in Poland and Ukraine. The OR for hyperactive behavior in the combined analysis was 3.1 (95% CI 1.3, 7.2) for the highest tertile compared to the lowest PFOA tertile. In Greenland, the ORs for hyperactivity were increased for the middle (OR=5.4, 95% CI 1.1, 25.6) and highest (OR=6.3, 95% CI 1.3, 30.1) tertiles (Høyer et al. 2015a).

Hoffman et al. (2010) examined the associations between perfluorochemicals, including PFOA, and diagnosis of ADHD using the NHANES data from 1999–2000 and 2003–2004. The study population comprised 571 children aged 12–15 years, including those who had been diagnosed as having ADHD (n = 48) and/or were taking ADHD medications (n = 21). Age, gender, and race/ethnicity were included as covariates; and socioeconomic status, health insurance coverage and having a routine health care provider, living with someone who smokes, birth weight, admittance to a neonatal intensive care unit, maternal smoking, and preschool attendance were confounders. Regression models were used to analyze the data. The median serum PFOA level was 0.0044 μg/mL and ranged from 0.0004 to 0.0217 μg/mL. Serum PFOA was positively associated with parental report of ADHD (OR=1.12, 95% CI 1.01–1.23). The OR for serum PFOA and parental report of ADHD and ADHD medication use was 1.19 (95% CI 0.95–1.49). Both PFOS and perfluorohexane sulfonate also were positively associated with parentally reported ADHD. Data interpretation was limited by the cross-sectional study design, random misclassification error resulting from using current PFOA levels as proxy measures of etiologically relevant exposures, and other confounders not included in the available data.

3.1.1.11 Postnatal Development

General population studies. Andersen et al. (2010) examined the association between maternal plasma PFOA concentration and offspring weight, length, and BMI at 5 and 12 months of age. The mothers (n = 1,010) reported the information during an interviews, and weight and length measurements were used to calculate BMI. The median PFOA level was 0.0052 µg/mL with a range of 0.0005–0.0219 µg/mL. Maternal plasma PFOA concentration was inversely associated with weight at 5 months (β -30.2, 95% CI -59.3–-1.1), BMI at 5 months (β -0.067, 95% CI -0.129–-0.004), weight at 12 months (β -43.1, 95% CI -82.9–-3.3), and BMI at 12 months in male children (β -0.078, 95% CI -0.144–-0.011) in models adjusted for maternal age, parity, prepregnancy BMI, smoking, gestational age at blood draw, socioeconomic status, and breast-feeding. Similar inverse associations were found with PFOS. No associations were observed between maternal plasma PFOA concentration and the endpoints for female children in the adjusted models.

The latest report on the Danish National Birth Cohort evaluated an association between maternal plasma PFOA levels and the children's BMI, waist circumference, and risk of being overweight at 7 years of age (Andersen et al. 2013). From the subset of 1,400 females who provided blood samples during their first trimester, children were included if they had weight and height information (n = 811) or waist measurements (n = 804) at age 7 years. The median PFOA level was 0.0053 µg/mL with a range of 0.0005–0.0219 µg/mL. Maternal PFOA levels were inversely associated with all of the children's anthropomorphic endpoints, but statistical significance was not attained and a dose response was not observed. Maternal PFOA (or PFOS) did not affect the risk of being overweight in either males or females.

The association between maternal PFOA (and PFOS) levels and prevalence of offspring that are overweight plus waist-to-height ratio >0.5 was investigated in a subset of the INUENDO birth cohort (Høyer et al. 2015b). Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,022 mother-child pairs at follow-up between January 2010 and May 2012, when the children were 7–9 years old. The study population consisted of 531 pairs from Greenland and 491 pairs from Ukraine. Maternal blood samples for measurement of plasma PFOA levels were taken at a mean gestational age of 24 weeks. Each child's weight and height were measured and BMI calculated. All analyses were performed on the entire cohort as well as by country.

The median maternal plasma PFOA level was 0.0018 µg/mL in pregnant females from Greenland and 0.0010 µg/mL in pregnant females from Ukraine. No associations were found between PFOA (and PFOS) levels and risk of being overweight in the combined analysis or in Ukraine. In Greenland, the risk of being overweight was slightly increased only for females (RR=1.81, 95% CI 1.04, 3.17). PFOA association with risk of having waist-to-height ratio >0.5 was slightly increased for the combined analysis (RR=1.30, 95% CI 0.97, 1.74), but statistical significance was not attained. PFOS levels were significantly associated with waist-to-height ratio >0.5 in the combined analysis (Høyer et al. 2015b).

Halldorsson et al. (2012) examined prenatal exposure to PFASs, including PFOA, and the risk of being overweight at 20 years of age in a prospective study. A birth cohort consisting of 665 mother-offspring pairs was recruited from a midwife center in Aarhus, Denmark. Maternal PFOA levels were measured in serum samples collected during week 30 of gestation for assessment of *in utero* PFOA exposure and offspring anthropometry at 20 years. The median PFOA concentration was 0.0037 ± 0.0020 µg/mL with quartiles of 0.0024 ± 0.0006, 0.0033 ±

0.0004, 0.0042 ± 0.0005, and 0.0058 ± 0.0019 μg/mL. Three PFASs, including PFOS, perfluorooctane sulphonamide, and perfluorononanoate, increased across quartiles of PFOA concentration, but eight other PFASs did not. In covariate-adjusted analyses, female offspring whose mothers were in the highest quartile had 1.6 kg/m^2 higher BMI (95% CI: 0.6, 2.6) and 4.3 cm larger waist circumference (95% CI: 1.4, 7.3) than offspring whose mothers were in the lowest quartile. Female offspring of mothers in the highest versus lowest PFOA quartile were also more likely to be overweight [RR 3.1 (95% CI: 1.4, 6.9)] and to have a waist circumference >88 cm at 20 years of age [3.0 (95% CI: 1.3, 6.8)]. Among female participants who provided blood samples at clinical examination (n = 252), maternal PFOA concentration was positively associated with insulin, leptin, and the leptin-adiponectin ratio; and inversely associated with adiponectin levels. PFOA was not associated with being overweight or obesity in male offspring. The other PFASs were not significantly associated with any endpoint after adjustment for PFOA.

Geiger et al. (2014b) used data from the NHANES to determine whether there was a relationship between serum PFOA levels and hypertension in children. A total of 1,655 participants (aged 12–18 years) from the 1999–2000 and 2003–2008 cycles of the survey who had PFOA measurements available were examined. Blood pressure was measured to determine the presence of hypertension, and linear regression modeling was used to study the association between increasing quartiles of serum PFOA and mean changes in systolic and diastolic blood pressures. Mean PFOA level was 0.0044 ± 0.0001 μg/mL. No association was found between serum PFOA (or PFOS) levels and hypertension in either unadjusted or multivariable-adjusted analyses. Compared with the lowest quartile, the multivariable-adjusted OR (95% CI) of hypertension in the highest quartile of exposure was 0.69 (0.41–1.17) (*P*-trend >0.30).

3.1.1.12 Summary and Conclusions from the Human Epidemiology Studies

Numerous epidemiology studies have been conducted of workers, a large highly exposed community (the C8 Health Project), and the general population to evaluate the association of PFOA exposure to a variety of health endpoints. Health outcomes assessed include blood lipid and clinical chemistry profiles, thyroid effects, diabetes, immune function, birth and fetal and developmental growth measures, and cancer.

Serum lipids. The association between PFOA and serum lipids has been examined in several studies in different populations. Cross-sectional and longitudinal studies in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b; Steenland et al. 2015) and in the high-exposure community (the C8 Health Project study population) (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009; Winquist and Steenland 2014a) generally observed positive associations between serum PFOA and TC in adults and children (aged 1–< 18 yrs); most of these effect estimates were statistically significant. Although exceptions to this pattern are present (e.g., some of the analyses examining incidence of self-reported high cholesterol based on medication use [Steenland et al. 2015; Winquist and Steenland 2014a]), the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (with means varying between 0.4 and > 12 μg/mL), and the mean serum levels in the C8 population studies were around 0.08 μg/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 0.002–0.007 μg/mL (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al 2014a; Nelson et al. 2010; Starling et al. 2014). The interpretation of results for these general population studies is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and

PFOA. Additionally, many of the C8 studies do not appear to have controlled for the impact of diet on serum lipids.

Liver disease and liver function. Few studies of the relationship between PFOA and liver disease are available, but the C8 Health Project did not observe associations with hepatitis, fatty liver disease, or other types of liver disease. In the studies of PFOA exposure and liver enzymes (measured in serum), positive associations were seen. The results of the occupational studies provide evidence of an association with increases in serum AST, ALT, and GGT, with the most consistent results seen for ALT. The associations were not large and might depend on the covariates in the models, including BMI, use of lipid lowering medications, and triglycerides (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b). Two population-based studies of highly exposed residents in contaminated regions near a fluorochemical industry in West Virginia have evaluated associations with liver enzymes, and the larger of the two studies reported associations of increasing serum ln ALT and ln GGT levels with increasing serum PFOA concentrations (Emmett et al. 2006; Gallo et al. 2012). A cross-sectional analysis of data from the NHANES, representative of the U.S. national population, also found associations with ln PFOA concentration with increasing serum ALT and ln GGT levels. Serum bilirubin was inversely associated with serum PFOA in the occupational studies. A U-shaped exposure-response pattern for serum bilirubin was observed among the participants in the C8 Health Project, which might explain the inverse associations reported for occupational cohorts. Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential of PFOA to affect liver function.

Immune function. Associations between prenatal, childhood, or adult PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen, although there was some indication of effect modification by gender (i.e., associations seen in female children but not in male children) (Fei et al. 2010a; Granum et al. 2013; Looker et al. 2014; Okada et al. 2012). Three studies have examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children (Grandjean et al. 2012; Granum et al. 2013) and in adults (Looker et al. 2014). The study in adults was part of the high-exposure community C8 Health Project. A reduced antibody response to one of the three influenza strains tested after subjects received the flu vaccine was seen with increasing levels of serum PFOA; these results were not seen with PFOS. The studies in children were conducted in general populations in Norway and in the Faroe Islands. Decreased vaccine response in relation to PFOA levels was seen in these studies, but similar results also were seen with correlated PFASs (e.g., PFOS).

Thyroid. Three large studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease in women or children, but not in men (Lopez-Espinosa et al. 2012; Melzer et al. 2010; Winquist and Steenland 2014b). In addition, associations between PFOA and TSH were seen in pregnant females with anti-TPO antibodies (Webster et al 2014). In contrast, generally null associations were found between PFOA and TSH in people who had not been diagnosed with thyroid disease.

Diabetes. No associations were observed between serum PFOA levels and type II diabetes incidence rate in general or worker populations with mean serum PFOA up to 0.0913–0.113 μg/mL (MacNeil et al. 2009; Steenland et al. 2015). PFOA was not associated with measures of metabolic syndrome in adolescents or adults (Lin et al. 2009). However, one study found an

increased risk for developing gestational diabetes in females with mean serum PFOA (measured preconception) of 0.00394 μg/mL (Zhang et al. 2015).

Fertility, pregnancy, and birth outcomes. There are no occupational exposure or general population studies examining pregnancy-related hypertension and preeclampsia in relation to PFOA exposure. The only data available come from the high-exposure C8 Health Project study population. Several studies, using different designs and exposure measures, have examined that outcome in this population (Darrow et al. 2013; Savitz et al. 2012a, 2012b; Stein et al. 2009). There is a progressively greater refinement and reduction in misclassification (or exposure and outcome) among this set of studies. Each of the studies provides some evidence of an association between PFOA exposure and risk of pregnancy-induced hypertension or preeclampsia, with the most robust findings from the methodologically strongest study (Darrow et al. 2013).

The association between PFOA and birth weight was examined in numerous studies. Most studies measured PFOA using maternal blood samples taken in the second or third trimester or in cord blood samples. Studies on the high-exposure C8 community population did not observe associations between PFOA and either birth weight among term births or the risk of low birth weight among all (singleton) births (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012a, 2012b; Stein et al. 2009). In contrast, several analyses of general populations indicate a negative association between PFOA levels and birth weight (Apelberg et al. 2007; Fei et al. 2007; Maisonet et al. 2012), while others did not attain statistical significance (Chen et al. 2012; Hamm et al. 2010; Monroy et al. 2008; Washino et al. 2009). A meta-analysis of many of these studies found a mean birth weight reduction of 19 g (95% CI: -30, -9) per each one unit (ng/mL) increase in maternal or cord serum PFOA levels (Johnson et al. 2014). It has been suggested that GFR can impact birth weight (Morken et al. 2014). Verner et al (2015) conducted a meta-analysis based on PBPK simulations and found that some of the association reported between PFOA and birth weight is attributable to GFR and that the actual association could be closer to a 7-g reduction (95% CI: -8, -6). Verner et al. (2015) showed that, in individuals with low GFR, there are increased levels of serum PFOA and lower birth weights. While there is some uncertainty in the interpretation of the observed association between PFOA and birth weight given the potential impact of low GFR, the available information indicates that the association between PFOA exposure and birth weight for the general population cannot be ruled out. In humans with low GFR (which includes females with pregnancy-induced hypertension or preeclampsia), the impact on body weight is likely due to a combination of the low GFR and the serum PFOA.

Two studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples in follow-up of pregnancy cohorts conducted in England (Christensen et al. 2011) and in Denmark (Kristensen et al. 2013). The results of these two studies are conflicting, with no association (or possible indication of an earlier menarche seen with higher PFOA) in Christensen et al. (2011), and a later menarche seen with higher PFOA in Kristensen et al. (2013). Another study examined PFOA exposure measured concurrently with the assessment of pubertal status (Lopez-Espinosa et al. 2011). An association between later age at menarche and higher PFOA levels was observed, but the interpretation of this finding is complicated by the potential effect of puberty on the exposure biomarker levels (i.e., reverse causality).

Studies found a positive association with ADHD in children in the highly exposed community (Stein et al. 2013) and the general population (Hoffman et al. 2010). No other behavior endpoints in children were associated with maternal PFOA levels in either population.

Limited data suggest a correlation between higher PFOA levels (>0.02 μg/mL) in females and decreases in fecundity and fertility (Fei et al. 2009; Vélez et al. 2015), but there are no clear effects of PFOA on male fertility endpoints (0.0035–0.005 μg/mL) (Joensen et al. 2009, 2013).

C8 Science Panel conclusions. As part of the C8 Health Project, the C8 Science Panel used epidemiological and other data available to them to assess probable links between PFOA exposure and disease (C8 Science Panel 2012). Analyses conducted by the C8 Science Panel used historical serum PFOA estimates over time, which were developed based on estimated intake of contaminated drinking water. The panel concluded that a probable link existed between PFOA exposure and ulcerative colitis, high cholesterol, pregnancy-induced hypertension, and thyroid disease.

The C8 Science Panel found no probable link between PFOA exposure and multiple other conditions, including birth defects, other autoimmune diseases (e.g., rheumatoid arthritis, lupus, type 1 diabetes, Crohn's disease, MS), type II diabetes, high blood pressure, coronary artery disease, infectious disease, liver disease, Parkinson's disease, osteoarthritis, neurodevelopmental disorders in children (e.g., ADHD, learning disabilities), miscarriage or stillbirth, chronic kidney disease, stroke, asthma or COPD, and preterm birth or low birth weight (C8 Science Panel 2012).

3.1.2 Cancer

Occupational exposure studies. Several occupational studies examining cancer mortality have been conducted at 3M's Cottage Grove facility in Minnesota and at the DuPont Washington Water Works plant in West Virginia. These studies have focused on kidney, bladder, liver, pancreatic, testicular, prostate, thyroid, and breast cancers. For cancers with a high survival rate (i.e., bladder, kidney, prostate, testicular, thyroid, and breast cancer), studies that use mortality data provide a more limited basis for drawing conclusions than studies that use incidence data. The discussion in this section summarizes the design and results of the available studies, focusing on the most recent update of occupational cohorts. Table 3-12 presents results for studies of kidney and testicular cancer.

Raleigh et al. (2014) is the latest update of the analyses of mortality in the 3M Cottage Grove workers, previously analyzed in Lundin et al. (2009) and Gilliland and Mandel (1993). Raleigh et al. (2014) followed 4,668 Cottage Grove workers through 2008, using an improved exposure reconstruction method and adding a nonexposed worker referent group from a different 3M plant. In addition to the mortality data, incidence data based on state cancer registries also were included. Exposure estimates for inhalation exposures were calculated from work history records and industrial hygiene monitoring data; blood levels were not included. No associations were found between PFOA exposure and the risk of dying from any cancer type (see Table 3-12 for bladder, kidney, and testicular cancer results). The mean age of the workers was 29 years at the start of employment and 63 years at the end of follow-up.

Table 3-12. Summary of PFOA Epidemiology Studies of Kidney and Testicular Cancer

Reference and Study Details	Analysis Group	Kidney	Testicular
Occupational Settings			
Raleigh et al. 2014 3M, Minnesota n = 4,668, follow-up through 2008 Mean age: 29 yrs at start of employment Mortality and incidence Comparison based on another (non-PFOA) 3M plant in Minnesota (n = 4,359) Cumulative exposure level based on industrial hygiene data (air monitoring), and PFOA production levels [update of Gilliland and Mandel 1993 and Lundin et al. 2009]	All (Minnesota referent) By quartile, mortality analysis 1 up to 0.000026 µg/m3-yr 2 up to 0.00014 3 up to 0.00073 4 maximum not reported By quartile, incidence analysis 1 up to 0.000029 µg/m3-yr 2 up to 0.00015 3 up to 0.00079 4 maximum not reported	0.53 (0.20, 1.16) (n = 6) Mortality 0.32 (0.01, 1.77) (n = 1) 0.74 (0.09, 2.69) (n = 2) 1.66 (0.08, 2.38) (n = 2) 0.42 (0.01, 2.34) (n = 1) Incidence 1.07 (0.36, 3.16) (n = 4) 1.07 (0.36, 3.17) (n = 4 0.98 (0.33, 2.92) (n = 4) 0.73 (0.21, 2.48) (n = 4)	n = 5 incident cases reported; no further analysis done
Steenland and Woskie 2012 DuPont Washington Water Works, West Virginia n = 5,791, follow-up through 2007 Mortality [update of Leonard et al. 2008, which did not include analysis by cumulative exposure] [Steenland and Woskie 2012 examined incidence of bladder, colorectal, and prostate cancers, and of melanoma]	DuPont referent (plants from 8 surrounding states) U.S. referent Cumulative exposure (ppm-yrs) 0 - < 904 904 - < 1,520 15,20 - < 2,720 ≥ 2720	1.28 (0.66, 2.24) (n = 12) 1.09 (0.56, 1.90) (n = 12) 1.07 (0.02, 3.62) (n = 1) 1.37 (0.28, 3.99) (n = 3) -- (0.0, 1.42) (n = 0) 2.66 (1.15, 5.24) (n = 8)	1.80 (0.05, 10.03) (n = 1)
High-Exposure Community			
Vieira et al. 2013 C8 Health Project population (Ohio and West Virginia) Incidence Modeled estimates for 1951–2008 using residence at time of diagnosis and emissions data and environmental characteristics	Total for 6 water districts (median serum level ranged from 5 to 125 µg/l) Annual serum levels (µg/l); assumed 10-year residence and 10-year latency (Ohio) Unexposed Low: 3.7 - 12. Medium: 12.9 - 30.7 High: 30.8 – 109 Very high: > 100	1.1 (0.9, 1.4) (n = 94) 1.0 (referent) 0.8 (0.4, 1.5) (n = 11) 1.2 (0.7, 2.0) (n = 17) 2.0 (1.3, 3.2) (n = 22) 2.0 (1.0, 3.9) (n = 9)	(0.6, 1.8) (n = 18) (referent) 0.2 (0.0, 1.6) (n = 1) 0.6 (0.2, 2.2) (n = 3) 0.3 (0.0, 2.7) (n = 1) 2.8 (0.8, 9.2) (n = 6)
Barry et al. 2013 C8 Health Project population (Ohio and West Virginia) Case-control (n varies by cancer) Incidence Modeled estimates for 1951–2008 using individual-level data on residential history, drinking water source, tap water consumption, emissions data, environmental characteristics, water pipe installation, PK data, and workplace water consumption (and for workers, workplace exposure based on job exposure matrix and modeling using serum samples from 1979–2004 and job history data.	Full sample Cumulative exposure, quartiles (cutpoints based on cancer-specific case distribution; approximate midpoints) 1 (30-50 µg/mL-yr) 2 (90-200 µg/mL-yr) 3 (800-1400 µg/mL-yr) 4 (100,000 µg/mL-yr)	1.09 (0.97, 1.21) (n = 105) (referent) 0.99 (0.53, 1.85) 1.69 (0.3, 3.07) 1.43 (0.76, 2.69) trend p = 0.34 community cohort; HR = 1.0, 0.94, 1.08,1.50, trend p = 0.02; worker cohort HR = 1.0, 1.22, 3.27, 0.99, trend p = 0.42	1.28 (0.95, 1.73) (n = 17) 1.0 (referent) 0.87 (0.15, 4.88) 1.08 (0.20, 5.90) 2.36 (0.41, 13.7) trend p = 0.02 15 of the cases from the community sample; HR = 1.0, 0.98, 1.54, 4.66, trend p = 0.02

Steenland and Woskie (2012) updated the cohort study by Leonard et al. (2008) of employees at the DuPont Washington Works plant in West Virginia (see Table 3-12 for bladder, kidney, and testicular cancer results). This study included 5,791 individuals who had worked at the DuPont West Virginia plant for at least 1 year between 1948 and 2002. Mean duration of employment was 19 years. Deaths through 2008 were ascertained through either the National Death Index or death certificate data. Exposure quartiles were assessed by estimated cumulative annual serum levels based on blood samples from 1,308 workers taken during 1979–2004 and time spent in various job categories (ppm-years). Referent groups included both nonexposed DuPont workers in the same region and the U.S. population. Overall, the mean cumulative exposure was 7.8 ppm-years and the estimated average annual serum level was 0.35 μg/mL. A significant positive trend was found for kidney cancer with the SMR=2.66 (n = 8; 95% CI 1.15, 5.24) for workers in the highest quartile. The most recent report on the same cohort included 6,026 workers evaluated for disease incidence, based on self-report with validation from medical records (Steenland et al. 2015). Lifetime serum cumulative dose was estimated by combining occupational and nonoccupational exposures. Median measured serum level was 0.113 μg/mL based on samples collected in 2005. Bladder cancer incidence (n = 29 cases) decreased with increased PFOA levels (RR 1.0, 0.55, 0.47, and 0.31 across quartiles, trend p = 0.03). Prostate cancer risk increased in Q1 compared to Q2 (n = 1.92), and remained at this level in the remaining quartiles (RR 1.89 and 2.15 in Q3 and Q4, respectively, trend p = 0.10).

Cholecystokinin (CCK) is a peptide hormone that stimulates the digestion of fat and protein, causes the increased production of hepatic bile, and stimulates contraction of the gall bladder. Research in rats suggests that pancreas acinar cell adenomas observed in rodents might be the result of increased CCK levels secondary to blocked bile flow (Obourn et al. 1997). CCK was measured in male workers (n = 74 males) at the 3M's Cottage Grove plant in 1997 as part of the medical surveillance program (Olsen et al. 1998, 2000). Employees' serum PFOA levels were stratified into three categories (<1, 1– <10, and ≥10 ppm). The mean CCK values for the three PFOA categories were 33.4, 28.0, and 17.4 pg/mL, respectively. The means in the two serum categories < 10 ppm were at least 50% higher than in the ≥ 10 ppm category. A statistically significant negative association between mean CCK levels and the three PFOA categories was observed (p = 0.03). A multiple regression model of the natural log of CCK and serum PFOA levels continued to display a negative association after adjusting for potential confounders. As stated previously (Olsen et al. 2000), no abnormal liver function, hypolipidemia, or cholestasis was observed in the workers. The authors suggested that the lack of a positive association between PFOA and CCK in workers could have resulted from serum PFOA levels too low to cause an increase in CCK provided that the same mechanism that increases CCK levels in rodents exists in humans.

High-exposure community studies. Vieira et al. (2013) investigated the relationship between PFOA exposure and cancer among the residents living near the DuPont plant in Parkersburg, West Virginia. This analysis included incident cases of 18 cancers diagnosed from 1996–2005 in five Ohio counties and eight West Virginia counties that included public water districts contaminated with PFOA. The dataset included 7,869 cases from Ohio geocoded to residence and 17,238 cases from West Virginia linked to water district. Exposure levels and serum PFOA concentrations were estimated based on residence at time of diagnosis, using modeled data based on previous work in the C8 study population (Shin et al. 2011). Individual-level exposure was categorized as very high, high, medium, low, or unexposed based on serum concentrations of >0.110 μg/mL, 0.0308–0.109 μg/mL, 0.0129–0.0307 μg/mL, 0.0037–0.0129 μg/mL, and unexposed (background levels not given), respectively. Logistic regression was applied to

individual-level data to calculate ORs and CIs for each cancer category. Data were first analyzed by water district. The adjusted ORs were increased for testicular cancer and for kidney cancer (OR: 5.1, 95% CI: 1.6, 15.6; n = 8 and OR: 1.7, 95% CI: 0.4, 3.3; n = 10, respectively) in the Little Hocking water district and for kidney cancer (OR: 2.0, 95% CI: 1.3, 3.1; n = 23) in the Tuppers Plains water district. Both districts are in Ohio. Residents of Little Hocking also had increased OR for non-Hodgkin lymphoma (OR: 1.6, 95% CI: 0.9, 2.8; n = 14) and prostate cancer (OR: 1.4, 95% CI: 0.9, 2.3; n = 36). The analysis by exposure level for kidney and testicular cancers is shown in Table 3-12. Kidney cancer was positively associated with very high and high exposure categories (OR: 2.0, 95% CI: 1.0, 3.9; n = 9 and OR: 2.0, 95% CI: 1.3, 3.2; n = 22, respectively), while ORs for medium and low exposure categories were close to the null when compared to the unexposed category. The largest OR was for testicular cancer with the very high exposure category (OR: 2.8, 95% CI: 0.8, 9.2; n = 6), but the estimate was imprecise because of the small numbers. ORs for the other exposure categories were all <1.0. Ovarian cancer, non-Hodgkin's lymphoma, and prostate cancer were positively associated with the very high exposure category, but showed weaker or negative associations for the other exposure categories (Vieira et al. 2013).

Barry et al. (2013) extended the study of cancer incidence in the C8 Health Project population in an analysis of data from 32,254 study participants; there is some overlap in the cases included in Vieira et al (2013) and in Barry et al. (2013). The cohort included 3,713 current and former DuPont Washington Works employees, but results for this subset were limited by the small sample size for cancers of interest. Median serum PFOA levels, measured in 2005–2006 at enrollment in C8, were 0.024 and 0.113 µg/mL for community and worker populations, respectively. A proportional hazard regression model was run for each cancer type with the cancer as the outcome, time-varying cumulative PFOA serum concentration as the independent variable, and age as the time scale. Cumulative PFOA serum concentrations were estimated based on historical regional monitoring data and individual residential histories. Self-reported cancers were validated through a cancer registry or medical record. Confounders included smoking, alcohol consumption, gender, education, and 5-year birth year period. Testicular cancer risk was significantly increased with an increase in the log of estimated cumulative PFOA serum level (HR: 1.34, 95% CI: 1.00, 1.79; n = 17). Using estimated cumulative PFOA serum concentration quartiles, a significant monotonic trend was found for testicular cancer. Slight nonsignificant increases were seen for kidney cancer (HR: 1.10, 95% CI: 0.98, 1.24; n = 105) and for thyroid cancer (HR: 1.10, 95% CI: 0.95, 1.26; n = 86) (Barry et al. 2013).

Members of the C8 Health Project were evaluated for an association between serum PFOA levels and incidence of colon or rectal cancer (Innes et al. 2014). This cross-sectional study compared serum PFOA (and PFOS) levels at enrollment with diagnosis of primary colorectal cancer; 47,151 cancer-free adults and 203 cases were included. Serum PFOA levels ranged from <0.0005 to 22.4 µg/mL, with an average of 0.0866 µg/mL. An inverse relationship was found between PFOA level and diagnosis of colorectal cancer with OR = 0.64 (95% CI 0.44, 0.94; highest to lowest quartile, p for trend = 0.002). A concentration-related inverse association also was found between PFOS and colorectal cancer.

In 2012, the C8 Science Panel concluded that there is a probable link between exposure to PFOA and testicular and kidney cancer, but no other types of cancers. Their conclusion was based on the studies presented above, other epidemiology studies on cancer incidence in the mid-Ohio population, worker cohorts, and published data. Panel studies addressed 21 different

categories of cancer and looked for positive trends with increasing exposure as measured by cumulative serum levels.

General population studies. Eriksen et al. (2009) examined the association between plasma PFOA concentration and the risk of cancer in the general Danish population. The study population was chosen from individuals (aged 50–65 years) who had enrolled in the prospective Danish cohort Diet, Cancer and Health study between December 1, 1993, and May 31, 1997. The Danish Cancer Registry and Danish Pathology Data Bank were used to identify cancer patients diagnosed between December 1, 1993, and July 1, 2006. The cancer patients (n = 1,240) consisted of 1,111 males and 129 females whose median age was 59 years and who had prostate cancer (n = 713), bladder cancer (n = 332), pancreatic cancer (n = 128), or liver cancer (n = 67). The individuals (n = 772) in the subcohort comparison group were randomly chosen from the cohort study and consisted of 680 males and 92 females whose median age was 56 years. The participants each answered a questionnaire upon enrollment in the cohort study, and data on known confounders were obtained from the questionnaires. The plasma PFOA concentrations, based on blood samples provided by cancer patients at enrollment (1993–1997) were as follows: males 0.0068 μg/mL, females 0.0060 μg/mL, prostate cancer 0.0069 μg/mL, bladder cancer 0.0065 μg/mL, pancreatic cancer 0.0067 μg/mL, and liver cancer 0.0054 μg/mL. The plasma PFOA concentrations for the subcohort comparison group were 0.0069, 0.0054, and 0.0066 μg/mL for males, females, and combined, respectively. IRRs, crude and adjusted for confounders, did not indicate an association between plasma PFOA concentration and prostate, bladder, pancreatic, or liver cancer (see Table 3-12 for bladder cancer results). The plasma PFOA levels in the population were lower than those observed in occupational cohorts. This study is novel in that it is the first to examine PFOA levels and cancer in the general population.

A subset of females enrolled in the Danish National Birth Cohort was evaluated for an association between plasma PFOA levels (as well as 15 other PFASs) measured during pregnancy and risk of breast cancer during a follow-up period of 10–15 years (Bonefeld-Jørgensen et al. 2014). A total of 250 females diagnosed with breast cancer were matched for age and parity with 233 controls. The mean PFOA level in the controls was 0.0052 μg/mL while levels in the cases were divided into quintiles ranging from <0.0037 up to >0.0065 μg/mL. No association was found between PFOA levels and breast cancer risk. A weak positive association was found only with perfluorooctane sulfonamide.

Hardell et al. (2014) investigated an association between prostate cancer and levels of perfluoroalkyl acids (PFAAs) in whole blood. Patients with newly diagnosed prostate cancer (n = 201) had median PFOA levels of 0.002 μg/mL while the case-control group (n = 186) had a median level of 0.0019 μg/mL. PFOA levels were not associated with higher risks of prostate cancer when compared to controls or when analyzed according to Gleason score (pathology grade) and prostate-specific antigen. A significantly higher risk for prostate cancer was found for PFOA levels above the median combined with a first-degree relative with prostate cancer, indicating a genetic risk factor.

Two studies found no differences in blood and tissue PFOA levels between cancer and noncancer patients; the types of cancer in the patients were not defined. Vassiliadou et al. (2010) found that median serum PFOA concentrations among 40 cancer patients (0.00227 μg/mL in males; 0.00185 μg/mL in females) were similar to two control groups (0.00314 and 0.00181 μg/mL in males; 0.0017 and 0.00171 μg/mL in females). Yeung et al. (2013) found similar PFOS levels in serum and liver tissue between controls and those with hepatocellular carcinoma. Median serum levels in controls (n = 25) and patients with liver cancer (n = 24) were 0.00234

and 0.0025 µg/mL, respectively, and liver tissue were 0.506 (n = 9) and 0.495 (n = 12) ng/g, respectively.

3.1.2.1 Summary and Conclusions from the Human Cancer Epidemiology Studies

Evidence of carcinogenic effects of PFOA in epidemiology studies is based on studies of kidney and testicular cancer. These cancers have relatively high 5-year survival rates of 73% for kidney cancer and 95% for testicular cancer (based on National Cancer Institute [NCI] Surveillance, Epidemiology, and End Results data for 2005–2011). Thus studies that examine cancer incidence are particularly useful for these types of cancer. The high-exposure community studies also have the advantage for testicular cancer of including the age period of greatest risk, as the median age at diagnosis is 33 years. The two occupational cohorts in Minnesota and West Virginia (most recently updated, respectively, in Raleigh et al. 2014 and Steenland and Woskie 2012) do not support an increased risk of these cancers, but each of them is limited by a small number of observed deaths and incident cases. Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment of 0.024 µg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013). There is some overlap in the cases included in these studies. None of the general population studies examined kidney or testicular cancer, but no associations were found in the general population between mean serum PFOA levels up to 0.0866 µg/mL and colorectal, breast, prostate, bladder, or liver cancer (Bonefeld-Jørgensen et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014).

As part of the C8 Health Project, the C8 Science Panel (2012) concluded that a probable link existed between PFOA exposure and testicular and kidney cancer.

A group of independent toxicologists and epidemiologists critically reviewed the epidemiological evidence for cancer based on 18 studies of occupational exposure to PFOA and general population exposure with or without coexposure to PFOS. The project was funded by 3M, but the company was not involved in the preparation or approval of the report. The authors evaluated the published studies based on the study design, subjects, exposure assessment, outcome assessment, control for confounding, and sources of bias. They followed the Bradford Hill guidelines on the strength of the association, consistency, plausibility, and biological gradient in reaching their conclusion. They found a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population. The discrepant findings across the study populations were described as likely due to chance, confounding, and/or bias (Chang et al. 2014).

3.2 Animal Studies

Acute and short-term studies in monkeys, rats, and mice provide data on systemic toxicity and MoA. Subchronic studies in monkeys and rats found decreased body weight, increased liver weight accompanied by microscopic lesions, and decreased serum cholesterol. The most prominent microscopic lesion of the liver in both monkeys and rats was centrilobular hepatocellular hypertrophy. Data from studies of inhalation and dermal exposures are limited.

Chronic exposure studies were conducted in monkeys, rats, and mice providing information on tumor incidences for both rats and mice. Effects on development and reproduction were found in both rats (a 2-generation study) and mice (male fertility) and included developmental delays and increased neonatal mortality. Many developmental studies focused on the impact of

gestational/lactational exposure on mammary gland development and effects observed in offspring at maturity.

3.2.1 Acute Toxicity

Oral Exposure

Dean and Jessup (1978) reported an oral lethal dose for 50% of animals (LD_{50}) of 680 mg/kg and 430 mg/kg PFOA for male and female CD rats, respectively. Glaza (1997) reported an oral LD_{50} of greater than 500 mg/kg in male Sprague-Dawley rats and between 250 and 500 mg/kg in females. Gabriel (1976a) reported an oral LD_{50} of less than 1,000 mg/kg for male and female Sherman-Wistar rats. According to the Hodge Sterner Scale, these LD_{50} values suggest that PFOA can be classified as moderately toxic after acute oral exposures.

Rigden et al. (2015) exposed groups of five male Sprague-Dawley rats to doses of 0, 10, 33, and 100 mg/kg/day for 3 days and maintained them for 4 additional days with daily body weight measurement and overnight collection of urine. Following the recovery period, the animals were sacrificed with collection of serum samples for analysis. Major organs were weighed, and the liver homogenized. The serum samples, liver homogenate, and supernatant were kept frozen at -80°C until they were analyzed. Phase I and II drug metabolizing enzymes and palmitoyl-coenzyme A (-CoA) oxidase were measured in the liver homogenate. Urine was analyzed for malondialdehyde (MDA) and 8-hydroxydeoxyguanine. The results for PFOA were compared with those for 100-mg/kg/day doses of di(2-ethylhexyl) phthalate (DEHP) and fenofibrate, known inducers of PPARα.

There was a dose-related statistically significant increase in palmitoyl-CoA oxidase and liver weight at all PFOA doses. The palmitoyl-CoA increase was not significant for DEHP and fenofibrate with 100-mg/kg doses; liver weight increased significantly for fenofibrate but not DEHP. The only serum parameter that showed a significant dose-related response with PFOA was a decrease in uric acid compared to controls. Serum was analyzed for several minerals, proteins, enzymes (e.g., ALP, AST, ALT), glucose, cholesterol, and triglycerides. Phase I drug metabolizing enzyme activities (ethoxyresorufin-O-deethylase [EROD] and pentoxyresorufin-O-dependentilase [PROD]) were significantly increased at the 100-mg PFOA/kg/day dose, and glutathione-S-transferase (GST) activity was significantly decreased at the two highest doses, but not in a dose-related fashion. UDP-glucuronyltransferase (UDP-GT) was significantly lower than controls at all doses, but the changes did not demonstrate a dose-related response. There were no dose-related significant changes for the other analytes. The 10-mg PFOA/kg/day dose administered for 3 days was a LOAEL for effects on the liver associated with PPARα activation and for a decrease in serum uric acid. PFOA at 10 mg/kg/day for 3 days had a stronger impact on liver weight and palmitoyl-CoA activation than 100 mg/kg/day of DEHP and fenofibrate for the same exposure duration (Table 3-13). The 10-mg/kg/day dose was a LOAEL for liver effects usually associated with PPARα activation. The PFOA response was stronger than that for a 100-g/kg/day dose for the two known activators of PPAR-α.

Table 3-13. Comparison of PPAR-α Related Effects in Rats for PFOA, DEHP, and Fenofibrate after a 3-day Exposure

Chemical	Dose mg/kg/day	Liver wt. g	Palm. CoA abs/min/g prot	EROD nmol/min/mg prot	PROD nmol/min/mg prot	UDP-GT nmol/min/mg prot	GST nmol/min/mg prot
Control	0	4.28 ± 0.20	1.02 ± 0.37	0.066 ± 0.022	0.045 ± 0.012	1.69 ± 0.12	1.21 ± 0.11
PFOA	10	5.73 ± 0.29*	3.17 ± 0.65*	0.096 ± 0.024	0.080 ± 0.024	0.88 ± 0.09*	1.11 ± 0.09
PFOA	33	6.40 ± 0.20*	4.89 ± 0.79*	0.080 ± 0.015	0.078 ± 0.031	1.00 ± 0.14*	0.88 ± 0.12*
PFOA	100	6.62 ± 0.47*	6.11 ± 1.51*	0.113 ± 0.025*	0.107 ± 0.029*	1.12 ± 0.20*	0.94 ± 0.19*
DEHP	100	4.14 ± 0.34	1.40 ± 1.09	0.060 ± 0.012	0.039 ± 0.031	1.45 ± 5031	1.27 ± 0.11
Feno-fibrate	100	5.73 ± 0.24*	1.71 ± 0.58	0.060 ± 0.016	0.046 ± 0.020	1.09 ± 0.14*	0.94 ± 0.13*

Notes: Mean ±SD
* Significant ANOVA followed by Dunnett post-hoc test $p < 0.05$.

Inhalation Exposure

Rusch (1979) reported no mortality in male or female Sprague-Dawley rats following inhalation exposure to 186,000 mg/m^3 PFOA for 1 hour. Kennedy et al. (1986) reported a 4-hour lethal concentration for 50% of animals (LC_{50}) of 980 mg/m^3 for groups of six male rats exposed to PFOA as a dust in air. As reported in a later publication (Kennedy et al. 2004), body weight loss, irregular breathing, and red discharge around the nose and eyes were observed. Corneal opacity and corrosion were seen at concentrations greater than or equal to 810 mg/m^3.

Dermal/Ocular Exposure

The dermal LD_{50} in New Zealand White rabbits was determined to be greater than 2,000 mg/kg (Glaza 1995). Kennedy (1985) determined a dermal LD_{50} of 4,300 mg/kg for rabbits, 7,000 mg/kg for male rats, and 7,500 mg/kg for female rats. The animals lost body weight and exhibited lethargy, labored breathing, diarrhea, and severe skin irritation (Kennedy et al. 2004). PFOA is an ocular irritant in rabbits when the compound is not washed from the eyes (Gabriel 1976b), but is not an irritant in rabbits when washed from the eye (Gabriel 1976c). Markoe (1983) found PFOA to be a skin irritant in rabbits, while Gabriel (1976d) did not conclude that PFOA is a skin irritant.

3.2.2 Short-Term Studies

Oral Exposure

Monkey. In a range-finding study, Thomford (2001) administered PFOA to male cynomolgus monkeys as an oral capsule containing 0, 2, and 20 mg/kg/day PFOA for 4 weeks. There were three monkeys in the 2- and 20-mg/kg/day groups and one monkey in the control group. Animals were observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Body weights were recorded weekly and food consumption was assessed qualitatively. The monkeys were fasted overnight and blood samples were collected 1 week prior to the start of the study and on day 30 for measurement of serum PFOA, clinical hematology, and clinical chemistry, plus analysis for hormones (estradiol, estrone, estriol, TSH, total and FT3, and total and FT4). Blood samples also were collected from each animal on day 2 (approximately 24 hours after the first dose) for clinical chemistry measurements.

At scheduled necropsy, liver samples were collected for palmitoyl-CoA oxidase activity (a biomarker for peroxisome proliferation) and serum PFOA. Liver, testes, and pancreas were collected and assayed for cell proliferation using antibodies to proliferating cell nuclear antigen (PCNA). Bile was collected from each animal for measurement of bile acid. The adrenals, liver, pancreas, spleen, and testes from each animal were examined microscopically.

All animals survived to scheduled sacrifice. There were no clinical signs of toxicity in the treated groups and there was no effect on body weight. Low or no food consumption was observed for one animal given 20 mg/kg/day. There were no effects on the hormones measured with the exception of estrone, which was notably lower in the 2- and 20-mg/kg/day PFOA groups. There was no evidence of peroxisome proliferation or cell proliferation in the liver, testes, or pancreas of the treated monkeys. No adverse effects were noted in either the gross or clinical pathology evaluations. Under the conditions of this study, the NOAEL was 20 mg/kg and no LOAEL was established.

Rat. Pastoor et al. (1987) dosed male Crl:CD (SD) BR rats (n = 6 per group) for 1, 3, and 7 days with 0 and 50 mg PFOA/kg. Liver sections were collected at necropsy and stained with hematoxylin and eosin. Sections also were examined by electron microscopy. DNA content was also determined for the livers of rats dosed for 7 days. Treatment with 50 mg PFOA/kg for 7 days caused a 17% decrease ($p<0.05$) in mean body weight. Pair-fed control rats had a 24% decrease in body weight. Body weight was no different in the rats treated for 1 and 3 days than in the control rats. Liver weight of rats treated for 1 day was no different than control liver weight. The relative liver weight of rats treated for 3 days was significantly increased ($p<0.05$) compared to control relative liver weight. Absolute and relative liver weights were significantly increased ($p<0.05$) after the 7-day treatment with PFOA. A 57% decrease ($p<0.05$) was observed in relative hepatic DNA/g liver, but no difference was observed between total amount of hepatic DNA/liver and total amount of DNA/liver in control rats.

The hepatocytes of rats treated with PFOA for 3 days were enlarged with partially occluded sinusoids, and had numerous basophilic granules, eosinophilic granular material in the cytoplasm, and fewer perinuclear glycogen vacuoles compared to control hepatocytes. Enlarged hepatocytes with hyperplastic smooth endoplasmic reticulum (ER), increased mitochondria, increased peroxisomes, decreased rough ER, and increased autophagosomes with electron-dense material also were observed in the hepatocytes.

Loveless et al. (2008) administered 0, 0.3, 1, 10, and 30 mg linear PFOA/kg by oral gavage to groups of male CD rats (n = 10 per group) for 29 days. Body weight was recorded on days 0, 3, and 6–28. At necropsy, blood was collected for hematology, clinical chemistry, and corticosterone (CORT) measurements. Tissues were collected for weight and microscopic examination. Body weight, weight gain, hematocrit, and hemoglobin were reduced at ≥10 mg PFOA/kg/day. Increased reticulocytes and hematopoieses were observed in the rats dosed with 30 mg PFOA/kg/day. Total and non-HDL cholesterol were significantly reduced at 0.3 and 1 mg/kg/day compared to control. HDL cholesterol was significantly decreased at 0.3, 1, and 10 mg/kg/day. Triglyceride levels were significantly decreased at all doses except 1 mg/kg. Absolute liver weight (≥1 mg/kg/day) and relative liver weight (≥10 mg/kg/day) were significantly increased. Hepatocellular hypertrophy was graded as minimum to mild (0.3–1 mg/kg/day) and moderate (≥10 mg/kg/day), and focal necrosis was present at doses ≥ 10 mg/kg/day. Although not statistically significant, serum CORT was increased at ≥ 10 mg/kg/day. The decrease in cholesterol and triglycerides at the lowest dose are not necessarily adverse. The 1-mg/kg/day dose is classified as the NOAEL and the 10-mg/kg/day

dose as the LOAEL based on the observations of increased liver weight, hepatocellular hypertrophy, and hepatic necrosis at that dose. Data on several immunological endpoints were reported as part of the Loveless et al. (2008) publication. The immunological data from that study are included in section 3.3.2 of this report.

Cui et al. (2009) exposed male Sprague-Dawley rats (10 per group) to PFOA (96% active ingredient) at 0, 5, and 20 mg/kg/day for 28 days by gavage once daily. The activity of the rats was observed over the course of the study. All rats were sacrificed after the final exposure. The rats dosed with 5 mg/kg/day exhibited hypoactivity, decreased food consumption, cachexia, and lethargy during the third week of the study. Rats dosed with 20 mg/kg/day also exhibited sensitivity to external stimuli. The visceral index (i.e., hepatic, renal, gonad weight/animal's body weight) used to evaluate hyperplasia, swelling, or atrophy was significantly increased in the treated animals compared to control animals. In the liver, treatment with 5 or 20 mg PFOA/kg caused hepatic hypertrophy, fatty degeneration, and acidophilic lesions as well as angiectasis (gross dilation) and congestion in the hepatic sinusoid or central vein. In the lung, treatment with 5 or 20 mg PFOA/kg caused pulmonary congestion and focal or diffuse thickened epithelial walls. No histopathologic lesions were observed in the kidneys of the low-dose animals, but turbidness and swelling in the epithelium of the proximal convoluted tubule were observed at 20 mg PFOA/kg. Under the conditions of this study, the LOAEL was 5 mg/kg/day based on increased visceral indices, and liver and pulmonary lesions; no NOAEL was established.

Male Sprague-Dawley rats (n = 10 per group) were fed diets containing 0 and 300 ppm PFOA for 1, 7, and 28 days in two studies (Elcombe et al. 2010). The mean daily intake for study 1 and study 2 were 19 and 23 mg/kg/d, respectively. A group of rats was fed diets containing 50 ppm Wyeth 14,643, a PPARα agonist, as a positive control. The animals were observed daily and body weights and food consumption were recorded. At necropsy, day 2, day 8, or day 29, the organs were weighed, examined for gross pathology and preserved for histopathology. In study 1, liver DNA content and concentration were determined, and plasma was collected for analysis of liver enzymes, cholesterol, triglycerides, and glucose. Hepatic cell proliferation and apoptosis also were determined.

In both studies, body weight significantly decreased ($p<0.05$) after 7 and 28 days on the PFOA diet. Body weight was not affected by Wyeth 14,643. Absolute liver weight was significantly increased ($p<0.05$) in rats fed PFOA diets for 7 days in the first study and in rats treated for 7 and 28 days in the second study (Table 3-14). The liver-to-body-weight ratio was significantly higher in rats fed PFOA diets for 7 and 28 days in both studies. Absolute liver weight and liver-to-body-weight ratios were significantly increased in rats fed the Wyeth 14,643 diet in both studies.

After 1 day of eating the PFOA diet, subjects' plasma AST was significantly decreased and triglycerides were significantly increased. After 7 and 28 days on the PFOA diet, TC, triglycerides, and glucose levels were significantly decreased. The AST response did not show a duration-related response because there was a significant decrease at 1 and 28 days, but not at 7 days on the PFOA diet. Liver DNA concentration was significantly decreased ($p<0.05$) in all PFOA-exposed rats except those treated for 1 day in the second study, but liver DNA content was not altered by PFOA, suggesting that the increase in volume was responsible for the change in concentration.

Table 3-14. Hepatic Effects of Rats Exposed to PFOA

		Study 1			Study 2		
	Day	Control	300 ppm PFOA (19 mg/kg/day)	50 ppm Wyeth 14,643	Control	300 ppm PFOA (32 mg/kg/day)	50 ppm Wyeth 14,643
Liver weight (g)	1	13.6 ± 1.3	14.1 ± 2.4	15.7 ± 1.2	15.2 ± 1.9	14.4 ± 0.9	15.8 ± 1.4
	7	15.3 ± 1.3	19.2 ± 3.1*	23.1 ± 3.1*	16.6 ± 1.7	22.8 ± 2.6*	23.4 ± 2.5*
	28	18.3 ± 2.5	20.8 ± 3.2	30.6 ± 3.2*	17.2 ± 2.0	24.6 ± 2.2*	29.2 ± 4.0*
Liver-to-bw (g/kg)	1	4.25 ± .34	4.39 ± 0.44	4.64± 0.17*	4.39 ± 0.36	4.27 ± 0.14	4.49 ± 0.23
	7	4.10 ± 0.26	5.83 ± 0.55*	6.26 ± 0.48*	4.28 ± 0.24	6.56 ± 0.38*	6.34 ± 0.33*
	28	3.96 ± 0.36	5.83 ± 0.56*	7.09 ± 0.42*	3.70 ± 0.21	6.13 ± 0.53*	6.65 ± 0.59*
Labeling index (%)	1	0.22 ± 0.17	0.74 ± 0.55*	2.10 ± 1.10*	1.02 ± 0.37	2.18 ± 0.73*	4.54 ± 1.03*
	7	1.42 ± 0.65	5.94 ± 2.12*	12.56 ± 6.42*	2.57 ± 1.31	13.18 ± 3.18*	23.85 ± 7.02*
	28	ND	2.08 ± 1.03	10.15 ± 2.69	0.66 ± 0.45	1.74 ± 0.96*	5.34 ± 2.79*

Source: Elcombe et al. 2010
Notes: *Significantly different from control ($p < 0.05$); ND = No Data.

After 1 day on the Wyeth 14,463 diet, subjects'AST and TC were significantly decreased. After 7 and 28 days on the Wyeth 14,643 diet, subjects' ALT, TC, triglycerides, and glucose levels were significantly decreased. AST was not significantly decreased in rats fed Wyeth 14,643 diets for 28 days, but it was after 7 days. In the Wyeth 14,643 rats, liver DNA concentration was significantly decreased after 1 and 7 days in the first study and 7 and 28 days in the second study. Total liver DNA content in the Wyeth 14,643-treated rats was significantly increased after 7 and 28 days in both studies.

Labeling indices for hepatic cell proliferation, as measured by bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) incorporation, was significantly increased after day 1 and 7 in study 1 in both PFOA ($p<0.05$) and Wyeth 14,643 ($p<0.01$) diet-fed rats. Samples from control livers at day 29 were not available for comparison. In study 2, labeling was significantly increased ($p<0.05$) at all time points in both groups of rats compared to labeling in control rats (Table 3-14). Apoptosis of hepatic cells was not altered by treatment with PFOA at any time point. In rats fed diets containing Wyeth 14,643 for 28 days, hepatic apoptosis was significantly decreased ($p<0.01$) compared to apoptosis observed in control livers.

Histological examination of the livers of PFOA and Wyeth 14,643 diet-fed rats showed decreased glycogen after 1, 7, and 28 days. An increase in hepatocellular hypertrophy was observed after 7 and 28 days on the diets, fatty vacuolation was observed after 7 days on the diets, and increased hepatocellular hyperplasia was observed after 28 days on the diets. The hepatic observations were similar in both studies, and findings in Wyeth 14,643 diet-fed rats were generally more pronounced or severe than those in PFOA diet-fed rats. Although there were many similarities in response to the PFOA and Wyeth 14,463 diets, the body weight and apoptosis responses differed.

Mouse. Kennedy (1987) fed male and female Crl:CD-1 mice diets containing 0, 30, 300, and 3,000 ppm PFOA for 14 days. At necropsy body weight and liver weight were recorded and analyzed. No histological evaluations were conducted. All mice died at 3,000 ppm. At 300 ppm, body weight was decreased and one female died. Both male and female mice had significantly increased absolute and relative liver weights at all doses ($p<0.05$) compared to the control. The

LOAEL was 30 ppm based on increased liver weight, and no NOAEL was established. Kennedy (1987) used lower doses in a follow-up study lasting 21 days. Male and female mice were fed diets containing 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 ppm PFOA. Absolute and relative liver weights for male and female mice were significantly increased ($p<0.05$) at ≥ 3 ppm PFOA. The LOAEL was 3 ppm based on increased liver weight, and the NOAEL was 1 ppm.

Loveless et al. (2008) administered 0, 0.3, 1, 10, and 30 mg linear PFOA/kg by oral gavage to groups of male CD-1 mice (n = 20 per group) for 29 days. Body weight was recorded on days 0, 3, and 6–28. At necropsy, blood was collected for hematology, clinical chemistry, and CORT measurements. Tissues were collected for weight and microscopic examination. Body weight was significantly reduced at 10 and 30 mg/kg/day. An increase in neutrophils and monocytes was observed at ≥10 mg/kg/day along with a decrease in eosinophils. Serum CORT levels were significantly increased in mice dosed with 10 mg/kg and elevated in those dosed with 30 mg/kg/day. Total serum cholesterol and triglycerides were significantly decreased at ≥10 mg/kg/day. HDL was significantly reduced at ≥1 mg/kg/day. In mice treated with 30 mg/kg and water, triglycerides and HDL levels were significantly decreased compared to control levels. Absolute and relative liver weights were significantly increased at ≥1 mg PFOA/kg/day. Increased incidences of microscopic lesions in the liver included mild hepatocellular hypertrophy at 0.3 mg/kg/day, moderate-to-severe hypertrophy and individual cell necrosis at ≥1 mg/kg/day, and increased hepatocellular mitotic figures, fatty changes, and bile duct hyperplasia at ≥10 mg/kg/day. The LOAEL for this study was 1 mg/kg/day based on increased liver weight, hepatocellular hypertrophy, and cell necrosis; the NOAEL was 0.3 mg/kg/day. Data on several immunological endpoints were reported as part of the Loveless et al. (2008) publication. The immunological data are included in section 3.3.2 of this report.

Some of the epidemiology studies report that evaluated serum lipids demonstrate a positive correlation between total serum cholesterol and triglycerides and serum PFOA. Tan et al. (2013) used male C57BL/6N mice to determine if dietary fat content could be an important variable influencing the impact of PFOA on serum lipids. Groups of seven or eight 4-month-old male mice were given either a liquid regular fat diet (RFD) or a high-fat diet (HFD), with or without PFOA, for 3 weeks. The RFD provided 12% and the HFD provided 35% of their calories from fat. Calories from protein (18%) were equivalent in both diets. The RFD provided 60% and the HFD provided 40% of their calories from carbohydrate. The fats were primarily monounsaturated (olive oil) or polyunsaturated (safflower and corn oil). PFOA was added to both diets for 3 weeks at a level that maintained a dose of 5 mg/kg/day to the mice. The PFOA-treated groups were fed *ad libitum*, and the control groups were given the amount consumed by the PFOA-treated groups the previous day. Body weight; liver weight; plasma ALT, AST, and ALP; total and direct bilirubin; free fatty acids and liver triglycerides; as well as subcutaneous and epididymal white adipose tissue were monitored. Statistical differences between groups ($p<0.05$) were determined using one-way ANOVA. Liver and epididymal white fat tissue samples were examined histologically.

The fat content of the diets alone resulted in significant differences in body weight and subcutaneous white adipose tissue, but not in liver weight. The addition of PFOA to the RFD resulted in significant increases in body weight, liver weight, ALT, ALP, and plasma free fatty acids, but not in AST or bilirubin. The addition of PFOA to both the RFD and HFD resulted in decreases in the mass of both epididymal and subcutaneous white fat deposits.

The HFD alone did not result in definitive alterations in liver histopathology. When PFOA was added to the RFD, indications of hepatocyte hypertrophy, necrosis, and inflammatory cell

infiltration were observed. The liver damage in the animals being fed the HFD with PFOA was increased more than in the RFD-PFOA animals, as indicated by higher levels of necrosis and inflammation accompanied, in this case, by lipid droplet accumulation and significantly increased liver triglycerides, but not liver cholesterol or free fatty acids. In the epididymal adipose tissues, adipocyte size was increased in the HFD control compared to the RFD control but decreased with the addition of PFOA compared to both the RFD and HFD controls. Inflammatory cell infiltration was observed in the epididymal adipose tissues when PFOA was added to the HFD but not the RFD. No data for the subcutaneous white fat tissues was provided.

The authors evaluated the hepatic expression of 84 genes involved in the regulation of fatty acid metabolism using RT^2 Profiler PCR Arrays. HFD and/or PFOA altered the expression of 33 genes (> 1.5 fold). PFOA alone upregulated 13 genes (>1.5) and downregulated 4 (>1.5) genes with fatty acid and triglyceride catabolism. Eight fatty acid transport-related genes were upregulated by PFOA and one was downregulated. The study demonstrates the importance of the fat content of the diet as a modulator of the effects of PFOA on the liver in animals. Damage to the liver tissues was intensified in the presence of the HFD.

Son et al. (2008) administered 0, 2, 10, 50, and 250 mg/L PFOA (0, 0.49, 2.64, 17.63, and 47.21 mg/kg PFOA, respectively) in the drinking water to 4-week-old male imprinting control region (ICR) mice for 21 days. Food and water consumption, and body weight were recorded daily. At sacrifice, blood was collected and the liver and kidneys were removed and weighed. Plasma from the blood was used to determine levels of ALT, AST, BUN, and creatinine. Sections of the liver and kidney were processed and stained with hematoxylin and eosin or stained for caspase 3 (a biomarker for apoptosis). Expression of mRNA for tumor necrosis factor-α, interleukin-1β, and transforming growth factor-β were determined using reverse transcription polymerase chain reaction (RT-PCR).

The mice exposed to 250 mg/L PFOA (47.21 mg/kg/day) had significantly reduced food and water consumption ($p<0.05$), and body weight gain ($p<0.05$) compared to the control mice. Body weight gain also was significantly reduced ($p<0.05$) in mice receiving 50 mg/L PFOA in the drinking water. In all PFOA-exposed mice, relative liver weight was significantly increased in a dose-dependent manner ($p<0.05$) compared to liver weight of the control mice. Relative kidney weight was not affected by PFOA exposure. At ≥ 10 mg/L PFOA (2.64 mg/kg/day), plasma ALT activity was significantly increased, and at ≥ 50 mg/L PFOA (17.63 mg/kg/day), plasma AST activity was significantly elevated compared to the activity level in the control mice. Exposure to PFOA did not affect BUN or creatinine.

The livers of mice exposed to ≥50 mg/L PFOA were characterized by enlarged hepatocytes with acidophilic cytoplasm and the presence of eosinophils. No apoptotic bodies were observed in the liver with staining for caspase 3. Exposure to PFOA did not affect kidney morphology and did not cause toxic damage or necrosis in the kidney. In the liver, tumor necrosis factor-α expression was significantly reduced at ≥ 50 mg/L PFOA, interleukin-1β expression was significantly reduced at 250 mg/L PFOA, and transforming growth factor-β expression was significantly elevated at ≥ 50 mg/L PFOA. Under the conditions of this study, the LOAEL was 2 mg/L (0.49 mg/kg/day) based on increased liver weight, and no NOAEL was established. The LOAEL for increased plasma ALT was 2.64 mg/kg/day.

Wolf et al. (2008a) gavage-dosed wild-type 129S1/SvlmJ mice (n = 7–8 per group) and PPARα-null mice (129S4/SvJae-$PPAR\alpha^{tm1Gonz}$/J, n = 6–8 per group) with 0, 1, 3, or 10 mg PFOA/kg or 50 mg Wyeth 14,643, a PPARα agonist, and wild-type CD-1 (n = 7–8 per group)

with 0, 1, and 10 mg PFOA/kg for 7 days to characterize hepatic effects resulting from exposure. The mice were sacrificed 24 hours following the last dosing. Blood was collected for serum, and the livers were removed and weighed. Liver sections were stained with hematoxylin and eosin for examination by light microscopy and with uranyl acetate for transmission electron microscopy. Liver sections were also processed for immunohistochemistry of PCNA. Hepatocyte hypertrophy and vacuolation, observed in both strains of wild-type mice, were assigned a score from 0 to 4 based on severity, with 0 being no lesions observed and 4 being panlobular hypertrophy with cytoplasmic vacuolation. Hepatic lesions in PPARα-null were assigned a score (0–4) based on cytoplasmic vacuolation as no hypertrophy was observed. The percentage labeling index was obtained by counting the number of positive PCNA cells in 900–1,000 hepatocyte nuclei per animal. Slides were read blind to treatment but with knowledge of genetic status.

Compared to control values, the absolute and relative liver weights, lesion score, and labeling index were significantly increased ($p<0.05$) in a dose-dependent manner in both strains of wild-type mice exposed to PFOA and also were significantly increased ($p<0.05$) in the wild-type 129S1/SvlmJ mice exposed to Wyeth 14,643 (see Table 3-15). The absolute and relative liver weights and lesion score were significantly increased ($p\leq0.05$) in a dose-dependent manner in all PFOA-exposed PPARα-null mice. The labeling index was significantly increased ($p<0.05$) in PPARα-null mice exposed to 10 mg PFOA/kg. Absolute and relative liver weights, lesion score, and labeling index of PPARα-null mice exposed to Wyeth 14,643 were no different from control values.

Table 3-15. Hepatic Effects in PFOA-Treated Mice

Group	Liver Weight (g)	Relative Liver Weight (%)	Lesion Score	Labeling Index
Wild-type CD-1 Mice				
Control	1.53 ± 0.14	4.5 ± 0.4	0.3 ± 0.5	0.6 ± 0.4
1 mg/kg/day PFOA	2.26 ± 0.24*	6.5 ± 0.5*	2.1 ± 0.9	0.7 ± 0.5
10 mg/kg/day PFOA	3.48 ± 0.54*	10.5 ± 0.8*	3.0 ± 0*	7.7 ± 3.0*
Wild-type 129S1/SvlmJ Mice				
Control	0.87 ± 0.08	3.3 ± 0.4	0.3 ± 0.5	0.3 ± 0.2
1 mg/kg/day PFOA	1.22 ± 0.22*	1.6 ± 0.2*	2.0 ± 0.0*	0.7 ± 0.6
3 mg/kg/day PFOA	1.70 ± 0.12*	6.4 ± 0.4*	2.0 ± 0.0*	1.0 ± 0.4
10 mg/kg/day PFOA	2.20 ± 0.23*	8.3 ± 0.2*	4.0 ± 0.0*	2.4 ± 0.9*
50 mg/kg/day Wyeth 14,643	1.5 ± 0.13*	5.6 ± 0.1*	3.3 ± 0.5*	2.1 ± 1.2*
PPARα-null Mice				
Control	0.92 ± 0.08	3.4 ± 0.4	1.1 ± 0.4	0.2 ± 0.2
1 mg/kg/day PFOA	1.2 ± 0.14*	4.5 ± 0.2*	1.9 ± 0.6*	0.6 ± 0.4
3 mg/kg/day PFOA	1.46 ± 0.21*	5. 8 ± 0.3*	3.0 ± 0.0*	0.6 ± 0.3
10 mg/kg/day PFOA	2.8 ± 0.18*	9.4 ± 0.6*	4.0 ± 0.0*	7.7 ± 3.0*
50 mg/kg/day Wyeth 14,643	1.07 ± 0.24	3.9 ± 0.5	1.4 ± 0.5	0.6 ± 0.5

Source: Wolf et al. 2008a
Note: * Statistically different from control, $p < 0.05$.

Ultrastructure evaluations were done on liver sections from wild-type 129S1/SvlmJ mice and PPARα-null mice, but not from CD-1 mice. There were the expected differences in the characteristics of hepatocytes from the control wild-type mice when compared to both the PFOA-treated and Wyeth 14,643 wild-type mice. In the PPARα-null mice, the responses of the control and Wyeth 14,643-dosed animals were similar, but the response of the PFOA-dosed animals differed. Table 3-16 summarizes the cellular characteristics of the hepatocytes for the control, POFA-treated, and Wyeth 14,643-treated wild-type and PPARα-null mice on the basis of their glycogen content, Golgi bodies and associated rough ER, mitochondria, peroxisomes, and lipid-like cytoplasmic vacuoles.

Table 3-16. Mouse Hepatocyte Ultrastructure After PFOA or Wythe 14,643 Treatment

	Characteristics				
Mouse/Treatment	**Glycogen**	**Golgi/ Rough ER**	**Mitochondria**	**Peroxisomes**	**Lipid-like Vacuoles**
Wild-type/Control	Prominent	Prominent	Numerous	Few	Rare
Wild-type/PFOA (10 mg/kg)	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
Wild-type/Wyeth	Negative	Nominal/ scarce ER	Numerous	Numerous	Scattered
PPARα-null/Control	Prominent	Prominent	Numerous	Absent	Scattered
PPARα-null/PFOA (10 mg/kg)	Limited	Limited	Not reported	Not reported	Numerous[a]
PPARα-null/Wyeth	Prominent	Prominent	Numerous	Absent	Scattered

Source: Wolf et al. 2008a

Note: [a] Described as electron-dense, nonmembrane-bound spaces morphologically consistent with lipids ranging from the size of mitochondria to the size of nuclei. The vacuoles were believed to be an accumulation of PFOA.

It is apparent from the data in Table 3-16 that PFOA and Wyeth 14,643 behaved similarly in the wild-type strains but differently in the PPARα-null mice. The hepatocytes of PFOA-dosed PPARα-null mice exhibited lower glycogen content, Golgi bodies, and associated rough ER than both the control and Wyeth 14,643 PPARα-null mice. In addition, the PFOA-dosed PPARα-null mice had numerous large nonmembrane-bound lipid-like vacuoles throughout the cytoplasm. At the high dose (10 mg/kg/day), there was an increase in the labeling index that was not observed with Wyeth 14,643. The authors concluded that the large lipid-like vacuoles in the hepatocytes of PFOA-dosed PPARα-null mice were likely accumulations of PFOA. Under the conditions of this study, the LOAEL was 1 mg/kg/day based on increased absolute and relative liver weight and hepatic morphology changes; no NOAEL was established.

Nakamura et al. (2009) investigated the functional difference in PFOA response between mice and humans using a humanized PPARα transgenic mouse strain (hPPARα). Humanized PPARα mice express a high level of human PPARα protein in the liver. Male 8-week-old wild-type (mPPARα) mice, PPARα-null mice, and hPPARα mice were gavage-dosed with 0, 0.1, and 0.3 mg/kg/day PFOA (n = 4–6 per group) for 2 weeks and sacrificed 18–20 hours following the last dose. Blood was collected and analyzed for triglyceride and cholesterol concentrations, and ALT measurements. Livers were collected and analyzed for triglyceride and cholesterol concentrations, plus histopathological changes. The differences in the observations for the three strains of mice are summarized in Table 3-17.

Table 3-17. Relative Response of hPPARα, mPPARα, and PPARα-null Mice to PFOA

Parameter	hPPARα	mPPARα	PPARα-null
Liver weight	ND	↑ compared to control (0.3 mg/kg/day)	↓ compared to control (0.1 mg/kg/day)
Liver/body weight ratio	ND	↑ compared to control (0.3 mg/kg/day)	ND
Hepatocyte hypertrophy	Mild (0.3 mg/kg/day)	Mild (0.3 mg/kg/day)	ND
ALT	ND	ND	ND
Plasma cholesterol	↑ compared to mPPARα (all doses)	ND	ND
Liver cholesterol	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day), mPPARα (0.3 mg/kg/day)	↑ compared to control (0.3 mg/kg/day)	ND
Plasma triglyceride	ND	ND	ND
Liver triglyceride	↓ compared to PPARα-null (0.3 mg/kg/day)	↓ compared to PPARα-null (0.1, 0.3 mg/kg/day; ↑ compared to control (0.3 mg/kg/day)	↑ compared to mPPARα (all doses)

Source: Nakamura et al. 2009
Notes:
hPPARα: transgenic mice (that express a high level of human PPARα protein in the liver); mPPARα: wild-type mice.
↑ = significant increase ($p < 0.05$).
↓ = significant decrease ($p < 0.05$)
ND = no differences.

Body weight of the hPPARα mice was slightly lower than the mPPARα and PPARα-null mice prior to PFOA treatment and remained lower throughout the dosing regimen. Treatment with PFOA did not affect plasma ALT or triglyceride concentrations in any group. The hPPARα mice differed from the wild-type mice in that their plasma cholesterol was significantly increased and their liver cholesterol and triglycerides significantly decreased at the highest dose (Table 3-17). In addition, the increases in absolute and relative liver weights were less than those observed in the wild-type mice. The PPARα-null mice differed from the wild-type in that liver triglycerides were significantly increased. Comparable to the Wolf et al. (2008a) report, the cytoplasmic vacuoles were larger in the PPARα-null mice than in the wild-type and hPPARα mice. There were no other significant differences between PPARα-null mice and wild-type mice.

Under the conditions of the study, the LOAEL for mPPARα mice was 0.3 mg/kg/day of PFOA based on increased liver weight and increased liver triglyceride and cholesterol concentrations. The NOAEL for mPPARα mice was 0.1 mg/kg/day of PFOA. The NOAEL for PPARα-null mice was 0.3 mg/kg/day because the changes in absolute liver weight were not dose-related and the increase in relative liver weight was not significantly different from the control. The NOAEL for hPPARα mice was 0.3 mg/kg/day of PFOA, the highest dose tested. However, a nonsignificant but dose-related increase was observed in plasma cholesterol.

Minata et al. (2010) examined hepatobiliary injury in mice treated with PFOA. Male wild-type 129S4/SvlmJ mice (n = 39) and PPARα-null (129S4/SvJae-Pparα^{tm1Gonz}/J mice (n = 40) were orally dosed with 0, 12.5, 25, and 50 μmol/kg/day of PFOA (equivalent to ~0, 5.4, 10.8, and 21.6 mg/kg/day of PFOA) for 4 weeks. At the end of 4 weeks, animals were sacrificed and blood, liver, and bile were collected for clinical chemistry analysis and determination of PFOA concentration. Sections of the liver were processed for histological examination, oxidative DNA damage, and multidrug resistance protein 2 (Mdr2) and tumor necrosis factor α (TNF-α) mRNA

expression. Bile acid and phospholipid contents in bile were determined as well as the protein expression of canalicular bile salt export pump (BSEP) and canalicular MRP2.

Absolute and relative liver weights in all PFOA treated wild-type and PPARα-null mice were significantly increased ($p<0.05$) at sacrifice compared to control liver weight. Plasma AST was significantly increased in wild-type mice at 25 and 50 μmol/kg/day (equivalent to 10.8 and 21.6 mg/kg/day) and in PPARα-null mice at 50 μmol/kg/day compared to the concentrations of their respective controls. Plasma ALT was no different from control in the treated mice. In wild-type mice, total bilirubin was significantly decreased at 12.5 μmol/kg/day and significantly increased at 50 μmol/kg/day. In PPARα-null mice, total bilirubin was significantly increased at 50 μmol/kg/day. Total bile acid was significantly increased at 50 μmol/kg/day in PPARα-null mice. TC was significantly decreased in wild-type mice at 25 and 50 μmol/kg/day, and total triglyceride was significantly increased at 12.5 and 25 μmol/kg/day. TC was significantly decreased at 12.5 and 25 μmol/kg/day and significantly increased at 50 μmol/kg/day in PPARα-null mice. In PPARα-null mice, total triglycerides were significantly increased at all doses.

Hepatocellular hypertrophy was observed in wild-type mice treated with 12.5, 25, and 50 μmol/kg/day (equivalent to 5.4, 10.8 and 21.6 mg/kg/day). A dose-dependent increase in eosinophilic cytoplasmic changes consistent with peroxisome proliferation was observed in liver parenchyma, but no fat droplets or focal necrosis were observed in wild-type mice. An increase in bile duct epithelium thickness suggested slight cholangiopathy in wild-type mice at 25 and 50 μmol/kg/day. Increased apoptosis in hepatic cells, hepatic arterial walls, and bile duct epithelium was observed at 25 and 50 μmol/kg/day in wild-type mice. Ultrastructure examination of livers from PFOA-treated wild-type mice showed decreased glycogen granules, degranulated or disrupted rough ER, nuclear vacuoles, extensive peroxisome proliferation, and slight mitochondria proliferation.

In PPARα-null mice treated with 12.5, 25, and 50 μmol/kg/day of PFOA (equivalent to 5.4, 10.8 and 21.6 mg/kg/day), hepatocellular hypertrophy, cytoplasmic vacuolation, and increased microvesicular steatosis were observed. These observations are consistent with Wolf et al. (2008a). At 50 μmol/kg/day, focal necrosis was observed. Areas of bile fibrosis and bile plaque and few inflammatory cells were observed in the bile ducts of PPARα-null mice at 25 and 50 μmol/kg/day. Increased apoptosis was observed in bile duct epithelium at 25 and 50 μmol/kg/day in PPARα-null mice. Ultrastructure examination of livers from PFOA-treated PPARα-null mice showed decreased glycogen granules, degranulated or disrupted rough ER, increased cytoplasmic lipid accumulation, mitochondria proliferation, and mitochondrial changes (e.g., swelling and decreased matrix density). Peroxisome proliferation was not observed. Ultrastructure of bile duct showed degradation of cytoplasmic structure, vacuolization, disintegration of nuclei and organelles, periductal infiltration of fibroblasts and macrophages, and fibrosis.

The marker for oxidative damage, 8-hydroxydeoxyguanosine (8-OH-dG), and TNF-α were not elevated or upregulated in wild-type mice. In PPARα-null mice, 8-OH-dG was elevated in the liver at 21.6 mg/kg/day and TNF-α mRNA was significantly increased at 10.8 and 21.6 mg/kg/day. The transporter Mdr2 moves biliary phospholipids from hepatocytes to bile and was significantly upregulated in wild-type mice at all doses, but only at 5.4 mg/kg/day in PPARα-null mice. The BSEP transports bile acid from hepatocytes to bile and was significantly decreased in wild-type mice at 21.6 mg/kg/day, significantly increased in PPARα-null mice at 5.4 mg/kg/day, and significantly decreased at 21.6 mg/kg/day. The transporter MRP2 also transports bile acid and was significantly decreased at 21.6 mg/kg/day in both groups of mice.

Under the conditions of the study, the LOAEL for male wild-type and PPARα-null mice was 5.4 mg/kg/day of PFOA based on increased liver weight. A NOAEL was not established. At the LOAEL, the difference between the PPARα-null mice and the wild-type mice was the presence of cytoplasmic vacuoles and microvesicular steatosis in addition to hypertrophy in the PPARα-null mice.

The effects of gavage exposure on groups of six male Klunming mice (8 weeks old) to doses of 0, 2.5, 5, and 10 mg PFOA/kg/day for 14 days on the testes and epididymis was examined by Liu et al. (2015). The lowest dose tested was a LOAEL for dose-related effects on decreased sperm count, testicular superoxide dismutase (SOD), catalase, nuclear respiratory factor 2 (NRF2), and BAX expression ($0<0.05$) plus increases in MDA, hydrogen peroxide, BAX and BCL expression ($p<0.05$). There was no effect on relative testes weight at any dose. Some effects were observed on testicular morphology at the lowest dose, including atrophy of the seminiferous tubules, depletion of spermatogonial cells, detachment of germ cells from the seminiferous epithelium, and decreased sperm production. The severity of the testicular morphological changes increased with dose. Six animals per dose group were used for the evaluation of testicular weight and 4 animals per dose group were used for the other assays. The increase in MDA and hydrogen peroxide accompanied by the decrease in SOD and carnitine acyltransferase (CAT) activity and NRF2 expression indicate that oxidative stress played a major role in the observed toxicity. NRF2 plays an important role as a messenger that upregulates genes involved in response to oxidative stress.

Lu et al. (2015) reported on the testicular effects of PFOA on the blood testes barrier after a 28-day exposure of BALBL/c male mice (14 days old) to gavage doses of 0. 1.25, 5, and 20 mg/kg/day (3–5 animals per dose group). The blood testes barrier divides the seminiferous epithelium into apical and basolateral compartments and plays an important role in germinal cell development and male fertility. The barrier prevents the passage of large molecules from one compartment to the other. At termination of the exposure, the animals were sacrificed and the testes recovered for analysis. A second component of this study examined the impact of the PFOA treatment on male fertility and is reported in section 3.2.6.

The blood testes barrier integrity was weakened at the lowest dose tested and in a dose-dependent manner as indicated by the passage of a red fluorescent dye injected into the interstitium and concentrations of IgG measure in gel electrophoresis columns visualized by chemiluminescence (three per dose group). Membrane integrity is dependent on coexisting tight junctions, basal ectoplasmic specializations, and gap junctions. Accompanying *in vitro* assays of cultured sertoli cells demonstrated downregulation of key proteins associated with the tight junction and gap junction intercellular communication (GJIC) and regulation of the ectoplasmic specialization protein N-cadhedran. Tumor necrosis factor actin protein in the testes increased in a dose-related fashion at 5 and 20 mg/kg/day on observation of three per dose group. The authors identified the 5-mg/kg/day dose as a LOAEL for PFOA effects on the blood testes barrier and the 1.25-mg/kg/day dose as a NOAEL, apparently based on the results for the key protein biomarkers for cellular intercommunication rather than the IgE and fluorescence results where 1.25 mg/kg/day was a LOAEL.

Li et al. (2011) investigated the involvement of mouse and human PPARα in PFOA-induced testicular toxicity. Wild-type, PPARα-null, and humanized PPARα male 129/Sv mice were given PFOA daily by gavage at doses of 0, 1, and 5 mg/kg/day for 6 weeks. Body weight and testis weight were not affected by treatment in any group. Absolute and relative-to-body weights of the epididymis and seminal vesicle plus prostate gland were decreased only in high-dose wild-type

mice compared to the wild-type controls. No effects on sperm count and motility were seen in any group. Sperm abnormalities were significantly increased in both treated groups of wild-type and humanized PPARα mice, but not in the PPARα-null mice. Plasma testosterone levels were slightly decreased in low-dose wild-type mice, and significantly decreased in high-dose wild-type and low- and high-dose humanized PPARα mice compared to the control groups. Testosterone levels were slightly reduced in a dose-related manner in the PPARα-null mice, but statistical significance was not attained.

Using real-time quantitative PCR, the mRNA levels for several genes associated with testicular cholesterol synthesis, transport, and testosterone biosynthesis were examined. Levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and aromatase were not changed after treatment in any group. Expression of steriodogenic acute regulatory protein (which transports cholesterol into mitochondria) was inhibited in wild-type mice at the high dose and in humanized PPARα mice at both doses; peripheral benzodiazepine receptor level was decreased only in high-dose humanized PPARα mice; cytochrome P450 side-chain cleavage enzyme was decreased in both groups of wild-type mice; cytochrome P450 17α-hydroxylase/C17-20 lyase was inhibited at the high dose in both wild-type and humanized PPARα mice; and 3β-hydroxysteroid dehydrogenase was decreased in both treated groups of humanized PPARα mice. Decreased expression of 17β-hydroxysteroid dehydrogenase was the only change found in treated PPARα-null mice.

In the mitochondria, carnitine palmitoyltransferase (CPT) was decreased in both groups of wild-type and high-dose humanized PPARα mice, and SOD levels were reduced in all treated wild-type and humanized PPARα mice. Histopathological lesions of the testes, including abnormal seminiferous tubules, lack of germ cells, or necrotic cells, were observed in high-dose wild-type and humanized PPARα mice. No morphological changes were observed in the testes from PFOA treatment in PPARα-null mice. The 1-mg/kg/day dose was the LOAEL for significant ($p<0.05$) sperm abnormalities, decreased testosterone, and several biochemical alterations in the PPARα and hPPARα mice, but not in the PPARα-null mice. There were dose-related decreases in testosterone in the PPARα-null mice, but they did not achieve statistical significance.

Inhalation Exposure

No data on the effects of short-term inhalation exposures to PFOA were identified in the literature.

Dermal Exposure

Fairley et al. (2007) investigated the role of dermal exposure to PFOA in an experiment to evaluate toxicity in BALB/c mice. The mice were exposed to 0, 0.01%, 0.1%, 0.25%, 0.5%, 1.0%, and 1.5% PFOA (equivalent to 0, 0.25, 2.5, 6.25, 12.5, 25, and 50 mg/kg PFOA). It was applied to the dorsal surface of both ears daily for 4 days. The mice were sacrificed 6 days later. Dermal PFOA exposure did not cause reductions in body weight or signs of inflammation at the application site. A significant increase in liver weight was observed in mice dosed with $\geq$ 6.25 mg/kg PFOA ($p<0.01$) compared to control liver weight. Under the conditions of the study, the LOAEL was 6.25 mg/kg PFOA based on increased liver weight, and the NOAEL was 2.25 mg/kg PFOA.

3.2.3 Subchronic Studies

Oral Exposure

Monkey. Goldenthal (1978) administered rhesus monkeys (two per gender per group) doses of 0, 3, 10, 30, and 100 mg/kg/day PFOA by gavage for 90 days. Animals were observed twice daily and body weights were recorded weekly. Blood and urine samples were collected once during a control period, and at 1 and 3 months for hematology, clinical chemistry, and urinalysis. Organs and tissues from animals that were sacrificed at the end of the study and from animals that died during the treatment period were weighed, examined for gross pathology, and processed for histopathology.

All monkeys in the 100-mg/kg/day group died between weeks 2–5 of the study. Signs and symptoms that first appeared during week 1 included anorexia, frothy emesis, swollen face and eyes, decreased activity, prostration, and body trembling. Three monkeys from the 30-mg/kg/day group died during the study. Beginning in week 4, all four animals showed slight to moderate, and sometimes severe, decreased activity. One monkey had emesis and ataxia, swollen face, eyes, and vulva. Beginning in week 6, two monkeys had black stools and one monkey had slight-to-moderate dehydration. No monkeys in the 3- or 10-mg/kg/day groups died during the study. One monkey in the 10-mg/kg/day group was anorexic during week 4, had a pale and swollen face in week 7, and had black stools for several days in week 12. Animals in the 3-mg/kg/day group occasionally had soft stools or moderate-to-marked diarrhea and frothy emesis.

Changes in body weight were similar to the controls for animals from the 3- and 10-mg/kg/day groups. Monkeys from the 30- and 100-mg/kg/day groups lost body weight after week 1. At the end of the study, this loss was statistically significant for the one surviving male in the 30-mg/kg/day group and reflected in body weight (2.30 kg versus 3.78 kg for the control). The results of the urinalysis, and hematological and clinical chemistry analyses were comparable for the control and the 3- and 10-mg/kg/day groups at 1 and 3 months.

At necropsy, there were significant decreases in the absolute heart and brain weight and relative liver weight in 10-mg/kg/day females. At 3 mg/kg/day, the relative pituitary weight in males was significantly increased. The biological significance of these weight changes is difficult to assess, as they were not accompanied by morphologic changes.

In animals that died, one male and two females from the 30-mg/kg/day group and all animals from the 100-mg/kg/day group had marked diffuse lipid depletion in the adrenal glands. All males and females from the 30- and 100-mg/kg/day groups also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. One female from the 30-mg/kg/day group and all animals in the 100-mg/kg/day group had moderate atrophy of the lymphoid follicles in the lymph nodes.

The one male in the 30-mg/kg/day group that survived until terminal sacrifice had slight-to-moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. Under the conditions of this study, the male LOAEL was 3 mg/kg/day based on increased relative pituitary weight, and no NOAEL was established. The female LOAEL was 10 mg/kg/day based on decreased heart and brain weight, and the NOAEL was 3 mg/kg/day.

Rat. In a dietary study reported by Perkins et al. (2004), male ChR-CD rats (45–55 per group) were administered concentrations of 1, 10, 30, and 100 ppm PFOA for 13 weeks. These doses are equivalent to 0.06, 0.64, 1.94, and 6.50 mg/kg/day. There were two control groups—a nonpair-fed control group and a pair-fed control group for the 100-ppm dose group); both were fed the basal diet. Following the 13-week exposure period, 10 animals per group were fed basal diet for an 8-week recovery period. The animals were observed twice daily for clinical signs of toxicity, and body weights and food consumption were recorded weekly. Food consumption was recorded daily for the pair-fed animals.

A total of 15 animals per group were sacrificed following 4, 7, and 13 weeks of treatment; 10 animals per group were sacrificed after 13 weeks of treatment and an 8-week recovery period. Serum samples collected from 10 animals per group at each scheduled sacrifice during treatment and from five animals per group during recovery were analyzed for estradiol, T, LH, and PFOA. The level of palmitoyl-CoA oxidase was analyzed from a section of liver that was obtained from five animals per group at each scheduled sacrifice. Weights of the brain, liver, lungs, testis, seminal vesicle, prostate, coagulating gland, and urethra were recorded, and these tissues also were examined histologically. In addition, the brain, liver, lungs, testis, seminal vesicle, and prostate were preserved in glutaraldehyde for electron microscopic examination.

In the analysis of the data, animals exposed to 1, 10, 30, and 100 ppm PFOA were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm PFOA. At 100 ppm, significant reductions in body weight and body weight gain were seen compared to the pair-fed control group during week 1 and the nonpair-fed control group during weeks 1–13. Body weight data in the other dosed groups were comparable to controls. At 10 and 30 ppm, mean body weight gains were significantly lower than for the nonpair-fed control group at week 2. These differences in body weight and body weight gains were not observed during the recovery period. Animals fed 100 ppm consumed significantly less food during weeks 1 and 2 than the nonpair-fed control group. Overall, there was no significant difference in food consumption. There were no significant differences among the groups for any of the hormones evaluated, although there was some indication of elevated estradiol for the 100 ppm group at week 4. The elevated estradiol for the high-dose group at week 4 should be interpreted with caution because most of the measurements for control and treated groups were below the level of detection at all other timepoints (Perkins et al. 2004).

Significant dose-related increases in absolute and relative liver weights and hepatocellular hypertrophy were observed at weeks 4, 7, and 13 in the 10, 30, and 100 ppm groups (Table 3-18). There was no significant evidence of any dose-related degenerative changes. Hepatic palmitoyl-CoA oxidase activity was significantly increased at weeks 4, 7, and 13 in the 30 and 100 ppm groups. At 10 ppm, hepatic palmitoyl-CoA oxidase activity was significantly increased at week 4 only. At 13 weeks, the palmitoyl-CoA oxidase activity was lower than it was at weeks 4 and 7 for the 10, 30, and 100 ppm dose groups, possibly suggesting attenuation of the peroxisomal response. Histologically, liver effects were limited to minimal or slight coagulative necrosis observed in 0/45, 1/45, 0/45, 2/45, and 3/44 in the control, 1, 10, 30, and 100 ppm groups, respectively.

Table 3-18. Liver Effects in Male Rats

Parameter	Week	Dose (mg/kg/day)					
		0 [a]	0 [b]	0.06 (1 ppm)	0.64 (10 ppm)	1.94 (30 ppm)	6.5 (100 ppm)
Palmitoyl-CoA Oxidase (IU/g)	4	8 ± 0.5	8 ± 0.4	9 ± 1.7	14 ± 3.6[c]	24 ± 11.4[c]	37 ± 14.8[c, d]
	7	7 ± 1.5	7 ± 1.5	7 ± 0.8	16 ± 5.5	32 ± 12.2[c]	54 ± 35.3[c, d]
	13	8 ± 0.9	5 ± 1.1	8 ± 1.9	10 ± 2.1	14 ± 3.4[c]	17 ± 4.5[cd]
Hepatocellular Hypertrophy	4	0/15	0/15	0/15	12/15	15/15	14/15
	7	0/15	0/15	0/15	12/15	15/15	15/15
	13	0/15	0/15	0/15	13/15	14/15	15/15
Hepatocellular Necrosis, Coagulative	4	0/15	1/15	0/15	0/15	1/15	2/14
	7	0/15	0/15	0/15	0/15	0/15	1/15
	13	0/15	0/15	1/15	0/15	1/15	0/15
Absolute Liver Weight (g)	4	16.34 ± 2.14	15.83 ± 1.13	15.45 ± 1.71	17.89 ± 2.13	23.23 ± 2.83[c]	25.44 ± 1.89[c]
	7	17.78 ± 2.12	16.91 ± 2.22	17.68 ± NA	19.42 ± 2.10	27.76 ± 3.51[c]	27.76 ± 3.51[c]
	13	19.73 ± 2.01	16.30 ± 1.62	18.03 ± 2.81	20.44 ± 2.87	22.74 ± 4.21	26.78 ± 5.47[c]
Mean Body Weight (g)	4	388 ± 21	365 ± 11	388 ± 23	383 ± 25	380 ± 27	356 ± 27[c]
	7	457 ± 29	434 ± 19	461 ± 30	458 ± 30	448 ± 31	432 ± 39[c]
	13	541 ± 41	508 ± 22	548 ± 37	551 ± 42	531 ± 46	494 ± 64[c]
Liver/Body Weight (%)	4	3.97 ± 0.37	4.07 ± 0.27	3.73 ± 0.23	4.49 ± 0.32[c]	5.77 ± 0.60[c]	6.73 ± 0.49[c]
	7	3.75 ± 0.29	3.76 ± 0.37	3.64 ± 0.33	4.12 ± 0.37	5.14 ± 0.53[c]	6.06 ± 0.72[c]
	13	3.53 ± 0.28	3.24 ± 0.23	3.24 ± 0.30[c]	3.69 ± 0.32	4.21 ± 0.56[c]	5.50 ± 0.84[c]

Source: Perkins et al. 2004
Notes: Mean ± SD; NA= not available.
[a] Nonpair-fed controls.
[b] Pair-fed controls.
[c] Statistically significant ($p < 0.05$) with nonpair-fed control.
[d] Statistically significant ($p < 0.05$) with pair-fed control.

Under the conditions of this study, the authors identified the LOAEL as 10 ppm (0.64 mg/kg/day) based on increases in absolute and relative liver weight and hepatocellular hypertrophy (Perkins et al. 2004). The NOAEL identified was 1.0 ppm (0.06 mg/kg/day). However, the liver weight and palmitoyl-CoA responses were associated with the activation of PPARα and were not accompanied by significant dose-related changes that would classify them as adverse for humans (e.g., fibrosis, macrovesicular steatosis, inflammation) as enumerated by Hall et al. (2012). Therefore, for the current assessment, the LOAEL is identified as 1.94 mg/kg/day based on a slight increased incidence of coagulative necrosis in the liver. The NOAEL is 0.64 mg/kg/day.

Serum samples were collected from 8 to 10 animals prior to each sacrifice. PFOA concentrations in serum increased with the dose, but all dose levels appeared to have reached steady state by the first sacrifice at week 4. Following the 8-week recovery period, serum levels were below detection for many animals and consistent with a half-life of about seven days in male ChR-CD rats.

Inhalation and Dermal Exposure

No data on the effects of subchronic inhalation or dermal exposures to PFOA were identified in the literature.

3.2.4 Neurotoxicity

Johansson et al. (2008) gave male neonatal Naval Medical Research Institute (NMRI) mice (3–4 litters, ~5–6 male pups per litter) a single gavage dose of 0, 0.58, and 8.7 mg PFOA/kg in a lecithin/peanut oil emulsion on PND 10, the approximate peak time of rapid brain growth in mice. Spontaneous behavior (e.g., locomotion, rearing, and total activity) and habituation in response to a placement in unfamiliar environment were tested in 10 mice in each group at ages 2 and 4 months. Each test period was divided into three 20-min periods. The habituation ratio was determined by dividing the activity for the third 20-min period by the activity for the first period. A high habituation ratio indicated that movement patterns of the exposed animals when placed in an unfamiliar test chamber differed from control by displaying comparatively low activity for the first 20 mins and comparatively higher activity for the last 20 mins.

Exposure to PFOA did not affect body weight or body weight gain in male NMRI mice following treatment. Compared to controls, the habituation ratio for rearing and locomotion in the high-dose animals was elevated compared to controls at 2 and 4 months, with a significantly higher ratio ($p<0.01$) at 4 months than at 2 months. At 4 months, the changes in activity patterns for the high dose were significant ($p<0.01$) compared to controls for locomotion, rearing, and total activity. The results at the low dose were less pronounced, with a significant impact on locomotion and slight changes in rearing behavior.

At 4 months of age, mice were tested for nicotine-induced behavior and behavior in the elevated plus maze. Increased activity is the expected response to nicotine injection (80 μg) as a result of stimulation of the cholinergic receptors in the brain. The activity responses of the PFOA-exposed animals to nicotine stimulation were significantly less than the response of the controls, but the differences were most pronounced in the high-dose animals.

The mice also were tested in an elevated plus maze, which determined whether they would select an enclosed environment (the expected response) over an open environment. No significant differences were observed in the PFOA-exposed mice in this test. Under the conditions of this study, the clear LOAEL was 8.7 mg/kg based on locomotion, rearing, and total activity; habituation ratio; and response to nicotine at 2 and 4 months after receiving a single gavage dose on PND 10. There were significant differences in locomotion and total activity at 4 months in the low-dose animals, which supports identifying the 0.58-mg/kg dose as a marginal LOAEL. However, the data at the low dose are less compelling than those at the high dose.

In a follow-up to their original study, Johansson et al. (2009) gave male neonatal NMRI mice (3–4 litters, ~5–6 male pups per litter) a single gavage dose of 0 and 8.7 mg PFOA/kg on PND 10. Protein levels of calcium/calmodulin-dependent protein kinase II (CaMKII), growth-associated protein-43 (GAP-43), synaptophysin, and tau protein were determined in the cerebral cortex and hippocampus. CaMKII regulates synapotogenesis and synaptic plasticity, GAP-43 modulates axon sprouting and growth, synaptophysin is a membrane glycoprotein in presynaptic vesicles, and tau protein is responsible for outgrowth of neuronal processes and microtubule assembly and maintenance.

Levels of CaMKII protein in the hippocampus were significantly higher (58%, $p\leq0.05$) in mice exposed to PFOA than levels in control mice, but unchanged in the cerebral cortex. Levels of GAP-43 protein in the hippocampus were significantly higher (17%, $p\leq0.05$) in PFOA-exposed mice than levels in control mice, but unchanged in the cerebral cortex. Synaptophysin levels in mice exposed to PFOA were significantly increased in the hippocampus (52%) and

cerebral cortex (82%). Tau protein levels in PFOA-exposed mice were increased 92% and 142% ($p \leq 0.001$) in the hippocampus and cerebral cortex, respectively, above levels in the control mice. The authors concluded that alterations of these proteins could be a factor in the altered behavior of adult mice that were exposed to PFOA as neonates because they are required for normal brain development.

Onishchenko et al. (2011) exposed pregnant C57BL/6/Bkl mice (n = 6 per group) to 0 and 0.3 mg PFOA/kg/day in the diet from GD 1 to the end of pregnancy. The behavior of the weaned offspring was analyzed in locomotor, circadian activity, elevated plus maze, and forced swim tests at 5–8 weeks of age. Muscle strength and motor coordination tests were given at 3–4 months of age. The distance traveled over 30 mins was registered in 5-min intervals in the locomotor test. For the circadian activity test, the activity of the mice in social groups was monitored for 48 hours after placement in new cages. Anxiety-like behavior was determined using the elevated plus maze. Depression-like behavior was determined in the forced swim test by tracking the time spent floating passively for 2 seconds or longer. Muscle strength (three trials) was measured by how long within 60 seconds it took the mouse to fall off an upside-down lid onto the cage floor. Motor coordination (four trials) was measured by how long the mice remained on a rotating drum as a rotarod accelerated from 4 to 40 rpm over 5 mins.

Prenatal exposure to PFOA did not alter offspring locomotor activity, anxiety-related behavior, depression-like behavior, or muscle strength. In the circadian activity tests, male offspring exposed to PFOA were significantly more active ($p = 0.013$) and the female offspring were significantly less active ($p = 0.036$) than control offspring during the first hour of the test. PFOA-exposed male offspring were significantly more active ($p<0.05$) than control males from the dark phase of day 1 through the dark phase day 2. Both male and female offspring exposed to PFOA had significantly less inactive periods ($p<0.05$) during the light phase compared to their respective controls. In the accelerating rotarod test, female offspring exposed to PFOA exhibited decreased fall latency over the four trials compared to control females, but no effect of treatment was observed in male offspring. The authors concluded that prenatal exposure to 0.3 mg/kg/day of PFOA resulted in gender-related postnatal alterations in offspring behavior and motor function at 3–4 months of age.

In vitro. Slotkin et al. (2008) characterized the neurotoxicity of PFOA using PC12 cells. The cells were derived from a neuroendocrine tumor of the rat adrenal medulla and serve as a model for neuronal development and differentiation. Exposure to nerve growth factors causes PC12 cells to differentiate into cells expressing either dopamine or acetylcholine phenotypes. The cells were incubated with 10, 50, 100, and 250 µmol PFOA. Synthesis of DNA, cell viability, cell growth, and lipid peroxidation were measured to determine if PFOA targets specific events in neural cell differentiation. Differentiation shifts towards or away from the dopamine and acetylcholine phenotypes were measured by assessing the activities of tyrosine hydroxylase (TH, dopamine) and choline acetyltransferase (ChAT, acetylcholine). The undifferentiated cells were evaluated after a 24-hour exposure to PFOA, and differentiating cells were evaluated after 4–6 days of exposure to PFOA.

Significant inhibition of DNA synthesis ($p<0.0001$) occurred in the undifferentiated cells after exposure to 250 µmol PFOA with no change in DNA content. Lipid peroxidation was significantly increased ($p<0.02$) after exposure to 10 µmol PFOA, and cell viability was significantly decreased ($p<0.03$) after a 24-hour exposure to 100 and 250 µmol PFOA.

In differentiating PC12 cells, exposure to 250 μmol PFOA caused decreased DNA content with no change in total protein/DNA content ratio or the membrane/total protein ratio. The lowest and highest PFOA concentrations caused a significant increase in lipid peroxidation ($p<0.007$), but no effect was observed in cell viability. TH activity was decreased ($p<0.05$) after exposure to 10 and 250 μmol PFOA, and the TH/ChAT ratio was decreased ($p<0.05$) at 10 μmol PFOA. The results suggest that PFOA exposure caused the differentiating cells to shift slightly to favor the acetylcholine phenotype.

3.2.5 Developmental/Reproductive Toxicity

Reproductive Effects

A comprehensive two-generation reproductive toxicity study was conducted in Sprague-Dawley Rats with publication of the results by Butenhoff et al. (2004a). A subsequent study by York et al. (2010) provided details of male reproductive organ histopathology. One study in mice examined the impact of mating exposed males with unexposed females on fertility and neonatal body weight (Lu et al. 2015).

Rat. A standard oral two-generation reproductive toxicity study of PFOA in Sprague-Dawley rats was conducted (Butenhoff et al. 2004a). Five groups of male and female SD rats (30 per gender per group) were administered PFOA by gavage at doses of 0, 1, 3, 10, and 30 mg/kg/day. The parental generation (F0) rats (n = 30 per gender per group) were dosed for 10 weeks prior to mating and until sacrificed (after mating for males; after weaning for females). F1 generation rats (n = 60 per gender per group) were dosed similarly, beginning at weaning. The F2 generation rats were maintained through LD 22. Reproductive parameters evaluated in the F0 and F1 generations included estrus cyclicity, sperm number and quality, mating, fertility, natural delivery, and litter viability and growth. Age at sexual maturation in F1 pups, anogenital distance in F2 pups, and presence of nipples (males) in F2 pups also were determined. Food consumption, body-weight gain, selected organ weights, gross pathology, and appropriate histopathology of reproductive organs were evaluated.

F0 Male Rats

One F0 male rat in the 30 mg/kg/day dose group was sacrificed on day 45 of the study because of adverse clinical signs. Statistically significant increases in clinical signs also were observed in male rats in the high-dose group, including dehydration, urine-stained abdominal fur, and ungroomed coat. Significant reductions in body weight were reported beginning on post-weaning day 50 at 3 mg/kg/day and for most of the study until termination in 10 and 30 mg/kg/day dose groups (6%, 11%, and 25% decrease from controls, respectively, at the end of premating; $p\leq0.05$). Absolute food consumption was significantly reduced to approximately 91% of the control level during the study in the 30-mg/kg/day dose group but not for the lower dose groups. Mean food consumption relative to body weight was increased in a dose-related manner for all treated males with statistical significance at ≥3 mg/kg/day; overall relative food consumption was 101, 105, 110, and 118% of the controls in the 1, 3, 10, and 30 mg/kg/day groups, respectively. The body weight and food consumption effects were not observed in female rats at any dose.

Organ weight data for the F0 male rats is shown in Table 3-19. The absolute and relative-to-body and -brain weights of the liver were statistically significantly increased in all dose groups. Absolute kidney weights were statistically significantly increased in the 1-, 3-, and 10-mg/kg/day dose groups, but significantly decreased in the 30-mg/kg/day group. Organ weight-to-terminal body weight ratios for the left and right kidney were statistically significantly increased in all treated groups. Kidney weight-to-brain weight ratios were significantly increased at 1, 3, and 10 mg/kg/day, but decreased at 30 mg/kg/day, following the trends in absolute weights. In the high-dose group, absolute and relative kidney weight changes occurred in a pattern typically associated with decrements in body weight. However, in the lower dose groups, consistent significant increases in absolute kidney weight and relative-to-body and -brain weights are a response to the challenge of providing transporters for renal removal of the foreign molecule. Increased kidney weight can be regarded as an adaptive response to the transport challenge. It is beneficial for the individual but adverse in the sense that it signifies the need to upregulate tubular transporters in the kidney to excrete the foreign material and a reflection of PFOA bioaccumulation in serum and tissues.

Table 3-19. Organ Weight Data from F0 Male Rats

	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Body weight (g)	581 ± 40	575 ± 48	542 ± 47**	513 ± 54**	432 ± 64**
Brain weight (g)	2.26 ± 0.17	2.28 ± 0.10	2.26 ± 0.12	2.24 ± 0.12	2.20 ± 0.14
Liver weight (g)	20.3 ± 2.5	24.3 ± 3.2**	27.7 ± 2.7**	28.7 ± 3.9**	27.5 ± 3.7**
Liver/body (%)	3.49 ± 0.29	4.22 ± 0.50**	5.13 ± 0.47**	5.61 ± 0.51**	6.42 ± 0.73**
Liver/brain (%)	903 ± 119	1066 ± 154**	1230 ± 120**	1285 ± 183**	1248 ± 144**
Rt. kidney (g)	2.19 ± 0.18	2.54 ± 0.30**	2.50 ± 0.18**	2.36 ± 0.25**	2.06 ± 0.20*
Rt. kidney/body (%)	0.379 ± 0.030	0.443 ± 0.048**	0.463 ± 0.039**	0.462 ± 0.034**	0.481 ± 0.051**
Rt. kidney/brain (%)	97.5 ± 9.9	111.6 ± 13.5**	111.0 ± 9.5**	105.6 ± 12.4**	93.5 ± 8.7
Lt. kidney (g)	2.19 ± 0.20	2.51 ± 0.28**	2.51 ± 0.21**	2.34 ± 0.24*	1.99 ± 0.19**
Lt. kidney/body (%)	0.378 ± 0.036	0.437 ± 0.047**	0.465 ± 0.043**	0.457 ± 0.040**	0.466 ± 0.054**
Lt. kidney/brain (%)	97.5 ± 10.7	110.1 ± 12.6**	111.7 ± 10.5**	104.6 ± 11.7*	90.4 ± 8.7*

Source: Butenhoff et al. 2004a
Not*es*: Mean±SD; n = 29–30; significantly different from control: *p < 0.05, **p < 0.01.

The only histologic finding was increased thickness and prominence of the zona glomerulosa and vacuolation in the cells of the adrenal cortex observed in 2/10 males in the 10-mg/kg/day dose group and 7/10 males in the 30-mg/kg/day dose group.

No treatment-related effects were reported at any dose level for any of the male reproductive parameters assessed. There was no evidence of altered testicular and sperm structure and function in PFOA-treated F0 rats with mean group serum PFOA concentrations of up to approximately 45 µg/mL (York et al. 2010). There was a significant dose-related increase in seminal vesicle weight ($p<0.05$) with and without fluid in the F1 males, but fertility of the exposed males in all generations was comparable to the controls.

No treatment-related effects were seen at necropsy or upon microscopic examination of the reproductive organs.

Under the conditions of the study, the LOAEL for F0 parental male rats is 1 mg/kg/day, the lowest dose tested, based on significant increases in absolute and relative liver and kidney weights. A NOAEL for the F0 parental males could not be determined.

F0 Female Rats

There were no treatment-related effects on clinical signs, body weight, food consumption, organ weights, or histology of the organs. There were no treatment-related effects on any of the reproductive parameters assessed, and no treatment-related effects were seen at necropsy other than slightly decreased liver weights ($p<0.05$) at doses of 3 and 10 mg/kg/day, but not 30 mg/kg/day. No abnormalities were seen with microscopic examination of the reproductive organs. The NOAEL for F0 parental females is 30 mg/kg/day, the highest dose tested.

F1 Generation

Pup body weight on a per-litter basis (genders combined) was significantly reduced ($p\leq0.01$) by 8–10% throughout the first 2 weeks of lactation in the 30-mg/kg/day group; at weaning, the mean body weight was reduced 4.5%, but the difference was not statistically significant. Although there were no effects on the viability and lactation indices, the total number of dead pups during lactation was increased in the 30-mg/kg/day groups; the difference was statistically significant on LDs 6–8. No other effects were noted, and there were no treatment-related findings for the pups necropsied at weaning. The offspring toxicity LOAEL is 30 mg/kg/day based on decreased body weight and an increase in the number of dead pups; the NOAEL is 10 mg/kg/day.

F1 Male Rats

Significant increases in treatment-related deaths (5/60 animals) were reported in F1 males in the high-dose group between days 2–4 postweaning. One rat was moribund sacrificed on day 39 postweaning and another was found dead on day 107 postweaning. Clinical signs included a significant increase in emaciation at 10 and 30 mg/kg/day, and in urine-stained abdominal fur, decreased motor activity, and abdominal distention at 30 mg/kg/day.

Mean body weight was significantly reduced in the 30-mg/kg/day group beginning on postweaning day 8, in the 10-mg/kg/day group beginning on postweaning day 36, and towards the end of the study in the 1- and 3-mg/kg/day groups. Terminal mean body weight was reduced in all treated groups at the time of sacrifice. For all groups, there was a significant, dose-related reduction in mean body weight gain for the entire dosing period (days 1–113). Absolute food consumption values were significantly reduced at 10 and 30 mg/kg/day during the entire precohabitation period (days 1–70 postweaning), while relative food consumption values were significantly increased.

Statistically significant delays in the average day of preputial separation ($p\leq 0.01$) were observed in high-dose animals versus concurrent controls (52.2 days of age versus 48.5 days of age, respectively). There were no other effects on any of the reproductive parameters assessed, and at necropsy no effects on reproductive organs or fertility were noted (York et al. 2010).

The absolute and relative weights of the liver were statistically significantly increased in all treated groups ($p \leq 0.01$). Treatment-related microscopic changes were described as diffuse hepatocellular hypertrophy in rats receiving doses of ≥ 3 mg/kg/day. At the same dose levels, there were scattered incidences of focal-to-multifocal necrosis and inflammation in the livers of the F1 male rats. As in the F0 males, the relative weight of the left and/or right kidneys was statistically significantly increased compared to controls for all dose groups, except for the right kidney at the high dose in which it was lower than for the controls (Table 3-20). Organ weight-to-terminal body weight and brain weight ratios for the kidney were statistically significantly

increased in all treated groups. All other organ weight changes observed (i.e., thymus, spleen, left adrenal, brain, prostate, seminal vesicles, testes, and epididymis) were probably due to decrements in body weight and not a reflection of target organ toxicity. Treatment-related microscopic changes were observed in the adrenal glands of high-dose animals (i.e., cytoplasmic hypertrophy and vacuolation of the cells of the adrenal cortex) and in the liver of rats treated with 3, 10, and 30 mg/kg/day (hepatocellular hypertrophy).

Table 3-20. Organ Weight Data from F1 Male Rats

	0 mg/kg/day	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30 mg/kg/day
Body weight (g)	560 ± 60	527 ± 55*	524 ± 48*	499 ± 64**	438 ± 42**
Brain weight (g)	2.34 ± 0.13	2.28 ± 0.16	2.31 ± 0.12	2.28 ± 0.10	2.18 ± 0.14**
Liver weight (g)	21.7 ± 3.2	24.6 ± 4.0**	28.2 ± 4.2**	29.3 ± 4.1**	29.7 ± 4.0**
Liver/body (%)	3.86 ± 0.32	4.65 ± 0.51**	5.41 ± 0.75**	5.90 ± 0.70**	6.79 ± 0.55**
Liver/brain (%)	927 ± 136	1075 ± 150**	1224 ± 179**	1285 ± 159**	1364 ± 166**
Rt. kidney (g)	2.24 ± 0.21	2.34 ± 0.28	2.48 ± 0.24**	2.33 ± 0.25	2.04 ± 0.21**
Rt. kidney/body (%)	0.402 ± 0.034	0.446 ± 0.041**	0.474 ± 0.041**	0.469 ± 0.050**	0.467 ± 0.036**
Rt. kidney/brain (%)	95.9 ± 9.1	102.6 ± 7.7**	107.4 ± 10.2**	102.3 ± 9.8*	93.6 ± 7.9
Lt. kidney (g)	2.21 ± 0.20	2.35 ± 0.26*	2.46 ± 0.20**	2.30 ± 0.22	2.03 ± 0.22**
Lt. kidney/body (%)	0.396 ± 0.031	0.446 ± 0.042**	0.472 ± 0.045**	0.464 ± 0.046**	0.465 ± 0.038**
Lt. kidney/brain (%)	94.8 ± 7.9	102.8 ± 7.6**	106.6 ± 9.1**	101.0 ± 7.9*	93.3 ± 10.0

Source: Butenhoff et al. 2004a
Notes: Mean±SD; n = 29-30; significantly different from control: *p<0.05, **p<0.01.

The LOAEL for adult systemic toxicity in the F1 males is 1 mg/kg/day based on significant, dose-related decreases in body weights and body weight gains, and in terminal body weights; and significant increases in absolute and relative kidney weights. A NOAEL for adult systemic toxicity in the F1 males could not be determined. Liver weights were significantly increased at all doses, but only accompanied by microscopic lesions at doses ≥ 3 mg/kg/day.

F1 Female Rats

A statistically significant increase in treatment-related mortality (6/60 animals) was observed in F1 females on postweaning days 2–8 at the highest dose of 30 mg/kg/day. No adverse clinical signs of treatment-related toxicity were reported. Statistically significant decreases in body weight were observed in high-dose rats on days 8, 15, 22, 29, 50, and 57 postweaning, during precohabitation (recorded on the day cohabitation began, when F1 generation rats were 92–106 days of age), and during gestation and lactation. Body weight gain was significantly reduced during days 1–8 and 8–15 postweaning. Statistically significant decreases in absolute food consumption were observed during days 1–8, 8–15, and 15–22 postweaning, during precohabitation, and during gestation and lactation in animals treated with 30 mg/kg/day. Relative food consumption values were comparable across all treated groups.

Statistically significant delays ($p \leq 0.01$) in sexual maturation (the average day of vaginal patency) were observed in high-dose animals versus concurrent controls (36.6 days of age versus 34.9 days of age, respectively). Prior to the rats mating, the study authors noted a statistically significant increase in the average numbers of estrous stages per 21 days in high-dose animals (5.4 versus 4.7 in controls). For this calculation, the number of independent occurrences of estrus in the 21 days of observation was determined. This calculation can be used as a screen for effects on the estrous cycle, but should be followed by a more detailed analysis.

Both 3M (2002, cited in USEPA [2005c]), and EPA (USEPA 2002b) conducted a more detailed analysis of the estrous cycle data. The 3M analysis of the data concluded that there were no differences in the number of females with ≥3 days of estrus or with ≥4 days of diestrus in the control and high-dose groups. This conclusion is consistent with that of EPA (USEPA 2002b). The cycles were evaluated as having either regular 4–5-day cycles, uneven cycling (defined as brief periods with irregular pattern) or periods of prolonged diestrus (defined as 4–6-day diestrus periods), extended estrus (defined as 3–4 days of cornified smears), possible pseudopregnancy (defined as 6 or more days of leukocytes), or persistent estrus (defined as 5 or more days of cornified smears). The two groups were not different in any of the parameters measured. Thus, the increase in the number of estrus stages per 21 days noted by the study authors was an outcome of the approach used for the calculations and is not biologically meaningful. There were no effects on the other reproductive parameters assessed, and at necropsy no effects on reproductive organs were noted.

No treatment-related effects were observed in the terminal body weights of the F1 female rats. The absolute weight of the pituitary, the pituitary weight-to-terminal body weight ratio, and the pituitary weight-to-brain weight ratio were statistically significantly decreased at 3 mg/kg/day and higher. Since there is not a clear dose-response relationship and histologic examination reveal no lesions, the biological significance of the pituitary weight data is problematic. No other differences were reported for the absolute weights or ratios for other organs evaluated. No treatment-related effects were reported following macroscopic and histopathologic examinations.

For F1 females, the LOAEL for developmental/reproductive toxicity was considered to be 30 mg/kg/day based on significantly reduced body weight and body weight gain during lactation, a delay in sexual maturation, and increased mortality during postweaning days 2–8; the NOAEL was 10 mg/kg/day. The NOAEL and LOAEL for adult systemic toxicity in F1 females are 10 and 30 mg/kg/day, respectively, based on statistically significant decreases in body weight and body weight gains.

F2 Generation Rats

There was a statistically significant increase ($p \leq 0.01$) in the number of pups found dead on LD 1 in the 3- and 10-mg/kg/day groups. An independent statistical analysis was conducted by EPA (USEPA 2002c), and no significant differences were observed between dose groups and the response did not have any trend in dose. There were no treatment-related effects on any of the developmental parameters assessed, and at necropsy, no treatment-related effects were noted. The NOAEL for developmental/reproductive toxicity in the F2 offspring was 30 mg/kg/day.

Mouse. In a follow-up to the 28-day component of the Lu et al. study (2015) of the impact on PFOA on the blood testes barrier (section 3.2.2), the authors examined the impact exposure to 0 and 5 mg/kg/day PFOA had on the fertility of the treated male mice (6–8 weeks old; 15 per dose group). Each treated male was mated with three virgin ICR females (8–10 weeks old). Successful mating was determined by the presence of a vaginal plug in the morning. The pregnant females were separated from the males and caged alone throughout the pregnancy; they were not dosed with PFOA. At parturition, the pups were counted and the litter body weight recorded. There was a statistically significant decrease in the number of mated females per male mouse and pregnant females per male mouse ($p<0.001$) for the exposed males compared to the controls. The average number of pups per litter was smaller for the exposed group (10.20 ± 0.72 versus 11.89 ± 0.54), but the difference was not significant. The average litter weight was

significantly lower for the offspring of the paternally exposed mice than of the paternal controls (16.17 ± 1.63 g versus 19.95 ± 0.80 g; $p<0.05$). The 5-mg/kg/day dose was a LOAEL for effects on male fertility and the significantly lower body weight of their progeny.

Developmental Effects

Standard developmental studies in rats and mice found impacts on pup body weight and developmental delays. Most studies used the oral route of exposure; one study used inhalation exposure to PFOA particulate matter. Some examined the developmental impact of PFOA associated with exposures that occurred only during gestation and lactation or during the prepubertal period and the association of PPARα with the developmental effect spectrum. A substantial number of studies in mice focused on PFOA's impact on mammary gland development.

3.2.6 Prenatal Development

Rat. Pregnant Sprague-Dawley rats were gavage-dosed with 0, 3, 10, and 30 mg/kg/day PFOA during days 4–10, 4–15, and 4–21 of gestation, or from GD 4 to LD 21 (Hinderliter et al. 2005; Mylchreest 2003). Clinical observations and body weights were recorded daily. On GDs 10, 15, and 21, five rats per group per time-point were sacrificed and the number, location, and type of implantation sites were recorded. Embryos were collected on day 10, and placentas, amniotic fluid, and embryos/fetuses were collected on days 15 and 21. Maternal blood samples were collected at 2 hours ± 30 mins post-dose. The remaining five rats per group were allowed to deliver. On LDs 0, 3, 7, 14, and 21, the pups were counted, weighed (genders separate), and examined for abnormal appearance and behavior. Randomly selected pups were sacrificed and blood samples were collected. On LDs 3, 7, 14, and 21, the dams were anesthetized and milk and blood samples were collected; dams were removed from their litters 1–2 hours prior to collection. Plasma, milk, amniotic fluid extract, and tissue homogenate (i.e., placenta, embryo, and fetus) supernatants were analyzed for PFOA concentrations by HPLC/MS.

All dams survived and there were no clinical signs of toxicity. In the 30-mg/kg/day group, mean body weight gain was approximately 10% lower than in the control group during gestation, and mean body weight was approximately 4% lower than for controls throughout gestation and lactation. The number of implantation sites, resorptions, and live fetuses were comparable among groups on days 10, 15, and 21 of gestation. One dam in the 3-mg/kg/day group and two dams in the 30-mg/kg/day group delivered small litters (3–6 pups per litter compared to 12–19 pups per litter in the control group); however, given the small sample size, the biological significance of this finding is unclear. There were no clinical signs of toxicity in the pups, and pup survival and body weights were comparable among groups. Under the conditions of this study, the maternal LOAEL was 30 mg/kg/day for decreased body weight gain during gestation, and the NOAEL was 10 mg/kg/day. The developmental NOAEL was 30 mg/kg/day.

Mouse. Lau et al. (2006) conducted a developmental toxicity study of PFOA in mice to ascertain whether there was a gender-related difference in the bioaccumulation of PFOA in the mouse and to evaluate the effects of PFOA on prenatal and postnatal development in offspring exposed during pregnancy. In that study, groups averaging 9–45 timed-pregnant CD-1 mice were given 0, 1, 3, 5, 10, 20, and 40 mg/kg PFOA daily by oral gavage on GDs 1–17. Maternal weight was monitored during gestation. Dams were divided into two groups. In the first group, dams were sacrificed on GD 18 and underwent maternal and fetal examinations that included measure of maternal liver weight and examination of the gravid uterus for numbers of live and dead fetuses

and resorptions. Maternal blood was collected and analyzed for PFOA serum concentration. PFOA levels in the fetuses were not examined. Live fetuses were weighed and subjected to external gross necropsy and skeletal and visceral examinations. In the second group of dams, an additional dose of PFOA was administered on GD 18. Dams were allowed to give birth on GD 19.

The day following parturition was designated as PND 1. Time of parturition, condition of newborns, and number of live offspring were recorded. The number of live pups in each litter and pup body weight were noted for the first 4 days after birth and then at corresponding intervals thereafter. Eye opening was recorded beginning at PND 12. Pups were weaned on PND 23 and separated by gender. The time to sexual maturity was determined by monitoring vaginal opening and preputial separation beginning on PND 24. Two to four pups per gender per litter were randomly selected for observation of postnatal survival, growth, and development. Estrous cyclicity was determined daily by vaginal cytology. After weaning, dams were sacrificed and the contents of the uteri examined for implantation sites. Postnatal survival was calculated based on the number of implantations for each dam.

Signs of maternal toxicity were observed following exposure to PFOA during pregnancy. Statistically significant dose-related increases ($p \leq 0.05$) in maternal liver weight also were observed, beginning at 1 mg/kg/day. Dose-related decreases in maternal weight gain during pregnancy were observed beginning at 5 mg/kg/day, with statistical significance ($p \leq 0.05$) seen in the 20- and 40-mg/kg/day dose groups. Under the conditions of the study, a maternal LOAEL of 1 mg/kg was indicated based on increased liver weight, and a NOAEL was not established. Signs of developmental toxicity were observed following *in utero* exposure to PFOA. The number of implantations in treated mice was comparable to control mice. Statistically significant increases ($p \leq 0.05$) in full-litter resorptions were reported at doses of ≥ 5 mg/kg/day, with complete loss of pregnancies at the highest dose group of 40 mg/kg/day. A 20% reduction ($p \leq 0.05$) in live fetal body weight at term was reported at 20 mg/kg/day. A statistically significant increase in prenatal loss was observed in the 20-mg/kg/day dose group. Ossification (number of sites) of the forelimb proximal phalanges was significantly reduced at all doses except 5 mg/kg. Ossification of hindlimb proximal phalanges was significantly reduced at all doses except 3 and 5 mg/kg. Reduced ossification ($p \leq 0.05$) of the calvaria and enlarged fontanel was observed at 1, 3, and 20 mg/kg and at ≥ 10 mg/kg in the supraoccipital bone. Statistically significant increases ($p \leq 0.05$) in minor limb and tail defects were observed in the fetuses at doses ≥ 5 mg/kg/day. Under the conditions of the study, a prenatal developmental LOAEL of 1 mg/kg was indicated based on increased skeletal defects, and the NOAEL was not established.

Slight, but statistically significant, increases ($p \leq 0.05$) in the average time to parturition were observed at 10 and 20 mg/kg/day. Increases ($p \leq 0.05$) in stillbirths and neonatal mortality (or decreases in postnatal survival) were observed at doses ≥ 5 mg/kg/day, with as much as a 30% increase in these effects seen in the 10- and 20-mg/kg/day dose groups. Postnatal survival and viability in the 1- and 3-mg/kg/day dose groups were comparable to controls. At doses ≥ 3 mg/kg/day, a trend in growth retardation (body weight reductions of 25–30%; $p \leq 0.05$), was observed in the neonates at weaning. Body weights were at control levels by 6 weeks of age for females and by 13 weeks of age for males. A trend for increasing body weight (~6–10% greater than controls) was observed in animals dosed with 5 mg/kg at 13 weeks and in animals dosed with 1 and 3 mg/kg at 48 weeks. Deficits in early postnatal growth and development also were manifested by significant delays ($p \leq 0.05$) in eye opening at doses ≥ 5 mg/kg/day. Slight delays

($p \leq 0.05$) in vaginal opening and in time to estrous were observed at 20 mg/kg/day in females; in contrast, significant accelerations ($p \leq 0.05$) in sexual maturation were observed in males, with preputial separation occurring 4 days earlier than controls at the 1-mg/kg/day dose and 2–3 days earlier in the 3–10-mg/kg/day dose groups, but the 20-mg/kg/day dose group was only slightly delayed compared to controls. Under the conditions of the study, a LOAEL for developmental toxicity of 1 mg/kg/day for males was indicated based on accelerated pubertal development, and a NOAEL was not established. For females, the developmental LOAEL was 3 mg/kg/day based on growth retardation, and the NOAEL was 1 mg/kg/day.

Values for the benchmark dose (BMD) and the lower 95th percentile confidence bound on the BMD (BMDL) for the maternal and developmental endpoints (BMD_5 and $BMDL_5$) were calculated by the study authors and reported in Lau et al. (2006). For maternal toxicity, BMD_5 and $BMDL_5$ estimates for decreases in maternal weight gain during pregnancy were 6.76 and 3.58 mg/kg/day, respectively. For increases in maternal liver weight at term, BMD_5 and $BMDL_5$ estimates were 0.20 mg/kg/day and 0.17 mg/kg/day, respectively. BMD_5 and $BMDL_5$ estimates for the incidence of neonatal mortality (determined by survival to weaning) at 5 mg/kg/day were 2.84 and 1.09 mg/kg/day, respectively. Significant alterations in postnatal growth and development were observed at 1 and 3 mg/kg/day, with BMD_5 and $BMDL_5$ estimates of 1.07 and 0.86 mg/kg/day, respectively, for decreased pup weight at weaning; and 2.64 and 2.10 mg/kg/day, respectively, for delays in eye opening. The BMD_5 and $BMDL_5$ estimates for reduced phalangeal ossification were < 1 mg/kg/day. BMD_5 and $BMDL_5$ estimates for reduced fetal weight at term were estimated to be 10.3 and 4.3 mg/kg/day, respectively.

Male and female 129S1/SvImJ and PPARα-null mice were used in studies to determine if PFOA-induced developmental toxicity was mediated by PPARα (Abbott et al. 2007). Pregnant 129S1/SvlmJ wild-type and PPARα-null mice were orally dosed from GD 1–17 with 0, 0.1, 0.3, 0.6, 1, 3, 5, 10, and 20 mg PFOA/kg/day. Heterozygous (HET) litters also were produced by mating wild-type and PPARα-null males with wild-type and PPARα-null dams to determine if genetic background affected survival. The HET litters were sacrificed on PND 15. Survival at birth was recorded and live offspring counted and weighed by gender. Litters were counted and offspring weighed on PND 1–10, 14, 17, and 22. Weaning occurred on PND 22, and all dams and one pup per litter were sacrificed. Blood was collected and the uteri were stained for implantation counts.

There was no effect of treatment on maternal weight or maternal weight gain (excluding nonpregnant females and those with full-litter resorptions), number of implants, or pup weight at birth. Wild-type dams exposed to ≥0.6 mg/kg/day and PPARα-null dams exposed to ≥5 mg/kg/day had a significantly greater percentage of litter loss compared to their respective controls. At ≥5 mg/kg/day in wild-type dams and 20 mg/kg/day in PPARα-null dams, 100% litter loss occurred. Relative liver weight was significantly increased in wild-type adult females dosed with ≥1 mg/kg/day and in PPARα-null adult females dosed with ≥3 mg/kg/day.

Body weight in wild-type offspring born of dams dosed with 1.0 mg/kg/day was significantly reduced ($p<0.05$) compared to control offspring body weight gain on PND 9, 10, and 22 (males) and PND 7–10 and PND 22 (females). No differences were observed between PPARα-null offspring body weight and control offspring body weight. Survival of pups from birth to weaning was significantly reduced ($p<0.05$) in wild-type litters exposed to ≥0.6 mg/kg/day, but was not affected in PPARα-null litters. Survival was significantly decreased ($p<0.05$) for wild-type and HET pups born to wild-type dams dosed with 1 mg/kg/day and for HET pups born to PPARα-null dams dosed with 3 mg/kg. Offspring born of wild-type dams showed a dose-related trend for

delayed eye opening compared to control offspring (significantly delayed at 1 mg/kg/day, $p<0.05$), but no difference in day of eye opening was observed in the offspring born of PPARα-null dams. At weaning, relative liver weight was significantly increased ($p<0.05$) in wild-type offspring gestationally exposed to ≥0.1 mg/kg/day and in PPARα-null offspring gestationally exposed to 3 mg/kg/day.

The authors concluded that survival of PPARα-null pups and deaths of HET pups born to PPARα-null dams indicates that expression of PPARα is required for PFOA-induced postnatal lethality; however, early prenatal lethality was independent of PPARα. Delayed eye opening and reduced postnatal weight gain appeared to be mediated by PPARα, but other mechanisms might also contribute. Under the conditions of the study, the maternal/reproductive LOAEL for wild-type mice was 0.6 mg/kg/day based on increased percentage of litter loss, and the NOAEL was 0.3 mg/kg/day. The developmental LOAEL for wild-type offspring was 0.1 mg/kg/day based on increased liver weight, and the NOAEL was not established. The maternal LOAEL for PPARα-null mice was 3 mg/kg/day based on increased liver weight, and the NOAEL was 1 mg/kg/day. The developmental LOAEL for PPARα-null offspring was 3 mg/kg/day based on increased liver weight, and the NOAEL was 1 mg/kg/day.

To further evaluate the developmental effects potentially mediated by PPARα, groups of female wild-type, PPARα-null, and PPARα-humanized mice were given 0 and 3 mg PFOA/kg on GDs 1–17 by oral gavage (Albrecht et al. 2013). Controls received the water vehicle. Females were either sacrificed on GD 18 (n = 5–8 per group) or allowed to give birth and then sacrificed, along with their litters (n = 8–14), on PND 20. Livers from dams, fetuses, and pups were weighed and collected for histopathological evaluation and RNA analysis. Gene expression results are given in section 3.3.4. Mammary gland whole mounts were prepared from female pups on PND 20 for quantification of ductal length and number of terminal end buds; these results are described below with other studies evaluating the effects of PFOA on mammary gland development.

Evaluation on GD 18 showed no effects of PFOA administration on maternal body weight, body weight gain, gravid uterine weight, number of implantations per dam, or number of resorptions per litter in dams of any genotype. For animals allowed to litter, the average day of parturition was slightly later in PFOA-treated humanized mice than in the controls. Body weight of dams during lactation, the number of pups born per litter, pup body weight during lactation, and the onset of pup eye opening were similar between treated and control groups for all genotypes. Offspring survival during PNDs 1–5 was significantly reduced in the wild-type PFOA-treated group, but not in the other genotypes.

Maternal liver weight was significantly increased in the treated groups of all genotypes on GD 18 and in wild-type animals on PND 20. Maternal liver weight was not affected on PND 20 in the PPARα-null or PPARα-humanized mice. Relative fetal liver weight on GD 18 was significantly increased in fetuses from treated wild-type and humanized dams. On PND 20, relative liver weight was increased only in pups from treated wild-type dams. Microscopic evaluation of the maternal liver showed centrilobular hepatocellular hypertrophy in all PFOA-treated groups on GD 18 and PND 20, with decreased incidence and severity by PND 20. On GD 18, the liver lesions were graded as mild in the wild-type mice, minimal-to-mild in the humanized mice, and minimal in the null mice. The morphological features of the liver lesions differed slightly between genotypes and are described in more detail in section 3.4.1. Only wild-type fetuses and pups from treated dams showed similar liver lesions.

Yahia et al. (2010) gavage-dosed pregnant ICR mice (n = 5 per group) with 0, 1, 5, and 10 mg PFOA/kg/day from GDs 0–17 or 0–18. The dams dosed from GDs 0–17 were sacrificed on GD 18, and the fetal skeletal morphology was evaluated. Dams dosed from GDs 0–18 were allowed to give birth and their offspring were either processed for pathological examination or observed for 4 days for neonatal mortality. Maternal liver, kidney, brain, and lungs were histologically examined after necropsy. Serum was collected for clinical chemistry and lipid analysis. Body weight was significantly decreased in dams receiving 10 mg/kg/day. Maternal absolute liver weight was significantly increased ($p<0.05$) at doses ≥ 5 mg/kg/day and relative liver and kidney weights were significantly increased at all doses. Hepatic hypertrophy was localized to the centrilobular region at the two lower doses and was diffuse at the highest dose. Renal cells in the outer medullar and proximal tubule were slightly hypertrophic at all doses. Treatment with 10 mg/kg/day caused a significant increase in AST, ALT, GGT, and ALP and a significant decrease in total serum protein, albumin, globulin, triglycerides, phospholipids, TC, and free fatty acids. At 5 mg/kg/day, total serum protein and globulin were significantly decreased, and phosphorus was increased. At 1 mg/kg/day, BUN and phosphorus were significantly increased. The maternal LOAEL was 1 mg/kg/day based on significantly increased relative liver and kidney weight, and no NOAEL was established.

Live fetal birth weight was significantly decreased at the two highest doses. There was no difference in the percentage of live fetuses between treated and control groups. At 10 mg/kg/day, increased incidence of cleft sternum, reduced phalanges ossification, and delayed eruption of incisors was observed. Delayed parturition was observed in dams treated with 10 mg/kg/day, and ~58% of all pups born to those dams were stillborn. Death occurred within 6 hours of delivery in the remaining pups, and whole body edema was observed in some of the pups. The body weight of the live pups born to dams treated with 5 or 10 mg/kg/day was significantly reduced compared to control pup body weight. By PND 4, 16% of offspring born to dams dosed with 5 mg/kg/day had died. No pathological differences were observed in the lungs or brains of treated and control offspring. The developmental LOAEL was 5 mg/kg/day based on decreased body weight and decreased survival rate, and the NOAEL was 1 mg/kg/day.

Suh et al. (2011) examined placental prolactin-family hormone and fetal growth retardation in mice treated with PFOA. Pregnant CD-1 mice (n = 10 per group) were treated with 0, 2, 10, and 25 mg/kg/day PFOA from GDs 11–16. Dams were sacrificed on GD 16 and uteri were removed and examined. Three placentas per group were analyzed histochemically and the numbers of glycogen trophoblast cell (GlyT) in the junctional zone plus sinusoidal trophoblast giant cells (S-TGC) in the labyrinth zone were counted and compared. Trophoblast cells express prolactin-family genes. mRNA from three placentas per group were analyzed using situ hybridization, northern blot hybridization, and RT-PCR for mouse placental lactogen- (mPL-) II, mouse prolactin-like protein- (mPLP-) E and F, Pit-1α, and β isotype (transacting factors of mPL and mPLP genes).

A significant difference in maternal body weight was observed from GD 13–16 in dams treated with 25 mg/kg/day of PFOA compared to controls. At ≥2 mg/kg/day of PFOA, placental weight was significantly decreased and the number of resorptions and dead fetuses was significantly increased. At ≥10 mg/kg/day of PFOA, fetal weight and the number of live fetuses were significantly decreased. There were no differences in the number of implantation sites among the groups, and postimplantation loss was 3.87, 8.83, 30.98, and 55.41% for the 0, 2, 10, and 25 mg/kg/day PFOA groups, respectively.

The placentas of dams dosed with ≥10 mg/kg/day of PFOA displayed necrotic changes. Parietal and S-TGC and GlyT cell frequency in the placental junctional and labyrinth zones was significantly decreased ($p<0.05$) in a dose-dependent manner in treated dams. At 25 mg/kg/day of PFOA, S-TGCs showed signs of atrophy with crushed cell nucleus. A significant dose-dependent decrease in mPL-II, mPLP-E, mPLP-F, and Pit-1α and β isotype mRNA and expression was observed. Correlation coefficients between fetal weight and maternal mPL-II, mPLP-E, and mPLP-F mRNA levels were positive ($p<0.001$). Based on the results, the authors suggested that inhibited prolactin-family gene expression could be secondary to insufficient trophoblast cell differentiation and increased cell necrosis. These effects reduced placental efficiency and contributed in part to fetal growth retardation. The 2-mg/kg/day dose was a LOAEL for increases in resorption and dead fetuses plus decreased placental weight. There was no NOAEL.

A meta-analysis was conducted to determine whether developmental exposure to PFOA was associated with fetal growth effects in animals (Koustas et al. 2014). Eight studies identified in the published literature met the criteria of the Navigation Guide systematic review methodology for inclusion in the analysis (Woodruff and Sutton 2014). The data sets included mouse gavage studies with offspring body weight at birth. Maternal PFOA doses ranged from 0.01 to 20 mg/kg/day. The results from the meta-analysis showed that a 1-mg/kg/day increase in PFOA dose was associated with a -0.023 g (95% CI -0.029, -0.016) difference in pup birth weight. All of the studies included in the analysis are described either above with the developmental toxicity studies or with the specialized developmental studies that follow.

3.2.7 Mammary Gland Development and Other Specialized Developmental Studies

The following studies used experimental study designs and/or examined endpoints not typically included in standard developmental toxicity studies. The studies were conducted to determine critical periods of exposure for outcomes that occurred later in life. A number of the studies focused on mammary gland development in dams and female offspring. Researchers focused on effects resulting from indirect exposure of offspring via treatment of pregnant animals and/or direct exposure of peripubertal animals starting about the time of weaning.

Indirect gestational and/or lactational exposures

Many studies evaluating indirect gestational and/or lactational exposure to PFOA are available and Table 3-21 provides an overview of experiments designed to assess the developmental effects of PFOA following exposures during gestation. Most of the studies focus on mammary gland effects as a consequence of gestational and lactational or prepubertal exposures in CD-1 mice. Some have included postnatal assessment of the liver. Additional details of the studies are described in the section following the table.

Timed pregnant CD-1 mice were given PFOA by gavage at doses of 0, 0.01, 0.1, 0.03, and 1 mg/kg/day from GD 1 through the end of lactation (PND 21) in a study by Quist et al. (2015). The litters were equalized on PND 4 to four females and six males. Only the females continued in the study after weaning. At the end of lactation, litters with less than four females were removed from the study. On PND 21, seven to 10 female pups per dose group were sacrificed by decapitation. The livers were removed for analysis.

Table 3-21. Studies of Pregnant CD-1 Mice Following Administration of PFOA

Dose (mg PFOA /kg/day)	Timing	Endpoints	Reference
0, 0.01. 0.1, 0.3, 1	GD 1 to PND 21	Liver histopathology; periportal inflammation; clinical chemistry; impact of postweaning HFD	Quist et al. 2015
0, 5	GD 1-17, 8-17, or 12-17	Body weight; mammary gland morphology GD 18 (dams) and PNDs 10 and 20 (dams, female pups)	White et al. 2007
0, 5 20	GD 7-17, 10-17, 13-17, 15-17 GD 15-17	Body weight; developmental landmarks and growth to PND 189; mammary gland morphology of female pups up to 18 months	White et al. 2009; Wolf et al. 2007
0, 3, 5	GD 1-17 Cross-fostered at birth	Body weight; developmental landmarks and growth to PND 245; mammary gland morphology of female pups up to 18 months	White et al. 2009; Wolf et al. 2007;
0, 5	GD 8-17 Cross-fostered at birth	Mammary gland morphology of dams and female pups on PNDs 1, 3, 5, and 10	White et al. 2009
0, 0.3, 1.0, 3	GD 1-17	Liver weight; mammary gland morphology of female pups on PNDs 7, 14, 21, 28, 42, 63, and 84	Macon et al. 2011
0, 0.01, 0.1, 1	GD 10-17		
0, 1, 5	GD 1-17	Body weight; reproductive parameters; mammary gland morphology of F0, F1, and F2 females	White et al. 2011
0, 1 + 5 ppb in drinking water to both groups	GD 1-17 Drinking water started on GD 7 and continued to F2 generation		
0, 3	GD 1-17	Wild-type, PPARα-null, and hPPARα vv/129 mice; pup body weight at PNDs 14 and 20 plus mammary gland structure	Albrecht et al. 2013
0, 0.01, 0.1, 0.3, 1	GD 1-17 Study included both CD-1 and C57BL/6 mice	Body weight; net body weight; absolute and relative liver weight on PNDs 21, 35, and 56; serum estradiol and progesterone; mammary gland morphology	Tucker et al. 2015

During the lactation period all pups received a Purina control diet with a normal fat content. At sacrifice on PND 21, there was a dose-related increase in relative liver weight at doses ≥0.3 mg/kg/day. When the animals from the same dose group were sacrificed on PND 91, there was no observed impact on relative liver weight.

Starting on PND 35, one pup from each of 20 dams was placed on a HFD (with 60% of the calories from fat) until sacrificed on PND 91. Half were fasted for 4 hours before blood collection and sacrifice. Another seven to 10 pups per dose group received the Purina control diet with lower fat content until their sacrifice on PND 91. Blood samples were collected at sacrifice for determination of ALP, AST, ALT, SDH, LDL, HDL, cholesterol, triglycerides, total bile acid (TBA), glucose, leptin and insulin. Liver sections were collected for histological analysis and graded 1 to 4 for lesion severity (1 = minimal; 4 = severe). Selected samples of the liver tissues (four from the HFD groups and four from the PFOA-exposed control diet group) were fixed in osmium trioxide and prepared for evaluation using transmission electron microscopy.

At PND 91, the animals on the PFOA + HFD weighed more than the Purina controls. The body weight for the group that received PFOA and the control diet did not differ from the untreated controls on the same diet. Serum samples from the PFOA-treated Purina controls, and the fasted high-fat and nonfasted high-fat groups were analyzed for LDL, HDL, TC, and leptin.

At PFOA doses < 0.3 mg/kg/day, the LDL and TC levels in the fasted and nonfasted HFD animals were greater than in the untreated Purina controls. There was no impact of PFOS on either parameter in animals on the PFOA plus Purina control diet, but both LDL and TC were statistically lower at the high PFOA dose than they were at the low doses for both parameters. A similar pattern was seen for the HDL levels.

The impact of PFOA dose on leptin was variable and not significant for the PFOA plus Purina control animals and the high-fat, fasted animals. For the high-fat, nonfasted animals, there was a trend towards decreasing leptin as the PFOA dose increased, which was significant at the high dose of 1 mg/kg/day ($P< 0.01$). In those animals, the liver showed chronic periportal inflammation and microvescicular intracytoplasmic lipid droplets. The transmission electron microscopy slides showed that the hypertrophic liver cells presented evidence of cellular damage and changes in both mitochondrial morphology and numbers. The observed mitochondrial abnormalities were not those generally associated with PPARα activation. The 0.01 mg/kg/day dose was a NOAEL. The LOAEL was 0.3 mg/kg/day for the effects on TC for animals receiving a HFD, but not for those receiving the PFOA plus Purina control diet.

Effects of PFOA exposure on mammary gland morphology of CD-1 mice were evaluated in a series of studies by the same researchers (Macon et al. 2011; Tucker et al. 2015; White et al. 2007, 2009, 2011). The focus was on mammary gland development of female pups, although limited evaluations were conducted on the dams. Mammary gland whole mounts were scored on a 1 to 4 subjective, age-adjusted, developmental scale (1 = poor development/structure; 4 = normal development/structure). Tissue was assessed qualitatively for the gross presence of several histological criteria by two independent scorers and a mean score calculated. Neither standardization of these subjective measures nor training of the scorers was described in the publications. Quantitative measures of longitudinal growth, lateral growth, and number of terminal end buds also were made in the Macon et al. (2011) and Albrecht et al. (2013) studies.

White et al. (2007) orally dosed pregnant CD-1 mice with 0 and 5 mg PFOA/kg/day on GD 1–17 (n = 14), 8–17 (n = 16), and 12–17 (n = 16) to determine if decreased neonatal body weights and survival were linked to gestational exposure or lactational changes in milk quantity or quality. The control mice (n = 14) were dosed with vehicle on GD1–17. A subset of dams was sacrificed on GD 18. The remaining dams were allowed to give birth, and pups were pooled and randomly redistributed among the dams of the respective treatment groups. Litters were equalized to 10 pups per litter. Half of the dams and litters were sacrificed on PND 10, and the remaining dams and litters were sacrificed on PND 20. Mammary glands were collected from dams and female pups at time of sacrifice.

Treatment with PFOA did not affect maternal weight gain, number of implants, or the number of live fetuses. There was a significant increase ($p<0.05$) in prenatal loss in dams exposed during GD1–17. Body weight of pups exposed gestationally to PFOA was significantly decreased ($p<0.05$) at all time points measured and for all dosing regimens. On GD 18, stunted alveolar development was observed in the mammary gland of dams treated with PFOA on GD 1–17 compared to the mammary glands of the control dams, which were saturated with milk-filled alveoli. Dams treated with PFOA on GD 1–17 or 8–17 exhibited significant mammary gland epithelial differentiation delays on PND 10 as evidenced by an excessive amount of adipose tissue. In comparison, mammary glands from control dams on PND 10 were well differentiated, full of alveoli filled with milk, and contained few apoptotic bodies and little adipose tissue. The mammary gland developmental score in dams treated on GD 12–17 was not

statistically different from control dams on PND 10. At PND 20, the mammary gland scores from all PFOA-treated dams were not significantly different from those of the control group.

The pups were impacted by their *in utero* PFOA exposure over all dosing intervals. Their mammary glands exhibited significantly stunted epithelial branching and longitudinal growth at PNDs 10 and 20; the resulting developmental scores were significantly less than those of controls. Very little mammary gland development occurred between PND 10–20 in the offspring of dams exposed to PFOA, even though postnatal growth and body weight gain increased in parallel to that of the controls. Thus, at the only dose tested, 5 mg/kg/day, effects were observed on the dam and pup mammary gland, accompanied by decreased pup body weight and decreased survival for the pups exposed during GD 1–17.

In the study by Wolf et al. (2007), CD-1 mice were orally dosed with 0 and 5 mg PFOA/kg/day on GD 7–17 (n = 14), 10–17 (n = 14), 13–17 (n = 12), and 15–17 (n = 12) or with 20 mg/kg on GD 15–17 (n = 6) to determine if there was a specific window during which PFOA exposure produced developmental effects. The developmental results from this study were published by Wolf et al. (2007) and the mammary gland effects were published by White et al. (2009). On PND 22, all dams and one male and female pup from each litter were necropsied. Blood samples were collected and livers from dams and offspring were removed and weighed. Uterine implantation sites were counted. The fourth and fifth inguinal mammary glands were removed from female offspring and analyzed at various intervals up to 18 months of age (White et al. 2009). Mammary gland whole mounts from female offspring between PNDs 22 and 32 were scored as described above; whole mounts from lactating dams and female offspring at 18 months were qualitatively examined with respect to concurrent controls.

Maternal weight gain was increased in dams exposed to PFOA beginning on GDs 7 and 10, but there was no effect on number of uterine implantation sites, litter loss, or number of pups per litter at birth. Male pup weight at birth was significantly decreased ($p<0.05$) in dams dosed with 5 mg/kg/day on GD 7–17 or 10–17 or with 20 mg/kg/day on GD 15–17. By PND 78, all male offspring had recovered to control body weight levels. On PND 161, the offspring of dams dosed during GD 13–17 weighed significantly more than control. Litters exposed to 20 mg/kg/day on GD 15–17 experienced decreased survival ($p<0.01$) during PND 1–22. Maternal relative liver weight was significantly increased in all PFOA-treated dams except those treated during GD 15–17. Relative liver weight in all male and female pups was significantly increased ($p<0.01$). Eye opening and growth of body hair were delayed in pups exposed GD 7–17 and 10–17 (Wolf et al. 2007).

Mammary gland developmental scores for all offspring of dams exposed to PFOA were significantly lower compared to scores from offspring of control dams at PND 29 and 32. Delayed ductal elongation and branching and delayed appearance of terminal end buds were characteristic of delayed mammary gland development at PND 32. At 18 months of age, mammary tissues were not scored due to a lack of a protocol applicable to mature animals. However, there were dark foci (composition unknown) in the mammary tissue that occurred at a higher frequency in the exposed animals compared to controls, but did not display a consistent response with dosing interval. Qualitatively, mammary glands from treated dams on LD 1 appeared immature compared with control dams (White et al. 2009). The 5-mg/kg/day dose was associated with increased maternal and pup liver weight, altered pup mammary gland development, and delayed pup eye opening and growth of body hair. The 20-mg/kg/day dose was associated with decreased postnatal pup survival.

The objective of a second component of the study by Wolf et al. (2007) and White et al. (2009) was to determine if postnatal body weight deficits, neonatal lethality, and developmental delays caused by PFOA exposure were the result of gestational exposure, lactational exposure, or a combination of gestational and lactational exposure. Pregnant CD-1 mice were orally dosed with 0 (n = 48), 3 (n = 28), and 5 (n = 36) mg PFOA/kg/day on GD 1–17 and their offspring cross-fostered at birth to create seven treatment groups: control, *in utero* exposure only (3U and 5U), lactation exposure only (PFOA stored in milk during gestation and released during lactation; 3L and 5L), and *in utero* and lactation exposure (3U+L and 5U+L). On PND 22, all dams and one male and female pup from each litter were necropsied. Blood samples were collected and the liver was removed from dams and offspring and weighed. Implantation sites were counted from the uteri of dams. The fourth and fifth inguinal mammary glands were removed from female offspring and analyzed at various intervals up to 18 months of age (White et al. 2009). Mammary gland whole mounts from female offspring between PND 22 and 63 were scored as described above; whole mounts from female offspring at 18 months were qualitatively examined with respect to concurrent controls due to lack of an applicable protocol for mature animals.

Maternal weight and weight gain were higher in PFOA-treated dams compared to control dams. Whole litter loss was significantly increased ($p<0.05$) at 5 mg/kg/day, but no differences in the number of implantation sites were observed between the treated and control mice. Absolute and relative liver weights of PFOA-treated dams from both dose groups were significantly increased ($p<0.001$) compared to absolute and relative liver weights of control dams 23 days after the last dose (PND 22). No difference in the number of live pups born per litter was found between treated and control mice, but male and female pup birth weight was reduced ($p<0.01$) in dams receiving 5 mg/kg/day (Wolf et al. 2007). The 3-mg/kg/day dose was a LOAEL for increases in liver weight in the dams while 5 mg/kg/day was a LOAEL for the pups, based on whole litter loss and significantly reduced male and female birth weight.

A dose-dependent increase of PFOA was observed in the serum of dams treated with PFOA, providing a reservoir for lactational transfer. The control dams that nursed offspring exposed *in utero* (3U and 5U) had low concentrations of PFOA in their serum that originated from maternal grooming behavior of the pups and allowed for low-level lactational transfer.

Body weight of male and female pups (3U+L, 5U, and 5U+L) was significantly reduced as early as PND 2 and 1, respectively, and remained reduced throughout the lactation period. Body weight recovery to control levels was reached by male offspring within 2 weeks of weaning, but recovery in female offspring in the 5U and 5U+L groups did not occur until after PND 85.

Postnatal survival in 5U+L pups was significantly decreased compared to control survival beginning at PND 4 and continuing throughout lactation. Survival in the other groups was no different than control survival. Eye opening and body hair growth were significantly delayed in the 3U+L, 5U, and 5U+L offspring. The relative liver weight was significantly increased in all offspring regardless of exposure scenario (Wolf et al. 2007).

All female offspring of PFOA-exposed dams had reduced mammary gland developmental scores at PND 22, except for females in the 3L group. At PND 42, mammary gland scores from females in the 3U+L group were the only ones not statistically different from control scores. This might have been due to interindividual variance and multiple criteria used to calculate mammary gland development scores. All offspring of dams exposed to PFOA exhibited delayed mammary gland development at PND 63, including those exposed only through lactation (3L and 5L). A

higher density of dark staining foci was observed in the mammary glands of these animals at 18 months of age (White et al. 2009).

White et al. (2009) also reported the results from pregnant CD-1 mice orally dosed with 0 (n = 56) and 5 (n = 56) mg PFOA/kg/day from GD 8–17 to determine the timing of the mammary gland development deficits observed following gestational or lactational exposure to PFOA. The groups were cross-fostered at birth to create four treatment groups: control, *in utero* exposure only (5U), lactation exposure only (5L), and *in utero* and lactation exposure (5U+L). Dams and litters were sacrificed on PNDs 1, 3, 5, and 10. Blood and liver samples were collected for PFOA analysis. The fourth and fifth inguinal mammary glands were collected from dams and female offspring and analyzed as described above; whole mounts from lactating dams were qualitatively examined with respect to concurrent controls.

Maternal weight gain in treated dams was significantly higher than control weight gain, but there were no effects of treatment on litter size or pup birth weight at PND 1. Significantly decreased body weight occurred in the pups of the 5U+L group on PND 3 and in all PFOA-exposed pups on PNDs 5 and 10. Relative liver weight of the treated dams was significantly increased ($p<0.05$) compared to relative liver weight of control dams. On PND 1, liver-to-body weight ratios were significantly increased ($p<0.05$) in pups exposed *in utero* (5U, 5U+L); serum PFOA levels were 65,000–70,000 ng/mL. The liver-to-body weight ratio was increased in pups exposed lactationally by PND 5; serum PFOA levels were approximately 15,000 ng/mL (White et al. 2009).

On PND 1, the mammary glands of PFOA-exposed dams were qualitatively similar to glands seen in late pregnancy, prior to parturition. In control dams nursing offspring from PFOA-exposed dams, reduced alveolar filling was noted as early as PND 3, presumably a result of exposure of the dam from maternal grooming behavior. The delayed lactational morphology in dams treated with PFOA and control dams nursing offspring from PFOA-treated dams was persistent up to PND 10 (terminal sacrifice). Reduced mammary gland developmental scores were observed as early as PND 1 in all female offspring from PFOA-exposed dams, including those exposed through lactation only (5L). Delayed mammary gland development persisted throughout the study duration (White et al. 2009).

Macon et al. (2011) gavage-dosed CD-1 mice with 0, 0.3, 1.0, and 3.0 mg PFOA/kg/day from GD 1–17 (n = 13 dams per group). Six offspring per group were sacrificed on PNDs 7, 14, 21, 28, 42, 63, and 84, and blood, liver, brain, and the fourth and fifth mammary glands were collected from female pups. Mammary gland developmental scores were not included in the published article, but were available in supplemental materials.

Body weight in male and female offspring was not affected through PND 84. Absolute liver weight was significantly increased at ≥0.3 mg/kg/day in females and at ≥1.0 mg/kg/day in males on PND 7, and at 3.0 mg/kg in females at PND 14. Relative liver weight was significantly increased at ≥0.3 mg/kg/day in males and females on PND 7, at ≥1.0 mg/kg/day in females on PND 14, and at 3.0 mg/kg/day in males and females on PNDs 14, 21, and 28. No dose-related differences were observed in absolute and relative brain weights.

Delayed mammary gland development of female pups was observed as early as PND 7 at ≥1.0 mg/kg/day and PND 14 at ≥0.3 mg/kg/day and persisted until the end of the study. However, the developmental scores did not show dose-related trends at each interval. The delayed development was characterized by reduced epithelial growth and the presence of

numerous terminal end buds. Photographs of the mammary gland whole mounts at PNDs 21 and 84 show differences in the duct development and branching pattern of offspring from dams given 0.3 and 1 mg/kg/day (offspring from high-dose dams not pictured). The LOAEL was 0.30 mg/kg/day based on significantly increased liver weight and delayed mammary gland development. No NOAEL was established. The lowest dose tested was a NOAEL at PND day 7 and is a LOAEL at day PND 14.

Macon et al. (2011) also gavage-dosed CD-1 mice with 0, 0.01, 0.1, and 1.0 mg PFOA/kg/day from GD 10–17 (n = 5–8 dams per group) to examine the effects of low doses of PFOA on mammary gland development. Female offspring (one from at least three litters per group) were sacrificed on PNDs 1, 4, 7, 14, and 21, and blood, liver, and the fourth and fifth mammary glands were collected. In addition to the qualitative mammary gland developmental scores, quantitative measurements of longitudinal growth, lateral growth, and numbers of terminal end buds and terminal ends were recorded. These data were presented only for animals sacrificed on PND 21.

No differences in body weight or brain weight were observed for male or female offspring. At 1 mg/kg, absolute and relative liver weights were significantly increased at PNDs 4 and 7. Relative liver weight also was significantly increased at PND 14. Mammary gland development was delayed by exposure to PFOA, especially longitudinal epithelial growth. At PND 21, all treatment groups had significantly lower developmental scores. At the highest dose, poor longitudinal epithelial growth and decreased number of terminal end buds were observed. As seen in Table 3-22, the quantitative measures were statistically significant only for the high dose compared to the controls, while the qualitative scores were significantly different from controls at all doses. The LOAEL was 0.01 mg PFOA/kg/day based on the qualitative / quantitative developmental score for mammary gland development and 1 mg/kg/day based on the quantitative score in the absence of the qualitative component. No NOAEL was established.

Table 3-22. Mammary Gland Measurements at PND 21 from Female Offspring of Dams Treated GD 10–17

Dose mg/kg/d	Score	Longitude µm	Lateral µm	Δ Longitude µm	Δ Lateral µm	TEBs #/gland	TEs #/gland
0 n = 5	3.3 ± 0.3	4321 ± 306	5941 ± 280	3394 ± 306	4358 ± 280	40 ± 4	81 ± 12
0.01 n = 4	2.2 ± 0,2*	3803 ± 386	5420 ± 326	3087 ± 386	3899 ± 326	33 ± 4	61 ± 8
0.1 n = 3	1.8 ± 0.3**	3615 ± 320	4822 ± 672	2370 ± 320	3035 ± 672	24 ± 4	58 ± 4
1.0 n = 5	1.6 ± 0.1***	2775 ± 285**	4822 ± 313**	1553 ± 301	3380 ± 313	15 ± 2***	47 ± 11

Longitude = longitudinal epithelial growth
Lateral = Lateral epithelial growth
Δ =change in
*= p<0.05, ** p<0.01, ***p<0.001

# = number
TEBs = terminal end buds
TE = differentiating duct ends

Source: Macon et al. 2011

White et al. (2011) examined the extended consequences of PFOA-induced altered mammary gland development in a multigenerational study in CD-1 mice. Pregnant mice (F0, n = 10–12 dams per group) were gavage-dosed with 0, 1, and 5 mg PFOA/kg/day from GD 1–17. A separate group of pregnant mice (n = 7–10 dams per group) was gavage-dosed with either 0 or 1 mg PFOA/kg/day from GD 1–17 and received drinking water containing 5 ppb PFOA beginning on GD 7. F1 females and F2 offspring from the second group continued to receive drinking water that contained 5 ppb PFOA until the end of the study, except during F1 breeding and early gestation, to simulate a chronic low-dose exposure. Only the F0 dams were given

PFOA by gavage. Total doses were not calculated for the groups receiving drinking water with 5 ppb PFOA. Table 3-23 shows the array of dosing regimens used in the study and the estimated average daily PFOA intake by F0 dams. The average daily intake from the chronic water exposures were calculated from total weekly water consumption, divided by the number of days per week (values given in supplemental materials; intake by the F1 animals was not calculated).

Table 3-23. Dosing Regimens Used in the Multigeneration Study of CD-1 Mice

	F0 Dams	F0 Dams → F1 Offspring		F1 Dams → F2 Offspring	F2 Offspring
Treatment	Gavage	Drinking water	Gavage + drinking water	Drinking water	Drinking water
Dose	0, 1, or 5 mg/kg/day	0+5 ppb	1+5 ppb	5 ppb	5 ppb
Duration Gavage Drinking water	 GD 1-17 None	 GD 1-17 GD 7-LD 22	 GD 1-17 GD 7-LD 22	 None Through LD 22	 None Through PND 63
Total Daily PFOA intake to dams from gavage and drinking water	Not relevant (0 mg/kg/day) 36 µg/day (1 mg/kg/day) 187 µg/day (5 mg/kg/day	0.054 µg/day (gestation) 0.105 µg/day (lactation)	37 + 0.051 µg/day (gestation) 0 + 0.130 µg/day (lactation)	Not calculated	Not calculated

Source: White et al. 2011

F0 females were sacrificed on PND 22. F1 offspring were weaned on PND 22 and bred at 7–8 weeks of age. F2 litters were maintained through PND 63. Groups of F1 and F2 offspring (n = 1–2 offspring per litter from 5–7 litters per group) were sacrificed on PND 22, 42, and 63. A group of F2 offspring (n = 6–10 per group) also was sacrificed on PND 10. A lactational challenge experiment was performed on PND 10 with F1 dams and F2 offspring. Mammary glands were evaluated from F0 dams on PND 22, from F1 dams on PNDs 10 and 22, and from F1 and F2 female offspring on PNDs 10 (F2 only), 22, 42, and 63. Mammary gland whole mounts were scored qualitatively as described above.

Exposure to 5 mg PFOA/kg/day significantly increased prenatal loss in F0 mice and significantly decreased the number of live offspring and the postnatal survival of the viable pups. Maternal weight gain and number of implants did not differ among F0 the groups. There was no indication of toxicity in F1 adult females. Exposure to PFOA did not affect prenatal loss or postnatal survival, although F1 females that had been exposed *in utero* to 5 mg/kg/day had significantly fewer implants.

On PND 22, F1 pup body weight was similar across all treated and control groups. F1 offspring body weight at PND 42 was significantly reduced for those whose dams received 5 mg/kg/day; at PND 63, body weight was significantly reduced for offspring from dams given 1 mg/kg/day plus 5 ppb in the drinking water compared to offspring from dams given 1 mg/kg/day. Liver-to-body weight ratios were significantly increased at 1 mg/kg/day on PND 22 and at 5 mg/kg on PNDs 22 and 42. For the F2 pups, a significant reduction in body weight was observed in control plus 5 ppb drinking water PFOA offspring on PND 1, but there was no difference by PND 3. F2 offspring from the 1 mg/kg/day and 1 mg/kg/day plus 5-ppb drinking water PFOA groups had increased body weight compared to controls on PNDs 14, 17, and 22. Liver-to-body weight ratios were no different across the groups.

Mammary gland developmental scores for the three generations of females are summarized in Table 3-24. At PND 22, control F0 dams displayed weaning-induced mammary involution. At PND 22, the mammary glands of all PFOA-exposed F0 dams, including the control dams receiving 5 ppb PFOA in drinking water, resembled glands of mice at or near the peak of lactation (~PND 10). The F1 dams examined on PNDs 10 and 22 had significantly lower developmental scores on PND 10, but that was no longer evident at PND 22, except for those exposed *in utero* to 5 mg/kg/day.

Table 3-24. Mammary Gland Scores from Three Generations of CD-1 Female Mice

Group	Control	Control +5 ppb	1 mg/kg/day	1 mg/kg/day + 5 ppb	5 mg/kg/day
F0 dams on PND 22	2.4 ± 0.2	3.4 ± 0.1*	3.0 ± 0.2*	3.2 ± 0.2*	3.9 ± 0.1*
F1 as pups PND 63	3.8 ± 0.2	2.6 ± 0.4*	2.9 ± 0.2*	2.0 ± 0.3*#	2.2 ± 0.2*
F1 as dams on PND 10	4.0 ± 0.0	2.8 ± 0.5*	2.5 ± 0.2*	2.0 ± 0.2*	2.5 ± 0.2*
F1 as dams on PND 22	2.1 ± 0.3	2.2 ± 0.2	1.9 ± 0.4	1.5 ± 0.2*	3.2 ± 0.3*
F2 PND 10	2.8 ± 0.3	3.0 ± 0.2	1.9 ± 0.3	2.6 ± 0.2	2.0 ± 0.2
F2 PND 22	3.1 ± 0.4	1.9 ± 0.3	2.3 ± 0.1	2.3 ± 0.2	2.0 ± 0.2
F2 PND 42	3.5 ± 0.2	2.5 ± 0.4*	3.4 ± 0.2	2.4 ± 0.2*#	3.3 ± 0.4
F2 PND 63	3.4 ± 0.2	3.5 ± 0.2	2.4 ± 0.2*	2.6 ± 0.5	2.6 ± 0.4

Notes: n = 4–11.
* $p<0.05$ compared with control.
# $p<0.05$ compared with 1 mg/kg/day.
F1 and F2 animals represented in each data set are different. They represent members of litters within each group at different stages of development.

In the F1 female offspring not used for breeding, the mammary glands of all mice exposed to PFOA were significantly delayed in development on PNDs 22, 42, and 63. For the F2 female offspring, some differences in mammary gland scores were observed between the groups, but most were not significantly different from controls.

In the lactational challenge experiment, dams were removed from their litters for 3 hours, then returned to their litters and allowed to nurse for 30 mins. The time from the dam's return to the litter and nursing initiation was recorded. The litters were weighed before and after nursing to estimate volume of milk produced. The results from the lactational challenge on PND 10 for the F1 dams showed a slight dose-related trend for decreased milk production (measured in grams) over a 30-min period (differences from controls not identified as significant), but no clear differences in time to initiate nursing (measured in seconds). As discussed above, morphological differences were seen in developmental scores for the treated F1 dams on PND 10 and were generally no longer evident at PND 22.

White et al. (2011) demonstrated that no significant dose-related differences were found in the ability of the CD-1 mice given 1 mg/kg/day to provide nourishment to their young as reflected in measurements of body weight in F1 and F2 pups across a 63-day postnatal period. There were body weight effects in the pups from dams given 5 mg/kg/day and in pups from dams that received 1 mg/kg/day by gavage with 5 ppb in the drinking water.

In the study by Albrecht et al. (2013) discussed earlier, groups of female wild-type, PPARα-null, and PPARα-humanized mice on a SV/129 genetic background were given 0 and 3 mg PFOA/kg on GD 1–17 by oral gavage. Controls received the water vehicle. The study was

designed with the goal of identifying the contribution of PPARα activation to the responses evaluated. Mammary gland structure was one of the endpoints evaluated. Females were either sacrificed on GD 18 (n = 5–8 per group) or allowed to give birth and then sacrificed, along with their litters (n = 8–14), on PND 20. The left and right fourth and fifth mammary glands were removed, spread on a glass slide, and stained. Ductal length and terminal end buds were quantified in the offspring of from three to nine dams. There was no significant difference in the measurements for either parameter at either timepoint for the offspring of PFOA-treated animals compared to the controls. In the case of the wild-type mice, the terminal end bud measurements were 2.1 ± 0.01 terminal end buds/gland for the control and 2.2 ± 0.2 terminal end buds/gland based on the mean for three control litters and four PFOA-exposed litters. For the ductal lengths, the values were 2.4 ± 0.3 millimeter (mm) for the control and 2.4 ± 0.4 mm for the PFOA-exposed animals. There was no qualitative component of the scoring approach used by Albrecht et al. (2013). The fewest number of terminal end buds and the longest ductal length measurement were those for the animals with the hPPARα.

To examine the impact of differences in mouse strains, Tucker et al. (2015) conducted a study of the effects of gestational exposure on mammary gland development as measured at prepubertal time points. Doses of 0, 0.01, 0.1, 0.3, and 1 mg/kg/day were administered to timed pregnant CD-1 and C57Bl/6 mice by gavage on GD 1–17. After parturition, the number of pups was reduced so that there were ultimately four to eight CD-1 litters per treatment block and three to seven B57BL/6 litters per treatment. Endpoints monitored included body weight; net body weight; absolute and relative liver weight on PNDs 21, 35, and 56; neonatal developmental endpoints (e.g., vaginal opening, first estrus); and serum estradiol and progesterone (P) measurement; and as well as mammary gland development scores. Qualitative assessment of mammary gland scores was as described above. Different treatment blocks monitored different endpoints at different times. Serum POA levels were measured at PNDs 21, 35, and 56 for the CD-1 mice (n = 4–12) and at PND 21 and 61 for the C57BL/6 mice (n = 2–6). At each time point, the serum concentration increased with dose and decreased with duration.

There were no measures that were significantly ($p<0.05$) different from controls for the CD-1 anthropometric parameters, except relative liver weight on PND 56 at 0.3 mg/g/day and on PND 21 at 1 mg/kg/day. Net body weight was significantly increased ($p<0.05$) at PNDs 21 and 35 in the 1-mg/kg/day group. No significant differences were observed in the C57Bl/6 mice at any dose or duration. There were no significant differences for postnatal developmental endpoints, estradiol, or P in either mouse strain. There was a trend towards decreasing mammary gland developmental scores with dose for both strains of mice. In the CD-1 mice, mammary gland developmental scores were significantly reduced at ≥0.01 mg/kg/day on PND 35 and at ≥0.1 mg/kg/day on PND 21 compared to scores in the controls. However, in the C57Bl/6 mice, mammary gland developmental scores were significantly reduced only at 0.3 and 1.0 mg/kg/day on PND 21 compared to scores in the controls.

Serum P was higher in the control and treated CD-1 mice on PND 56 than at the other two time points but lacked dose response; estradiol was relatively consistent across time points. For the C57BL/6 mice, the estradiol levels at PND 61 were higher in all treated groups but lacked dose-response; P changed little with time and was similar between treated and control groups. The LOAEL was 0.01 mg/kg/day for aberrant mammary gland development in the CD-1 mice and 0.3 mg/kg/day for the C57BL/6 mice. The CD-1 mice lacked a NOAEL. The NOAEL for the C57/BL/6 mice was 0.1 mg/kg/day. Although both strains experienced delayed prepubertal mammary gland development, there were no significant changes in other postnatal

developmental events. The relevance of the mammary gland changes at maturity in the absence of any postlactational PFOA exposure is uncertain, especially as it relates to humans.

Direct peripubertal exposures

C. Yang et al. (2009) gavage-dosed 21-day-old female BALB/c mice (5 per group) with 0, 1, 5, and 10 mg PFOA/kg/day for 5 days per week for 4 weeks to determine the effects of peripubertal PFOA exposure on puberty and mammary gland development. At necropsy, uteri and livers were weighed and processed for histological examination. Mammary glands were collected and processed for histological and whole-mount examination. A significant decrease in body weight was observed following exposure to 10 mg/kg/day. The mammary glands of female BALB/c mice treated with 5 or 10 mg/kg/day had reduced ductal length, decreased number of terminal end buds, and decreased stimulated terminal ducts compared to the mammary glands of control mice. BrdU incorporation into the mammary gland revealed a significantly lower number of proliferating cells in the ducts and terminal end buds/terminal ducts at 5 mg/kg/day (not tested at 10 mg/kg/day). Absolute and relative liver weight was significantly increased in all treated BALB/c mice. The absolute and relative uterine weight was significantly decreased in all treated mice compared to uterine weight in control mice. Vaginal opening was significantly delayed in mice dosed with 1 mg/kg/day and did not occur at 5 or 10 mg/kg/day. The LOAEL was 1 mg/kg/day based on delayed vaginal opening, increased liver weight, and decreased uterine weight; and no NOAEL was established.

C. Yang et al. (2009) also dosed 21-day-old female C57BL/6 mice in the same manner as the BALB/c mice and examined the effects of PFOA on mammary gland development and vaginal opening. The body weight effects were similar in both strains with 10 mg/kg/day causing significantly reduced body weight. At 5 mg/kg/day, PFOA had a stimulatory effect on the mammary glands. There was a significant increase in the number of terminal end buds and stimulated terminal ducts. Ductal length was not affected. Mammary gland development was inhibited in mice dosed with 10 mg/kg/day, with no terminal end buds or stimulated terminal ducts present and very little ductal growth. Absolute and relative liver weight was significantly increased in all treated mice. The absolute and relative uterine weight was significantly increased in C57BL/6 mice dosed with 1 mg/kg/day and significantly decreased in C57BL/6 mice dosed with 10 mg/kg/day. There was no difference in uterine weights between mice treated with 5 mg/kg/day and control mice. Vaginal opening was delayed in C57BL/6 mice dosed with 5 mg/kg/day and did not occur in mice dosed with 10 mg/kg/day. The LOAEL was 1 mg/kg/day based on increased liver and uterine weights, and no NOAEL was established.

Y. Zhao et al. (2010) conducted several experiments in C57BL/6 mice to determine the potential mechanism by which peripubertal PFOA exposure resulted in the stimulation of mammary gland development observed by C. Yang et al. (2009). In experiments to determine if PFOA has a hormonal effect on mammary gland development, C57BL/6 mice (n = 10 per group) were OVX at 3 weeks of age, allowed 1 week to recover, and treated with 0 and 5 mg PFOA/kg bw/day for 4 weeks. Abdominal and inguinal mammary glands were collected at sacrifice, prepared as whole mounts, and scored for growth and development. The mammary glands of the OVX control and PFOA-treated OVX mice were similarly stunted in growth as evidenced by no outgrowth of ducts or presence of terminal end buds. This was in contrast to the stimulatory effect of PFOA observed by C. Yang et al. (2009) in intact mice.

In experiments to determine if PFOA-affected mammary glands respond to hormone treatment, intact C57BL/6 mice were dosed with 0 or 5 mg/kg bw/day of PFOA for 4 weeks

starting at 21 days of age. After the last dose, the mice were OVX, allowed to recover for 1 week, and injected subcutaneous for 5 days with E2 (1 μg/0.2 ml per mouse), P (1 mg/0.2 ml per mouse), or both (E+P, 1 μg+1 mg/0.2 ml per mouse). The mice were sacrificed 24 hours after the last hormone injection. Abdominal and inguinal mammary glands were collected at sacrifice, prepared as whole mounts, and scored for growth and development. In the mammary glands of mice treated with PFOA and estradiol, stimulated terminal ducts were observed, and in PFOA-treated mice given P or E+P, stimulated terminal ducts and an increased number of side branches were observed. The results showed that PFOA increased the mouse mammary gland response to exogenous estrogen and P.

In experiments to determine if PFOA-induced mammary gland development stimulation is related to PPARα expression and the impact of PFOA on steroid hormones and growth factors, female C57BL/6 and PPARα-null C57BL/6 mice (n = 5–10 mice per group) were gavage-dosed with 0 or 5 mg/kg bw/day of PFOA 5 days per weeks for 4 weeks starting at 21 days of age (Y. Zhao et al. 2010). Vaginal opening was monitored daily and estrous cycle state was determined at sacrifice after 4 weeks of treatment. At necropsy, blood was collected for measurement of serum steroid hormones and binding proteins. Portions of the mammary glands, ovaries, and livers were collected and processed for histological examination. RNA was extracted from the livers for quantitative RT-PCR and PCR array for selected genes related to metabolism of drugs, toxic chemicals, hormones, and micronutrients. Portions of mammary glands were used in western blot analysis of several enzymes, local growth factors, and receptors, including aromatase—which aids in converting testosterone to estradiol and androstenedione to estrone, hydroxysteroid 17β dehydrogenase 1 (HSD17β1)—which aids in converting estrone to estradiol, and hydroxysteroid 3β dehydrogenase 1 (HSD3β1)—which aids in converting pregnenolone to P and androstenedione to testosterone. Growth factors critically involved in mammary gland development, including amphiregulin (Areg), insulin like growth factor I (IGF-I), and hepatocyte growth factor (HGFα), and markers of cell proliferation (e.g., cyclin D1 and PCNA) were analyzed by western blot. Areg mediates estrogen receptor α (ERα) function and is a ligand for the epidermal growth factor receptor (EGFR). These receptors also were analyzed by western blot.

The mammary glands of PPARα-null mice treated with PFOA had an increased number of terminal end buds and stimulated terminal ducts compared to control PPARα-null mice. Protein levels of Areg, IGF-I, HGFα, ERα, and EGFR were significantly increased ($p<0.05$) in PFOA-treated C57BL/6 mice; and Areg, HGFα, ERα, and EGFR were significantly increased ($p<0.05$) in PFOA-treated PPARα-null mice. Cyclin D1 and PCNA were significantly increased ($p<0.05$) in C57BL/6 and PPARα-null mice treated with PFOA compared to levels in control mice. Immunofluorescent staining of the mammary glands for ERα and Areg showed a significant increase ($p<0.05$) in Areg positive luminal epithelial cells and Areg and ERα double positive staining cells in C57BL/6 and PPARα-null mice treated with PFOA compared to control mice. The results show that the stimulatory effect of PFOA on mammary gland development is independent of PPARα expression and suggest that PFOA increases the levels of steroid hormones, growth factors, and receptors, which promote mammary gland cell proliferation.

Estradiol levels were similar between intact control and treated wild-type mice, but P levels were significantly increased ($p<0.05$) in PFOA-treated mice in proestrus and estrus compared to control mice in the same stages of the estrous cycle. Serum SHBG and albumin levels were not significantly changed by treatment with PFOA.

The effect of PFOA on aromatase, HSD17β1, and HSD3β1 activity in the ovaries of C57BL/6 PPARα-null mice was examined. In C57BL/6 mice, HSD17β1 and HSD3β1 proteins were significantly increased ($p<0.05$), and in PPARα-null mice, HSD17β1 protein was significantly increased. Aromatase levels were not affected by PFOA. The results suggest that PFOA might increase serum steroid hormone levels in the ovaries.

Due to the increased P levels observed in PFOA-treated mice, the expression of liver metabolic enzymes was analyzed. Liver metabolic function might affect steroid hormone serum levels, which play a role in mammary gland development. In PPARα-null and C57BL/6 mice treated with PFOA, detoxification enzymes in the liver, including glutathione s-transferase α1, μ3, and μ4, were upregulated ($p<0.05$). Expression of liver drug metabolic enzymes, including CYP1a1, CYP1a2, and HSD17β2, was significantly downregulated ($p<0.05$) in C57BL/6 mice treated with PFOA, but expression in PFOA-treated PPARα-null mice was comparable to that in control mice. Hydroxysteroid 17β dehydrogenase 4, an enzyme that converts estradiol to estrone, was significantly upregulated ($p<0.05$) in C57BL/6 mice treated with PFOA. The results suggested that PFOA-induced expression changes in liver enzymes might not contribute to PFOA-induced mammary gland development stimulation.

Inhalation Exposure

Staples et al. (1984) exposed Sprague-Dawley rats to PFOA using whole-body dust inhalation for 6 hours per day on GD 6–15. The MMAD of the particles ranged from 1.4 to 3.4 μm and the GSD ranged from 4.3 to 6.0. The study was carried out in two trials with each trial including two experiments. In experiment 1, the dams were sacrificed on GD 21 prior to parturition, and in experiment 2, the dams were allowed to litter and were sacrificed on PND 23; offspring were sacrificed on PND 35. In the trial 1 (both experiments), dams (n = 12) were exposed to 0, 0.1, 1, and 25 mg/m^3. In trial 2, the high dose was reduced to 10 mg/m^3. In experiment 1 of trial 2, dams numbered 12–15 per group and two additional groups (6 dams per group) were added and were pair-fed at 10 and 25 mg/m^3. In experiment 2 of trial 2, only six control and six dams dosed at 10 mg/m^3 were allowed to litter.

In experiment 1, the dams were weighed on GDs 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded, and the reproductive status of each animal was evaluated. The ovaries, uterus, and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions, and implantation sites. Pups (live and dead) were counted, weighed and sexed, and examined for external, visceral, and skeletal alterations. The heads of all control and high-dosed group fetuses were examined for visceral alterations and macroscopic and microscopic evaluations were conducted of the eyes.

Treatment-related clinical signs of maternal toxicity occurred at 10 and 25 mg/m^3 and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m^3 (on GDs 12, 13, and 17). Food consumption was significantly reduced at 10 and 25 mg/m^3; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight also were observed at these concentrations, with statistical significance at the high concentration only. Likewise, statistically significant increases in mean liver weights ($p<0.05$) were seen in the high-concentration group. The NOAEL and LOAEL for maternal toxicity were 1 and 10 mg/m^3, respectively.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25 mg/m^3 PFOA group ($p = 0.002$) and in the pair-fed control group ($p = 0.001$). Interpretation of the decreased fetal body weight is difficult given the high incidence of mortality in the dams. The NOAEL and LOAEL for developmental toxicity were 10 and 25 mg/m^3, respectively.

In experiment 2, in which the dams were allowed to litter, the procedure was the same as for experiment 1 until GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated PND 1. Dams were weighed and examined for clinical signs on PNDs 1, 7, 14, and 22. On PND 23 all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. At birth, each pup was subsequently weighed and then inspected for adverse clinical signs on PNDs 4, 7, 14, and 22. The eyes of the pups were also examined on PNDs 15 and 17. Pups were sacrificed on PND 35 and examined for visceral and skeletal alterations.

Clinical signs of maternal toxicity seen at 10 and 25 mg/m^3 were similar in type and incidence to those described for trial 1. Maternal body weight gain during treatment at 25 mg/m^3 was less than controls, although the difference was not statistically significant. In addition, two out of 12 dams died during treatment at 25 mg/m^3. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. The NOAEL and LOAEL for maternal toxicity were 1 and 10 mg/m^3, respectively.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on PND 1 (6.1 g at 25 mg/m^3 versus 6.8 g in controls, $p = 0.02$). On PNDs 4 and 22, pup body weight continued to remain lower than controls, although the difference was not statistically significant. No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. The NOAEL and LOAEL for developmental toxicity were 10 and 25 mg/m^3, respectively.

Dermal Exposure

No data on the developmental effects of dermal exposures to PFOA were identified in the literature.

3.2.8 Chronic Toxicity

Oral Exposure

Monkey. Male cynomolgus monkeys (n = 4 or 6 per dose) were administered PFOA by oral capsule containing 0, 3, 10, or 30/20 mg/kg/day for 26 weeks (Butenhoff et al. 2002). Dosing of animals in the 30-mg/kg/day dose group ceased after 12 days and decreased to 20 mg/kg/day when reinstated on day 22 because of low food consumption, decreased body weight, and decreased feces. Sacrifice of the surviving monkeys, except for two control monkeys and two monkeys from the mid-dose group (recovery animals) occurred at 26 weeks. The animals in the recovery groups were sacrificed 13 weeks later.

Animals were observed twice daily for mortality and moribundity and were examined at least once daily for signs of poor health or abnormal behavior. Ophthalmic examinations were performed before treatment began and at weeks 26 and 40. Body weight, food consumption, clinical hematology, clinical chemistry, urinalysis, serum hormone levels, and PFOA levels in

blood and tissue were assessed throughout the study. One animal from the 30/20-mg/kg/day dose group was sacrificed in moribund condition on day 29 with signs of dosing injury and liver lesions. One animal from the 3-mg/kg/day dose was sacrificed (day 137) with signs of hind limb paralysis, ataxia and hypoactive behavior, few feces, and no food consumption. Treatment of the remaining three animals given 30/20 mg/kg/day was halted on days 43, 66, and 81, respectively, because of thin appearance, few or no feces, low or no food consumption, and weight loss, but the animals appeared to recover from compound-related effects within 3 weeks after cessation of treatment. No significant changes in mean body weight were observed at doses of 3 or 10 mg/kg/day.

Serum hormone levels (i.e., estrone, estradiol, estriol, testosterone, TSH, FT4, total T4, and CCK) were not significantly altered throughout the study. However, FT3 and total T3 levels were significantly decreased ($p<0.05$) from weeks 5 to 10 and at week 27 in the 30/20-mg/kg/day dose group compared to controls.

At terminal sacrifice (26 weeks), mean absolute liver weight was significantly increased in all dose groups and the relative liver-to-body weight ratio was significantly increased for the High-Dose Group. Final Body Weight And Liver Weight Data Are Presented In Table 3-25.

Table 3-25. Liver Weight Data in Monkeys Administered PFOA for 6 Months

Dose	Body Weight	Absolute Liver Wt (g)	Relative Liver Wt (%)
0 mg/kg (n = 4)	3947 ± 591	60.2 ± 6.9	1.5 ± 0.1
3 mg/kg (n = 3)	4486 ± 30	81.8 ± 2.8*	1.8 ± 0.1
10 mg/kg (n = 4)	4447 ± 498	83.2 ± 9.7*	1.9 ± 0.1
30/20 mg/kg (n = 2)	3925 ± 583	90.4 ± 4.2*	2.4 ± 0.5*

Source: Butenhoff et al. 2002
Note: * Significantly different from control, $p<0.01$.

The cause of the increase in liver weight was suggested to be hepatocellular hypertrophy (indicated by decreased hepatic DNA content), which was hypothesized to result from mitochondrial proliferation based on an increase in hepatic succinate dehydrogenase activity. The two animals given 20 mg/kg/day had significantly decreased hepatic DNA content, and increased succinate dehydrogenase and palmitoyl-CoA oxidase activities; glucose-6-phophatase activity was slightly decreased in all treated groups, but a dose-response was not shown. These data are shown in Table 3-26. Succinate dehydrogenase activity was highly variable in animals given 3 mg/kg/day despite this group having the most consistent liver PFOA concentrations. Although serum steady-state had been attained by 4-6 weeks of dosing (Table 3-26 (see section 2.2, Distribution), liver PFOA levels ranged from 11.3-18.5, 6.29-21.9, and 16-83.3 μg/g tissue in the 3, 10, and 20 mg/kg/day groups, respectively.

Because administration of PFOA to rats has been shown to result in liver, Leydig cell tumors (LCTs), and pancreatic acinar cell tumors (PACTs), Butenhoff et al. (2002) analyzed markers of tumor formation in the monkey study just described. In the liver, a twofold increase in hepatic palmitoyl-CoA oxidase activity was observed in the 30/20-mg/kg/day group, consistent with reports for species that are not particularly responsive to PPARα agonists. Replicative DNA synthesis in the liver, an indication of cell proliferation, was not altered in the treated animals. It also has been proposed that changes associated with the PACTs in rats include increased serum CCK concentrations and indications of cholestasis, including increases in ALP, bilirubin, and bile acids. None of these changes were observed in the cynomolgus monkeys. There were also no significant changes in estradiol, estriol, or testosterone in the monkeys. Each of these factors

is associated with LCTs in rats. There were no changes in replicative DNA synthesis in the pancreas or testes.

Table 3-26. Subcellular Liver Enzyme Activities and Liver PFOA Concentrations

Endpoint	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	20 mg/kg/day
DNA (mg/g liver)	1.44 ± 0.28	1.23 ± 0.89	1.25 ± 0.37	1.02 ± 0.17*
Succinate dehydrogenase (µmol cytochrome c reduced/min/g liver)	0.21 ± 0.15	1.77 ± 1.59	0.55 ± 0.14	1.37 ± 0.73*
Palmitoyl-CoA oxidation (µmol/min/g liver)	0.53 ± 0.12	0.47 ± 0.13	0.90 ± 0.29	1.36 ± 0.34*
Acid phosphatase (µmol/min/g liver)	0.78 ± 0.10	0.81 ± 0.11	0.80 ± 0.14	0.55 ± 0.10
Glucose-6-phophatase (µmol/min/g liver)	12.32 ± 3.11	6.02 ± 0.33*	10.17 ± 0.63	8.83 ± 1.41
PFOA liver level (µg/g tissue) (individual animal)	0.09 <LOQ 0.23 <LOQ	15.2 18.5 11.3 18.3 (sacrificed day 137)	21.9 6.29 8.86 18.8	16.0 83.3

Source: Butenhoff et al. 2002

Notes:
* Significantly different from control, $p<0.05$.
<LOQ: less than the lower LOQ of 0.019 µg/g.

After a 13-week recovery period, there were no treatment-related effects on terminal body weights or on absolute or relative organ weights, suggesting that the treatment-related liver weight changes were reversible. There were no treatment-related macroscopic or microscopic changes at the recovery sacrifice. Toxicological significance of the parameters in Table 3-26 are difficult to evaluate because of the small number of animals and the high variability in the enzyme measurements and the liver PFOA levels.

Rat. The chronic toxicity of PFOA was investigated in a 2-year study in rats by Butenhoff et al. (2012); this study was conducted from April 1981 through May 1983. Sprague-Dawley (Crl:CD BR) rats (50 per gender) were fed diets containing 0, 30, and 300 ppm PFOA (0, 1.3, and 14.2 mg/kg/day for males; 0, 1.6, and 16.1 mg/kg/day for females). Groups of 15 additional rats per gender were fed 0 or 300 ppm PFOA and evaluated at the 1-year interim sacrifice. All animals were observed daily throughout the 2-year dosing period. Periodic observations included body weights and feed consumption, hematology, serum chemistry, urinalysis, gross pathology, organ weights, and histopathology. Animals were sacrificed after 1 and 2 years of dosing. Organ weights were determined after each sacrifice and tissues subjected to histological examination.

No dose- or treatment-related clinical signs of toxicity were observed in males or females. Significantly decreased body weight gains occurred in high-dose male and female rats compared to the controls. The body weight changes correlated with slight decreases in feed consumption during the study. Survival rate was increased for high-dose males and females compared with their respective controls, likely because of the lower body weights for these treated groups. No consistent dose-related changes over time were observed in hematology parameters of males and females. Clinical chemistry changes included slight (less than twofold) but significant increases in ALT, AST, and ALP in both treated male groups from 3 to 18 months, but only in the high-dose males at 24 months (Table 3-27); no differences were observed in females. No dose- or treatment-related differences in absolute and relative organ weights were found between the treated and control groups at 2 years.

Table 3-27. Clinical Chemistry Values from Male Rats Given PFOA for 2 Years

Endpoint	Diet Level (ppm)	3 Months	6 Months	12 Months	18 Months	24 Months
ALT (IU/L)	0	21.4 ± 2.67	24.1 ± 3.75	33.5 ± 19.45	34.1 ± 10.68	33.4 ± 8.1
	30	34.5 ± 15.33*	53.3 ± 29.34*	77.6 ± 56.59*	59.7 ± 33.41*	42.5 ± 10
	300	31.9 ± 21.94*	54.8 ± 29.26*	106.1 ± 70*	84.3 ± 55.95*	61.8 ± 20.13*
AST (IU/L)	0	45.3 ± 7.26	49.7 ± 14.98	79.1 ± 44.61	99.1 ± 68.14	64.9 ± 25.76
	30	59.7 ± 22.47	92.1 ± 45.6*	124.4 ± 94.04*	116.4 ± 57.99	68.0 ± 17.64
	300	58.2 ± 27.23	87.8 ± 34.83*	132.7 ± 76.84*	123.3 ± 62.98	95.7 ± 29.76*
ALP (mg/dL)	0	91.1 ± 26.22	97.1 ± 40.41	150.8 ± 43.94	85.2 ± 33.76	70.1 ± 25.53
	30	138.7 ± 33.14*	146.9 ± 37.13*	128.3 ± 41.75	112.5 ± 32.61	81.2 ± 26.2
	300	153.5 ± 31.84*	147.3 ± 34.85*	166.5 ± 59.28*	184.4 ± 73.37*	113.5 ± 22.84*

Source: Butenhoff et al. 2012
Note:
* Significantly different from control, $p \leq 0.05$.

Incidence of selected microscopic lesions is detailed in Table 3-28; severity scores were not given for any type of lesion. Significantly increased incidence of lesions in the liver was observed in the high-dose male group. At 1 year, diffuse hepatocellular hypertrophy, portal mononuclear cell infiltration, and hepatocellular necrosis were seen. At 2 years, significant increases in hepatocellular hypertrophy were observed in the males and females in the high-dose group. Hepatic cystoid degeneration, a condition characterized by areas of multilocular microcysts in the liver parenchyma, also was significantly increased in high-dose males. The incidence of hepatocellular necrosis did not increase for the high-dose males at the end of the study compared with the interim rate.

Among the high-dose males, histological changes were noted in tissues other than the liver. Small but statistically significant increases in vascular mineralization of the testes and of pulmonary hemorrhage probably were not caused by treatment with PFOA. In the lung, while the incidence of alveolar macrophages was increased, that of perivascular mononuclear infiltrate and of pneumonia were decreased and vascular mineralization was a common finding in treated and control animals.

The LOAEL for male rats is 300 ppm (14.2 mg/kg/day) based on a decrease in body weight gain and histological changes in the liver. The LOAEL for female rats is 300 ppm (16.1 mg/kg/day) based on decreased body weight gain. The NOAEL for both genders is 30 ppm (1.3 mg/kg/day for males and 1.6 mg/kg/kg for females).

Biegel et al. (2001) conducted a 2-year mechanistic study in which male Crl:CD BR (CD) rats (n = 156 per group) were fed a diet containing 0 or 300 ppm PFOA (0 or 13.6 mg/kg/day). Interim sacrifices were conducted every 3 months up to 21 months for measurements of liver and testes weights, peroxisome proliferation, and cell replication. Serum samples were collected and reproductive hormones measured.

Table 3-28. Incidence of Nonneoplastic Lesions in Rats Given PFOA for 2 Years

Lesion	0 ppm	30 ppm	300 ppm
Males			
Liver			
Cystoid degeneration	4/50	7/50	28/50*
Hepatocellular hypertrophy	0/50	6/50	40/50*
[incidence at 1 year]	[0/15]	[-]	[12/15]
Mononuclear cell infiltrate	37/50	32/50	48/50*
[incidence at 1 year]	[7/15]	[-]	[13/15]
Necrosis	3/50	5/50	5/50
[incidence at 1 year]	[0/15]	[-]	[6/15]
Lung			
Alveolar macrophages	10/49	16/50	31/49*
Hemorrhage	10/49	14/49	22/50*
Mononuclear infiltrate	21/49	3/49*	7/50*
Testes			
Vascular mineralization	0/49	3/50	9/50*
Females			
Liver			
Cystoid degeneration	0/50	0/50	0/50
Hepatocellular hypertrophy	0/50	1/50	8/50*
Mononuclear cell infiltrate	19/50	11/50	19/50
Necrosis	5/50	6/50	2/50

Source: Butenhoff et al. 2012
Notes:
* Significantly different from control, $p \leq 0.05$.
- Not examined; interim sacrifice not done on animals at 30 ppm.

Body weight was significantly decreased from days 8 through 630 in PFOA-exposed rats. In the treated group, relative liver weights and hepatic β-oxidation activity were statistically significantly increased at all time points between 1 and 21 months when compared to the controls. Absolute testis weights were significantly increased only at 24 months. No hepatic or Leydig cell proliferation was observed at any sampling times. The incidence of Leydig cell hyperplasia was significantly increased in PFOA-exposed rats (46% versus 14% in the control group). Pancreatic acinar cell proliferation was significantly increased at 15, 18, and 21 months. The incidence of acinar cell hyperplasia was 30/76 (39%) compared to the incidence in the control group of 14/80 (18%). There were no significant differences in serum testosterone or prolactin in the PFOA-treated rats when compared to the controls. Serum FSH was significantly increased at 6 months, and LH was significantly increased at 6 and 18 months. There were significant increases in serum estradiol concentrations in the treated rats at 1, 3, 6, 9, and 12 months.

3.2.9 Carcinogenicity

Oral Exposure

Rat. Tissues from the animals in the Butenhoff et al. study (2012) were evaluated for neoplastic and preneoplastic formations; this study was conducted from April 1981 through May 1983. Hepatocellular carcinomas were observed at 6% (3/49), 2% (1/50), and 10% (5/50) in the control, low-, and high-dose male rats, respectively. None were observed in females in the control and low-dose groups, but a 2% (1/50) incidence was observed for female rats in the high-

dose group. The differences between the treated and control groups were not significantly different. No liver adenomas were observed.

At the 1-year sacrifice, testicular masses were found in 7/50 (14%) high-dose and 2/50 (4%) low-dose rats, but not in any of the controls. A significant increase ($p<0.05$) in the incidence of testicular (Leydig) cell adenomas was observed in the high-dose male rats at the end of the study. The LCT incidence in the control, low-, and high-dose groups was 0/50 (0%), 2/50 (4%), and 7/50 (14%), respectively. The increase also was statistically significant when compared to the historical control incidence of 0.82% observed in 1,340 Sprague-Dawley control male rats used in 17 carcinogenicity studies (Chandra et al. 1992). In a published workshop report on LCTs, Clegg et al. (1997) identified the spontaneous incidence of LCTs in 2-year-old Sprague-Dawley rats as approximately 5%.

A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats at the 2-year sacrifice. The incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. The biological significance of this effect at the time of the initial evaluation was unknown, as there was no evidence of progression to tumors.

Slides of the ovaries from the Butenhoff et al. study (2012)—originally conducted from April 1981 through May 1983—were reevaluated by Mann and Frame (2004) with emphasis placed on the proliferative lesions of the ovary. Using more recently published nomenclature, the ovarian lesions were diagnosed and graded as gonadal stromal hyperplasia and/or adenomas, which corresponded to the diagnoses of tubular hyperplasia or tubular adenoma by the original study pathologist. The data are summarized in Table 3-29. No statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined were seen in treated groups compared to controls. There was some evidence of an increase in size of stromal lesions observed at the 300-ppm group; however, adenomas occurred in greater incidence in the control group than in either of the treated groups. Results of this follow-up evaluation indicated that rats sacrificed at the 1-year interim sacrifice, as well as rats that died prior to the interim sacrifice, were not considered at risk for tumor development.

Table 3-29. Incidence of Ovarian Stromal Hyperplasia and Adenoma in Rats

Group	0 ppm	30 ppm	300 ppm
No. examined	45	47	46
Hyperplasia (Total)	8	16	15
Grade 1	6	7	5
Grade 2	2	3	1
Grade 3	0	5	6
Grade 4	0	1	3
Adenoma	4	0	2
Adenoma and/or Hyperplasia	12	16	17

Source: Mann and Frame 2004

Mammary gland tumors also were observed in the Butenhoff et al. (2012) bioassay. In the original analysis of mammary tissues from female rats, the incidence of fibroadenoma of the mammary gland in the female 300-ppm group (48%) was greater than that in either of the concurrent control groups (22%). It also was similar to the incidence in the 30-ppm group (42%), but considered to be within the norm for background variation of this lesion in Sprague-Dawley

rats based on the published literature. As a result of questions raised about this conclusion, a pathology working group (PWG) was commissioned to review the female mammary tumor findings, blinded to treatment status, using current diagnostic criteria (Hardisty et al. 2010). Table 3-30 compares the original mammary gland tumor findings to those of the PWG.

Table 3-30. Mammary Gland Tumor Incidence Comparison

	0 ppm		30 ppm		300 ppm	
	Butenhoff	**Hardisty**	**Butenhoff**	**Hardisty**	**Butenhoff**	**Hardisty**
Number necropsied	50	50	50	50	50	50
Lobular hyperplasia (%)	6 (12%)	0 (0%)	3 (6%)	2 (4%)	2 (4%)	0 (0%)
Adenocarcinoma (%)	8 (16%)	9 (18%)	14 (28%)	16 (32%)	5 (10%)	5 (10%)
Fibroadenoma[a] (%)	10 (20%)	18 (36%)	19 (38%)	22 (44%)	21 (42%)	23 (46%)
Adenoma (%)	3 (6%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Source: Hardisty 2005; Hardisty et al. 2010
Notes:
[a] Includes fibroadenoma, multiple counts.

The principal differences between the original reported findings and the PWG results relate to changes in the mammary gland that were initially reported as lobular hyperplasia, which the PWG felt had features more characteristic of mammary gland fibroadenoma (Table 3-30). As a result, the numbers of rats with benign tumors (adenoma and fibroadenoma) were reclassified from 13 to 19 in the control group, from 19 to 22 in the 30-ppm group, and from 21 to 23 in the 300-ppm group. Although the incidence of neoplasms varied among the control and treated groups, there were no statistically significant differences when evaluated using the Fisher's exact test for pairwise comparison for fibroadenoma, adenocarcinoma, total benign neoplasms, and total malignant neoplasms. The morphologic appearance, overall incidence, and distribution of the neoplasms observed in treated and control groups were similar, resulting in a conclusion that they are not related to compound administration.

A 2-year mechanistic study in male Crl:CD BR (CD) rats (Biegel et al. 2001; Cook et al. 1992) resulted in liver tumors, LCTs, and PACTs. The rats (n = 156 per group) were fed diets containing 0 ppm (*ad libitum* control and control pair-fed to the PFOA-exposed rats) or 300-ppm PFOA (13.6 mg/kg intake). Rats were euthanized at interim time points of 1, 3, 6, 9, 12, 15, 18, and 21 months. All rats surviving the 24-month test period were necropsied for microscopic examination of various organs (e.g., kidneys, liver, testes, brain, heart, spleen). The incidence of liver adenomas in the *ad libitum* control, pair-fed control, and treated groups was 3% (2/80), 1% (1/79), and 13% (10/76), respectively. In the Butenhoff et al. study (2012), no hepatic adenomas were observed. The incidence for liver carcinomas was 0% (0/80), 3% (2/79), and 0% (0/76) in the *ad libitum* control, pair-fed control, and treated groups, respectively.

There was a significant increase in the incidence of Leydig cell adenomas in the treated rats—11% (8/76) when compared to the pair-fed control rats (3%, 2/78)—supporting the observations from the Butenhoff et al. study (2012). The incidence in *ad libitum* control rats was 0% (0/80). In addition, the treated group had a significant increase in the incidence of liver adenomas and pancreatic acinar cell adenomas when compared to the pair-fed and *ad libitum* control groups. The incidence for the pancreatic acinar cell adenomas was 0% (0/80) in the

treated rats, 1% (1/79) in the pair-fed control rats, and 9% (7/76) in the control rats. The incidence of pancreatic acinar cell carcinoma was 1% (1/76) in the treated rats, 0% (0/79) in the pair-fed control rats, and 0% (0/80) in the control rats.

In Butenhoff et al. (2012), there was no reported increase in the incidence of PACTs. However, the incidence of pancreatic acinar hyperplasia in the male rats was 0/33, 2/34, and 1/43 in the control, 30-, and 300-ppm groups, respectively. To resolve this discrepancy, the histological slides from both studies were reviewed by independent pathologists. This review of the microscopic lesions of the pancreas in the two studies indicated that PFOA produced increased incidence of proliferative acinar cell lesions of the pancreas in the rats of both studies at the dietary concentration of 300 ppm. The differences observed were quantitative rather than qualitative; more and larger focal proliferative acinar cell lesions and greater tendency for progression of lesions to adenoma of the pancreas were observed in the Biegel et al. study (2001) than in the Butenhoff et al. study (2012). The difference between pancreatic acinar hyperplasia (Butenhoff et al. 2012) and adenomas (Biegel et al. 2001) in the rat was a reflection of arbitrary diagnostic criteria and nomenclature by different pathologists. The basis for the quantitative difference in the lesions observed is not known, but was believed most likely to have been caused by the difference in the diets used in the two laboratories (Frame and McConnell 2003).

Mouse. Filgo et al. (2015) reported on tumor development in females from three strains of mice (CD-1, SV-139, and SV-129 PPARα knock-out [KO]) at 18 months with exposures that occurred only during development (gestation and lactation). The animals were from separate experiments initially carried out by EPA and published as Hines et al. (2009) and Abbott et al. (2007). The Filgo et al. (2015) analysis focused on the mature offspring from the earlier publications and was carried out at the National Institute for Environmental Health Sciences (NIEHS). Dosing regimens differed for the individual strains as did the doses and the number of animals per dose group. Some of the animals in the original studies had died before the 18-month sacrifice at NIEHS. After sacrifice, the livers were recovered for analysis. The tissue sections were reviewed by a team of board-certified veterinary pathologists. Table 3-31 summarizes the tumor results.

Table 3-31. Liver Tumors in Three Strains of Mice at 18 Months with Exposure to PFOA Only during Gestation and Lactation

Strain	0 mg/kg/day	0.01 mg/kg/day	0.1 mg/kg/day	0.3 mg/kg/day	0.6 mg/kg/day	1 mg/kg/day	3 mg/kg/day	5 mg/kg/day
	Number of Tumors / Total Number Tested Tumor Type							
CD-1	1/29 L	1/29 HCA	1/37 HCA	6/26 HCA(4), HCC, L	NT	2/31 HcyS, L	NT	6/21 HmS(2), HCA, HCC, HcyS, L
SV-129	0/10	NT	1/10 HcyS	0/8	0/6	0/8	NT	NT
SV-129-PPARαKO	0/6	NT	1/10 HCA	2/10 HCA. ICT	NT	1/9 ICT	2/9 HCA	NT

Notes:
HCA = hepatocellular adenoma, HCC = hepatocellular carcinoma, HcyS = histocytic sarcoma, HmS = hemangeosarcomas, ICT = Ito cell tumor, L = lymphoma, NT = not tested.

It is difficult to draw conclusions regarding the carcinogenicity of PFOA in mice based on the data collected because of the small number of animals evaluated in both studies of SV-129 mice and the lack of PFOA exposure between PND 21 and 18 months for all dose groups. As was the case for liver tumors in the Butenhoff et al. study (2012), there is a lack of dose-response for total liver tumors, although the four hepatocellular adenomas seen at 0.3 mg/kg/day in CD-1 mice were significantly greater ($p<0.05$) than the control. Tumor types varied across the dose groups. The authors also reported on preneoplastic basophilic, and eosinophilic foci were observed in the CD-1 mice but did not show a response to dose.

An interesting histological finding in both the CD-1 and SV-129 mice was a trend for increased Ito cell atrophy and lesion severity across the doses (Filgo et al. 2015). Since Ito cells accumulate fat in the liver sinusoids, this observation provides additional support for hepatic steatosis as a condition of concern following developmental PFOA exposure. There was an increase in severity with dose for the Ito cell fat deposits for all but the high-dose group. The Ito cell lesion was present in the SV-129 mice, but was not associated with tumors. CD-1 mice had a significant increase in Ito cell hypertrophy at 5 mg/kg/day compared to controls, but there was a lack of dose-response. The authors concluded that liver damage from PFOA exposure occurring early in development is not totally linked to PPAR-α and could progress as animals aged without continued dosing, thus compromising liver function and possibly leading to tumor development.

Inhalation and Dermal Exposures

No data on the tumorigenic effects of chronic inhalation or dermal exposures to PFOA were identified in the literature.

3.3 Other Key Data

3.3.1 Mutagenicity and Genotoxicity

PFOA has been tested for genotoxicity in a variety of *in vivo* and *in vitro* assays. The data from the *in vitro* studies are summarized in Table 3-32.

PFOA was tested in a cell transformation and cytotoxicity assay conducted in $C_3H10T_{½}$ mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry and Nelson 1981).

PFOA was tested twice (Lawlor 1995, 1996) for its ability to induce mutation in the *Salmonella – E. coli*/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen in *S. typhimurium* TA1537 when tested without metabolic activation was not reproducible. PFOA did not induce mutation in either *S. typhimurium* or *E. coli* when tested either with or without metabolic activation. PFOA did not induce chromosomal aberrations in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli 1996a; NOTOX 2000). Sadhu (2002) reported that PFOA did not induce gene mutation when tested with or without metabolic activation in the K-1 line of CHO cells in culture.

Table 3-32. Genotoxicity of PFOA *In Vitro*

Test System	End-point	With Activation	Without Activation	Reference
$C_3H10T_{½}$ mouse embryo fibroblasts	Cell Transformation	NA	-	Garry and Nelson 1981
$C_3H\ 10T_{½}$ mouse embryo fibroblasts	Cytotoxicity	NA	-	Garry and Nelson 1981
S. typhimurium TA1537	Gene Mutation	-	+ (not reproducible)	Lawlor 1995, 1996
E. coli	Gene Mutation	-	-	Lawlor 1995, 1996
CHO cells	Chromosomal Aberrations	+, +	+, -	Murli 1996b, 1996c
CHO cells	Polyploidy	+, +	+, -	Murli 1996b, 1996c
Human lymphocytes	Chromosomal Aberrations	-	-	Murli 1996c; NOTOX 2000
K-1 CHO cells	Gene Mutation	-	-	Sadhu 2002
S. typhimurium TA98, TA100, TA102, TA104	Gene Mutation	-	-	Freire et al. 2008

Note: NA= not applicable.

Murli (1996b, 1996c) tested PFOA twice for its ability to induce chromosomal aberrations in CHO cells. In the first assay, PFOA induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, PFOA induced significant increases in chromosomal aberrations and in polyploidy (Murli 1996b). The effects were observed only at toxic concentrations (EFSA 2008).

PFOA did not display mutagenic activity with or without metabolic activation in *S. typhimurium* strains TA98, TA100, TA102, or TA104 (Freire et al. 2008).

The data summarized in Table 3-32 suggest that PFOA is not a mutagen. A single positive result in *S. typhimurium* was not reproducible by the same authors and was not replicated in other studies. Potential chromosomal effects were found in CHO cells at toxic concentrations, but not in human lymphocytes.

Governini et al. (2015) collected semen samples from 59 healthy nonsmoking patients attending a Center for Couple Sterility conference at the University in Siena, Italy. The subjects were divided into those that were normozoospermic (13) and those that were oligoasthenoterato-zoospermic (46). PFOA was present in 75% of the seminal plasma samples and only 16% of the blood samples. Conversely, PFOS was present in 25% of the seminal plasma samples and 84% of the serum samples. Sperm were evaluated for the presence of aneuploidy and diploidy, and sperm DNA was evaluated for fragmentation using the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. The frequencies of aneuploidy and diploidy were significantly greater in the PFAS-positive samples than in the PFAS-negative samples ($P<0.001$ and $P<0.05$, respectively), suggesting the possibility for errors in cell division. The levels of fragmented chromatin were significantly increased ($P<0.001$) for the PFAS-positive group compared with the PFAS-negative group.

PFOA was tested twice in the *in vivo* mouse micronucleus assay. PFOA did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli 1995, 1996d).

G. Zhao et al. (2010) used A_L cells to determine the mutagenicity of PFOA to mammalian cells. A_L cells are a human-hamster hybrid containing CHO-K1 chromosomes and a single copy of human chromosome 11. The significance of human chromosome 11 is that it encodes for expression of the human cell surface protein CD59. A_L and mitochondria-deficient A_L cells were incubated with 0, 1, 10, 100, and 200 μmol PFOA for up to 16 days and used in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) viability, mutation, or caspace assays. Reactive oxygen species (ROS), nitric oxide, and superoxide anion production were measured in the cells, and the effects of ROS/reactive nitrogen species quenchers [0.5% dimethyl sulfoxide (DMSO) and 0.2 mM NG-methyl-$_L$-arginine, respectively] on mutagenicity and caspace activities were determined. At 100 and 200 μmol PFOA, A_L cell viability was significantly decreased after incubation for 1, 4, 8, and 16 days. CD59 mutation frequencies were increased in A_L cells after a 16-day incubation with 200 μmol PFOA. There was no increase in mutations in mitochondria-deficient A_L cells after incubation with 100 or 200 μmol PFOA.

Production of ROS, nitric oxide, and superoxide anion was significantly increased at 100 and 200 μmol PFOA after incubation of A_L cells for 1, 4, and 16 days. Incubation with DMSO to inhibit ROS production significantly decreased the CD59 mutation frequency caused by 200 μmol PFOA after the 16-day incubation. In contrast, mitochondria-deficient A_L cells had no increase in ROS or superoxide production after incubation with up to 200 μmol PFOA for 16 days.

To assess whether PFOA could induce the apoptotic pathway, caspase-3/7 and caspase-9 were examined in intact A_L cells (mitochondria-deficient cells were not examined). The highest concentration significantly increased caspase 3/7 and 9 activities after 1- and 4-day incubations. Incubation with 0.5% DMSO and 0.2 mM NG-methyl-$_L$-arginine significantly decreased the increased caspace activity induced by 200 μmol PFOA. The results led the authors to suggest that mitochondrial-dependent ROS might play an important role in PFOA-induced mutagenicity and that induction of caspase activities might be mediated by reactive oxygen and nitrogen species.

3.3.2 Immunotoxicity

The impact of PFOA on the immune system has been the subject of considerable research, primarily in mice. A number of the early studies by Yang et al. (2000, 2001, 2002a) used high-dose exposures of 0.02% to 0.05% PFOA. Later studies by DeWitt et al. (2008, 2009, 2015) and Loveless et al. (2008) used a range of doses from < 1 mg/kg/day to 30 mg/kg/day. Most of the studies focused on responses associated with the spleen and thymus. Some of the effects observed were PPARα-associated, but others are totally or partially independent. There is evidence for full or partial reversal of effects in those studies that incorporated a recovery phase. One study of immunotoxicity used the dermal route of exposure (Fairley et al. 2007).

Rat. Loveless et al. (2008) administered 0, 0.3, 1, 10, and 30 mg linear PFOA/kg by oral gavage to groups of male CD rats (n = 10 per group) for 29 days. The animals received a dose of SRBC on day 23. A separate group of high-dose rats were injected with water instead of SRBC. Rat body weight was recorded on days 0, 3, and 6–28. At necropsy, blood was collected for evaluation of immune system parameters. Cell counts were determined for the thymus and

spleen. Total spleen and thymocyte cell counts and organ weights in exposed rats were comparable to control. Microscopic examination of the thymus, mesenteric lymph nodes, and popluteal lymph nodes revealed no effects in treated rats resulting from PFOA exposure. There was no difference observed in immunoglobulin (IgM) titers between treated and control rats. The immunological NOAEL was 30 mg/kg/day.

Mouse. Yang et al. (2000, 2001, 2002a, 2002b) completed a series of studies investigating the immunotoxic effects of PFOA. In the first study, Yang et al. (2000) examined the liver, spleen, and thymus effects of several known PPARα activators, including PFOA. The researchers administered 0.02% PFOA (~40 mg/kg/day) to male C57BL/6 mice in the diet for 2, 5, 7, and 10 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were weighed. Administration of PFOA resulted in a significant increase in liver weight relative to control even at day 2. Following 5 days of administration, significant decreases in thymus and spleen weight were noted.

A second component of the Yang et al. study (2000) examined the effect of 0.02% PFOA in the diet on the cellularity, cell surface phenotype, and cell cycle of thymocytes and splenocytes. After 7 days, significant decreases in the total number of thymocytes (85%) and splenocytes (80%) were observed. There is a pattern to the development of thymocytes that should be considered when evaluating the impact of chemicals on their differentiation. Early thymocytes formed in the bone marrow do not express CD4 or CD8 ($CD4^{-}CD8^{-}$). In the thymus, they differentiate and express both CD4 and CD8 ($CD4^{+}CD8^{+}$). They also undergo proliferation and downregulation of either the CD4 or CD8 protein expression to become either a CD4 or CD8 thymocyte (Yang et al. 2000). Following exposure to PFOA, the number of thymocytes expressing neither CD4 nor CD8 decreased by 57%; the number expressing both CD4 and CD8 decreased by 95%; the number expressing only CD4 decreased by 64% while those expressing only CD8 decreased by 72%. As detected by cell cycle flow cytometry analyses, thymocyte proliferation was inhibited based on the number of cells in each stage of the cell cycle.

T-cells ($CD3^{\pm}$) and B-cells ($CD19^{\pm}$) in the spleen decreased by 75% and 86%, respectively. Splenic T-cells are lymphocytes produced in the thymus that carry the $CD3^{+}$ surface protein marking them as T-cells for exportation to the spleen. There are several classes of T-cells that are characterized by surface proteins. Yang et al. (2000) found significant decreases in helper $CD3^{+}$T-cells with $CD4^{+}$ surface proteins (78%) and cytotoxic $CD3^{+}$T-cells with $CD8^{+}$ surface proteins (74%). The authors suggested that, unlike the $CD3^{+}$T-cells that originate in the thymus, the decrease in $CD19^{+}$B-cells of the spleen reflects decreased differentiation and maturation in the bone marrow where they are formed.

In the final phase of the Yang et al. study (2000), the effects of *in vitro* exposure of thymocytes and splenocytes to PFOA were examined. The *in vitro* studies showed spontaneous apoptosis occurring in splenocytes and thymocytes after 8 or 24 hours of culturing in the presence of varying concentrations of PFOA (50, 100, and 200 μmol). However, under the exposure conditions, PFOA did not appear to significantly alter the cell cycle. The only dose tested (~40 mg/kg/day) was a LOAEL for its effects on the immunoactive products of the thymus and spleen. Recovery can occur with the cessation of exposure as illustrated by the Yang et al. study (2001) described below.

Yang et al. (2001) reported on their examination of the immunosuppressive effects of PFOA. As was the case in their earlier publication (Yang et al. 2000), the 2001 report includes several components. A diet of 0.02% PFOA (~40 mg/kg/day) was fed to C57BL/6 mice for 2–10 days.

One group of animals was exposed to PFOA each day until day of sacrifice on days 2, 5, 7, and 10. At sacrifice, body, liver, and spleen weights were recorded. A second group of animals was dosed according to the same schedule, but dosing ceased after day 7, and the animals were fed normal diets for 2–10 days to monitor recovery from the effects of exposure. In the recovery group, animals were sacrificed after 2-, 5-, and 10-day recovery periods.

The mice that received 0.02% PFOA for up to 10 days experienced significant increases in liver weight compared to controls beginning at day 2. Significant decreases in thymus and spleen weights were observed starting on day 5. Body weight increased for the first 2 days of the study and decreased continuously for the remainder of the exposure period. The activity of palmitoyl-CoA and lauryl-CoA, biomarkers for PPARα activation and peroxisome proliferation, also were increased significantly and increasingly across the exposure period. The impact of PFOA exposure was similar to that observed in the Yang et al. study (2000). After administration for 7 days, the number of thymocytes expressing neither CD4 nor CD8 decreased by 65% following exposure to PFOA; the number expressing both CD4 and CD8 decreased by 95%; and the number expressing either CD4 or CD8 decreased by 65% and 75%, respectively. T-cell ($CD3^+$) splenocytes and B-cell ($CD19^+$) splenocytes decreased by 65% and 75%, respectively. As detected by cell cycle flow cytometry analyses, thymocyte but not splenocyte proliferation was inhibited.

The animals that participated in the recovery portion of this study rapidly regained their body weight starting on the second day after withdrawal of PFOA. However, the liver weight failed to recover even after 10 days. Thymus weight recovery started on day 2 and was completed by day 10. The spleen weights returned to normal by day 2 post-withdrawal. The increases in thymus and spleen weight during recovery were paralleled by increases in total thymocyte and splenocyte counts. Thymocyte recovery was apparent on day 5 and complete by day 10, although during the first two days of the recovery period, further decreases in the $CD4^+CD8^+$, $CD4^+$ and $CD8^+$ cells were observed. Flow cytometry evaluation of the distribution of the cells across the cell cycle in the recovery group animals demonstrated increases in cell proliferation following removal of PFOA from the diet. However, final cell counts did not reach the control values for the thymocyte ($CD4^+$ and $CD8^+$) or splenocyte ($CD3^+$ and $CD19^+$) populations evaluated.

In the second component of the Yang et al. study (2001), C57BL/6 mice were administered diets consisting of 0.001%–0.05% PFOA (w/w) for 10 days. These doses are equivalent to approximately 2.0–100 mg/kg/day. There was a dose-related decrease in spleen and thymus weights and a dose-related increase in liver weights accompanied by a corresponding increase of palmitoyl-CoA and lauryl-CoA activity. Enzyme activity was significantly increased for all doses. Spleen and thymus weights were significantly decreased at doses ≥ 0.01% and higher but not at the lower doses; the increases in liver weights were significantly increased for the 0.02% and 0.05% doses. With the testing of a broader range of doses, ~20 mg/kg/day was found to be a LOAEL for effects on the thymus and spleen and the ~6 mg/kg/day dose a NOAEL.

Yang et al. (2002a) examined the possible involvement of PPARα in the immunomodulation exerted by PFOA. This study made use of transgenic PPARα-null mice (Sv/129), which are homozygous with regards to a functional mutation in the PPARα gene. These mice do not exhibit peroxisome proliferation or hepatomegaly and hepatocarcinogenesis even after exposure to peroxisome proliferators. The mice were fed a diet consisting of 0.02% PFOA (w/w) (~40 mg/kg/day) for 7 days. At the end of the feeding period, mice were sacrificed and the liver, spleen, and thymus were removed and weighed. The effect of PFOA on peroxisome proliferation, cell cycle, and lymphoproliferation was ascertained.

The results showed that, in contrast to wild-type mice, feeding PPARα-null mice PFOA resulted in no significant decrease in body weight. Liver weight in PPARα-null mice fed the PFOA diet was significantly increased when compared to control PPARα-null mice, but not when compared to wild-type PFOA-exposed mice. Peroxisome proliferation, as measured by fatty acid oxidation, was totally lacking in PPARα-null mice. Also, in contrast to wild-type mice, feeding PPARα-null mice PFOA resulted in no significant decrease in the weight of the spleen or the number of splenocytes.

There was a decrease in weight and cellularity of the thymus in the PPARα-null mice compared to the PPARα-null control mice, but it was not as dramatic as that in the PFOA-exposed wild-type mice. In addition, the decreases in the size of the CD4+CD8+ population of thymus cells and the number of thymus cells in the S and G2/M phases of the cell cycle were lower in PPARα-null mice than they were in the PFOA-exposed wild-type mice, but higher than in the PPARα-null control mice. PFOA treatment caused no significant change in splenocyte proliferation in PPARα-null mice in response to mitogen exposure, but did show a response in the PFOA-exposed wild-type mice as described above.

The series of studies published by Yang et al. (2000, 2001, 2002a) link many of the effects of the liver, thymus, and spleen in PFOA-exposed mice to the activation of PPARα. However, there were some impacts on the thymus and liver that were independent of PPARα receptor activation. PPARα-null mice still showed increases in liver weight and effects on the thymus (small decrements in organ weight, thymocyte cellularity, and proliferative cell cycle) following a 7-day exposure to approximately 40 mg/kg/day PFOA that were independent of PPARα.

Yang and colleagues extended their studies of the immunotoxicity of PFOA in a feeding study designed to examine the effects of PFOA on specific humoral immune responses in mice (Yang et al. 2002b). For this study, 0.02 % PFOA was administered to male C57BL/6 mice for 10 days. The animals were then evaluated via plaque-forming cell (PFC) and serum antibody assays for their ability to generate an immune response to horse red blood cells (HRBCs). *Ex vivo* and *in vitro* splenic lymphocyte proliferation assays also were performed. The results showed that mice fed normal chow had a strong humoral response to challenge the HRBCs, as measured by the PFC assay. In contrast, mice fed PFOA responded to HRBC immunization with no increase in HRBC-specific PFCs, relative to unimmunized controls. However, in experiments where PFOA-treated mice received normal chow following HRBC immunization, there was a significant recovery of the numbers of specific PFCs stimulated. The suppression of the humoral immune response by PFOA was confirmed by analysis of the serum anti-HRBC response.

In *ex vivo* experiments, splenocytes isolated from control mice responded to both concanavalinA (ConA) and lipopolysaccharide (LPS) with lymphocyte proliferation, as measured by thymidine incorporation. However, treating mice with 0.02% PFOA for 7 days attenuated the proliferation. In a set of *in vitro* experiments, PFOA (1–200 μmol) added to the culture medium of splenocytes cultured from untreated mice did not cause an alteration of lymphocyte proliferation in response to LPS or ConA.

DeWitt et al. (2008) expanded the repertoire of studies of the immunological effects of PFOA by examining various aspects of humoral (antibody production) and cellular immunity. The first component of their publication had many similarities with the Yang et al. study (2001). Adult female C57BL/6J mice (n = 40 per endpoint and 8 per group) were exposed to a single daily dose of 30 mg PFOA/kg/day in distilled water by gavage for 10 continuous days. After 10 continuous days of exposure, half of the mice continued receiving PFOA from day 11 through

day 15 (constant group) while the other half received distilled water from day 11 through day 15 (recovery group). On day 11, 16 mice per group were immunized with sheep red blood cells (SRBC) and eight mice per group were injected with BSA. Sacrifices took place on day 16 (1 day postexposure period) and day 31 (15 days postexposure period). Vehicle and cage controls also were included in the study. All groups were monitored for the following effects:

- Body weight and organ weights (day 16, day 31)
- Serum IgM levels (day 16)
- Delayed-type hypersensitivity (DTH) foot-pad response to BSA (day 26)
- Serum IgG levels after booster immunization with SRBC on day 20 (day 31)

The results for body and organ weights were comparable to those in the Yang et al. study (2001). Body weight was significantly decreased from days 8–11 for both PFOA-treated groups and on day 16 for mice in the constant exposure group. By day 31, there were no body weight differences between the groups. Relative liver weight was significantly elevated in both PFOA-treated groups on days 16 and 31. Absolute and relative spleen and thymus weights of animals in both PFOA groups were significantly decreased compared to control groups on day 16. By day 31, thymus and spleen weights were not statistically different between control and treated mice. IgM levels following immunization with SRBC were reduced by up to 20% compared to controls on postexposure day 1 in both the recovery and constant exposure groups. There were no significant differences from controls for SRBC-specific IgG levels and for DTH foot-pad responses to the BSA challenge.

The C57BL/6 mice used for the continuous-dosing versus recovery component of the DeWitt et al. study (2008) were found to develop ulcerative dermatitis following the PFOA exposure. It was determined that this effect was a genetic susceptibility in the strain, and they were not used for the dose-response component of the study; the C57BL/6N strain was used in its place.

Two studies of dose-response were included in the DeWitt et al. (2008) publication. Groups of 16 female C57BL/6N mice were given 0, 3.75, 7.5, 15, and 30 mg PFOA/kg/day in the drinking water for 15 days during the first experiment. In the second experiment, the doses were 0, 0.94, 1.88, 3.75, and 7.5 mg PFOA/kg/day administered for 15 days in the drinking water. The immunological sensitization and postdose monitoring were identical to that used in the constant-dosing versus recovery experiment.

In the first experiment, body weight was significantly decreased from day 8–16 at 30 mg/kg PFOA and on day 16 at 15 mg/kg PFOA. As observed previously, liver weights were significantly elevated at day 16 and day 31 at all doses. Absolute and relative spleen and thymus weights were significantly decreased at ≥15 mg/kg PFOA on day 16. With the exception of the absolute thymus weight at 15 mg/kg PFOA, all spleen and thymus weights were similar to weights in controls 15 days after dosing. The IgM response to SRBC was significantly reduced at ≥3.75 mg/kg PFOA in a direct dose-related manner. The IgG response to SRBC challenge was slightly but significantly elevated at 3.75 and 7.5 mg/kg PFOA but similar to that of the control level at the higher doses. Thus, there was a direct response of IgM, but not IgG, to dose across the dose levels. There was no significant change in the DTH response at any dose. The LOAEL from the first experiment was 3.75 mg/kg/day dose based on decreased IgM and increased IgG response to SRBC immunization and increased liver weights ($p<0.05$).

The second dose-response experiment confirmed the 3.75 mg/kg/day dose as the immunological LOAEL on the basis of significantly decreased spleen weight, decreased IgM

levels on day 16, and increased IgG levels on day 31. The immunological NOAEL was 1.88 mg/kg/day. BMD analysis of IgM serum titer data gave a lower bound 95% confidence limit of 1.75 mg/kg/day on a BMD (one SD) of 3.06 mg/kg/day. Liver weight was significantly increased at all doses on days 16 and 31. The LOAEL for increased liver weight was 0.94 mg/kg PFOA.

As mentioned earlier, some of the immunological responses observed in the studies of immunotoxicity are linked to PPARα activation by PFOA. DeWitt et al. (2015) published results for a study in female PPARα KO mice (B6.129S4-*Ppar*^{tm1Gonz}N12 mice) and compared them to the response of female C57BL/6-Tac wild-type mice. Both T-cell-dependent and T-cell-independent antibody production were evaluated. The doses used in the study of the T-cell-dependent responses were 0, 7.5, and 30 mg PFOA/kg/day dissolved in deionized drinking water for 14 days. On day 11, the animals were injected with SRBCs to stimulate an immune response. PFOA dosing continued for 4 more days (15 days dosed); the following day, the animals were sacrificed. Body weight was significantly decreased only in wild-type mice at 30 mg/kg/day. Relative spleen weights were significantly decreased ($P<0.05$) in the wild-type but not the KO mice at 30 mg/kg/day of PFOA. Relative thymus weights were significantly decreased in the wild-type mice at 7.5 mg/kg/day, but not in the KO mice at either dose or the wild-type mice receiving 30 mg/kg/day. There was a significant ($P<0.05$) reduction in the IgM antibody response to the SRBC injection at 30 mg/kg/day for both the wild-type and KO animals ($n = 6$), indicating that the response was not totally related to PPAR-α activation. The NOAEL in wild-type and KO animals was 7.5 mg/kg/day and the LOAEL 30 mg/kg/day based on decreased T-cell-dependent IgM antibody response to SRBC.

To evaluate T-cell-independent responses to PFOA, groups of eight C57BL/6N mice were given doses of 0, 0.94, 1.88, 3.75, and 7.5 mg/kg/day in their drinking water. On day 11, they were injected with the T-cell-independent antigen dinitrophenyl ficol. At sacrifice (day 16), blood was collected for analysis of IgM antibody titers. There was a significant decrease ($p<0.05$) in the antibody response by 9.4–10.7% in the animals receiving doses ≥ 1.88 mg/kg/day of PFOA. The NOAEL for the T-cell-independent response to dinitrophenyl ficol was 0.94 mg/kg/day of PFOA and the LOAEL was 1.88 mg/kg/day. Thus, suppression of the T-cell-independent response occurred at a lower dose (1.88 mg/kg/day) than the dose resulting in suppression of the T-cell-dependent response (7.5 mg/kg/day).

The authors looked at changes in lymphocyte populations at 10, 13, and 15 days of exposure in the female C57BL/6N mice and saw no significant dose-dependent changes in lymphocyte cell types. They concluded that both sets of responses were due to changes in cellular function rather than lymphocytotoxicity (DeWitt et al. 2015).

Loveless et al. (2008) administered 0, 0.3, 1, 10, and 30 mg linear PFOA/kg by oral gavage to groups of male CD-1 mice ($n = 20$ per group) for 29 days. The animals received a dose of SRBC on day 24. A separate group of high-dose mice was injected with water instead of SRBC. Mice were weighed daily. At necropsy, blood was collected for evaluation of immunity parameters. Cell counts were determined for the thymus and spleen.

Absolute and relative spleen and thymus weights were significantly decreased at ≥10 mg/kg/day. The relative spleen weight of mice dosed with 1 mg/kg/day also was significantly decreased compared to control animals. Spleen and thymus cell counts were significantly decreased and minimal to severe lymphoid depletion/atrophy of the thymus was observed at ≥10 mg/kg/day. IgM titers were significantly decreased at ≥10 mg/kg/day. Serum

CORT was significantly increased at 10 mg/kg/day and elevated (not statistically significant) at 30 mg/kg/day. When IgM and CORT were plotted against each other, a negative correlation coefficient suggested that increasing CORT levels decreased the ability to make SRBC antibody. The LOAEL was 1 mg/kg/day based on decreased spleen weight, and the NOAEL was 0.3 mg/kg/day. Mice appeared to be more susceptible than rats to immunosuppression from PFOA.

Loveless et al. (2008) hypothesized that at least a portion of the thymic response to PFOA might be related to physiological stress and increased levels of CORT hormones. DeWitt et al. (2009) investigated this hypothesis by comparing the immunological response of adrenalectomized (ADX) C57BL/6N female mice to that of sham-operated female mice from the same strain. The animals were dosed with 0, 3.75, 7.5, and 15 mg PFOA/kg/day in the drinking water for 10 days. Body weight was recorded on dosing days 0, 4, and 8, plus 2 and 5 days postdosing. On exposure days 5 and 10, blood and serum were collected for analysis of a broad array of clinical chemistry parameters, including activity of liver enzymes indicative of cellular damage (e.g., ALP, AST, ALT, LDH, GGT, and SDH), serum lipids (cholesterol and triglycerides), and CORT. A baseline measure of CORT was determined from serum samples collected before dosing began. One day after cessation of exposure, the mice were immunized with SRBC. Four days later, serum was collected and the levels of CORT and IgM were determined.

Body weight in the sham-operated mice declined during dosing in the highest dose group but recovered by 5 days postdosing. In the ADX mice, body weight declined during dosing at 7.5 and 15 mg PFOA/kg/day, but recovered in mice receiving 7.5 mg PFOA/kg/day by 5 days postdosing. There were significant increases in ALT, AST, LDH, and SDH at the highest dose for the ADX mice indicative of damage to hepatic cell membranes (Table 3-33).

Serum levels of triglycerides significantly decreased compared to controls, with all doses for the sham-operated mice on day 5 of dosing but only for the 7.5- and 15-mg/kg/day doses in the ADX mice. Cholesterol levels were significantly decreased ($p<0.05$) in the sham-operated mice at the highest dose, but no differences in cholesterol levels were observed in the ADX mice.

Table 3-33. Selected Clinical Chemistry Parameters in Mice Treated with PFOA for 5 Days

Dose (mg/kg/day)	ALT	AST	LDH	SDH
Sham-Operated				
0	39.52±2.50	121.56±17.96	320.57±29.84	46.43±1.03
3.75	43.88±0.93	104.07±10.24	293.92±68.65	39.31±3.32
7.5	56.96±6.78	95.55±10.22	262.71±35.60	39.02±7.77
15	62.57±3.15	89.07±1.30	191.76±22.25	46.87±1.46
ADX				
0	26.96±1.78	73.53±4.70	176.50±19.32	33.05±1.58
3.75	29.67±1.62	76.58±3.38	222.69±19.18	37.95±2.35
7.5	39.04±2.59	83.79±8.94	320.45±53.34	46.35±1.42
15	94.23±31.66*	126.47±16.39*	435.57±81.42*	77.61±19.89*

Source: DeWitt et al. 2009
Note: * = $p<0.05$ versus corresponding sham control or ADX control group.

After 10 days, there were no significant differences in liver enzymes for the ADX or sham mice. However, there was a dose-related trend towards increased levels of liver enzymes for the PFOA-exposed sham-operated animals and for LDH in the PFOA-exposed ADX animals (Table 3-34).

Table 3-34. Selected Clinical Chemistry Parameters in Mice Treated with PFOA for 10 Days

Dose (mg/kg/day)	ALT	AST	LDH	SDH
Sham-Operated				
0	51.51±14.62	93.30±6.33	333.48±86.86	54.60±16.72
3.75	79.26±33.87	123.73±15.20	404.14±59.89	45.50±10.15
7.5	135.57±38.18	142.66±15.59	490.44±69.14	80.71±14.59
15	344.53±235.63	242.92±117.62	595.01±137.37	89.20±26.03
ADX				
0	128.22±24.80	106.00±8.86	236.96±30.23	61.88±8.87
3.75	282.23±193.54	217.10±3.48	379.61±80.67	68.78±24.88
7.5	89.79±21.54	99.78±12.59	574.65±236.38	52.07±11.98
15	261.14±75.95	181.40±32.94	614.05±144.95	101.93±24.00

Source: DeWitt et al. 2009

At the end of dosing, corticosteroid levels in the sham-operated animals were greatly elevated compared to the levels in the control animals at all doses, and the difference was statistically significant at the highest dose. By 5 days postdosing, the CORT levels had declined for all doses but were still elevated compared to controls for the 7.5- and 15-mg/kg/day groups. In the animals lacking their adrenal glands, there were no statistically significant differences in the hormone levels. IgM levels were significantly lower than controls at the highest dose for the sham-operated animals and at the two highest dose groups for the ADX mice. However, when comparing the sham mice to the ADX mice, the only significant difference in IgM was found for the 7.5-mg/kg/day animals. On the basis of data, it appears that stress-related CORT production did not have a major impact on the IgM response to the SRBC inoculation.

Son et al. (2009) administered 0, 2, 10, 50, and 250 ppm PFOA (equivalent to 0, 0.49, 2.64, 17.63, and 47.21 mg/kg PFOA) in the drinking water to 4-week-old male ICR mice for 21 days to determine if PFOA alters T lymphocyte phenotypes and cytokine expression in mice. The spleen, thymus, and trunk blood were collected at sacrifice. Sections of the spleen and thymus were processed for histological examination. Splenic and thymic expression of mRNA from proinflammatory cytokines—including tumor necrosis factor-α, interleukin-1β, and interleukin-6, and the proto-oncogene c-myc—were analyzed using RT-PCR. Flow cytometry was used to phenotype the splenic and thymic lymphocyte populations.

Spleen and thymus weights were slightly decreased in mice treated with PFOA. Enlargement with marked hyperplasia of the white pulp and increased cellular density of the lymphoid follicles were observed in spleens at 250 ppm. In the thymus, decreased cortex and medulla thickness and densely arranged cortex lymphoid cells were observed at 250 ppm. Tumor necrosis factor-α, interleukin-1β, interleukin-6, and c-myc expression were significantly elevated at 250ppm in the spleen. Interleukin-1β expression also was elevated at 50 ppm in the spleen. In the thymus, c-myc expression was significantly elevated by treatment with 50 and 250 ppm PFOA.

The splenic and thymic lymphocyte population was altered by PFOA treatment, as shown in Table 3-35. A 50% decrease was observed in splenic $CD8^+$ lymphocytes at all PFOA doses, and increases in splenic $CD4^+$ lymphocytes of 43% and 106% at 50 and 250 ppm PFOA, respectively, were observed. In the thymus, a 110% increase was observed in thymic $CD8^+$ lymphocytes at 250 ppm, but thymic $CD4^+$ lymphocyte populations were not affected and $CD4^+CD8^+$ populations were decreased at 50 and 250 ppm PFOA. The lowest dose tested (0.49 mg/kg/day) was a LOAEL for $CD4^-$ and $CD8^+$ splenocytes.

Table 3-35. Impact of PFOA on Splenic and Thymic Lymphocyte Populations

	Dose (mg/kg/day)			
Spleen	0.49	2.64	17.63	47.21
$CD4^-CD8^-$	↑	-	-	↓
$CD4^+CD8^-$	-	-	↑	↑
$CD4^-CD8^+$	↓	↓	↓	↓
$CD4^+CD8^+$	↓	↓	-	-
Thymus				
$CD4^-CD8^-$	-	-	-	↑
$CD4^+CD8^-$	-	-	-	-
$CD4^-CD8^+$	-	-	-	↑
$CD4^+CD8^+$	-	-	↓	↓

Source: Son et al. 2009
Notes:
↑ Significantly increased compared to control ($p < 0.05$).
↓ Significantly decreased compared to control ($p < 0.05$).
- Not significantly different from control.

Qazi et al. (2009) investigated the impact of PFOA on the innate immune system. Adult male C57BL/6 ($H\text{-}2^b$) mice were administered 0.001% and 0.02% PFOA (~2 or 40 mg/kg) in the diet (w/w) for 10 days. After the last dose, all mice were sacrificed. Sacrifice was delayed for a subset of the animals until 2 hours after they had received an LPS injection to stimulate an immunological response. Blood, peritoneal exudate cells, liver, epididymal fat, spleen, thymus, and bone marrow were recovered. The blood, peritoneal exudate, bone marrow, and spleen were evaluated for total and differential white blood cell counts and concentrations of tumor necrosis factor (TNF-α) and interleukin 6 (IL-6).

Consistent with other studies of the 0.02% dose, there was a significant increase in liver weight after the 10-day exposure. Body weight, thymus weight, spleen weight, and epididymal fat depots were decreased. Food consumption in these animals was reduced by 35%, which might have played a role in the reduced body, organ, and tissue weights. Compared to the controls, there was a significant decrease in total white cells, lymphocytes, and neutrophils at 0.02% PFOA. This same dose was associated with a decrease in total white cell count in bone marrow and spleen, and an increase in the proportion found as macrophages in the bone marrow, spleen, and peritoneal cavity. Although the total number of macrophages was not reduced in the peritoneal cavity and spleen, it was reduced in the bone marrow. The increase in the proportion of macrophages reflects a decrease in other white cell populations. There was significant increase in the concentration of IL-6 in all of the 0.02 % dosed animals, but only those receiving the LPS injections showed a significant increase in TNF-α. The 0.001% dose (about 2 mg/kg/day) was a NOAEL.

The data available on immunological responses of animals following oral exposure to PFOA are extensive, especially as they apply to mice. A number of the studies used doses of about 40 mg/kg/day. However, studies conducted at lower doses find effects on the spleen and/or thymus at doses from 0.5 to 2 mg/kg/day. Activation of PPARα appears to augment the response based on studies in PPARα-null mice but is not necessary (Yang et al. 2002a). There are differences between mice and rats with mice showing a response at a lower dose (Loveless et al. 2008). Cessation of dosing can reverse some of the observed effects in mice (Yang et al. 2001).

Inhalation Exposure

No data on the effects of inhalation exposure on immunological endpoints were identified in the literature.

Dermal Exposure

Fairley et al. (2007) carried out a complex study of toxicity and respiratory hypersensitivity to ovalbumin (OVA) as impacted by dermal exposure to PFOA dissolved in acetone compared to acetone alone. There were several phases to the study. In the first phase, a range-finding study, PFOA was applied to each ear of female BALB/c mice (n = 5 per group) at doses of 0, 0.01%, 0.1%, 0.25%, 0.5%, 1.0%, and 1.5% PFOA (equivalent to 0, 0.25, 2.5, 6.25, 12.5, 25, and 50 mg/kg/day) for 4 days. Six days after last inoculation, the animals were sacrificed. The liver, spleen, and thymus were recovered and weighed. A significant increase in liver weight was observed at ≥6.25 mg/kg PFOA. Spleen weight was significantly decreased in mice dosed with 25 mg/kg and 50 mg/kg PFOA, and thymus weight was significantly decreased in mice at the highest dose ($p<0.05$). The cell counts in the spleen were significantly decreased compared to control at all doses and for the highest two doses in the thymus. The LOAEL was 6.25 mg/kg/day based on a statistically significant increase in liver weight ($p<0.01$), and the NOAEL was 2.5 mg/kg/day.

In the second phase of the Fairley et al. study (2007), groups of 5–15 animals were dosed dermally on the ears for 4 days with doses of 0, 0.5%, 0.75%, 1.0%, and 1.5% PFOA (equivalent to 0, 12.5, 18.75, 25, and 50 mg/kg/day). On days 1 and 10, they were injected i.p. with either 2.0 mg alum or 7.5 μg OVA and 2.0 mg alum in a phosphate-buffered saline solution (100μL). Four days after the last inoculation, the animals were sacrificed and blood was collected by cardiac puncture. Liver, spleen, and thymus were recovered and weighed; spleen and thymus cellularities were determined. A significant ($p<0.01$) increase in liver weight and decrease in spleen weight and spleen cellularity occurred at all doses. Thymus weight and cellularity were significantly decreased ($p<0.01$) at ≥18.75 and ≥ 25 mg/kg/day, respectively. There were no significant differences in the $CD4^+$, $CD8^+$, $CD4^-8^-$, or CD3e T-cells. CD3e protein is expressed by both thymocytes and mature T-cells.

Levels of IgE and OVA-specific IgE were measured in the control and dosed animals by enzyme-linked immunosorbent assay. IgE is the immunoglobulin that is best correlated with respiratory allergic responses. It functions to stimulate mast cells and basophils to release histamine and other mediators of inflammation (Saladin 2004). The IgE response was increased in a dose-related fashion compared to the OVA control for all the PFOA-treated animals; the increase was significant ($p<0.05$ or 0.01) at doses ≥18.75 mg/kg/day. The OVA-specific IgE response did not demonstrate a direct response to dose, but there was a significant increase ($p<0.05$) for the 18.75- and 25-mg/kg/day groups. The OVA-specific response for the three highest dose groups was practically indistinguishable from the OVA control.

The dermal LOAEL was 12.5 mg/kg/day based on increased liver weight and decreased spleen weight and cellularity. No NOAEL was established.

In the third part of the Fairley et al. study (2007), mice (n = 5) were dosed dermally via their ears for 4 days as described above (0, 12.5, 18.75, 25, and 50 mg/kg/day). On days 19 and 26 after the start of dosing, they were challenged by pharyngeal aspiration of 250 μg OVA in the phosphate-buffered saline vehicle and sacrificed 24 hours after the last challenge. There was a dose-related decrease in number of splenocytes carrying the $B220^{+}$ marker (expressed on B-cells, activated B-cells, and subsets of T- and natural killer- [NK-] cells) compared to the OVA controls. The changes were significantly different for the 25-mg/kg/day ($p<0.05$) and 37.5-mg/kg/day ($p<0.01$) groups.

After the day 19 challenge, the mice (n = 5) were placed in a plethysmography chamber for measurement of enhanced pause airway respiration (PenH) values. PenH values reflect volume of air in the lungs. Once in the chamber, they were challenged with nebulized methacholine for 3 mins followed by 2 mins of fresh air. The PenH measures were recorded every 30 seconds over the next 5 hours. The area under the plasma concentration time curve (AUC) for the PenH measures was determined after correction for baseline (acetone control, no OVA or PFOA). An AUC of 1.6 was considered to be a positive response. Twenty-four hours later, blood was drawn from the abdominal artery and the mice were sacrificed. The lungs were recovered for histological analysis. An increase in antigen-specific hyperactivity response to PFOA, in both the PenH values and the number of animals responding, was observed at doses up to about 25 mg/kg/day. The PenH AUC was significantly ($p<0.05$) increased in mice treated with 25 mg/kg/day PFOA and OVA compared to the OVA control mice, but there was no significant difference between the OVA control and the animals exposed to 50 mg/kg/day PFOA and OVA. The LOAEL for the PenH response was 25 mg/kg/day, and the NOAEL was 18.75 mg/kg/day.

Histopathological evaluation of the lungs revealed macrophage and eosinophil infiltration in response to the challenge with 250 μg OVA by pharyngeal aspiration. The severity of the response increased with increasing concentrations of PFOA. Eosinophils and macrophages were found in the interstitial, peribronchiole, and perivascular areas. Neutrophils, lymphocytes, and some multinucleated giant cells also were observed. Increased secretory matter, sloughing of epithelium, and secretory cell necrosis were observed in mice exposed to all concentrations of PFOA and OVA. The response was not observed in the mice exposed to only PFOA. The authors concluded that dermal exposure to PFOA was immunotoxic and enhanced the airway hypersensitivity response to OVA suggesting that PFOA may augment the IgE response to environmental allergens.

In vitro. In a pilot study, Brieger et al. (2011) examined the effects of PFOA on human leukocytes. Peripheral blood mononuclear cells (PBMCs) were obtained from the blood of 11 voluntary donors (n = 6 females, 5 males). PBMCs were incubated with varying concentrations of PFOA followed by assays for cell viability, proliferation, and NK cell activity. The human promyelocytic leukemia cell line, HL-60, was used in cell viability and monocyte differentiation assays. The various components of the assays employed are identified as follows:

- In the cell viability assay, the PBMCs were incubated with 0–500 μg/mL for 24, 48, and 72 hours, and HL-60 cells were incubated with 0–125 μg/mL PFOA for 24 hours.
- In the proliferation assay, the PBMCs were incubated with 0–100 μg/mL PFOA for 24 hours, labeled with 6-carboxyfluorescein succinimidyl ester (CFSE), stimulated with

ConA, a T-cell mitogen (5 μg/mL to half of all samples), and incubated for an additional 72 hours.

- For the NK assays, PBMCs were incubated with 0–100 μg/mL PFOA for 24 hours followed in incubation of 3 hours with K562 target cells (12.5:1 ratio) labeled with CFSE.
- In the monocyte differentiation assay, HL-60 cells were incubated with 0–100 μg/mL PFOA for 72 hours. Half of each sample was stimulated with 25 nmol calcitrol, 1α,25-dehydroxyvitamin D3 ($1,25D_3$) 24 hours into the incubation period. Expression of CD11b and CD14 were measured as markers of differentiation.
- Whole blood was incubated with 0–100 μg/mL PFOA in the presence or absence of 25 μg/mL phytohemagglutinin (PHA), T-cell cytokine secretion stimulator for 48 hours in quantification assays for the cytokines TNF-α and IL-6. LPS (0 or 250 ng/mL) was added to whole blood incubated with 0.1–100 μg/mL PFOA either 4 or 24 hours prior to the end of the 48-hour incubation period to determine TNF-α and IL-6 release.

The plasma concentrations of PFOA were 3.3, 1.56, and 4.19 ng/mL for all, female, and male volunteers, respectively. Exposure to 31.3 and 62.5 μg/mL PFOA significantly increased PBMC viability at the 72-hour endpoint, and 62.5 μg/mL PFOA significantly increased cell viability at 24 hours. Exposure to 250 and 500 μg/mL PFOA significantly decreased cell viability at all time endpoints. Exposure to PFOA did not affect HL-60 cell viability. A trend towards slightly augmented proliferation was observed following incubation with PFOA. Of the nine samples used, cells from six donors had slightly increased proliferation and t had no response. In cells incubated with ConA and 100 μg/mL PFOA, a nonsignificant decrease in the number of proliferating cells was observed. PFOA decreased NK cell activity approximately 16% (not statistically significant). In the presence of $1,25D_3$, 100 μg/mL PFOA significantly increased the percentage of HL-60 cells expressing CD11b and CD14. There were no differences in monocyte differentiation in the absence of $1,25D_3$.

In whole blood, exposure to PFOA for 48 hours caused a slight increase in TNF-α and IL-6 levels. In the presence of PHA, a slight dose-dependent decrease in TNF-α and IL-6 was observed. There was a slight dose-dependent decrease in TNF-α release when LPS was added 4 hours before the end of the incubation period and a slight dose-dependent increase in IL-6 release when LPS was added 24 hours prior to the end of incubation. The authors also looked at the correlation between basal PFOA concentration and cytokine release. A significant association was observed between PFOA concentration and the release of LPS-induced TNF-α and IL-6 by peripheral monocytes. The authors suggested that the trends observed at the lower concentrations might show an impact on human immunity with a larger population.

Ahuja et al. (2009) examined the effects of PFOA on the production and activation of human monocyte-derived dendritic cells. These cells are responsible for a primary immune system response by activating lymphocytes and secreting cytokines. Peripheral monocytes and immature dendritic cells were incubated with 200 μmol PFOA from day 0 to day 6 or 8 to determine the impact on phenotype and cytokine secretion. Maturation stimulus (i.e., prostaglandin E2, tumor necrosis factor, interleukin 1β, and IL-6) was added during the last 48 hours of incubation to induce dendritic cell maturation. Mixed leukocyte reaction assays were conducted to determine if immature dendritic cells could stimulate T-cells. Cytokine (HLA-DR, CD25, CD80, CD83, and CD86) expression was measured as a determination of maturity. HLA-DR is a cytokine that presents antigens to elicit T-cell response. CD25, 80, 83, and 86 are cell receptors that act as co-receptors in T-cell activation; and interleukin 12p40 and 10 stimulate T-cells. Mature cytokine-

activated dendritic cells secrete interleukin 12p40 and interleukin 10 as antiinflammatory cytokines.

In peripheral monocytes incubated with only PFOA from day 0–6 or day 0–8, expression of HLA-DR and CD86 was increased compared to expression in control cells, indicating that immature dendritic cells were present. In the mixed leukocyte reaction assay, the ability to stimulate T-cells was not different between immature dendritic cells generated in the absence or presence of PFOA.

To determine if PFOA impacted the differentiation of immature dendritic cells to mature dendritic cells, immature dendritic cells were incubated with 200 μmol of PFOA for 6 days and the maturation stimulus was added for the final 2 days of incubation. There were no differences in cytokine (CD25, CD80, CD83, and CD86) expression between cells incubated with PFOA and control cells. Expression of interleukin 12p40 and interleukin 10 was significantly inhibited by PFOA in mature cytokine-activated dendritic cells, even in the presence of maturation stimulus during the last 48 hours of incubation. The result suggested that exposure to PFOA during generation of dendritic cells affected the phenotype and cytokine production of human dendritic cells and could lead to immunomodulation in the development of the immune response.

3.3.3 Hormone Disruption

Thyroid. Martin et al. (2007) administered 20 mg PFOA/kg to adult male Sprague-Dawley rats (n = 4 or 5) for 1, 3, or 5 days by oral gavage and determined the impact of PFOA on hormone levels. Blood was collected via cardiac puncture and the serum was analyzed for cholesterol, testosterone, FT4 and total T4, and total T3. RNA extracted from the livers was used for gene expression profiling, genomic signatures, and pathway analyses to determine a mechanism of toxicity.

Following a 1-day, 3-day, and 5-day dose, a significant decrease ($p<0.05$) was observed in serum cholesterol (~↓45-72%), total T4 (~↓83%), FT4 (~↓80%), and total T3 (~↓25-48%). Serum testosterone was significantly decreased ($p<0.05$, ~↓70%) following a 3-day and 5-day PFOA dose. PFOA treatment was matched to hepatotoxicity-related genomic signatures, as well as signatures for hepatocellular hypertrophy, hypocholesterolemia, hypolipidemia, and peroxisome proliferation. PPARα nuclear regulated genes were induced by PFOA treatment. Genes associated with the thyroid hormone release and synthesis pathway including *Dio3,* which catalyzes the inactivation of T3, and *Dio1,* which deiodinates prohormone T4 to bioactivate T3, were affected by PFOA. Treatment with PFOA resulted in significantly upregulated expression of *Dio3* and downregulated expression of *Dio1* ($p<0.05$). Expression of HMG-CoA reductase (involved in cholesterol biosynthesis) was significantly upregulated and cholesterol biosynthesis was downregulated in a manner consistent with PPARγ agonists. The authors suggested a link between PFOA, PPAR, and thyroid hormone homeostasis based on work by Miller et al. (2001), who observed decreased serum T4 and T3 levels and increased hepatic proliferation following exposure to peroxisome proliferators. They also noted that PFOA exhibited similarities to compounds that induce xenobiotic metabolizing enzymes through PPARγ and CAR. The 20-mg/kg/day dose was a LOAEL for the effects monitored after a 5-day exposure.

Reproductive Hormones. Cook et al. (1992) gavage-dosed male CD rats (n = 15 per group) for 14 days with 0, 1, 10, 25, and 50 mg PFOA/kg/day to examine the possibility that an endocrine-related mechanism might explain Leydig cell adenomas observed in rats. A separate control group was pair-fed to the 50-mg/kg/day group. Blood and testicular interstitial fluid were

collected at necropsy for hormone analysis including testosterone, estradiol, and LH. A separate group of rats was dosed with 0 and 50 mg PFOA/kg/day for 14 days and challenged with 100 IUs of human chorionic gonadotropin (hCG) or 2 mg naloxone/kg 1 hour prior to necropsy to induce testosterone concentrations. Blood was collected and analyzed for testosterone and LH. Serum from rats challenged with 100 IUs hCG also was analyzed for P, 17 α-hydroxyprogesterone, and androstenedione.

The relative liver weight at 10, 25, and 50 mg PFOA/kg/day was significantly increased ($p<0.05$). The accessory sex organ unit relative weight was significantly decreased ($p<0.05$) at 25 and 50 mg PFOA/kg/day compared to those weights in control rats. The relative weights of the liver, accessory sex organ unit, and ventral prostate were significantly decreased at the highest dose compared to the pair-fed control.

Serum estradiol was significantly increased at ≥10 mg PFOA/kg compared to the control. No differences were observed in testosterone and LH between the treated rats and control. In the challenge experiment, serum testosterone was significantly decreased ($p<0.05$) by treatment with 50 mg PFOA/kg after challenge with 100 IUs hCG. No differences in testosterone concentration were observed in the naloxone-challenged rats, and no differences in LH were observed after either challenge. In the hCG-challenged rats, androstenedione was significantly reduced at 50 mg PFOA/kg, but no differences in concentrations were observed in P or 17 α-hydroxyprogesterone between control and treated rats. The authors suggested that the observed decreased serum testosterone levels could be due to decreased conversion of 17 α-hydroxyprogesterone to androstenedione as a result of increased serum estradiol levels. The LOAEL was 10 mg/kg based on increased liver weight and increased serum estradiol levels, and the NOAEL was 1 mg/kg.

Biegel et al. (1995) conducted *in vitro, in vivo,* and *ex vivo* studies to determine the effects of PFOA on Leydig cell function. In the *in vitro* study, Leydig cells were cultured with ± 2 IUs hCG (for final 3 hours) and 0, 100, 200, 250, 500, 700, and 1000 µmol PFOA for a total of 5 hours and then analyzed for testosterone concentration. Leydig cells also were incubated ±500 µmol PFOA and analyzed for testosterone and estradiol at 0, 0.5, 1, 3, 6, 12, 24, and 48 hours.

In the *in vitro* studies, there was no effect of PFOA treatment on testosterone in Leydig cells cultured without hCG. In cells cultured with hCG, PFOA caused a dose-dependent decrease in testosterone production. At 100 µmol PFOA plus hCG, the testosterone concentration was significantly increased compared to cells treated with only 100 µmol PFOA. Cytotoxicity occurred at ≥ 750 µmol PFOA. In the time course experiment, 500 µmol PFOA significantly inhibited hCG-stimulated release of testosterone at time points of at least 3 hours compared to control. Estradiol levels of PFOA-treated Leydig cells at 48 hours were statistically greater than the control.

Male CD rats were gavage-dosed for 14 days with 0, 0 pair-fed, or 25 mg PFOA/kg and necropsied on day 15. Blood and testicular interstitial fluid were collected for hormone analysis. Liver samples were collected for analysis of peroxisomal β-oxidation and microsomal aromatase activities. Serum estradiol was significantly increased ($p<0.05$) by 25 mg PFOA/kg when compared to the *ad libitum* and pair-fed control rats. Testicular interstitial fluid testosterone concentration was significantly decreased ($p<0.05$) and microsomal aromatase activity, and peroxisomal β-oxidation activity were significantly increased ($p<0.05$) in PFOA-treated rats compared to the pair-fed control rats.

Leydig cells from the treated rats in the *in vivo* study were isolated and cultured for analysis of testosterone concentration for the *ex vivo* study. An increase of 8.6-fold in testosterone production ($p<0.05$) was observed in Leydig cells isolated from PFOA-treated rats. The authors suggested that the increased serum estradiol levels resulted from liver aromatase induction by PFOA, and that PFOA could directly affect Leydig cell function.

Liu et al. (1996) treated adult male Crl:CD(BR) rats (n = 15 per group) with 0, 0 pair-fed, 0.2, 2, 20, and 40 mg PFOA/kg for 14 days by oral gavage to determine the impact of PFOA on aromatase activity. At necropsy on day 15, blood was collected for serum estradiol determination. Liver samples were collected for determination of microsomal aromatase activity and total P450 concentration. The testes were collected and testicular aromatase was determined.

The body weight of rats treated with ≥ 20 mg PFOA/kg was significantly decreased ($p<0.05$) compared to the control rats. Pair-fed control rats also had significantly decreased body weight compared to the control rats. Body weight was not different between the pair-fed control rats and rats dosed with 40 mg/kg PFOA. Absolute and relative liver weights were significantly increased ($p<0.05$) at ≥ 2 mg PFOA/kg. Relative testes weight was significantly increased at ≥20 mg PFOA/kg, but the differences were due to decreased body weight. There were no differences observed in testicular aromatase activity. In the remaining analysis, the pair-fed control group was similar to the *ad libitum* control group. The protein yield of hepatic microsomes was significantly increased at ≥0.2 mg PFOA/kg, and hepatic aromatase activity, total hepatic aromatase activity adjusted for liver and body weight effects, and serum estradiol were significantly increased ($p<0.05$) at ≥2 mg PFOA/kg. The maximum increase in total hepatic aromatase activity was 16-fold and the increase was twofold for serum estradiol. A significant correlation ($p<0.0001$) was observed between total hepatic aromatase activity and serum estradiol. The aromatase activity in liver microsomes isolated from control rats and incubated for 2 hours with PFOA was significantly decreased at ≥ 100 µmol. The authors estimated the half maximal effective concentration (EC_{50}) values for the outcomes, and they are shown in Table 3-36. Liu et al. (1996) concluded that the PFOA-increased protein yields suggested induction of the ER resulting in aromatase induction, which led to increased serum estradiol. However, PFOA also inhibited aromatase activity, which would explain why serum estradiol was only increased up to twofold.

Table 3-36. Estimated EC_{50} Values

Parameters	EC_{50} (mg PFOA/kg)
Hepatic microsome protein yield	0.53
Hepatic microsomal aromatase activity	0.76
Absolute liver weight	1.07
Relative liver weight	1.56
Serum estradiol	3.24
Terminal body weight	11.65

Source: Liu et al. 1996
Note: EC_{50}= half-maximum response.

A separate component of the Liu et al. study (1996) examined the effect of PFOA on aromatase activity in cultured hepatocytes and is discussed below. Aromatase is a cytochrome P450 enzyme localized to the ER that catalyzes the conversion of androgens to estrogens. The cultured hepatocytes isolated from control male CD rats were incubated with 0–1000 µmol PFOA and the aromatase activity was evaluated after 18, 42, and 66 hours (Liu et al. 1996).

Compared to aromatase activity in time-matched control cultures, PFOA caused a decrease in aromatase activity after 18 and 42 hours incubation with hepatocytes and an increase after the 66-hour incubation period.

In their study examining the impact of PFOA on aromatase activity, Liu et al. (1996) also examined the impact of PFOA on peroxisome β-oxidation and cytochrome P450 activities. Male Crl:CD BR (CD) rats (n = 15 per group) were orally dosed with 0, 0 pair-fed, 0.2, 2, 20, and 40 mg PFOA/kg for 14 days. Liver samples were collected for determination of microsomal total cytochrome P450 concentration and peroxisome β-oxidation activity. Total cytochrome P450 was significantly increased ($p<0.05$) at ≥20 mg PFOA/kg and β-oxidation activity was increased at ≥2 mg PFOA/kg. The estimated EC_{50}s for total cytochrome P450 and β-oxidation were 18.18 and 2.19 mg PFOA/kg, respectively. The LOAEL was 2 mg/kg based on increased liver weight, serum estradiol, and hepatic aromatase activity, and the NOAEL was 0.2 mg/kg.

Hines et al. (2009) examined the roles that exposure to PFOA and ovarian hormones might play in animals exposed during gestation compared to during their adult years. Timed-pregnant CD-1 mice were gavage-dosed in two blocks on GDs 1–17, but not thereafter. Block 1 animals were dosed with 0, 1, 3, and 5 mg PFOA/kg, and block 2 animals were dosed with 0, 0.01, 0.1, 0.3, 1, and 5 mg PFOA/kg/day. At birth, pups were pooled within each block and dose group and randomly redistributed among the dams (10 pups per litter). Offspring were weaned at 3 weeks, and a subset of females from each dose group (0, 0.01, 0.1, 0.3, 1, and 5 mg PFOA/kg/day) was OVX at weaning or the day after weaning. All animals were observed until they reached 18 months of age.

Body weight was recorded weekly for the first 9 months of age, followed by monthly body weight recordings over the next 9 months. As the animals matured, they were evaluated for the endpoints listed in Table 3-37. A group of naive 8-week-old adult mice were dosed for 17 days with 0, 1, and 5 mg PFOA/kg/day to compare the impact of exposure in adult animals to those occurring during gestation. At 18 months of age, the mice were sacrificed. Blood, retroperitoneal abdominal fat, interscapular brown fat, organs, and abnormal growths were collected at necropsy.

Table 3-37. Data Collection for Female Mice Gestationally Exposed to PFOA

Test	Age at Test	Dose (mg/kg/day)	Group
Glucose tolerance test	15-16 weeks	0, 1, 5	Intact
Serum leptin and insulin	21-33 weeks	0, 0.01, 0.1, 0.3, 1	Intact, OVX
Body mass composition	42 weeks	0, 0.01, 0.1, 0.3, 1	Intact
Glucose tolerance test	17 months	0, 0.1, 1, 5	Intact
Food consumption	17 months	0, 0.1, 1, 5	Intact
Serum estradiol	18 months	0, 0.01, 0.1, 0.3, 1, 5	Intact

Source: Hines et al. 2009

Body weight of offspring born to dams exposed to 5 mg PFOA/kg was significantly decreased ($p<0.05$) on PND 1 and through 18 months of age compared to control offspring body weight. At weaning, the body weight of offspring born to dams exposed to 1 mg PFOA/kg/day was significantly decreased ($p<0.05$) compared to control offspring body weight. A significant increase ($p<0.05$) in body weight, due to more rapid weight gain after week 10, compared to intact control body weight, was observed in intact mice exposed to 0.01–0.3 mg PFOA/kg/day

during gestation. Body weight of intact mice gestationally exposed to 0.01–0.3 mg PFOA/kg/day was comparable to body weight of control mice at 18 months.

Due to the increased weight gain observed in intact mice exposed to PFOA during gestation, glucose tolerance tests were carried out along with determination of serum insulin concentration. In cases of insulin resistance, plasma glucose and insulin levels are elevated and the insulin response is lessened. Insulin resistance also has been associated with excess abdominal fat. Serum leptin levels also were determined as increased leptin levels have been associated with a leptin-resistance mechanism of action (MOA) for increased weight gain in humans. Body mass composition was used to determine if there were differences in body fat between the intact groups, and feed consumption was recorded to determine if consumption played a role in body weight differences in intact control and intact gestationally exposed mice. Serum estradiol was measured to determine if PFOA impacted hormone levels at 18 months in intact control and intact gestationally exposed mice.

Glucose tolerance testing showed no statistically significant differences in baseline glucose or response to glucose challenge at 15–16 weeks or at 17 months. At 21 and 31 weeks of age, a significant increase in serum leptin and insulin levels was observed in intact mice exposed to 0.01 and 0.1 mg PFOA/kg/day. No statistically significant difference was observed between the fat-to-lean ratio of intact control and intact gestationally exposed animals at 42 weeks of age. No significant difference was observed in food consumption between intact control and intact gestationally exposed animals at 42 weeks of age. Serum estradiol levels were not different between intact control and intact gestationally exposed animals at 18 months.

Exposure to PFOA as an adult did not result in body weight differences among the groups at 18 months of age. The body weight of intact mice gestationally exposed to 1 mg PFOA/kg/day was significantly increased ($p<0.05$) compared to adult mice exposed to 1 mg PFOA/kg/day. No other differences in body weight among the groups were observed.

No significant differences among the groups were observed in survival during the 18-month study. At necropsy, abdominal white fat was significantly decreased ($p<0.05$) at 1 and 5 mg PFOA/kg/day in gestationally exposed intact mice compared to intact control mice. Interscapular brown fat was significantly increased ($p<0.05$) at 1 and 3 mg PFOA/kg/day in gestationally exposed intact mice and in gestationally exposed OVX mice at 1 mg PFOA/kg/day. Relative spleen weight was significantly decreased ($p<0.05$) at 3 mg PFOA/kg/day in gestationally exposed intact mice and at 1 and 5 mg PFOA/mg ($p = 0.05$-0.07) in gestationally exposed OVX mice. Relative liver weight was not different between the groups. No differences were observed at 18 months of age in tissue weight in mice exposed to PFOA as adults. At 1 mg PFOA/kg/day, white and brown fat weight was significantly increased in gestationally exposed intact mice compared to adult-exposed mice exposed to 1 mg PFOA/kg/day.

The authors concluded that developmental exposure to low doses and high doses of PFOA resulted in different phenotypes in mice. At low doses, increased weight, increased serum insulin, and increased serum leptin were observed in adult mice. At high doses the animals displayed decreased weight in early and late life, decreased white fat, increased brown fat, and decreased spleen weight. Under the conditions of the study, the developmental LOAEL was 0.01 mg PFOA/kg based on increased weight gain and increased serum insulin and leptin levels. No developmental NOAEL was established. The adult NOAEL was 5 mg PFOA/kg, and no LOAEL was established.

Adrenal Hormones. Thottassery et al. (1992) exposed intact or ADX male Sprague-Dawley rats to a single dose of 150 mg/kg PFOA in corn oil to determine the role of adrenal hormones on liver enlargement and peroxisomal proliferation. ADX rats were dosed 2 days after surgery with PFOA (ADX PFOA), CORT (ADX CORT), or both (ADX CORT PFOA). A group of intact and ADX rats received only the vehicle and served as controls. The animals were sacrificed 48 hours after dosing with PFOA or vehicle. Assays were conducted to determine DNA levels and changes in peroxisomal β-oxidation, catalase, and ornithine decarboxylase activities. An increase in ornithine decarboxylase activity has been associated with proliferation of many different cell types. An increase of ornithine decarboxylase in the livers of animals exposed to PFOA would suggest that the increased liver weight observed in PFOA-exposed animals was the result of hyperplasia. Ornithine decarboxylase was determined by measuring liberated CO_2 from DL-[1-^{14}C] ornithine hydrochloride in all animals except those in the ADX CORT PFOA group.

Relative liver weight in intact rats treated with PFOA was significantly increased compared to control (36%, $p<0.05$). Relative liver weight in rats in the ADX PFOA group was significantly increased compared to rats in the ADX vehicle group (16%, $p<0.05$). Relative liver weight in rats in the ADX CORT PFOA group was significantly increased compared to rats in the ADX CORT group (32%, $p<0.05$). Hepatic DNA levels were significantly decreased $p<0.001$) in intact rats treated with PFOA and in rats in the ADX CORT PFOA group.

Ornithine decarboxylase activity was significantly increased in the rats in the ADX PFOA group compared to rats in the ADX group (170.5 pmole CO_2/hr/mg protein, versus 30.5 pmole CO_2/hr/mg protein, $p<0.001$), but no different between the intact rats treated with PFOA and the intact rats treated with the vehicle.

PFOA increased whole liver peroxisomal β-oxidation activity by a similar amount and was not different among the groups. In intact rats and rats in the ADX CORT PFOA group, exposure to PFOA increased whole liver catalase activity, but exposure did not increase activity in the rats in the ADX PFOA group. Based on the results, the authors concluded that adrenal hormones were not required to induce peroxisomal β-oxidation activity in PFOA-exposed rats, but are required to increase catalase activity. They also concluded that the enlarged livers of PFOA-exposed animals were the result of hypertrophy rather than hyperplasia based on decreased hepatic DNA content and lack of increased ornithine decarboxylase activity.

3.3.4 Physiological or Mechanistic Studies

Gene Expression. Rosen et al. (2007) examined the gene expression profile in the lung and liver of mouse fetuses exposed to PFOA. Pregnant CD-1 mice were gavage-dosed with 0, 1, 3, 5, and 10 mg PFOA/kg/day on GD 1–17. Dams were sacrificed on GD 18, and three fetuses per litter were processed for total RNA from portions of the liver and lung. Global gene expression was analyzed using Affymetrix gene chips.

A dose-related increase was observed in the number of genes altered by PFOA exposure in both the liver and lung. A greater number of genes in the liver were altered compared the number of genes altered in the lung. Analysis of the genes by canonical pathway or biological function showed that most of the altered genes in both the liver and lung were associated with lipid homeostasis. In the fetal lung, the two highest doses of PFOA altered genes associated with fatty acid catabolism. In the fetal liver, all doses of PFOA were associated with genes involved in fatty acid catabolism, lipid transport, cholesterol biosynthesis, bile acid biosynthesis, lipoprotein metabolism, steroid metabolism, retinol metabolism, inflammation, phospholipid metabolism,

glucose metabolism, proteosome activation, and ketogenesis. Although PPARα is known to at least partly regulate the expression of genes for the pathways or biological functions involved in lipid homeostasis, PFOA might independently activate other nuclear receptors, influencing the metabolic responses observed.

Rosen et al. (2008a) described the gene profiles in liver tissue from wild-type 129S1/SvlmJ mice (7–8 per group) and PPARα-null mice (129S4/SvJae-PPARαt^{m1Gonz}/J, 6–8 per group) dosed for 7 days with 0, 1, and 3 mg PFOA/kg or 50 mg Wyeth 14,643, a PPARα agonist (Wolf et al. 2008a). RNA was isolated from the tissues and gene expression analyzed using Applied Biosystems Mouse Genome Survey Microarrays. RT-PCR was used to evaluate selected genes.

In both wild-type and PPARα-null mice exposed to PFOA, the number of significant and fully annotated genes used to evaluate the data for relevance to canonical pathway or biological function were fewer at 1 mg/kg than at 3 mg/kg PFOA. However, 85% of the altered genes at 1 mg/kg PFOA also were altered at 3 mg/kg PFOA.

PPARα target genes including acyl-CoA oxidase 1 (Acox1), Me1, Slc27a1, Hsd17b4, Hadha, Hadhb, and Pdk4 were upregulated in PFOA- and Wyeth 14,643-treated wild-type mice, but not in PPARα-null mice. Pdk4 was downregulated in PPARα-null mice exposed to PFOA but not in PPARα-null mice exposed to Wyeth 14,643. Principal components analysis showed that genes activated in PFOA-treated PPARα-null mice were similar to those in PFOA-treated wild-type mice at 3 mg PFOA/kg, suggesting that many of the responses were not completely linked to PPARα.

In wild-type PFOA- and Wyeth 14,643-treated mice, alterations were observed in genes associated with fatty acid metabolism (mostly upregulated), inflammatory response (mostly downregulated), cell cycle control (mostly upregulated), peroxisome biogenesis (mostly upregulated), and proteasome structure and organization (mostly upregulated). In genes associated with xenobiotic metabolism, the response was different between PFOA- and Wyeth 14,643-treated wild-type mice. Many of the Cyp2 genes were upregulated by PFOA and downregulated by Wyeth 14,643. In PPARα-null PFOA-treated mice, genes associated with fatty acid metabolism, inflammation, xenobiotic metabolism, and cell cycle control were altered in a manner similar to the changes observed in PFOA-treated wild-type mice.

RT-PCR generally revealed good agreement with microarray analysis. However, expression of Ehhadh, a PPARα-regulated gene, was upregulated in PFOA-treated wild-type mice but not in PFOA-treated PPARα-null mice in microarray analysis. In contrast, expression of Ehhadh was upregulated in all PFOA-treated mice in RT-PCR analysis. The authors concluded that PFOA induces transcriptional changes mediated through PPARα activation, and it also alters gene expression independently of PPARα. They noted that PFOA had multiple modes of action and can function as a biologically active xenobiotic in the absence of PPARα.

Rosen et al. (2008b) described the transcript profiles in the livers of adult mice exposed to PFOA. Tissues from several different studies were analyzed. The samples included liver tissue from:

- male wild-type (strain 129S1/SvlmJ) and PPARα-null (strain 129S4/SvJae) mice dosed with 3 mg/kg/day PFOA for 7 days (from Wolf et al. 2008a);
- male wild-type and PPARα-null mice (strain SV129/C57BL/6) gavage-dosed or fed diets containing Wyeth 14,643 (PPARα agonist);

- female wild-type and CAR-null (strain C57BL/6x129Sv) gavage-dosed with CAR activators phenobarbital (PB) or 1,4-bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP); and
- wild-type and Nrf-null ICR mice gavage-dosed with the Nrf activator dithiol-3-thione.

RNA was isolated from the tissues and gene expression was analyzed using Affymetrix full genome mouse chips. Rosetta Resolver software was used to identify significantly altered genes.

Exposure to 3 mg/kg PFOA for 7 days upregulated 641 genes and downregulated 451 genes in wild-type mice compared to 104 upregulated genes and 52 downregulated genes in PPARα-null mice. A total of 117 genes were regulated similarly in both strains, and 29 upregulated genes and 11 downregulated genes were unique to PPARα-null mice.

The gene expression profile of wild-type and PPARα-null mice exposed to PFOA for 7 days or Wyeth 14,643 for 12 hours, or 3 or 7 days were compared. Four groups of altered genes were identified based on their expression in wild-type and PPARα-null PFOA-exposed mice compared to genes from Wyeth 14,643-treated mice. The first group consisted of genes (397) regulated by both PFOA and Wyeth 14,643 in wild-type mice. They had a common direction and magnitude of change and were characterized as being involved in lipid homeostasis, inflammation, cell proliferation, or proteome maintenance genes. Group II consisted of genes in wild-type mice (51) regulated solely by PFOA; most were involved in amino acid metabolism. Of the 81 genes altered by PFOA exposure in PPARα-null mice (Group III), 62 had similar expression in wild-type mice and many were involved in lipid metabolism. Regulation of these genes also was observed in Wyeth 14,643 wild-type mice. Group IV genes (19) were altered by PFOA only in PPARα-null mice; most were xenobiotic metabolizing enzymes.

By comparing the gene expression patterns between PFOA and Wyeth 14,643, the authors concluded that:

- PPARα is required for a majority of the transcriptional changes observed in the mouse liver following PF also are regulated by other peroxisome proliferators in a PPARα-dependent manner; and
- PFOA impacts some PPARα-independent genes including ones involved in lipid homeostasis (upregulated), amino acid metabolism (downregulated), and xenobiotic metabolism (upregulated).

The transcription profiles of PFOA exposed wild-type and PPARα-null mice were compared to the transcription profile of PB- or TCPOBOP-exposed wild-type and CAR-null mice and dithiol-3-thione-exposed wild-type and Nrf2-null mice to determine if PFOA activated CAR or Nrf2. A similar pattern was observed in the modified gene expression of PFOA-exposed PPARα-null mice and PB- (0.86 Pearson's correlation) or TCPOBOP- (0.84 Pearson's correlation) exposed wild-type mice, but no pattern was observed in gene expression of dithiol-3-thione-exposed mice (≤0.06 Pearson's correlation) and PFOA-exposed PPARα-null mice. These results suggest that some genes altered by PFOA exposure in PPARα-null mice are regulated by CAR but not by Nrf2.

Bjork and Wallace (2009) examined the PPARα-dependent transcriptional activation potential of PFOA in rodent and human hepatic liver cells. Primary rat and human hepatocytes and HEPG2/C3A cells were incubated with 0, 5, 10, 20, 30, 50, 100, and 200 μmol PFOA for 24 hours. Expression of Acox, Cyp4a1 (rat), Cyp4a11 (human), acyl-CoA thioesterase (Cte–rat, Acot1–human), and DNA damage inducible transcript (Ddit3) were determined by quantitative

RT-PCR. These genes are inducible by peroxisome proliferators, except Ddit3, which is induced in the presence of direct or indirect DNA damage. Exposure to ≥ 100 μmol PFOA significantly increased Ddit3 mRNA expression in primary rat hepatocytes. At the highest dose, Ddit3 was significantly increased in human hepatocytes and HepG2/C3A cells. Expression of Acox was significantly induced by 5, 10, 20, and 30 μmol PFOA, and Cte/Acot1 was significantly induced at ≥20 μmol PFOA in rat hepatocytes only. Expression of Cyp4a1/11 was significantly induced in rat hepatocytes at 5–-50 μmol and in human hepatocytes at 20–50 μmol. The authors concluded that induction of peroxisome-related fatty acid oxidation gene expression is not observed in primary human liver cells or in transformed human liver cells *in vitro.*

Nakamura et al. (2009) investigated the differences in PFOA response between mice and humans using a humanized PPARα transgenic mouse line (hPPARα). The study design and whole animal toxicity data are described in section 3.2.2. Male 8-week-old wild-type (mPPARα) mice, PPARα-null mice, and hPPARα mice were gavage-dosed with 0, 0.1, and 0.3 mg/kg PFOA (n = 4–6 per group) for 2 weeks and sacrificed 18–20 hours following the last dose. Livers were collected and analyzed for mRNA (RT-QPCR) and protein levels (western blot analysis) of PPARα and related genes (retinoid X receptor alpha [RXRα], peroxisomal bifunctional protein [PH], peroxisomal thiolase [PT], very long chain acyl-CoA dehydrogenase [VLCAD], medium chain acyl-CoA dehydrogenase [MCAD], and cytochrome P450 4a10 [CYP4A10]). RXRα forms a heterodimer with PPARα to control transcription of genes affecting lipid metabolism. CYP4A10 also plays a role in lipid metabolism. Treatment with peroxisome proliferators caused an increase in both PH and PT. MCAD and VLCAD are mitochondrial fatty acid metabolizing enzymes whose gene expression is mediated by PPARα (Aoyama et al. 1998). The results of mRNA expression impacted by PFOA exposure are shown in Table 3-38.

Treatment with PFOA did not alter mRNA expression or protein expression of PPARα, RXRα, or MCAD in mPPARα mice. At 0.1 mg/kg PFOA, mRNA expression of CYP4A10 was significantly increased ($p<0.05$) in mPPARα mice compared to control mPPARα mice. Treatment with 0.3 mg/kg PFOA resulted in significantly increased ($p<0.05$) mRNA expression of CYP4A10 and mRNA and protein expression of PH, PT, and VLCAD in mPPARα mice when compared to control mPPARα mice.

Table 3-38. mRNA Expression of Hepatic PPARα and Related Genes

	mPPARα			PPARα-null			hPPARα		
	0 mg/kg	0.1 mg/kg	0.3 mg/kg	0 mg/kg	0.1 mg/kg	0.3 mg/kg	0 mg/kg	0.1 mg/kg	0.3 mg/kg
PPARα	-	-	-	NA	NA	NA	-*↑	-*↑	-*↑
RXRα	-	-	-	-	-	-	-	-	-
PH	-	-	+↑	-	-*↓	-*↓	-	-*↓	-*↓
PT	-	-	+↑	-	-*↓	-*↓	-*↑	-	-
VLCAD	-	-	+↑	-*↓	-*↓	-*↓	-	-*↓	-*↓
MCAD	-	-	-	-	-*↓	-	-	-*↓	+↑
CYP4A10	-	+↑	+↑	-	-*↓	-*↓	-*↑	-*↓	-*↓

Source: Nakamura et al. 2009
Notes:
- Not different from respective control.
+ Significantly different from respective control.
* Significantly different from mPPARα mice treated with same concentration.
↓ Decreased expression relative to respective control or mPPARα mice at same concentration.
↑ Increased expression relative to respective control or mPPARα mice at same concentration.

As expected, mRNA and protein expression of PPARα was absent in PPARα-null mice. Treatment with 0.1 and 0.3 mg/kg PFOA did not alter mRNA or protein expression for any genes investigated compared to control PPARα-null mice. VLCAD mRNA expression and PT protein expression in control PPARα-null mice was significantly decreased ($p<0.05$) compared to mPPARα control mice. VLCAD mRNA and protein expression of PFOA treated PPARα-null mice was significantly decreased ($p<0.05$) compared to mPPARα mice treated with the same doses. Following treatment with 0.1 mg/kg PFOA, MCAD mRNA expression was decreased ($p<0.05$) compared to mPPARα mice treated with the same dose. When compared to mPPARα mice treated with the same dose, mRNA and protein expression of PH and PT was significantly decreased ($p<0.05$) in PPARα-null mice, as was CYP4A10 mRNA expression.

Treatment with PFOA did not alter mRNA or protein expression of PPARα, RXRα, PH, PT, or VLCAD in hPPARα mice compared with their respective controls. Expression of CYP4A10 mRNA also was not altered by PFOA treatment. MCAD mRNA and protein expression were significantly increased ($p<0.5$) in hPPARα mice treated with 0.3 mg/kg PFOA compared to hPPARα control mice. Expression of PPARα mRNA and protein levels were significantly higher ($p<0.05$) in all hPPARα mice than in mPPARα mice given the same concentration of PFOA. Treatment of hPPARα mice with 0.1 and 0.3 mg/kg PFOA caused a decrease ($p<0.05$) in mRNA expression of PH, VLCAD, and CYP4A10 compared to mPPARα mice at the same dose. Only hPPARα mice treated with 0.3 mg/kg PFOA had decreased protein expression of PH and VLCAD compared to mPPARα mice given the same treatment. An important finding from this study was the significant downregulation of some genes in PPARα-null and hPPARα mice that are significantly upregulated by PPARα in the control animals. In the animals with the humanized PPARα gene or no PPARα gene, there was a response, but the response was the opposite of what occurred with normal mouse PPARα activation. In the null and humanized mice, the significantly decreased alterations in gene expression occurred at 0.1 mg/kg/day; this dose level had no change in expression for all but one gene in the normal mice and increased expression, rather than decreased expression, at 0.3 mg/kg/day (see Table 3-38).

Treatment with 0.3 mg/kg PFOA caused activation of PPARα in mouse, but not in humanized PPARα mice. The results suggest that the functional activation of human PPARα could be weaker than that of mice as expression of human PPARα in mice was greater than the expression of mouse PPARα. Higher concentrations of PFOA might be needed to cause activation of human PPARα in hPPARα mice.

To further evaluate the developmental effects potentially mediated by PPARα, groups of female wild-type, PPARα-null, and PPARα-humanized mice were given 0 and 3 mg PFOA/kg on GDs 1–17 by oral gavage (Albrecht et al. 2013). The study design and developmental toxicity data are described in section 3.2.5. Females were either sacrificed on GD 18 (n = 5–8 per group) or allowed to give birth and then sacrificed, along with their litters (n = 8–14), on PND 20. Livers from dams, fetuses, and pups were collected for measurement of mRNAs encoding the PPARα target genes Cyp4a10 and Acox1, the CAR target gene (Cyp2b10), and the PXR target gene (Cyp3a11).

On GD 18, maternal liver samples from treated groups showed increased expression of Acox1 in wild-type mice and Cyp4a10 in wild-type and humanized mice. Expression of Cyp2b10 and Cyp3a11 were increased following PFOA administration in all three genotypes. On PND 20, maternal liver samples from treated groups showed increased expression of Acox1 in wild-type mice; expression of Cyp2b10 was unchanged in all groups; and expression of Cyp3a11 was increased following PFOA administration in all three genotypes.

For fetuses on GD 18, liver samples from treated groups showed increased expression of Acox1 and Cyp4a10 in wild-type and humanized mice. Expression of Cyp2b10 was unchanged following maternal PFOA administration in all three genotypes, while expression of Cyp3a11 was increased in humanized fetal liver. On PND 20, pup liver samples from treated dams showed increased expression of Acox1 and Cyp4a10 in wild-type mice; expression of Cyp2b10 was increased in all genotypes; and expression of Cyp3a11 was increased following maternal PFOA administration in wild-type and humanized pups. Thus, expression of PPARα target genes that modulate lipid metabolism was increased in both wild-type and humanized mice coincident with increased liver weight and microscopic lesions; however, the neonatal mortality was observed only in wild-type offspring (Albrecht et al. 2013).

Walters et al. (2009) examined the impact of PFOA on mitochondrial biogenesis and gene transcription in adult male Sprague-Dawley rats orally dosed with 0 or 30 mg/kg PFOA for 28 days. At sacrifice, a portion of the midlobe region of the livers was collected. Liver DNA and RNA were isolated for RT-PCR of genes in the peroxisome proliferator-activated receptor gamma coactivator 1α- (Pgc-1α-) mediated pathway of mitochondrial biogenesis: Pgc-1α, estrogen-related receptor α (Errα), nuclear respiratory factor 1 (Nrf1) and Nrf2, transcription factor A (Tfam), cytochrome c oxidase subunit II and IV (Cox II and Cox IV), NADH dehydrogenase 2 (Nd2), and NADH dehydrogenase iron-sulfur protein 8 (Ndufs8). In mitochondrial biogenesis, Pgc-1α and Errα increase expression of the transcription factors Nrf1 and Nrf2. The Nrf transcription factors promote expression of Tfam, which is required for mitochondrial DNA replication and transcription. Within the mitochondrial membrane, oxidative phosphorylation proteins (Cox II and IV, Nds, and Ndufs8) catalyze the transfer of electrons and/or pump protons from the matrix to the intermembrane space. Western blotting was used to analyze protein expression of Pgc-1α, Tfam, Cox II, and Cox IV.

Mitochondrial DNA in rats treated with PFOA was significantly increased ($p<0.05$) compared to control rats. In PFOA-treated rats, the expression of Pgc-1α, Errα, Nrf1, Nrf2, and Tfam was significantly increased 1.3–2.2-fold ($p<0.05$), and expression of Cox II, Cox IV, Nd2, and Ndufs8 was significantly increased 2–9-fold ($p<0.05$) compared to controls. Protein expression of Pgc-1α was increased, and expression of Cox II and Cox IV were decreased in PFOA-treated rats. Protein expression of Tfam was not affected by treatment with PFOA. The results suggested that PFOA induced mitochondrial biogenesis at the transcriptional level by activation of the Pgc-1α pathway, confirming the potential for effects on mitochondria but not clarifying whether those effects are in some way linked to PPARα activation.

Elcombe et al. (2010) examined the expression of some cytochrome P450 isoforms in the livers of male Sprague-Dawley rats fed diets containing 300 ppm PFOA or 50 ppm Wyeth 14,643 for 1, 7, or 28 days. The isoforms included those involved in activation of PPARα (CYP4A1), CAR (CYP2B1/2), and PXR (CYP3A1). All three isoforms were induced by PFOA exposure. CYP2B1/2 and CYP4A1 were induced after 1 day of exposure to PFOA. CYP3A1 was induced in all PFOA-exposed rats after 7 days of exposure. Treatment with Wyeth 14,643 caused the induction of CYP4A1 only.

PPAR Activation. Takacs and Abbott (2007) evaluated the potential for PFOA to activate PPARs, using a transient transfection cell assay. Cos-1 cells, derived from the kidney cells of the African green monkey, were transfected with mouse or human PPARα, PPARβ/δ, or PPARγ reporter plasmids and exposed to 0.5–100 μmol PFOA or 0.5–100 μmol PFOA and MK-886 (PPARα antagonist) or GW9662 (PPARγ antagonist). An antagonist for PPARβ/δ was not available. The three types, PPARα, β/δ, and γ, are encoded by different genes, expressed in many

tissues, and have specific roles during development as well as in the adult. The results are shown in Table 3-39. PFOA activated PPARα in a dose-dependent manner with a significant increase in activity observed at 10, 20, 30, and 40 µmol for the mouse receptor and 30 and 40 µmol for the human receptor compared to the negative control. The presence of the PPARα antagonist MK-886 prevented the activity increase resulting from PFOA exposure alone in mouse and human PPARα constructs.

Table 3-39. Activation of Mouse and Human PPAR by PFOA

PPARα			PPARβ/δ			PPARγ		
PFOA (µm)	Mouse	Human	PFOA (µm)	Mouse	Human	PFOA (µm)	Mouse	Human
0	-	-	0	-	-	0	-	-
0.5	-	-	10	-	-	1	-	-
1	-	-	15	-	-	5	-	-
3	-	-	20	-	-	10	-	-
5	-	-	30	-	-	20	-	-
10	+	-	40	+	-	30	-	-
15	-	-	50	+	-	40	-	-
20	+	-	60	+	-	50	-	-
30	+	+	70	+	-	75	-	-
40	+	+	80	+	-	100	-	-

Source: Takacs and Abbott 2007
Notes:
+ Significant increase in activity between treated and control.
- No difference in activity between treated and control.

Activity of mouse PPARβ/δ was significantly increased after exposure to 40–80 µmol PFOA compared to the negative control. Activity of human PPARβ/δ was not increased by PFOA exposure. Activity of mouse and human PPARγ were not increased by exposure to PFOA. PFOA was found to activate mouse and human PPARα and mouse PPAR β/δ under the conditions in this study.

Biomarkers for Peroxisome Proliferation. Pastoor et al. (1987) dosed male Crl:CD (SD) BR rats for 1, 3, and 7 days with 0 or 50 mg PFOA/kg/day. Hepatic DNA content, cytochrome P450 content, UDP-glucuronyltransferase, glutathione S-transferase, benzphetamine N-demethylase activity (marker for smooth ER proliferation), and ethoxyresorufin O-deethylase activity (marker for cytochrome P450 induction via the aryl hydrocarbon receptor) were measured from rats dosed 1 and 3 days. Liver microsomes were prepared from rats dosed for 3 days for CAT and CPT activity assays. CAT served as a marker for peroxisome proliferation and CPT was a marker for mitochondrial proliferation. Incorporation of [^{14}C]acetate into hepatic lipids was used to determine the effect of PFOA on hepatic lipid metabolism. Plasma TC and triacylglycerides was determined from rats dosed for 7 days.

Hepatic DNA content was not increased in treated rats when compared to content in control rats. Cytochrome P450 was significantly increased ($p<0.05$) and ethoxyresorufin O-deethylase activity was significantly decreased ($p<0.05$) after treatment for 1 and 3 days. Benzphetamine N-demethylase activity was significantly increased ($p<0.05$) after treatment with PFOA for 3 days. CAT activity increased 12-fold ($p<0.05$) and CPT increased twofold ($p<0.05$) after a 3-day treatment with 50 mg PFOA/kg. No differences were observed among the groups for the

other enzymes. No differences were observed between rats treated for 7 days and control rats in plasma TC or triacylglycerol. Although a significant increase ($p<0.05$) was observed for [^{14}C]acetate incorporation into triacylglycerols, cholesteryl esters, and polar lipids, there was no difference in the distribution of the incorporated label between control and treated rats. The authors concluded that the lack of increased DNA content, proliferation of smooth ER, and peroxisome proliferation pointed to increased liver weight due to hepatocyte hypertrophy.

Gap Junction Intercellular Communication. Upham et al. (1998, 2009) examined the effects of perfluorinated fatty acids on gap junction intercellular communication (GJIC) in male Fischer 344 rats fed diets containing 0 or 0.02% PFOA (intake 37.9 mg/kg/day) for 1 week and in WB-F344 rat liver epithelial cells. The chain lengths of the perfluorinated fatty acids ranged from 2–10, 16, and 18 carbons. Liver weight in the rats fed diets containing 0.02% PFOA was significantly increased compared to control rat liver weight. No differences were observed in serum AST, ALT, and ALP. PFOA significantly inhibited GJIC in the livers of rats after treatment for 1 week. In WB-F344 cells, GJIC was inhibited by perfluorinated fatty acids with 7–10 carbons within 15 mins of incubation. The inhibition was reversible with full recovery occurring within 30 mins of PFOA removal from media. Extracellular receptor kinase was activated by PFOA within 5 mins of incubation in the cells. Preincubation of cells with the phosphatidylcholine-specific phospholipase C inhibitor D609 partially prevented GJIC inhibition by PFOA. The authors concluded that PFOA, having an 8-carbon chain, inhibited GJIC by activation of extracellular receptor kinase and phosphatidylcholine-specific phospholipase C, but noted that other mechanisms might be involved.

Production of ROS. Takagi et al. (1991) fed male Fischer 344 rats diets containing 0, 10, and 20 mg PFOA/kg for 2 weeks to determine the formation of 8-OH-dG (marker of oxidative DNA damage). Livers and kidneys were removed at necropsy and DNA was isolated from each organ and analyzed. The relative liver and kidney weights were significantly increased ($p<0.05$) in the treated rats compared to the control. A significant increase in 8-OH-dG liver levels was observed at ≥ 10 mg PFOA/kg. There were no significant differences in 8-OH-dG kidney levels between PFOA-treated and control rats. The authors concluded that PFOA could cause organ-specific oxidative DNA damage.

Hu and Hu (2009) exposed human hepatoma cells, HepG2, to PFOA to evaluate cytotoxic effects. Cells also were exposed to a mixture of PFOA and PFOS to determine antagonistic or synergic effects. The cells were exposed to 0, 50, 100, 150, and 200 µmol PFOA or to 0, 50, 150, and 200 µmol each of PFOA and PFOS. A group of cells also were exposed to 0, 50, 100, 150, and 200 µmol PFOS. The cells were cultured for 24, 48, and 72 hours. Cell viability, apoptosis, ROS, mitochondrial membrane potential, antioxidant enzymes, glutathione content, and differential expression of apoptosis gene regulators p53, Bax, Bcl-2, caspace-3, and caspace-9 genes were evaluated.

Exposure to PFOA or PFOS caused a dose-dependent decrease in viability of HepG2 cells. A nonsignificant dose-dependent increase in apoptosis was observed in the cells cultured with PFOA. However, the combination of PFOA and PFOS showed a significant dose-dependent increase ($p<0.05$) in apoptosis. Intracellular ROS were significantly increased ($p<0.05$) in cells cultured with 100, 150, and 200 µmol PFOA or PFOS. HepG2 cells exposed to the mixture of 100 and 200 µmol PFOA and PFOS exhibited a decline in fluorescence intensity in the mitochondrial membrane potential assay, indicating that mitochondrial pathways were involved in the apoptosis observed. Exposure to 100 µmol PFOA significantly decreased ($p<0.05$) glutathione concentration and glutathione peroxidase activity; and 150 µmol PFOA significantly

increased ($p<0.05$) the activities of SOD, catalase, and glutathione reductase, and significantly decreased ($p<0.05$) glutathione peroxidase activity, and glutathione concentration in HepG2 cells. The trend was the same at 200 μmol PFOA, with the exception of GST activity being significantly decreased ($p<0.05$).

Exposure to PFOA did not change p53, Bax, or caspace-3 expression in HepG2 cells. Expression of Bcl-2 was downregulated and caspace-9 was upregulated in a dose-dependent manner in HepG2 cells following exposure to 50–200 μ/Mol PFOA. The authors proposed that PFOA and PFOS induced cell apoptosis by overwhelming the homeostasis of antioxidative systems, increasing ROS, impacting mitochondria, and changing gene expression of apoptosis gene regulators.

Eriksen et al. (2010) examined ability of PFOA to generate ROS and induce oxidative DNA damage in human HepG2 cells. Cell were incubated with 0, 0.4, 4, 40, 200, 400, 1,000, and 2,000 μmol PFOA and 2',7'-dichlorofluorescein diacetate. Hydrogen peroxide, H_2O_2, was used as a positive control. A fluorescence spectrophotometer was used to measure ROS production every 15 mins during the 3-hour incubation period in all cultures. The comet assay was used to measure DNA damage in cells exposed to 0, 100, and 400 μmol PFOA for 24 hours. Cytotoxicity was determined by measuring the level of lactate dehydrogenase activity in the cell medium. Exposure to PFOA caused a dose-independent increase (all doses $p<0.05$) in ROS production in HepG2 cells. Compared to ROS production in negative control cells, PFOA induced a 1.52-fold increase in production. There was no difference in oxidative DNA damage and lactase dehydrogenase activity between PFOA-treated cells and negative control cells. The authors concluded that oxidative stress and DNA damage were probably not relevant to potential adverse effects of PFOA.

Protein Binding. The ability of PFOA to bind to serum proteins for distribution is discussed in section 2.2. PFAS protein binding also can impact cellular function in cases in which the proteins in question are transporters (serum albumin and fatty acid binding protein) or enzymes (lysine decarboxylase) as well as membrane receptors (e.g., members of the PPAR family) and thyroid hormone receptors. The mechanistic studies of the nuclear PPARα membrane receptors are described in section 3.3.4.

Ren et al. (2015) examined the relative binding affinities of 16 PFASs for the human thyroid hormone receptor's α ligand binding domain (TRα-LBD) using a fluorescence competitive binding assay. Solutions of 1 μmol TRα-LBD were prepared in DMSO. Changes in TRα-LBD tryptophan fluorescence after binding to 10-μmol T3 in the absence or presence of the PFAS was used to determine the binding properties of the PFAS. IC_{50} values were calculated by linear extrapolation between two responses located in the vicinity of a 50% inhibition level. All the PFASs had a lower affinity for the receptor than T3. Affinity of PFOA was less than that for PFDA, PFUnA, PFNA, and PFOS.

ToxCast Assay Results. The Toxicity Forecaster (ToxCast) database is a large high-throughput screening compilation of public *in vitro* and *in vivo* assays on over 9,000 chemicals (USEPA 2015). PFOA was tested in 1,084 assays and was active in 40 (USEPA 2015). Assays with less than 50% efficacy reported or overfitting issues are not included in the summary of results that follows.

Three of the acceptable ToxCast active cytotoxicity assays evaluated the impact of PFOA. All three of these assays are based on one cell type. If there was no cytotoxicity reported for a specific cell type, the AC50 (the minimum concentration with 50% cytotoxicity activity) was used for comparison when reporting the ToxCast results. The lowest recorded AC50 (109 μmol) measured the degradation of microtubules in liver cells at 109 μmol and the highest recorded (123 μmol) measured general cytotoxicity in liver cells.

PFOA activated two of the 21 estrogen related assays in ToxCast; both were ESR1-related. Estrogen and its receptors are essential for sexual development and reproductive function, but also play a role in other tissues such as bone. PFOA induced estrogen response element and inhibited ESR1 at concentrations lower than their AC50 values with concentrations of 33.8 μmol and 47.4 μmol, respectively. This implies that PFOA could have some estrogenic potential; however, due to the small fraction of estrogenic assays activated (10%), any activity is likely weak.

PFOA activated PPARs, PXR, CAR, and retinoic acid receptor (RAR) assays within the ToxCast program. From the PPAR assays, PFOA induced the DNA sequences for PPARα, PPARγ, and the peroxisome proliferator hormone response element (PPRE) and antagonized the PPARγ receptor. The only PPAR assay AC50 value that was above the cell-specific AC50 was that for PPARγ antagonism at 5.91 μmol. However, it is possible that cytotoxicity occurs due to PPAR induction, or that PPAR cytotoxicity leads to PPAR induction confounding interpretation of the outcome. PFOA induced DNA sequences for PXR (AC50 9.42 μmol) at a concentration lower than the cell-specific AC50. CAR and RAR alpha antagonism also was observed, but the concentrations of 17.57 μmol and 28.45 μmol, respectively, were not below the cell-specific cytotoxicity value. PPAR, PXR, CAR, and RAR pathways are all nuclear receptors that can form heterodimers with one another to induce translation of various genes. Some of these genes are important for development, reproduction, and waste degradation, and could play a role in PFOA-induced cancer.

The ToxCast program examined Cytochrome P450 (CYP) activation associated with PFOA exposure. Although PFOA is not metabolically active, it was found to activate four CYPs: CYP2C18, CYP2C19, and CYP2C9 in human cells and CYP2C11 in rat cells. All of the CYP assays were activated at concentrations lower than the lowest AC50 (109 μmol) but lacked cell-specific AC50s. The CYP2C class of CYPs is involved in the metabolism of xenobiotics such as the following drugs: the antiseizure medication diazepam, beta blocker propranolol, and selective serotonergic reuptake inhibitor citalopram. Though there is no evidence of metabolism of PFOA by these CYPs, it is possible that it acts as a competitive or allosteric inhibitor for known substrates of the CYPs activated. This coupled with PFOA's high affinity for binding to albumin could significantly alter the PKs of various pharmaceutical bound to serum albumin, thus potentially playing a role in increasing systemic toxicity of some pharmaceuticals by increasing the free serum concentration.

PFOA failed to cause toxicity in the *in vivo* fish model for neurological and developmental toxicity. This is important because PFOA induces developmental toxicity in mice and rats *in vivo*.

3.3.5 Structure-Activity Relationship

Bjork and Wallace (2009) compared the PPARα-dependent transcriptional activation potential of linear perfluorocarboxylic and sulfonic acids in rodent and human hepatic liver cells. The PFAAs tested included perfluorinated carboxylic acids with carbon chain lengths of 2–8 and perfluorinated sulfonic acids with chain lengths of 4–8. Primary rat and human hepatocytes and HEPG2/C3A cells were incubated with 0 and 25 μmol perfluorinated compounds for 24 hours. Expression of Acox, Cyp4a1 (rat), Cyp4a11 (human), Cte/Acot1, and Ddit3 (GADD153) transcripts were determined by quantitative RT-PCR. All the genes are inducible by peroxisome proliferators except Ddit3, which is induced in the presence of direct or indirect DNA damage.

Perfluorinated compounds induced mRNA expression of either Acox or Cte/Acot1 only in rat hepatocytes, and the degree of stimulation of gene expression increased with increasing carbon number. The Cyp4a11 gene was not expressed or stimulated by any of the PFAAs in HepG2/C3A cells. However, this gene expression was stimulated by perfluorinated exposure in both rat and human hepatocytes with the perfluorocarboxylates showing a chain-length-dependent structure activity relationship. The study results suggest that the PPARα-related changes in gene expression induced by perfluorinated compounds in primary rat hepatocytes are directly related to the carbon chain length and appear to be stronger for the carboxylic acids (i.e., PFOA) than the sulfonates (i.e., PFOS). There was no induction in expression of Acox and Cte/Acot 1 in either primary or transformed human liver cells in culture. The authors suggested that the PPARα mediated peroxisome proliferation observed in rodent liver might not be relevant as an indicator to human risk.

Wolf et al. (2008b) tested PFAAs, including PFOA, to determine if mouse and human PPARα activity could be induced in transiently transfected COS-1 cell assays. COS-1 cells were transfected with either a mouse or human PPAR-α receptor-luciferase reporter plasmid and, after 24 hours, were exposed to either negative controls (water or 0.1% DMSO), a positive control (Wyeth 14,643), or PFOA at 0.5–100 μmol. Other concentrations of PFAAs were used but not provided in this report. At the end of 24 hours of exposure, the luciferase activity was measured. The positive and negative controls had the expected results. A lowest observed effect concentration (LOEC) and no observed effect concentration (NOEC) were determined for each PFAA. In the study, the mouse PPARα was more responsive than the human. Also, carboxylates induced higher mouse and human PPARα activity than the sulfonates. In this study, the NOEC for PFOA was 0.5 μmol in the mouse and 5 μmol in humans; the LOEC was 1 μmol (0.43 μg/mL) in the mouse and 10 μmol (4.3 μg/mL) in humans.

A similar study included additional PFAAs (Wolf et al. 2012). Transfected cells were incubated with PFAAs at concentrations of 0.5 to 100 μmol, vehicle (water or 0.1% DMSO as negative control) or with 10 μmol Wyeth 14,643 (positive control) on each plate. Assays were performed with three identical plates per compound per species with nine concentrations per plate and eight wells per concentration. Cell viability was assessed using the Cell Titer Blue cell viability kit and read in a fluorometer. The positive and negative controls had the expected results. All cells transfected with either human and mouse PPARα responded to the PFAAs. Again, the carboxylates were stronger inducers than the sulfonates, and the mouse PPARα was more reactive than the human PPARα. The study also provided the C_{20max} values for each PFAA (the concentration at which the PFAA produced 20% of the maximal response elicited by the most active PFAA). For PFOA, this was 6 μmol in mouse PPARα and 7 μmol in human PPARα. For comparison, PFOS was 94 μmol and 262 μmol, respectively.

3.4 Hazard Characterization

3.4.1 Synthesis and Evaluation of Major Noncancer Effects

Serum Lipids. Because of the structural similarities between linear perfluorinated acids and the short- and medium-chain fatty acids, the potential for these chemicals to cause elevated serum lipids has been an area of considerable interest. High levels of serum lipids (TC and LDL) are risk factors for cardiovascular disease in humans, including IHD, a condition in which blood flow to the heart is decreased through the development of atherosclerotic plaque or clots in the cardiac arteries.

The association between PFOA and serum lipids has been examined in several studies in different populations. Cross-sectional and longitudinal studies in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b; Steenland et al. 2015) and in the high-exposure community (the C8 Health Project study population) (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009; Winquist and Steenland 2014a) generally observed positive associations between serum PFOA and TC in adults and children (aged 1–< 18 yrs); most of these effect estimates were statistically significant. Although exceptions to this pattern are present (i.e., some of the analyses examining incidence of self-reported high cholesterol based on medication use in Winquist and Steenland 2014a and in Steenland et al. 2015), the results are relatively consistent and robust. Similar associations were seen in analyses of LDL, but were not seen with HDL. The range of exposure in occupational studies is large (means varying between 0.4 and > 12 μg/mL), and the mean serum levels in the C8 population studies were around 0.08 μg/mL. Positive associations between serum PFOA and TC (i.e., increasing lipid level with increasing PFOA) were observed in most of the general population studies at mean exposure levels of 0.002–0.007 μg/mL (Eriksen et al. 2013; Fisher et al. 2013; Geiger et al. 2014a; Nelson et al. 2010; Starling et al. 2014). The interpretation of these general population results is limited, however, by the moderately strong correlations (Spearman r > 0.6) and similarity in results seen for PFOS and PFOA. The most recent update of disease incidence in workers in the C8 Health Project study population identified 35 cases of nonhepatitis liver disease (with medical validation) (Steenland et al. 2015); no association was seen with cumulative exposure when analyzed without a lag (HRs by quartile 1.0, 0.58, 1.43, 1.20; trend p = 0.86 for log cumulative exposure), but there was a possible trend in the analysis using a 10-year lag (HRs by quartile 1.0, 1.46, 2.13, and 2.02; trend p = 0.40).

Cholesterol and/or triglycerides were monitored in only a few of the animal studies, which did not all measure concurrent serum PFOA levels. Information on serum lipids from animal studies has received less attention than in the human population because of the fact that decreases in triglycerides, cholesterol, and lipoprotein complexes are an expected consequence of PPARα activation in rodents. The PPARα response in animals tends to lower rather than raise serum cholesterol and associated lipid levels. Peroxisomes are subcellular organelles that increase beta oxidation of long-chain fatty acids using a beta oxidation pathway that is not linked to adenosine triphosphate (ATP) production and release the shortened fatty acids to the cytosol as an endproduct for export in VLDLs or hepatic ATP-production via mitochondrial beta oxidation (Garrett and Grisham 1999). PPARα activation also stimulates metabolic changes that lower hepatic cholesterol. The effects of human PPARα activation are much less pronounced than those in rats and mice.

Nakamura et al. (2009) and Minata et al. (2010) examined the lipid endpoints relative to the mouse strain's PPARα status and PFOA exposure. Nakamura et al. (2009) found that mice with a normal PPARα receptor had significantly increased levels of cholesterol and triglycerides in liver but not plasma at a LOAEL of 0.3 mg/kg/day. However, there were no differences in serum or liver cholesterol or triglycerides between PFOA-treated mice with a humanized PPARα receptor or PPARα-null mice (NOAEL= 0.3 mg/kg/day) and their respective controls. The study by Minata et al. (2010) used higher doses than Nakamura et al. (2009) and found that TC was significantly decreased (LOAEL= 10.8 mg/kg/day; whole blood 47 μg/mL) and total triglycerides significantly increased (LOAEL= 5.4 mg/kg/day; whole blood 21 μg/mL) in wild-type mice. In the PPARα-null mice, the TC was significantly decreased for the 5.4- and 10.8-mg/kg/day doses but significantly increased for a 21.6-mg/kg/day dose while total triglycerides were significantly increased at all doses; these doses corresponded to whole blood PFOA levels of 13, 36, and 71 μg/mL, respectively. Rosen et al. (2007) found that PFOA activated genes for fatty acid catabolism, cholesterol biosynthesis; bile acid biosynthesis; and lipoprotein, steroid, and glucose metabolism in fetal livers. When comparing the response in PPARα wild-type to null mice (Rosen et al. 2008b), 62 of 81 activated genes were the same for both strains and were ones involved with lipid metabolism.

Martin et al. (2007) identified a 45–72% decrease in serum cholesterol after treatment of male Sprague-Dawley rats with 20 mg PFOA/kg/day for up to 5 days (serum PFOA 245 μg/mL after 3 days), and Loveless et al. (2008) reported decreased TC, HDL, and non-HDL in male CD rats after doses of 0.3 and 1 mg/kg/day for 28 days. Triglycerides were decreased in the rats at ≥0.3 mg/kg/day. De Witt et al. (2009) found a dose-dependent decrease in triglyceride levels in female C57BL/6N mice exposed to 0, 7.5, and 15 mg PFOA/kg bw in drinking water for 10 days. In male CD-1 mice, TC, HDL, and triglycerides were decreased at 10 and 30 mg/kg/day (Loveless et al. 2008). In pregnant female ICR mice, triglyceride, TC, and free fatty acids were significantly decreased at 10 mg/kg (Yahia et al. 2010). Elcombe et al. (2010) found a significant decrease in cholesterol in male Sprague-Dawley rats following a 7- or 28-day exposure to 300 ppm PFOA in the diet with a resulting serum level of 252 μg/mL at 28 days. Accordingly, there is not a high degree of concordance between the lipidemic effects of PFOA as noted in human epidemiology studies and those seen in animals.

Filgo et al. (2015) found a trend for increased liver Ito (fat) cell atrophy and lesion severity across the doses in CD-1 and SV-129 mice at 18 months. PFOA exposure occurred only through the dam during gestation and lactation in this study. This observation suggests that liver steatosis could be a concern late in life for animals exposed to PFOA gestationally and during their early postnatal period. However, the 18-month fat accumulation could also be related to normal aging and/or dietary fat intakes across the animal's lifetime (Quist et al. 2015). Tan et al. (2013) found that the fat content of the diet was an important variable in determining the impact of PFOA (5 mg/kg/day) on liver and serum lipids. Intake of an HFD plus PFOA increased liver triglycerides and serum free fatty acids compared to an RFD plus PFOA but had no impact on liver cholesterol concentrations. Serum cholesterol was not monitored.

Hepatic Effects. Both the human and animal studies suggest effects on the liver as indicated by increases in liver enzymes. The results of the occupational studies provide evidence of an association with increases in serum AST, ALT and GGT, with the most consistent results seen for ALT. The associations were not large and could depend on the covariates in the models, such as BMI, use of lipid-lowering medications, and triglycerides (Costa et al. 2009; Olsen et al. 2000, 2003; Olsen and Zobel 2007; Sakr et al. 2007a, 2007b). Two population-based studies of highly

exposed residents in contaminated regions near a fluorochemical industry in West Virginia have evaluated associations with liver enzymes, and the larger of the two studies reported associations of increasing serum ln ALT and ln GGT levels with increasing serum PFOA concentrations (Emmett et al. 2006; Gallo et al. 2012). A cross-sectional analysis of data from NHANES, representative of the U.S. national population, also found associations with ln PFOA concentration with increasing serum ALT and ln GGT levels. Serum bilirubin was inversely associated with serum PFOA in the occupational studies. A U-shaped exposure-response pattern for serum bilirubin was observed among the participants in the C8 Health Project, which might explain the inverse associations reported for occupational cohorts. Overall, an association of serum PFOA concentration with elevations in serum levels of ALT and GGT has been consistently observed in occupational, highly exposed residential communities, and the U.S. general population. The associations are not large in magnitude, but indicate the potential to affect liver function.

The data from animal studies for increases in ALT and AST support the findings in human epidemiology studies; however, the animal studies for both aminotransferases lacked serum PFOA measurements for comparison with the human serum data. Concurrent with the evidence in animals of damage to liver cells, levels of some membrane transport proteins were altered. In mice, the increased expression of MRP3 and MRP4 (Maher et al. 2008) and the decreased expression of OATPs (Cheng and Klaassen 2008) favor excretion of PFOA into the bile. Competition of PFOA with bile acids for transport could alter the excretion of the cholesterol derivatives excreted in bile.

In animal studies, serum levels of ALT and/or AST were significantly increased indicating apoptosis or necrosis of liver cells (Butenhoff et al. 2012; Minata et al. 2010; Son et al. 2008). Increased levels of ALT were observed at a LOAEL of 2.65 mg/kg/day in ICR mice by Son et al. (2008). Yahia et al. (2010) reported significantly increased ALT, GGT, AST, and ALP in PFOA-exposed (10 mg/kg) pregnant ICR mice. Total protein, albumin, and globulin were significantly decreased in the same mice.

No evidence of liver damage has been found in the human epidemiology studies with the exception of the few enzyme changes discussed above. In most PFOA animal studies (e.g., monkeys, rats, and mice), short-term and chronic exposure caused a dose-related increase in liver weight as at least one of the co-occurring effects (Butenhoff et al. 2002, 2004a, 2012; DeWitt et al. 2009; Elcombe et al. 2010; Minata et al. 2010; Pastoor et al. 1987; Perkins et al. 2004; Son et al. 2008; Wolf et al. 2008a). Increased liver weights were observed in mice that are both active and null for PPARα activation (Albrecht et al. 2013; Minata et al. 2010; Wolf et al. 2008a). The histological characteristics of the liver differed in the mice with and without the PPARα receptor, but the liver weight increase was the same. Liver effects were seen in mice with an active PPARα receptor at doses as low as 0.3 mg/kg/day (Nakamura et al. 2009) and 1 mg/kg/day in the null mice (Wolf et al. 2008a).

Histological examination of liver tissues from PFOA-exposed wild-type mice and PPARα-null mice were distinctly different from their respective controls (Minata et al. 2010; Wolf et al. 2008a). In the case of the wild-type PFOA-exposed mice, there was less rough ER than in controls and more lipid-like vacuoles scattered throughout the cytoplasm. The PFOA-exposed PPARα-null mice had proliferation of smooth ER and limited rough ER and Golgi bodies compared to their controls. The PPARα-null control mice had the scattered lipid-like vacuoles seen in the wild-type PFOA exposed mice; however, their lipid-like vacuoles were considerably larger than those seen in the wild-type animals and occupied a considerable volume within the

cytoplasm. The vacuoles in the PPARα-null PFOA-exposed mice were hypothesized to be filled with PFOA as a consequence of its uptake into the cell without dispersion or assimilation.

Similarly, Albrecht et al. (2013) observed centrilobular hepatocellular hypertrophy in mouse dams given 3 mg/kg on GDs 1–17, but the morphological features differed slightly between wild-type, PPARα-humanized, and PPARα-null mice. In wild-type mice, hypertrophy was characterized primarily by centrilobular hepatocytes with increased amounts of densely eosinophilic and coarsely granular cytoplasm consistent with increased peroxisomes. In null mice, hypertrophy was generally less prominent than seen in wild-type mice, and affected hepatocytes had pale eosinophilic, finely granular-to-amorphous cytoplasm. The morphological features of centrilobular hepatocytes in humanized mice were intermediate between those observed in wild-type and null mice. The lesion was graded as mild in wild-type mice, minimal in null mice, and minimal or mild in humanized mice. An additional finding in PFOA-treated null and humanized mice, but not in wild-type mice, was the presence of few clear, discrete vacuoles within the cytoplasm of centrilobular hepatocytes.

Hepatocellular hypertrophy and an increased liver-to-body weight ratio are common findings in rodents when PPARα activation leads to peroxisome proliferation and these effects are considered nonadverse in wild-type strains when they occur. Hepatic necrosis, effects on bile ducts, and other signs of liver damage unrelated to PPARα activation observed in conjunction with the increased liver weight and hepatpcellular hypertrophy are sufficient to justify the liver weight and hypertrophy as adverse (Hall et al. 2012). Low-level necrotic cell damage was observed in the Perkins et al. (2004) rat study and in the Loveless et al. study (2008) in CD rats at 10 mg/kg/day and CD1 mice at 1 mg/kg/day. In the Perkins et al. study (2004), there was a slight increase in coagulative necrosis at 1.94 and 6.5 mg/kg/day when compared to the control and lower doses. Some hepatocellular necrosis also was observed in conjunction with hepatocellular hypertrophy and increased liver weight in F1 male rats from the Butenhoff et al. (2004a) two-generation study at 3 mg/kg/day.

Minata et al. (2010) reported degenerative histological changes in the bile ducts of PPARα-null mice at doses ≥10.6 mg/kg/day and Loveless et al. (2008) observed bile duct hyperplasia in CD1 mice at doses ≥10 mg/kg/day. PPARα-null mice had an increased hepatocyte PCNA labeling index at a dose of 10 mg/kg/day (Wolf et al. 2008a). When considering the studies in animals with and without the active PPARα receptor, it is clear that PFOA has some effects of potential toxicological significance that appear to be independent of PPARα activation.

Kidney and Other Organ Effects. Overall, studies of occupational cohorts (Costa et al. 2009), a highly exposed community (Steenland et al. 2010; Watkins et al. 2013), and the U.S. general population (Shankar et al. 2011) that evaluated uric acid levels or eGFR as measure of kidney function found associations with decreased function. Reverse causality as an explanation cannot be ruled out in studies using serum PFOA as a biomarker of exposure, as a low GFR would diminish the removal of PFOA from serum for excretion by the kidney.

Some studies in animals have shown effects on the kidney, mainly increased organ weight in male rats, but the studies lacked concurrent PFOA serum levels and histological examination of the kidney tissues. In general, kidney effects in rats occurred at doses similar to those resulting in liver effects.

Increases in absolute and relative-to-body kidney weights occurred in rats given 5 mg/kg/day (lowest dose tested) for 28 days (Cui et al. 2009). In a two-generation study, F0 and F1 males

had significantly increased absolute kidney weight at 1 and 3 mg/kg/day, but significantly decreased organ weight at 30 mg/kg/day. Organ weight-to-terminal body weight ratios for the kidney were statistically significantly increased at ≥1 mg/kg/day. Kidney weight-to-brain weight ratios were significantly increased at 1, 3, and 10 mg/kg/day, but decreased at 30 mg/kg/day, following the trends in absolute weights (Butenhoff et al. 2004a). In the high-dose group, absolute and relative kidney weight changes occurred in a pattern typically associated with decrements in body weight. However, in the lower dose groups kidney weight, consistently displayed an increase (absolute and relative to body and brain weights), suggesting an induction of transporters for renal removal of the foreign molecule. The differential expression of transporters in the kidney of rats has been shown to be under hormonal control with males having lower levels of export transporters compared with females (Kudo et al. 2002).

In both the Cui et al. (2009) and Butenhoff et al. (2004a) studies, PFOA was administered by daily gavage. No changes in kidney weight were found with dietary administration with a resulting dose of 14.2 mg/kg/day to male rats for 2 years (Butenhoff et al. 2012).

In general, effects on organs other than the liver tend to occur at doses higher than those that affect the liver. Lung effects, including pulmonary congestion, have been observed in male Sprague-Dawley rats (LOAEL = 5 mg/kg/day) (Cui et al. 2009). Increased thickness and prominence of the adrenal zona glomerulosa and vacuolation in the cells of the adrenal cortex were observed in male rats fed 10 mg/kg/day for approximately 56 days (Butenhoff et al. 2004a).

Thyroid Effects. Three large studies provide support for an association between PFOA exposure and incidence or prevalence of thyroid disease in female adults or children, but not in males (Lopez-Espinosa et al 2012; Melzer et al. 2010; Winquist and Steenland 2014b). In addition, associations between PFOA and TSH were also seen in pregnant females with anti-TPO antibodies (Webster et al. 2014). However, generally null associations were found between PFOA and TSH or thyroid hormones (T4 or T3) in people who have not been diagnosed with thyroid disease.

Effects of PFOA on thyroid hormones in animals are generally not as well characterized as those of PFOS. Butenhoff et al. (2002) evaluated the toxicity of PFOA in male cynomolgus monkeys during 6 months of oral administration and reported that levels of total T3 and FT3 in circulation were reduced significantly in the 30/20 mg/kg/day treatment group. The effect seen as early as 5 weeks after initiation of treatment, 2 weeks after the dose was lowered to 20 mg/kg/day. Recovery of T3 deficits was noted upon cessation of chemical treatment once the serum level of PFOA returned to baseline 90 days later. Serum total T4, FT4, and TSH were not altered throughout the study. The preferential effects of PFOA on serum T3 and a lack of a TSH compensatory response are similar to those observed with PFOS.

Martin et al. (2007) showed that serum total T4 and FT4 were markedly and abruptly depressed (~ 80%) in adult male rats 1 day after oral gavage treatment with PFOA (20 mg/kg); serum T3 was also reduced (25%), although to a lesser extent. These findings were confirmed when both male and female rats were given PFOA (10 mg/kg) daily for 3 weeks and serum thyroid hormones were monitored (Lau, personal communication) (Martin et al. 2007). Serum total T4 and FT4 were profoundly depressed (>85%) and T3 less so (~ 25%) in male rats, but serum TSH levels were not altered significantly. These hormonal changes were noted when serum PFOA level reached about 67 µg/ml. The dose-response relationship of serum total T4 with PFOA exposure has yet to be fully evaluated and the lowest effective dose remains unknown.

None of the thyroid hormones were affected by PFOA in mature female rats, primarily because these animals were able to clear the chemical effectively (with half-life estimate of 2–4 hours compared to that of 6–7 days for male rats). This suggests that the thyroid disrupting effects of PFOA are directly related to endogenous accumulation of the chemical and could be relevant to humans because of the long PFOA human half-life.

Displacement of T4 from binding to TTR has been proposed as a possible mechanism to account for the hypothyroxinemia in rats. However, although PFOA binds to human TTR, its binding affinity is only one-fifteenth of that of the natural ligand T4 (Weiss et al. 2009). Based on a toxicogenomic analysis of rat liver after an acute exposure to PFOA, Martin et al. (2007) suggested a possible role of peroxisome proliferators in the thyroid hormone imbalance, although this hypothesis has yet to be explored in detail.

Hyperglycemia. Several human epidemiology studies have examined PFOA in relation to diabetes (incidence or prevalence) or measures of hyperglycemia These studies do not show a pattern of results that suggest an association between PFOA and diabetes or hyperglycermia in occupational settings (Costa et al. 2009; Olsen et al. 2000, 2003; Sakr et al. 2007a; Steenland et al. 2015), in the high-exposure community population (MacNeil et al. 2009), or in the general population (Lin et al. 2009; Nelson et al. 2010).

Hines et al. (2009) found no differences in glucose tolerance tests at 15–16 weeks and at 17 months of age in PFOA-exposed CD-1 mice, but did observe significantly increased serum leptin and insulin levels at 21 and 31 weeks of age, suggesting that the insulin resistance mechanistic pathway could be affected by PFOA. Conversely, Quist et al. (2015) found no dose-related impact on serum leptin in CD-1 pups from the Hines et al. study (2009) when examined on PND 91 for the mice on an RFD and on an HFD fasted for 4 hours before serum collection. In the animals on a HFD that did not fast before serum collection, there was a trend towards a dose-related decrease in serum leptin. Thus, the fat content of the diet and the timing of serum collection are important variables that can influence study results relative to leptin levels and indicators of insulin resistance.

Nervous System Effects. The data pertaining to neurotoxicity (including neurodevelopmental effects) of PFOA are limited, but do not indicate the presence of associations between PFOA and a variety of outcomes. Fei et al. (2008b) found no association between maternal serum PFOA concentrations and fine motor skills, gross motor skills, and cognitive abilities of children aged 6 and 18 months. Fei and Olsen (2011) found no association between behavioral or coordination problems in children aged 7 years and prenatal PFOA exposure. Epidemiology studies of children derived from the NHANES and C8 populations found a weak statistical association between serum PFOA with parental reports of ADHD (Hoffman et al. 2010; Stein et al. 2013).

One animal study (Johansson et al. 2009) suggests a potential effect on habituation and activity patterns in NMRI mice treated on PND 10 with a single dose of PFOA and evaluated at and 2 and 4 months of age (LOAEL=0.58 mg/kg). The *in vivo* observations were supported by changes in the expression of a variety of neurologically active brain proteins in the treated pups (Johansson et al. 2009). The offspring of C57BL/6/Bkl dams fed 0.3 mg PFOA/kg/day throughout gestation had detectable levels of PFOA in their brains at birth (Onishchenko et al. 2011). Behavioral assessments of the offspring starting at 5 weeks of age revealed gender-related differences in exploratory behavior patterns. In the social group setting, the PFOA-exposed males were more active and PFOA-exposed females were less active than their respective controls. The PFOA-exposed males also had increased activity counts compared to control males

in circadian activity experiments. The results of an *in vitro* study of hippocampal synaptic transmission and neurite growth in the presence of long-chain perfluorinated compounds showed that 50 and 100 μmol PFOA increased spontaneous synaptic current and had an equivocal impact on neurite growth (Liao et al. 2009a, 2009b). These data suggest a need for additional studies of the effects of PFASs, including PFOA, on the brain.

Reproductive and Developmental Effects. There have been numerous human studies examining PFOA exposure and reproductive and/or developmental effects in both humans and animals. A series of studies in the high-exposure C8 Health Project study population have reported associations between PFOA exposure and pregnancy-induced hypertension or preeclampsia (Darrow et al. 2013; Savitz et al. 2012a, 2012b; Stein et al. 2009). Each of these studies provides evidence of an association between PFOA exposure and risk of pregnancy-induced hypertension or preeclampsia, with the most robust findings from the methodologically strongest study (Darrow et al. 2013).

The association between PFOA and birth weight has been examined in numerous human studies. Most studies measured PFOA using maternal blood samples taken in the second or third trimester or in cord blood samples. Studies on the high-exposure C8 community population (Darrow et al. 2013; Nolan et al. 2009; Stein et al. 2009; Savitz et al. 2012a, 2012b) have not observed associations between PFOA and either birth weight among term births or the risk of low birth weight among all (singleton) births. In contrast, several analyses of general populations indicate a negative association between PFOA levels and birth weight (Apelberg et al. 2007; Fei et al. 2007; Maisonet et al. 2012), while others did not attain statistical significance (Chen et al. 2012; Hamm et al. 2010; Monroy et al. 2008; Washino et al. 2009). A meta-analysis of many of these studies found a mean birth weight reduction of 19 g (95% CI: -30, -9) per each 1-unit (ng/mL) increase in maternal or cord serum PFOA levels (Johnson et al. 2014). However, when low GFR was accounted for in PBPK simulations by Verner et al. (2015), the association reported between PFOA and birth weight is less than that found in their meta-analysis of the epidemiology data. The study authors reported that the actual association might be closer to a 7-g reduction (95% CI: -8, -6). Verner et al. (2015) also showed that, in individuals with low GFR, there are increased levels of serum PFOA and lower birth weights. This suggests that a portion of the association between PFOA and birth weight could be confounded by low maternal GFR under conditions such as preeclampsia and pregnancy-induced hypertension. While there is some uncertainty in the interpretation of the observed association between PFOA and low GFR with birth weight, given the available information, the association between PFOA exposure and reduced birth weight observed for the general population is plausible. In humans with low GFR, the impact on body weight is likely due to a combination of the low GFR and the serum PFOA.

Two studies examined development of puberty in females in relation to prenatal exposure to PFOA as measured through maternal or cord blood samples in follow-up of pregnancy cohorts conducted in England (Christensen et al. 2011) and in Denmark (Kristensen et al. 2013). The results of these two studies are conflicting, with no association (or a possible indication of an earlier menarche seen with higher PFOA) in Christensen et al. (2011), and a later menarche seen with higher PFOA in Kristensen et al. (2013). Another study examined PFOA exposure measured concurrently with the assessment of pubertal status (Lopez-Espinosa et al. 2011). An association between later age at menarche and higher PFOA levels was observed, but the interpretation of this finding is complicated by the potential effect of puberty on the exposure biomarker levels (i.e., reverse causality).

Limited data suggest a correlation between higher PFOA levels (>0.02 μg/mL) in females and decreases in fecundity and fertility (Fei et al. 2009; Vélez et al. 2015), but there are no clear effects of PFOA on male fertility endpoints (0.0035–0.005 μg/mL) (Joensen et al. 2009, 2013).

Knox et al. (2011) found that the odds of having experienced menopause were significantly higher in the highest PFOA quintile group relative to the lowest PFOA group. Two studies found delayed puberty in females (Kristensen et al. 2013; Lopez-Espinosa et al. 2011), but reverse causality needs to be considered. However, Christensen et al. (2011) found no association between puberty and PFOA exposure in children of the Avon Longitudinal Study of Parents and Children in the United Kingdom. Removal of PFOA with the start of monthly menstruation and the cessation of this route with menopause or hysterectomy are additional factors that can influence serum PFOA levels that are the result of the developmental milestones rather than a cause (Taylor et al. 2014; Wong et al. 2014). Costa et al. (2009) found no association between serum PFOA concentration and estradiol or testosterone in workers at a PFOA production plant.

Measures of postnatal development and behavior in children were not associated with PFOA levels in the mother (0.001–0.0057 μg/mL) (Andersen et al. 2010, 2013; Fei et al. 2008b; Fei and Olsen 2011; Høyer et al. 2015a, 2015b). Fei et al. (2008b) found no association between maternal PFOA concentration and fine motor skills, gross motor skills, and cognitive skills in offspring at 6 and 18 months of age. Fei and Olsen (2011) also found no association between prenatal PFOA exposure and behavior or coordination problems in children aged 7 years. The age at which children reached developmental milestones did not show any relationship to maternal plasma PFOA concentration. Halldorsson et al. (2012) found that low-dose developmental exposures to PFOA resulted in obesogenic effects in female offspring at 20 years.

Among the animal studies, there was no effect of PFOA on reproductive or fertility parameters in rats (Butenhoff et al. 2004a; York et al. 2010), but effects on male fertility were observed in male mice (Lu et al. 2015). In mouse gavage studies, decreased body weight and decreased neonatal survival were observed at ≥ 1 mg/kg/day, increased full litter resorptions and increased stillbirths were observed at ≥ 5 mg/kg/day, increased time to parturition was observed at ≥10 mg/kg/day, and decreased maternal weight gain was observed at ≥20 mg/kg/day for exposures lasting from GD1–17 (Abbott et al. 2007; Lau et al. 2006; White et al. 2007; Wolf et al. 2007).

Postnatal development also has been studied extensively in rats and mice as discussed below. A separate group of studies in mice focused on mammary gland development in dams and female offspring. Both species showed some indication of potential developmental toxicity. Doses that elicited a response were higher in rats compared with in mice. The species differences in dose response are likely related to half-life differences of hours for the female rat and days-to-weeks for the female mouse.

Reduced postnatal growth leading to developmental delays was observed in both rats and mice. A two-generation diet study in rats resulted in significantly decreased body weight gain prior to weaning and delayed sexual maturity in the first generation males and females at 30 mg/kg/day (Butenhoff et al. 2004a). For treatment beginning on PND 21, delayed vaginal opening was also observed in BALB/c mice at ≥1 mg/kg/day and in C57BL/6 mice at ≥5 mg/kg/day, although body weight was not decreased until doses of ≥10 mg/kg/day in both strains (C. Yang et al. 2009). Cross-fostering studies in mice showed that gestational PFOA exposure maximized decreased postnatal body weight, delayed eye opening, delayed body hair growth, and decreased survival in the offspring (Wolf et al. 2007). Restricted exposure studies

showed that gestational exposure to PFOA over differing gestational time periods led to differing offspring effects (Wolf et al. 2007). The longer the gestational exposure, the greater the body weight deficit in the male and female pups over PND 2–22. In males, the difference in body weight persisted until PND 92. Delayed eye opening and body hair growth were observed at 5 mg/kg/day in offspring exposed GD 7–17 or 10–17, but decreased postnatal survival was observed at the same dose in offspring exposed GD 15–17.

Two developmental studies compared wild-type mice with PPARα-null mice, but results are inconclusive. One study revealed that the litter resorptions were independent of PPARα expression (≥5 mg/kg), while decreased neonatal survival (0.6 mg/kg) and delayed eye opening (1 mg/kg) were dependent upon PPARα expression (Abbott et al. 2007). These results are only partially supported by Albrecht et al. (2013), who used a single dose of 3 mg/kg. They found decreased pup survival only in wild-type mice, but no differences in litter resorptions or eye opening between wild-type and null mice. Albrecht et al. (2013) did not find effects on pup survival in PPARα-humanized mice, suggesting that the mouse PPARα could be involved in the etiology of PFOA-induced neonatal mortality.

Qualitative assessment found delayed mammary gland development of female CD1 mouse pups following maternal doses ≥ 0.01 mg PFOA/kg in Macon et al. (2011) and Tucker et al. (2015). Macon et al. (2011) also found significant differences from controls in quantitative measures of longitudinal and lateral growth and numbers of terminal end buds at 1 mg/kg/day. However, Albrecht et al. (2013) found no significant differences in the average length of mammary gland ducts and the average number of terminal end buds per mammary gland per litter in female pups of PPARα wild-type, PPARα-null, or hPPARα sv/129 mice following a maternal dose of 3 mg/kg, using an approach to scoring that lacked a qualitative component adjustment such as that used by Macon et al. (2011).

The approach to scoring mammary gland development was not consistent across studies, and little information was provided on the qualitative components of the scores. This makes comparisons across studies difficult. Statistical significance was attained at higher dose levels for the quantitative portion of the Macon et al. (2011) scoring protocol than for the qualitative component of the score. The process used to score the qualitative developmental score by Macon et al. (2011) was not described. Tucker et al. (2015) found that CD-1 mice were considerably more sensitive to effects on mammary gland development (LOAEL = 0.01 mg/kg/day) than C57BL/6 mice (NOAEL 0.1 mg/kg/day). Scoring was conducted using the Macon et al (2011) approach.

White et al. (2011) used doses of 0 and 1 mg PFOA/kg/day to F0 dams throughout gestation with and without the addition of drinking water containing 5 ppb PFOA beginning on GD 7 and continuing the contaminated drinking water during the production of two more generations; no persistent significant differences were found in the body weights of the pups in the F1 and F2 generations for the pups receiving 1 mg/kg/day, indicating a poor correlation between mammary duct branching patterns and the ability to support pup growth during lactation. The 5 mg/kg/day dose did have an impact on body weight. Albrecht et al. (2013) also found no significant impacts on pup body weight in their one-generation assay at a dose of 3 mg/kg/day. Despite the diminished ductal network assessed in the qualitative mammary gland developmental score of the dams in White et al. (2011), milk production was sufficient to nourish growth in the exposed pups as reflected in the body weight measurements compared to controls. The MoA for PFOA-induced delayed mammary gland development is unknown and requires further investigation.

At doses of 5 and 10 mg/kg/day, mammary gland development was delayed in BALB/c mice (C. Yang et al. 2009). In C57BL/6 mice, mammary gland development was accelerated at 5 mg/kg/day, but delayed at 10 mg/kg/day, indicating strain differences in pubertal mammary gland development following a dose of 5 mg/kg/day. Y. Zhao et al. (2010) showed that 5 mg PFOA/kg/day stimulates mammary gland development in C57BL/6 mice by promoting steroid hormone production in the ovaries and increasing mammary gland growth factor levels.

Immune Effects. Associations between prenatal, childhood, or adult PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been consistently seen, although there was some indication of effect modification by gender (i.e., associations seen in female children but not in male children) (Fei et al. 2010a; Granum et al. 2013; Looker et al. 2014; Okada et al. 2012).

The WHO guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications (WHO 2012). Three studies have examined associations between maternal and/or child serum PFOA levels and vaccine response (measured by antibody levels) in children (Grandjean et al. 2012; Granum et al. 2013) and adults (Looker et al. 2014). The study in adults was part of the high-exposure community C8 Health Project; a reduced antibody response to one of the three influenza strains tested after receiving the flu vaccine was seen with increasing levels of serum PFOA; these results were not seen with PFOS. The studies in children were conducted in general populations in Norway and in the Faroe Islands. Decreased vaccine response in relation to PFOA levels was seen in these studies, but similar results also were seen with correlated PFASs (e.g., PFOS).

Several animal studies demonstrate effects on the spleen and thymus as well as their cellular products (B lymphocytes and T-helper cells) in several strains of mice. Studies by Yang et al. (2000, 2001, 2002b) and DeWitt et al. (2008) were conducted using relatively high PFOA doses (~30–40 mg/kg/day). In each study, the PFOA-treated animals exhibited significant decreases in spleen and thymus weights as well as in splenocyte and thymocyte populations at various stages of differentiation. Recovery usually occurred within several days of cessation of PFOA dosing. However, when the response of C57BL/6 Tac PPARα mice was compared to wild-type of the same strain, the KO mice showed no response of both spleen and thymus weights at 30 mg/kg/day, whereas there was a response in the wild-type strain (DeWitt et al. 2015). Both strains showed an increase in IgM in response to a SRBC injection. The 30-mg/kg/day dose was the LOAEL for the KO mice and 7.5 mg/kg/day was the response level for the wild-type strain. Thus the suppression of the immune system is not totally a PPARα-related response. In a similar experiment (Yang et al. 2002a), no significant changes in spleen weight or cellularity were observed in PPARα-null mice as compared to wild-type mice, but there was a small and significant decrease in thymus weight and cellularity compared to controls.

DeWitt et al. (2008) used different functionality assays in their study in C57Bl/6 mice. The IgM response to SRBC was suppressed by 20% when mice were immunized immediately after exposure to the initial dose of 30 mg PFOA/kg/day ceased. However, there was no significant increase in the response to BSA 4 days post-PFOA exposure or in the IgG response to SRBC 15 days post-PFOA exposure. These results are indicative of recovery once PFOA exposures have ceased.

DeWitt et al. (2008) followed their initial study of PFOA with one designed to examine the dose response for a 15-day drinking water exposure in a slightly different mouse strain, C57Bl/6N. The study design examined the spleen and thymus weights, splenocyte and thymocyte numbers, and IgM response of the immune system to the immunological challenges as described above. The LOAEL was 3.75 mg/kg/day based on a significant decrease in IgM response, and the NOAEL was 1.88 mg/kg/day.

In one component of the Yang et al. study (2002b), the functional impact of changes in spleen and thymus were evaluated through the response of treated mice to HRBCs. The control mice responded to the HRBC exposure with an increased plaque-forming response; however, the PFOA-treated mice did not have an increased plaque-forming response when tested (Yang et al. 2002b). In addition, when blood from PFOA-treated mice was evaluated posttreatment, there was no increase in lymphocyte proliferation in response to the addition of Con-A and LPS to the test media. The control mice responded with the expected lymphocyte proliferation after the addition of Con-A and LPS antigens.

Loveless et al. (2008) looked at the IgM response to SRBC in male CD rats and CD-1 mice following a 29-day exposure to 0–30 mg PFOA/kg/day. The thymus and spleen cell counts and organ weights and the IgM titers were not altered by PFOA treatment in rats. In mice, however, thymus and spleen weights, thymus and spleen cell counts, and IgM titers were decreased at ≥10 mg PFOA/kg/day. CORT also was increased in mice at the same doses.

The data collected from the immunotoxicity studies support a MoA through which PFOA interferes with splenocyte and thymocyte precursor cells in the bone marrow as well as maturation of the cells once they have been transported to their respective organs. Examination of cell populations at different stages of development reveals lower numbers of the $CD4^{-}CD8^{-}$ cells formed in bone marrow as well as decreased populations of splenocyte and thymocyte cells at different stages of expressing the surface proteins that mark them as functional beta lymphocytes (thymus) or T-helper cells (spleen) (Son et al. 2009). Although the studies that measured the splenocyte and thymocyte populations were carried out at doses higher than the 3.75 mg/kg/day LOAEL observed by DeWitt et al. (2008), the fact that the IgM response to an antigenic material was decreased at that dose indicates an inability to produce antibodies at adequate levels when exposed to a challenge.

Loveless et al. (2008) hypothesized that the observed effects on serum lymphocytes could be the result of adenocorticotropic steroids in a response to stress. A study by DeWitt et al. (2009) in which the immunological response of ADX mice treated with PFOA were compared to sham-operated controls did not support the Loveless et al. (2008) hypothesis.

Data from PPARα-null mice suggest that rodents might be more susceptible to the immunosuppressive impacts of PFOA than humans. However, the fact that there were still effects on the thymus weight and cellularity even in the PPARα-null mouse strain indicate the potential for an inadequate humoral response in exposed populations.

3.4.2 Synthesis and Evaluation of Carcinogenic Effects

Evidence of carcinogenic effects of PFOA in epidemiology studies is based primarily on studies of kidney and testicular cancer. These cancers have relatively high survival rates (e.g., 2005–2011 5-year survival rates 73% and 95%, respectively, for kidney and testicular cancer based on NCI Surveillance, Epidemiology and End Results data). Thus studies that

examine cancer incidence are particularly useful for these types of cancer. The high-exposure community studies also have the advantage, for testicular cancer, of including the age period of greatest risk, as the median age at diagnosis is 33 years. The two occupational cohorts in Minnesota and West Virginia (most recently updated in Raleigh et al. 2014 and Steenland and Woskie 2012) do not support an increased risk of these cancers, but each of these is limited by a small number of observed cases (six kidney cancer deaths, 16 incident kidney cancer cases, and five inciden testicular cancer cases in Raleigh et al. 2014; and 12 kidney cancer deaths and 1 testicular cancer death in Steenland and Woskie 2012). Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 0.024 μg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013); there is some overlap in the cases included in these studies. No associations were found in the general population between mean serum PFOA levels up to 0.0866 μg/mL and colorectal, breast, prostate, bladder, and liver cancer (Bonefeld-Jørgensen et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014); none of these studies examined kidney or testicular cancer.

Two animal carcinogenicity studies indicate that PFOA exposure can lead to liver adenomas (Biegel et al. 2001), Leydig cell adenomas (Biegel et al. 2001; Butenhoff et al. 2012), and PACTs (Biegel et al. 2001) in male Sprague-Dawley rats. Liver adenomas were observed in the Biegel et al. study (2001) at an incidence of 10/76 (13%) at 20 mg/kg/day. The incidence in the control group was 2/80 (3%). Although no liver adenomas were observed in Butenhoff et al. (2012), carcinomas were identified in the male controls, males in the low-dose group (2 mg/kg/day), and male and female rats in the high-dose group (20 mg/kg/day). The differences from control were not significant in either study, but the carcinoma incidence among the Butenhoff et al. (2012) high-dose males (10/50) was similar to that for the adenomas in the Biegel et al. study (2001) (10/76). Liver lesions were identified in the males and females at the 1- and 2-year sacrifices (Butenhoff et al. 2012). An increased incidence of diffuse hepatomegalocytosis and hepatocellular necrosis occurred at 20 mg/kg/day. At the 2-year sacrifice, hepatic cystoid degeneration (characterized by areas of multilocular microcysts in the liver parenchyma) was observed in 8, 14, and 56% in males of the control, 2-, and 20-mg/kg/day dose groups, respectively. Hyperplastic nodules in male livers were increased in the high-dose group (6% versus 0% in control rats).

Filgo et al. (2015) examined the livers of three strains of mice exposed only during gestation/lactation for tumors when they were sacrificed at 18 months. Liver tumors were found in each dose group, but tumor types varied and the data did not display any evidence of dose response. The animals were survivors from two different projects and the number per dose group was small. Thus, the data are not adequate for determining whether PFOA is a carcinogen in mice.

Testicular LCTs were identified in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The tumor incidence was 0/50 (0%), 2/50 (4%), and 7/50 (14%) for the control, 2.0-, and 20-mg/kg/day dose groups, respectively (Butenhoff et al. 2012). The Biegel et al. study (2001) included one dose group (20 mg/kg/day); the tumor incidence was 8/76 (11%) compared to 0/80 (0%) in the control group. LCT incidence at 20 mg/kg/day was comparable between the two studies (11 and 14%).

PACTs were only observed in the Biegel et al. study (2001). The incidence was 8/76 (11%; 7 adenoma, 1 carcinoma) at 20 mg/kg/day while none were observed in the control animals. Although no PACTs were observed by Butenhoff et al. (2012), pancreatic acinar hyperplasia was observed at 2 and 20 mg/kg/day at incidences of 2/34 (6%) and 1/43 (2%), respectively, which

lacked dose response. Reexamination of the pancreatic lesions in Butenhoff et al. (2012) and Biegel et al. (2001) resulted in the conclusion that 20 mg/kg/day increased the incidence of proliferative acinar cell lesions in both studies. Some lesions in the Biegel et al. study (2001) had progressed to adenomas.

The initial findings from the Butenhoff et al. study (2012) were equivocal for mammary fibroadenomas in female rats. However, a reexamination of the tissues by a PWG found no statistically significant differences in the incidence of fibroadenomas or other neoplasms of the mammary gland between control and treated animals (Hardisty et al. 2010). The PWG used the diagnostic criteria and nomenclature of the Society of Toxicological Pathologists for the reexamination. Under those criteria, there was an increase in the number of tumors documented in the control group, especially fibroadenomas originally classified as lobular hyperplasia. The reclassification led to a loss of significance when the tumors in the treated animals were compared to tumors in the control animals.

Ovarian tubular hyperplasia and adenomas also were observed in female rats (Butenhoff et al. 2012). Mann and Frame (2004) reexamined the ovarian lesions using an updated nomenclature system, which resulted in some of the hyperplastic lesions being reclassified. The ovarian lesions originally described as tubular hyperplasia or tubular adenomas were regarded as gonadal stromal hyperplasia and/or adenomas. After the reclassification, there were no statistically significant increases in hyperplasia (total number), adenomas, or hyperplasia/adenoma combined in treated groups compared to controls.

Mutagenicity studies of PFOA using the *S. typhimurium* (Friere et al. 2008; Lawlor 1995, 1996) and *E. coli* (Lawlor 1995, 1996) system have resulted in negative results in the presence and absence of activation. One mutagenicity study (Lawlor 1995, 1996) in *S. typhimurium* gave a positive result, but it was not reproducible. Clastogenicity studies in CHO by Murli (1996b, 1996c) were positive with activation for chromosomal abnormalities and polyploidy and equivocal in the absence of activation. Micronucleus assays by Murli (1995, 1996d) were negative.

A significant increase in 8-OH-dG liver levels, a biomarker for oxidative stress, was observed at ≥ 10 mg PFOA/kg in the liver but not the kidney of Fischer 344 male rats by Takagi et al. (1991). Work with HepG2 cells by Hu and Hu (2009) suggested that PFOA could induce apoptosis by overwhelming the homeostasis of antioxidative systems, increasing ROS, impacting mitochondria, and changing expression of apoptosis gene regulators. Eriksen et al. (2010) observed a PFOA-induced increase in ROS production in HepG2 cells, but no PFOA-induced oxidative DNA damage or cytotoxicity.

3.4.3 Mode of Action and Implications in Cancer Assessment

The modes of toxicological/carcinogenic action of PFOA are not clearly understood. However, available data suggest that the induction of tumors is likely due to nongenotoxic mechanism involving membrane receptor activation, perturbations of the endocrine system, and/or the process of DNA replication and cell division. PFOA lacks the ability to react with and modify DNA, although its electrostatic properties would permit interaction with chromosomal histone proteins with a net positive surface charge.

Rat Liver Tumors. PPARα agonism has been proposed as a potential MOA for the liver carcinomas and adenomas in rats following chronic PFOA exposure (Maloney and Waxman 1999; Klaunig et al. 2003, 2012). In the PPARα agonism MOA, binding of PFOA to the PPARα receptor results in increased peroxisome proliferation and cell replication. PPARα is primarily expressed in the liver, but also is present in the kidney, intestines, heart, and brown adipose tissue (Hall et al. 2012).

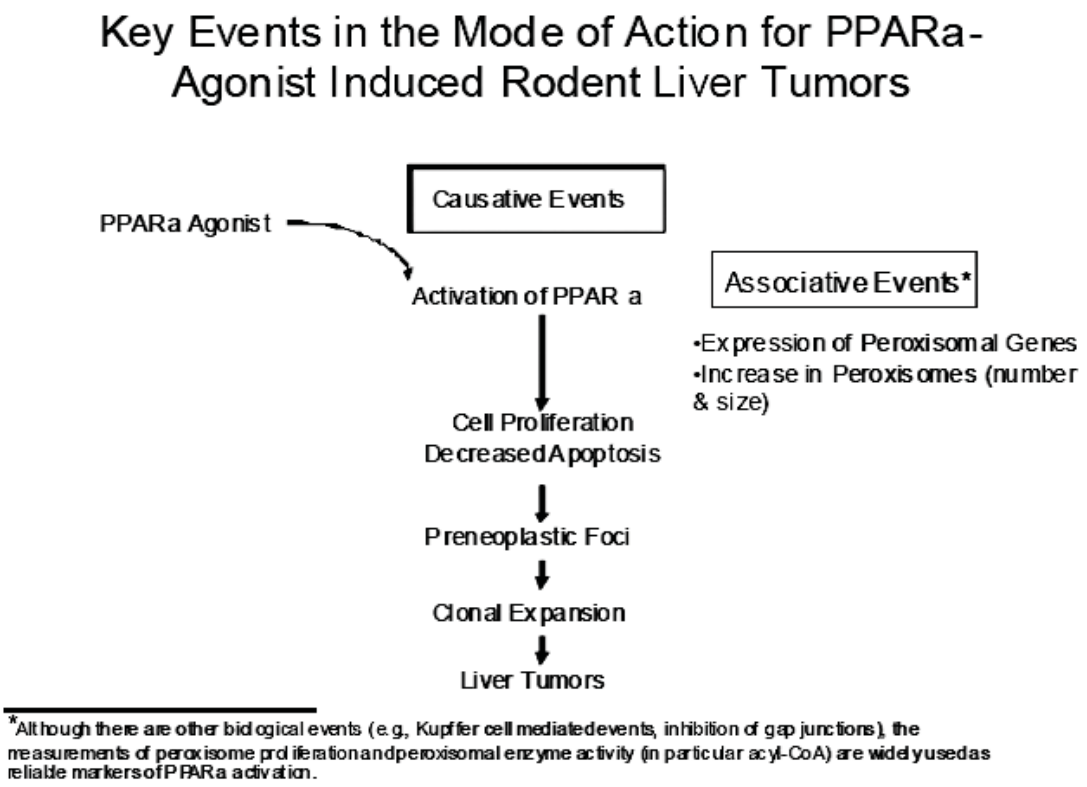
Peroxisomes are single-membrane organelles found in a number of plant and animal cells that have the capacity to carry out beta oxidation of long-chain fatty acids and other substrates through hydrogen peroxide-generating pathways and without the generation of ATP (Goodrich and Sul 2000). Peroxisomes metabolize the long-chain fatty acids via both beta and omega oxidation pathways (Fielding 2000), but are unable to metabolize fatty acid chains of eight carbons or less (Garrett and Grisham 1999). The shorter chain fatty acids are exported to the cytosol and taken up by mitochondria for further degradation via beta oxidation with resultant production of acetyl-CoA and ATP.

When a chemical binds to and activates the PPARα receptor, it forms a heterodimer with the retinoid-X receptor and binds to the peroxisome proliferator response element found in the promoter region of selected genes (Spector 2000). In addition to a variety of xenobiotic chemicals, there are a number of endogenous substances in animals and humans that can activate the PPARα receptor, including unsaturated C18 fatty acids, metabolites of arachidonic acid, and the prostaglandin metabolite PGJ2 (Spector 2000). PPARα activation is accompanied by upregulation of many genes associated with catabolism of fatty acid and cholesterol biosynthesis and lipid transport (Hall et al. 2012; Rosen et al. 2008a).

There are four key events in the PPARα-agonist MOA for liver tumors (Klaunig et al. 2003, 2012) (see Figure 3-1). The first key event is activation of PPARα. Increased palmitoyl-CoA oxidase activity is used in many studies as a biomarker for PPARα activations. Other associated indicators are hepatocellular hypertrophy and increased liver weight. However, these indicators alone are not sufficient to establish a PPARα MOA because they also are caused by chemicals that have no influence on PPARα.

The primary data that demonstrate PFOA activation of the PPARα receptor are those from Rosen et al. (2008a, 2008b) that examined the transcript profiles in the livers of wild-type and PPARα-null mice dosed with 1, 3, and 10 mg/kg/day PFOA for up to 7 days. The data from the wild-type mice were compared to those from the known PPARα gene activator Wyeth 14,643 and PPARα-null mice. Based on the analysis of gene regulation, it was clear that PPARα activation was required for a majority of the transcriptional changes observed in the mouse liver following PFOA or Wyeth 14,643 exposure. The data from this study demonstrate the ability of PFOA to act as a PPARα agonist.

Multiple studies in both rats and mice provide evidence that PFOA induces peroxisome proliferation in the liver (Elcombe et al. 2010; Minata et al. 2010; Pastoor et al. 1987; Wolf et al. 2008b; Yang et al. 2001). PFOA also was found to activate mouse and human PPARα using a transient transfection cell assay (Takacs and Abbott (2007). Maloney and Waxman (1999) also demonstrated that 5–10 μmol PFOA (2 to 4 mg/L) activated mouse PPARα using COS1 cells (kidney fibroblast-derived cells) transfected with a luciferase reporter gene.



Source: USEPA 2005c

Figure 3-1. PPARα Agonist MoA for Liver Tumors

In rodents, hepatic physical and biochemical changes observed after activation are highly correlated with liver tumors leading to the hypothesis that a > 3-fold increase in peroxisomes and > 1.5 fold increase in liver weights in short-term studies are sufficient to cause liver cancers in long-term studies (Hall et al. 2012). The temporal and dose-response relationship of measures of peroxisome proliferation, hepatocellular hypertrophy, liver weight, and liver histopathology were examined in male Sprague-Dawley rats following 4, 7, and 13 weeks of administration of dietary PFOA at doses ranging from 0–6.5 mg/kg/day (Perkins et al. 2004). There was no evidence of peroxisome proliferation, hepatocellular hypertrophy, or liver weight increases at 0.06 mg/kg/day. However, at 13 weeks, the 6.5-mg/kg/day dose had an increase in palmitoyl-CoA oxidase activity (an indicator for peroxisomes) that was 3.4 times greater than that for the pair-fed control. The absolute liver weight was 1.6 times greater than the pair-fed control. At the lower 1.94 mg/kg/day doses, the increases were 2.8 and 1.4 for the palmitoyl-CoA and liver weight, respectively

There are indications that PFOA also acts through PPARα-independent mechanisms associated with CAR and PXR receptors. Martin et al. (2007) examined the genomic signature from PFOA-treated Sprague-Dawley rats (up to 5-day exposure) using microarray expression profiling, pathway analysis, and quantitative PCR. The animal responses were consistent with PPARα agonism, but there was also evidence of PPARγ agonism (downregulation of cholesterol synthesis) and activation of CAR- and PXR-related genes. CAR activation can lead to hepatocyte proliferation and hepatocarcinogenesis in animals. However, the human CAR receptor is relatively resistant to mitogenic effects and less likely to induce cancers through this mechanism (Hall et al. 2012). In rodents, the PXR receptor can interact with PPARα in the coordination of

hepatocyte proliferation, but there are differences in the amino acid composition of the ligand binding domain of the mouse receptor and the human receptor (10% homology) (Hall et al. 2012). Accordingly, although the line of evidence is strongest for PPARα activation as the initiator for the downstream events in the PFOA cancer MOA, there can be involvement from other membrane receptors other than PPARα.

The second step in the PPARα MoA calls for evidence for increased cell proliferation and decreased apoptosis. Few studies examined the occurrence of these events with PFOA. Son et al. (2008) saw evidence of decreased apoptosis in liver and kidney cells stained for caspace3 in 4-week-old male ICR mice treated for 21 days at a dose of about 20 mg/kg/day. However, Elcombe et al. (2010) failed to see a significant decrease in male Sprague-Dawley rats with a 28-day exposure to a diet containing 300 ppm (~20 mg/kg/day) PFOA (comparable to the high dose in both cancer studies). In wild-type 129S4/SvlmJ mice, Minata et al. (2010) observed increased apoptosis in hepatocytes, arterial walls, and bile duct epithelium and in the bile duct epithelium of PPARα-null mice at 10.8 and 21.6 mg/kg PFOA. Thus, the apoptosis data for PFOA are not consistently supportive of the key step in this proposed MoA (i.e., a decrease in apoptosis).

Using a BrdU labeling technique, Elcombe et al. (2010) observed significant increases in cell proliferation in male Sprague-Dawley rats after 1, 7, and 28 days of exposure to a 300-ppm PFOA dietary dose. The highest increase was observed after 7 days of treatment (a fivefold increase) and declined to a twofold increase after 28 days of dosing. The liver results from the Biegel et al. (2001) mechanistic study were negative for cell proliferation in male Sprague-Dawley rats exposed to the same dietary concentration (20 mg/kg/day) and sacrificed at 1, 3, 6, 9, 12, and 15 months. However, based on the Elcombe et al. (2010) observations, the timing of the interim sacrifice would have missed the peak of the proliferative response. The Butenhoff et al. study (2012) identified hyperplastic nodules in 3/50 high-dose males and 2/50 high-dose females at 20 mg/kg/day; 5/50 males and 1/50 females had hepatocellular carcinomas.

The study by Wolf et al. (2008a) looked at the labeling index in 129S1/SvlmJ mice and PPARα-null mice and found a difference in their dose response. In the wild-type mice, the labeling index was increased at all doses ≥ 1 mg/kg/day; however, in PPARα-null mice, the labeling index was increased only at the highest dose, 10 mg/kg/day.

There were no studies identified that focused specifically on preneoplastic foci and clonal expansion of altered cells after PPAR activation. Minata et al. (2010) observed a dose-dependent increase in eosinophilic cytoplasmic changes consistent with peroxisome proliferation in liver parenchyma, but found no focal necrosis at doses ≤ 21.6 mg/kg/day in wild-type 129S4/SvlmJ mice.

Klaunig et al. (2003, 2012) concluded that, based on the available data, PFOA-induced liver tumors in Sprague-Dawley rats can be attributed to a PPARα MOA since there are data available addressing most of the key steps in this proposed MoA. However, some data gaps exist for key events and other mechanisms that might be involved. Overall, the tumor response observed in the available studies was not strong and did not demonstrate a dose-related response in males (3/49, 1/50, and 5/50 hepatocellular carcinomas in the control, 2-mg/kg/day, and 20-mg/kg/day dose groups, respectively) and a single carcinoma in females at the high dose. Biegel et al. (2001) did not identify any liver carcinomas (0/76) in males at their 20-mg/kg dose, but there were 10/76 males with adenomas. This is consistent with a hyperplastic tissue response rather than the loss of cell cycle control characteristic of cancer. The data from the Butenhoff et al. (2012) and

Biegel et al. (2001) studies suggest that PFOA is not a potent hepatic carcinogen based on the low tumor incidence and finding of hyperplastic nodules.

Leydig Cell Tumors (LCT). LCTs were observed in both the Butenhoff et al. (2012) and Biegel et al. (2001) studies. The LCT incidence was 0/49, 2/50, and 7/50 at doses of 0, 2, and 20 mg/kg/day, respectively, in Butenhoff et al. (2012) and 2/78 (pair-fed control) and 8/76 at 20 mg/kg/day in Biegel et al. (2001).

A large number of nongenotoxic compounds of diverse chemical structures have been reported to induce LCTs in rats, mice, or dogs. LCTs also occur in humans but are relatively rare at about 1–3% of human testicular tumors, which also are infrequent (1%) (Carpino et al. 2007). A workshop report (Clegg et al. 1997) on the MOA for LCT classified the chemicals that caused LCT in animal studies into seven MOA categories. The postulated MOAs support the following hormonal steps to the process:

1. A xenobiotic chemical inhibits the production of testosterone, leading to low serum levels.
2. Low serum testosterone levels signal the hypothalamus to produce gonadotropin releasing hormone (GnRH).
3. GnRH signals the pituitary to release LH.
4. LH signals the Leydig cells to produce testosterone.
5. LH causes Leydig cell proliferation.

Several of the available PFOA studies support an impact of PFOA on decreased testosterone production. Studies conducted by Cook and colleagues (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996) found that adult male rats administered PFOA by gavage for 14 days had decreased serum testosterone and increased serum estradiol levels (Cook et al. 1992). These endocrine changes correlated with its potency to induce LCTs (Biegel et al. 2001).

Subsequent experiments demonstrated that PFOA increased levels of estradiol by inducing cytochrome P450 CYP19 (aromatase). Aromatase converts androgens to estrogens, including the conversion of testosterone to estradiol. PFOA directly inhibits testosterone production when incubated with isolated Leydig cells and *ex vivo* studies demonstrate that this inhibition is reversible (Biegel et al. 1995). However, in the mechanistic bioassay by Biegel et al. (2001), serum testosterone and LH levels were not significantly altered at the levels of PFOA that resulted in LCTs (20 mg/kg/day).

This inhibition of testosterone biosynthesis can be mediated by PPARα (Gazouli et al. 2002). Support for PPARα-mediated inhibition of testosterone production is found in Li et al. (2011). Lower testosterone concentrations, reduced reproductive organ weights, and increased sperm abnormalities were found in PFOA-treated male PPARα wild-type and humanized PPARα mice but not in PPARα-null mice. Similarly, disruption of testosterone biosynthesis by lowering the delivery of cholesterol into the mitochondria and decreasing the conversion of cholesterol to pregnenolone and androstandione in the testis was noted in wild-type and humanized PPARα mice. These effects were not seen in PPARα-null mice. Decreased serum testosterone was noted after oral exposure to PFOA in studies by Biegel et al. (1995, 2001) and Cook (1992).

The induction of LCTs by PFOA also can be attributed to a hormonal mechanism whereby PFOA either inhibits testosterone biosynthesis and/or lowers testosterone by increasing its conversion to estradiol through increased aromatase activity in the liver. Both of these mechanisms appear to be mediated by PPARα. However, data are not currently sufficient to

demonstrate that the other key steps in the postulated MOA are present in PFOA-treated animals following exposures that lead to tumor formation. Studies are needed to demonstrate the increase of GnRH and LH in concert with the changes in aromatase and estradiol. There was also no indication of increased Leydig cell proliferation at the doses that caused adenomas in the Biegel et al. study (2001). Thus, additional research is needed to determine if the hormone testosterone-estradiol imbalance is a key factor in development of LCTs as a result of PFOA exposure.

Two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrollment 0.024 μg/mL) and kidney and testicular cancers (Vieira et al. 2013; Barry et al. 2013). This contributed to the EPA conclusion that PFOA can be classified as having *suggestive evidence* for carcinogenicity.

Pancreatic Acinar Cell Tumors. The 2-year bioassay by Biegel (2001) identified PACTs in 7/6 rats receiving a 20-mg/kg dose for 2 years compared to 1/79 in the pair-fed controls. As with LCTs, the MOA for PACTs is not understood. These tumors are most commonly identified in rats, but do occur in other animal species (e.g., mice, hamsters) and in humans (Wisnoski et al. 2008). Males are more susceptible to pancreatic tumors than females. Two hypothetical MOAs have been proposed and are as follows (Klaunig et al. 2003, 2012; Obourn et al. 1997):

- There is a change in the bile acid flow or composition that leads to cholestasis, thereby causing an increase in CCK activating a feedback loop resulting in proliferation of the secretory pancreatic acinar cells. CCK is a peptide hormone that stimulates the digestion of fat and protein, causes the increased production of hepatic bile, and stimulates contraction of the gall bladder. An HFD, trypsin inhibition, and changes in bile composition are proposed initiators for this sequence of events.
- Increased levels of testosterone support the growth of acinar cell preneoplastic foci, leading to the development of carcinomas.

There is minimal information on the relationship of PFOA exposure to either of the proposed MOAs. Obourn et al. (1997) studied the impact of PFOA on CCK and trypsin using *in vitro* assays and found that PFOA was not an agonist for the CCKA receptor that activates CCK release. PFOA also had no inhibitory action on trypsin at levels 1,000 times greater (0.31 μg/mL) than the positive control.

The Obourn et al. study (1997) also looked at Wyeth 14,643, a peroxisome proliferator, in these same assays and found results similar to those for PFOA. When they conducted an *in vivo* study with 100 ppm Wyeth 14,643, they found a small but significant increase ($p<0.05$) in bile flow per unit body weight, a decrease ($p<0.05$) in bile flow per unit liver weight, and a small decrease ($p<0.05$) in the total bile acid concentration following a 6-month dietary exposure.

There is the potential for PFOA to change the composition of bile because of its competition with bile acids for biliary transport. In mice, increased expression of MRP3 and MRP4 transporters (Maher et al. 2008) and decreased expression of OATPs (Cheng and Klaassen 2008) favor excretion of PFOA into the bile. Minata et al. (2010) found the levels of PFOA in bile from wild-type male mice to be considerably higher than those in PPARα-null mice, suggesting a link to PPARα. In the same study, male wild-type and PPARα-null mice were orally dosed with ~0, 5.4, 10.8, and 21.6 mg/kg/day of PFOA for 4 weeks. Total bile acid was significantly increased at the highest dose in PPARα-null mice suggesting that, in the presence of PFOA, activation of PPARα increases PFOA excretion, a scenario that could possibly decrease the flow of bile acids competing for the same transporters. In the Butenhoff et al. study (2012), there was a lack of

PACT tumors but an increase in proliferative lesions of the acinar cells. One hypothesis offered for the difference in results was differences in the diets used in the two studies (Chang et al. 2014).

PFOA appears to suppress testosterone production through the induction of aromatase (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996) and to increase the estradiol. Accordingly, the second proposed MOA for PACTs does not appear to apply to PFOA.

The data on a PPARα-linked MoA are strongest for the liver tumors. Some data also provide a link of PPARα to the Leydig cell and PACT tumors observed in the rat 2-year bioassays. They are not as strong and identify a need for additional research justifying the suggestive evidence finding. However, when integrated with the metabolic inertness of PFOA in animals and humans, a linear response to dose is not likely. This is consistent with the tumor data. Thus a nonlinear MOA is likely and the remaining challenge is to identify the critical event in each MOA that leads to development of the tumors.

Other Potential Modes of Action. There are other potential MOAs that could apply to PFOA. They include interruption of intercellular communication, mitochondrial effects, and hormonal effects. None of these mechanisms are considered to be key steps in the MOAs discussed above.

GJIC, a process by which cells exchange ions, messages, and other small molecules, is important for normal growth, development, and differentiation as well as for maintenance of homeostasis in muticellular organisms. Because tumor formation requires loss of homeostasis and many tumor promoters inhibit GJIC, it has been hypothesized that GJIC might play a role in carcinogenesis (Trosko et al. 1998). PFOA has been demonstrated to inhibit GJIC in liver cells *in vitro* and *in vivo* (Upham et al. 1998, 2009). However, inhibition of GJIC is a widespread phenomenon, and the effect by PFOA was neither species- nor tissue-specific. In addition it was reversible. Thus, the significance of GJIC inhibition in regard to the mode of carcinogenic action of PFOA is unknown.

Several chemicals structurally related to PFOA have been shown to manifest their toxicity by interfering with mitochondria biogenesis and bioenergetics. Walters et al. (2009) found evidence supporting mitochondrial proliferation in Sprague-Dawley rats receiving 30 mg/kg/day of PFOA for 28 days as reflected in measurements of mitochondrial DNA, transcription factors, and other biomarkers for mitochondrial effects. Dietary PFOA also was demonstrated to uncouple oxidative phosphorylation in mitochondria of the liver from rats exposed via their diet (Keller et al. 1992). At high concentrations, PFOA caused a small increase in resting respiration rate and slight decreases in the membrane potential. The observed effects were attributed to a slight increase in nonselective permeability of the mitochondria membranes caused by PFOA's surface-active properties (Starkov and Wallace 2002). Quist et al. (2015) found evidence of mitochondrial proliferation in the liver of CD-1 mice pups from dams exposed to 1 mg/kg/day during gestation and lactation when tissues were examined using transmission electron microscopy at PND 21 and 91.

3.4.4 Weight of Evidence Evaluation for Carcinogenicity

The findings for cancer in humans provide support for an association between PFOA and kidney and testicular cancers; however, the number of independent studies examining each of these is limited. The support comes from high-exposure community studies examining cancer incidence and covering children and young adults (Barry et al. 2013; Vieira et al. 2013); there is

some overlap in the cases included in these studies. The two occupational cohorts in Minnesota and West Virginia (most recently updated in Raleigh et al. 2014 and Steenland and Woskie 2012) do not support an increased risk of kidney or testicular cancer, but are limited by a very small number of observed cases. None of the general population studies examined these cancers, but associations were not seen in the general population studies addressing colorectal, breast, prostate, bladder, and liver cancer, with mean serum PFOA levels up to 0.0866 µg/mL (Bonefeld-Jørgensen et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014).

The only chronic bioassays of PFOA were conducted in rats (Butenhoff et al. 2012; Biegel et al. 2001). The two studies support a positive finding for the ability of PFOA to be tumorigenic in one or more organs of male, but not female, rats. There are no carcinogenicity data from a second animal species. There are some data that provide support for the hypothesis that the PPARα agonism MOA is wholly or partially linked to each of the observed tumor types. The data support a PPARα MOA for the liver tumors and thus are indicative of lack of relevance to humans. PPARα activation also could play a role in the other tumor types observed, but more data to support intermediate steps in the proposed MOAs are needed.

The mutagenicity data on PFOA are largely negative, although there is some evidence for clastogenicity in the presence of microsomal activation and at cytotoxic concentrations. Given the chemical and physical properties of PFOA—including the fact that it is not metabolized, binds to cellular proteins, and carries a net negative electrostatic surface charge—the clastogenic effects are likely the result of an indirect mechanism. PFOA has the potential to interfere with the process of DNA replication because of its protein binding properties and the fact that histone proteins, spermine and spermidine, carry a net positive surface charge. Involvement of ROS in the MOA as a result of PFOA alone is unlikely because of its metabolic stability. Conditions leading to ROS would be a function of metabolic responses perturbed by PFOA rather than PFOA alone. A compound that is not metabolized will not be able to covalently alter the structure of DNA or intercalate because of electrostatic repulsion between the aromatic base pi bond electrons with the partial negative charges on the PFOA fluoride atoms. Due to its protein binding properties, PFOA could have an impact on one or more of the proteins involved in the process of DNA replication or cell division (cytoskeletal proteins); however, no mechanistic studies were identified that examined the biochemical effects of PFOA on DNA replication or cell division. There are no data that support the clastogenic MOA.

Despite the limitations in the data for the LCTs and PACTs, under the *U.S. EPA Guidelines for Carcinogen Risk Assessment (USEPA 2005a) there is suggestive evidence of carcinogenic potential* of PFOA in humans.

3.4.5 Potentially Sensitive Populations

Human biomonitoring studies do not suggest major differences between serum PFOA levels in males and females. However, the worker populations that are those most likely to demonstrate such differences because of their higher exposures were predominantly male.

Some animal species have gender differences that affect toxicity of PFOA. Sexually mature female rats excreted almost all of a 10-mg/kg dose of PFOA within 48 hours compared to only 19% excreted by male rats. Male hamsters excrete PFOA faster than female hamsters, and female rabbits excrete PFOA slightly faster than male rabbits. Male and female mice excrete PFOA at approximately the same rate (Hundley et al. 2006). Studies of the transporters involved in the toxicokinetics of PFOA demonstrate that they are differentially impacted by the presence

of male and female sex hormones influencing tissue persistence (Cheng et al. 2006; Kudo et al. 2002). As studied in rats (Kudo et al. 2002), the male sex hormones increased half-life (decreased excretion) of PFOA while the female hormones were associated with shorter half-lives (increased excretion). The gender differences in mice are not as pronounced as those in rats. Work by Cheng et al. (2006) and Cheng and Klaassen (2009) demonstrated that these hormones impact transporters in the liver and kidney.

In studies in which both male and female rats were used, the males were more sensitive to toxicity than were the female rats (Butenhoff et al. 2004a). Mice displayed similar sensitivities following PFOA exposure (Kennedy 1987). In the monkey studies, the number of animals per gender per dose group was too small to reveal a difference related to gender.

Unfortunately, much work remains to be done to determine whether the gender difference seen in rats is relevant to humans. Similarities are possible because the long half-life in humans suggests that they might be more like the male rat than the female rat. There is a broad range of half-lives in human epidemiology studies, suggesting a variability in human transport and binding capabilities resulting from genetic variations in transporter structures and, consequently, in function. Genetic variation in human OATs and OATPs has been identified as described in a review by Zaïr et al. (2008).

Neonates, Infants, and Fetuses

The developing fetus might be sensitive to effects of PFOA. The observed effects on birth weight in animals are supported by evidence of an association between PFOA and low birth weight in humans (Johnson et al. 2014). There is some uncertainty related to the interpretation of the small change in birth weight observed in humans. Specifically, it has been suggested that low GFR also can impact birth weight (Morken et al. 2014). Verner et al (2015) conducted a meta-analysis based on PBPK simulations and found that, in individuals with low GFR, there are increased levels of serum PFOA as well as lower birth weights. Thus, while there is some uncertainty in the interpretation of the observed association between PFOA and low GFR and birth weight given the available information, the data indicate that PFOA exposure does impact birth weight in the general population. In humans with low GFR (which includes females with pregnancy-induced hypertension or preeclampsia) who also are exposed to PFOA, the effect on body weight is likely due to a combination of both.

Several animal studies have examined potential MoAs for developmental effects following prenatal exposure to PFOA. PFOA exposure during development in rats and mice resulted in increased resorptions (mouse), increased fetal skeletal variation (rat, mouse), decreased neonatal survival (rat, mouse), decreased postnatal body weight (mouse), delayed eye opening and body hair growth (mouse), delayed vaginal opening (mouse), accelerated preputial separation (mouse), and delayed mammary gland development (mouse dam and offspring) (Abbott et al. 2007; Butenhoff et al. 2004a; Lau et al. 2006; Macon et al. 2011; Tucker et al. 2015; White et al. 2007, 2009, 2011; Wolf et al. 2007). Other long-term effects observed in the surviving offspring included increased body weight gain, serum leptin, and serum insulin levels along with changes in adipose tissue (Hines et al. 2009). The MOAs for these developmental effects are unknown, but several potential MoAs have been investigated.

Wolf et al. (2007) restricted mouse prenatal PFOA exposures to 3–11-day periods during gestation to determine if PFOA was affecting a certain stage of organogenesis resulting in the observed developmental effects. Decreased postnatal survival was observed at the highest dose

used (20 mg/kg/day). Eye opening and body hair growth were delayed in offspring exposed for the longest periods of time (GD 7–17 and GD 10–17) and might have been the result of a higher cumulative dose or greater sensitivity during early gestation. A cross-fostering paradigm was used to determine if the developmental effects were the result of gestational exposure, lactational exposure, or a combination of both. Postnatal survival was decreased in offspring exposed through gestation and lactation (5 mg/kg/day). Eye opening and body hair growth were delayed and body weight was reduced in offspring exposed during gestation (5 mg/kg/day), and gestation and lactation (3 and 5 mg/kg/day). No developmental delays in eye opening and body hair growth were observed in offspring exposed via lactation only, indicating that, for these developmental endpoints, PFOA alters growth regulation in the developing fetus that persists as growth continues postnatally.

Both gestational and lactational exposures contribute to the impact of PFOA on body weight during early life as illustrated by cross-fostering control unexposed female pups with those dosed with PFOA. Three cross-fostering combinations were evaluated by White et al. (2009): control pups nursed by exposed dams, exposed pups nursed by control dams, and exposed pups nursed by exposed dams. Two doses were evaluated: 3 and 5 mg/kg/day, but the body weight data was only provided for the 5-mg/kg/day dose group for PND 1–10. PFOA exposures significantly reduced pup body weights and increased liver weights. The body weight deficits compared to control were greatest for the gestation and lactation exposure combination and lowest for the lactation-only group.

Abbott et al. (2007) examined activation of PPARα as a factor in the developmental toxicity of PFOA. Wild-type and PPARα-null mice experienced full litter resorptions following gestational (GD 1–17) PFOA exposure (≥5 mg/kg/day), indicating that the mechanism of PFOA-induced resorptions was independent of PPARα expression. These resorptions could be due to insufficient trophoblast cell type differentiation and/or increased trophoblast cell necrosis (Suh et al. 2011). Postnatal survival was significantly decreased in wild-type offspring but not in PPARα-null offspring, indicating that PPARα expression was required for postnatal lethality (Abbott et al. 2007). Eye opening was significantly delayed in wild-type offspring, but not in PPARα-null offspring, although a trend was observed in those offspring for later eye opening. The results indicated that PPARα expression was important for eye opening, but other PPARα-independent factors also might play a role in its mechanism. Takacs and Abbott (2007) showed that PFOA can activate mouse PPARβ/δ, which is expressed in developing tissue, and suggested that inappropriate activation of PPARβ/δ could cause adverse effects. Further research needs to be conducted to fully elucidate the mechanism.

Mouse mammary gland development was another endpoint examined in prenatally PFOA-exposed offspring. White et al. (2007) found that dams dosed with 5 mg PFOA/kg/day on GD 1–17 and GD 8–17 had significantly delayed mammary gland development (full of alveoli, visible adipose tissue, not well differentiated) at PND 10, which is at the peak of lactation in rodents. The delayed dam mammary gland development could play a role in the observed reduced offspring body weight gain if the quantity or quality of the milk is altered by PFOA (Abbott et al. 2007; Lau et al. 2006; White et al. 2007; Wolf et al. 2007).

Restricted gestational exposure and cross-fostering studies showed that delayed offspring mammary gland development observed PND 1–63 occurred regardless of exposure duration or timing (gestation versus lactation exposure; maternal dose of 1 mg/kg/day). The developmental delays persisted even as the internal PFOA dose decreased (Macon et al. 2011; White et al. 2007, 2009, 2011). More studies need to be conducted to elucidate the MOA for dam and offspring

mammary gland effects and its potential functional consequences for lactating humans. White et al. (2011) conducted a multigeneration study of the effects of PFOA on mammary gland development and found no dose-related effects on the pup body weights nourished by dams with lower mammary gland scores than the controls. Tucker et al. (2015) demonstrated that a dose-response for developmental mammary gland effects varies by more than an order of magnitude, depending on the strain of mouse studied. CD-1 mice are more sensitive than C57BL/6 mice (Tucker et al. 2015).

Mammary gland development also was affected by peripubertal exposure to PFOA (C. Yang et al. 2009, Y. Zhao et al. 2010). Low doses (5 mg/kg/day) of PFOA from 3 to 7 weeks of age caused accelerated mammary gland development in C57BL/6 mice, but delayed mammary gland development in BALB/c mice, suggesting strain-related differences.

Experiments examining the mechanism for accelerated mammary gland development showed that PFOA promotes steroid hormone production in the ovaries and increases the levels of several mammary gland growth factors in C57BL/6 wild-type and PPARα-null mice. The mechanism for delayed mammary gland development following a peripubertal PFOA exposure needs to be examined.

Hines et al. (2009) found that low doses of PFOA given during gestation to CD-1 mice resulted in significant weight gain and increased serum insulin and leptin levels of the offspring in mid-life. The increased leptin levels, as well other hormone perturbations, might place PFOA into the environmental endocrine disruptor obesogen category similar to diethylstilbestrol (Newbold et al. 2007). However, in a study by Quist et al. (2015) using the mature animals from the Hines et al. study (2009), there was no dose-related impact on serum leptin in CD-1 pups gestationally exposed across a dose range of 0–1 mg/kg/day when examined on PND 91, except in the group given an HFD and not fasted before serum collection. For those animals, there was a dose-related decrease in leptin. Other mice on an HFD that were fasted for 4 hours before serum collection in the same study lacked a dose-related leptin response. In humans, increased leptin levels are associated with increased body fat and suggestive of a leptin-resistance MOA for being overweight (Considine et al. 1996). A similar relationship might occur in prenatally PFOA-exposed mice; however, the Quist et al. study (2015) suggests that the fat content of the diet and the time of serum collection are important variables that need to be considered. Studies determining MOAs need to be conducted to determine relevance of the mammary gland effects to animal and human health.

Diet might influence the risk associated with PFOA exposures. Animal studies demonstrate an increased risk for liver steatosis in animals on an HFD (Quist et al. 2015; Tan et al. 2013) and possibly for insulin resistance (Hines et al. 2009). The epidemiology data are not supportive of a correlation with insulin resistance, but the observations of elevated serum triglycerides, especially among a highly exposed population, could be viewed as a risk factor for steatosis. Most of the epidemiology studies did not evaluate dietary factors as part of the study design for either birth weight or serum lipids (e.g., cholesterol, triglycerides, LDL).

4 DOSE-RESPONSE ASSESSMENT

4.1 Dose-Response for Noncancer Effects

An RfD or reference concentration (RfC) is used as a benchmark for the prevention of long-term toxic effects other than carcinogenicity. RfD/RfC determination assumes that thresholds exist for toxic effects, such as cellular necrosis, significant body or organ weight changes, blood disorders, and so forth. The RfD is expressed in terms of mg/kg/day and the RfC is expressed in milligrams per cubic meter (mg/m^3). The RfD and RfC are estimates (with uncertainties spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

4.1.1 RfD Determination

The derivation of the RfD for PFOA presented a number of challenges due to the toxicokinetic complexity of PFOA, variability in half-life between species, and metabolic inertness of PFOA in living organisms. The toxicokinetic features of PFOA lead to differences in half-lives across species and in the case of rats, and possibly humans, differences between genders. Toxicokinetics also influence intraindividual and lifestage variability in response to dose. Additionally there were inconsistencies across the epidemiology studies and the effects observed in animal studies, and a number of animal studies lacked a NOAEL. Each of these factors highlights the importance of having measures of internal dose for quantification of an RfD and supports the utilization of a PK model as a component of the dose-response assessment.

Human Data. Key studies examined occupational and residential populations at or near large-scale PFOA production plants in the United States in an attempt to determine the relationship between serum PFOA concentration and various health outcomes suggested by the standard animal toxicological database. Health outcomes assessed include blood lipid and clinical chemistry profiles, reproductive parameters, thyroid effects, diabetes, immune function, birth and fetal and developmental growth measures, and cancer.

Epidemiology studies examined workers at PFOA production plants, a high-exposure community population near a production plant in the United States (i.e., the C8 cohort), and members of the general population in the United States, Europe, and Asia. These studies examined the relationship between serum PFOA concentration (or other measures of PFOA exposure) and various health outcomes. Exposures in the highly-exposed C8 community are based on the concentrations in contaminated drinking water and serum measures. Exposures among the general population typically included multiple PFAS as indicated by serum measurements. The correlation among these compounds is often moderately strong (e.g., Spearman $r > 0.6$ for PFOA and PFOS in the general population). Mean serum levels among the occupational cohorts ranged approximately 1–4 μg/mL and in the C8 cohort they ranged 0.01–0.10 μg/mL. Geometric mean serum values for the NHANES general population (≥ age 12; 2003–2008) were 0.0045 μg/mL for males and 0.0036 μg/mL for females (Jain 2014).

These studies have generally found positive associations between serum PFOA concentration and TC (i.e., increasing lipid level with increasing PFOA) in the PFOA-exposed workers at mean serum levels 0.4 to >12 μg/mL and the high-exposure community at mean serum about 0.08 μg/mL; similar patterns are seen with LDLs but not with HDLs. These associations also

were seen in most of the general population studies (mean serum 0.002–0.007 μg/mL), but similar results were seen with PFOS and the studies did not adjust for these correlations. Associations between PFOA exposure and elevations in serum levels of ALT and GGT, were consistently observed in occupational cohorts, the high-exposure community, and the U.S. general population at serum PFOA concentrations also associated with increased TC. The associations are not large in magnitude, but they indicate the potential to affect liver function.

Thyroid disease incidence was associated with PFOA in women and girls in the high-exposure C8 study population and in women with background exposure at mean serum concentrations of 0.026–0.123 μg/mL. Changes in thyroid hormones were not consistently associated with PFOA concentration.

Associations between PFOA exposure and risk of infectious diseases (as a marker of immune suppression) have not been found, but a decreased response to vaccines in relation to PFOA exposure was reported in studies in adults in the high-exposure community population (median 0.032 μg/mL) and in studies in children in the general population (mean 0.004 μg/mL). In the latter studies, it is difficult to distinguish associations with PFOA from those of other correlated PFAAs. Increased risk of ulcerative colitis was reported in the high-exposure community study and in a study limited to workers in that population.

Studies in the high-exposure community reported an association between serum PFOA at approximately 0.01–0.02 μg/mL and risk of pregnancy-related hypertension or preeclampsia. This outcome has not been examined in other populations. An inverse association between maternal PFOA (measured during the second or third trimester) or cord blood PFOA concentrations and birth weight was seen in several studies, but the magnitude was small. It has also been suggested that low GFR can impact birth weight (Morken et al. 2014). Verner et al. (2015) conducted a meta-analysis based on PBPK simulations and found that some of the association reported between PFOA and birth weight could be partially attributable to low GFR. However, the study authors demonstrated that in individuals with low GFR there also are increased levels of serum PFOA. Thus, while there is some uncertainty in the interpretation of the observed association between PFOA and low GFR and birth weight given the available information, the data indicate that PFOA exposure does impact birth weight in the general population.

The epidemiology studies have not found associations between PFOA and diabetes, neurodevelopmental effects, or preterm birth and other complications of pregnancy. Developmental outcomes including delayed puberty onset in girls has been reported; however, in the two studies examining prenatal PFOA exposure in relation to menarche, conflicting results were found (i.e., no association or a possible indication of an earlier menarche seen with higher maternal PFOA levels in one study and a later menarche seen with higher maternal PFOA levels in the other study).

Animal Data—Long Term Studies. Some of the effects in animal studies are associated with activation of the PPARα receptor leading to peroxisome proliferation. These include increased liver weight; decreases in serum triglycerides, cholesterol, and lipoproteins; and increases in ALT, AST, or both. However, although the mechanisms for other effects, such as decreased body weight, immunological effects, and developmental delays are unknown, they might be relevant to human health risk assessment.

As an initial step in the dose-response assessment, EPA identified a suite of animal studies with NOAELs, LOAELs, or both that identified the studies as candidates for development of a chronic RfD. These studies are listed in Table 4-1. The candidate studies were selected based on their NOAEL, LOAEL, or both; a duration of ≥ 7 weeks; use of a control; and two or more doses. Table 4-1 does not include the data from human epidemiology studies because, although they include information on serum levels, they do not identify exposure sources or external doses.

Table 4-1. NOAEL/LOAEL Data for Oral Subchronic and Chronic Studies of PFOA

Species	Study Duration	NOAEL mg/kg/day	LOAEL mg/kg/day	Critical Effects (s)	Reference
Monkey Male	90 days	none	3	↑ relative pituitary weight	Goldenthal 1978
Monkey Female	90 days	3	10	↓ absolute and relative heart weight	Goldenthal 1978
Monkey Male	26 weeks	none	3	↑ absolute liver weight (hepatocellular hypertrophy) and mean liver-to-body weight percentages	Butenhoff et al. 2002
Rat Male	13 weeks	0.64	1.94	↑ absolute and relative liver weight with hepatocellular hypertrophy accompanied by a slight, but not significant, increase in hepatic coagulative necrosis	Perkins et al. 2004
Rat Male F0 generation	84 days	none	1	↑ absolute and relative liver and kidney weight accompanied by ↓ body weight	Butenhoff et al. 2004a; York et al. 2010
Rat Male F1 generation	16 weeks	none	1	↓ body weights and weight gains; ↑ absolute and relative liver weights, liver hypertrophy; ↑ absolute and relative kidney weights	Butenhoff et al. 2004a; York et al. 2010
Rat Female F0 generation	127 days	30	none	No significant effects observed	Butenhoff et al. 2004a; York et al. 2010
Rat Female F1 generation	10 weeks	10	30	Delay in sexual maturity, ↓ body weight and weight gain	Butenhoff et al. 2004a; York et al. 2010
Rat Male and Female	2 years	1.3 (m) 1.6 (f)	14.2 (m) 16.1 (f)	M: ↓ body weight gain; histopathology lesions in liver, testes, and lungs. F: ↓ body weight gain	Butenhoff et al. 2012

When examining the effects associated with the LOAELs summarized in Table 4-1, changes in relative liver weight, absolute liver weight, or both appear to be a common denominator for monkeys and rats (Butenhoff et al. 2002, 2004a; Perkins et al. 2004) with or without other hepatic indicators of adversity. Serum PFOA levels, where available, associated with increased liver weight were 81 and 41 µg/mL for the male monkey and rat, respectively. However, the increases in liver weight and hypertrophy are effects associated with activation of cellular PPARα receptors, making it difficult to determine whether this change is totally a reflection of the PPARα activation or PFOA toxicity and meet the Hall et al. (2012) criteria for establishing adversity for a PPARα-activating chemical. Studies in PPARα null mice and animals with a

human PPARα receptor (Li et al. 2011; Minata et al. 2010; Nakamura et al. 2009; Wolf et al. 2008b), along with studies of hepatic gene activation by PFOA (Albrecht et al. 2013; Bjork and Wallace 2009; Nakamura et al. 2009; Rosen et al. 2008a, 2008b), suggest that the increase in liver weight is at least partially due to cellular impacts that are not controlled by PPARα receptors. However, it remains difficult to separate the impact of PPARα activation from the direct effects of PFOA in the candidate studies.

According to Hall et al. (2012), increases in liver weight can be considered adverse when accompanied by cellular necrosis, inflammation, fibrosis of the liver, and/or macrovesicular steatosis. There was some evidence of hepatic necrosis in the studies of Perkins et al. (2004) and in the male F1 generation adult rats from the Butenhoff et al. study (2004a), but the incidences were not statistically significant or described in detail. To the extent that adverse lesions reflect sensitivity in the animals impacted, they are used in the assessment to reflect that the liver hypertrophy and increased liver weight are adverse in individual animals where they are accompanied by necrosis.

Body weight effects were seen in several studies (Butenhoff et al. 2004a, 2012) and are a more toxicologically-relevant endpoint, especially in the cases where they were not accompanied by decreased food intake and when found in neonates (Butenhoff et al. 2004a). There were developmental delays for males and females in the two-generation study published by Butenhoff et al. (2004a). Testicular effects were observed by Butenhoff et al. (2012) and in the chronic one-dose study by Biegel et al. (2001). There was evidence of increased kidney weight in male F1 Sprague-Dawley rats (Butenhoff et al. 2004a) confounded by decreases in body weight at higher doses, but at lower doses the kidney weight effect is likely a reflection of tissue adaptation as a result of the requirement for upregulation of tubular transporters to facilitate urinary excretion using transporters developed for excretion of endogenous and dietary substrates rather than PFOA.

Four of the longer term studies in Table 4-1 lack a NOAEL and have LOAELs that range 1–3 mg/kg/day. The NOAELs for the remaining 7 studies range from 0.64 (male rats) to 30 mg/kg/day (female rats). Male monkeys and rats appear to respond at doses that are lower than their female counterparts. No long-term studies in mice were identified. Since NOAELs and LOAELs are to some extent the product of concentration or dose level selection, examination of the dose information in Table 4-1 suggests that several of the data sets that have serum data to inform modeling of internal doses have the potential to be co-critical in the dose-response evaluation.

Animal Data—Short Term Studies. A number of studies identified adverse effects following low dose exposures over durations of 7 to 38 days. The studies fall into two clusters, those evaluating developmental or reproductive effects and those with a focus on immunological effects. The critical shorter-term studies in rats and mice are summarized in Table 4-2. Although the exposure duration is shorter in developmental studies than the two-generation study, the developmental studies are important in quantification of dose-response because the exposures occur during critical windows of development and predicate effects that can occur later in life.

Table 4-2. Shorter-term and Developmental Oral Exposure Studies

Species	Study Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical Effect(s)	Reference
Rat					
Male	29 days	1	10	Increased absolute and relative liver weight, focal liver necrosis	Loveless et al. 2008
Male	14 days	0.2	2	↑ liver weight, ↑ serum estradiol and hepatic aromatase	Liu et al. 1996
Mouse					
Female offspring	17 days	none	1	Delayed mammary gland development in dams during lactation	White et al. 2011
Male & Female	38 days	1	5	↑ Ito cell hypertrophy at 18 months. Dosing occurred during gestation and lactation only.	Filgo et al. 2015
Female	38 days	0.01	0.03	↑ TC at PND 91 for fasted and nonfasted animals receiving a HFD but not those receiving the standard fat content control diet. Exposure occurred only during gestation and lactation.	Quist et al. 2015
Male	29 days	0.3	1	↑ absolute and relative liver weight, ↓ relative spleen weight, moderate-severe liver hypertrophy with single cell and focal necrosis	Loveless et al. 2008
Male	28 days	none	5	Significantly ↓ fertility based on pregnant females per male mouse, and ↓ pup birth weight.	Lu et al. 2015
Female	17 (pups) /18 (dams) days	none	1	↑ absolute maternal liver weight, ↓ ossification (calvarin, enlarged fontanel), accelerated onset of puberty in male offspring.	Lau et al. 2006
Female	17 days GDs 1–17	none	3	↑ absolute and relative maternal liver weight, delayed offspring eye opening and body hair growth, ↑ offspring relative liver weight, ↓ offspring body weight, delayed mammary gland development (female offspring).	White et al. 2009; Wolf et al. 2007
Female CD1	17 days GDs 1–17	none	0.3	Delayed mammary gland development	Macon et al. 2011
Female CD-1	17 days	none	0.01	Delayed mammary gland development at PND 56. Exposure occurred only during gestation.	Tucker et al. 2015
Female C57BL6	17 days	0.1	0.3	Delayed mammary gland development at PND 61. Exposure occurred only during gestation.	Tucker et al. 2015
Female	15 days	1.88	3.75	↓ IgM (1 day post-dose), increased IgG (15 days post-dose), ↓absolute and relative spleen weight (1 day post-dose)	DeWitt et al. 2008

Species	Study Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical Effect(s)	Reference
Male	15 days	7.5	30	↓ sheep red blood cell IgM response in PPAR null mice indicate the response not completely PPAR dependent	DeWitt et al. 2015
Male	14 days	2.5	5	↓ sperm count, changes in testicular morphology, evidence of ↑ free radical oxidation	Liu et al. 2015
Male	14 days	0.2	2	↑ liver weight, serum estradiol and hepatic aromatase activity	Liu et al. 2015
Female	11 days GDs 7–17	none	5	↑ maternal and pup relative liver weight, delayed offspring eye opening and hair growth, ↓ male offspring body weight, delayed mammary gland development (female offspring)	White et al. 2009; Wolf et al. 2007
Female CD 1	8 days GDs 10–17	none	0.01	Delayed mammary gland development on PND 21 (female offspring)	Macon et al. 2011
Female CD 1	17 days GDs 1–17	none	0.3	Delayed mammary gland development on PND 14 (female offspring)	Macon et al. 2011

All but two of the short term studies used mice as the target species. Mice differ from rats in that the toxicokinetics of the males and females are similar. The half-life of PFOA in male rats is much longer than that in females, favoring higher serum levels in males after equivalent exposures. The difference in the excretion kinetics is a consequence of differences in renal transporters between male and female rats that appear to be under hormonal control. Several of the short term studies include serum data to support PK modeling of internal dose-response (DeWitt et al. 2008; Lau et al. 2006; Macon et al. 2011).

As was the case with the longer-term studies, increased liver weight was a common effect among the shorter-term studies. Increases in absolute or relative liver weights were reported in six of the studies that provided dose-response data from short term exposures (Lau et al. 2006; Liu et al. 1996, 2005; Loveless et al. 2008; White et al. 2009; Wolf et al. 2007) (Table 4-2). In some of the remaining studies, liver weight was not monitored as a variable. The Loveless et al. study (2008) identified significant focal liver necrosis in rats at the 10 mg/kg/day LOAEL, and both single cell and focal liver necrosis in mice at a LOAEL of 1 mg/kg/day. This might indicate that mice are more susceptible to necrosis than rats. Hepatic necrosis was reported in the longer duration Perkins et al. study (2004) of male rats and in the male F1 generation adult rats from the Butenhoff et al. study (2004a), but hepatic necrosis was present in few animals and not evaluated for statistical significance.

The co-occurring effects at the LOAEL were effects on spleen, thymus, liver, and/or developmental endpoints. Four of the studies involved exposures that occurred only during gestation and lactation and resulted in effects that were observed in the mature offspring. The hepatic and serum cholesterol effects in Quist et al. (2015) at PND 91 at a LOAEL of 0.03 mg/kg/day were present only in animals with elevated intakes of dietary fat. In adult animals with the same gestation/lactation only exposures, Filgo et al. (2015) identified a LOAEL of 5 mg/kg/day for accumulation of fat deposits in the liver Ito cells (steatosis). The study did not

provide information on the intakes of dietary lipid that could be compared with the data from Quist et al. (2015) to determine whether the effects were correlated with postnatal dietary fats or from the exposure during gestation and lactation.

A NOAEL of 2.5 mg/kg/day and a LOAEL of 5 mg/kg/day was reported for sperm counts and testicular morphology after a14-day exposure by Liu et al. (2015). No impact on male fertility was observed in Sprague-Dawley rats in the two-generation Butenhoff et al. study (2004a), in contrast to the Lu et al. study (2015) where male fertility was decreased in mice at a dose of 5 mg/kg/day for 28 days. A 14-day exposure to 2 mg/kg/day (Liu et al. 2015) led to significantly increased serum estradiol and increased hepatic aromatase activity.

The developmental impacts of PFOA exposure ranged from delayed mammary gland development in pups (Albrecht et al. 2013; Macon et al. 2011; Tucker et al. 2015; White et al. 2009, 2011; Wolf et al. 2007) to delays in attaining developmental milestones such as ossification, eye opening, and hair growth (Lau et al. 2006; Wolf et al. 2007). The LOAEL for developmental effects on mammary glands in female offspring from dams given 0.01 mg/kg/day for 8 days from Macon et al. (2011) is of unknown biological significance. In the same study, no effects on offspring body weight were found at maternal doses up to 3 mg/kg/day for 17 days (Macon et al. 2011). Data from White et al. (2011) showed no significant effects on body weight gain in pups nursing from dams treated with 1 mg/kg/day despite these dams having less fully-developed mammary glands compared to controls. Similarly, no differences in response to lactational challenge were seen in PFOA-exposed dams with morphologically delayed mammary gland development (White et al. 2011).

The studies that looked at the delays in other developmental milestones including eye opening, hair growth, and bone ossification all lacked a NOAEL. In Lau et al. (2006), the LOAEL was 1 mg/kg/day for reduced ossification of the proximal phalanges (forelimb and hindlimb). In the Wolf et al. (2007) study, delays in eye opening and hair growth occurred at a LOAEL of 5 mg/kg/day for gestational exposures of both 1–17 days and 7–17 days. Attainment of sexual maturity in males from the Lau et al. study (2006), rather than being delayed, was accelerated, at the LOAEL of 1 mg/kg/day.

The LOAEL for the mammary gland developmental effects in female offspring from dams given 0.01 mg/kg/day for 8 days from Macon et al. (2011). In the same study, no effects on offspring body weight were found at maternal doses up to 3 mg/kg/day for 17 days (Macon et al. 2011). Data from White et al. (2011) showed no significant effects on body weight gain in pups nursing from dams treated with 1 mg/kg/day despite these dams having less fully-developed mammary glands compared to controls. Similarly, no differences in response to lactational challenge were seen in PFOA-exposed dams with morphologically-delayed mammary gland development (White et al. 2011). Given that milk production was sufficient to nourish growth in exposed pups, there is uncertainty related to the functional impact of this endpoint and thus it was not considered quantitatively.

A NOAEL of 2.5 mg/kg/day and a LOAEL of 5 mg/kg/day were reported for sperm counts and testicular morphology after a 14-day exposure by Liu et al. (2015). No impact on male fertility was observed in Sprague-Dawley rats in the two-generation Butenhoff et al. study (2004a) in contrast to the Lu et al. study (2015) where male fertility was decreased in mice at a dose of 5 mg/kg/day for 28 days. A 14-day exposure to 2 mg/kg/day (Liu et al. 2015) lead to significantly-increased serum estradiol and increased hepatic aromatase activity.

The studies by DeWitt et al. (2008, 2015) demonstrate effects of PFOA on spleen and thymus weights and the immunoglobulin response to SRBC or dinitrophenyl ficol in wild-type and PPARα null mice. The DeWitt et al. (2015) data indicate that some but not all of the response is related to PPARα activation. As supported by the epidemiology data, suppression of the immune system response to a challenge because of PFOA exposure is an area of concern for humans as well as animal species.

Six of the twelve studies lacked a NOAEL. For those studies with a NOAEL, the value ranged 0.01–7.5 mg/kg/day, while the LOAELs ranged 0.01–30 mg/kg/day. The range of values across studies is narrow, with overlap between the NOAELs and LOAELs. In all instances, the durations of exposure in shorter-term studies were less than 39 days, suggesting that physiological responses to PFOA occur early in the exposure continuum and at doses, but not necessarily average serum levels, comparable to those observed in the long term studies.

4.1.1.1 PK Model approach

In linking chemical exposure to toxic endpoints, careful consideration of PKs is crucial. This is especially true for PFOA, where inter-species and gender variation in CL half-life can vary by several orders of magnitude. If the toxicological endpoints are assumed to be driven by internal concentrations, the internal exposure needs to be calculated and considered across species. Differences in PKs (e.g., male rats excrete PFOA more slowly than females) and differences across species produce differences in the external dose needed to achieve the same internal dose. The use of the animal data and the available PK model allows for the incorporation of species differences in saturable renal resorption, dosing duration, and serum measurements for doses administered to determine HEDs based on average serum concentration and CL.

Candidate studies for RfD development were restricted to those of adequate duration (preferably > 7 weeks), multiple dose groups, use of a concurrent control, and with serum data amenable for modeling that showed the most sensitive effects following exposure to PFOA. Those studies included the subchronic study by Perkins et al. (2004), the two-generation study by Butenhoff et al. (2004a), both conducted in rats, and the Butenhoff et al. study (2002) in monkeys. Also included are the developmental studies of Lau et al. (2006) and Wolf et al. (2007)/White et al. (2009), and the DeWitt et al. study (2008) of immunotoxicity in mice that showed effects of lifetime concern despite their briefer exposure durations. Together these studies encompassed the range of doses evaluated and the LOAELs observed in other studies that lacked serum data.

The Butenhoff et al. study (2002) was included as it is the only longer-term study in a nonhuman primate and had serum PFOA data available. The dose of 3 mg/kg/day was a LOAEL for increased liver weight in the absence of clear adverse effects. The small number of animals per group (2–4) made evaluation of accompanying liver effects difficult to evaluate against the Hall et al. (2012) criteria. Thus, while included in the model for comparison of serum levels, data from Butenhoff et al. (2002) will not be considered further in the quantitative RfD assessment.

PFOA has dose-dependent kinetics. Although repeated doses rapidly result in quasi-equilibrium blood concentrations, a single dose results in a much longer half-life than is consistent with a rapid approach to quasi-equilibrium (Andersen et al. 2006; Lou et al. 2009). Using a simple, linear PK model (e.g., a one- or two-compartment model) to predict internal dose resulted in estimated HEDs from repeated exposures that were greater than the external doses supported by the experimental data (Butenhoff et al. 2004a; Lou et al. 2009). Application

of a saturable renal resorption model (Andersen et al. 2006) predicted average serum values at the time of sacrifice and the duration necessary to reach steady state.

PK data (serial blood concentrations following treatment with known quantities of PFOA) were collected for three species: cynomolgus monkey (Butenhoff et al. 2004b), Sprague-Dawley rat (Kemper 2003), and mice. Data were available for two strains of mice and these were analyzed separately: CD1 (Lou et al. 2009) and C57BL6/N (DeWitt et al. unpublished, cited in DeWitt et al [2008]). Due to the pronounced difference in the PKs of male and female rats, the two genders were fit separately. For mice, only female data were used. For monkeys a limited amount of female data was used jointly with male data, assuming the only difference between the genders for monkey was bodyweight.

For each study with a toxicological endpoint and LOAEL, the AUC and final serum concentrations were determined for the exposure duration investigated in that study. These values are summarized in Table 4-3 for rats, Table 4-4 for mice, and Table 4-5 for monkeys. In order to make a rough assessment of the validity of the model predictions, a final serum concentration was predicted for each treatment so that it could be compared to measured serum values. The predicted final serum concentration is the estimate for serum concentration at the time of sacrifice. They differed by a factor of four when strains were different and closer to a factor of two when predicted using parameters from the same strain. Because these predictions do not perfectly match the measured serum concentrations, there remains uncertainty about the exposure estimates, and this uncertainty has not been fully characterized.

Table 4-3. Predicted Final Serum Concentration and Time-Integrated Serum Concentration (AUC) for Studies in Rats

Study	Species/ Strain	Exposure Duration	Oral Doses mg/kg/day	Measured Final Serum value µg/ml	Species/ Strain Used for Prediction	Predicted Final Serum Value µg/ml	Predicted AUC mg/L*h
Perkins et al. 2004	Rat (M) ChR-CD	13 weeks Diet	0.06 0.64 1.94 6.50	7.1 (1.2) 41 (13) 70 (16) 138 (34)	Rat (M) Sprague-Dawley	3.8 (0.0955) 34.8 (0.865) 79.5 (3.84) 139 (13.1)	7230 (181) 69100 (1540) 168000 (6520) 326000 (27100)
Butenhoff et al. 2004a	Rat (M) Sprague-Dawley	F0M: 10 wk pre mating-mating Gavage	1 3 10 30	NT NT 51.5 [s] 45.3	Rat (M) Sprague-Dawley	49.9 (1.53) 102 (6.5) 153 (17.3) 169 (27.7)	92500 (2600) 204000 (10900) 345000 (34200) 412000 (70500)

Notes: Numbers in parentheses indicate SD
M = male; s = serum; NT = not tested
Since the Kemper (2003) data were not tied to toxicological endpoints and were only used in model development, they are not included in this table.

Table 4-4. Predicted Final Serum Concentration and Time-Integrated Serum Concentration (AUC) for Studies in Mice

Study	Species/ Strain	Exposure Duration days	Oral Doses mg/kg/d	Measured Serum Value µg/ml	Species/ Strain Used for Prediction	Predicted Final Serum Value µg/ml	Predicted AUC mg/L*h
White et al. 2009; Wolf et al. 2007	Mouse (F) CD-1	GDs 1–17[a] Gavage	3 5	NT NT	Mouse (F) CD-1	25 (2.22) 25.6 (2.26)	33,700 (1,860) 40,700 (2,170)
		GDs 7–17 Gavage	5	24.8	Mouse (F) CD-1	29 (2.55)	25,400 (1,320)
DeWitt et al. 2008	Mouse (F) C57BL/6N	15 days Drinking water	0.94 1.88 3.75 7.5 15 30	NTb NTb 35.3 42.8 50.0 162.6	Mouse (F) C57BL/6N	29.7 (1.58) 51.9 (1.89) 70.2 (2.57) 81.4 (3.91) 94.7 (11.8) 117 (29.3)	7,300 (541) 13,800 (951) 22,400 (1,290) 30,500 (1,540) 40,100 (4,720) 56,000 (12,300)
Lau et al. 2006	Mouse (F) CD-1	GDs 1–17 Gavage	1 3 5 10 20 40	21.9c 40.5 c 71.9 c 116 c 181 c 271 c	Mouse (F) CD-1	57.6 (3.82) 87.2 (7.93) 95.2 (7.41) 106 (5.84) 121 (11) 148 (30.2)	16,400 (606) 33,600 (1,930) 40,700 (2,180) 49,600 (1,980) 61,400 (5,050) 80,100 (12,700)

Notes: Numbers in parentheses indicate SD
NA = not applicable; could not be determined
F = female; GD = gestation day; NT = not tested
[a] Sacrificed on PND 22.
[b] DeWitt et al. (2008) had 0.94 and 1.88 mg/kg/day dose groups in a second experiment.
[c] The Lau et al. (2006) serum data were provided by the author for animals treated GDs 1–17.

Table 4-5. Predicted Final Serum Concentration and Time-Integrated Serum (AUC) in Studies of Monkeys

Study	Species/ Strain	Exposure Duration	Oral Doses mg/kg/day	Measured Serum value µg/ml	Species/ Strain Used For Prediction	Predicted Final Serum Value µg/ml	Predicted Exposure (AUC) mg/L*h
Butenhoff et al. 2002, 2004b	Monkey (M) Cyno-molgus	26 weeks Oral capsule	3 (n = 3) 10 (n = 4) 30/20 (n = 3)	117.9 (87.6-141) 77.35 (55.4- 96.5) 283.2 (61.7-489)	Monkey Cyno-molgus	89.1 (12.4) 121 (14) 149 (31)	380,000 (50,100) 553,000 (62,800) 710,000 (144,000)

Notes: Numbers in parentheses indicate SD
M = male

The average serum concentration for the LOAEL or NOAEL was determined through numeric simulation. The average or mean value has the advantage of normalizing the serum concentration across the exposure durations to generate a uniform metric for internal dose in situations where the dosing durations varied and serum measurements were taken immediately prior to sacrifice. The averaged serum concentration is a hybrid of the AUC and the maximum serum concentration. Compared across studies, PFOA average serum concentration appears to be a stable reflection of internal dosimetry.

Table 4-6 provides the AUC from the model, the dosing duration from each of the modeled studies, and the resultant average serum concentration. The data from the monkey study (Butenhoff et al. 2002, 2004b) were not used because of the small number of animals evaluated

and the wide variability in the responses. The internal doses associated with the effect levels (LOAELs) differ by less than an order of magnitude (13.1–96.2 mg/L), while the AUC values differ by over two orders of magnitude (5,360–380,000 mg/L*h). Given the differences in external doses, the projected serum levels are proportionally quite similar.

Table 4-6. Average Serum Concentrations Derived from the AUC and the Duration of Dosing

Study	Dosing duration days	NOAEL mg/kg/day (AUC mg/L*h)	NOAEL (Av serum mg/L)	LOAEL mg/kg/day (AUC mg/L*h)	LOAEL (Av serum mg/L)
DeWitt et al. 2008: mice; ↓ IgM response to SRBC	15	1.88 (13,800)	38.2 (2.63)	3.75 (22,400)	61.9 (3.58)
Lau et al. 2006: mice reduced pup ossification (m, f), accelerated male puberty	17	None	None	1 (16,400)	38.0 (1.4)
Perkins et al. 2004: rats; ↑liver weight/necrosis	91	0.64 (69,100)	31.6 (0.073)	1.94 (168,000)	77.4 (2.98)
Wolf et al. 2007: mice; GDs 1–17 ↓Pup body weight[a]	17	None	None	3 (33,700)	77.9 (4.3)
Wolf et al. 2007: mice; GDs 7–17 ↓Pup body weight[a]	11	None	None	5 (25,400)	87.9 (4.57)
Butenhoff et al. 2004a: ↓relative body weight/↑ relative kidney weight and ↑kidney:brain weight ratio in F0 and F1 at sacrifice	84	None	None	1 (92,500)	45.9 (1.29)

Notes: Significance $p < 0.05$ or < 0.01
m = male; f = female; SRBC = Sheep Red Blood Cell
[a] serum from pups on PND 22

Table 4-6 identifies serum values of 38, 45.9, and 61.9 mg/L as the lowest concentrations associated with adverse effects in the Lau et al. (2006), Butenhoff et al. (2004a), and DeWitt et al. (2008) studies, respectively. Thus, it appears that the LOAELs are roughly consistent across gender, species, and treatment with respect to average serum concentration. Assuming that MoA, susceptibility to toxicity, or both do not vary and that PKs alone explains variation, it is reasonable to expect similar concentrations to cause similar effects in humans.

The Andersen et al. (2006) model, used to make the predictions in Tables 4-3, 4-4, and 4-5 can be solved analytically to predict the steady-state concentration (C_{ss}) resulting from a fixed infusion DR (in units of µmol/h):

$$C_{ss} = \frac{DR}{free * Q_{fil}}\left(1 + \frac{T_{max}}{Q_{fil} * k_T + \mathrm{DR}}\right)$$

Although the assumption of a constant infusion exposure simplifies the actual dose regimen used, this assumption permits rapid calculation and analysis of C_{ss}. Using this equation, one can calculate a range of C_{ss} values for each DR. The range of C_{ss} values are derived from the species-specific combinations of parameters from the Bayesian analysis of the available PK data. This result for C_{ss} depends nonlinearly on DR. The PFOA toxicity studies used discrete, daily doses; these doses were converted into rates by dividing daily dose by 24 hours. In Table 4-7, the C_{ss} is compared with the average serum concentration predicted. The fraction of C_{ss} is calculated, indicating that the studies range 36%–91% of C_{ss}.

For human exposure to PFOA, one needs to rely on steady-state calculations since there is a lack of both the sufficient PK and exposure knowledge to make more complicated estimates. The average serum concentrations of the LOAEL in Table 4-7 are all within roughly one order of magnitude (12.4–87.9 mg/L).

Table 4-7. Comparison of Average Serum Concentration and Steady-State Concentration

Study	Dosing duration days	LOAEL mg/kg/day	C_{ss} (mg/L) for constant infusion of LOAEL	Average Serum Conc. for Study (mg/L)	Fraction of C_{ss} (Average / C_{ss})
Perkins et al. 2004	91	1.94	84.4 (3.81)	77.4 (2.98)	0.913 (0.00746)
Butenhoff et al. 2004a	84	1	52.5 (1.72)	45.9 (1.29)	0.874 (0.00776)
Wolf et al. 2007; GDs 7–17	11	5	107 (6.8)	87.9 (4.57)	0.82 (0.0117)
Wolf et al. 2007; GDs 1–17	17	3	95.9 (6.73)	77.9 (4.3)	0.813 (0.0148)
DeWitt et al. 2008	15	3.75	84.1 (4.5)	61.9 (3.58)	0.736 (0.0233)
Lau et al. 2006	17	1	67.8 (4.39)	38 (1.4)	0.561 (0.0277)

Notes: Average serum concentrations from PK simulations of toxicity study treatment regimens and Css were both predicted using species-specific parameter distributions (i.e., draws from the Markov Chain determined by analyzing the available PK data for the appropriate species). The number in parentheses is the SD.

The predicted average serum concentrations can be converted into an oral equivalent dose by recognizing that, at steady state, CL from the body should equal dose to the body. CL can be calculated if the rate of elimination (derived from the half-life) and the V_d are both known. In making the calculation based on CL, it is important also to consider whether the exposure to PFOA has lasted long enough to reach steady state. Four of the endpoints modeled represent serum values that are greater than 80% of steady state, but none represent steady-state concentrations. Those endpoints representing lower percentages of steady state require consideration of the uncertainty resulting from use of a projection that is not representative of a steady-state concentration (UF_S) when establishing an RfD for a chronic lifetime exposure endpoint.

Measures of half-life in humans have been determined for both workers and the general population (section 2.6.2). Olsen et al. (2007) gives the human half-life as 3.8 years for PFOA in an occupationally-exposed U.S. cohort. Bartell et al. (2010) determined a value of 2.3 years based on the decline in serum levels among members of the general population exposed via drinking water in the area near the DuPont Works plant in Washington, West Virginia after the drinking water concentrations decreased. EPA chose to use the Bartell et al. (2010) half-life value because it is the one most relevant to scenarios where exposures result from ingestion of contaminated drinking water by members of the general population.

Thompson et al. (2010) gives a V_d of 0.17 L/kg_{bw} (section 2.6.3). The V_d is defined as the total amount of PFOA in the body divided by the blood or serum concentration. The V_d was calibrated using human serum concentrations and exposure data from NHANES and assumes that most PFOA intake came from contaminated drinking water. The value for V_d was calibrated so that the model prediction of elevated blood levels of PFOA was consistent with the values from NHANES. The V_d value determined by Thompson et al. (2010) did not consider PFOA contributions from sources other than drinking water. However this estimate is not radically different from the 0.198 L/kg_{bw} determined for the monkey in the study by Butenhoff et al. (2004b).

The half-life ($t_{½}$) and V_d are utilized to calculate the CL for PFOA according to the following equation assuming first order kinetics for CL (Medinsky and Klaassen 1996):

$$CL = V_d \times (\ln 2 \div t_{½}) = 0.17\ L/kg_{bw} \times (0.693 \div 839.5\ days) = 0.00014\ L/kg\ bw/day$$

Where:

V_d = 0.17 L/kg
ln 2 = 0.693
$t_{½}$ = 839.5 days (2.3 years x 365 days/year = 839.5 days)

These values combined give a CL of 1.4×10^{-4} L/kg bw/day.

Scaling the derived average serum concentrations (in mg/L) for the NOAELs and LOAELs in Table 4-6 yields the predicted oral HED in mg/kg/day for each corresponding serum measurement. The HED values are the predicted human oral exposures necessary to achieve serum concentrations equivalent to the LOAEL (and NOAEL where available) in the animal toxicity studies. Note that this scaling assumes linear first-order human kinetics in contrast to the nonlinear phenomena observed at high doses in animals. It is justifiable at the lower dose NOAEL and LOAEL concentrations from the animal studies that that demonstrate the first-order, linear response to dose necessary to calculate CL.

Thus, HED = average serum concentration (in mg/L) x CL

Where:

Average serum is from model output in Table 4-8
CL = 0.00014 L/kg bw/day

Table 4-8. HEDs Derived from the Modeled Animal Average Serum Values

Study	Dosing duration days	NOAEL mg/kg/d	NOAEL Av serum mg/L	HED mg/kg/d	LOAEL mg/kg/d	LOAEL (Av serum) mg/L	HED mg/kg/d
DeWitt et al. 2008: mice; ↓ IgM response to SRBC	15	1.88	38.2	0.0053	3.75	61.9	0.0087
Lau et al. 2006: mice reduced pup ossification (m,f), accelerated male puberty	17	None	-	-	1	38.0	0.0053
Perkins et al. 2004: rats; ↑liver weight/necrosis	91	0.64	31.6	0.0044	1.94	77.4	0.0108
Wolf et al. 2007: mice; GDs 1–17 ↓pup body weight	17	None	-	-	3	77.9	0.0109
Wolf, et al. 2007: mice; GDs 7–17 ↓pup body weight[1]	11	None	-	-	5	87.9	0.0123
Butenhoff et al. 2004a: ↓F0 body weight/↑ absolute and relative kidney weight	84	None	-	-	1	45.9	0.0064
Macon et al. (2011) GDs 1–17 ↓mammary gland development[2]	17	-	-	-	0.3	12.4	0.0017

Notes: Significance $p < 0.05$ or < 0.01
m = male; f = female; SRBC = Sheep Red Blood Cell
[1] serum from pups on PND 22
[2] serum from pups on PND 7

4.1.1.2 RfD Quantification

The subset of studies amenable for use in derivation of HED based on average serum measurements from the PK model is based solely on results from studies that have dose and species-specific serum values for model input, as well as exposure durations of sufficient length to achieve values near to steady-state projections or applicable to developmental endpoints with lifetime consequences following short term exposures.

As explained previously, human data identified significant relationships between serum levels and specific indicators of adverse health effects but lacked the exposure information for dose-response modeling. For this reason none of the human studies provided an appropriate POD for RfD derivation. The pharmacokinetically-modeled average serum values from the animal studies are restricted to the animal species selected for their low dose response to oral PFOA intakes. Extrapolation to humans adds a layer of uncertainty that needs to be accommodated in deriving the RfD.

HED PODs. The HEDs derived from Perkins et al. (2004), DeWitt et al. (2008), Lau et al. (2006), and Butenhoff et al. (2004a) were each examined as the potential basis for the RfD. Only Perkins et al. (2004) and DeWitt et al. (2008) identified a NOAEL from which the HED could be derived. The Lau et al. (2006) and Wolf et al. (2007)/White et al. (2009) LOAEL HEDs included developmental effects in the offspring as accompanied by the increased liver weight that is an accepted biomarker for PFOA exposure. These are developmental exposure studies that carry lifetime consequences for a less-than-lifetime exposure. The Butenhoff et al. study (2004a) included significant decreased body weight (not confounded by reduced food intake) in F0 males accompanied by increased kidney weight (consistent with the need for renal tubular transport) as co-critical at the LOAEL. The DeWitt et al. study (2008) has a LOAEL for decreased IgM, increased IgG, and increased absolute and relative spleen weight after a 15-day exposure.

Table 4-9 provides the calculations for potential RfDs using the HEDs derived from PK modeling based on the serum values collected at animal sacrifice. The table applies UFs to each POD and illustrates the array of potential RfD outcomes. Each POD is impacted by the doses utilized in the subject study, the endpoints monitored, and the animal species/gender studied. Thus, the array of outcomes, combined with knowledge of the individual study characteristics, helps to inform selection of an RfD that will be protective for humans.

The potential lifetime RfD values in Table 4-9 differ by about an order of magnitude (0.00002–0.00015 mg/kg/day) but so do the UFs applied to the POD. These results demonstrate the ability of the model to normalize the animal data across species, strain, gender, and exposure duration. The UFs applied in the derivation of the potential RfDs alter the first-order, direct relationship between the animal serum measurements associated with the animal studies and the resultant RfD. Accordingly, the resultant RfD cannot be extrapolated to a corresponding human serum value equivalent to the RfD using the CL value applied when calculating the HED from the animal serum.

Table 4-9. The Impact of Quantification Approach on the RfD Outcomes for the HEDs from the PK Model Average Serum Values

POD	Value mg/kg/day	UF_H	UF_A	UF_L	UF_S	UF_D	UF_{total}	Candidate RfD mg/kg/day
$PK\text{-}HED_{NOAEL\ Perkins}$ rats; ↑liver weight/necrosis	0.0044	10	3	-	-	-	30	0.00015
$PK\text{-}HED_{LOAEL\ Wolf\ GD\ 1–17}$ mice; ↓pup body weight	0.0109	10	3	10	-	-	300	0.00004
$PK\text{-}HED_{LOAEL\ Wolf\ GD\ 7–17}$ mice; ↓pup body weight[a]	0.0123	10	3	10	-	-	300	0.00004
$PK\text{-}HED_{NOAEL\ DeWitt}$ mice; ↓ IgM response to SRBC	0.0053	10	3	-	10	-	300	0.00002
$PK\text{-}HED_{LOAEL\ Lau}$ mice reduced pup ossification (m,f), accelerated male puberty	0.0053	10	3	10	-	-	300	0.00002
$PK\text{-}HED_{LOAEL\ Butenhoff}$ ↓F0 body weight/↑ absolute and relative kidney weight	0.0064	10	3	10	-	-	300	0.00002

Notes: m = male; f = female; SRBC = Sheep Red Blood Cell
[a] serum from pups on PND 22

The Perkins et al. (2004) and Butenhoff et al. (2004a) studies were conducted in male Sprague-Dawley rats with durations of 91 days via diet and 84 days via gavage, respectively. Both were associated with increased relative liver weight accompanied by some hepatic necrosis in a small number of animals. The Butenhoff et al. study (2004a) also observed a significant decrease in body weight compared to controls for the F0 male rats at the end of the 84-day exposure. The studies by Lau et al. (2006) and Wolf et al. (2007)/White et al. (2009) were conducted in pregnant female mice with a 17-day average exposure via gavage, resulting in increased liver weights in the dams and low body weights and developmental delays in offspring.

Uncertainty Value Application

A UF for intraspecies variability (**UF_H**) of 10 is assigned to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic genetic, life stage, health status) and extrinsic (life style) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics among humans other than variability in serum levels measured among populations residing in common geographical locations with presumably fairly similar exposures.

A UF for interspecies variability (**UF_A**) of three was applied to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). The 3-fold factor is applied to account for toxicodynamic differences between the animals and humans. The HEDs were derived using average serum values from a model to account for PK differences between animals and humans.

A UF for LOAEL to NOAEL extrapolation (**UF_L**) of 10 was applied to all PODs other than the Perkins et al. study (2004) to account for use of a LOAEL for the POD. The POD for the Perkins et al. study (2004) is a NOAEL.

A UF for extrapolation from a subchronic to a chronic exposure duration (**UF_S**) is one, because the PODs are based on average serum concentrations and determined to represent > 80% of steady state for each study (81%–91%) except for the Lau et al. (2006) developmental study (56%). The Lau et al. (2006) developmental HED was not adjusted for lifetime exposures, because the average serum values associated with the developmental studies are more protective than those for the longer-term studies of systemic toxicity. A UF_S of 10 was applied to the DeWitt et al. study (2008) serum-derived HED reflecting 73% of steady state because the data suggest that longer-term exposures to the same dose have the potential to increase serum values beyond the levels indicated by the 15-day exposure to mice. In addition, the NOAEL for immunological effects (0.94 mg/kg/day) was a LOAEL for effects on liver weight in the absence of histological evaluation on both days 16 and 31 following a 15-day exposure. Thus, there is a potential that lifetime exposures at serum steady state could impact the liver, increasing the risk for tissue damage.

A database UF (**UF_D**) of one was applied to account for deficiencies in the animal study database for PFOA. There are extensive human data from epidemiology studies in the general population, as well as in worker cohorts. The epidemiology data provide strong support for the identification of hazards observed following exposure to PFOA in the laboratory animal studies and the human relevance of the critical effects. However, uncertainties in the use of the available epidemiology data precluded their use at this time in the quantification of an RfD. There are extensive data from short term, subchronic, chronic, reproductive, developmental, and mechanistic studies in laboratory animals that support the endpoints used in calculating the potential RfD. The potential RfD is the one applicable to those most at risk from exposure to PFOA present in drinking water, the fetus and young infants. The alternative identical RfDs are values that could be more appropriate for other exposure scenarios and endpoint concerns.

4.1.2 RfD Selection

The candidate RfDs in Table 4-9 range 0.00002–0.00015 mg/kg/day. The RfD of 0.00002 mg/kg/day calculated from HED average serum values from Lau et al. (2006) was selected. The RfD based on Lau et al. (2006) is derived from reduced ossification of the proximal phalanges (forelimb and hindlimb) and accelerated puberty in male pups (4 days earlier than controls) as the critical effects. The selected RfD from the Lau et al. study (2006) is supported by the RfD for effects on the response of the immune system (DeWitt et al. 2008) to external challenges as observed following the short-term 15-day exposures to mature mice and effects on organ and body weights in F1 adult males observed following chronic exposure.

Decreased pup body weights were also observed in studies conducted by Wolf et al. (2007)/White et al. (2009) and Lu et al. (2015) using mice receiving external doses within the same order of magnitude (1, 3, and 5 mg/kg/day respectively) as those in the chosen study for the RfD. The selected RfD from the reproductive and developmental study is supported by the longer-term RfD for effects on the response of the immune system (DeWitt et al. 2008) to external challenges as observed following the short-term exposures to mature mice and the effects on kidney weight observed at the time of sacrifice in the F0 parental males in the Butenhoff et al. study (2004a) that provided the modeled serum data.

Support for the selected RfD is provided by other key studies with NOAELs and LOAELs similar to those used for quantification, yet lacked serum data that could be used for modeling. There were effects on liver weight and hepatic hypertrophy in the Perkins et al. (2004) and

DeWitt et al. (2008) studies that were not considered in the identification of the study LOAEL because of a lack of data to demonstrate adversity as determined by the Hall criteria (Hall 2012). The LOAEL for evidence of hepatic necrosis and other signs of tissue damage in the F1 male rats from the Butenhoff et al. study (2004a) was 3 mg/kg/day, and the NOAEL was 1 mg/kg/day. In the Loveless et al. study (2008), for male rats 1 mg/kg/day was a NOAEL for increased relative liver weight and focal liver necrosis was seen at a LOAEL of 10 mg/kg/day, while in male mice the NOAEL was 0.3 mg/kg/day for the increased liver weight and focal liver necrosis was at a LOAEL of 1 mg/kg/day following a 29-day exposure. In the study by Tan et al. (2013) the degree of damage to the liver at 5 mg/kg/day became more severe with increased necrosis, inflammation, and steatosis when animals were given an HFD. The HED modeled from the average serum value in mice for the LOAEL of 3 mg/kg/day from Wolf et al. (2007)/White et al. (2009) was 0.0109 mg/kg/day, about twice that of the 0.0053 mg/kg/day for the rats in the Lau et al. study (2006) at a LOAEL of 1 mg/kg/day. Both studies lacked a NOAEL.

Using the PK model of Wambaugh et al. (2013), average serum PFOA concentrations were derived from area under the curve (AUC) considering the number of days of exposure before sacrifice. The predicted serum concentrations were converted as described above to oral HEDs in mg/kg/day for each corresponding serum measurement. The POD for the derivation of the RfD for PFOA is the HED of 0.0053 mg/kg/day that corresponds to a LOAEL that represents approximately 60% of steady-state concentration. An UF of 300 (10 UF_H, 3 UF_A, and 10 UF_L) was applied to the HED LOAEL to derive an RfD of 0.00002 mg/kg/day.

There are extensive human data from epidemiology studies on the general population, as well as worker cohorts. The epidemiology data provide support for the human relevance of the hazards identified in the laboratory animals. However, they lack the quantitative information on the human exposures (doses and durations) responsible for the human serum levels. Although some associations show a relationship between effects and serum measures, the serum measures are lower than the PODs from the animal studies and some associations are confounded by reverse causality. Data supporting a first-order kinetic relationship between dose/duration and serum concentrations are needed before the human data can be used in a manner comparable to the process utilized in the RfD derivation.

4.1.3 RfC Determination

Limited data from human epidemiology and animal toxicity studies were available with which to evaluate the potential health effects resulting from continuous inhalation exposure to PFOA. The available data base, summarized below for human and animal data, does not provide the minimum data needed for derivation of the RfC as discussed in USEPA (1994b). Thus, the RfC for PFOA is not recommended or derived.

Human Data. Studies have examined occupational and residential populations at or near large-scale PFOA production plants in the United States in an attempt to determine the relationship between serum PFOA concentration and various health outcomes suggested by the standard animal toxicological database. While inhalation is an important route of exposure to workers, drinking water was identified as a contributor to exposure in the general population. In all of the epidemiology studies, wide ranges of serum levels were reported that are likely a reflection, not only of intra-human toxicokinetic variability, but also of diversity in external exposure sources and routes of exposure. Thus, the data cannot be clearly applied to quantification of dose-response via inhalation.

Animal Data. Inhalation toxicity data in laboratory animals were limited to acute, single, and repeated exposures for PK studies, and a developmental toxicity study in rats. No subchronic or chronic inhalation toxicity studies in animals were available for assessment. Generally, adverse effects observed following inhalation exposure to PFOA were similar to effects following exposure to an irritating dust. For male rats exposed to PFOA as a dust in air, the 4-hour LC_{50} was 980 mg/m^3, with adverse clinical signs of body weight loss, irregular breathing, red discharge around the nose and eyes, and corneal opacity and corrosion (Kennedy et al. 1986, 2004).

Distinct toxicokinetic differences between male and female rats were found following single and repeated inhalation exposures. Sprague-Dawley rats were exposed nose-only to PFOA aerosols of 0, 1, 10, or 25 mg/m^3 for 6 hours or for 6 hours/day, 5 days/week, for 3 weeks (Hinderliter 2003). Absorption was indicated in both males and females after single and repeated exposures with plasma PFOA concentrations proportional to exposure concentration. The C_{max} values were approximately 2–3 times higher in males than in females and persisted for up to 6 hours in males compared to just 1 hour in females. Similarly, the elimination of PFOA was rapid by females at all exposure levels, and by 12 hours after exposure the plasma levels had dropped below the analytical LOQ (0.1 µg/ml). In males, the plasma concentration remained approximately 90% of the peak concentration at all exposure levels at 24 hours after exposure, and steady state was reached following repeated exposures. While these results clearly show toxicokinetic differences between male and female rats, toxicity data were not included, limiting use of the information in a quantitative risk assessment.

In a developmental toxicity study, pregnant Sprague-Dawley rats were exposed whole-body to PFOA dust concentrations of 0, 0.1, 1, 10, or 25 mg/m^3 for 6 hours/day on GDs 6–15 (Staples et al. 1984). Dams were either sacrificed on GD 21 or allowed to litter and rear their offspring until PND 35. Maternal toxicity at 10 and 25 mg/m^3 consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, lethargy (high-concentration group only), and decreased body weight and food consumption. Five out of 24 dams died during treatment at 25 mg/m^3. Significantly increased mean liver weight ($p<0.05$) was seen at 25 mg/m^3. No effects were observed on the maintenance of pregnancy or fetal and pup survival. At 25 mg/m^3, mean offspring body weight was lower than that of controls on GD 21 and throughout lactation.

4.2 Dose-Response for Cancer Effects

As discussed in section 3.4.5, there is equivocal evidence that PFOA exposure might be associated with an increased risk for cancer from the human epidemiology database and animal studies. Only one study in highly exposed worker populations showed a positive association between death from cancer and PFOA exposure. In that study, a significant increase in mortality due to kidney cancer was found for workers in the highest quartile of cumulative PFOA exposure; the estimated average mean serum level was 0.35 µg/mL (Steenland and Woskie 2012). Mortality from cancer in PFOA workers was not elevated in several other studies (Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014). Serum levels were not available in studies reporting only mortality. No association was found between PFOA level and cancer incidence rate in workers with mean serum of 0.113 µg/mL (Steenland et al. 2015).

No associations were found in the general population between mean serum PFOA levels up to 0.0866 µg/mL and colorectal, breast, prostate, bladder, and liver cancer (Bonefeld-Jørgensen

et al. 2014; Eriksen et al. 2009; Hardell et al. 2014; Innes et al. 2014). In contrast, two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrolment 0.024 μg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013).

The only chronic bioassays of PFOA were conducted in rats (Biegel et al. 2001; Butenhoff et al. 2012). The two studies support a positive finding for the ability of PFOA to be weakly tumorigenic in one or more organs of male but not female rats. There are no carcinogenicity data from a second animal species. The study by Butenhoff et al. (2012) examined male and female rats; the Biegel et al. study only evaluated males. The tumor types observed were:

- Liver (Butenhoff et al. 2012).
- Leydig Cell (Biegel et al. 2001; Butenhoff et al. 2012).
- Pancreatic Ascinar Cell (Biegel et al. 2001).

The dose response information and tumors incidence data from the Butenhoff et al. (2012) and Biegel et al. (2001) studies are summarized in Table 4-10. The data are limited in that only Butenhoff et al. tested more than one dose. Only one tumor-type (Leydig cell adenoma) demonstrated a dose-response relationship.

Table 4-10. Summary of Tumor Data from Animal Studies

Tissue	Concentration in Diet (ppm)			Tumor Type	Reference
	0[a]	30	300		
Liver Male	7/50	2/50	10/50	Hepatocellular carcinoma	Butenhoff et al. 2012
Liver Male	0/80	NT	0/76	Hepatocellular carcinoma	Biegel et al. 2001
Liver Male	2/80	NT	10/76	Hepatocellular adenoma	Biegel et al. 2001
Liver Female	0/50	0/50	2/50	Hepatocellular carcinoma	Butenhoff et al. 2012
Testes Male	0/50	2/50	7/50	Leydig Cell adenomas	Butenhoff et al. 2012
Testes Male	0/80	NT	8/76	Leydig Cell adenomas	Biegel et al. 2001
Pancreas Male	1/80	NT	0/76	Acinar Cell carcinoma	Biegel et al. 2001
Pancreas Male	0/80	NT	7/76	Acinar Cell adenoma	Biegel et al. 2001

Notes: [a] The value reported is for the ad libitum control
NT = Not tested

There are some data that provide support for the hypothesis that the PPARα agonism is the MOA for the observed liver tumors in rats. PPARα is found in human livers and, when activated, is linked through activation to a number of metabolic responses but not to the large-scale peroxisome proliferation associated with tumors in rats and other rodent species. The data support a PPARα MOA for the liver tumors and, thus, are indicative of lack of relevance to humans.

PPARα activation might also play a role in the other tumor types observed. However for the Leydig tumors the PPARα involvement is indirect. The favored hypothesis for the DNA replication errors responsible for induction of Leydig tumors are postulated to be a consequence of the following sequence of events:

- Decreased testosterone synthesis.
- Increased GnRH and increased levels of LH leading to chronic stimulation of Leydig cells by growth-stimulating mediators including IGF-1, TGF-α, leukotrienes and various free radicals (Clegg et al. 1997; Li et al. 2011).

There are some experimental data that demonstrate systemic effects of PFOA leading to decreased testosterone and increased estradiol as a result of increased activity of aromatase, the cellular enzyme responsible for the metabolic conversion of testosterone to estradiol (Biegel et al. 1995). However, more data to support the relationship of PFOA to intermediate steps in the proposed MOAs are needed.

Current MOA theories for the PACT tumors are linked to the impact of either the mitogenic effects of elevated testosterone levels or intestinal tissue hormones (CCK, gastrin, or both) in promoting proliferation of acinar cell preneoplastic foci (Klaunig et al. 2003; Obourn et al. 1997). PACT tumors are most commonly found in rats but also occur in humans. Because PFOA is associated with decreased rather than increased levels of testosterone, the mechanistic link between PFOA exposure and PACT is more likely associated with gastric hormone changes, possibly associated with alterations in bile composition. Some of the membrane transporters that are impacted by PFOA function in transport of bile components from the liver to the gallbladder and thereby to the intestines. Cholecystokinen and gastrin stimulate contraction of the gallbladder and release of bile into the intestines. Data to support this hypothesis are not available for PFOA. Obourn et al. (1997) studied the impact of PFOA on CCK using *in vitro* assays and found that it was not an agonist for the CCKA receptor that activates CCK release.

The increase in hepatocellular tumors did not show a direct relationship to dose in male rats and was not significantly elevated in either males or females at the high dose when compared to controls.

There was a dose-related significant increase in LCTs in male rats in the Butenhoff et al. study (2012), which was confirmed by the high dose in the single-dose mechanistic study by Biegel et al. (2001). At the high dose (300 ppm in the diet; 14.2 mg/kg/day), tumors were found in 14% of the male rats at the end of 2 years in the Butenhoff et al. study (2012) and 4% at the low dose (1.3 mg/kg/day). In the Biegel et al. study (2001), 11% were affected at a dose of 300 ppm in the diet (13.6 mg/kg/day). In each case, there were no LCTs in the controls. The PACT tumors, only detected in the single dose Biegel et al. study (2001), do not support quantification.

Under the EPA 2005 cancer guidelines, the evidence for the carcinogenicity of PFOA is considered *suggestive* because only one species has been evaluated for lifetime exposures and the tumor responses occurred primarily in males. Dose-response data are only available for the LCTs in one study. However, two studies involving members of the C8 Health Project showed a positive association between PFOA levels (mean at enrolment of 0.024 μg/mL) and kidney and testicular cancers (Barry et al. 2013; Vieira et al. 2013). Therefore, the data on LCTs from Butenhoff et al. (2012) were modeled to provide a perspective on the magnitude of the potential cancer risk as it compares with the level of protection provided by the RfD.

The dose-response for the LCTs from Butenhoff et al. (2012) was modeled using EPA's Benchmark Dose Software (BMDS) Version 2.3.1. The multistage cancer model predicted the dose at which a 4% increase in tumor incidence would occur. The 4% was chosen as the low-end of the observed response range within the Butenhoff et al. (2012) results. Both the first and second degree polynomials gave identical goodness-of-fit criteria (p value and Akaike's Information Criterion [AIC]). Results are shown in Table 4-11 and Figure 4-1 and details of the model run are given in Appendix A.

Table 4-11. Multistage Cancer Model Dose Prediction Results for a 4% Increase in LCT Incidence

	BMD (mg/kg/day)	BMDL (mg/kg/day)
First Degree Polynomial Fit	3.51	1.99
Second Degree Polynomial Fit	3.51	1.99
AIC = 62.6936	P = 0.2245	

Source: Butenhoff et al. (2012)

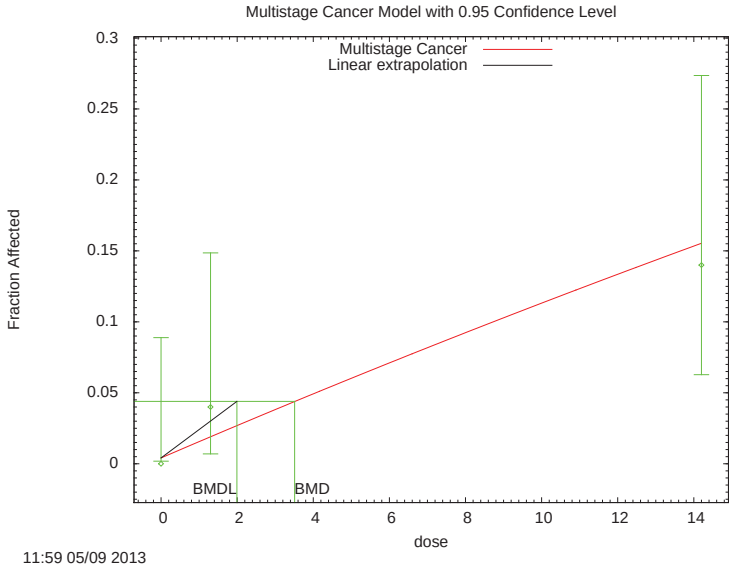


Figure 4-1. BMD Model Results for LCTs (Butenhoff et al. 2012)

The CSF for PFOS is derived from the $BMDL_{04}$ of 1.99 mg/kg/day after converting the animal BMDL to a HED using body weights to the ¾ power. The HED is calculated as follows[2]:

$$HED = \text{Animal BMDL} \times (\text{animal body weight})^{1/4} \div (\text{human body weight})^{1/4}$$

$$HED = 1.99\ mg/kg/day \times [(0.523\ kg)^{1/4} \div (70\ kg)^{1/4}] = 1.99\ mg/kg/day \times 0.29 = 0.58\ mg/kg/day$$

Where:

1.99 mg/kg/day	=	$BMDL_{04}$ for LCTs
0.29	=	The dosimetric adjustment factor

The CSF is calculated from the $BMDL_{04}$ HED as follows

$$CSF = \text{response} \div BMDL_{04}\ HED$$

$$CSF = 0.04 \div 0.58\ mg/kg/day = 0.07\ (mg/kg/day)^{-1}$$

[2] Body weight for male Sprague-Dawley rats (chronic Exposures) USEPA 1988

The CSF should not be used at doses > 0.58 mg/kg/day, the HED corresponding to the POD for the 4% incidence of LCTs following lifetime exposure to PFOA. The observed dose-response relationships do not continue linearly above this level, and the fitted dose-response models better characterize the dose-response for the higher exposures. The calculated concentration in drinking water with one-in-a-million risk for an increase in testicular tumors at levels greater than background is 0.0005 mg/L.

$$10^{-6}\ \text{Cancer Risk Concentration} = 0.000001 \text{ x } 80\ \text{kg} \div [0.07\ (\text{mg/kg/day}) - 1 \text{ x } 2.5\ \text{L/day}] = 0.000457\ \text{mg/L (rounded to 0.0005 mg/L)}.$$

The equivalent concentration derived from the RfD for noncancer effects using default adult body weight of 80 kg and a default DWI rate of 2.5 L/day (USEPA 2011) and a 20% relative source contribution (RSC) to account for the contribution of drinking water to the total exposure is 0.0001 mg/L. The 0.0001 mg/L concentration derived for an adult based on the RfD for noncancer effects with a 20% RSC for drinking water is lower than the concentration of 0.0005 mg/L associated with a one-in-a-million risk for testicular cancer also derived with adult default values indicating that a guideline derived from the developmental endpoint will be protective for the cancer endpoint.

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Appendix A: Literature Search Strategy Developing the Search

The literature search strategy was planned with input from EPA library services staff. Chemical Abstracts Service (CAS) numbers served as the basis for identification of relevant search terms. Trial searches were conducted, and results were evaluated to refine the search strategy (e.g., to pevent retrieval of citations unrelated to health and occurrence). The search string was refined to improve the relevancy of the results. All searches were conducted in the PubMed database, which contains peer-reviewed journal abstracts and articles on various biological, medical, public health, and chemical topics. The first search string (as well as future iterations) is presented below.

Every 2 weeks, a search was run in PubMed and a bibliography of the search results was compiled.

In 2012, the State of New Jersey Department of Environmental Protection (NJDEP) initiated a monthly search in PubMed for emerging literature on perfluorinated chemicals, primarily from the carboxylic acid and sulfonate families. These searches were provided to the EPA on a monthly basis. There was a high degree of overlap with the results from the EPA search, thus increasing the confidence in the search strategy.

In 2013, the EPA search strategy was expanded to cover other members of the perfluorocarboxylic acids (C-4 to C-12) and sulfonate families (C-4, C-6, C-8). The search string was altered in June of 2013 to rely more on the search features offered by PubMed.

A change in the PubMed database structure in 2015 required additional modifications to the search strategy.

The NJDEP search terms did not change from 2012 to 2015. All search iterations are noted below. However, the reports shared with EPA were streamlined to remove information on analytical methods and other nonhealth related citations that were not consistent with the criteria presented previously in the backgorund section of this document.

Search Strategy Examples: (Arranged from most recent to oldest).

2015

Search: perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkyl acids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctane sulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants”

Filters: English.
Frequency: Every 2 weeks

September 2013

Search: perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkyl acids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctane sulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants”

Filters: English.
Frequency: Every 2 weeks

June 2013

Search: (PFOA[tw] OR perfluorooctanoic acid[tw] OR 335-67-1[tw] OR PFBA[tw] OR perfluorobutanoate[tw] OR 3794-64-7[tw] OR PFDA[tw] OR perflurordecanoic acid[tw] OR 335-76-2[tw] OR PFHpA[tw] OR perfluoroheptanoic acid[tw] OR 375-85-9[tw] OR PFHxA[tw] OR perfluorohexanoic acid[tw] OR 307-24-4[tw] OR PFNA[tw] OR perfluorononanoic acid[tw] OR 375-95-1[tw] OR PFPtA[tw] OR perfluoropentanoic acid[tw] OR 2706-90-3[tw] OR PFPA[tw] OR pentafluoropropionic acid[tw] OR 422-64-0[tw]) AND (human* [tw] OR mammal*[tw]) NOT (environment* OR ecolog*)

Filters: English.
Frequency: Every 2 weeks

February, 2013

Search: perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkyl acids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctane sulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants”

Filters: English.
Frequency: Every 2 weeks

June 2011

Search (perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkylacids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctanesulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants” OR 335-67-1 [rn])

Limits: Publication Date—Dates will change for each search, English Language only

June 2009

Search (perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkylacids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctanesulfonate OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants” OR 335-67-1 [rn] OR 1763-23-1 [rn])

Limits: Entrez Date from 2009/04/07 to 2009/04/12

New Jersey Search Terms

Search: perfluorinated OR perfluorooctanoate OR perfluorononanoate OR perfluorooctanesulfonate OR perfluorooctanesulphonate OR perfluoroalkylated OR perfluoroalkyl OR polyfluoroalkyl OR polyfluorinated OR PFBA OR PFBS OR PFDA OR PFHA OR PFHPA OR PFHXA OR PFHXS OR PFNA OR PFOA OR PFOAs OR PFOS OR PFUNDA OR “perfluorooctanoic acid” OR “perfluoro octanoic acid” OR “perfluorooctane sulfonate” OR “perfluorooctane sulfonic acid” OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulphonate” OR “perfluorooctanyl sulfonate” OR “perfluorobutanoic acid” OR “perfluoroalkyl acids” OR “perfluorononanoic acid” OR “perfluorohexanoic acid” OR “perfluorohexane sulfonate” OR “perfluorohexane sulphonate” OR perfluorobutanoate OR “perfluoro butanoate” OR perfluorohexanoate OR “perfluoro hexanoate”

Filters: 1

Appendix B: Studies Evaluated Since August 2014

The tables that follow identify the papers that were retrieved and reviewed for inclusion following the August 2014 peer review for the draft PFOS Health Effects Support Document. The papers listed include those recommended by the peer reviewers or public commenters, as well as those identified from the literature searches between the completion of the peer review draft and December 2015. The review of papers recommended by the commenters and their potential impact on the updates to the draft assessments was facilitated by publications such as the critical review of the recent literature by Post et al. (2012). Post et al. (2012) provides an in-depth analysis of the available health effects literature for PFOA. Papers included in the final HESD are noted and reasons provided for those that were not included in the final document.

The tables for document retrieval and review are followed by updated versions of the summaries of the epidemiology summary tables from the peer reviewed draft as recommended by the peer reviewers. They are a useful tool to facilitate a high level comparison of the study outcomes for each of the epidemiological study groupings.

The criteria utilized in determining the papers that were included in the HESD after the peer review and presented in the Background were the following:

1. The study examines a toxicity endpoint or population that had not been examined by studies already present in the draft assessment.
2. Aspects of the study design, such as the size of the population exposed or quantification approach, make it superior to key studies already included in the draft document.
3. The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
4. There are elements of the study design that merit its inclusion in the draft assessment based on its contribution to the mode of action or the quantification approach.
5. The study elucidates the mode of action for any toxicity endpoint or toxicokinetic property associated with PFOA exposure.
6. The effects observed differ from those in other studies with comparable protocols.

Table B-1. PFOS Epi Papers—Post Peer Review (Retrieved and Reviewed)

Authors and Year	Topic—Keywords	Status/Notes
Andersen et al. 2013	Postnatal growth	Added PFOA/PFOS
Back et al. 2015	Time to pregnancy	Added PFOA
Barrett et al. 2015	Ovarian hormone	Not Added—No association observed for PFOA; PFOS was not included in the assessment
Berg et al. 2015	Thyroid	Added PFOA/PFOS
Bonefeld-Jørgenson et al. 2014	Breast cancer	Added PFOA/PFOS
Bonefeld-Jørgenson et al. 2011	Breast cancer	Added PFOA/PFOS
Brieger et al. 2011	Immune effects	Already presented in PFOS/PFOA
Buck Louis et al. 2015	Semen quality	Added PFOA/PFOS
Chang et al. 2014	Analysis of human cancer studies	Added PFOA in the cancer weight of evidence section
Chen et al. 2015	Birth weight	Added PFOS
Dankers et al. 2013	Blood-testis barrier	Reviewed,—not added; Study of an assay that used PFOA as one chemical in the test battery

Authors and Year	Topic—Keywords	Status/Notes
Darrow et al. 2013	Reproductive outcome	Added PFOA/PFOS
Darrow et al. 2014	Miscarriage	Added PFOA/PFOS
Donauer et al. 2015	Infant Neurobehavior	Not added—negative for PFOS; No statistical differences in PFOA levels during pregnancy and any neuro endpoint. Better studies.
Eriksen et al. 2013	Total cholesterol—Danish	Added PFOA/PFOS
Fitz-Simon et al. 2013	Serum lipids	Added PFOA/PFOS
Fisher et al. 2013	Plasma lipids	Added PFOA/PFOS
Fletcher et al. 2013	Cholesterol–genes	Added PFOA/PFOS
Fu et al. 2014	Serum lipids in Chinese subjects	Not added: Chinese population, dataset available on U.S. population. More branched chain isomers found among the people in China.
Geiger et al. 2014a	Lipids/children	Added PFOA/PFOS
Geiger et al. 2014b	Hypertension/children	Added PFOA/PFOS
Ghisari et al. 2014	Breast cancer—Inuit	Not added; same population as Bonefeld-Jørgensen et al. 2014; this study focuses on gene polymorphisms
Governini et al. 2015	DNA effects in sperm	Added PFOA/PFOS
Grandjean and Clapp 2015	Health Risks	Not added; the primary studies are already included in the documents.
Granum et al. 2013	Immune children	Added PFOA/PFOS
Hardell et al. 2014	Prostate cancer	Added PFOA/PFOS
Høyer et al. 2015a	Human weight	Added PFOA/PFOS
Høyer et al. 2015b	Behavior motor development	Added PFOA/PFOS
Humblet et al. 2014	Asthma	Added PFOA/PFOS
Jain 2014	NHANES	Added PFOA/PFOS
Innes et al. 2014	Colorectal cancer	Added PFOA/PFOS
Joensen et al. 2013	Sperm	Added PFOA/PFOS
Kerger et al. 2011	Cholesterol C8	Added; demographics for cholesterol and PFOS in summary section of epi studies
Kjeldsen and Bonefeld-Jørgensen 2013	Sex hormones	Covered multiple PFAS *in vitro* no impact on weight of evidence
Kristensen et al. 2013	Prenatal female repro	Added PFOA/PFOS
Liew et al. 2014	Cerebral palsy children	Added PFOA/PFOS
Looker et al. 2014	Immune	Added PFOA/PFOS
López-Doval et al. 2014	Male repro	Added PFOS
Maisonet et al. 2015	Gestational diabetes	Added PFOA/PFOS
Maisonet et al. 2012	Birth weight	Added PFOA/PFOS
Mørck et al. 2015	PFAS levels in children	Not added; No significant impact
Okada et al. 2014	Allergy children	Added PFOS
Osuna et al. 2014	Antibodies PFOS PFOA	Not added; focus more on methylHg and PCB than PFAS; only n = 38 as preliminary study
Roth and Wilks 2014	Neurodevelopmental	. Not added; no significant impact
Shrestha et al. 2015	Thyroid	Added PFOA/PFOS

Authors and Year	Topic—Keywords	Status/Notes
Starling et al. 2014	Plasma lipids	Added PFOA/PFOS
Steenland et al. 2015	Workers	Added PFOA
Stein et al. 2009	Pregnancy	Added PFOA
Taylor et al. 2014	Menopause	Added PFOA/PFOS
Vanden Heuvel 2013	Serum lipids	Not added; is a rebuttal of Fletcher et al. 2013 conclusions. No significant impact
Vassiliadou et al. 2010	PFOS in cancer vs non-cancer patients	Added PFOA/PFOS
Vélez et al. 2015	Fertility	Added PFOA/PFOS
Verner et al. 2015	Fetal growth GFR	Added PFOA/PFOS
Verner and Longnecker 2015	Menstruation/excretion	Added PFOS
Vested et al. 2013	Semen quality and hormones	Added PFOS/PFOS
Vesterinen et al. 2014	Fetal Growth GFR	Added PFOA/PFOS
Wang et al. 2013	Thyroid	Added PFOA/PFOS
Watkins et al. 2013	Kidney function	Added PFOA/PFOS
Webster et al. 2014	Maternal thyroid	Added PFOA/PFOS
Webster et al. 2015	Thyroid—iodine statue	Added PFOS
Wen et al. 2013	Thyroid	Added PFOA/PFOS
Yeung et al. 2013	Liver cancer	Added PFOA/PFOS
Zhang et al. 2015	Gestational diabetes	Added PFOA/PFOS

Table B-2. PFOA Post Peer Review Animal Toxicity Studies

Authors and Year	Topic	Action Notes
Bjork et al. 2011	Nuclear receptor activation	In vitro, mechanistic findings comparable to studies already included
Corsini et al. 2014	Immune data review	Not added; no significant impact
Corsini et al. 2012	Immune in vitro data review	Not added; no significant impact
Dewitt et al. 2015	Immunotoxicity	Added PFOA
Fenton 2015	Repro editorial	Not added
Filgo et al. 2015	Liver tumors in females developmentally exposed	Added PFOA
Hall et al. 2012	PPARα and cancer	Cited in synthesis. Paper on adversity of liver hypertrophy PFOA/ PFOS
Koustas et al. 2014	Fetal growth (animal studies) navigation guide	Added PFOA
Liu et al. 2015	Testes	Added PFOA
Long et al. 2013	Neurotoxicity adult PFOS	Added PFOS
Lu et al. 2015	Testes	Added PFOA
Ngo et al. 2014	Tumors mice Min/+ PFOS	Added PFOS
Post et al. 2012	Review paper	Not added. Key studies included in the document; no significant impact
Quist et al. 2015	Liver histopathology/high fat diet post weaning exposure	Added PFOA
Rigden et al. 2015	Acute liver effects	Added PFOA
Shabalina et al. 2015	Brown fat uncoupling protein 1	Not added. Mechanistic; no significant impact

Authors and Year	Topic	Action Notes
Sheng et al. 2016	Binding to liver fatty acid binding protein	Not added; no significant impact, topic covered by other papers
Tan et al. 2012	Gene activation	Added PFOA/PFOS
Tan et al. 2013	Gene activation dietary fat	Added PFOA
Tucker et al. 2015	Mammary gland	Added PFOA
Wallace et al. 2013	Mitochodrial respiration	Not added. No significant impact, topic covered by other papers
Wan et al. 2014b	Glucose metabolism	Added PFOS
Wan et al. 2012	Hepatic steatosis	Added PFOS
Wan et al. 2014a	Sertoli cells	Added PFOS
F. Wang et al. 2015	MiRNA liver PFOS early life	Not added; no significant impact
S. Wang et al. 2014	Lysine decarboxylase	Added PFOA/PFOS
L. Wang et al. 2014	Inhibition of LDL	Added PFOS
Y. Wang et al. 2015	Special learning and memory	Added PFOS
Yan et al. 2015	Glucose homeostasis	Not added. Dose-response in Wan (2014b) presented (more robust). Single dose for whole animal
Yu et al. 2015	Thyroid PFOS isomers	Added PFOS
Zeng et al. 2014	Mitochondrial mediated apoptosis of the heart	Added PFOS
L. Zhang et al. 2013	Fatty acid binding protein	Added PFOA/PFOS
Y. Zhang et al. 2013	Biological half-life	Added PFOA/PFOS
W. Zhang et al. 2014	Breast cancer cell invasion—mechanistic	Not added; *in vitro*, no significant impact
Zhao et al. 2014	Testosterone reduction in Leydig cells PFOS	Added PFOS

Table B-3. Toxicokinetics: Post Peer Review

Authors and Year	Topic	Action Notes
D'Alessandro et al. 2013	Serum albumin	Added PFOS
Augustine et al. 2005	Transporter expression testes	Not added background paper on testes transporters –no relationship to PFOA PFOA or any PFAS
Beesoon et al. 2011	Isomer profile	Added PFOA
Beesoon and Martin 2015	Albumin binding	Added PFOA
Cui et al. 2010	Excretion subchronic	Added PFOA/PFOS
Fàbrega et al. 2014	PK model	Added PFOA/PFOS
Kerstner-Wood et al. 2003	Plasma protein binding	Added—PFOA/PFOS
Klaassen and Aleksunes 2010	Transporter paper—Provided diagram of kidney transporters	Added PFOA
Loccisano et al. 2013	PK model—Human	Added PFOA/PFOS
Mondal et al. 2014	Breast milk	Added PFOS/PFOA
Ospinal-Jimenez and Pozzo 2012	Protein denaturation	Added PFOS
Pérez et al. 2013	Human tissue levels	New PFOA/PFOS
Ren et al. 2015	Thyroid hormone receptor binding (*in vitro*)	Added PFOA/PFOS
Rigden et al. 2015	Liver and excretion	Added PFOA
Shabalina et al. 2015	Brown fat	Not added; No information on MOA for body weight effects in the animal or human studies
Slitt et al. 2007	Transporter expression PFOA	Not added. Reported on transporters during extrahepatic cholestasis. No data on PFOA and PFOS. No significant impact.
Tucker et al. 2015	Menstruation-excretory route	Added PFOA
Verner and Longnecker 2015	Excretion PFOS	Added PFOS
Wambaugh et al. 2013	PK model	Added PFOA/PFOS
Wong et al. 2014	Menstrual blood as excretory route	Added PFOA/PFOS
T. Zhang et al. 2014	Excretion general population and pregnancy	Added PFOA/PFOS
L. Zhang et al. 2014	PPAR gamma	Added PFOS
Y. Zhang et al. 2013	Excretion, half-life	Added PFOA/PFOS
T. Zhang et al. 2013	Maternal transfer	Added PFOA/PFOS

Tables B-4 through B-8 provide updated versions of the epidemiology summary tables from the peer-reviewed draft, as recommended by the reviewers. They are a useful tool to facilitate a high-level comparison of the study outcomes for each of the epidemiology study groupings.

Table B-4. Association between Serum PFOA and Serum Lipids and Uric Acid

Reference	Study Type	n	Mean Serum PFOA	TC	VLDL	LDL	HDL	Non-HDL	TG	UA
Occupational Populations										
Olsen et al. 2000	Cross-sectional	111 (1993) 80 (1995) 74 (1997)	0–80 µg/mL 0–114 µg/mL 0.1–81 µg/mL	↔ ↔ ↔	NM	↔ ↔ ↔	↔ ↔ ↔	NM	↔ ↔ ↔	NM
Olsen et al. 2001b, 2003	Cross-sectional	206 (Antwerp) 215 (Decatur)	1.03 µg/mL 1.90 µg/mL	↑	NM	NM	↔	NM	↑	NM
Olsen et al. 2001c, 2003	Longitudinal; ~5 years	175 (Decatur and Antwerp combined for analysis)	1.36–1.41 µg/mL (1995 baseline) 1.49–1.77 µg/mL (2000 follow-up)	↑	NM	NM	↔	NM	↑	NM
Sakr et al. 2007a	Cross-sectional	1,025	0.428 µg/mL	↑	↑	↑	↔	NM	↔	↑
Sakr et al. 2007b	Longitudinal	454 (23-yr follow-up)	1.04 µg/mL (first) 1.16 µg/mL (last)	↑	NM	↔	↔	NM	↔	NM
Olsen and Zobel 2007	Cross-sectional	506 (Antwerp, Cottage Grove, Decatur combined)	2.21 µg/mL	↔	NM	↔	↓	NM	↑	NM
Costa et al. 2009	Cross-sectional	34 workers 107 controls	4.02 µg/mL	↑	NM	NM	↔	NM	↔	↑
General Populations										
Emmett et al. 2006	Cross-sectional	371	0.354 µg/mL	↔	NM	NM	NM	NM	NM	NM
Steenland et al. 2009	Cross-sectional (C8)	46,294	0.08 µg/mL	↑	NM	↑	↔	↑	↑	NM
Steenland et al. 2010	Cross-sectional (C8)	53,458	0.086 µg/mL	NM	NM	NM	NM	NM	NM	↑

Reference	Study Type	n	Mean Serum PFOA	TC	VLDL	LDL	HDL	Non-HDL	TG	UA
Winquist and Steenland 2014a	Cross-sectional (C8)	32,254	0.0261 µg/mL	↑	NM	NM	NM	NM	NM	NM
Frisbee et al. 2010	Cross-sectional (C8, children and adolescents)	6,536 children 5,934 adolescents	0.0777 µg/mL 0.0618 µg/mL	↑	NM	↑	↔	NM	↑	NM
Fitz-Simon et al. 2013	Longitudinal; 4.4 years (C8)	521	0.140 µg/mL (baseline) 0.068 µg/mL (follow-up)	↔	NM	↔	↔	NM	↔	NM
Nelson et al. 2010	Cross-sectional (NHANES)	1,445	0.0046 µg/mL	↑	NM	↔	↔	↑	NM	NM
Eriksen et al. 2013	Cross-sectional	753	0.0071 µg/mL	↑	NM	NM	NM	NM	NM	NM
Starling et al. 2014	Cross-sectional (maternal at 14–26 weeks gestation)	891	0.00225 µg/mL	↔	NM	↔	↑	NM	↔	NM
Fisher et al. 2013	Cross-sectional	2,700	0.0025 µg/mL	↔	NM	↔	↔	NM	NM	NM

Notes: ↑ = positive association; ↓ = negative association; ↔ = no association; TC = total cholesterol; VLDL= very low density lipoprotein; LDL= low-density lipoprotein; non-HDL= TC(VLDL,IDL, LDL)-HDL; HDL= high-density lipoprotein; TG = triglycerides; UA = uric acid; NM = not measured

Table B-5. Association of Serum PFOA and Biochemical and Hematological Measures

Reference	Study Type	n	Mean Serum PFOA	Liver enzymes	Bilirubin	Renal Enzymes/Function	Glucose	Hematology
Occupational Populations								
Olsen et al. 2000	Cross-sectional	111 (1993) 80 (1995) 74 (1997)	0–80 µg/mL 0–114 µg/mL 0.1–81 µg/mL	↔ ↔ ↔	↔ ↔ ↔	↔ ↔ ↔	↔ ↔ ↔	↔ ↔ ↔
Olsen et al. 2001b, 2003	Cross-sectional	206 (Antwerp) 215 (Decatur)	1.03 µg/mL 1.90 µg/mL	↔ ↔	↔ ↔	↔ ↔	↔ ↔	↔ ↔
Olsen et al. 2001c, 2003	Longitudinal; ~5 years	175 (Decatur and Antwerp combined for analysis)	1.36–1.41 µg/mL (1995 baseline) 1.49–1.77 µg/mL (2000 follow-up)	↔ ↔	↔ ↔	↔ ↔	↔ ↔	↔ ↔
Sakr et al. 2007a	Cross-sectional	1025	0.428 µg/mL	↑ (GGT only)	↔	NM	↔	↔
Sakr et al. 2007b	Longitudinal	454	1.04 µg/mL (first) 1.16 µg/mL (last)	↑ (AST only)	↓	NM	NM	NM
Olsen and Zobel 2007	Cross-sectional	506 (Antwerp, Cottage Grove, Decatur combined)	2.21 µg/mL	↑ (ALP, ALT, GGT Decatur only)	↓	NM	NM	NM
Costa et al. 2009	Cross-sectional	56 workers	4.02 µg/mL	↑ (GGT, ALP, ALT)	↓	↔	↔	↔
General Populations								
Emmett et al. 2006	Cross-sectional	371	0.354 µg/mL	↔	NM	↔	NM	↔
Lin et al. 2010	Cross-sectional (NHANES)	1076 men 1140 women	0.00505 µg/mL 0.00406 µg/mL	↑ (ALT, GGT)	↔	NM	NM	NM
Gallo et al. 2012	Cross-sectional (C8)	47,092	0.028 µg/mL	↑ (ALT)	↔	NM	NM	NM
Shankar et al. 2011	Cross-sectional (NHANES)	4587	0.0059 µg/mL	NM	NM	↑ (chronic kidney disease)	NM	NM
Watkins et al. 2013	Cross-sectional (C8)	9,660 (children)	0.0283 µg/mL	NM	NM	↑ (decreased eGFR)	NM	NM

Notes: ↑ = positive association; ↓ = negative association; ↔ = no association; ALP = alkaline phosphatase; eGFR = estimated glumerular filtration rate; GGT = gamma-glutamyl transpeptidase; AST = aspartate aminotransferase; ALT = alanine transaminase; NM = not measured

Table B-6. Association between PFOA level and prevalence of thyroid disease and thyroid hormone levels

Study	Study Type	Population (n)	Mean Serum PFOA (µg/mL)	Thyroid Disease	TSH	T3	T4
Occupational Populations							
Olsen et al. 1998	Cross-sectional	111 and 80 Adult workers	10–30 >30	NM NM	↑ ↔	NM	NM
Olsen et al. 2001b, 2003	Cross-sectional	Adult workers 215 (Decatur) 206 (Antwerp)	 1.9 1.03	NM	↔	↔	↔
Sakr et al. 2007a	Cross-sectional	1,025 Adult workers	0.428	NM	↔	↔	↔
Costa et al. 2009	Cross-sectional	56 Adult workers	4.02	NM	↔	↔	↔
Olsen and Zobel 2007	Cross-sectional	506 Adult workers	2.21	NM	↔	↑	↔ serum ↓ free
General Populations							
Emmett et al. 2006	Cross-sectional	40 (thyroid disease) 331 (no thyroid disease)	0.387 0.451	↔	NM	NM	NM
Pirali et al. 2009	Cross-sectional	28 Adults	2.0 ng/g thyroid tissue	↔	NM	NM	NM
Bloom et al. 2010	Cross-sectional	31 Adults	0.00133	NM	↔	NM	↔
Shrestha et al. 2015	Cross-sectional	51 men 36 women	0.0104	↔	↔	↑	↑
Winquist and Steenland 2014b	Cross-sectional	32,254 (C8)	0.0261	↔ (men) ↑ (women)	NM	NM	NM
Lopez-Espinosa et al. 2012	Cross-sectional	10,725 children (C8)	0.0293	↑	↔	NM	↔
Melzer et al. 2010	Cross-sectional	3,966 Adults (NHANES)	0.025 (men) 0.019 (women)	↔ (men) ↑ (women)	NM	NM	NM
Wen et al. 2013	Cross-sectional	1,181 (NHANES)	0.00415	NM	↔	↔	↔
de Cock et al. 2014	Cross-sectional	83 newborns	0.000943 (cord)	NM	NM	NM	↔ boys ↑ girls
Lin et al. 2013	Cross-sectional	545	0.00267	NM	↔	NM	↔
Chan et al. 2011	Cross-sectional	271 Pregnant women	0.00135	NM	↔	NM	↔
Wang et al. 2013	Cross-sectional	903 women at gestation week 18	0.0022	NM	↔	NM	NM

Study	Study Type	Population (n)	Mean Serum PFOA (µg/mL)	Thyroid Disease	TSH	T3	T4
Berg et al. 2015	Cross-sectional	375 women at gestation week 18, day 3 and week 6 after delivery (Norwegian Mother/Child Cohort)	0.00153	NM	↔	↔	↔
Webster et al. 2014	Cross-sectional	152 women at gestation week 15–18	0.0017	NM	↔	↔	↔

Notes: ↑ = positive association; ↓ = negative association; ↔ = no association; NM = Not Measured

Table B-7. Association between Serum PFOA and Markers of Immunotoxicity

Study	Study Type	Population (n)	Mean or Median Serum PFOA (µg/mL)	Disease Prevalence	Vaccine Response
Steenland et al. 2015	Cross-sectional	Workers (6,027)	0.113	↑ ulcerative colitis ↑ rheumatoid arthritis ↓ asthma	NM
Okada et al. 2012	Prospective cohort	Maternal, third trimester (343)	0.0014	↔ up to 18 months old	NM
Fei et al. 2010b	Cross-sectional	Maternal, first trimester (1,400)	0.0056	↔ early childhood	NM
Grandjean et al. 2012	Prospective cohort	Maternal at gestation week 32 (587)	0.0032	NM	↓ (antibody titer)
Grandjean et al. 2012	Prospective cohort	Children age 5 years (587)	0.00406	NM	↓ (antibody titer)
Granum et al. 2013	Prospective cohort	Women at delivery (56)	0.0011	↔	↔
Dong et al. 2013	Cross-sectional	Children age 10–15 years (231 asthmatics and 225 controls)	0.0015 (asthmatics) 0.0010 (nonasthmatics)	↑ for asthma	NM
Humblet et al. 2014	Cross-sectional	Children age 12–19 years (1,877)	0.0043 (asthmatics) 0.0040 (nonasthmatics)	↑ for asthma	NM
Looker et al. 2014	Cross-sectional	Adults (411)	0.0337	NM	↓ (antibody titer)

Notes: ↑ = positive association; ↓ = negative association; ↔ = no association; NM = Not Measured

Table B-8. Association between Serum PFOA and Reproductive and Developmental Outcomes

Study	Study Type	n	Mean Serum PFOA	Outcome	Measures at Birth	Growth/ Development	Fecundity/ Fertility
Reproductive Outcome, Anthropometric Measures at Birth							
Fei et al. 2007, 2008a, 2009, 2010a	Cross-sectional	1,400	0.0056 µg/mL	↔ (gestation length) ↓ (length of breastfeeding)	↓ (weight) ↓ (size) ↔ (Apgar score)	NM	↑ (TTP) ↑ (infertility)
Vélez et al. 2015	Cross-sectional	1,743	0.00166 µg/mL	NM	NM	NM	↑ (TTP) ↑ (infertility)
Nolan et al. 2009, 2010	Cross-sectional	1,555	0.00678 µg/mL	↔ (preterm birth, congenital anomalies, labor/delivery complications, maternal risk)	↔ (weight)	NM	NM
Stein et al. 2009	Cross-sectional (C8)	1,505	0.0488 µg/mL	↔ (miscarriage)	↔ (low weight)	NM	NM
Darrow et al. 2013, 2014	Cross-sectional (C8)	1,330 and 1,129	0.031–0.0337 µg/mL	↔ (preterm, miscarriage)	↔ (low weight, birth weight)	NM	↑ (hypertension)
Apelberg et al. 2007	Cross-sectional	293	0.0016 µg/mL (cord blood)	↔ (gestational age)	↓ (weight, head circumference, ponderal index)	NM	NM
Monroy et al. 2008	Cross-sectional	101	0.00254 µg/mL (maternal at 24-28 weeks) 0.00224 µg/mL (maternal at delivery) 0.0019 µg/mL (umbilical cord blood)	NM	↔ (weight)	NM	NM
Washino et al. 2009	Cross-sectional	428	0.0014 µg/mL	NM	↔ (weight and size)	NM	NM
Hamm et al. 2010	Cross-sectional	252	0.0021 µg/mL	↔ (gestation length)	↔ (weight)	NM	NM
Whitworth et al. 2012	Cross-sectional	849	0.0021 µg/mL	NM	↔ (birth weight)	NM	NM
Maisonet et al. 2012	Cross-sectional	395	0.0037 µg/mL	NM	↓ (birth weight)	NM	NM
Chen et al. 2012	Cross-sectional	429	0.0018 µg/mL	NM	↔ (birth weight)	NM	NM

Study	Study Type	n	Mean Serum PFOA	Outcome	Measures at Birth	Growth/ Development	Fecundity/ Fertility
Male Fertility							
Joensen et al. 2009 (PFOA/PFOS combined)	Cross-sectional	105	0.0049 µg/mL	NM	NM	NM	↓ (normal sperm) ↔ (testosterone)
Joensen et al. 2013	Cross-sectional	247	0.0035 µg/mL	NM	NM	NM	↔ (semen parameters) ↔ (testosterone, hormones)
Buck Louis et al. 2015	Cross-sectional	462	0.00429–0.00509 µg/mL	NM	NM	NM	↑ (lower % sperm with coiled tail) (total of six PFAS associated with changes in sperm quality)
Neurodevelopmental Endpoints							
Fei et al. 2008b	Cross-sectional	1,400	0.0056 µg/mL	NM	NM	↔ (motor skills and mental develop. at 6 and 18 months)	NM
Lieu et al. 2014	Cross-sectional	156 cases 550 controls	0.00456 µg/mL	NM	NM	↑ (cerebral palsy in boys)	NM
Fei and Olsen 2011	Cross-sectional	787 (behavior) 537 (coordina-tion)	0.0057 µg/mL	NM	NM	↔ (behavior and coordination at 7 years)	NM
Høyer et al. 2015a	Cross-sectional	1,106	0.0014 µg/mL	NM	NM	↔ (motor skills) ↑ (hyperactivity)	NM
Stein et al. 2013	Cross-sectional (C8)	321	0.0351 µg/mL (child)	NM	NM	↔ (behavioural problems) ↑ (executive function; ADHD from mother, not teacher)	NM
Hoffman et al. 2010	Cross-sectional (NHANES)	571 children	0.0044 µg/mL	NM	NM	↑ (ADHD)	NM

Study	Study Type	n	Mean Serum PFOA	Outcome	Measures at Birth	Growth/ Development	Fecundity/ Fertility
Postnatal Development							
Andersen et al. 2010	Cross-sectional	1,010	0.0052 µg/mL	NM	NM	↓ (weight and BMI in boys at 5 and 12 months)	NM
Andersen et al. 2013	Cross-sectional	811 (children at age 7 years)	0.0053 µg/mL	NM	NM	↔ (height, weight, waist measurement, risk of overweight)	NM
Høyer et al. 2015b	Cross-sectional	1,022	0.001–0.0018 µg/mL	NM	NM	↔ (overweight) ↑ (waist-to-height ratio)	NM
Lopez-Espinosa et al. 2011	Cross-sectional (C8)	3,076 boys 2,931 girls	0.02–0.026 µg/mL	NM	NM	↑ (delayed puberty in girls)	NM
Christensen et al. 2011	Cross-sectional	448 girls	0.0036–0.0039 µg/mL (maternal)	NM	NM	↔ (age at menarche)	NM
Kristensen et al. 2013	Cross-sectional	343 women	0.0036 µg/mL (maternal)	NM	NM	↑ (delayed puberty)	NM
Vested et al. 2013	Cross-sectional	169 men	0.0038 µg/mL (maternal)	NM	NM	↑ (lower sperm conc and total count)	NM
Halldorsson et al. 2012	Cross-sectional	665	0.0037 µg/mL	NM	NM	↑ (overweight in females at 20 years)	NM

Notes: ↑ = positive association; ↓ = negative association; ↔ = no association; NM = Not Measured

Appendix C: Multistage Model for Leydig Cell Tumors

```
 ================================================================
  Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
  Input Data File: C:/1Data/MyFiles/PFOA-PFOS/PFOA Docs/msc_Leydig_Opt.(d)
  Gnuplot Plotting File:  C:/1Data/MyFiles/PFOA-PFOS/PFOA Docs/msc_Leydig_Opt.plt
                                        Thu May 09 11:59:27 2013
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]

   The parameter betas are restricted to be positive

   Dependent variable = Col2
   Independent variable = Col1

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008


Default Initial Parameter Values
    Background   =   0.0132945
       Beta(1)   =   0.0097738
       Beta(2)   =           0


Asymptotic Correlation Matrix of Parameter Estimates

 ( *** The model parameter(s)  -Beta(2) have been estimated at a boundary point, or
have been specified by the user, and do not appear in the correlation matrix)

                                  Background       Beta(1)
                   Background              1         -0.64
                   Beta(1)             -0.64             1


                              Parameter Estimates

                                        95.0% Wald Confidence Interval
    Variable       Estimate       Std. Err.      Lower Conf. Limit  Upper Conf. Limit
 Background       0.00409839          *                  *                  *
 Beta(1)           0.0116288          *                  *                  *
 Beta(2)                   0          *                  *                  *

* - Indicates that this value is not calculated.
```

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.3468	2	1.40286	1	0.2362
Reduced model	-34.0451	1	10.7995	2	0.004518

AIC: 62.6936

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0041	0.205	0.000	50	-0.454
1.3000	0.0190	0.952	2.000	50	1.084
14.2000	0.1557	7.784	7.000	50	-0.306

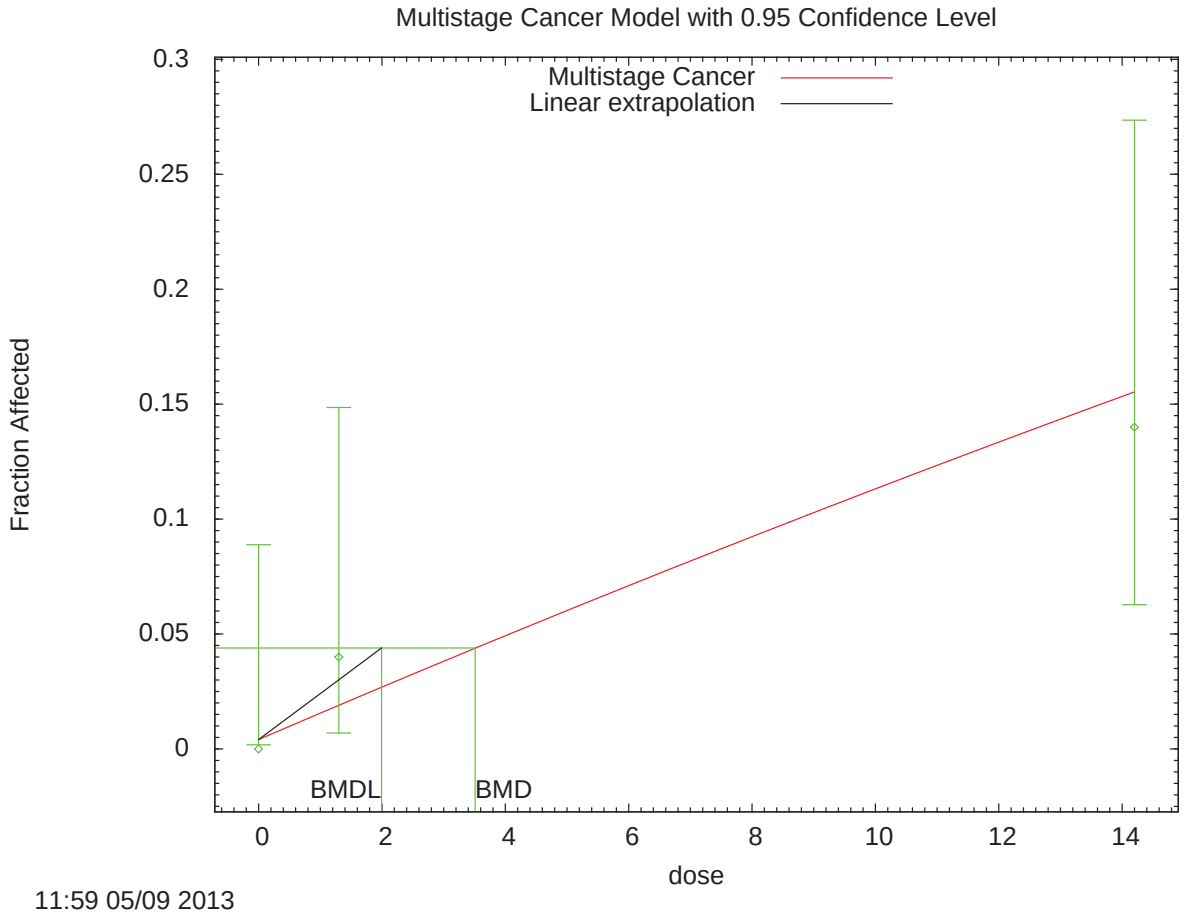
Chi^2 = 1.48 d.f. = 1 P-value = 0.2245

Benchmark Dose Computation

Specified effect = 0.04
Risk Type = Extra risk
Confidence level = 0.95
BMD = 3.51044
BMDL = 1.99346
BMDU = 10.7788

Taken together, (1.99346, 10.7788) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0200656




```
 ================================================================
Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
Input Data File: C:/1Data/MyFiles/PFOA-PFOS/PFOA Docs/msc_Leydig_Opt.(d)
Gnuplot Plotting File:  C:/1Data/MyFiles/PFOA-PFOS/PFOA Docs/msc_Leydig_Opt.plt
                                          Thu May 09 12:05:42 2013
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

   The parameter betas are restricted to be positive


   Dependent variable = Col2
   Independent variable = Col1

  Total number of observations = 3
  Total number of records with missing values = 0
  Total number of parameters in model = 2
  Total number of specified parameters = 0
  Degree of polynomial = 1


Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008
```

Default Initial Parameter Values
Background = 0.0132945
Beta(1) = 0.0097738

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.64
Beta(1)	-0.64	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.00409839	*	*	*
Beta(1)	0.0116288	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.6454	3			
Fitted model	-29.3468	2	1.40286	1	0.2362
Reduced model	-34.0451	1	10.7995	2	0.004518

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Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0041	0.205	0.000	50	-0.454
1.3000	0.0190	0.952	2.000	50	1.084
14.2000	0.1557	7.784	7.000	50	-0.306

Chi^2 = 1.48 d.f. = 1 P-value = 0.2245

Benchmark Dose Computation

Specified effect = 0.04
Risk Type = Extra risk
Confidence level = 0.95
BMD = 3.51044
BMDL = 1.99346
BMDU = 8.7003

Taken together, (1.99346, 8.7003) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0200657

Attachment

1D

4

United States Environmental Protection Agency | **Office of Water Mail Code 4304T** | **EPA 822-R-16-002 May 2016**

Health Effects Support Document for Perfluorooctane Sulfonate (PFOS)

Health Effects Support Document

for

Perfluorooctane Sulfonate (PFOS)

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

http://www.epa.gov/dwstandardsregulations/drinking-water-contaminant-human-health-effects-information.

EPA Document Number: 822-R-16-002
May 2016

BACKGROUND

The Safe Drinking Water Act (SDWA), as amended in 1996, requires the Administrator of the U.S. Environmental Protection Agency (EPA) to establish a list of unregulated microbiological and chemical contaminants known or anticipated to occur in public water systems and that might require control in the future through national primary drinking water regulations. The SDWA also requires the Agency to make regulatory determinations on at least five contaminants on the Contaminant Candidate List (CCL) every 5 years. For each contaminant on the CCL, before EPA makes a regulatory determination, the Agency needs to obtain sufficient data to conduct analyses on the extent to which the contaminant occurs and the risk it poses to populations via drinking water. Ultimately, this information will assist the Agency in determining the most appropriate course of action in relation to the contaminant (e.g., developing a regulation to control it in drinking water, developing guidance, or deciding not to regulate it).

The PFOS health assessment was initiated by the Office of Water, Office of Science and Technology in 2009. The draft *Health Effects Support Document for Perfluorooctane Sulfonate Acid (PFOS)* was completed in 2013 and released for public comment in February 2014. An external peer-review panel meeting was held on August 21 and 22, 2014. The final document reflects input from the panel as well as public comments received on the draft document. Both the peer-reviewed draft and this document include only the sections of a health effects support document (HESD) that cover the toxicokinetics and health effects of PFOS. If a decision is made to regulate the contaminant, this document will be expanded.

One of the challenges inherent in conducting this assessment was the wealth of experimental data published before and during its development. This section provides a synopsis of the approach used in identifying and selecting the publications reflected in the final assessment.

Data were identified through the following:

Monthly/bimonthly literature searches conducted by EPA library staff (2009–2015) and New Jersey Department of Environmental Protection library staff (2012–2015).

- Papers identified by EPA internal and external peer reviewers.
- Papers identified through public comments on the draft assessments.
- Papers submitted to EPA by the public.

In mid-2013, the EPA library searches were expanded to cover other members of the perfluorocarboxylic acids (C-4 to C-12) and sulfonate families (C-4, C-6, C-8). Appendix A describes the literature search strategy used by the libraries. Through the literature search, documents were identified for retrieval, review, and inclusion in the HESD using the following criteria:

- The study examines a toxicity endpoint or population not examined by studies already included in the draft document.
- Aspects of the study design such as the size of the population exposed or quantification approach make it superior to key studies already included in the draft document.
- The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
- Elements of the study design merit its inclusion in the draft document based on its contribution to the mode of action or the quantification approach.

- The study elucidates the mode of action for any toxicity endpoint or toxicokinetic property associated with PFOS exposure.
- The effects observed differ from those in other studies with comparable protocols.

In addition to each publication being evaluated against the criteria above, the relevance of the study to drinking water exposures and to the U.S. population also were considered.

The studies included in the final draft were determined to provide the most current and comprehensive description of the toxicological properties of PFOS and the risk it poses to humans exposed to it in their drinking water. Appendix B summarizes the studies evaluated for inclusion in the HESD following the August 2014 peer review and identifies those selected for inclusion in the final assessment. Appendix B includes epidemiology data that provide a high-level summary of the outcomes across the studies evaluated.

Development of the hazard identification and dose-response assessment for PFOS has followed the general guidelines for risk assessment forth by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (USEPA 2014a). Other EPA guidelines used in the development of this assessment include the following:

- *Guidelines for the Health Risk Assessment of Chemical Mixtures* (USEPA 1986a)
- *Guidelines for Mutagenicity Risk Assessment* (USEPA 1986b)
- *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (USEPA 1988)
- *Guidelines for Developmental Toxicity Risk Assessment* (USEPA 1991)
- *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies* (USEPA 1994a)
- *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (USEPA 1994b)
- *Use of the Benchmark Dose Approach in Health Risk Assessment* (USEPA 1995)
- *Guidelines for Reproductive Toxicity Risk Assessment* (USEPA 1996)
- *Guidelines for Neurotoxicity Risk Assessment* (USEPA 1998)
- *Science Policy Council Handbook*: *Peer Review (2nd edition)* (USEPA 2000a)
- *Supplemental Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (USEPA 2000b)
- *A Review of the Reference Dose and Reference Concentration Processes* (USEPA 2002)
- *Guidelines for Carcinogen Risk Assessment* (USEPA 2005a)
- *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (USEPA 2005b)
- *Science Policy Council Handbook: Peer Review* (USEPA 2006a)
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (USEPA 2006b)
- *Highlights of the Exposure Factors Handbook* (USEPA 2011)
- *Benchmark Dose Technical Guidance Document* (USEPA 2012)
- *Child-Specific Exposure Scenarios Examples* (USEPA 2014b)

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CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

ACh	acetylcholine
ADHD	attention deficit hyperactivity disorder
ALP	alkaline phosphatase
ALT	alanine transaminase
ANOVA	analysis of variance
AP	activation protein
AST	aspartate aminotransferase
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose – lower 95th percentile confidence bound
BMI	body mass index
BUN	blood urea nitrogen
°C	degrees Celsius
CAR	constitutive androstane receptor
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CCL	Chemical Contaminants List
CD	circular dichroism
CFSE	6-carboxyfluorescein succinimidyl ester
CHMS	Canadian Health Measures Survey
CI	confidence interval
CL	clearance
CoA	coenzyme A
Conc.	Concentration
C_{ss}	Steady-state concentration
CSF	cerebrospinal fluid
CSM	cholestyramine
Cte	acyl CoA thioesterase
d	day
DA	dansylamide
DAUDA	11-(5-dimethylaminoapthalenesulphonyl)-undecanoic acid
DCDQ	Developmental Coordination Disorder Questionnaire
DIO1	type 1 deiodinase
DIO3	type 3 deiodinase
dL	deciliter
DMEM	Dulbecco’s Minimal Essential Medium
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNBC	Danish National Birth Cohort
DP	dansyl-L-proline
DR	dose rate
E	estradiol
EAA	excitatory amino acid
EC_{50}	half maximal effective concentration
ECF	Electro-Chemical Fluorination
ED	equilibrium dialysis

eGFR	estimated glomerular filtration rate
EMM	Estimated Marginal Mean
EPA	U.S. Environmental Protection Agency
FABP	fatty acid binding proteins
FAI	free androgen index
FR	fecundability ratio
FSH	Follicle-stimulating hormone
FT	free testosterone
FT3	free triiodothyronine
FT4	free thyroxin
g	gram
GABA	gamma-aminobutyric acid
GAP-43	growth-associated protein-43
GD	gestation day
GFAP	glial fibrillary acidic protein
GFR	glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
GI	gastrointestinal
GJIC	gap junction intercellular communication
GLP	good laboratory practice
Glu	glutamate
GS	glutamine synthetase
HDL	high density lipoprotein
HED	human equivalent dose
HESD	Health Effects Support Document
HL-60	human promyelocytic leukemia cell line
HMG-CoA	3-hydroxy-3-methylglutaryl coenzyme A
HOMA	homeostatic model assessment
HPT	hypothalamic-pituitary-thyroid
HPLC/MS/MS	High-performance liquid chromatography/tandem mass spectrometry
h	hour
HSDB	Hazardous Substances Database
HSI	hepatosomatic index
I_{Ca}	inward calcium currents
IC_{50}	half-maximal Inhibiting Concentration
ICR	imprinting control region
IgE	Immunoglobulin E
IL	interleukin
INUENDO	Biopersistent Organochlorines in Diet and Human Fertility study
IQR	interquartile range
IRR	incidence rate ratio
IU	international unit
IV	intravenous
K_{ow}	octanol-water partition coefficient
K_t	affinity constant
kg	kilogram
KO	knockout
L	liter
LBW	low birth weight

LC_{50}	Lethal concentration for 50% (statistical median) of animals
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LD	lactation day
LD_{50}	Lethal dose for 50% (statistical median) of animals
LDL	low density lipoprotein
L-FABP	liver fatty acid binding protein
LI	labeling index
LIFE	Longitudinal Investigation of Fertility and the Environment
LLOQ	lower limit of quantitation
LOAEL	lowest observed adverse effect level
LOEC	lowest observed effect concentration
LOQ	Limit of quantitation
LPS	Lipopolysaccharide
m	meter
MDA	malondialdehyde
ME	malic enzyme
µg	microgram
mg	milligram
min	minute
mL	milliliter
mmol	millimole
µmol	micromole
MOA	mode of action
mol	mole
MRP	multidrug resistance-associated protein
NA	not applicable
NCEH1	Neutral Cholesterol Ester Hydrolase 1
ND	not detected or not determined
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIS	sodium iodide symporter
NJDEP	New Jersey Department of Environmental Protection
NK	natural killer
nmol	nanomole
NMRI	Naval Medical Research Institute
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NR1H3	Nuclear Receptor Subfamily 1, Group H, Member 3
NS	no sample
NSP	newborn screening program
NT	not tested
OAT	organic anion transporter
OATp	organic anion transporting peptide
OR	odds ratio
p	probability
PB	phenobarbital
PBDE	polybrominated diphenyl ether
PBMC	peripheral blood mononuclear cells
PBPK	physiologically-based pharmacokinetic

PCB	polychlorinated biphenyl
PCNA	proliferating cell nuclear antigen
PCoAO	palmitoyl CoA oxidase
PFAS	perfluoroalkyl substance
PFBA	perfluorobutyric acid
PFBS	perfluorobutane sulfonate
PFHxS	Perfluorohexanesulfonic acid
PFNA	perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	perfluoroocatane sulfonate
PFOSA	perfluorooctane sulfamide
pg	picogram
PI	proliferation index
PK	pharmacokinetic
pKa	acid dissociation constant
pmol	picomole
PND	postnatal day
POD	point of departure
POSF	perfluorooctanesulfonyl fluoride
PPAR	peroxisome proliferator activated receptor
ppb	parts per billion
ppm	parts per million
mPSC	miniature post-synaptic current
mRNA	messenger ribonucleic acid
PTU	propylthiouracil
PUFA	polyunsaturated fatty acid
PXR	pregnane X receptor
Q	flow in and out of tissues
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RIA	radio immunoassay
RNA	ribonucleic acid
RR	rate ratio
RSI	renal-somatic index
SD	standard deviation
SDQ	Strengths and Difficulties Questionnaire
SDWA	Safe Drinking Water Act
SHBG	sex hormone-binding globulin
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
SPC	saponin compound
SRBC	sheep red blood cells
Syn 1	synapsin 1
Syp	synaptophysin
T	total testosterone
T-AOC	total antioxidation capability
T3	triiodothyronine

T4	thyroxine
$t_{1/2}$	chemical half-life
$T_{1/2}$	elimination half-time
T_m	transport maximum
TBG	thyroxine-binding globulin
TC	total cholesterol
TG	triglycerides
TH	thyroid hormone
TNF-α	tumor necrosis factor-α
TNP	trinitrophenol
TPO	thyroid peroxidase
TPOAb	thyroid peroxidase antibody
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone
TSHR	thyroid stimulating hormone receptor
TT3	total triiodothyronine
TT4	total thyroxin
TTP	time to pregnancy
TTR	thyroid hormone transport protein, transthyretin
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
UCB	umbilical cord blood
UF	uncertainty factor
UGT	uridine diphosphoglucuronosyl transferase
UK	United Kingdom
U.S.	United States
V_d	volume of distribution
VLDL	very low density lipoprotein
WHO	World Health Organization

EXECUTIVE SUMMARY

Perfluorooctane sulfonate (PFOS) is a fluorinated organic compound with an eight-carbon backbone and a sulfonate functional group. PFOS-related chemicals are used in a variety of products, including surface treatments for soil/stain resistance; surface treatments of textiles, paper, and metals; and in specialized applications such as firefighting foams. Because of strong carbon-fluorine bonds, PFOS is stable to metabolic and environmental degradation and is resistant to biotransformation. Data in humans and animals demonstrate ready absorption of PFOS and distribution of the chemical throughout the body by noncovalent binding to serum albumin and other plasma proteins. Both experimental data and pharmacokinetic models show higher levels of PFOS in fetal serum and brain compared with the maternal compartments. PFOS is not readily eliminated from humans as evidenced by the estimated average half-life values of 4.1–8.67 years. In contrast, half-life values for the monkey, rat, and mouse are 121 days, 48 days, and 37 days, respectively. The long half-lives appear to be the result of saturable resorption from the kidney. In other words, after initial PFOS removal from blood by the kidney, a substantial fraction of what would normally be eliminated in urine is resorbed from the renal tubules and returned to the blood. A number of published toxicokinetic models use saturable resorption as a basis for predicting serum values in animals and humans, including one developed by the U.S. Environmental Protection Agency (EPA) to support this assessment.

Peroxisome proliferation as a result of binding to and activation of peroxisome proliferator-activated receptor-alpha (PPARα), is usually associated with hepatic lesions in the rat, but some uncertainties exist as to whether this is true for liver effects induced by PFOS. Increased hepatic lipid content in the absence of a strong PPARα response is a characteristic of exposure to PFOS. In two studies, mice administered PFOS showed differential expression of proteins mainly involved in lipid metabolism, fatty acid uptake, transport, biosynthetic processes, and response to stimulus. Many of the genes activated by PFOS are associated with nuclear receptors other than PPARα.

Numerous epidemiology studies have examined occupational populations at large-scale PFOS production plants in the United States and a residential population living near a PFOA production facility in an attempt to determine the relationship between serum PFOS concentration and various health outcomes. Epidemiology data report associations between PFOS exposure and high cholesterol and reproductive and developmental parameters. The strongest associations are related to serum lipids with increased total cholesterol and high density lipoproteins (HDLs). Data also suggest a correlation between higher PFOS levels and decreases in female fecundity and fertility, in addition to decreased body weights in offspring, and other measures of postnatal growth. Several human epidemiology studies evaluated the association between PFOS and cancers including bladder, colon, and prostate, but these data present a small number or cases and some are cofounded by failure to adjust for smoking. The associations for most epidemiology endpoints are mixed. While mean serum values are presented in the human studies, actual estimates of PFOS exposure (i.e., doses/duration) are not currently available. Thus, the serum level at which the effects were first manifest and whether the serum had achieved steady state at the point the effect occurred cannot be determined. It is likely that some of the human exposures that contribute to serum PFOS values come from PFOS derivatives or precursors that break down metabolically to PFOS. These compounds may originate from PFOS in diet and materials used in the home, thus, there is potential for confounding. Additionally, most of the subjects of the epidemiology studies have many perfluoroalkyl substances (PFAS), other contaminants, or both in their blood. Taken together, the weight of evidence for human

studies supports the conclusion that PFOS exposure is a human health hazard. At this time, EPA concludes that the human studies are adequate for use qualitatively in the identification hazard and are supportive of the findings in laboratory animals.

Short-term and chronic exposure studies in animals demonstrate increases in liver weight consistently at doses generally ≥ 0.5 milligrams per kilogram per day (mg/kg/day). Co-occurring effects in these studies include decreased cholesterol, hepatic steatosis, lower body weight, and liver histopathology.

One and two generation toxicity studies also show decreased pup survival and body weights. Additionally, developmental neurotoxicity studies show increased motor activity and decreased habituation and increased escape latency in the water maze test following in utero and lactational exposure to PFOS. Gestational and lactational exposures were also associated with higher serum glucose levels and evidence of insulin resistance in adult offspring. Limited evidence suggests immunological effects in mice.

EPA derived a reference dose (RfD) for PFOS of 0.00002 mg/kg/day based on decreased neonatal rat body weight from the two-generation study by Luebker et al. (2005b). A pharmacokinetic model was used to predict an area under the curve (AUC) for the no observed adverse effect level (NOAEL) and used to calculate a human equivalent dose $(HED)_{NOAEL}$. The total uncertainty factor (UF) applied to the HED_{NOAEL} from the rat study was 30, which included a UF of 10 for intrahuman variability and a UF of 3 to account for toxicodynamic differences between animals and humans. The HED for effects on pup body weight in the two generation study is supported by comparable values derived from the lowest observed adverse effect level for the same effect in the one-generation study and the NOAEL for effects seen in a developmental neurotoxicity study.

Applying the U.S. EPA Guidelines for Carcinogen Risk Assessment, there is *suggestive evidence of carcinogenic potential* for PFOS (USEPA 2005a). In a chronic oral toxicity and carcinogenicity study of PFOS in rats, liver, thyroid, and mammary fibroadenomas were identified. The biological significance of the mammary fibroadenomas and thyroid tumors was questionable as a linear response to dose was not observed. The liver tumors also showed a slight, but statistically-significant increase only in high-dose males and females. The liver tumors most found were adenomas (7/60 and 5/60 in high-dose males and females, respectively, versus none in the controls of either sex). Only one hepatocellular carcinoma was found in a high-dose female. The genotoxicity data are uniformly negative. Human epidemiology studies did not find a direct correlation between PFOS exposure and the incidence of carcinogenicity in worker-based populations. Although one worker cohort found an increase in bladder cancer, smoking was a major confounding factor, and the standardized incidence ratios were not significantly different from the general population. Other worker and general population studies found no statistically-significant trends for any cancer type. Thus, the weight of evidence for the carcinogenic potential to humans was judged to be too limited to support a quantitative cancer assessment.

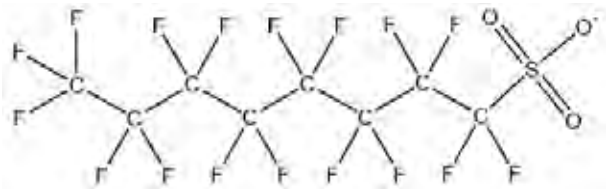
1. IDENTITY: CHEMICAL AND PHYSICAL PROPERTIES

Perfluorooctane sulfonate, commonly known as PFOS, and its salts are fluorinated organic compounds and are part of the group of chemicals called perfluoroalkyl substances (PFAS). The two most widely known PFAS have an eight-carbon backbone with either a sulfonate (PFOS) or carboxylate (perfluorooctanoic acid, PFOA) attached (Lau et al. 2007). PFOS-related chemicals are used in a variety of products including surface treatments for soil/stain resistance, coating of paper as a part of a sizing agent formulation, and in specialized applications such as firefighting foams. PFOS is produced commercially from perfluorooctanesulfonyl fluoride (POSF), which is primarily used as an intermediate to synthesize other fluorochemicals.

POSF is manufactured through a process called Simons Electro-Chemical Fluorination (ECF) in which an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedback of 1-octanesulfonyl fluoride, causing the carbon-hydrogen bonds on molecules to be replaced with carbon-fluorine bonds (OECD 2002). This process yields a mixture of linear and branched chain isomers (Beesoon and Martin 2015). The isomer ratio is about 70% linear and 30% branched chain. Yu et al. (2015) measured the isomer profiles of drinking water samples collected from 10 locations in China and found that the levels of the branched isomers accounted for 31.8% to 44.6% of the PFOS present using limits of quantification (LOQ) that ranged from 0.04 to 0.06 nanograms per liter (ng/L). Some systems had 1-methyl and 6-methyl isomers that were > 2% of the total. Levels of the other isomers were lower. Isomer concentrations are important because half-life decreases as the percentage of branched isomers increases.

A second process for preparing PFOS is called telomerization. It produces linear chains and was the favored process in the United States until the time 3M voluntarily ceased production in 2002 (Beesoon et al. 2011). PFOS can also be formed in the environment by the degradation of other POSF-derived fluorochemicals such as N-methyl or N-ethyl perfluorooctane sulfonamides (PFOSAs) often referred to as precursors.

Because of strong carbon-fluorine bonds, PFOS is stable to metabolic and environmental degradation. It is a solid at room temperature and has a low vapor pressure. Because of the surface-active properties of PFOS, it forms three layers in octanol/water making determination of an n-octanol/water partition coefficient (K_{ow}) impossible. No direct measurement of the acid dissociation constant (pKa) of the acid has been located; however, the chemical is considered to have a low pKa and exist as a highly dissociated anion. The chemical structure is provided in Figure 1-1, and the physical properties for PFOS are provided in Table 1-1.



Source: Environment Canada (2006)

Figure 1-1. Chemical Structure of PFOS

The branched chain isomers have a 7 carbon linear chain with methyl groups located on carbons 1, 3, 4, 5, or 6 (Beesoon and Martin 2015).

Table 1-1. Chemical and Physical Properties of PFOS

Property	PFOS, acidic form*	Source
Chemical Abstracts Service Registry Number (CASRN)	1763-23-1	
Chemical Abstracts Index Name	1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonic acid	
Synonyms	Perfluorooctane sulfonic acid; heptadecafluoro-1-octane sulfonic acid; PFOS acid	
Chemical Formula	$C_8HF_{17}O_3S$	
Molecular Weight (grams per mole [g/mol])	500.13	Lewis (2004); Hazardous Substances Database (HSDB) (2012); SRC (2016)
Color/Physical State	White powder (potassium salt)	OECD (2002)
Boiling Point	258–260 ^oC	SRC (2016)
Melting Point	No data	
Vapor Pressure	2.0 x 10^{-3} milligrams Mercury (mm Hg) at 25 ^oC (estimate)	HSDB (2012)
Henry's Law Constant	Not measureable	ATSDR (2015)
K_{ow}	Not measurable	EFSA (2008); ATSDR (2015)
organic carbon water partitioning coefficient (K_{oc})	2.57	Higgins and Luthy (2006)
Solubility in Water	680 mg/L	OECD (2002)
Half-life in Water	Stable	UNEP (2006)
Half-life in Air	Stable	UNEP (2006)

Notes: *PFOS is commonly produced as a potassium salt (CASRN 2795-39-3). Properties specific to the salt are not included. This CASRN given are for linear PFOS, respectively, but the toxicity studies are based on a mixture of linear and branched, and thus the RfD applies to the total linear and branched.

2. TOXICOKINETICS

Because of strong carbon-fluorine bonds, PFOS is stable to metabolic and environmental degradation. It is not readily eliminated and can have a long half-life in humans and animals. However, the toxicokinetic profile and the underlying mechanism for the chemical's long half-life are not completely understood. In the case of another perfluorinated compound (PFAS), PFOA, membrane transporter families appear to play an important role in absorption, distribution, and excretion. The transporter families identified for PFOA include organic anion transporters (OATs), organic anion transporting peptides (OATps), multidrug resistance-associated proteins (MRPs), and urate transporters. Transporters play a critical role in gastrointestinal absorption, uptake by the tissues, and excretion via the kidney. Limited data are available regarding the transporters and PFOS, however the toxicokinetic properties of PFOS suggest facilitated transport functions in tissue uptake and renal resorption. Hepatic OATp1, OATp2, and MRP2 messenger ribonucleic acid (mRNA) respond to PFOA exposure in a dose-related manner. Some inhibition studies suggest that PFOS with its similar chain length, renal excretion properties and liver accumulation could involve the same transporters. However, transporter-specific data related to PFOS are minimal.

Animal studies indicate that PFOS is well-absorbed orally and distributes primarily to the blood and liver. While PFOS can form as a metabolite from other perfluorinated compounds, PFOS itself does not undergo further metabolism after absorption takes place. PFAS are known to activate peroxisome proliferator activated receptor (PPAR) pathways by increasing transcription of mitochondrial and peroxisomal lipid metabolism, as well as sterol and bile acid biosynthesis based on transcriptional activation of many genes in PPARα-null mice, the effects of PFAS involve more than activation of PPAR receptors (Andersen et al. 2008). A summary of toxicokinetic data are provided in Appendix C, Table C-1.

2.1 Absorption

The absorption process requires transport across the tissue interface with the external environment. PFOS displays both hydrophobic and oleophobic properties, indicating that movement across the membrane surface is likely to be associated with transporters rather than simple diffusion. Unfortunately no information on the interaction of PFOS with intestinal, lung, or skin transporters in mammals was identified.

While there are no absorption studies available that quantify absorption in humans, extensive data on serum PFOS demonstrate uptake from the environment but not the exposure route. Studies that provide the basis for human half-life estimates rely on changes in serum levels over time. Section 2.5.2 of this document provides serum levels measured in humans.

2.1.1 Oral Exposure

Chang et al. (2012) administered a single dose of 4.2 milligrams per kilogram (mg/kg) of PFOS-^{14}C in solution to 3 male rats. At 48 hours after dosing, 3.32% of the total dose was found in the digestive tract and 3.24% in the feces, indicating that most of the dose had been absorbed with some of the unabsorbed material excreted in fecal matter (Table 2-1).

Table 2-1. Mean % (± SE) of ^{14}C-K+PFOS in Rats after a Single Dose of 4.2 mg/kg

Compartment	% ^{14}C of dose recovered	
	24 hr	48 hr
carcass	79.0 ± 1.8	94.2 ± 5.1
digestive tract	3.58 ± 0.23	3.32 ± 0.12
feces	1.55 ± 0.15	3.24 ± 0.08
urine	1.57 ± 0.25	2.52 ± 0.31
plasma	11.02 ± 0.64 (estimated)*	10.01 ± 0.62(estimated)*
red blood cell (RBC)	2.29 ± 0.18 (estimated)*	3.25 ± 0.92 (estimated)*
Total	99.0	116.5

Source: Data from Chang et al. 2012
Note: *A mean body weight of 300g was used to estimate the red blood cell (RBC) and plasma volume.

2.1.2 Inhalation Exposure

An acute lethal concentration for 50% (statistical median) of animals (LC_{50}) study in rats indicates that PFOS absorption occurs after inhalation exposures. However, pharmacokinetic data were not included in the published report (Rusch et al. 1979). The analytical methods for measuring PFOS in animals were limited at the time the study was conducted.

2.1.3 Dermal Exposure

No data are available on dermal absorption of PFOS.

2.2 Distribution

PFOS is distributed within the body by non-covalently binding to plasma proteins, most commonly albumin. The *in vitro* protein binding of PFOS in rat, monkey, and human plasma at concentrations of 1–500 parts per million (ppm) PFOS was investigated by Kerstner-Wood et al. (2003). PFOS was bound to plasma protein in all three species at all concentrations with no sign of saturation (99.0–100%). When incubated with separate human-derived plasma protein fractions, PFOS was highly bound (99.8%) to albumin and showed affinity for low density lipoproteins (LDLs, formerly beta-lipoproteins) (95.6%) with some binding to alpha-globulins (59.4%) and gamma-globulins (24.1%). Low levels of binding to alpha-2-macroglobulin and transferrin were measured when the protein concentrations were approximately 10% of physiological concentration (Table 2-2).

Table 2-2. Percent (%) Binding of PFOS to Human Plasma Protein Fractions

Fraction	~ 10% Physiological Concentration (Conc.)	100% Physiological Conc.
Albumin	99.0	99.8
Gamma-globulin	6.3	24.1
Alpha-globulin	49.9	59.4
Fibrinogen	< 0.1	< 0.1
Alpha-2-macroglobulin	12.5	< 0.1
Transferrin	7.2	< 0.1
LDLs	90.1	95.6

Source: Data from Kerstner-Wood et al. 2003.

Zhang et al. (2009) used equilibrium dialysis, fluorophotometry, isothermal titration calorimetry and circular dichroism (CD) to characterize interactions between PFOS and serum albumin and deoxyribonucleic acid (DNA). Solutions containing known amounts of serum albumin or DNA were placed in dialysis tubing and suspended in solutions with varying concentrations of PFOS. The solutions were allowed to equilibrate while measuring the change in the PFOS concentration in the dialysis solution. During dialysis, the PFOS concentration in the solution decreased reflecting its ability to cross the dialysis membrane and bind to the biopolymer within the dialysis bag. Based on the data, the serum albumin could bind up to 45 moles of PFOS per mole of protein and 0.36 moles per base pair of DNA. The binding ratio increased with increasing PFOS concentrations and decreasing solution pH (i.e., capable of promoting protein and DNA denaturation), thus providing an increased number of binding sites. It is important to remember that these studies were conducted *in vitro* and may not reflect *in vivo* situations.

The authors concluded that the interactions between serum albumin and PFOS were the results of surface electrostatic interactions between the sulfonate functional group and the positively charged side chains of lysine and arginine. Hydrogen binding interactions between the negative dipoles (fluorine) of the PFOS carbon-fluorine bonds could also play a role in the non-covalent bonding of PFOS with serum albumin. Intrinsic fluorescence analysis of tryptophan residues in serum albumin suggested a potential interaction of PFOS with tryptophan, an amino acid likely to be found in a hydrophobic portion of the albumin. In the case of DNA, the authors postulated that the interaction with PFOS occurred along the major or minor grooves of the double helix and was stabilized by the hydrogen bonding and van der Waals interactions.

Serum albumin plays an important role in the transport of a number of endogenous and exogenous compounds, such as fatty acids, bile acids, some medications and pesticides (Zhang et al. 2009). Accordingly, changes in conformation could change its transporting activity. CD spectrometry was used to determine if PFOS changed the conformation of the albumin or DNA in solution. The results of both analyses indicated conformational changes as a result of PFOS binding. However, the CD results did not demonstrate whether there was a change in transport function as a result of the conformational change.

Binding of five perfluoroalkyl acids, including PFOS, to human serum albumin was investigated by using site-specific fluorescence (Chen and Guo, 2009). Intrinsic fluorescence of trytophan-214 in human serum albumin was monitored upon addition of the perfluoroalkyl acids. PFOS induced fluorescence quenching indicative of binding. A binding constant of $2.2 \times 10^4\ M^{-1}$ and a binding ratio of PFOS to human albumin of 14 moles PFOS/mole albumin were calculated.

Human serum albumin has two high-affinity drug binding sites which are known as Sudlow's drug Site I and Site II. Past experiments have shown that two fluorescence probes, dansylamide (DA) and dansyl-L-proline (DP), are specific for the two drug binding sites on human serum albumin. Alone these two probes emit negligible fluorescence; after binding with albumin, fluorescence increases. The titration of PFOS into human serum albumin pretreated with DA (site I), showed that at low concentrations of PFOS (0.07 mmol), DA emission increased as the PFOS concentration increased until it was at 140% the original intensity. At the higher PFOS concentrations (0.7–4 mmol), however, the fluorescence dropped. The author speculated that the rise in fluorescence was induced by the conformational changes of the protein after PFOS binds to it at a site different from Site I, and the decrease at higher concentrations was from displacement of DA by PFOS. For Site II, PFOS caused a fluorescence reduction that was quick at first, but then became more gradual suggesting the possibility that PFOS was binding to this

site with two different affinities. The binding constant calculated at Site II was 7.6×10^6 M^{-1}. These findings indicate PFOS has binding sites that are similar to those identified for fatty acids.

Structure and the energy of PFOS binding sites were determined for human serum albumin using molecular modeling (Salvalaglio et al. 2010). Calculations were based on a compound approach docking, molecular dynamics simulations, and estimating free binding energies by adopting the weighted histogram analysis method umbrella sampling and semiempirical methodology. The binding sites impacted were ones identified as human serum albumin fatty acid binding sites. The PFOS binding site with the highest energy (−8.8 kilocalories per mole [kcal/mol]) was located near the tip of the tryptophan-214 binding site, and the maximum number of ligands that could bind to human serum albumin for PFOS was 11. The most populated albumin binding site for PFOS was dominated by van der Waals interactions. The author indicated that eleven PFOS molecules were adsorbed on the surface of the albumin.

PFOS binding to bovine serum albumin was evaluated using electrospray ionization mass spectrometry by D'Alessandro et al. (2013). Using this approach, the estimate for the maximum number of PFOS binding sites was also 11, but the data on collision-induced PFOS removal was more consistent with 7 binding sites. Two of the potential binding sites (Sudlow's sites I and II) are binding sites for a number of pharmaceuticals.

D'Alessandro et al. (2013) also examined whether PFOS could prevent binding of ibuprofen to its Sudlow II site and whether it was also able to displace bound ibuprofen. The study showed that PFOS competes with ibuprofen for its site when the PFOS:ibuprofen ratio is ≥ 0.5 moles:1 mole. In addition, when the binding site is occupied by PFOS, ibuprofen is unable to bind. Zhang et al. (2009) conducted a similar study of the impact of PFOS on the ability of serum albumin to bind vitamin B_2 (riboflavin). The study found that at normal physiological conditions, 1.2 mmol/L of PFOS decreased the binding ratio of serum albumin for riboflavin *in vitro* by > 30%. These data suggest that PFOS can alter the pharmacokinetics and pharmacodynamics of medicinal and natural substances that share a common site on albumin.

Beesoon and Martin (2015) examined differences in the binding of the linear and branched chain isomers to serum albumin and human serum proteins. The linear PFOS molecule was found to bind more strongly to calf serum albumin than the branched chain isomers. When arranged in order of increasing binding the order was 3m < 4m < 1m < 5m < 6m (iso) <linear. In the isomer-specific binding to spiked total human serum protein, the 1m appeared to bind most strongly and the 4m the least. Binding was estimated based on the concentrations in the ultrafiltrate after spiking with 5 to 60 mg/L technical PFOS. The human serum was diluted ten-fold before spiking.

2.2.1 Oral Exposure

PFOS entry from serum into tissues appears to be controlled by several families of membrane transporters based on PFOA studies. Yu et al. (2011) administered PFOS to rats and extracted the mRNAs for OATp1, OATp2, and MRP2 from the liver to determine if they were involved in hepatic uptake. Approximately six female Wistar rats per group were administered vehicle (0.5% Tween 20), or PFOS at 0.2, 1.0, or 3.0 mg/kg in Tween 20 once daily by gavage for 5 consecutive days. Blood, bile, and liver tissue were collected 24 hours after the last dose. Exposure to 3.0 mg/kg of PFOS increased hepatic OATp2 mRNA expression (1.43 times control) while MRP2 was increased approximately 1.80 and 1.69 times that of controls in the

1.0 and 3.0 mg/kg groups, respectively. No effect with treatment was observed on OATp1. No additional information on PFOS tissue transport was identified.

Humans. In humans, PFOS distributes mostly to the liver and blood. Olsen et al. (2003a) sampled both liver and serum from cadavers for PFOS. There was a good correlation between samples from the same subject. There was no difference in the PFOS concentrations identified in males and females or between age groups. Kärrman et al. (2010) identified PFOS in postmortem liver samples (n = 12; 6 males and 6 females 27–79 years old) with a mean concentration of 26.6 ng/g tissue.

Pérez et al. (2013) collected tissue samples from 20 adult subjects (aged 28–83) who had been living in Catalonia, Spain for 10 years and died of a variety of causes. Autopsies and tissue collection (liver, kidney, brain lung, and bone) were carried out in the first 24 hours after death. The tissues were analyzed for 21 perfluorinated compounds. PFOS was present in 90% of the samples but could be quantified in only 20% (median 1.9 ng/g). PFOS accumulated primarily in the liver (104 ng/g), kidney (75.6 ng/g), and lung (29.1 ng/g), and it was low in brain (4.9 ng/g) and bone (not detected) based on the mean wet weight tissue concentration. Detection levels varied with the tissue evaluated.

Stein et al. (2012) compared PFAS levels in maternal serum and amniotic fluid paired samples from 28 females in their second trimester of pregnancy. PFOS (0.0036–0.0287 μg/mL) was detected in all serum samples and in nine amniotic fluid samples (0.0002–0.0018 μg/mL). The Spearman correlation coefficient between the serum and amniotic fluid levels was 0.76 and is significant ($p = 0.01$), indicating a direct relationship between the levels in blood and amniotic fluid. The median ratio of maternal serum:amniotic fluid concentration was 25.5:1. Based on a simple regression between the levels in each compartment, PFOS was rarely detected in amniotic fluid unless the serum concentration was ≥ 0.0055 μg/mL.

Harada et al. (2007) obtained cerebrospinal fluid (CSF) from seven patients (6 males and 1 female; aged 56–80) to evaluate the partitioning of PFOS between serum and the CSF. The median concentration of PFOS in the serum was 0.0184 μg/mL, compared to the concentration in the CSF (0.00010 μg/mL). The CSF to serum ratio was 9.1×10^{-3}. The levels identified indicate that PFOS does not easily cross the adult blood-brain barrier.

PFOS has been detected in both umbilical cord blood and breast milk indicating that maternal transfer occurs (Apelberg et al. 2007; Von Ehrenstein et al. 2009; Völkel et al. 2008). Kärrman et al. (2010) identified PFOS in breast milk samples from healthy females (n = 10; females 30–39 years old). The levels in milk (mean 0.12 ng/mL) were low compared to liver levels.

Animals

Monkey. Seacat et al. (2002) administered 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS orally in a capsule by intragastric intubation to six young-adult to adult cynomolgus monkeys/sex/group, except for the 0.03 mg/kg/day group which was 4/sex, daily for 26 weeks (182 days). Serum and tissues were collected at the time of sacrifice. The dosing was followed by a 52-week recovery period in 2 animals in the control, 0.15 and 0.75 mg/kg/day groups. Levels of PFOS were recorded in the serum and liver. Serum PFOS measurements demonstrate a linear increase with dosing duration in the 0.03 and 0.15 mg/kg/day groups and a non-linear increase in the 0.75 mg/kg/day group. Levels in the high-dose group appeared to plateau after about 100 days (14 weeks). Serum levels of PFOS decreased with recovery in the two highest

dosed groups. The average percent of the cumulative dose of PFOS in the liver at the end of treatment ranged from 4.4% to 8.7% with no difference by dose group or gender. The concentration of PFOS in the liver decreased during the recovery period. Serum levels are provided in Table 2-3.

Table 2-3. Average PFOS Level (µg/mL or ppm) in Serum of Monkeys

Time (weeks)	Group 1 0.0 milligram (mg)/kilogram (kg)/day		Group 2 0.03 mg/kg/day		Group 3 0.15 mg/kg/day		Group 4 0.75 mg/kg/day	
	Males	Females	Males	Females	Males	Females	Males	Females
1	< LOQ	< LOQ	0.869 ± 0.147	0.947 ± 0.110	4.60 ± 0782	3.71 ± 0.455	21.0 ± 1.57	20.4 ± 2.71
4	< LOQ	< LOQ	3.20 ± 0.577	3.40 ± 0.291	17.8 ± 1.68	16.5 ± 1.87	95.3 ± 70.4	92.7 ± 39.6
16	0.04 ± 0.01	0.04 ± 0.008	11.2 ± 2.44	10.5 ± 1.90	56.2 ± 5.84	42.1 ± 4.04	189 ± 15.9	162 ± 19.3
27	0.05 ± 0.01	0.04 ± 0.01	15.9 ± 5.54	11.1 ± 1.52	68.1 ± 5.75	58.5 ± 4.67	194 ± 8.93	160 ± 23.9
35	0.05 ± 0.003	0.07 ± 0.004	Not Determined	Not Determined	84.5 ± 12.0	74.7 ± 9.53	181 ± 19.5	171 ± 10.1
57	0.03 ± 0005	0.0445 ± 0.00385	Not Determined	Not Determined	30.2 ± 2.36	32.3 ± 1.34	78.0 ± 16.3	106 ± 3.84
79	0.02 ± 0.003	0.02 ± 0.003	Not Determined	Not Determined	19.1 ± 0.805	21.4 ± 2.01	41.1 ± 25.9	41.4 ± 1.15

Source: Data from p. 304 in OECD 2002.
Note: LOQ = limit of quantitation (value not stated)

At the two low doses, serum levels were comparable in the males and females, whereas at the high dose, the levels were higher in the males than females. Only for the highest dose group did the animals appear to reach serum steady state (week 16 for both males and females). In the lower dose groups, the serum levels continued to increase with dose across the dosing period. Once dosing ceased serum levels declined in all animals monitored.

Rat. Martin et al. (2007) administered 10 mg PFOS/kg to adult male Sprague-Dawley rats (n = 5) for 1, 3, or 5 days by gavage and determined the liver and serum levels. Blood was collected via cardiac puncture and PFOS concentration was determined by high-performance liquid chromatography-electrospray tandem mass spectrometry. The mean liver PFOS concentration was 83 ± 5, 229 ± 10, and 401 ± 21 µg/g after 1, 3, or 5 daily doses, respectively. The mean serum concentration was 23 ± 2.8 and 87.7 ± 4.1 µg/mL, after 1 and 3 days of dosing, respectively. Serum PFOS concentration was not determined after 5 days of dosing due to sample unavailability (not further explained by the authors).

Yu et al. (2011) administered the doses of 1, 0.2, 1.0, or 3.0 mg PFOS/kg dissolved in 0.5% Tween 20 as the vehicle to 6 female Wistar rats/group once daily by gavage for 5 consecutive days as part of a study of the effects of PFOS on the thyroid. Blood and bile were collected 24 hours after the last dose (Table 2-4). The data demonstrate a dose-related distribution to both serum and bile.

Table 2-4. Levels of PFOS in Serum and Bile of Rats Treated for 5 Days

PFOS (mg/kg bw)	Serum PFOS (microgram [µg]/milliliter [mL])	Bile PFOS (µg/mL)
0.0	< LOQ	< LOQ
0.2	1.09 ± 0.12	1.51 ± 0.42
1.0	8.20 ± 0.13	3.58 ± 0.66
3.0	33.5 ± 1.79	6.51 ± 0.67

Source: Data from Table 2 in Yu et al. 2011.
Note: LOQ = limit of quantification, 0.5 µg/L

Groups of 15 Sprague-Dawley rats/sex/group were administered 0, 20, 50 or 100 mg PFOS/kg diet (Curran et al. 2008). Tissues were analyzed for PFOS residue by liquid chromatography negative electrospray tandem mass spectrometry. Distribution of PFOS is provided in Table 2-5 and indicates that the highest levels were distributed to the liver and spleen. There were no consistent differences between sexes for the liver tissues, however levels in the spleen and heart tended to be higher in females (F) than males (M) at all doses. The levels in the liver were considerably higher than those in the heart and spleen in both sexes for all doses.

Table 2-5. Mean (± SD) Daily PFOS Consumption and Tissue Residue Levels in Rats Treated for 28 Days

	0 mg/kg diet		2 mg/kg diet		20 mg/kg diet		50 mg/kg diet		100 mg/kg diet	
Parameter	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
PFOS consumption (mg/kg bw/day)	0	0	0.14 ± 0.02	0.15 ± 0.02	1.33 ± 0.24	1.43 ± 0.24	3.21 ± 0.57	3.73 ± 0.57	6.34 ± 1.35	7.58 ± 0.68
Serum (µg PFOS/g serum)	0.47 ± 0.27	0.95 ± 0.51	0.95 ± 0.13	1.50 ± 0.23	13.45 ± 1.48	15.40 ± 1.56	20.93 ± 2.36	31.93 ± 3.59	29.88 ± 3.53	43.20 ± 3.95
Liver (µg PFOS/gram (g) liver)	0.79 ± 0.49	0.89 ± 0.44	48.28 ± 5.81	43.44 ± 6.79	560.23 ± 104.43	716.55 ± 59.15	856.90 ± 353.83	596.75 ± 158.01	1030.40 ± 162.80	1008.59 ± 49.41
Ratio liver:serum PFOS	2.04 ± 1.39	1.30 ± 1.32	51.34 ± 9.20	29.99 ± 8.11	42.10 ± 9.20	46.81 ± 5.26	41.42 ± 16.95	20.23 ± 7.50	35.23 ± 8.50	23.48 ± 1.98
Spleen (µg PFOS/g spleen)	0.27 ± 0.36	2.08 ± 4.17	6.07 ± 1.85	7.94 ± 3.76	45.27 ± 2.16	70.03 ± 36.66	122.51 ± 7.83	139.45 ± 15.44	230.73 ± 11.47	294.96 ± 26.66
Heart (µg PFOS/g heart)	0.10 ± 0.14	1.42 ± 2.91	4.67 ± 1.73	6.54 ± 3.07	33.00 ± 3.44	54.65 ± 30.89	90.28 ± 4.95	107.53 ± 6.24	154.13 ± 11.78	214.45 ± 17.58

Source: Data from Table 1 on in Curran et al. 2008
Note: M = male; F = female; SD = standard deviation

Ten three-month old male Sprague-Dawley rats/group were administered 0 (Milli-Q water only), 5, or 20 mg/kg/day of PFOS by oral gavage for 28 days (Cui et al. 2009). Rats were sacrificed after the exposure and blood and tissue samples obtained. Concentrations identified in rat whole blood and various tissues at the end of the exposure are provided in Table 2-6. The study indicated that the highest levels of PFOS were identified in the liver after 28 days of exposure.

Table 2-6. Concentrations of PFOS in Male Rats' Whole Blood (µg/mL) and Various Tissues (µg/g) after 28 Days

Tissues	Controls	5 mg/kg/day PFOS	20 mg/kg/day PFOS
blood	ND	72.0 ± 25.7	No sample
liver	ND	345 ± 40	648 ± 17
kidney	ND	93.9 ± 13.6	248 ± 26
lung	ND	46.6 ± 17.8	228 ± 122
heart	ND	168 ± 17	497 ± 64
spleen	ND	38.5 ± 11.8	167 ± 64
testicle	ND	39.5 ± 10.0	127 ± 11
brain	ND	13.6 ± 1.0	146 ± 34

Source: Data from Table 1 in Cui et al. 2009.
Note: ND = not detected

A combined chronic toxicity/carcinogenicity good laboratory practice (GLP) study was performed in 40–70 male and female Sprague-Dawley Crl:CD (SD)IGS BR rats administered 0, 0.5, 2, 5, or 20 ppm of PFOS for 104 weeks (Thomford 2002/Butenhoff et al. 2012[1]). Doses were approximately 0, 0.018–0.023, 0.072–0.099, 0.184–0.247 and 0.765–1.1 mg/kg/day. A recovery group was administered the test substance at 20 ppm for 52 weeks and observed until sacrifice at 106 weeks. Serum and liver samples were obtained during and at the end of the study to determine the concentration of PFOS. Dose-dependent increases in the PFOS level in the serum and liver were observed, with values slightly higher in females. Further study details are described in section 3.2.7 Chronic Toxicity. Levels of PFOS identified in the liver and serum are included in Table 2-7.

Table 2-7. PFOS Levels in the Serum and Liver of Rats

Timepoint (weeks)	0 ppm PFOS (0 mg/kg/day)		0.5 ppm (0.024–0.029 mg/kg/day)		2 ppm (0.098–0.120 mg/kg/day)		5 ppm (0.242–0.299 mg/kg/day)		20 ppm (0.984–1.251 mg/kg/day)	
	M	F	M	F	M	F	M	F	M	F
Serum PFOS levels (µg/mL)										
0	< LOQ*	0.0259	0.907	1.61	4.33	6.62	7.57	12.6	41.8	54.0
14	< LOQ**	2.67	4.04	6.96	17.1	27.3	43.9	64.4	148	223
53	0.0249	0.395							146	220
105	0.0118	0.0836	1.31	4.35	7.60		22.5	75.0	69.3	233
106									2.42[a]	9.51[a]
Liver PFOS levels (µg/g)										
0	0.104	0.107	11.0	8.71	31.3	25.0	47.6	83.0	282	373
10	0.459	12.0	23.8	19.2	74.0	69.2	358	370	568	635
53	0.635	0.932							435	560
105	0.114	0.185	7.83	12.9	26.4		70.5	131	189	381
106									3.12[a]	12.9[a]

Source: Data from Tables 4 and 5 on pp. 38 and 39 in OECD 2002
Notes: [a] These samples were obtained from the recovery group administered 20 ppm for 52 weeks and then observed until death.
*LOQ = limit of quantification = 0.00910 picogram (pg)/mL
**LOQ = 0.0457 pg/mL
M = male; F = female

[1] Thomford (2002) is unpublished, but it contains the raw data. Butenhoff et al. (2012) is the published study.

Mouse. Adult male C57/BL6 mice (3 mice/group) were administered ^{35}S-PFOS in the feed at a low and high dose for 1, 3, and 5 days. The dose equivalents were 0.031 mg/kg/day in the low dose group and 23 mg/kg/day in the high dose group. Tissue contents were determined by liquid scintillation (Bogdanska et al. 2011). At 23 mg/kg/day after 5 days, mice had hypertrophy of the liver, atrophy of fat pads, and atrophy of epididymal fat when compared to the mice at 0.013 mg/kg/day at 5 days. To determine the amount of radioactivity recovered that was due to blood in the tissues, the hemoglobin content was determined in all of the samples. By correcting for PFOS in the blood, the actual tissue levels were then calculated.

At both doses and at all time-points, the liver contained the highest amount of PFOS. At the low dose, the liver PFOS level relative to blood concentration increased with time, whereas at the high dose, the ratio plateaued after three days. The autoradiography indicated that the distribution within the liver did not appear to favor one area to a greater extent than any other. The liver contained 40% to 50% of the recovered PFOS at the high dose. The authors hypothesized that this could possibly reflect high levels of binding to tissue proteins.

In the high dose mice, the next highest level was found in the lungs. Distribution was fairly uniform with some favoring of specific surface areas. The tissue to blood ratio for the lung was greater than that for all other tissues except the liver. The lowest PFOS levels were in the brain and fat deposits.

While the levels in Table 2-8 report the PFOS in the whole bone, when the authors did a whole body autoradiogram of a mouse 48 hours after a single oral dose of ^{35}S-PFOS (12.5 mg/kg), the results indicated that most PFOS was found in the bone marrow and not the calcified bone. Levels for the kidney roughly equal those values observed in the blood at both concentrations and all timepoints (see Table 2-8).

Table 2-8. Mean Concentration of PFOS (± SD) in Various Tissues of Mice

Tissues	1 day	3 days	5 days
Dose of 0.013 mg/kg/day (PFOS in tissue reported as picomole [pmol]/g)			
Blood	61(6)	129 (41)$^{\#}$	99 (21)
Liver	114 (13)**	343 (24)$^{**\#}$	578 (39)$^{**\#}$
Kidney	38 (19)	65 (13)	93 (11)$^{\#}$
Lung	39 (29)	88 (6)$^{\#}$	141 (10)$^{*\#}$
Whole bone	113 (15)**	98 (24)	109 (6)
Dose of 23 mg/kg/day (PFOS in tissue reported as nanomole [nmol]/g)			
Blood	67 (4)	171 (21)$^{\#}$	287 (9)$^{\#}$
Liver	246 (31)**	698 (71)$^{**\#}$	1044 (114)$^{**\#}$
Kidney	62 (3)	166 (8)$^{\#}$	233 (12)$^{**\#}$
Lung	135 (18)**	336 (69)$^{*\#}$	445 (42)$^{**\#}$
Whole bone	55 (6)*	155 (17)$^{\#}$	207 (8)$^{**\#}$

Source: Data from Tables 2 and 3 in Bogdanska et al. 2011
Notes: *significantly different ($p < 0.05$) than blood at the same time-point as evaluated by an independent t-test
**significantly different ($p < 0.01$) than blood at the same time-point as evaluated by an independent t-test
$^{\#}$significantly different ($p < 0.05$) from the value for the same tissue at day 1 as determined by one-way analysis of variance (ANOVA) followed by Duncan's test

In an immunotoxicity study, four to six C57BL/6 male mice/group were administered diets with 0% to 0.02% PFOS for 10 days. Levels in the serum increased as the concentration increased (Table 2-9) (Qazi et al. 2009a).

Table 2-9. Levels of PFOS (Means ± SE) in Mouse Serum Following Treatment for 10 Days

Dietary level (% w/w)	Number of mice	ppm
PFOS (0)	4	0.0287 ± 0.01
PFOS (0.001%)	4	50.8 ± 2.5
PFOS (0.005%)	4	96.7 ± 5.2
PFOS (0.02%)	4	340 ± 16

Source: Data from study report by Qazi et al. 2009a

Distribution during Reproduction and Development

The availability of distribution data from pregnant females plus animal pups and neonates is a strength of the PFOS pharmacokinetic database, because it helps to identify those tissues receiving the highest concentration of PFOS during development. For this reason the information on tissue levels during reproduction and development are presented separately from those that are representative of other life stages.

Humans. T. Zhang et al. (2013) recruited pregnant females for a study to examine the distribution of PFOS between maternal blood, cord blood, the placenta, and amniotic fluid. Thirty two females from Tianjin, China volunteered to take part in the study. Samples were collected at time of delivery. Maternal ages ranged from 21 to 39 years, and periods ranged from 35 to 37 weeks. It was the first child for 26 of the females and the second child for 6. The study yielded 31 maternal whole blood samples, 30 cord blood samples, 29 amniotic fluid samples, and 29 placentas. The maternal blood contained variable levels of 10 PFAS, 8 acids and 2 sulfonates. The mean maternal blood concentration was highest for PFOS (14.6 ng/mL), followed by PFOA (3.35 ng/mL). In both cases, the mean was greater than the median, indicating a distribution skewed toward the higher concentrations.

PFOS was found in all fluids/tissues sampled. It was transferred to the amniotic fluid to a lesser extent than PFOA based on their relative proportions in the maternal blood and cord blood (21% versus 58%, respectively). Compared to the mean PFOS value in maternal blood, the mean levels in the cord blood, placenta, and amniotic fluid were 21%, 56%, and 0.14% of the mean levels in the mother's blood, respectively. The correlation coefficients between the maternal PFOS blood levels and placenta, cord blood, and amniotic fluid levels were good (0.7 to 0.9), and the relationships were statistically-significant ($p < 0.001$).

Rat. To determine the dose-response curve for neonatal mortality in rat pups born to PFOS exposed dams and to investigate associated biochemical and pharmacokinetic parameters, 5 groups of 16 female Sprague-Dawley Crl:CD(SD)IGS VAF/Plus rats each were administered 0, 0.1, 0.4, 1.6, or 3.2 mg PFOS/kg bw/day by oral gavage beginning 42 days prior to cohabitation and continuing through gestation day (GD) 14 or 20 (Luebker et al. 2005b). Eight rats from each group were randomly chosen and sacrificed on GD 15, followed by Caesarean removal of the pups. All remaining animals were sacrificed and C-sectioned on GD 21. Urine and feces were collected overnight from dams on the eve of cohabitation day 1 and during GDs 6–7, 14–15, and 20–21. Serum samples were collected just prior to cohabitation and on GD 7, GD 15, and GD 21. Fetal liver and blood samples were obtained on GD 21 and pooled by litter.

The urine, feces, and liver of the control animals all contained PFOS at small concentrations. In treated rats, the highest concentration of PFOS was in the liver. Serum levels in the dams for each dose were consistent between GD 1 and GD 15, indicating achievement of steady state prior to conception (Table 2-10). The GD 21 levels in the dams had dropped below those observed earlier in the pregnancy. Serum levels in the GD,21 fetuses were higher than those in the dams. In contrast, the liver levels in the dams on GD 21 were about three times higher than in the fetuses. Fecal excretion was greater than urinary excretion by the dams.

Table 2-10. PFOS Concentrations (Mean ± Standard Deviation [SD]) in Samples from Pregnant Dams and Fetuses (GD 21 Only) in µg/mL (ppm) for Serum and Urine and µg/g for Liver and Feces

Parameter	Dose (mg/kg/day)	GD 1	GD 7	GD 15	GD 21	
					Dams	Fetuses
Serum	0.1	8.90 ± 1.10	7.83 ± 1.11	8.81 ± 1.47	4.52 ± 1.15	9.08
	0.4	40.7 ± 4.46	40.9 ± 5.89	41.4 ± 4.80	26.2 ± 16.1	34.3
	1.6	160 ± 12.5	154 ±14.0	156 ± 25.9	136 ± 86.5	101
	3.2	318 ± 21.1	306 ± 32.1	275 ± 26.7	155 ± 39.3	164
Liver	0.1	-	-	-	29.2 ± 10.5	7.92
	0.4	-	-	-	107 ± 22.7	30.6
	1.6	-	-	-	388 ± 167	86.5
	3.2	-	-	-	610 ± 142	230
Urine	0.1	0.05 ± 0.02	0.06 ± 0.03	0.07± 0.04	0.06 ± 0.01	-
	0.4	0.28 ± 0.19	0.31 ± 0.20	0.53 ± 0.23	0.55 ± 0.16	-
	1.6	0.96 ± 0.39	1.10 ± 0.57	0.36 ± 0.35	2.71 ± 2.07	-
	3.2	1.53 ± 0.87	1.60 ± 0.97	0.52 ± 0.28	1.61 ± 0.53	-
Feces	0.1	0.50 ± 0.14	0.49 ± 0.11	0.66 ± 0.10	0.42 ± 0.10	-
	0.4	2.42 ± 0.49	2.16 ± 0.43	2.93 ±0.62	2.39 ± 1.21	-
	1.6	10.3 ± 3.01	9.20 ± 2.68	11.1 ± 3.28	9.94 ± 4.51	-
	3.2	23.9 ± 4.16	33.0 ± 10.0	29.5 ± 8.92	20.1 ± 4.21	-

Source: Data from Luebker et al. 2005b
Note: - = no sample obtained

This same study also included a subset of dams allowed to litter naturally and dosed through lactation day (LD) 4. Liver and serum samples were collected from dams and pups on LD 5. In this sampling, serum PFOS levels were similar between the dam and offspring, but the liver values were now higher in the neonates than in their dams.

Twenty five female Sprague-Dawley rats/group were administered 0, 0.1, 0.3, or 1.0 mg/kg/day potassium PFOS by gavage from GD 0 through postnatal day (PND) 20. An additional 10 mated females served as satellite rats to each of the four groups and were used to collect additional blood and tissue samples. Further details from this study are provided in section 3.2.6 as reported in Butenhoff et al. (2009). Samples were taken from the dams, fetuses, and pups for serum and tissue PFOS concentrations and the results reported by Chang et al. (2009). The blood and tissue sampling results are provided in Table 2-11.

Table 2-11. Mean PFOS (± Standard Error) Concentrations in Serum, Liver, and Brain Tissue in Dams and Offspring

Time	Dose (mg/kg)	Serum PFOS (µg/mL)		Liver PFOS (µg/g)		Brain PFOS (µg/g)	
		Dam	Offspring	Dam	Offspring	Dam	Offspring
GD 20[a]	Control	< LLOQ	0.009 ± 0.001	< LLOQ	< LLOQ	< LLOQ	< LLOQ
	0.1	1.722 ± 0.068	3.906 ± 0.096	8.349 ± 0.344	3.205 ± 0.217	0.151 ± 0.012	1.233 ± 0.067
	0.3	6.245 ± 0.901	10.446 ± 0.291	21.725 ± 0.721	5.814 ± 0.245	0.368 ± 0.043	3.126 ± 0.238
	1.0	26.630 ± 3.943	31.463 ± 1.032	48.875 ± 72.733	20.025 ± 2.021	0.999 ± 0.083	12.984 ± 1.122
PND 4[a]	Control	0.008 ± 0.000	< LLOQ	NS	< LLOQ	NS	< LLOQ
	0.1	3.307 ± 0.080	2.236 ± 0.070	NS	9.463 ± 0.512	NS	0.680 ± 0.033
	0.3	10.449 ± 0.234	6.960 ± 0.163	NS	20.130 ± 0.963	NS	1.910 ± 0.074
	1.0	34.320 ± 31.154	22.440 ± 0.723	NS	50.180 ± 1.124	NS	6.683 ± 0.428
PND 21	Control	0.007 ± 0.000	< LLOQ – m/f	NS	< LLOQ – m/f	NS	< LLOQ – m/f
	0.1	3.159 ± 0.081	1.729 ± 0.079 (M) 1.771 ± 0.076 (F)	NS	5.980 ± 0.614 (M) 5.278 ± 0.174 (F)	NS	0.220 ± 0.014 (M) 0.229 ± 0.011 (F)
	0.3	8.981 ± 0.275	5.048 ± 0.108 (M) 5.246 ± 0.138 (F)	NS	14.780 ± 0.832 (M) 13.550 ± 0.298 (F)	NS	0.649 ± 0.053 (M) 0.735 ± 0.039 (F)
	1.0	30.480 ± 1.294	18.611 ± 1.011 (M) 18.010 ± 0.744 (F)	NS	44.890 ± 2.637 (M) 41.230 ± 2.295 (F)	NS	2.619 ± 0.165 (M) 2.700 ± 0.187 (F)
PND 72	Control	NA	< LLOQ – m/f	NA	< LLOQ – m/f	NA	NS – M/F
	0.1	NA	0.042 ± 0.004 (M) 0.207 ± 0.042 (F)	NA	0.981± 0.091 (M) 0.801 ± 0.082 (F)	NA	NS – M/F
	0.3	NA	0.120 ± 0.009 (M) 0.556 ± 0.062 (F)	NA	2.464 ± 0.073 (M) 2.252 ± 0.095 (F)	NA	NS – M/F
	1.0	NA	0.560 ± 0.105 (M) 1.993 ± 0.293 (F)	NA	7.170 ± 0.382 (M) 7.204 ± 0.414 (F)	NA	NS – M/F

Source: Data from Table 2 in Chang et al. 2009
Notes: [a] Data are from samples pooled by litters in the fetuses/pups
< LLOQ = sample less than lower limit of quantitation, serum = 0.01 µg/mL; liver = 0.05 µg/g; brain = 0.025 µg/g
NS = no sample obtained
NA = not applicable; all dams sacrificed on PND 21
m = male; f = female

On GD 20, PFOS concentration in maternal serum, liver, and brain correlated with the daily doses administered. Maternal liver-to-serum PFOS ratios ranged from 1.8 to 4.9, while the maternal brain-to-serum ratios were 0.04 to 0.09 (Chang et al. 2009). The concentrations in the brains of fetuses was about ten times higher than in their dams for all doses.

Based on the maternal and offspring data on GD 20, there is placental transfer of PFOS from rat dams to developing fetuses. Serum values were approximately 1–2 times greater in the fetuses than in the dams at GD 20. The concentration of PFOS in fetal liver was less than that of dams, and the brain values were much higher; this is possibly due to the lack of development of the blood-brain barrier at this stage of offspring development. PFOS serum concentrations in the offspring were lower than those for the dams on PND 4 and continued to drop through PND 72. However, based on the concentrations still present in the neonate serum, lactational transfer of PFOS was occurring. At PND 72, the males appeared to be eliminating PFOS more quickly as the serum values were lower than those in the females; this difference was not observed at earlier time-points. In the liver, PFOS was the greatest in the offspring at PND 4 and decreased significantly by PND 72. Liver values were similar at all time-points between males and females. On GD 20, the brain levels for the pups were ten-fold higher than those for the dam. The levels in pup brain gradually declined between PND 4 and PND 21.

In a study by Zeng et al. (2011) ten pregnant Sprague-Dawley rats/group were administered 0, 0.1, 0.6, or 2.0 mg/kg/day of PFOS by oral gavage in 0.5% Tween 80 from GD 2 to GD 21. On GD 21, dams were monitored for parturition, and the day of delivery was designated PND 0. On PND 0, five pups/litter were sacrificed and the trunk blood, cortex, and hippocampus were collected for examination. The other pups were randomly redistributed to dams within the dosage groups and allowed to nurse until PND 21, when they were sacrificed with the same tissues collected as previously described. PFOS concentration in the hippocampus, cortex, and serum increased in a dose-dependent manner but overall was lower in all tissues on PND 21 when compared to PND 0. Levels of PFOS are included in Table 2-12.

Table 2-12. PFOS Contents in Serum, Hippocampus, and Cortex of Offspring (n = 6)

Time	Dose group (mg/kg/day)	Serum (µg/mL)	Hippocampus (µg/g)	Cortex (µg/g)
PND 0	Control	ND	ND	ND
	0.1	1.50 ± 0.43*	0.63 ± 0.19*	0.39 ± 0.09*
	0.6	24.60 ± 3.02**	7.43 ± 1.62*	5.23 ± 1.58**
	2.0	45.69 ± 4.77**	17.44 ± 4.12*	13.43 ± 3.89**
PND 21	Control	ND	ND	ND
	0.1	0.37 ± 1.12*	0.25 ± 0.14*	0.06 ± 0.04*
	0.6	1.86 ± 0.35**	1.59 ± 0.78**	1.03 ± 0.59**
	2.0	4.26 ± 1.73***	6.09 ± 1.30***	3.69 ± 0.95***

Source: Data from Table 2 in Zeng et al. 2011
Notes: ND = not detected
* $p < 0.05$ compared with control in the same day
** $p < 0.05$ compared with 0.1 mg/kg group in the same day
*** $p < 0.05$ compared with 0.6 mg/kg group in the same day

Sprague-Dawley rats were administered PFOS in 0.05% Tween (in deionized water) once daily by gavage from GD 1 to GD 21 at 0, 0.1, or 2.0 mg/kg/day. There was a postnatal decline in the serum and brain PFOS levels between PND 0 and PND 21. PFOS concentrations were higher in the serum when compared to the lung in offspring on both PND 0 and 21 (Chen et al. 2012) (see Table 2-13).

Table 2-13. Mean PFOS Content in Serum and Lungs of Rat Offspring (n = 6)

Age	Treatment	PFOS in serum (µg/mL)	PFOS in lung (µg/g)
PND 0	0 mg/kg/day	ND	ND
	0.1 mg/kg/day	1.7 ± 0.35*	0.92 ± 0.04*
	2.0 mg/kg/day	47.52 ± 3.72*	22.4 ± 1.03*
PND 21	0 mg/kg/day	ND	ND
	0.1 mg/kg/day	0.41 ± 0.11*	0.21 ± 0.04*
	2.0 mg/kg/day	4.46 ± 1.82**	3.16 ± 0.11**

Source: Data from Table 2 in Chen et al. 2012
Notes: ND = not detected
* $p < 0.05$ compared with control
** $p < 0.01$ compared with control

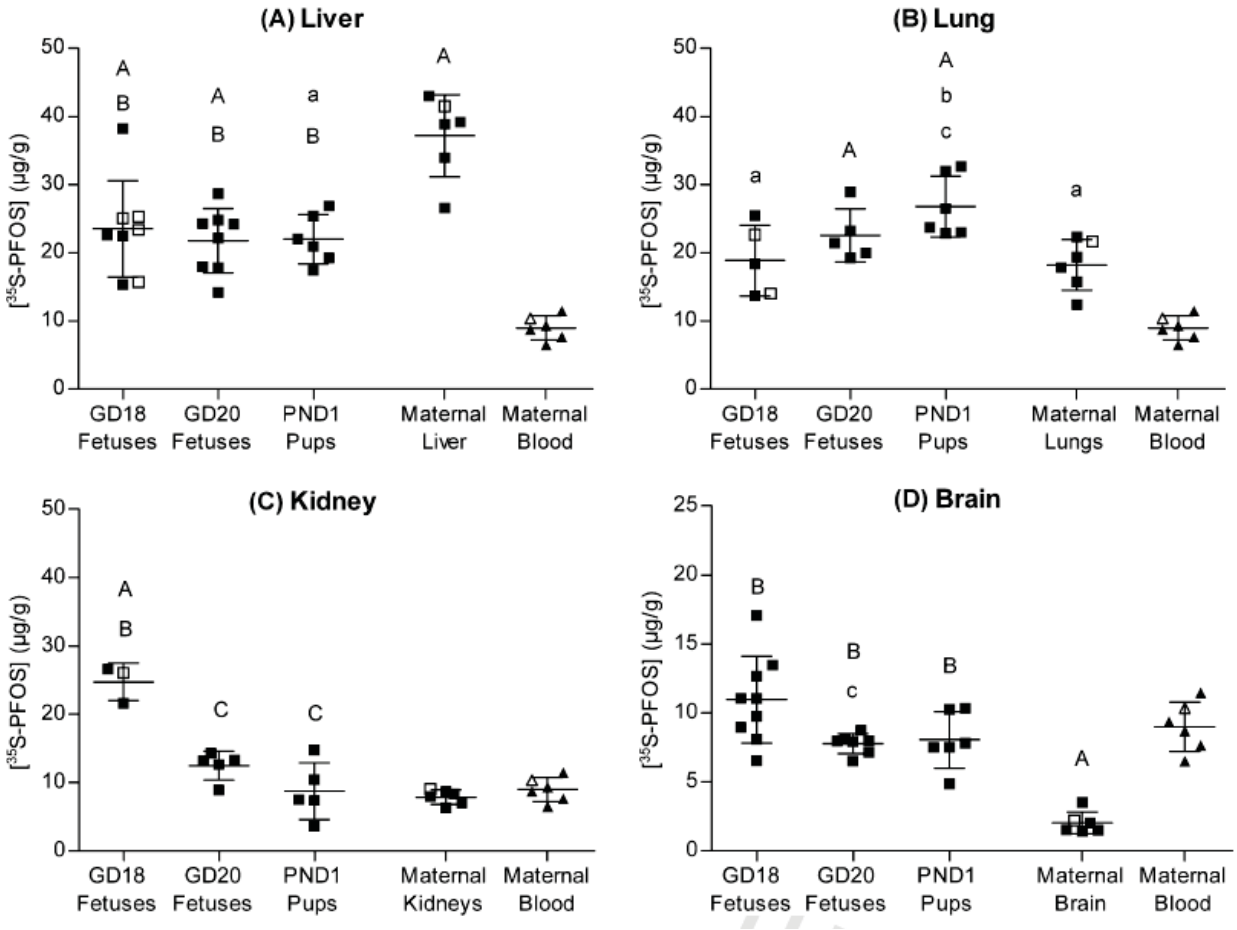
Mouse. Borg et al. (2010) administered a single dose of 12.5 mg/kg ^{35}S-PFOS by intravenous injection (n = 1) or gavage (n = 5) on GD 16 to C57Bl/6 dams. Using whole-body autoradiography and liquid scintillation, counting distribution of PFOS was determined for the dams/fetuses (GD 18 and 20) and the neonates on PND 1. Distribution in the dams was similar regardless of the route of exposure, with the hepatic level being approximately four times greater than the serum. Maternal PFOS levels were highest in the liver and lungs at all timepoints. In dams, the concentration of PFOS in the liver was approximately 4 times and in the lung was approximately 2 times the blood concentrations, respectively. The distribution of PFOS in the kidneys was similar and the amount in the brain was lower than that of the blood. In the fetuses, the highest concentrations of PFOS were found in the kidneys and liver. In the fetuses on GD 18, values in the lungs were similar to the maternal lungs, and this value increased by GD 20. In the kidneys, the highest concentration of PFOS was observed in the fetuses on GD 18 (3 times higher than maternal levels)

In the offspring at all timepoints, PFOS was homogeneously distributed in the liver at a level 2.5 times higher than maternal blood and 1.7 times lower than maternal liver. In pups on PND 1, PFOS was mostly concentrated in the lungs and liver. Pups on PND 1 had PFOS levels that were 3 times higher in the lungs, compared to maternal blood with a heterogeneous distribution. In the kidneys, the levels in pups on PND 1 were similar to their dams despite being higher on GD 18. Levels in the brain were similar at all timepoints in the offspring and higher than in the maternal brain, likely due to an immature brain-blood barrier. Select data are provided in Table 2-14 and Figure 2-1.

Table 2-14. Ratios (Means ± SD) Between the Concentrations of ^{35}S-Labeled PFOS in Various Organs and Blood of Mouse Dams, Fetuses, and Pups versus the Average Concentration in Maternal Blood

	$[^{35}S\text{-}PFOS]_{organ}/[^{35}S\text{-}PFOS]_{maternal\ blood}$				
Subject	Liver (n = 6–8)	Lungs (n = 5–6)	Kidneys (n = 3–6)	Brain (n = 6–9)	Blood (n = 1–6)
Dams	4.2** ± 0.7	2.0* ± 0.4	0.9 ± 0.1	0.2** ± 0.05	1.0
Fetus on GD 18	2.6** ± 0.8	2.1* ± 0.6	2.8** ± 0.3	1.2 ± 0.3	2.3
Fetus on GD 20	2.4** ± 0.5	2.5** ± 0.4	1.4 ± 0.2	0.9 ± 0.1	1.1 ± 0.04
Pups on PND 1	2.4* ± 0.4	3.0** ± 0.5	1.0 ± 0.5	0.9 ± 0.2	1.7** ± 0.3

Source: Data from Table 1 in Borg et al. 2010
Notes: *Statistically-significant ($p \leq 0.01$) in comparison to maternal blood
**Statistically-significant ($p \leq 0.001$) in comparison to maternal blood



Filled symbols are representative after oral exposure; open after intravenous exposure.
A = $p \leq 0.001$ and a = $p \leq 0.01$, compared to maternal blood
B = $p \leq 0.001$ and b = $p \leq 0.05$, compared to maternal tissue
C = $p \leq 0.001$ and c = $p \leq 0.05$, comparing between fetuses/pups on GD 20/PND 1 with corresponding value on GD 18;

Figure 2-1. Distribution of Radiolabeled PFOS in Dams and in Fetuses/Pups in the Liver, Lung, Kidney, and Brain
(Figure from Borg et al. 2010)

2.2.2 Inhalation and Dermal Exposure

No data on distribution following inhalation or dermal exposures were identified.

2.2.3 Other Routes of Exposure

Male and female mice were administered PFOS by subcutaneous injection one time on PNDs 7, 14, 21, 28, or 35 at concentrations of 0 or 50 mg/kg bodyweight (bw) (Liu et al. 2009). Animals were killed 24 hours after treatment and the PFOS concentration levels obtained. The percent distribution found in the blood, brain, and liver are provided in Table 2-15. The distribution shows that beyond PND 14 the levels in the liver are approximately two to four times greater than those found on PND 7.

Table 2-15. Percent Distribution (%) of PFOS in Mice after a 50 mg/kg Subcutaneous Injection

PND	Males			Females		
	Blood	Brain	Liver	Blood	Brain	Liver
7	11.78 ± 2.88	5.04 ± 1.49	14.84 ± 4.01	10.77 ± 1.16	4.17 ± 1.17	16.23 ± 4.84
14	13.78 ± 1.52	1.61 ± 0.80**	26.50 ± 7.36	12.31 ± 2.24	3.26 ± 0.58	26.30 ± 4.54
21	9.85 ± 2.74	2.40 ± 0.60**	51.35 ± 11.06**	12.37 ± 3.80	2.14 ± 0.38**	51.48 ± 3.44**
28	9.89 ± 2.94	0.85 ± 0.19**	63.39 ± 19.78**	12.16 ± 2.32	2.10 ± 0.73**	51.05 ± 10.59**
35	13.33 ± 0.89	1.02 ± 0.28**	73.68 ± 6.86**	11.54 ± 1.28	0.90 ± 0.23**	69.92 ± 18.52**

Source: Data from Table 4 in Liu et al. 2009.
Note: **Statistically significant from PND 7 ($p < 0.01$)

2.3 Metabolism

No studies on the metabolism of PFOS were identified as it does not appear to be further metabolized once absorbed. However, electrostatic interactions with biopolymers are indicated by the Kerstner-Wood et al. (2003) data on binding to plasma proteins, in addition to the Zhang et al. (2009) and Chen and Guo (2009) data from albumin-binding investigations. PFOS binding to other serum and intracellular proteins also occurs.

Weiss et al. (2009) screened the binding of PFOS to the thyroid hormone transport protein transthyretin (TTR) in a radioligand-binding assay to determine if it could compete with thyroxine (T4), the natural ligand of TTR. Human TTR was incubated with ^{125}I-labeled T4, unlabeled T4, and 10–10,000 nanomoles (nmol) competitor (PFOS) overnight. The unlabeled T4 was used as a reference compound, and the levels of T4 in the assay were close to the lower range of total T4 measured in healthy adults. PFOS had a high binding potency to TTR. The 50% inhibition concentration was 940 nmol. The authors concluded that PFOS demonstrates an affinity to TTR and had a greater affinity than the compounds with shorter chain lengths.

Luebker et al. (2002) investigated the possibility that PFOS could interfere with the binding affinity of liver-fatty acid binding protein (L-FABP), an intracellular lipid-carrier protein, with long chain fatty acids (e.g., palmitic and oleic acid). This study was performed *in vitro* with a fluorescent fatty acid analogue 11-(5-dimethylaminoapthalenesulphonyl)-undecanoic acid (DAUDA). The concentration that can inhibit fifty percent of specific DAUDA-L-FABP binding (half-maximal Inhibiting Concentration, or IC_{50}) was determined. PFOS demonstrated inhibition of L-FABP in competitive binding assays; with 10 micromoles (µmols) PFOS added, 69% of specific DAUDA-L-FABP binding was inhibited with the calculated IC_{50} of 4.9 µmol.

L. Zhang et al. (2013) cloned the human L-FABP gene and used it to produce purified protein for evaluation of the binding of PFOA and other PFASs. Nitrobenzoxadizole-labeled lauric acid was the fluorescent substrate used in the displacement assays. IC_{50} values and dissociation constants were generated for the PFAS studied. Oleic and palmitic acids served as the normal substrates for L-FABP binding. The nitrobenzoxadiazole labeled lauric acids indicated that there were two distinct binding sites for fatty acids in human FABP, with the primary site having a 20-fold higher affinity than the secondary site. The IC_{50} value for PFOS was 3.3 ±1 µmol, suggesting that it has a higher binding affinity than PFOA.

2.4 Excretion

2.4.1 Oral Exposure

Humans. Urinary excretion of PFOS in humans is impacted by the isomeric composition of the mixture present in blood and the gender/age of the individuals. The half-lives of the branched chain PFOS isomers are shorter than those for the linear molecule, an indication that renal resorption is less likely with the branched chains.

Y. Zhang et al. (2013) determined half-lives for PFOA isomers based on paired serum samples and early morning urine samples collected from healthy volunteers in two large Chinese cities. Half-lives were determined using a one compartment model and an assumption of first order clearance. The volume of distribution (V_d) applied in the analysis as determined by Thompson et al. (2010) was 170 mL/kg. Clearance was estimated from the concentration in urine normalized for creatinine and assuming excretion of 1.2 and 1.4 L/day of urine and 0.9 and 1.1 mg creatinine/day for males and females, respectively. The mean half-life for the sum of all PFOS isomers in younger females (n = 20) was 6.2 years (range: 5.0–10 years), while that for all males and older females (n = 66) was 27 (range: 14–90 years); the medians were 6.0 and 18 years, respectively.

The mean half-life values for the six branched chain isomers of PFOS were lower than the value for the linear chain with the exception of the 1-methyl heptane sulfonate, suggesting that resorption transporters may favor uptake of the linear chain and 1-methyl branched chain over the other isomers. Older females and males have longer half-lives than young females, suggesting the importance of monthly menstruation as a pathway for excretion (Y. Zhang et al. 2013). The mean half-life for the 1-methylheptane sulfonate in the males and older females (n = 43) was considerably greater than that for the sum of all isomers (90 years versus 27 years). For males and older females there were considerable inter-individuals differences, with 100-fold differences between the minimum and maximum values among the males and older females compared to < 10-fold differences for the younger females.

T. Zhang et al. (2014) derived estimates for PFOA's urinary excretion rate using paired urine and blood samples from 54 adults (29 male, 25 female) in the general population and 27 pregnant females in Tainjin, China The age range for the general population was 22–62 and that for the pregnant females was 21–39. Urinary excretion was calculated based on the concentration in the urine times volume of urine, wherein a urinary volume of 1200 mL/day was applied to all females and 1600 mL/day applied to all males. Urine samples were first draw morning samples. Total daily intakes for PFOS were calculated from the concentration in blood using first order assumptions, a half-life of 5.4 years (Olsen et al. 2007) and a volume of distribution of 170 mL/kg (Thompson et al. 2010; Egeghy and Lorber 2011). Urinary elimination rate was then calculated from the urine levels and the modeled total daily intake. Total daily intake, and thus the urinary elimination rate, was not calculated for pregnant females due to the highly variable blood levels of PFOS during pregnancy. PFOS was detected in the blood samples for all participants but only for 48% of the urine samples from the general population and 11% of samples from the pregnant females. Unfortunately the urinary PFOS was below detection for most of the females in the study.

The calculated geometric mean total daily intake for PFOS was 89.2 ng/day for the adult general population, resulting in a daily urinary excretion rate of 16% of the estimated intake; there was no significant difference between males and females. From the limited number of urine

samples available, the urine:blood ratio was lower for pregnant females than nonpregnant females (0.0004 versus 0.0013) suggesting other removal pathways such as placenta and cord blood. There was a difference between the younger menstruating females (21–50 years versus 51–61 years), with a higher ratio for the younger females (0.0018 versus 0.0006). There is no indication that data were collected from the participants relative to menstruation status on the day of blood and urine collection. There was a significant difference between PFOS urinary excretion in older adults compared to younger adults ($p = 0.015$), with a higher elimination rate in the younger adults compared to the older age group.

Wong et al. (2014) looked at the role of menstrual blood as an excretory pathway to explain the shorter half-life of PFOS in females than males. They fit a population-based pharmacokinetic model to six cross-sectional National Health and Nutrition Examination Survey (NHANES) data sets (1999–2012) for males and females. They concluded that menstruation could account for about 30% of the elimination half-life difference between females and males. Verner and Longnecker (2015) suggested a need to consider the nonblood portion of the menstrual fluid and its albumin content in the Wong et al. (2014) estimate for the menstrual fluid volume. A yearly estimate for serum loss of 868 mL/year by Verner and Longnecker (2015) compared to the 432 mL/year estimate of Wong et al. (2014) suggests that the menstrual fluid loss can account for > 30% of the difference in the elimination half-life between females and males.

Harada et al. (2007) obtained serum and bile samples from patients (2 male and 2 female; aged 63–76) undergoing gallstone surgery to determine the bile to serum ratio and biliary resorption rate. The median concentration for PFOS in the serum was 23.2 ng/mL (0.023 ppm), compared to the bile, 27.9 ng/mL (0.028 ppm). The fact that the levels in bile concentrations are higher than in serum is supportive of bile as a route of excretion. The biliary resorption rate was 0.97, which could contribute to the long half-life in humans. Method of exposure to PFOS was unknown.

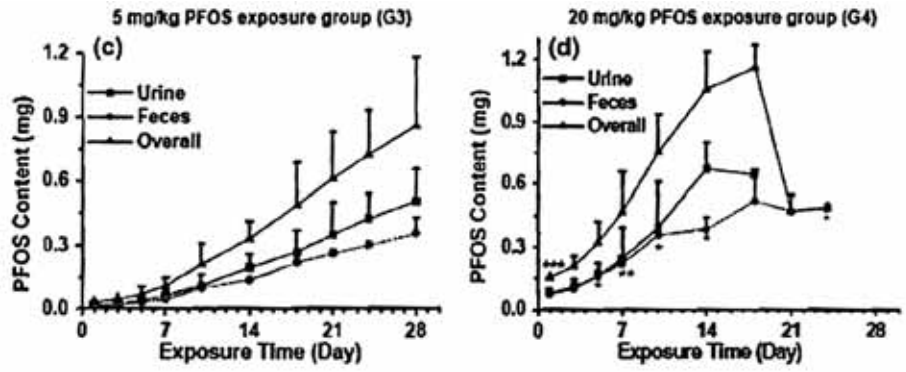
Biliary excretion in humans and the potential for resorption from bile discharged to the gastrointestinal (GI) tract is supported by the Genuis et al. (2010) self-study of the potential for cholestyramine to lower the levels of PFAS in blood. Ingestion of 4 g/day cholestyramine (a bile acid sequestrant) in three doses for 20 weeks decreased the PFOS serum levels from 23 ng/g serum to 14.4 ng/g serum.

Animals. In a study by Chang et al. (2012), three Sprague-Dawley rats/sex/timepoint were administered ^{14}C-PFOS as the potassium salt, one time by oral gavage at a dose of 4.2 mg/kg. Urine and feces were collected after 24 and 48 hours. The amounts recovered in urine and feces were approximately equivalent at each time point: 1.57% and 1.55%, respectively, at 24 hours and 2.52% and 3.24%, respectively, at 48 hours.

Ten male Sprague-Dawley rats (~ 9 weeks old)/group were administered 0, 5, or 20 mg/kg/day of either PFOA or PFOS by gavage once daily, 7 days a week for 4 weeks (Cui et al. 2010). The dose groups were identified as the following: Group (G) 0 = ultrapure water; G1 = 5 mg/kg/day PFOA; G2 = 20 mg/kg/day PFOA; G3 = 5 mg/kg/day PFOS; and G4 = 20 mg/kg/day PFOS. Urine and fecal samples were obtained after the daily gavage by placing the rats in metabolism cages for 24 hour intervals on the following days: prior to treatment (day 0), day 1, and days 3, 5, 7, 10, 14, 18, 21, 24, and 28. Urine was collected three times daily, and the volume of the urine sample and weight of the fecal sample were recorded. Samples were stored at −40 degrees Celsius (°C) prior to analyzing. Target analytes were

determined by using a high-performance liquid chromatography-electrospray tandem mass spectrometry system with separation of PFOS and PFOA achieved by the analytical column.

An upward trend of increased excretion was observed in the rats administered 5 mg/kg/day PFOS during the study and a similar trend was observed in the rats administered 20 mg/kg/day PFOS. However, in the third week, mortalities occurred. By study day 24, there were only 2 out of 10 rats in the 20 mg/kg/day group surviving. The range of PFOS excreted in urine by rats treated with 20 mg/kg/day was 0.080 mg on day 1 to 0.673 mg on day 14. In the feces, the lowest amount of PFOS was at 5 mg/kg/day on day 1 (0.0015 mg) and the highest on day 28 (0.355 mg). A similar trend in feces was observed in the rats treated with 20 mg/kg/day until the deaths occurred; however, the fecal excretion reached a steady state after a maximum on day 18 (0.519 mg). This steady state could have been the result of lower feces volume because the rats had decreased food intake as well. The mean fecal excretion rates of PFOS between the two dose groups was comparable as 1.2% and 1.3% of the oral doses were eliminated by fecal excretion in the 5 mg/kg/day and 20 mg/kg/day groups on day 1, respectively, indicating a majority of the dose was absorbed. Overall, more PFOS was eliminated in the urine rather than the feces, but there was not a notable difference in total excretion between the two PFOS dose groups. When the average elimination rates (urinary, fecal, and overall) of PFOA versus PFOS were compared, the amount of PFOA being eliminated was higher than PFOS, especially on the first day. The elimination rates on the first day were 2.6% and 2.8% in rats at 5 mg PFOS/kg/day and 20 mg PFOS/kg/day, respectively (see Figure 2.2).



Notes: No urine was available after day 18 in the 20 mg/kg/day group due to high mortality in this group.
*Statistically-significant at $p < 0.05$
**Statistically-significant at $p < 0.01$

Figure 2-2. PFOS Contents in Urine, Feces, and Overall Excretion in Male Rats Treated for 28 Days

Five groups of 16 female Crl:CD(SD)IGS VAF/Plus rats each were administered 0, 0.1, 0.4, 1.6, or 3.2 mg PFOS/kg bw/day by oral gavage beginning 42 days prior to cohabitation and continuing through GD 14 or 20 (Luebker et al. 2005b). Urine and feces were collected overnight from dams on the eve of cohabitation day 1 and during GDs 6–7, 14–15, and 20–21. The concentrations in the feces were consistently about 5 times greater than in the urine (see Table 2-10).

2.4.2 Inhalation Exposure

In a case report, a 51-year old asymptomatic male researcher lived in a home with carpet flooring that had been treated intermittently with soil/dirt repellants. The carpeting also had an

in-floor heating system under the carpets (Genuis et al. 2010). Because of his work, the man knew that he had an unusually high amount of PFASs in his serum, primarily perfluorohexanesulfonic acid (PFHxS), PFOS, and PFOA. The level of PFOS in his serum was 26 ng/g, the level in his urine was < 0.50 ng/mL, and it was < 0.50 ng/g in sweat and stool samples. The man began treatment with two bile acid sequestrants, cholestyramine (CSM) and saponin compounds (SPCs) to see if they would lower the serum PFAS levels. Stool samples were evaluated for PFOS levels after administration of each compound. The concentration of PFOS was increased after CSM treatment, suggesting that it may help with removing PFOS that gains access to the GI tract with bile. The first stool sample after approximately 20 weeks of CSM treatment showed PFOS levels of 9.06 ng/g and the second, 7.94 ng/g. The treatment with SPCs did not increase the PFOS found in the stool. Serum levels of PFOS decreased to 15.6 ng/g after 12 weeks of treatment with CSM and to 14.4 ng/g after 20 weeks of treatment even though the man's exposure at his home had not changed.

2.5 Pharmacokinetic Considerations

2.5.1 Pharmacokinetic models

Toxicokinetic models that can accommodate half-life values that are longer than would be predicted based on standard absorption, distribution, metabolism, and excretion concepts have been published as tools to estimate internal doses for humans, monkeys, and rats. The underlying assumption for all of the models is saturable resorption from the kidney filtrate, which consistently returns a portion of the excreted dose to the systemic circulation and prolongs both clearance from the body (e.g., extends half-life) and the time needed to reach steady state.

One of the earliest physiologically-based pharmacokinetic (PBPK) models (Andersen et al. 2006) was developed for PFOS using two dosing situations in cynomolgus monkeys. In the first, three male and three female monkeys received a single intravenous dose of potassium PFOS at 2 mg/kg (Noker and Gorman 2003). For oral dosing, groups of four to six male and female monkeys were administered daily oral doses of 0, 0.03, 0.15, or 0.75 mg/kg PFOS for 26 weeks (Seacat et al. 2002).

This model was based on the hypothesis that saturable resorption capacity in the kidney would account for the unique half-life properties of PFOS across species. The model structure (Figure 2-3; Andersen et al. 2006) was derived from a published model for glucose resorption from the glomerular filtrate via transporters on the apical surface of renal tubule epithelial cells.

The model was parameterized using the body weight and urine output for cynomolgus monkeys (Butenhoff et al. 2002, 2004) and a cardiac output of 15 liters (L)/hour (h)-kg from the literature (Corley et al. 1990). Other parameters were assumed or optimized to fit the best for monkeys. In the intravenous time course data, some time and/or dose-dependent changes occurred in distribution of PFOS between the blood and tissue compartments, and these changes were less noticeable in the females, therefore, only the female data were used. The simulation captured the overall time course scenario but did not provide good correspondence with the initial rapid loss from plasma and the apparent rise in plasma concentrations over the first 20 days. For the oral dosing, the 0.15 mg/kg dose simulation was uniformly lower, and the 0.75 mg/kg dose simulation was higher than the data. When compared to PFOA, PFOS had a longer terminal half-life and more rapid approach to steady-state with repeated oral administration.

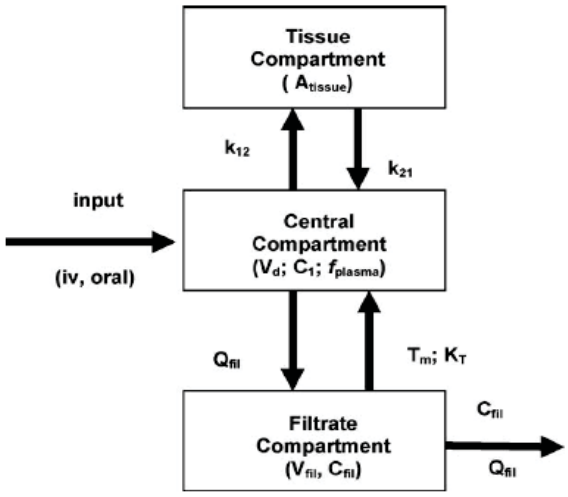
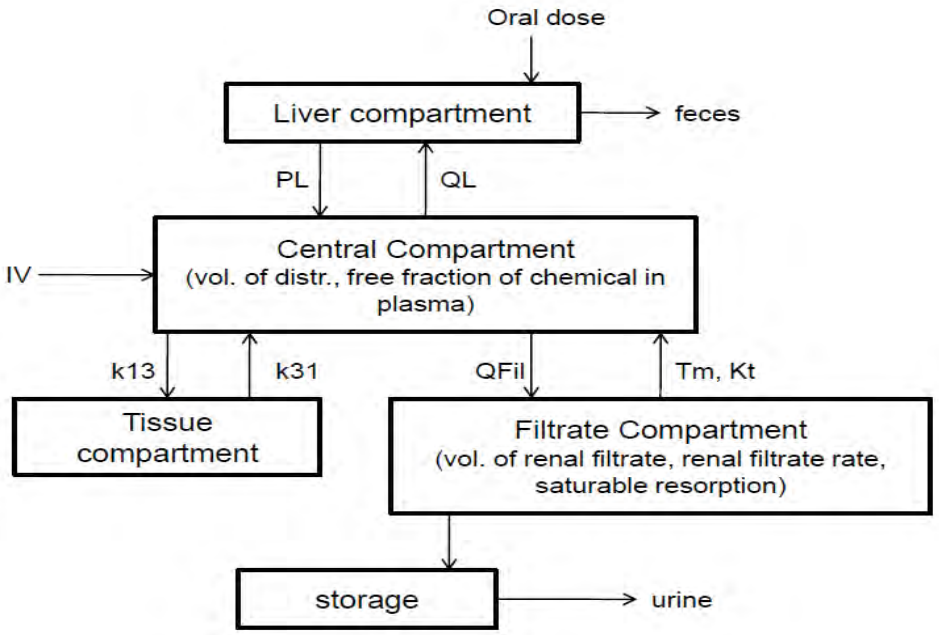


Figure 2-3. Schematic for a Physiologically-Motivated Renal Resorption Pharmacokinetic Model

Tan et al. (2008) developed a physiologically-based pharmacokinetic model by modifying the model by Andersen et al. (2006). The new model included time-dependent descriptions and a liver compartment for rats and monkeys to simulate the data on plasma and urine concentrations of PFOS in male and female cynomolgus monkeys after a single intravenous (IV) injection of 2 mg PFOS/kg bw (Noker and Gorman, 2003), and to simulate the time course data on plasma concentrations of PFOS in rats after single oral dosing (see Figure 2-4 below). Only one time-dependent function (protein binding) was needed to fit the plasma data from male monkeys exposed to PFOS, while two functions (protein binding and volume of distribution) were needed to fit the male rat data. The PFOS retention in the liver appeared to occur only in male rats but not in male monkeys because of the higher liver:blood partition coefficient and additional binding in the rat liver. The liver:blood partition coefficient was 1 in the monkey and 6.51 in the rat. Comparing the renal resorption parameters, the transport maximum (T_m) was about 1,500 times higher in the monkey than the rat. Comparing PFOA and PFOS, the model suggested that PFOS was retained in the tissues longer by the higher liver:blood partition coefficient and renal filtration. The author stated that development of a human model was feasible.

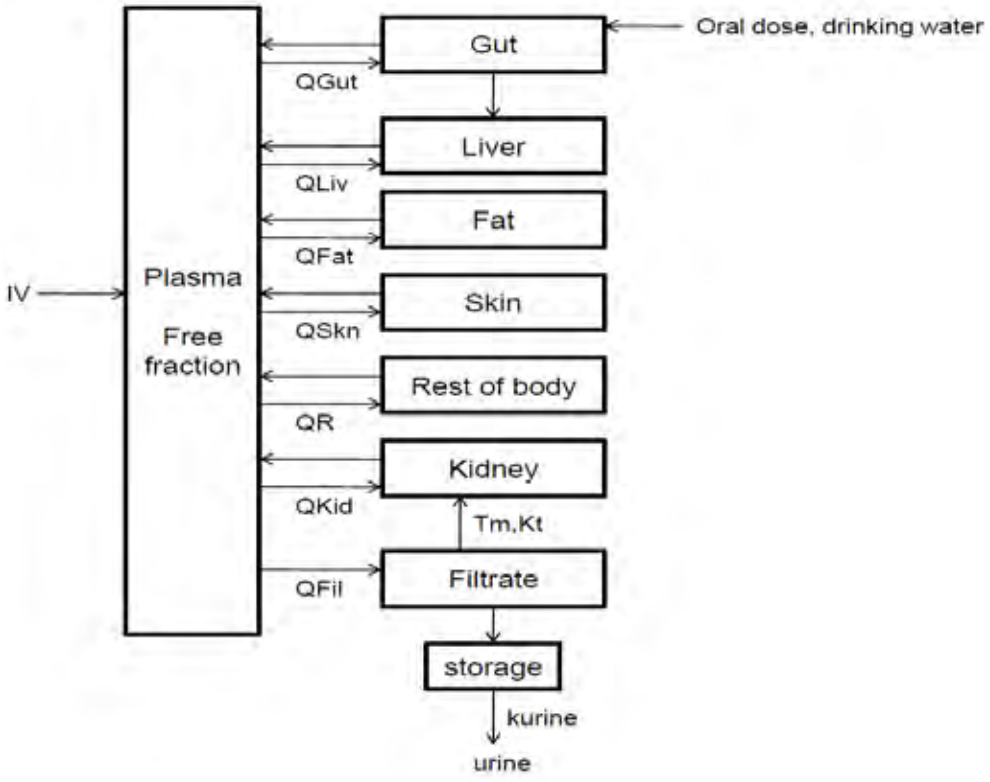
Loccisano et al. (2011) developed a PFOS PBPK model for monkeys based on the Andersen et al. (2006) and Tan et al. (2008) models, and they extrapolated it for use in humans (Figure 2-5). The model reflects saturable renal absorption of urinary PFOS by the proximal tubule of the kidney. This is represented in Figure 2-5 by the interactions between the plasma and kidney, plus the interaction of the filtrate compartment with both plasma and kidney. A second route for PFOS resorption is represented by the gut plasma interaction that allows for resorption of PFOS from bile secreted into the gastrointestinal tract.

The fraction of PFOS free in plasma and available for glomerular filtration was based on data fit and was considered to decrease over time. Lacking primary data on transporter resorption kinetics, the rate was based on the best fit to the plasma/urine data. Binding to serum albumin allowed for less than a tenth of the plasma concentration to be available for glomerular filtration. A storage compartment was added to the model between the filtrate compartment and urine because PFOS appears in the urine at a slower rate than it disappears from the plasma.



Notes: T_m = transport maximum, K_t = affinity constant, and Q = flow in and out of tissues

Figure 2-4. Structure of Model for PFOS in Rats and Monkeys



Notes: T_m = transport maximum, K_t = affinity constant, and Q = flow in and out of tissues

Figure 2-5. Structure of the PFOS PBPK Model in Monkeys and Humans

Existing data sets for the cynomolgus monkey were used to develop the monkey model. The IV data came from monkeys administered a single dose of 2 mg/kg, and the concentrations in plasma and urine were monitored for up to 161 days after dosing (Noker and Gorman 2003). The repeat-dose oral data were from Seacat et al. (2002) with exposures to 0, 0.03, 0.15, or 0.75 mg/kg by capsule for 26 weeks with follow-up monitoring of plasma levels in two monkeys per group at the two highest doses for a year after the cessation of dosing. Both data sets show that the plasma and liver are the primary target tissues for PFOS. The model projections for the repeat dose oral study were in good agreement with the Seacat et al. (2002) data for the 0.15 mg/kg dose, but overestimated the plasma values for the 0.75 mg/kg/day dose. The model projected a sharper rise in plasma levels with achievement of steady state more rapidly than indicated by the experimental results.

Human data for PFOS are limited, although serum concentrations were collected from retired workers (Olsen et al. 2007) and from residents (n = 25) in Little Hocking, Ohio. The structure of the human model was similar to that used for the monkeys (Loccisano et al. 2011). The fact that the serum data applied to measurements made following uncertain exposure routes and uncertain exposure durations presented a challenge in the assessment of model fit. The human half-life used for the model (5.4 years) came from an occupational study (Olsen et al. 2007, see section 2.5.2). No measures of PFOS concentration were available for the drinking water at Little Hocking, so the authors estimated the value that could account for the average population serum concentration. The value for the drinking water was estimated to be 0.34 parts per billion (ppb). The model results can be characterized as good when compared to the reported average serum measurements. The average daily exposure, consistent with the serum value, was estimated as 0.003 µg/kg/day during the period from 1999 to 2000, and about 0.002 µg/kg/day for the 2003 to 2009 time period. The authors concluded that in order to refine the human model more data are needed on the kinetics of renal transporters and intrahuman variability, as well as definitive information on exposures.

Additional projections of human exposures consistent with measured average serum levels from selected human populations have also been published (Egeghy and Lorber 2011; Thompson et al. 2010). Both papers used a first-order, one-compartment model to assess PFOS exposure from both an intake and body burden perspective using the following equations to determine clearance (CL) with information on V_d and chemical half-life ($t_{½}$).

$$CL = V_d \times (\ln 2 \div t_{½})$$

$$\text{Human dose} = \text{average serum concentration} \times CL$$

Egeghy and Lorber (2011) estimated PFOS exposures from both intake and serum measurements for both typical and contaminated scenarios for adults and children, using available data from peer-reviewed publications. A range of intakes was estimated from the PFOS serum concentrations reported by NHANES, as well as published concentrations in various media including dust, air, water, and foods. In the absence of human data, high and low bounding estimates of 3 L/kg and 0.2 L/kg were used for volume of distribution. Total PFOS intakes over all pathways were estimated to be 160 and 2,200 ng/day for adults and 50 and 640 ng/day for children in typical and contaminated scenarios, respectively, with food ingestion being the main exposure source in adults and food and dust ingestion being the two main sources in children. Based on the model predictions, the range of intake of PFOS consistent with the serum levels was 1.6 to 24.2 ng/kg-bw/day for adults, assuming a 70 kg body weight.

Thompson et al. (2010) predicted PFOS concentration in blood serum as a function of dose, elimination rate, and volume of distribution. The volume of distribution in this study, 0.23 L/kg bw, was adjusted by 35% from the calibrated data for PFOA in accordance with the differences in PFOA and PFOS volumes of distribution calculated by Andersen et al. (2006). The volume of distribution from PFOA was obtained by calibrating human serum and exposure data collected from two communities in the Little Hocking, Ohio area (see section 2.5.3). Applying the volume of distribution and elimination rate values for PFOS calculated from the Little Hocking population to serum data collected from members of the Australian population, the predicted intake by the Australian population was calculated to be 1.7 to 3.6 ng/kg bw/day.

Fàbrega et al. (2014) adapted the Loccisano et al. (2011) model to include compartments for the brain and lung and remove the skin. They applied the adjusted model to humans by using intake and body burden data from residents in Tarragona County, Spain. Food and drinking water were the major vehicles of exposure. Body burden information came from blood samples from 48 residents, and tissue burdens came from 99 samples of autopsy tissues. The adjusted model over-predicted serum levels by a factor of about two for PFOS but under-predicted the levels in both liver (slightly) and kidney (by a factor of about 4).

The authors also looked at the value of using partition coefficients from human tissues in place of the Loccisano et al. (2011) rat data. The PFOS simulation values were closer to the human experimental data when using the human partition coefficients values for liver, brain, and kidney but not for the lung PFOS results. However, the Loccisano et al. (2011) model demonstrated better performance overall. The authors suggested that both saturable resorption and variations in protein binding are important parameters for pharmacokinetic models. With the exception of serum albumin, the existing models have not considered protein binding constants within tissues. Even though the use of human partition coefficients improved the steady state predictions overall for tissues there were still considerable differences between the experimental values and the predictions for both models.

Loccisano et al. (2012a) utilized the saturable resorption hypothesis and pharmacokinetic data from Chang et al. (2012), 3M Environmental Laboratory (2009), and Seacat et al. (2003) for adult Sprague-Dawley rats to develop the model depicted in Figure 2-6. The structure of the model is similar to that for the monkey/human model depicted in Figure 2-5 but lacks the fat and skin compartments and includes a storage compartment to accommodate fecal loss of unabsorbed dietary PFOS as well as that from biliary secretions. Partition coefficients for liver:plasma, kidney:plasma, and rest of the body:plasma were derived from unpublished data on mice by DePierre (2009) through personal communication to authors (Loccisano et al. 2012a); most of the other kinetic parameters were based on values providing the best fit to the experimental data. The free fraction in plasma was allowed to decrease with time suggesting a strong binding to serum proteins.

The agreement between the experimental data and the model output was good but requires additional data from experimental studies on plasma binding and renal tubular transporters to support further refinement of the parameters derived from model fit. In general, liver and plasma concentrations after daily dosing were overestimated by a factor of about two. Male and female rats did not differ significantly in their ability to move PFOS from tissues to urine or in resorption capability. PFOS appeared to have a greater capacity to bind to sites in the liver than PFOA.

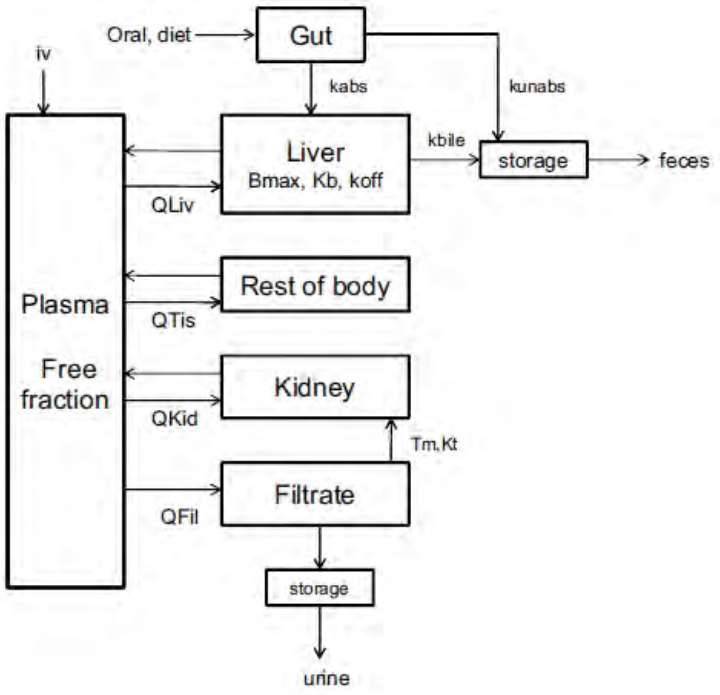


Figure 2-6. Structure of the PBPK Model for PFOS in the Adult Sprague-Dawley Rat

Loccisano et al. (2012b) expanded their adult Sprague-Dawley rat model described above to cover gestational and lactational exposure to the fetus and pups. The data from Thibodeaux et al. (2003) and Chang et al. (2012) for GDs 0 to 20 were used in model development. Both studies used multiple dose levels in addition to data on serum and selected tissue concentrations (liver, brain) from the dams and fetuses at one or more time points. The gestational model structure for the dams is similar to Figure 2-6. The model was expanded to include the fetuses linked to the dams by way of the placenta. Uptake from the placenta was described by simple diffusion; the fetal plasma compartment was separate from the dams as was distribution to fetal tissues and amniotic fluid. The model allowed for saturable binding of PFOS within the liver and to serum proteins. Model performance was judged by its ability to predict 24-hour area under the curve (AUC) for plasma, liver, and brain for both the fetus and dam. Brain data were only available from the Chang et al. (2012) study.

According to the model, liver concentrations for the dam are six to seven times greater than those for the fetus, and the brain levels for the fetus about eight times greater than those for the dam. Model performance in comparison to the experimental data was judged to be good. The model was used to project the maternal and fetal plasma levels expected at the doses employed in the Butenhoff et al. (2009), Luebker et al. (2005a, 2005b), Yu et al. (2009a), and Lau et al. (2003) studies as depicted in Figure 2-7.

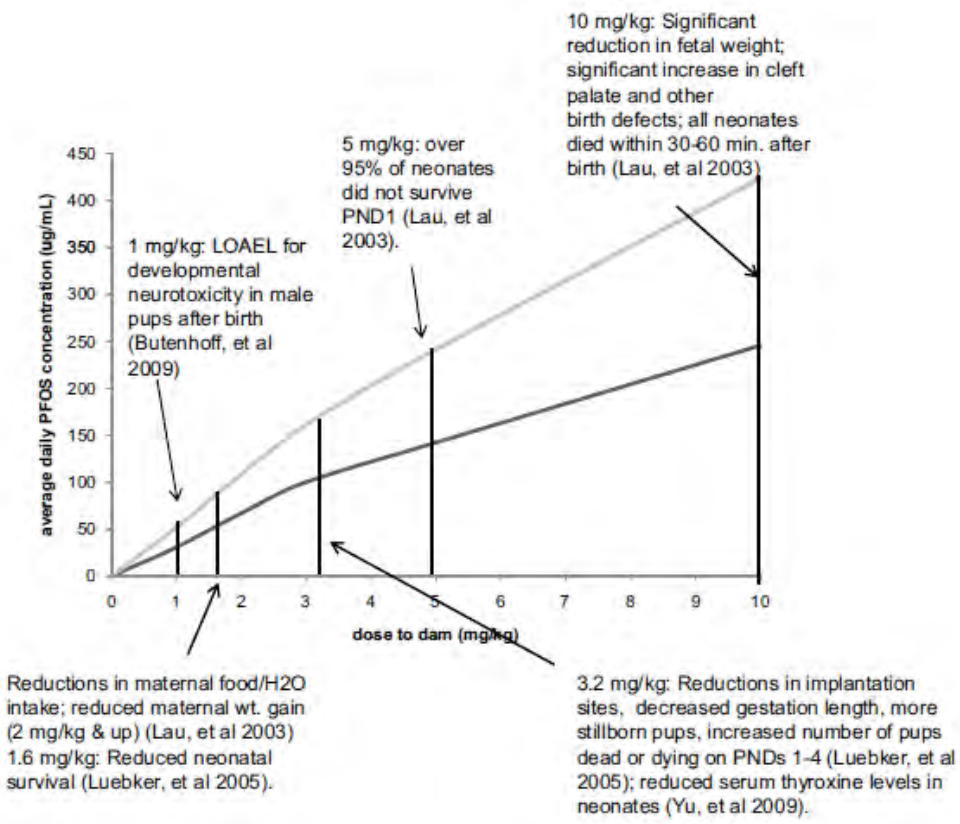


Figure 2-7. Predicted Daily Average Concentration of PFOS in Maternal (Black Line) and Fetal (Gray Line) Plasma at External Doses to the Dam

The lactational component of the Loccisano et al. (2012b) model allowed for PFOS transport to neonates via mammary-tissue secretion and consequent ingestion by the pups. Pup tissues included in the lactational model included the gut, liver, kidney, and the remainder of the body. A renal filtrate compartment linked to plasma and the kidney allowed for neonate PFOS resorption. PFOS transfer to milk via the mammary gland was assumed to be controlled by simple diffusion. Pup urine returned PFOS from the kidney filtrate to the dam.

Loccisano et al. (2013) extended their model development to cover humans during pregnancy and lactation, building on the work done with rodents and recognizing the limitations of the human data available for evaluating the model predictions. Figure 2-8 illustrates the structure of the model used. The basic structure was derived from the rat model discussed above. Some of the key features of the model are summarized below:

- The fetus is exposed via the placenta through simple bidirectional diffusion.
- Transfer rates to the fetus from the amniotic fluid are governed by bidirectional diffusion.
- Transfer from the fetal plasma to tissues is flow-limited.
- Maternal plasma is directly linked to the milk compartment and considered to be flow limited; only the free fraction in plasma is transferred to maternal milk.
- The neonate is exposed to PFOS only via maternal milk for the first 6 months postpartum.
- The infant in the model is treated as one compartment with a volume of distribution.

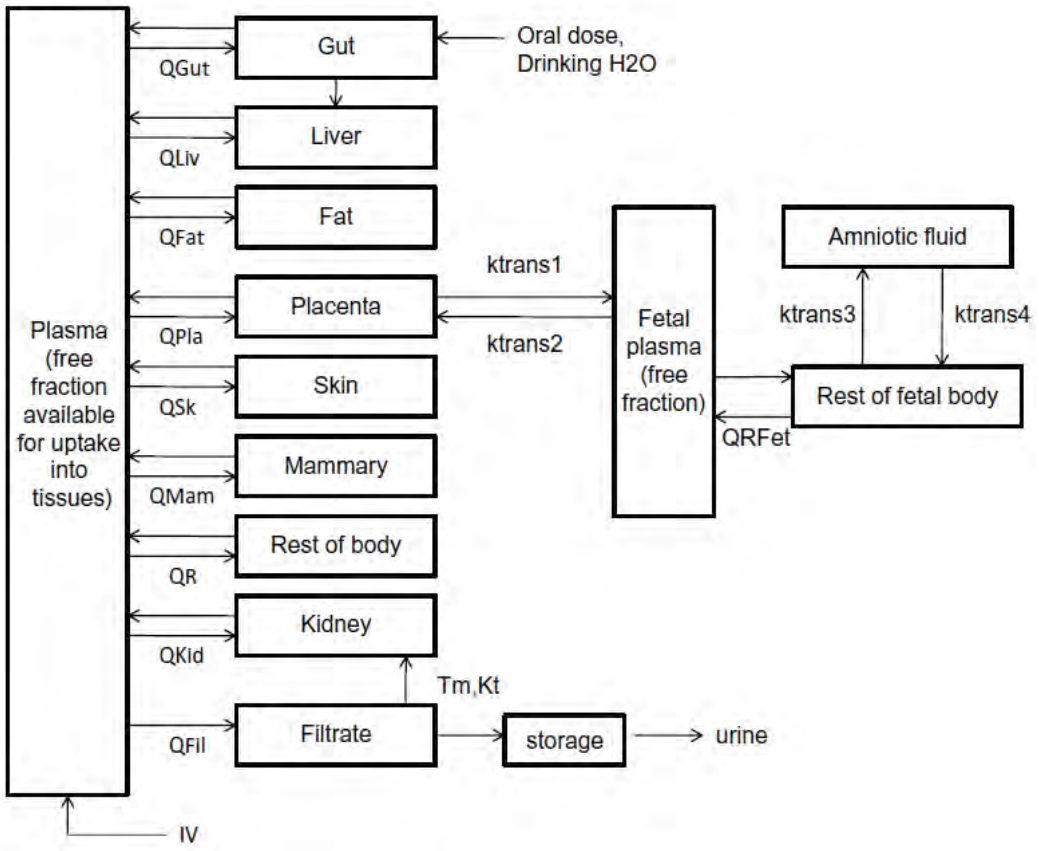


Figure 2-8. PBPK Model Structure for Simulating PFOA and PFOS Exposure During Pregnancy in Humans (Maternal, Left; Fetal, Right)

Limitations to the model are acknowledged and attributed primarily to lack of data to support a more mechanistic approach. Physiological parameters applicable to a pregnant or lactating woman, the fetus, and the nursing infant were obtained from a variety of referenced publications.

In order to obtain a plasma value at the time of conception, the model was run until it reached a prepregnancy steady state concentration. The model predicted 30 years as the exposure necessary to reach steady state (1×10^{-4} to 2×10^{-3} µg/kg bw/day) for the general female population. The model performance simulations for PFOS were run using an exposure of 1.35×10^{-3} µg/kg bw/day. Projections were developed for maternal plasma, fetal plasma, infant plasma, and maternal milk. Agreement between the observed concentrations (µg/L) and the predicted values was considered satisfactory if the predicted value was within 1% of the observed value. Model output was compared to maternal and fetal plasma values at delivery or at specific time points, and for the infant plasma and milk data where available. Predicted maternal:fetal plasma (cord blood) concentration ratios were more variable for PFOS than PFOA in the comparisons to the published data. The projections for fetal internal dose were reasonable, and there was good agreement between the model and the available human lactation data. When modeled, the maternal plasma was 14 µg/L at conception, slowly decreased across the gestation period, and increased slightly at delivery. For the most part the modeled results fell within ± 1 SD of the observed data.

During lactation there was a gradual, very-slight, decline in maternal plasma across the six months of lactation. Thereafter, plasma values slowly increased and stabilized at about 12.5 μg/L at six months postpartum. The fetal plasma was about 6.5 μg/L at the start of gestation, and declined to about 5.5 μg/L at the time of delivery. Maternal plasma values are about twice those for the fetus. During the lactation period, the infant plasma increased in a linear fashion to a terminal value of about 13 μg/L. Milk concentrations declined very slightly across the lactation period with an initial concentration of 0.16 μg/L and a final value of 0.15 μg/L. These concentrations were estimated from the graphic data presentation.

The projections for PFOS differed from those for PFOA in several respects. Most importantly maternal and fetal plasma values were similar for PFOA but for PFOS, maternal levels were approximately two-fold greater than fetal levels. Compared with PFOS, there was a much greater decline in maternal PFOA plasma values during lactation accompanied by a comparable decline in the PFOA concentration in milk. The increase in infant plasma across the lactation period was comparable for PFOA and PFOS with the concentration at 6 months postpartum about 2.5 times higher than that at 1 month.

The authors compared the human pregnancy lactation model results to published data, and they identified several important research needs as follows:

- Are there differences in the transporter preferences and transfer rates for the individuals PFASs? Do those differences correlate with half-life differences?
- Are there qualitative or quantitative differences between the transporters favored by PFOS compared to PFOA?
- What physiological factors influence clearance for the mother, the fetus, and the infant during gestation and lactation?
- Are placental transport processes active, facilitated, or passive?

These research needs are more pronounced for PFOS than PFOA, because the information supporting renal resorption and tissue uptake via membrane transporters for PFOS is very limited. Most models infer that PFOS and PFOA are similar based on their half-lives rather than on published research on transporters.

The authors acknowledged the lack of primary experimental data on PFOS transport and potential transporters. Similarity to PFOA was assumed in model development, and PFOS was transparently described as lacking supporting transporter data. The authors concluded that additional research on PFOS binding to serum proteins and liver tissues, its biliary excretion and resorption, and information on renal resorption transporters in dams and pups are needed to accomplish further refinements to the published model (Loccisano et al. 2012b, 2013).

Building on the work of other researchers, Wambaugh et al. (2013) developed and published a pharmacokinetic model to support the development of an EPA reference dose for PFOS. The model was applied to data from studies conducted in monkeys, rats, or mice that demonstrated an assortment of systemic, developmental, reproductive, and immunological effects. A saturable renal resorption pharmacokinetic (PK) model was again used. This concept has played a fundamental role in the design of all of the published PFOS models summarized in this section. In this case, an oral dosing version of the original model introduced by Andersen et al. (2006) and summarized early in section 2.5.3 was selected for having the fewest number of parameters that would need to be estimated. A unique feature of the Wambaugh et al. (2013) approach was to use a single model for all species in the toxicological studies in order to examine the

consistency in the average serum values associated with effects and with no effects from 13 animal studies of PFOS. The model structure is that depicted in Figure 2-3 with minor modifications.

Wambaugh et al. (2013) placed bounds on the estimated values for some parameters of the Andersen et al. (2006) model to support the assumption that serum carries a significant portion of the total PFOS body load. The Andersen et al. (2006) model is a modified *two-compartment model* in which a primary compartment describes the serum and a secondary deep tissue compartment acts as a specified tissue reservoir. Wambaugh et al. (2013) constrained the total volume of distribution to a value of not > 100 times that in the serum. As a result, the ratio of the two volumes (serum versus total) was estimated in place of establishing a rate of transfer from the tissue to serum.

A nonhierarchical model for parameter values was assumed. Under this assumption a single numeric value represents all individuals of the same species, gender, and strain. The gender assumption was applied to the monkeys and mice, while male and female rats were treated separately because of the established gender toxicokinetic differences. Body weight, the number of doses, and magnitude of the doses were the only parameters to vary. In place of external doses, serum concentrations as measured at the time of euthanasia were used as the metric for dose magnitude. Measurement errors were assumed to be log-normally distributed. Table 2-16 provides the estimated and assumed PK parameters applied in the Wambaugh et al. (2013) model for each of the species evaluated.

The PK data that supported the analysis were derived from two PFOS PK *in vivo* studies. The monkey PK data were derived from Seacat et al. (2002) and Chang et al. (2012). Data for the rats (male/females) and mice were both from Chang et al. (2012). The data were analyzed within a Bayesian framework using a Markov Chain Monte Carlo sampler implemented as an R package developed by EPA to allow predictions across species, strains, and genders and identify serum levels associated with the no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) external doses. The model chose vague, bounded prior distributions on the parameters being estimated allowing them to be significantly informed by the data. The values were assumed to be log normally distributed constraining each parameter to a positive value.

The model predictions were evaluated by comparing each predicted final serum concentration to the serum value in the supporting animal studies. The predictions were generally similar to the experimental values. There were no systematic differences between the experimental data and the model predictions across species, strain, or gender, and median model outputs uniformly appeared to be biologically plausible despite the uncertainty reflected in some of the 95th percentile credible intervals. The application of the model outputs in the derivation of a human RfD is the focus of section 4 of this document.

Table 2-16. Pharmacokinetic Parameters from Wambaugh et al. (2013) Meta-Analysis of Literature Data

Parameter	Units	CD1 Mouse (F)[a]	CD1 Mouse (M)[a]	Sprague-Dawley Rat (F)[a]	Sprague-Dawley Rat (M)[a]	Cynomolgus Monkey (M/F)[a]
Bodyweight[b]	kg	0.02	0.02	0.203	0.222	3.42
Cardiac Output[c]	L/h/kg$^{0.74}$	8.68	8.68	12.39	12.39	19.8
k_a	L/h	1.16 (0.617–42,400)	433.4 (0.51–803.8)	4.65 (3.02–1,980)	0.836 (0.522–1.51)	132 (0.225–72,100)
V_{cc}	L/kg	0.264 (0.24–0.286)	0.292 (0.268–0.317)	0.535 (0.49–0.581)	0.637 (0.593–0.68)	0.303 (0.289–0.314)
k_{12}	L/h	0.0093 (2.63 x e^{-10} – 38,900)	2,976 (2.8 x e^{-10} –4.2 x e^{4})	0.0124 (3.1 x e^{-10} –46,800)	0.00524 (2.86 x e^{-10}–43,200)	0.00292 (2.59 x e^{-10}–34,500)
$R_{V2:V1}$	Unitless	1.01 (0.251–4.06)	1.29 (0.24–4.09)	0.957 (0.238–3.62)	1.04 (0.256–4.01)	1.03 (0.256–4.05)
T_{maxc}	µmol/h	57.9 (0.671–32,000)	1.1 x e^{4} (2.1–7.9 x e^{4})	1,930 (4.11–83,400)	1.34 x e^{-6} (1.65 x e^{-10}–44)	15.5 (0.764–4,680)
K_T	µmol	0.0109 (1.44 x e^{-5} –1.45)	381 (2.6 x e^{-5}–2.9 x e^{3})	9.49 (0.00626–11,100)	2.45 (4.88 x e^{-10} –60,300)	0.00594 (2.34 x e^{-5}–0.0941)
Free	Unitless	0.00963 (0.00238–0.0372)	0.012 (0.0024–0.038)	0.00807 (0.00203–0.0291)	0.00193 (0.000954–0.00249)	0.0101 (0.00265–0.04)
Q_{filc}	Unitless	0.439 (0.0125–307)	27.59 (0.012–283)	0.0666 (0.0107–8.95)	0.0122 (0.0101–0.025)	0.198 (0.012–50.5)
V_{filc}	L/kg	0.00142 (4.4 x e^{-10} –6.2)	0.51 (3.5 x e^{-10} –6.09)	0.0185 (8.2 x e^{-7} –7.34)	0.000194 (1.48 x e^{-9}–5.51)	0.0534 (1.1 x e^{-7}–8.52)

Notes: Means and 95% confidence interval (in parentheses) from Bayesian analysis are reported. For some parameters the distributions are quite wide, indicating uncertainty in that parameter (i.e., the predictions match the data equally well for a wide range of values).

[a] Data sets modeled for the mouse and rat were from Chang et al. 2012 and for the monkey from Seacat et al. 2002 and Chang et al. 2012

[b] Average bodyweight for species:individual-specific bodyweights

[c] Cardiac outputs obtained from Davies and Morriss 1993

Q_{filc} = median fraction of blood flow to the filtrate

T_{max} = time of maximum plasma concentration

M = male; F = female

2.5.2 Half-life data

Differences between species were observed in studies determining the elimination half-life ($T_{1/2}$) of PFOS in rats, mice, monkeys, and humans. Gender differences in rats do not appear to be as dramatic for PFOS as they are for PFOA (Loccisano et al. 2012a, 2012b).

Humans

Occupational Population. Blood sampling was performed on retirees from the 3M plant in Decatur, Alabama where PFOS was produced. These samples were taken approximately every 6 months over a 5-year period to predict the half-life of PFOS. Results ranged from approximately 4 years to 8.67 years (3M Company 2000; Burris et al. 2002). Both of these studies exhibited some deficiencies in sample collection and methods.

More recently, Olsen et al. (2007) obtained samples from 26 retired fluorochemical production workers (24 males and 2 females) from the 3M plant in Decatur, Alabama to determine the half-life of PFOS. Periodic serum samples (total of 7–8 samples per person) were collected over a period of 5 years, stored at −80 °C, and at the end of the study, High-performance liquid chromatography/mass spectrometry was used to analyze the samples. The study took place from 1998 to 2004. The mean number of years worked at the plant was 31 years (range: 20–36 years), the mean age of the participants at the initial blood sampling was 61 years (range: 55–75 years), and the average number of years retired was 2.6 years (range: 0.4–11.5 years). The initial arithmetic mean serum concentration of PFOS was 0.799 μg/mL (range: 0.145–3.490 μg/mL), and when samples were taken at the end of the study the mean serum concentration was 0.403 μg/mL (range: 0.037–1.740 μg/mL). Semi-log graphs of concentration versus time for each of the 26 individuals were created, and individual serum elimination half-lives were determined using first-order elimination. The arithmetic and geometric mean serum elimination half-lives of PFOS were 5.4 years (95% confidence interval [CI]: 3.9–6.9 years) and 4.8 years (95% CI: 4.1–5.4 years), respectively.

General Population. No data on the half-life of PFOS in the general population were identified.

Infants. Newborn Screening Programs (NSPs) collect whole blood as dried spots on filter paper from almost all infants born in the United States. One hundred and ten of the NSPs collected in the state of New York from infants born between 1997 and 2007 were analyzed for PFOS (Spliethoff et al. 2008). The analytical methods were validated by using freshly drawn blood from healthy adult volunteers. The mean whole blood concentration for PFOS ranged from 0.00081 to 0.00241 μg/mL. The study grouped the blood spots by two different time-points; those collected in 1999–2000 and in 2003–2004, which corresponded to the intervals reported by NHANES. The PFOS concentrations decreased with a mean value of 0.00243 μg/mL reported in 1999–2000 and 0.00174 μg/mL in 2003–2004. The study authors determined the half-life of PFOS using the regression slopes for natural log blood concentrations versus the year 2000 and after. The calculated half-life for PFOS was 4.1 years.

Animal Data

A series of studies was performed to determine the pharmacokinetic parameters of PFOS in rats, mice, and monkeys following administration of single doses (Chang et al. 2012). Another study provided half-life information from monkeys administered PFOS for 26 weeks (Seacat et al. 2002). Minimal gender-related differences were observed in the species examined.

Monkeys. In the study by Chang et al. (2012), three male and three female monkeys were administered a single IV dose of PFOS of 2 mg/kg and followed for 161 days. All monkeys were observed twice daily for clinical signs, and body weights were obtained weekly. Urine and serum samples were taken throughout the study. There was no indication that elimination was different from males versus females. Serum elimination half-lives ranged 122–146 days in male monkeys and 88–138 days in females. Mean values are shown in Table 2-17. The V_d values suggest that distribution was predominately extracellular.

Table 2-17. PFOS Pharmacokinetic Data Summary for Monkeys

Species	Time evaluated after last dose	Route	Sex	Amount K^+PFOS (mg/kg)	Mean serum $T_{1/2}$ by sex (days)	Mean serum $T_{1/2}$ by species (days)	Mean serum V_d by sex (mL/kg)
Cynomolgus monkeys	23 weeks	IV	M	2	132.0 ± 7	120.8	202
			F	2	110.0 ± 15		274

Source: Data from Chang et al. 2012
M = male; F = female

Seacat et al. (2002) administered 0, 0.03, 0.15, or 0.75 mg/kg/day potassium PFOS orally in a capsule by intragastric intubation to 6 young-adult to adult cynomolgus monkeys/sex/group, except for the 0.03 mg/kg/day group which had 4/sex, daily for 26 weeks (182 days) in a GLP study. Two monkeys/sex/group in the control, 0.15, and 0.75 mg/kg/day groups were monitored for 1 year after the end of the treatment period for reversible or delayed toxicity effects. The elimination half-life for potassium PFOS in monkeys was estimated from the elimination curves as approximately 200 days. This value is consistent with that reported by Chang et al. (2012) above.

Rats. Chang et al. (2012) conducted a series of pharmacokinetic studies in rats (Table 2-18). First, a single oral dose of 4.2 mg ^{14}C-K^+PFOS/kg was administered to male Sprague-Dawley rats (3/timepoint). Urine and fecal samples were collected for 24 and 48 hours. Interim sacrifices to obtain plasma samples were obtained at 1, 2, 6, 12, 24, 48, 96, and 144 hours post-dosing. In the next study, 3 rats/sex were administered 2.2 mg PFOS/kg once by oral gavage or IV administration. The rats had a jugular cannula in place and serum samples from it were obtained at 0.25, 0.5, 1, 2, 4, 8, 18, and 24 hours post-dosing. The $T_{1/2}$ values should be viewed with caution because the blood samples were limited to a 24-hour post-dose observation period in contrast to the 144-hour (6-day) period from the first study.

In a third study, serum uptake and elimination of PFOS were evaluated at two dose levels: 2 mg/kg and 15 mg/kg. PFOS was administered as a single oral dose in a 0.5% Tween 20 vehicle to 3 rats/sex or 5/sex at the low and high dose, respectively. Periodic serum, urine, and fecal samples were taken for up to 10 weeks. Liver concentrations were evaluated at termination. Half-life estimates (Table 2-18) did not differ significantly with dose, but there was a difference by sex, with values for the males about half those for the females. There were also gender related differences in the volume of distribution values. PFOS concentrations in the liver exceeded those for paired serum concentrations.

The studies by Chang et al. (2012) described above are limited in that they each reflect pharmacokinetic features associated with a single dose. In an unpublished study by 3M (Butenhoff and Chang 2007), 5 rats/sex were administered 1 mg/kg/day of PFOS orally for 28 days. Interim blood, urine, and feces were obtained for up to 10 weeks. There was no effect on body weight, and PFOS elimination was more prominent in the urine than the feces. The elimination of PFOS in this study approximated first order kinetics with a ‘stair-stepping’ pattern. Using nonlinear, noncompartmental software for computation, the half-lives for males ranged 35–53 days and that for females ranged 33–55 days.

Table 2-18. PFOS Pharmacokinetic Data Summary for Rats

Species	Time evaluated after last dose	Route	Sex	Amount K^+PFOS (mg/kg)	Mean serum $T_{1/2}$ by dose (days)	Mean serum $T_{1/2}$ by sex (days)	Mean serum $T_{1/2}$ by species (days)	Mean serum V_d by dose (mL/kg)
SD rats	144 hours	Oral	M	4.2	8.2 ± 1.5			275
SD rats	24 hours	Oral	M	2.2	3.1[ab]	Not determined due to study design.		765[a]
			F		1.9[b]			521
		IV	M		8.0[b]			649
			F		5.6[a]			586[a]
SD rats	10 weeks	Oral	M	1 x 28 days	35–53	48.2	47.6	-
			F	1 x 28 days	33–55	46.9		-
SD rats	10 weeks	Oral	M	2	38.3 ± 2.3	39.8	53.3	1,228
				15	41.2 ± 2.0			666
			F	2	62.3 ± 2.1	66.7		484
				15	71.1 ± 11.3			468

Source: Data from Chang et al. 2012 and Butenhoff and Chang 2007
Notes: [a] Data reflected a single value derived from one rat only
[b] Within limits of the study design and a follow-up duration of only 24 hours
NA= not available
M = male; F = female

Mice. CD-1 male and female mice were administered PFOS as a single oral dose of 1 or 20 mg/kg (Chang et al. 2012). At designated times (2, 4, 8 hours and 1, 8, 15, 22, 36, 50, 64, and 141 days) post-dosing, four mice/sex were sacrificed and blood, kidneys, and liver samples were obtained. Urine and feces were collected for each 24-hour period up until sacrifice. At the end of the observation period, the daily urinary and fecal excretion was < 0.1% of the administered dose. Results are shown in Table 2-19. Serum elimination values were similar for males and females, independent of dose administered (distribution appeared to be mostly extracellular).

Table 2-19. PFOS Pharmacokinetic Data Summary for Mice

Species	Time evaluated after last dose	Route	Sex	Amount K^+PFOS (mg/kg)	Mean serum $T_{1/2}$ by dose (days)	Mean serum $T_{1/2}$ by sex (days)	Mean serum $T_{1/2}$ by species (days)	Mean serum V_d by dose (mL/kg)
CD-1 mice	20 weeks	Oral	M	1	42.8	39.6	36.9	290.0
				20	36.4			263.0
			F	1	37.8	34.2		258.0
				20	30.5			261.0

Source: Data from Chang et al. 2012
M = male; F = female

Table 2-20 summarizes the half-life data from the studies discussed above. Despite the limitation that the half-life values from most animal studies were derived from administration of only one dose (Chang et al. 2012), consistency was found in the half-lives for males and females for the monkeys, rats, and mice. In rats, this is in contrast to the results observed for PFOA, where there is a much longer half-life in males than in females. However, similar to PFOA, the half-life of PFOS in humans is much greater than that in laboratory animals. A measure of PFOS

half-life in a retired worker population is 5.4 years (Olsen et al. 2007), compared with several months in the laboratory animals.

Table 2-20. Summary of Half-Life Data

Source	Human	Monkey	Rat	Mouse	Strain
Spliethoff et al. 2008	4.1 years	ND	ND	ND	Infants
3M Company 2000	4–8.67 years	ND	ND	ND	Occupational
Olsen et al. 2007	5.4 years	ND	ND	ND	Occupational
Butenhoff and Chang 2007	ND	ND	48.2 days (M) 46.9 days (F)	ND	SD; 28 days oral
Chang et al. 2012	ND	ND	39.8 days (M) 66.7 days (F)	ND	SD; single oral dose
	ND	ND	ND	39.6 days (M) 34.2 days (F)	CD-1; single oral dose
	ND	132 days (M) 110 days (F)	ND	ND	Cynomolgus; single IV dose
Seacat et al. 2002	ND	200 days (M/F)	ND	ND	Cynomolgus; oral, 182 days

Note: ND = No Data
M = male; F = female

The animal data summarized in Table 2-20 show fairly consistent half-life values following single and multiple dosing regimens in both the rat and monkey, probably due to the relatively long follow-up in both species after the last dosing was given. In the rat, half-lives for males and females were nearly identical at 48.2 and 46.9 days, respectively, after 28 days of dosing and 10 weeks of follow-up (Butenhoff and Chang 2007). These results for rats were more consistent between sexes than those half-life values calculated after a single oral dose (Chang et al. 2012). In male and female monkeys, half-life values were similar for either a single intravenous dose (Chang et al. 2012) or repeated oral dosing for 182 days (Seacat et al. 2002). Half-life values for male and female monkeys from Chang et al. (2012) were calculated from the serum concentrations measured over 23 weeks, while the value from Seacat et al. (2002) was estimated from the elimination curves.

2.5.3 Volume of Distribution Data

Humans. None of the available studies provide data for calibration of volume of distribution of PFOS in humans. However, several researchers have attempted to characterize PFOS exposure and intake in humans (Thompson et al. 2010; Egeghy and Lorber 2011) through pharmacokinetic modeling. In the models discussed below, volume of distribution was defined as the total amount of PFOS in the body divided by the blood or serum concentration.

Both research groups defined a volume of distribution for humans using a simple, first-order, one-compartment pharmacokinetic model (Thompson et al. 2010; Egeghy and Lorber 2011). The models developed were designed to estimate intakes of PFOS by young children and adults (Egeghy and Lorber 2011) and the general population of urban areas on the east coast of Australia (Thompson et al. 2010). In both models, the volume of distribution was calibrated using human serum concentration and exposure data from NHANES, and it was assumed that most PFOS intake was from contaminated drinking water. Thus, the value for volume of distribution was calibrated so that model prediction of elevated blood levels of PFOS matched those seen in the study population.

Thompson et al. (2010) used a first-order, one-compartment pharmacokinetic model, as described previously, to predict PFOS concentration in blood serum as a function of dose, elimination rate, and volume of distribution. The volume of distribution was first obtained for PFOA by calibrating human serum and exposure data. The volume of distribution for PFOS (230 mL/kg) was adjusted from the calibrated PFOA data by 35% in accordance with the differences in PFOA and PFOS volumes of distribution calculated by Andersen et al. (2006). The original Andersen et al. (2006) model was developed from oral data in monkeys and optimized a volume of distribution of 220 mL/kg for PFOS and 140 mL/kg for PFOA. Thus, the volume of distribution in monkeys for PFOS was approximately 35% greater than that for PFOA in the optimized models. Therefore, Thompson et al. (2010) used a volume of distribution of 230 mL/kg for humans in their model.

Egeghy and Lorber (2011) used high and low bounding estimates of 3,000 mL/kg and 200 mL/kg for volume of distribution since data in humans were not available. The two separate estimates of volume of distribution were used in a first-order, one-compartment model to estimate a range of intakes of PFOA. They concluded that the volume of distribution was likely closer to the lower value based on a comparison of predicted modeled intake with estimates of intakes based on exposure pathway analyses. Use of the lower value gave a modeled intake prediction similar to that obtained by a forward-modeled median intake based on an exposure assessment. The authors concluded that the lower value of 200 mL/kg was appropriate for their analysis.

Both of the models described above used a volume of distribution calibrated from actual human data on serum measurements and intake estimates. A calibration parameter obtained from human studies, where constant intake was assumed and blood levels were measured, is considered a more robust estimate for volume of distribution than that optimized within a model developed from animal data.

Animals. The Chang et al. (2012) series of pharmacokinetic studies on rats, mice, and monkeys described above, included volume of distribution calculations. Values for all species were calculated following a single oral or IV dose of PFOS. As discussed below, the volume of distribution values reported for male and female monkeys, female rats, and male and female mice were reasonably similar.

The volume of distribution was 202 and 274 mL/kg, for male and female cynomolgus monkeys, respectively (Table 2-17), following a single IV dose of 2 mg/kg (Chang et al. 2012). Animals were evaluated up to 23 weeks after dosing, and the resulting volumes of distribution are similar to the 230 mL/kg calibrated from human data by Thompson et al. (2010) described above.

The Chang et al. (2012) volume of distribution findings for rats are in Table 2-18. Those values calculated from a follow-up duration of only 24 hours are not considered reliable. In studies with a longer follow-up after dosing, the values for male rats were 275, 666, and 1228 mL/kg and, for female rats, values were 468 and 484 mL/kg. The volume of distribution was notably greater for male rats than that of female rats or other species including humans, with the exception of one value. The authors could not explain the higher value for the male rat but concluded that the volume of distribution for monkeys, rats, and mice is likely in the range of 200–300 mL/kg.

Data for mice (Chang et al. 2012) are shown in Table 2-19. For males and females the volume of distribution was 263–290 mL/kg and 258–261 mL/kg, respectively, following a single oral dose.

Pharmacokinetic models based on animal data described previously in this section generally optimized the value for volume of distribution based on model output. The original Andersen et al. (2006) model was developed using data from Seacat et al. (2002) on serum PFOS concentrations in cynomolgus monkeys following oral dosing. The volume of distribution in this model was 220 mL/kg.

2.6 Toxicokinetic Summary

Uptake and egress of PFOS from cells is largely regulated by transporters in cell membranes based on data collected for PFOA, a structurally similar chemical. On the basis of the tissue concentrations found in the pharmacokinetic studies (Cui et al. 2009; Curran et al. 2008), PFOS is absorbed from the gastrointestinal tract, as indicated by the serum measurements in treated animals, and distributed to the tissues. The highest tissue concentrations are usually those in the liver. Post mortem tissues samples collected from 20 adults in Spain found PFOS in liver, kidney, and lung (Pérez et al. 2013). The levels in brain and bone were low. In serum, PFOS is electrostatically bound to albumin occupying up to eleven sites (Weiss et al. 2009). Linear PFOS chains display stronger binding than branched chains (Beesoon and Martin 2015). Binding causes a change in the conformation of serum albumin (Weiss et al. 2009) thereby changing its affinity for the endogenous compounds also transported by serum albumin. PFOS binds to other serum proteins including immunoglobulins and transferrin (Kerstner-Wood et al. 2003). It is not metabolized, thus any effects observed in toxicological studies are not the effects of metabolites.

Electrostatic interactions with proteins are an important toxicokinetic feature of PFOS. Studies demonstrate binding or interactions with nuclear receptors (e.g., PPARα), transport proteins (e.g., transthyretin [TTR]), FABP), and enzymes (Luebker et al. 2002; Ren et al. 2015; L. Wang et al. 2014; Weiss et al. 2009; Wolf et al. 2008; L. Zhang et al. 2013, 2014). Saturable renal resorption of PFOS from the glomerular filtrate via transporters in the kidney tubules is believed to be a major contributor to the long half-life of this compound. No studies were identified on specific renal tubular transporters for PFOS, but many are available for PFOA. All toxicokinetic models for PFOS and PFOA are built on the concept of saturable renal resorption first proposed by Anderson et al. (2006). Some PFOS is removed from the body with bile (Chang et al. 2012; Harada et al. 2007), a process that is also transporter-dependent. Accordingly, the levels in fecal matter represent both unabsorbed material and that discharged with bile.

The arithmetic mean half-life in humans for occupationally exposed workers (Olsen et al. 2007) was 5.4 years (95% CI: 3.9–6.9 years). Half-lives from animals include 120.8 days for monkeys, 33–35 days for male and female Sprague-Dawley rats, and 36.9 days for male and female CD1 mice (Chang et al. 2012). The half-life differences between male and female rats observed for PFOA were not observed with PFOS. This indicates a lack of sex-related differences in renal excretion in rats and implies that the renal excretion and/or resorption transporters for PFOS differ from those for PFOA. No comprehensive studies of PFOS transporters in humans or laboratory animals were identified.

3. HAZARD IDENTIFICATION

The Hazard Identification section provides a summary and synthesis of the data from a large number of human epidemiology studies accompanied by studies in laboratory animals designed to identify both the dose-response and critical effects that result from exposures to PFOS and to examine the mode of action leading to toxicity.

3.1 Human Effects

There is a substantial body of research on the adverse effects of PFOS in both humans and animals. The human database lacks data on acute effects and short term exposures, but it includes many epidemiology studies. The database of human studies is large, in part, due to the extensive research program conducted by the C8 Science Panel on residents of communities in Ohio and West Virginia that were impacted by PFOA discharges from the DuPont Washington Works plant in Parkersburg, West Virginia. The purpose of the C8 Health Project is to assess if there are any *probable links* between PFOA (and PFOS) exposure and disease. During the period August 2005–July 2006, about 69,000 study participants were identified. Eligible participants included those who had consumed drinking water for at least one year up to and including December 4, 2004 from the (1) Lubeck and Mason County water districts in West Virginia; (2) the Belpre, Little Hocking, Tuppers Plains-Chester, and Pomeroy water districts in Ohio; or (3) private water source within the geographical boundaries of the public water sources. The participants (n = 69,030; 33,242 males, 35,788 females; aged < 10 to 70 years and older) donated a blood sample, filled out an extensive questionnaire, and received $400 in compensation. Although the project was designed to examine the impact of PFOA on health effects among residents of the impacted community, the serum was analyzed for other perfluorochemicals, including PFOS. Medical records were used to validate diseases reported by participants. The C8 Science panel studies were funded by DuPont under a consent decree. Some of the studies evaluated the impact of PFOS (or PFOA) on outcome.

Commercial use of PFOS and other PFASs began over 60 years ago, resulting in global release of this family of compounds. As a result, population monitoring of serum is widespread and has supported multiple epidemiological investigations of the general population within the United States and abroad. Occupational epidemiology studies are available from 3M, a U.S. manufacturer of PFOS. Studies investigating the association between PFOS levels and health effects in the U.S. general population have also been conducted using the NHANES data set. The NHANES examined representative members of the U.S. population through their surveys focusing on different health topics. These studies consist of an interview (demographic, socioeconomic, dietary, and medical questions) and examination (medical including blood and urine collection, dental, and physiological parameters).

A study by Jain (2014) examined the influence of diet and other factors on the levels of serum PFOS and other PFASs using the NHANES 2003–2004, 2005–2006, and 2007–2008 data. Significantly higher serum PFOS levels were found in males (0.020 μg/mL) compared to females (0.014 μg/mL). There was a significant decreasing trend in serum PFOS concentration between 2003 and 2008. There was a positive association of PFOS with increases in serum cholesterol ($p < 0.01$) and serum albumin ($p < 0.01$) in the 5,591 records used for the assessment. Intakes of meat and fish were positively associated with serum PFOA ($p < 0.01$).

3.1.1 Long-Term Noncancer Epidemiological Studies

3.1.1.1 Serum Lipids and Cardiovascular Diseases

Occupational studies. Cross-sectional, as well as a longitudinal analyses of medical surveillance data from the 3M Decatur, Alabama and Antwerp, Belgium plants were conducted to evaluate possible associations between PFOS levels and hematology, clinical chemistry, and hormonal parameters (Olsen et. al 2001a, 2001b, 2003b). In the cross-sectional study, male (n = 215) and female (n = 48) volunteers working at the Decatur plant and male (n = 206) and female (n = 49) volunteers working at the Antwerp plant underwent clinical chemistry tests to evaluate hepatic enzyme activity, renal function, thyroid activity, and cholesterol levels. Data on employees from both plants appeared to be combined for the regression analyses; however, it was not clear whether females were included or whether the analyses only included males. The mean PFOS level in all employees from the Decatur and Antwerp plants was 1.40 μg/mL (range: 0.11–10.06 μg/mL) and 0.96 μg/mL (range: 0.04–6.24 μg/mL), respectively. Positive significant associations were reported between serum PFOS and cholesterol (probability [p] = 0.04) and between serum PFOS and triglycerides (p = 0.01); similar results were found for PFOA. Age was also significant in both analyses. Alcohol consumed per day was significant in the cholesterol model, while body mass index (BMI) and cigarettes smoked per day was significant for triglycerides. PFOS was positively associated with alkaline phosphatase (ALP). Hepatic enzymes and bilirubin were not associated with PFOA. However, there were many limitations to combining and comparing the data from the two plants.

A longitudinal analysis of the above data was performed to determine whether occupational exposure to fluorochemicals over time was related to changes in clinical chemistry and lipids (Olsen et al. 2001b, 2003b). The medical surveillance data from 175 individuals who had participated in two or more medical exams in 1995, 1997, and 2000 were analyzed using multivariable regression. Mean PFOS levels at the beginning and end of the surveillance period were 2.62 μg/mL and 1.67 μg/mL, respectively, in Decatur employees and 1.87 μg/mL and 1.16 μg/mL, respectively, in Antwerp employees. When male employees from both plants were combined, no statistically-significant ($p < 0.05$) associations were observed over time between PFOS and serum cholesterol or triglycerides. There were no significant associations between PFOS and changes over time in HDL, ALP, gamma-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), or alanine transaminase (ALT) activities, total bilirubin, or direct bilirubin. PFOA was positively associated with cholesterol and triglycerides in the Antwerp employees.

High-exposure community studies. The C8 Health Project conducted in 2005–2006 on approximately 69,000 residents in Ohio and West Virginia evaluated general population exposures to PFOS and other perfluorochemicals. Public drinking water was contaminated in six water districts surrounding the plant (≥ 0.05 ng/mL of PFOA). Residents were eligible to participate in the study if they had consumed water from any of the 6 water districts for at least one year prior to the study. Blood samples were collected from the participants to determine PFOA and PFOS serum levels and clinical chemistry was performed. Extensive questionnaires were administered as well. The levels of PFOA were elevated, however, levels of PFOS in this population were similar to those reported in the general U.S. population (median 0.02 μg/mL).

Steenland et al. (2009) examined serum PFOS and PFOA levels and lipids among 46,294 residents, ≥ 18 years old, participating in the C8 Health Project. The mean serum PFOS level among participants was 0.022 μg/mL, with a range of 0.00025–0.7592 μg/mL. Lipid outcomes

(total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides) were examined in relation to PFOS and PFOA serum levels. All lipid outcomes, except for HDL, showed significant increasing trends with increasing PFOS levels (similar for PFOA). The predicted increase in cholesterol from lowest to highest PFOS decile was 11–12 mg/deciliter (dL). Logistic regression analyses indicate statistically-significant incidence of hypercholesterolemia (≥ 240 mg/dL) with increasing PFOS serum levels. Cholesterol levels ≥ 240 mg/dL are characterized as high, and medical intercession is recommended. The odds ratios (ORs) across quartiles for cholesterol ≥ 240 mg/dL were 1.00, 1.14 (95% CI: 1.05–1.23), 1.28 (95% CI: 1.19–1.39) and 1.51 (95% CI: 1.40–1.64). The cross-sectional design of this study, as well as the lack of cumulative exposure measurements, are limitations in the study design.

Frisbee et al. 2010 evaluated 12,476 children ≤ 18 years old who lived in the C8 Health Project communities for total cholesterol, LDLs, HDLs, and fasting triglycerides. The mean level of PFOS was 0.023 μg/mL. PFOS was significantly associated with increased total cholesterol, HDL-cholesterol, and LDL- cholesterol in a linear regression analysis after adjustment for co-variables. A statistically-significant increased risk of high total cholesterol [OR 1.6 (1.4–1.9)] and LDL-cholesterol [OR 1.6 (1.3–1.9)] was also observed between the first and fifth quintiles of PFOS serum levels. No trends were observed with triglycerides. Total cholesterol, LDL, and triglycerides were also positively associated with serum PFOA concentration. As with the other C8 project data, the authors acknowledge that the cross-sectional nature of this study limits causal inference.

A cohort of 521 adult members of the C8 Health Project was evaluated for an association between changes in serum PFOS levels and changes in serum LDL-cholesterol, HDL-cholesterol, total cholesterol, and triglycerides over a 4.4-year period (Fitz-Simon et al. 2013). Linear regression models were fit to the logarithm (base 10) of ratio change in each serum lipid measurement in relation to the logarithm of ratio change in PFOS. Mean serum PFOS concentration decreased by approximately one-half between baseline (0.023 ± 0.014 μg/mL) and follow-up (0.011 ± 0.007 μg/mL). No corresponding changes in serum lipids were found. However, those individuals with the greatest declines in serum PFOS had a tendency for a slight decrease in LDL-cholesterol. Similar results were found with PFOA.

A subset of 290 individuals in the C8 Health Project was evaluated for evidence that PFOS exposure can influence the transcript expression of genes involved in cholesterol metabolism, mobilization, or transport (Fletcher et al. 2013). Ribonucleic acid (RNA) was extracted from whole blood samples taken from 144 males and 146 females aged 20–60 years; serum collected at the same time was used to measure PFOS concentration. The association between candidate gene expression levels and PFOS levels was assessed by multivariable linear regression with adjustments for confounders. A positive association was seen between PFOS and a transcript involved in cholesterol mobilization (Neutral Cholesterol Ester Hydrolase 1 [NCEH1]; $p = 0.018$), and a negative relationship with a transcript involved in cholesterol transport (Nuclear Receptor Subfamily 1, Group H, Member 3 [NR1H3]; $p = 0.044$). When sexes were analyzed separately, PFOS was positively associated with expression of genes involved in cholesterol mobilization and transport in females (NCEH1 and Peroxisome Proliferator-Activated Receptor alpha [PPARα]; $p = 0.003$ and 0.039, respectively), but no effects were evident in males. Similar associations were also found for PFOA.

General population studies. Nelson et al. (2010) used NHANES 2003–2004 data to analyze PFOS and three other perfluorinated chemicals and total cholesterol, HDLs, non-HDL lipoproteins, and LDL. LDL was available only for a subsample of the fasting population and

was not measured directly, but was estimated by the Friedewald formula[2] as recommended by Centers for Disease Control and Prevention. Homeostatic model assessment (HOMA) was used to assess insulin resistance (calculated from fasting insulin and fasting glucose measurements collected in NHANES). BMI and waist circumference were used to measure body size. Exclusion criteria included current use of cholesterol-lowering medications, participants over the age of 80, pregnant/breastfeeding females or insulin use. After exclusion criteria, approximately 860 participants were included in the analyses. The mean PFOS serum concentration for participants 20–80 years old was 0.025 μg/mL (range: 0.0014–0.392 μg/mL).

A positive association was identified between total serum cholesterol and serum PFOS concentrations. When analyzed by PFOS serum quartiles, adults in the highest PFOS quartile had total cholesterol levels of 13.4 mg/dL (95% CI: 3.8–23.0), higher than those in the lowest quartile. As expected, non-HDL cholesterol accounted for most of the total cholesterol. Consistent trends were not observed for HDL or LDL. Adjusting the cholesterol models for serum albumin produced similar results. Body weight and insulin resistance were not consistently associated with serum PFOS levels. Similar results were found for PFOA.

Lin et al. (2009) explored associations of serum lipid levels with NHANES PFOA data from 1999–2000 and 2003–2004. Serum HDL was inversely associated with serum PFOS concentration OR ((95% CI): 1.61 (1.15–2.26), $p < 0.05$). Triglycerides did not show an association with PFASs.

Effects of PFOS on plasma lipid levels in the Inuit population of Northern Quebec were examined in a cross-sectional epidemiology study (Château-Degat et al. 2010). The relationship between consumption of PFOS-contaminated fish and wild game with blood lipids was assessed in 723 Inuit adults (326 man and 397 females). This traditional diet is also rich in n-3-polyunsaturated fatty acids (n-3 PUFAs) which are known to have hypolipidemic effects; therefore, the n-3 PUFAs were considered as a confounder in the analyses. Multivariate linear regression modeling was used to evaluate the relationship of PFOS levels and blood lipids, including total cholesterol (TC), HDL cholesterol, LDL cholesterol, and triacylglycerols. Plasma levels of HDL cholesterol were positively associated with PFOS levels, even after adjustment for circulating levels of n-3 PUFAs, but the other blood lipids were not associated with PFOS levels. The geometric mean level of PFOS in plasma for females and males was 0.019 μg/mL.

Eriksen et al. (2013) examined the association between plasma PFOS levels and total cholesterol levels in a middle-aged Danish population. This cross-sectional study included 663 males and 90 females aged 50–65 years who were enrolled in the Danish Diet, Cancer and Health cohort. Generalized linear models were used to analyze the association between PFOS and total cholesterol levels and adjusted regression analyses were performed. The mean plasma PFOS level was 0.0361 μg/mL. A significant, positive association was found between PFOS (and PFOA) and total cholesterol such that in the fully adjusted model, a 4.6 mg/dL (95% CI: 0.8–8.5) higher concentration of total cholesterol was found per interquartile range of plasma PFOS. The quartiles of PFOS used in the analyses were not defined and no comparison was made for cholesterol levels between the highest and lowest PFOS quartile.

[2] Friedewald formula: [LDL-cholesterol] = [total cholesterol] – [HDL-cholesterol] – [triglycerides/5]. All values are expressed in mg/dL units.

A cross-sectional study of 891 pregnant females evaluated the association between plasma PFOS levels and plasma lipids (Starling et al. 2014). Six other perfluoroalkyl substances were also quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study, and the majority of blood samples were drawn during weeks 14–26 of gestation. Weighted multiple linear regression was used to estimate the association between PFOS level and each lipid level. The median plasma PFOS level was 0.013 μg/mL. No association was observed between PFOS and triglycerides. PFOS was positively associated with total cholesterol, HDL-cholesterol, and LDL-cholesterol, although confidence intervals were broad for all associations. Each ln-unit increase in PFOS was associated with an increase of 8.96 mg/dL (95% CI: 1.70–16.22) in total cholesterol and for each interquartile range (IQR)-unit increase in the ln-PFOS concentration, total cholesterol increased by 4.25 mg/dL (95% CI: 0.81–7.69). With HDL-cholesterol, each IQR-unit increase in ln-PFOS was associated with an increase of 2.08 mg/dL (95% CI: 1.12–3.04). For LDL-cholesterol, each IQR-unit shift in ln-PFOS was associated with a change of 3.07 mg/dL LDL (95% CI: −0.03–6.18). Five of the seven PFASs studied were positively associated with HDL cholesterol, and all seven had elevated HDL associated with the highest quartile.

Fisher et al. (2013) examined the association of plasma PFAS levels, including PFOS, with metabolic function and plasma lipid levels. This cross-sectional study included 2,700 participants, aged 18–74 years (approximately 50% male), in the Canadian Health Measures Survey. Multivariate linear and logistic regression models were used for analyses of associations between PFOS levels and cholesterol outcomes, metabolic syndrome, and glucose homeostasis. The geometric mean PFOS concentration was 0.0084 ± 0.002 μg/mL. In weighted analyses, no association was found between PFOS (or PFOA) and total cholesterol, HDL- and LDL-cholesterol, and metabolic syndrome and glucose homeostasis parameters. Hypercholesterolemia (cholesterol greater than 240 mg/dL), was associated with PFOS exposure in unadjusted analyses of this cohort.

Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration (Table 3-1). These studies provide support for an association between PFOS and small increases in total cholesterol in the general population at mean serum levels of 0.0224–0.0361 μg/mL (Frisbee et al. 2010; Nelson et al. 2010; Eriksen et al. 2013). Hypercholesterolemia, (clinically defined as cholesterol greater than 240 mg/dL), was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009). Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003b). Evidence for associations between other serum lipids and PFOS is mixed, including HDL cholesterol, LDL, very low density lipoprotein (VLDL), non-HDL cholesterol, and triglycerides. The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults, but some studies exist on children and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the United Kingdom (UK), to Scandinavian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design. Studies also included populations with known elevated exposure to other environmental chemicals including PFOA in the C8 population or polybrominated diphenyl ethers (PBDEs) and other persistent organic compounds among the Inuit population. Overall, the epidemiologic evidence supports an association between PFOS and increased total cholesterol.

Table 3-1. Association of Serum PFOS with Serum Lipids

Reference and Study Details	PFOS Level (µg/mL)	Total Cholesterol (TC)	Low Density Lipoprotein (LDL)	High Density Lipoprotein (HDL)	Triglycerides (TG)
Occupational Populations					
Olsen et al. 2001a, 2003b Cross-sectional from manufacturing plant workers n = 263 (Decatur) n = 255 (Antwerp)	Mean 1.40 Decatur Mean 0.96 Antwerp	Beta = 0.010 (95% CI) (−0.005, 0.025)	NM	No association	Beta = 0.025 (95% CI) (−0.015, 0.065)
Olsen et al. 2001b, 2003b Longitudinal; ~ 5 years n = 175 (Decatur and Antwerp combined for analysis)	Mean 2.62 (baseline) 1.67 (follow-up) (Decatur) 1.87 (baseline) 1.16 (follow-up) (Antwerp) PFOS Quartiles Q1: 0.04–0.42 Q2: 0.43–0.81 Q3: 0.82–1.68 Q4: 1.69–10.06 ppm	TC by quartile of PFOS mean (SD): Q1: 214 (41) Q2: 214 (43) Q3: 215 (39) Q4: 222 (44)	NM	HDL by quartile of PFOS mean (SD): Q1:54 (15) Q2:47 (11) Q3:48 (13) Q4: 48 (15)	TG by quartile of PFOS mean (SD): Q1:131 (95) Q2: 155 (102) Q3: 169 (123) Q4: 177 (123) p < 0.05 Q4 v Q1
General Populations with high environmental exposure to other PFASs					
Steenland et al. 2009 Cross-sectional (C8), Logistic regression analysis, 2005–2006 n = 46,294 Age: 18–80 yrs (not taking cholesterol-lowering medications) Mean duration: not provided Linear regression, quartiles and continuous	Mean 0.022 Quartiles of PFOS (ng/mL): Q1: 0–13.2 Q2: 13.3–19.5 Q3: 19.6–28.0 Q4: ≥ 28.1	Odds Ratio (95% CI) for high cholesterol by 1 IQR increase in PFOS Q1: 1 (referrant) Q2: 1.14 (1.05, 1.23) Q3: 1.28 (1.19, 1.39) Q4: 1.51 (1.40, 1.64) Beta 0.02660 (SD 0.00140) [log PFOS and lipids]	Nearly monotonic increase in association with PFOS Beta 0.04176 (SD 0.00221) [log PFOS and lipids]	Null associations Beta 0.00355 (SD 0.00173) [log PFOS and lipids]	Increased Beta 0.01998 (SD 0.00402) [log PFOS and lipids]

Reference and Study Details	PFOS Level (µg/mL)	Total Cholesterol (TC)	Low Density Lipoprotein (LDL)	High Density Lipoprotein (HDL)	Triglycerides (TG)
Fitz-Simon et al. 2013 Longitudinal (C8); n = 521 Duration: 4.4 years Within-individual changes in PFOS & lipids over time, 2005–2006 versus 2010 serum concentrations. Linear regression fit to log of ratio change in lipid in relation to change in PFOS	0.023 (baseline) 0.011 (follow-up) Tertiles of PFOS ng/ml (ratio follow up/baseline) T1: < 0.4 T2: 0.4–0.54 T3: > 0.54	Geometric mean (mg/dL): baseline, follow-up 192.5, 192.8 Percent decrease (95% CI) in lipid per halving PFOS 3.20 (1.63–4.76)	Geometric mean (mg/dL): baseline, follow-up 107.8, 109.2 Percent decrease (95% CI) in lipid per halving PFOS 4.99 (2.46–7.44)	Geometric mean (mg/dL): baseline, follow-up 48.6, 47.2 Percent decrease (95% CI) in lipid per halving PFOS 1.28 (−0.59–3.12)	Geometric mean (mg/dL): baseline, follow-up 144.1, 146.9 Percent decrease (95% CI) in lipid per halving PFOS 2.49 (−2.88–7.57)
Nelson et al. 2010 Cross-sectional (NHANES), USA. n = 860 (20–80 yrs old) Linear regression analysis for PFOS and serum lipids	0.025 Serum PFOS by quartile Q1: 1.4–13.6 Q2: 13.8–19.7 Q3: 19.8–28.1 Q4: 28.2–392.0	TC by PFOS Quartile (mg/dl): Q1: 198.6 Q2: 201.6 Q3: 202 Q4: 205.7 Beta 0.27 (95% CI; 0.05–0.48)	LDL by PFOS Quartile (mg/dl): Q1: 113.6 Q2: 116.4 Q3: 113.4 Q4: 123.1 Beta 0.12 (95% CI; −0.17–0.41)	HDL by PFOS Quartile (mg/dl): Q1: 54.3 Q2: 56.0 Q3: 52.7 Q4 : 55.2 Beta 0.02 (95% CI; −0.05–0.09)	NM
Château-Degat et al. 2010 Cross-sectional, Inuit population (Quebec). PFOS effect on total lipids. Effect modification of n-3 PUFAs, which can be hypolipidemic n = 723 Multiple linear regression modeling	0.019 Geometric mean (95% CI) µg/L Women: 16.8 (15.8–17.8) Men: 20.4 (19.1–21.8)	Adjusted models R^2, Beta (p value) 0.17, 0.0009 (0.086)	Adjusted models R^2, Beta (p value) 0.17, −0.0020 (0.242)	Adjusted models R^2, Beta (p value) Women: 0.12, 0.0042 (0.001) Men: 0.12, 0.0016 (< 0.001)	Adjusted models R^2, Beta (p value) Women: 0.20, −0.0014 (0.04) Men: 0.16, −0.0009 (0.162)

Reference and Study Details	PFOS Level (µg/mL)	Total Cholesterol (TC)	Low Density Lipoprotein (LDL)	High Density Lipoprotein (HDL)	Triglycerides (TG)
Eriksen et al. 2013 Cross-sectional, Middle aged Danish population n = 753 (663 men and 90 women) Generalized linear models used for analysis	0.036	Differences in TC (mg/dl) per 1 IQR increase Beta (95% CI): Total population: 3.7 (0.1, 7.3) Women: 11.7 (−0.2, 23.6) Men: 2.9 (−0.9, 6.7)	NM	NM	NM
Fisher et al. 2013 Cross-sectional, 2007–2009, Canadian Health Measures Survey (CHMS) Cycle 1. n = 2700 (aged 18–74) Used multivariate linear and logistic regression models to assess associations between PFOS and serum lipids.	0.0084	Unadjusted OR for high cholesterol compared to Q1 of PFOS exposure: OR (95% CI) Q1: Referrent Q2: 1.12 (0.89, 1.41) Q3: 1.15(0.91, 1.45) Q4: 1.66 (1.32, 2.09) p trend = 0.03 Null effects in adjusted model	Null effects	Null effects	NM
Children and Adolescents					
Frisbee et al. 2010 Cross-sectional (C8, children) GLM Analysis, n = 12,476 Differences of Estimated Marginal Mean (EMM) between Q1 and Q5 and regression analysis for Q trend	0.023	Differences in Estimated Marginal Mean (EMM), Beta (SE), p for trend: Age 1 to < 12: 5.5, 1.3 (0.3), < 0.001 Age 12 to < 18: 9.5, 2.1 (0.4), < 0.001	Differences in EMM, B(SE) p for trend: Age 1 to < 12: 3.4, 0.9 (0.3), .002 Age 12 to < 18: 7.5, 1.7 (0.2), < 0.001	Differences in EMM, B(SE), p for trend: Age 1 to < 12: 1.6, 0.3 (0.1), 0.007 Age 12 to < 18: 1.5, 0.4 (0.1), 0.001	Differences in EMM, B(SE), p for trend: Age 1 to < 12: 2.8, 0.1 (1.4), 0.99 Age 12 to <1 8: 2.8, −0.1 (1.0), 0.90
Geiger et al. 2014a Cross-sectional, NHANES, 1999–2008, dyslipidemia (TC, LDL, HDL, TG). n = 815 (Age ≤ 18) Multivariate regression analysis. n = 815	T1: < 12.1 T2: 12.1–21.8 T3: > 21.8 ppb	TC (mg/dL) association with PFOS by tertiles T1: 1 T2: 1.73 (−2.89, 6.36) T3: 3.91 (−1.32, 9.14) p trend: 0.15 log transformed PFOS Beta 0.04 (95% CI: 0.00─ 0.08)	Association between PFOS and LDL: T1: 1 (referent) T2: 0.49 (−3.41, 4.38) T3: 4.59 (−0.17, 9.35) P trend: 0.0632 log transformed PFOS Beta 2.83 (95% CI: 0.03─ 5.37)	Association between PFOS and HDL: T1: 1 T2: 2.86 (0.44, 5.28) T3: 1.11 (−0.93, 3.15) P trend: 0.2931	Association between PFOS and TG: T1: 1 T2:−8.13 (−15.50, −0.77) T3: −8.89 (−15.67, −2.11) P trend: 0.0126 log transformed PFOS Beta −3.90 (95% CI: −7.72 to −0.08)

Reference and Study Details	PFOS Level (µg/mL)	Total Cholesterol (TC)	Low Density Lipoprotein (LDL)	High Density Lipoprotein (HDL)	Triglycerides (TG)
Lin et al. 2009 Cross-sectional, NHANES, 1999–2000, 2003–2004. Adolescents and adults aged ≥ 12 yrs n = 3,685	Mean (SEM) Log PFOS 12 to < 20 yrs olds: 3.11 (0.05) ng/mL 20 yrs old and older: 3.19 (0.04) ng/mL	NM	NM	OR (95% CI), p 1.61 (1.15–2.26), p < 0.05 in those 20 yrs or older	Null findings
Maisonet et al. 2015 Avon Longitudinal Study of Parents and Children. Prenatal PFOS compared to serum lipids in female offspring. n = 111 (age 7), n = 88 (age 15)	Mean (SD) 22.2 (11.4) mg/dl	Non-linear associations of TC with PFOS.	Non-linear associations of LDL with PFOS.	Null findings	Null findings
Timmermann et al. 2014 Danish children, aged 8–10 years old. Linear regression models. 1997. n = 499	Median 41.5 ng/mL	Null findings in normal weight children.	Null findings in normal weight children.	Null findings in normal weight children.	Null findings in normal weight children. In overweight children, 10 ng increase PFOS/mL plasma associated with 8.6% (95% CI: 1.2%–16.5%) higher triglyceride concentrations
Pregnant Women					
Starling et al. 2014 Cross-sectional (maternal at 14–26 weeks gestation), Norwegian Mother and Child Cohort (MoBa) 2003–2004. n = 891	0.013 Quartiles (ng/mL): Q1: < 10.31 Q2: 10.31–13.03 Q3: 13.04–16.59 Q4: > 16.60	B (95% CI) PFOS (ng/ml) and TC (mg/dL). Q1: Referrent Q2: −3.35 (−10.34, 3.64) Q3: 3.06 (−4.93, 11.05) Q4: 7.59 (−0.42, 15.60) TC change per IQR change in PFOS: 4.25 (0.81, 7.69)	B (95% CI) PFOS (ng/ml) and LDL(mg/dL). Q1: referrent Q2: −3.23 (−9.28, 2.83) Q3: 2.60 (−4.49, 9.70) Q4: 5.51 (−1.62, 12.64) LDL change per IQR PFOS change: 3.07 (−0.03, 6.18)	B (95% CI) PFOS (ng/ml) and HDL (mg/dL). Q1: Referrent Q2: 1.96 (−0.39, 4.31) Q3: 2.49 (0.00, 4.97) Q4: 4.45 (2.04, 6.86) HDL change per IQR change in PFOS: 2.08 (1.12, 3.04)	B (95% CI) PFOS (ng/ml) and TG (mg/dL). Q1: Referrent Q2: 0.00 (−0.06, 0.07) Q3: −0.03 (−0.10, 0.05) Q4: 0.00 (−0.07, 0.07) TG change per IQR PFOS change: −0.01 (−0.04, 0.02)

NM = Not Measured

Some of the studies that examined serum LDL and HDL cholesterol also found significant increases these measures. Neither of these lipoprotein complexes is a stand-alone indicator for cardiovascular decrease risk. Rather, it is the relationship across the lipoprotein complexes within the same individuals that is important with HDLs considered as protective and LDLs a biomarker for potential atherosclerosis. Relatively few studies of triglycerides noted a significant increase with the serum PFOS levels.

3.1.1.2 Liver Enzymes and Liver Disease

Cross-sectional studies and longitudinal studies of PFOS and liver enzymes in various populations are described below and summarized in Table 3-2.

Table 3-2. Summary of Epidemiology Studies of PFOS and Liver Enzymes

Reference and Study Details	PFOS Level (µg/mL)	Results
Lin et al. 2010 n = 2,216 adults (1,076 men and 1,140 women) Age: > 20 years old Data from 1999–2000 and 2003–2004 NHANES Regression models used to analyze data and adjust for confounders	Mean levels Women: 0.0222 Men: 0.0274	Linear regression coefficients (standard error), p-value (adjusted for age, gender, race, lifestyle, measurement data, etc.) ALT (U/L): 1.01 (0.53), 0.066 (slight pos. association) GGT (U/L): 0.01 (0.03), p = 0.81 Total bilirubin (µmol): 0.30 (0.24), p = 0.22
Gallo et al. 2012 n = 47,092 Data from those enrolled in C8 Health Project Linear and logistic regression models used.	Mean level: 0.0233	Linear regression coefficients, (partial R^2) Ln-ALT: 0.020, 95% CI: 0.014–0.026 (< 0.001) Raised ALT in logistic regression odds ratio, (p-value) OR: 1.13, 95% CI: 1.07–1.18 (p < 0.001) GGT: no association Direct bilirubin: less consistent results

Lin et al. (2010) investigated the association between low-dose serum PFOS (along with three other individual PFAS) and liver enzymes in the adult population of the United States by analyzing data from the 1999–2000 and 2003–2004 NHANES. The study population included 2,216 adults (1,076 males, 1,140 females) older than 20 years who were not pregnant or nursing; had fasted > 6 hours at the time of examination; were negative for hepatitis B or C virus; had body weight, height, educational attainment, and smoking status data available; and had serum tests for PFAS, liver function, and metabolic syndrome. Regression models were used to analyze the data and adjust for confounding factors. Mean PFOS levels were 0.0274 and 0.0222 µg/mL for males and females, respectively.

Serum PFOS concentration was divided into quartiles. Unadjusted liver enzymes, serum ALT, and log-GGT increased across quartiles of PFOS ($p \leq 0.03$), but total bilirubin showed no trend. The linear regression models were adjusted for:

- Age, gender, and race/ethnicity.
- Lifestyle (smoking status, drinking status, education level).
- Biomarker data (BMI, metabolic syndrome, iron saturation status, insulin resistance).

In the fully adjusted model, a slight positive association was found between serum PFOS concentration and serum ALT (p = 0.066). A positive association was also found between serum PFOA concentration and serum ALT and PFOA concentration and serum GGT. Data interpretation was limited by the cross-sectional study design, and the fact that other environmental chemicals (possible covariates or explanatory variables) and medication use were not included in the study.

Gallo et al. (2012) investigated the correlation between serum PFOS levels and liver enzymes in a total of 47,092 samples collected from members enrolled in the C8 Health Project. The association of ALT, GGT, and direct bilirubin with PFOS was assessed using linear regression models adjusted for age, physical activity, body mass index, average household income, education level, race, alcohol consumption, and cigarette smoking. Median PFOS level was 0.0233 μg/mL with an interquartile range of 0.0137–0.0294 μg/mL. The ln-transformed values of ALT were significantly associated with ln-transformed PFOS levels (and PFOA) and showed a steady increase in fitted levels of ALT per decile of PFOS, leveling off after approximately 0.030 μg PFOS/mL. Fitted values of GGT showed no overall association with ln-transformed PFOS levels. A positive association was seen with direct bilirubin and PFOS levels in linear regression models, but this was not evident with logistic regression models. Limitations of the study include the cross-sectional design and self-reported lifestyle characteristics. Only a small number of ALT values were outside the normal range, making the results difficult to interpret in terms of health.

The epidemiological data supporting liver damage based on serum ALT and GGT as reported by Gallo et al. (2012) are not strong enough to support an association of serum PFOS alone with liver damage in humans, because in most of the epidemiology studies the serum contains a mixture of PFASs and possibly other exogenous chemicals.

3.1.1.3 Biomarkers of Kidney Function and Kidney Disease

Epidemiology studies of PFOS and kidney function and biomarkers in various populations are described below and summarized in Table 3-3.

Shankar et al. (2011) used data from the NHANES to determine whether there was a relationship between serum PFOS levels and chronic kidney disease. A total of 4,587 adult participants (51.1% females) with PFOS measurements available from the 1999–2000 and 2003–2008 cycles of the survey were examined. Chronic kidney disease was defined as glomerular filtration rate (GFR) < 60 mL/minute (min)/1.73 m^2. Serum PFOS levels were categorized into quartiles: quartile 1 = < 0.012 μg/mL; quartile 2 = 0.012–0.019 μg/mL; quartile 3 = 0.019–0.030 μg/mL; quartile 4 = > 0.030 μg/mL. The multivariable odds ratio for chronic kidney disease for individuals in quartile 4 was 1.82 (95% CI: 1.01–3.27; p for trend = 0.019) compared with individuals in quartile 1. This association was shown to be independent for confounders of age, sex, race/ethnicity, body mass index, diabetes, hypertension, and serum cholesterol level. However, the authors noted that because of the cross-sectional nature of the study, the possibility of reverse causality could not be excluded. A low GFR would diminish the removal of PFOS from serum for excretion by the kidney, thus increasing the serum PFOS levels.

Table 3-3. Summary of Epidemiology Studies of PFOS and Measures of Kidney Function

Reference and Study Details	PFOS levels (µg/mL)	Results
Shankar et al. 2011 USA, NHANES n = 4587 adults PFOS from 1999–2000 and 2003–2008	Quartiles, µg/mL, n 1: < 0.012 µg/mL, 1,152 2: 0.012–0.019 µg/mL, 1,151 3: 0.019–0.030 µg/mL, 1,137 4: > 0.030 µg/mL, 1,147	Estimated glomerular filtration rate (eGFR) Chronic kidney disease defined as eGFR < 60 mL/minute/1.73 m^2 Quartile, OR (95% CI) 1: Referent 2: 1.12 (0.64, 1.99) 3: 1.53 (0.87, 2.67) 4: 1.82 (1.01, 3.27) p = 0.02 Logistic regression adjusting for age, gender, race/ethnicity, education, smoking, alcohol, BMI, systolic blood pressure, diastolic blood pressure, diabetes, serum total cholesterol and glycohemoglobin Adjustment for PFOS did not alter association with PFOA Multivariate regression of association PFOS with eGFR among subjects with and without chronic kidney disease β (SE) with −1.8 (0.8) and without −3.2 (0.6) chronic kidney disease (p < 0.05)
Steenland et al. 2010 USA, C8 Health Project participants n = 54,591 (≥ 20 yrs old)	Mean: 0.0234 ± 0.0161	Increased predicted uric acid of 0.2 to 0.3 µg/dL with increasing deciles of PFOS. Odds Ratio, p-value Hyperuricemia (> 6.0 mg/dL for women and > 6.8 mg/dL for men): 1.00 1.02 (95% CI: 0.95−1.10), p < 0.0001 1.11 (95% CI: 1.04−1.20), p < 0.0001 1.19 (95% CI: 1.11−1.27), p < 0.0001 1.26 (95% CI: 1.17−1.35), p < 0.0001 Trend for increase uric acid more prominent with PFOA
Children		
Watkins et al. 2013 USA, C8 Health Project participants n = 9,660 (1 to < 18 yrs old)	Median: 0.020	β (95% CI) change in unit eGFR (mL/min/1.73 m^2) per ln serum PFOS, −1.10 (−1.66 to −0.53), p = 0.0001 Linear regression adjusting for age, gender, race, smoking, and household income.
Geiger et al. 2014b USA, NHANES n = 1644 (12–18 yrs old)	Mean: 0.018 ± 0.005	Multivariable-adjusted OR (95% CI) between PFOS and hypertension Quartile 1: 1 (referent) Quartile 2: 0.99 (0.55, 1.78) Quartile 3: 0.73 (0.36, 1.61) Quartile 4: 0.77 (0.37, 1.61) p = 0.36 Log transformed PFOS = 0.83 (0.58, 1.19)

Steenland et al. (2010) reported on another analysis of the C8 Health Project participants ≥ 20 years old (n = 54,591) for a possible association between PFOS (and PFOA) serum levels and uric acid. Elevated uric acid is a risk factor for hypertension and may be an independent risk factor for stroke. The mean PFOS level was 0.0234 ± 0.0161 μg/mL. A statistically-significant ($p < 0.0001$) trend was observed between increasing PFOS levels (untransformed) and uric acid levels. A 0.2–0.3 μg/dL increase in uric acid was associated with an increase from the lowest to highest PFOS decile (0.010–0.050 μg/mL). Hyperuricemia (> 6.0 mg/dL for females and > 6.8 mg/dL for males) risk by quintiles increased slightly with PFOS levels (OR 1.00, 1.02, 1.11, 1.19, and 1.26). The serum of C8 study participants included several PFASs; PFOA appeared to have a greater influence on uric acid trends than PFOS in the models employed by Steenland et al. (2010).

Children. Watkins et al. (2013) evaluated the cross-sectional association between PFOS exposure and kidney function among children aged 1 to <18 years (mean 12.4 ± 3.8 years) enrolled in the C8 Health Project. A total of 9,660 participants had data available on serum PFOS (median = 0.020 μg/mL), serum creatinine, and height, which were used to calculated an estimated glomerular filtration rate (eGFR). Linear regression was used to evaluate the association between quartiles of measured serum PFOS concentration and eGFR. A shift from the lowest to the highest quartile of measured, natural log–transformed concentrations of PFOS in serum [IQR ln-(PFOS) = 0.64] was associated with a decrease in eGFR of 1.10 mL/min/1.73 m^2 (95% CI: −1.66 to −0.53; $p = 0.0001$) adjusting for age, sex, race, smoking status, and household income. With increasing quartile of serum PFOS concentrations, eGFR decreased monotonically with a decrease of 2.3, 2.6, and 2.9 mL/min/1.73 m^2 for the second, third, and fourth quartile of serum PFOS, respectively, compared with the lowest quartile (p for trend across quartiles = 0.0001).

Geiger et al. (2014b) used data from the NHANES to determine whether there was a relationship between serum PFOS levels and hypertension in children. A total of 1,655 participants (aged 12–18 years) with PFOS measurements available from the 1999–2000 and 2003–2008 cycles of the survey were examined. Blood pressure was measured to determine the presence of hypertension and linear regression modeling was used to study the association between increasing quartiles of serum PFOS and mean changes in systolic and diastolic blood pressures. Mean PFOS level was 0.018 ± 0.005 μg/mL. No association was found between serum PFOS levels and hypertension in either unadjusted or multivariable-adjusted analyses. Compared with the lowest quartile, the multivariable-adjusted odds ratio (95% confidence interval) of hypertension in the highest quartile of exposure was 0.77 (0.37–1.61) (p-trend > 0.30).

3.1.1.4 Reproductive Hormones and Reproductive/Developmental Studies

Many of the studies of PFOS focused on pregnancy-related outcomes, including measures of fetal growth retardation, puberty, and other developmental endpoints, as well as pregnancy-related hypertension, preeclampsia, and gestational diabetes. Reproductive outcomes such as measures affecting fertility were also evaluated. Within each section, the discussion is divided into occupational exposure studies (if applicable) and general population studies. Epidemiology studies of PFOS and pregnancy-related outcomes in various populations are described below and summarized in Table 3-4.

Table 3-4. Summary of Epidemiology Studies of PFOS and Pregnancy Outcomes

Study	PFOS level (µg/mL)	Results
Stein et al. 2009 United States (C8 Health Project) n = 5,262 pregnancies Self-reported pregnancy outcomes in mid-Ohio Valley in 2000–2006.	Median: 0.014	OR (95% CI), preeclampsia per IQR(lnPFOS) increase in PFOS: 1.1 (0.9, 1.3) < 50th percentile 1.0 (referent) ≥ 50th percentile 1.3 (1.1, 1.7) < 50th percentile 1.0 (referent) 50th–<75th percentile 1.3 (1.0, 1.7) 75th–90th percentile 1.1 (0.8, 1.6) ≥ 90th percentile 1.6 (1.2, 2.3)
Darrow et al. 2013 United States (C8 Health Project) n = 1,630 live births from 1,330 women after January 1, 2005	Geometric mean: 0.0132	Pregnancy induced hypertension OR (95% CI) per log unit increase in PFOS: 1.47 (1.06, 2.04) By quintile: Q1 up to 0.0086 µg/mL 1.0 (referent) Q2 0.0086– < 0.0121 1.46 (0.69, 3.11) Q3 0.0121– < 0.0159 2.71 (1.33, 5.52) Q4 0.0159– < 0.0214 2.21 (1.07, 4.54) Q5 ≥ 0.0214 1.56 (0.72, 3.38) Q1 up to 0.0086 µg/mL 1.0 (referent) First pregnancy after PFOS measure 2.02 (1.11, 3.66)
Zhang et al. 2015 n = 258 women as part of LIFE study. Blood samples taken during 2005–2009.	Mean: 0.0131 with gestational diabetes and 0.012 without	Gestational diabetes OR (95% CI) associated with SD increment of preconception PFOS log-transformed concentration OR 1.13 (0.75, 1.72) (fully adjusted for age, BMI, smoking, etc.)

Pregnancy-related Outcomes. Stein et al. (2009) examined serum levels of PFOS and self-reported pregnancy outcomes of a population of females (5,262 pregnancies; aged 15–55 years) in the mid-Ohio Valley in 2000–2006. These females were enrollees in the C8 Health Project, a community health study of residents near a chemical plant that used PFOA in the manufacture of fluoropolymers. Pregnancies within the 5 years preceding the exposure measurements were analyzed. The mean level of PFOS in the serum of these females was 0.014 µg/mL. There was no association between PFOS levels and miscarriages. PFOS was associated with preeclampsia (adjusted odds ratio = 1.3; 95% CI: 1.1–1.7). Similarly, PFOA was not associated with miscarriage and only weakly associated with preeclampsia. The self-reported nature of pregnancy outcomes is a recognized limitation with uncertain impact on study results.

Darrow et al. (2013; 2014) analyzed pregnancy outcomes for the five years following enrollment in the C8 Health Project. Among the 69,030 females who provided serum for PFOS measurement in 2005–2006, 32,354 provided follow-up interviews on reproductive histories. After exclusions, 1,630 singleton live births from 1,330 females after January 1, 2005 were linked to birth records to identify outcomes of preterm birth (i.e., < 37 gestational weeks), pregnancy-induced hypertension, low birth weight (LBW) (i.e., < 2500 grams), and birth weight among full-term infants (Darrow et al. 2013). Effects on fetal growth measures are described in that section below. Another subset of 1,129 females with a total of 1,438 pregnancies was evaluated for an association between PFOS levels and miscarriage (Darrow et al. 2014). The baseline mean PFOS level for these females was 0.016–0.017 µg/mL. Confounders that were adjusted in each model for every outcome in the 2013 Darrow et al. study included maternal age,

educational level, smoking status, parity, BMI, self-reported diabetes, time between conception and serum measurement. Parity was excluded, and race was included in the miscarriage analysis (Darrow et al. 2014).

An increased risk of pregnancy-induced hypertension was detected per log unit increase in PFOS (OR = 1.47; 95% CI: 1.06−2.04) and PFOA (OR = 1.27; 95% CI: 1.05−1.55). Although monotonicity was not evident, consistently increased odds were found across all upper PFOS (OR range: 1.46–2.72) and PFOA (OR range: 2.39–3.43) quintiles.

The odds of miscarriage per each log unit increase in PFOS was 1.21 (95% CI: 0.94−1.55) for all reported prospective pregnancies and 1.34 (95% CI: 1.02−1.76) when restricted the analysis to each woman's first pregnancy. Miscarriage results were comparable across all PFOS quintiles in the primary analysis (OR range: 1.34–1.59) and those restricted to first pregnancy (OR range: 1.68–1.94). PFOA was not associated with miscarriage and was not a confounder of the observed association with PFOS. To address the potential for reverse causality related to PFAS levels decreasing from prior pregnancies, analyses were restricted to nulliparous and nulligravid females. Adjusted odds ratios were higher across all four quintiles for nulliparous (OR range: 1.88–3.08) and nulligravid females (OR range: 2.04–3.73). These studies represent prospective assessment of PFASs in relation to adverse pregnancy outcomes, which address some of the limitations in the available cross-sectional studies. The impact of measurement error resulting from unknown critical exposure windows and the time lag (> 99% of births were within 3 years) between the estimated conception date and the serum collection is unclear in these studies.

Preconception serum levels of PFOS (and other PFASs) were evaluated in females attempting pregnancy in relation to risk of developing gestational diabetes (Zhang et al. 2015). The 258 participants were members of the Longitudinal Investigation of Fertility and the Environment (LIFE) study with blood samples taken during 2005–2009. The ORs and 95% CIs of gestational diabetes associated with each SD increment of preconception serum PFOS concentration (log-transformed) (and six other PFAS) were estimated with the use of logistic regression after adjusting for age, pre-pregnancy body mass index, smoking, and parity, each conditional on the number of times a woman had been pregnant. Preconception mean serum PFOS levels were 0.0131 μg/mL in females with gestational diabetes and 0.012 μg/mL in females without gestational diabetes (p-value for mean difference = 0.10). A positive association was found between PFOS and risk of gestational diabetes in the fully adjusted model (OR = 1.13; 95% CI: 0.75−1.72). PFOA was the only PFAS that was significantly associated with developing gestational diabetes in this analysis.

Fetal Growth. Many different measures of fetal growth can be used in epidemiology studies. Birth weight is widely available (as it is routinely collected in medical records and birth certificates). LBW (defined as < 2500 g) can be a proxy measure for preterm birth (particularly when accurate gestational age dating is not available). Other measures of fetal growth, such as small for gestation age, tend to more accurately reflect fetal growth retardation. Epidemiology studies of PFOS and fetal growth are described below and summarized in Table 3-5.

Table 3-5. Summary of Epidemiology Studies of PFOS and Fetal Growth

Study	PFOS level (µg/mL)	Results
Grice et al. 2007 United States (C8 Health Project) n = 263 women reporting 429 births Self-reported pregnancy outcomes in workers associated with perfluorinated chemical production factory.	Exposure to PFOS was based on job assignment and varied Never exposed: 0.11–0.29 ppm Low exposure: 0.39–0.89 ppm High exposure: 1.30–1.97 ppm	No association between PFOS exposure and mean birth weight Regression coefficients for birth weight compared to never-exposed pregnancies, 95% CI (adjusted for maternal age, smoking, gravidity) Ever exposed, low exposure −0.08 (−0.25, 0.09) Ever exposed, high exposure 0.07 (−0.14, 0.28) High exposure, > 1 yr 0.11 (−0.11, 0.33) Low or high exposure, > 1 yr −0.03 (−0.19, 0.13) Ever exposed, low or high - 0.05 (−0.20, 0.11)
Apelberg et al. 2007 United States (Baltimore) n = 293 newborns born between November 2004 and March 2005 Cord blood samples	Geometric mean: 0.005	Change in birth weight (g) per log unit increase (95% CI) −69 (−149, 10)
Fei et al. 2007 n = 1,400 women and their infants randomly selected from the group enrolled in the DNBC	Mean: 0.035	LBW OR (95% CI) for LBW by quartile Q1 0.0064 to 0.026 µg/dL 1.0 (referent) Q2 0.026 to 0.033 µg/dL 3.5 (0.37, 31.16) Q3 0.033 to 0.043 µg/dL 6.0 (0.73, 49.34) Q4 ≥ 0.043 µg/dL 4.8 (0.56, 41.16) Trend: p = 0.13
Andersen et al. 2010 n = 1,010 women and their infants randomly selected from the group enrolled in the DNBC	Median: 0.0334 (range: 0.0064–0.1067)	PFOS concentrations per each 0.001 µg/mL increase inversely associated with: birth weight in girls: Beta = −3.2; 95% CI: −6.0 to −0.3 weight at 12 months in boys: Beta = −9.0; 95% CI: −15.9 to −2.2
Monroy et al. 2008 n = 101 pregnant women as part of a larger cohort study conducted at McMaster University Medical Center	Mean: 0.0183 in maternal serum (24–28 wks) 0.0162 in maternal serum at delivery 0.0072 in umbilical cord blood	No association between PFOS levels and infant birth weight Change in PFOS per g change in birth weight Beta = 0.000853 (p = 0.73)
Washino et al. 2009 Japan n = 428 women and their infants between July 2002 and October 2005	Mean: 0.006	Change in birth weight per log unit increase (95% CI) For all: Beta = −149 g (−297.0, −0.5) For female infants: Beta = −269.4 g (−465.7, −73.0)
Hamm et al. 2010 Canada n = 252 women with blood samples taken between December 2005 and June 2006	Mean: 0.009	Change in birth weight per Ln unit increase (95% CI) 31.3 g (−43.3, 105.9)
Stein et al. 2009	Mean: 0.014	OR (95% CI), birth weight < 5.5 lbs. per IQR(lnPFOS) increase in PFOS: 1.3 (1.1, 1.6) < 50th percentile 1.0 (referent) ≥ 50th percentile 1.5 (1.1, 1.9) < 50th percentile 1.0 (referent) 50th–<75th percentile 1.3 (0.9, 1.8) 75th–90th percentile 1.6 (1.1, 2.3) ≥ 90th percentile 1.8 (1.2, 2.8)

Study	PFOS level (µg/mL)	Results
Darrow et al. 2013 United States (C8 Health Project) n = 1,630 live births from 1,330 women after January 1, 2005	Geometric mean: 0.0132	LBW OR (95% CI) per LBW (< 2,500 g) per log unit increase: 1.12 (0.75, 1.67) By quintile: Q1 up to 0.0086 µg/mL 1.0 (referent) Q2 0.0086– < 0.0121 1.48 (0.71, 3.08) Q3 0.0121– < 0.0159 1.23 (0.57, 2.65) Q4 0.0159– < 0.0214 1.31 (0.59, 2.94) Q5 ≥ 0.0214 1.33 (0.60, 2.96) First pregnancy after PFOS measure 0.97 (0.61, 1.54)

An occupational cohort study by Grice et al. (2007) examined the relationship between PFOS exposure and self-reported adverse pregnancy outcomes in employees at a perfluorinated chemical production facility in Decatur, Alabama. Current and former female employees of the facility completed a questionnaire and provided a brief pregnancy history. The level of exposure was categorized according to a job-specific exposure matrix. A total of 263 females participated (participation rate = 73%) and reported 439 births, of which there were 421 live births, 14 stillbirths, and 4 with missing outcome data. The birth weight models of single births were adjusted for maternal age, smoking status, and gravidity. No associations were detected between PFOS exposure and the pregnancy outcomes that were examined (i.e., stillbirth and mean birth weight).

Apelberg et al. (2007) measured PFOS in the cord blood of 293 newborns (singleton births without congenital anomalies) born November 26, 2004 through March 16, 2005 at Johns Hopkins Hospital in Baltimore, Maryland. Maternal and infant data, including maternal birth cohort, social class, place of residence, past pregnancies, insurance type, BMI, age, race, education, marital status, parity, gestational age, smoking status, and infant sex were collected from the hospital database and forms filled out at time of delivery. PFOS was found in > 99% of the cord blood samples (geometric mean 0.005, range < level of detection [0.2]–0.035 µg/mL). PFOS concentrations were evenly distributed across larger maternal age categories. The non-smoker and passively exposed individuals (5.2 ng/mL) had higher mean PFOS levels than smokers (4.1 ng/mL), as did Asians (6.5 ng/mL) and Blacks (5.2 ng/mL) compared to Caucasians (4.5 ng/mL). No associations were observed between PFOS and maternal age, gestational age, BMI, or various socioeconomic measures (e.g., education, insurance, marital status, living in Baltimore City). Birth weight, head circumference, and ponderal index were inversely associated with both cord PFOS and PFOA levels. For example, large deficits in mean birth weight per one ln-unit increase were found for both PFOS (β = −69; 95% CI: −149–10) and PFOA (β = −104 g; 95% CI: −213–5).

A series of longitudinal, population-based studies was conducted in a subset of 91,827 females aged 25–35 enrolled in the Danish National Birth Cohort (DNBC) from March 1996 to November 2002 (Andersen et al. 2013; Fei et al. 2007, 2008a, 2008b, 2009, 2010a). This prospective birth cohort was comprised of a random sample of 1,400 females who were recruited through general practitioners around weeks 6–12 of gestation to investigate the association between blood levels of perfluorinated chemicals and adverse reproductive and developmental outcomes in the females and their children. This subset was sampled from 43,035 females with singleton live births without congenital malformation who provided the first blood sample between gestational weeks 4 and 14 and who responded to all four telephone interviews. Study data were collected by telephone interviews at 12 and 30 weeks of gestation, approximately

6 and 18 months after birth, and when the children were 7 years of age. A food frequency questionnaire was filled out at home during approximately week 25 of pregnancy. Maternal blood samples were taken in the first and second trimester, and infant cord blood was sampled just after birth. Only blood results from the 1,400 females in the first trimester were reported. Mean plasma PFOS levels by age groups were: < 25 years: 0.039 μg/mL; 25–29 years: 0.037 μg/mL; 30–34 years: 0.034 μg/mL and ≥ 35 years: 0.033 μg/mL.

Potential confounders for which adjustments were made included: maternal age, maternal occupation and educational status, parity, pre-pregnancy BMI, smoking/alcohol consumption during pregnancy, gestational weeks at blood draw, child's sex, child's age at interview with mother, breast-feeding > 6 months (for 18-month interview), out-of-home child care, hours mother spent with child per day, and home density (the total number of rooms divided by the total number of people in the household). Although dietary data were available for at least 80% of the births, it is unclear why some of these studies did not examine these data as confounders (e.g., Fei et al. 2009). Although the DNBC had a low participation rate (31%), a previous study of various exposures in relation to three different outcomes (preterm birth, small-for-gestational-age, infancy and antepartum stillbirth) did not provide any evidence of non-participation bias (Nohr et al. 2006).

Using data from the DNBC, Fei et al. (2007) investigated the association between plasma levels of PFOS in pregnant females, length of gestation, preterm birth (i.e., < 37 gestational weeks), and infant birth weight. The average PFOS levels in maternal plasma were 0.035μg/mL (range: 0.0064–0.107 μg/mL). The data were adjusted for confounding factors that might also influence fetal growth or length of gestation and analyzed by analysis of variance and linear regression using both continuous PFOS concentrations and PFOS quartiles. No associations between PFOS and birth weight were found. PFOA concentrations based on the continuous exposure measures were inversely associated with birth weight (β= −10.6; 95% CI: −20.8 to −0.5) following adjustment for confounding (unadjusted β= −20.5; 95% CI: −31.5 to −9.6). Although most were not statistically-significant, ORs for preterm birth were consistent in magnitude (OR range: 1.43–2.94) across both the upper three PFOS and PFOA quartiles. Consistently elevated ORs were also detected (OR range: 3.39–6.00) for LBW across the upper three PFOS and PFOA quartiles, but all of these analyses were limited by very small cell sizes given low incidence of these outcomes. Although these ORs often lacked statistical significance due to low statistical power, the elevated odds detected between PFOS levels and various outcomes including preterm delivery and LBW warrant further research, especially given the potential generalizability limitations of this low-risk study population.

Fei et al. (2008a) also investigated the association between PFOS levels and placental weight, birth length, and head and abdominal circumference in the DNBC study population. Maternal PFOS levels were not associated with any of the fetal growth indicators when the lowest quartile was compared to the highest. In a stratified analysis of PFOS, inverse associations were found with birth length for post-term and pre-term infants and with ponderal index (relationship between mass and height) in multiparous females. In nulliparous females the association was positive. These associations were not statistically-significant.

Andersen et al. (2010) examined the association between maternal plasma PFOS concentration and offspring weight, length, and BMI at 5 and 12 months of age from participants in the DNBC. The mothers (n = 1,010) reported the information during an interview and weight and length measurements were used to calculate BMI. Median maternal plasma PFOS level was 0.0334 μg/mL with a range of 0.0064–0.1067 μg/mL. PFOS concentrations (per each 0.001

μg/mL increase) were inversely associated with birth weight in girls (β= −3.2; 95% CI: −6.0 to −0.3), weight at 12 months in boys (β= −9; 95% CI: −15.9 to −2.2), and BMI at 12 months in boys (β= −0.017; 95% CI: −0.028 to −0.005) in models adjusted for maternal age, parity, prepregnancy BMI, smoking, gestational age at blood draw, socioeconomic status, and breastfeeding. Similar inverse associations were found with PFOA only in boys.

Monroy et al. (2008) examined the relationship between the maternal serum levels of PFOS and PFOA and infant birth weight from neonates born to 101 pregnant females enrolled in a large cohort study, the Family Study, conducted at McMaster University Medical Center in Ontario, Canada. Linear regression analyses were adjusted for parity, gestational length, BMI, gender, and smoking status as confounding factors. PFOS was measured in maternal serum from 24–28 weeks of gestation and at delivery and in umbilical cord blood (UCB) from 105 babies. PFOS was detected in all of the collected samples with mean levels of 0.0183, 0.0162, and 0.0072 μg/mL in maternal serum at 24–28 weeks, maternal serum at delivery, and in UCB, respectively. The concentration of PFOS in maternal serum was significantly higher than in UCB (mean ratio of UCB/maternal serum at delivery was 0.45). No statistically-significant associations were detected between levels of PFOS in the maternal serum or UCB and infant birth weight. Maternal PFOS levels were also not associated with maternal body mass index, gestational length, or gender. Results were similar for PFOA.

A prospective cohort study was conducted on birth weight between July 2002 and October 2005 at the Sapporo Toho Hospital in Hokkaido, Japan that included 428 native Japanese females and their infants (Washino et al. 2009). Females enrolled were at 23–35 weeks of gestation with a mean age of 30.5 years. Exclusion criteria included maternal pregnancy-induced hypertension, diabetes mellitus, fetal heart failure, and multiple births (i.e., restricted to singletons). A self-administered questionnaire survey after the second trimester of pregnancy was used by the subjects to report dietary habits, smoking status, alcohol consumption, caffeine intake, household income, and educational level. Other potential confounding factors collected from medical records included prepregnancy BMI, pregnancy complications, gestational age, infant sex, parity, infant disease, birth weight, and birth size. A blood sample was collected for measurement of PFOS and PFOA during the second trimester when the questionnaire was administered or after pregnancy for anemic mothers. The mean concentration of PFOS in the females was 0.006 μg/mL with detection in 100% of samples. The highest PFOS concentration identified was 0.016 μg/mL. The results indicated that large reductions in mean birth weight (β = −149 g; 95% CI: −297.0 to −0.5) were detected for each log-10 change in maternal serum PFOS exposure, especially among female infants (β = −269.4 g; 95% CI: −465.7 to −73.0). Large birth weight deficits were also detected per each unit increase in PFOA for both males (−68.1 g; 95% CI: −246.2–110.0) and females (−76.7 g; 95% CI: −234.7–81.3), with an overall change in mean birth weight of 75 grams (95% CI: −191.8–41.6).

A cohort study on pregnant females (≥ 18 years old) at 15–16 weeks gestation in the city of Edmonton, Alberta, Canada was undertaken to examine a possible association between perfluorinated chemicals, fetal growth, and gestational age (Hamm et al. 2010). The study population included 252 pregnant females who elected to undergo a second trimester prenatal *triplescreen* at 15–16 weeks of gestation for Down's syndrome, trisomy 18, and open spina bifida. This population was restricted to mothers > 18 years of age who gave birth to live singletons without evidence of malformations, and who delivered at greater than or equal to 22 weeks of gestation. Serum samples collected from December 2005 to June 2006 during the second trimester had PFOS levels ranging from nondetectable to 0.035 μg/mL, with the mean

and geometric mean being 0.009 μg/mL and 0.0074 μg/mL, respectively. Potential confounders included maternal age, maternal weight, maternal height, maternal smoking status, maternal race, gravida, gestational age at the time of serum collection, infant sex, infant birth weight, and infant gestational age at birth. Overall, there was no association with the level of PFOS and birth weight or length of gestation. Mean birth weight increased slightly by increasing PFOS tertiles (3,278 g for < 0.006 μg/mL; 3,380 g for 0.006–0.010 μg/mL; 3,387 g for > 0.010–0.035 μg/mL). The mean length of gestation for all groups was 38 weeks; the preterm delivery percentage was similar between groups. Similar associations were found for other PFASs, which were correlated with serum PFOS including PFOA (Spearman correlation coefficient = 0.52) and perfluorohexane sulfonate (Spearman correlation coefficient = 0.54).

In addition to the pregnancy-related outcomes discussed previously, Stein et al. (2009) examined fetal growth outcomes among females enrolled in the C8 Health Project. Pregnancies within the 5 years preceding the exposure measurements were analyzed. The mean level of PFOS in the serum of these females was 0.014 μg/mL at the time of measurement. There was no association between PFOS levels and preterm births. PFOS was, however, associated with an increased risk above the median (adjusted odds ratio = 1.5: 95% CI: 1.1–1.9) for LBW, and a dose-response relationship was reported for the 50^{th}–75^{th}, 75^{th}–90^{th} and > 90^{th} percentile serum PFOS exposure concentrations (adjusted ORs = 1.3, 1.6, and 1.8, respectively). Similarly, PFOA was not associated with LBW and preterm birth. The self-reported nature of pregnancy outcomes is a recognized limitation with uncertain impact on study results. Although this 5-year window was intended to ensure that measured PFAS values at the time of study enrollment reflected exposure level at the time of pregnancy, this could have resulted in exposure misclassification given changes in maternal PFAS levels that could have occurred between the time of serum collection and pregnancy and lactation because measures had been implemented to decrease population exposures.

Darrow et al. (2013, 2014) analyzed pregnancy outcomes for the 5 years after enrollment in the C8 Health Project. Among the 69,030 females who provided serum for PFOS measurement in 2005–2006, 32,354 provided follow-up interviews on reproductive histories. After exclusions, 1,630 singleton live births from 1,330 females after January 1, 2005 were linked to birth records to identify outcomes of preterm birth (i.e., < 37 gestational weeks), LBW, and birth weight among full-term infants (Darrow et al. 2013). Another subset of 1,129 females with a total of 1,438 pregnancies was evaluated for an association between PFOS levels and miscarriage (Darrow et al. 2014). The baseline mean PFOS level for these females was 0.016–0.017 μg/mL. Confounders that were adjusted in each model for every outcome in the 2013 Darrow et al. study included maternal age, educational level, smoking status, parity, BMI, self-reported diabetes, and time between conception and serum measurement. Parity was excluded and race was included in the miscarriage analysis (Darrow et al. 2014). Maternal serum PFOS levels were not associated with preterm birth or LBW. An inverse association was found between PFOS and mean birth weight in full-term infants (−29 g per log unit increase; 95% CI: –66–7). PFOA was not associated with mean birth weight, and therefore was not a confounder of this association. These studies represent prospective assessments of PFASs in relation to adverse pregnancy outcomes thereby avoiding some of the limitations of the cross-sectional studies. The impact of measurement error resulting from unknown critical exposure windows and the time lag (> 99% of births were within 3 years) between the estimated conception date and the serum collection is unclear.

Preeclampsia is a condition where the pregnant female is hypertensive because of reduced renal excretion associated with a decrease in GFR. Preecampsia is often accompanied by LBW (Whitney et al. 1987). Morken et al. (2014) used a subset of the Norwegian Mother and Child Cohort to evaluate the relationship between GFR and fetal size. Participants included 470 preeclamptic patients and 483 non-preeclamptic females; plasma creatinine measured during the second trimester was used to estimate GFR. For the overall cohort, for each mL/min increase in GFR, infant weight at birth increased 0.73–0.83 g depending on the method used to calculate GFR. The increases in body weight with increased GFR were greater, and statistically-significant, in females with preeclampsia. Differences were not statistically-significant for the nonpreeclamptic group. Morken et al. (2014) was not a study of perfluorochemicals, and there were no serum measurements of any PFAS. However because PFOA/PFOS serum levels are expected to be higher with a lower GFR, the finding stimulated examination of the GFR as it relates to serum PFAS levels and the LBW identified in the epidemiology studies (Vesterinen et al. 2014; Verner et al. 2015).

Evidence for an inverse association between PFAS levels and birth weight raised the question of reverse causality linked to maternal GFR. PFOS excretion by the kidney is dependent, in part, by the GFR. Conditions that result in impairment of GFR (and, thus, increased serum PFOS) and are also related to fetal growth restriction could result in a confounded observation of an association between PFOS and decreased birth weight. Vesterinen et al. (2014), using the Navigation Guide systematic review methods, examined evidence pertaining to the relation between fetal growth and maternal GFR. They identified relevant studies that met the Navigation Guide criteria for inclusion in the analysis; none included consideration of PFOS or other PFASs. All studies were rated as *low* or *very low* quality leading to the conclusion that data were *inadequate* to determine an association between fetal growth and GFR.

Verner et al. (2015) modified the PK model of PFOS during pregnancy by Loccisano et al. (2013) described in section 2.5.1 to evaluate the association between GFR, serum PFOS levels and birth weight. When low GFR was accounted for in the model simulations, the reduction in birth weight associated with increasing serum PFOS was less than that found by the author's meta-analysis of the same data. This finding suggests that a portion of the association between prenatal PFOS and birth weight could be confounded by maternal GFR differences within the populations studied. The true association for each 1 ng/mL increase in PFOS could be closer to a 2.72 g reduction (95% CI: −3.40 to −2.04) in body weight compared to the 5.00 g reduction (95% CI: −21.66 to −7.78) predicted by meta-analysis of the epidemiology data without a correction for low GFR.

Other Developmental Effects. Fei et al. (2010a) reported on the effects of PFOS and PFOA on the length of breastfeeding. Self-reported data on the duration of breastfeeding were collected during the telephone interviews at 6 and 18 months after birth of the child. Statistically-significant higher levels of PFOS were associated with a shorter duration of breastfeeding following adjustment for confounding. This is an expected consequence because PFOS is transferred from the mother during breast feeding; thus, the shorter the lactation period the greater the proportion of the serum PFOS at the time of birth remains with the mother. A 20% increase risk for the mother in weaning before 6 months was noted in both primiparous [OR = 1.20; 95% CI: 1.04–1.37] and multiparous females, [OR = 1.20; 95% CI: 1.06–1.37]) for each 0.010 μg/mL increase in PFOS concentration in the maternal blood.

A dose-response relationship was noted only among multiparous females (OR range: 1.55–2.64) based on categorical PFOS exposures, as only the highest PFOS quartile showed an elevated effect estimate [OR = 1.52; 95% CI: 0.89–2.60]) among primiparous females. For analyses based on termination of exclusive breastfeeding before 4 months, associations were only seen among multiparous females for both PFOS and PFOA exposures. Given that the associations between length of breastfeeding and PFOA and PFOS exposures were largely only seen among multiparous females, reverse causality is a possible explanation since reductions of current PFOS and PFOA levels may have resulted from longer lactation periods for previous children.

Andersen et al. (2013) evaluated the association between maternal plasma PFOS levels and the children's body mass index, waist circumference, and risk of being overweight at 7 years of age. From the subset of 1,400 randomly selected females from the DNBC who provided blood samples during their first trimester, only those children with weight and height information (n = 811) or waist measurements (n = 804) at age 7 years were included in the analysis. Maternal plasma PFOS levels were evaluated as both continuous and categorical exposures. Maternal PFOS concentrations were inversely associated with all of the children's anthropometric endpoints, but statistical significance was not attained and a dose-response relationship was not observed. Neither maternal PFOS nor PFOA levels were associated with anthropometric measures in either boys or girls at age 7 in this prospective birth cohort.

A case-cohort study from the DNBC population was used to evaluate the relationship between prenatal PFAS exposure and the risk of congenital cerebral palsy (Liew et al. 2014). From a source population of 83,389 mother-child pairs, 156 cases of cerebral palsy were identified and matched to 550 randomly selected controls (including 440 boys). Stored maternal plasma samples collected in early or mid-pregnancy were analyzed for 16 PFAS; six compounds were quantifiable in > 90% of the samples. For the cerebral palsy cases and matched controls, median maternal PFOS levels were 0.0289 and 0.0276 µg/mL, respectively, for boys and 0.0275 and 0.0262 µg/mL, respectively, for girls. A statistically-significant increased risk of developing cerebral palsy in boys (rate ratio [RR] = 1.7; 95% CI: 1.0–2.8) was detected per each natural-log unit increase in maternal PFOS level. A dose-response relationship between cerebral palsy and categorical PFOS exposures was detected in boys. Positive associations were also found with PFOA and perfluoroheptanesulfonate (PFHpS), and the results for PFOS remained unchanged after adjusting for multiple PFAS in the regression models. No association was found between any PFAS level and risk of cerebral palsy in girls, although this analysis was much more limited by smaller numbers.

Fei and Olsen (2011) examined the association between prenatal PFOS (and PFOA) exposure and behavior or coordination problems in children aged 7 enrolled in the DNBC study. Behavioral problems were assessed using the Strengths and Difficulties Questionnaire (SDQ), and coordination problems were assessed using the Developmental Coordination Disorder Questionnaire (DCDQ) completed by the mothers. A total of 787 mothers completed the SDQ and 537 completed the DCDQ for children aged 7.01–8.47 years (mean age 7.15 years). The mean maternal PFOS concentration was 0.036 µg/mL, and PFOS levels were divided into quartiles: <LLOQ–0.00395, 0.00396–0.00532, 0.00535–0.00711, and 0.00714–0.02190 µg/mL. The primary analyses of dichotomized outcomes were examined using logistic regression. Linear regression and ordinal logistic regression were also used to examine the full scale of behavioral scores. There were no statistically-significant associations detected between 4th quartile PFAS exposures and various outcomes, including total difficulties, emotional symptoms, hyperactivity

score, conduct problems, or peer problems. Odds ratios adjusted for different outcomes were adjusted for the following confounders: parity, maternal age, pre-pregnancy BMI, smoking and alcohol consumption during pregnancy, sex of the child, breastfeeding, birth year, housing density, gestational age at blood draw, and parental behavioral problem scores during their childhood. Overall, no associations between behavioral or coordination problems in children 7 years of age and prenatal PFOS (and PFOA) exposure were found.

A prospective birth cohort study called INUENDO[3] was designed to examine biopersistent organochlorines in diet and human fertility (Høyer et al. 2015b). Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,106 mother-child pairs at follow-up between January 2010 and May 2012 when the children were 7–9 years old. The study population consisted of 526 pairs from Greenland, 89 from Poland, and 491 from Ukraine. Since maternal blood samples for measurement of plasma PFOS levels were taken any time during pregnancy, median gestational age at time of collection varied by country (range: 23–33). Behavior of children was assessed with SDQ score, and logistic regression models were used in the analyses of PFOS tertiles and behavioral problems. Motor development was assessed with DCDQ score, and linear regression was used for analyses. All analyses were performed on the entire cohort, as well as by country although not all analyses could be performed on the Polish subset due to the small number of cases. Analyses were adjusted for the following potential confounders: maternal cotinine level during pregnancy, maternal alcohol consumption at conception, maternal age at pregnancy, gestational age at blood-sampling, and child gender.

The median maternal plasma PFOS level was 0.01 μg/mL for the combined population and 0.02, 0.005, and 0.008 μg/mL for the pregnant females from Greenland, Ukraine, and Poland, respectively. No associations were found between PFOS (and PFOA) levels and motor development score. Total SDQ score was not associated with PFOS levels; however, PFOS concentrations were associated with higher total SDQ score only in Greenland. The highest PFOS tertile was associated with a 0.5 point higher hyperactivity scores in the combined analysis in Greenland (0.3) and Poland (1.3), but no association was found in Ukraine. The adjusted OR for hyperactive behavior in the combined analysis was 1.4 (95% CI: 0.4–4.9) for the highest tertile compared to the lowest PFOS tertile, with comparable results found for Greenland and Ukraine. Although statistical adjustment in the regression models included country of participant, inter-country differences complicate interpretation of the study results especially given variability in exposure data collection periods and vastly different participation rates (e.g., 37% in Poland and 86% in Greenland). In addition to the potential for selection and information biases, the unknown critical exposure window(s), including the impact of unmeasured post-natal exposures, for these outcomes increases the uncertainty of these study results.

Fei et al. (2008b) examined the association between plasma levels of PFOS in pregnant females and the motor and mental development in their children. The developmental measures examined in the infants included Apgar score of child at birth and maternal reported questionnaire responses about child development milestones at 6 and 18 months. Using linear regression, no significant association between PFOS and Apgar score was observed after adjustment for potential confounders (OR = 1.20; 95% CI: 0.57–2.25). Although these data were limited by maternal reporting of the outcome data, there was no association between PFOS levels and motor or mental development as reported in the questionnaire at 6 months. In children at 18 months, mothers with higher PFOS levels were slightly more likely to report that their babies

[3] Biopersistent Organochlorines in Diet and Human Fertility study.

started sitting without support at a later age and "did not use word-like sounds to tell what he/she wants." No statistically-significant associations were found with PFOA.

Hoffman et al. (2010) examined the associations between perfluorochemicals, including PFOS, and diagnosis of attention deficit hyperactivity disorder (ADHD) using the NHANES data from 1999–2000 and 2003–2004. The study population included 571 children aged 12–15 years including those who had been diagnosed as having ADHD (n = 48) and/or taking ADHD medications (n = 21). Various potential confounders were considered, including birth weight, admittance to a neonatal intensive-care unit, socioecomonic status, health insurance coverage, having a routine health care provider, preschool attendance, and lead exposure. NHANES sample cycle, age, sex, race/ethnicity, living with a smoker, and maternal smoking were adjusted for in the logistic regression models. The median serum PFOS levels were 0.023 μg/mL and ranged from 0.002 to 0.09 μg/mL. Serum PFOS was positively associated with parental report of ADHD (OR = 1.03, 95% CI: 1.01–1.05). The adjusted odds ratio per each 1 μg/L increase in serum PFOA for parental report of ADHD and ADHD medication use was 1.05 (95% CI: 1.02–1.08). Both PFOA and perfluorohexane sulfonate were also positively associated with parentally-reported ADHD. Data interpretation were limited by the cross-sectional study design, other potential confounders (e.g., alcohol consumption) that were not included in the available data, and measurement error resulting from using current PFOS levels as proxy measures of etiologically relevant exposures.

In a prospective study, Halldorsson et al. (2012) examined prenatal exposure to PFASs, including PFOS, and the risk of being overweight at 20 years of age. A birth cohort consisting of 965 singleton pregnancies were recruited from a midwife center in Aarhus, Denmark. Maternal PFOS levels were measured in serum samples collected during week 30 of gestation for assessment of *in utero* PFOS exposure and offspring anthropometry at 20 years of age. Among the 965 study subjects, 915 of their offspring were located and 665 agreed to participate. The median PFOS concentration was 0.0215 ± 0.0019 μg/mL with quartiles of 0.016 ± 0.0056, 0.0202 ± 0.0057, 0.0236 ± 0.0068, and 0.0285 ± 0.0021 μg/mL. Four PFASs, including PFOA, PFOS, PFOSA, and perfluorononanoic acid (PFNA) exhibited sufficient contrasts to examine quartiles of exposure; while eight of the other quantified PFASs did not. PFOS was positively associated with female offspring BMI at 20 years. Maternal PFOS concentrations were not associated with offspring anthropometry at 20 years. Associations of PFOS and other variables including smoking status; waist circumference; or insulin, leptin, or adiponectin concentrations at 20 years were not reported. Therefore, possible confounding cannot be assessed. Study strengths include a high rate of participation (69%) in the offspring analysis and for sample collection from the original cohort (72%).

The relationship between maternal PFOS (and PFOA) levels and prevalence of offspring overweight and waist-to-height ratio > 0.5 was investigated in a subset of the INUENDO (biopersistent organochlorines in diet and human fertility) prospective birth cohort (Høyer et al. 2015a). Pregnant females were enrolled between May 2002 and February 2004 with a total of 1,022 mother-child pairs at follow-up between January 2010 and May 2012 when the children were 7–9 years old. The study population consisted of 531 pairs from Greenland and 491 from Ukraine. The maternal blood samples for measurement of plasma PFOS levels were taken at a mean gestational age of 24 weeks, but there was a substantial range of collection windows in both Greenland (5–42 weeks) and Ukraine (9–40 weeks). The child's weight and height were measured and used to calculate BMI. All analyses were performed on the entire cohort as well as by country.

The median maternal plasma PFOS level was 0.0202 μg/mL in the pregnant females from Greenland and 0.0050 μg/mL in the pregnant females from Ukraine. No associations were found between PFOS (and PFOA) levels and risk of being overweight in the combined analysis or in Ukraine. No associations were observed between PFOS and BMI score in either country. In the combined analysis, an association was detected for having waist-to-height ratio > 0.5 and the continuous (per each ln-unit increase) exposure (RR = 1.38, 95% CI: 1.05–1.82). Comparable results were noted for PFOA also and waist-to-height ratio > 0.5 in the combined analysis (Høyer et al. 2015a), although this was not statistically-significant (RR = 1.30, 95% CI: 0.97–1.74).

Reproductive Outcomes in Females. Using the C8 Health Project data, blood samples from a population of females aged 18–65 years (n = 25,957) were analyzed to determine whether the onset of menopause, levels of serum estradiol, and the amount of PFAS in the blood were inter-related (Knox et al. 2011). These data were cross-sectional, with a one-time serum measurement collected for participants. The mean PFOS level of all the females was 0.018 μg/mL. The analyses of menopause excluded participants who reported undergoing a hysterectomy. Logistic regression models were adjusted for age, smoking, alcohol consumption, BMI, and exercise. The analysis for menopause was determined upon three groups of females: childbearing (aged 30–42), perimenopausal (aged > 42–51) and menopausal (aged > 51– ≤ 65). These same groups were used for the estradiol concentrations except the childbearing group was extended to include those > 18 years; exclusions for this analyses included pregnant females, females with a full hysterectomy, or females taking hormones, fertility drugs, or selective estrogen receptor modulators.

Among females aged 51–65, statistically-significant ORs for menopause were detected across PFOS quintiles, including a monotonic dose-response relationship. Similar results were found with PFOA quintiles (OR range: 1.5–1.7). Although dose-response relationships were not evident, consistent ORs for menopause were detected among the perimenopausal age group, as well for both PFOS and PFOA exposures (OR range: 1.2–1.4). Inverse associations were detected between estradiol concentrations and PFOS in the perimenopausal group ($\beta = -3.65$; $p < 0.0001$) and menopausal group ($\beta = -0.83$; $p < 0.007$). Serum PFOA and estradiol concentrations were not associated. Despite the contaminated water supplies, the PFOS exposure levels were comparable to those from NHANES and likely represented general population levels. A study limitation was the one-time serum measurement and cross-sectional study design; thus, exposure misclassification is likely despite long half-lives reported for PFAS. The level of PFOS was significantly higher in the set of females that had undergone a hysterectomy. Menopause and having undergone a hysterectomy, therefore, may be associated with increased serum PFAS due to the loss of menstruation as a route for removing PFOS with the associated menstrual blood loss. Thus, reverse causation cannot be ruled out as an alternative explanation for the study findings.

Lopez-Espinosa et al. (2011) evaluated the relationship between pubertal timing and PFOS levels among 2,931 girls and 3,076 boys aged 8–18 years from the C8 study. A high proportion of available participants provided serum biomarkers among both boys (66%) and girls (67%). The median serum PFOS level was 0.018 μg/mL among these female participants, and exposures were examined continuous and categorical (quartiles) variables. Pubertal development was based on hormone levels (total > 50 ng/dL and free > 5 pg/mL testosterone in boys and estradiol > 20 pg/mL in girls) or onset of menarche. although participant age at survey and time of day of blood sampling were the only confounders that were identified and adjusted for, other covariates considered as potential confounders included BMI z-score, height annual household family

income, ethnicity, ever smoking, and ever alcohol consumption. A reduced odds of having reached puberty was found with increasing PFOS levels, with girls having a difference of 138 days between the highest and lowest PFOS quartile. A reduced odds of postmenarche was found for both PFOS (138 days of delay) and PFOA (130 days of delay).

Christensen et al. (2011) used data from a prospective cohort study in the United Kingdom to conduct a nested case-control study examining the association between age at menarche and gestational exposure to perfluorinated chemicals including PFOS and PFOA. The study population from the Avon Longitudinal Study of Parents and Children included single-birth female subjects who had completed at least two puberty staging questionnaires between the ages of 8 and 13 years and whose mothers provided at least one prenatal serum sample. If more than one serum sample were available, the earliest sample provided was used for analysis. The study does not provide information as to when samples were collected. The females were divided into two groups, including those who experienced menarche prior to age 11.5 years (n = 218 cases), and a sample of those who experienced menarche after age 11.5 (n = 230 controls) from the 5,756 female offspring enrolled in the Avon study. Confounders including the mother's pre-pregnancy BMI, age at delivery, age at menarche, educational level, and the child's birth order and ethnic background were included in linear and logistic regression models used to analyze the data. The median maternal serum PFOS concentrations were 0.019 and 0.02 μg/mL for the early menarche and non-early menarche groups, respectively.

Although not statistically-significant, decreased adjusted odds ratios for earlier age at menarche were found for the prenatal PFOS examined as a continuous [OR = 0.68; 95% CI: 0.40–1.13] and the categorical [OR = 0.83; 95% CI: 0.56–1.23] exposure dichotomized as the median value (0.0198 μg/mL). Results were null for the continuous PFOA exposure measure and slightly elevated for the categorical exposure [OR = 1.29; 95% CI: 0.86–1.93] above the median value of 0.0037 μg/mL. The limitations of the study included having a small sample size, using a single maternal gestational serum sample for perfluorinated chemical measurement, and the self-reported nature of some covariates including menarche status and age at menarche.

The relationship between prenatal exposure to PFOS (and PFOA) and female and male reproductive function was evaluated in 343 females and 169 males whose mothers participated in an Aarhus, Denmark cohort in 1988–1989 (Kristensen et al. 2013; Vested et al. 2013). Maternal blood samples were collected during week 30 of gestation. Follow-up was initiated in 2008 when the offspring were approximately 20 years old. Median serum PFOS level was 0.0211 μg/mL for the mothers with daughters evaluated. Potential confounders adjusted for included maternal smoking during pregnancy, social class, and daughter's BMI. No statistically-significant association was found between prenatal exposure to PFOS and age of menarche. In adjusted regression analysis, daughters from mothers in the highest PFOA tertile had a later age at menarche compared with those in the lowest tertile. No statistically-significant relationships were found between PFOS (or PFOA) exposure and cycle length, reproductive hormone levels, and number of follicles assessed by ultrasound (Kristensen et al. 2013). Study limitations included retrospective collection of some health outcome data, such as age of the menarche, which was queried 2–10 years afterward.

Fei et al. (2009) evaluated associations with PFOS levels and fecundity as indicated by the time to pregnancy (TTP) in the DNBC study population. In females who had a planned pregnancy (n = 1,240), there was a longer TTP with higher levels of PFOS ($p < 0.001$). PFOS was also associated with irregular menstrual periods (11.6% in the lowest quartile versus 14.2% in the upper three exposure quartiles). The proportion of females with infertility

(TTP > 12 months) was higher in the upper three quartiles of PFOS versus the lowest quartile. These trends were statistically-significant. In females who had planned pregnancies (n = 1,240), there was a longer TTP with higher levels of PFOS ($p < 0.001$). Females with longer TTP were also older and had a history of spontaneous miscarriages or irregular menstrual cycles. The biological mechanism by which PFOS may reduce fecundity is unknown. Both TTP and infertility were also positively associated with serum PFOA levels. The selection of females who gave birth among only those with planned or partly planned pregnancies may limit study generalizability. Selection bias is also possible if excluded fertile females who did not plan their pregnancy had differentially higher or lower PFAS exposures. Additional analyses of unplanned pregnancies actually resulted in stronger association between PFAS levels and TTP.

Participants enrolled in the Maternal-Infant Research on Environmental Chemicals Study, a Canadian pregnancy and birth cohort, were evaluated for an association between serum PFOS levels (as well as PFOA and PFHxS) and TTP (Vélez et al. 2015). Females (n = 1,743) recruited from prenatal clinics across 10 Canadian cities between 2008 and 2011 (39% participation rate) were included in this analysis if they provided a first trimester blood sample collected between 6 and 14 gestational weeks. Infertility was defined as having a TTP of > 12 months or requiring infertility treatment for the current pregnancy. The geometric mean plasma PFOS level was 0.00459 µg/mL. No statistically-significant associations with fecundity were observed, although an increased risk was observed for infertility (OR = 1.14; 95% CI: 0.98–1.34) per one SD increased in PFOS. In contrast, statistically-significant associations were detected for infertility and reduced fecundity and both PFOA and PFHxS.

Reproductive Outcomes in Males. Lopez-Espinosa et al. (2011) also included 3,076 boys aged 8–18 years from the C8 database in their analysis, with a high proportion of available participants providing serum biomarkers (66%). The median serum PFOS level was 0.020 µg/mL among these male participants. Pubertal development was based on hormone levels (total > 50 ng/dL and free > 5 pg/mL testosterone). Reduced odds of reaching puberty in boys (i.e., raised testosterone) was detected with increasing PFOS (delay of 190 days between the highest and lowest quartile).

Reproductive function and other reproductive endpoints also were evaluated in the sons of the mothers who participated in the Aarhus, Denmark cohort (Kristensen et al. 2013). The median (25^{th}–75^{th} percentile) serum PFOA level was 0.0212 µg/mL (0.017.4–0.026.5 ng/mL) for the mothers with sons who were evaluated. PFOS was not associated with any outcome of reproductive function analyzed with multivariable regression models. No associations were found between PFOS (and PFOA) levels and percentage of progressive sperm, sperm morphology, semen volume, or testicular volume. Monotonic exposure-response relationships were detected for *in utero* PFOA exposure and sperm concentration, total sperm count, and percentage of progressive spermatozoa (based on the computer-assisted semen analysis), and positive associations for follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were associated with PFOA (Vested et al. 2013).

Joensen et al. (2009) investigated the relationship between PFAS and semen quality in a cross-sectional study of 105 Danish males. The study participants were recruited in 2003 from a sample of 546 males from a compulsory medical examination for all young Danish males being considered for military service. They represented the individuals with the lowest and highest testosterone levels in that study population. Nine PFAS were measured from frozen, archived (5 years) samples, while the semen samples were collected during the 2003 examination. Confounders adjusted for in the various regression models included duration of abstinence and

time between ejaculation and semen analysis. The median PFOS serum level in the 105 study participants was 0.025 μg/mL. Males with high combined levels of PFOA/PFOS had a median level of 6.2 million morphologically normal spermatozoa compared to 15.5 million in males with low PFOA/PFOS levels ($p = 0.030$).

There was no statistically significant association between testosterone levels and PFAS exposures and no difference in PFAS levels between high and low testosterone groups. To address previous study limitations and expand the generalizability of the findings, a later study by Joensen et al. (2013) was conducted to investigate the associations between serum PFOS concentration and reproductive hormones and semen quality. Study participants included a random sample of 247 healthy young Danish males (mean age 19.6 years) recruited in 2008–2009 from the same study population. Serum samples were analyzed for PFOS, as well as total testosterone (T), estradiol (E), sex hormone-binding globulin (SHBG), LH, FSH, and inhibin-B. Semen samples were collected the same morning as the blood samples, and self-administered questionnaires were also completed by the study participants. Confounders adjusted for in the various regression models included time to semen analysis, abstinence time, BMI, and smoking. The mean PFOS level was 0.0085 μg/mL. Inverse associations were detected for PFOS and various outcomes including T, calculated free T (FT), free androgen index (FAI), and ratios of T/LH, FT/LH, and FAI/LH (all p-values ≤ 0.05). PFOS was also inversely associated with estradiol, T/E ratio, and inhibin-B/FSH ratio, and positively associated with SHBG, LH, FSH, and inhibin-B, although statistical significance was not attained. No associations were detected between PFOS levels and any semen quality parameters. Study strengths included improved generalizability due to the random selection of subjects from the general population and a higher participation rate was (30%) compared to other population-based semen quality studies.

The relationship between serum PFOS exposures and 35 semen quality parameters was evaluated in 462 males enrolled in the LIFE Study cohort (Buck Louis et al. 2015). The males were recruited from 501 couples discontinuing contraception for the purposes of becoming pregnant and residing in 16 counties from Michigan and Texas. Forty-two percent of eligible couples enrolled in the study, and the 462 males provided at least one semen sample. Linear mixed models were adjusted for age, BMI, smoking, abstinence time, sample age, and study site. The study participants had a mean age of 31.8 years and mean PFOS levels were 0.017 μg/mL for Michigan residents and 0.021 μg/mL for Texas residents. Statistically-significant associations were detected between PFOS exposures and for a lower percentage of sperm with coiled tails; no associations were found for any other endpoint. In total, six PFAS (including PFOS) were associated with changes in 17 semen quality endpoints. Study strengths included improved generalizability, since participants were from the general population and had a higher participation rate (42%) compared to other population-based semen quality studies. A key study limitation of this and many of these types of epidemiology studies is the uncertainty related to the critical exposure window(s) relative to timing of the collected samples and the multiple comparisons ($n = 245$) that were examined.

Raymer et al. (2012) conducted a cross-sectional study of the relationships between PFAS and semen quality and reproductive hormones. The study population included 256 males recruited between 2002 and 2005 from Duke University Medical Center's IVF Clinic. Reproductive health questionnaires were administered to participants. Blood and semen samples were used to detect PFAS and were both collected at the time of evaluation. Linear and logistic regression models were used to calculate effect estimates and were adjusted for age, period of abstinence, and tobacco use. The average PFOS levels in plasma were 0.0374 μg/mL and

0.0008 µg/mL in semen. The strongest correlations detected between PFAS and hormones were between plasma PFOS and LH (r = 0.12), plasma PFOA and LH (r = 0.16), plasma PFOS and triiodothyronine (r = 0.14), as well as semen PFOS and FSH (r = 0.13). No statistically-significant associations were detected between PFOS and PFOA concentrations and reproductive hormones or different semen quality outcomes. The older population (mean age = 42 years) may limit comparability with previous studies and generalizability of study findings.

The INUENDO prospective birth cohort study of persistent organic pollutants and fertility was used to examine the relationship between PFAS and semen quality parameters (Toft et al. 2012). The study population included 588 males (97%) from Greenland, Poland and Ukraine who provided a semen sample among the underlying 607 male partners of 1710 pregnant females. PFOS levels were quantified from serum samples; these were categorized into tertiles and also examined as continuous exposures. Linear regression models and categorical analyses were adjusted for the following potential confounders: age, abstinence time, spillage, smoking, urogenital infections, BMI, and country of origin. For the categorical analysis combining the three cohorts, compared to the first tertile, the percent of normal sperm cells was decreased in the upper two serum PFOS tertiles with a decrease of 22% (95% CI: 1%–44%) and 35% (95% CI: 4%–66%) in the second and third PFOS tertiles, respectively. Exposure-response relationships were detected for the overall population based on the continuous PFOS exposure data, although this was only evident among the Polish and Ukrainian populations. No other associations between PFOS exposure and semen quality parameters were noted. The variable participation rates across study sites and potential for participation bias (i.e., if participation was related to fertility status and exposure levels) complicate interpretation of these results. The cross-sectional nature of this study also limits the ability to draw causal inference from these types of studies, especially since temporality could not be established some of the study population based on the timing of the blood and semen samples (e.g., nearly 60% of the Greenland samples were collected approximately a year before the semen samples).

Summary. Fetal growth retardation was examined through measures including mean birth weight, LBW, and small for gestational age. Mean birth weight examined as a continuous outcome was the most commonly examined endpoint for epidemiology studies of serum/cord PFOS exposures. Although three studies were null (Fei et al. 2008a; Hamm et al. 2010; Monroy et al. 2008), birth weight deficits ranging from 29 to 149 grams were detected in five studies (Apelberg et al. 2007; Chen et al. 2015; Darrow et al. 2013; Maisonet et al. 2012; Washino et al. 2009). Larger reductions (from 69 to 149 grams) were noted in three of these studies (Apelberg et al. 2007; Chen et al. 2015; Washino et al. 2009) on the basis of per unit increases in serum/cord PFOS exposures, while the lone categorical data showed an exposure-response deficit in mean birth weight up to 140 grams across the PFOS tertiles (Maisonet et al. 2012). Two (Chen et al. 2015; Whitworth et al. 2012) out of four (Fei et al. 2007; Hamm et al. 2009) studies of SGA and serum/cord PFOS exposures showed some suggestion of increased ORs (range: 1.3–2.3), while three (Chen et al. 2012; Fei et al. 2007; Stein et al. 2009) out of four (Darrow et al. 2014) studies of LBW showed increased risks (OR range: 1.5–4.8). Although a few of these studies showed some suggestion of dose-response relationships across different fetal growth measures (Fei et al. 2007; Maisonet et al. 2012; Stein et al. 2009), study limitations, including the potential for exposure misclassification, likely precluded the ability to adequately examine exposure-response patterns. While there is some uncertainty in the interpretation of the observed association between PFOS and birth weight given the potential impact of low GFR, the available information indicates that the association between PFOS exposure and birth weight for the general population cannot be ruled out. In humans with low GFR (which includes females

with pregnancy induced hypertension or preeclampsia) the impact on body weight is likely due to a combination of the low GFR and the serum PFOS.

A small set of studies observed an association with gestational diabetes (preconception serum PFOS; Zhang et al. 2015), pre-eclampsia (Stein et al. 2009) and pregnancy-induced hypertension (Darrow et al. 2013) in populations with serum PFOS concentrations of 0.012 – 0.017 μg/mL. Zhang et al. (2015) and Darrow et al. (2013) used a prospective assessment of adverse pregnancy outcomes in relation to PFASs which addresses some of the limitations the available cross-sectional studies. Associations with these outcomes and serum PFOA also were observed.

Although there was some suggestion of an association between PFOS exposures and semen quality parameters in a few studies (Joensen et al. 2009; Toft et al. 2012), most studies were largely null (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013). For example, morphologically abnormal sperm associated with PFOS were detected in three (Buck Louis et al. 2015; Joensen et al. 2009; Toft et al. 2012) out of nine (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013) studies.

Small increased odds of infertility was found for PFOS exposures in studies by Jørgensen et al. (2014) [OR = 1.39; 95% CI: 0.93–2.07] and Vélez et al. (2015) [OR = 1.14; 95% CI: 0.98–1.34]. Although one study was null (Vestergaard et al. 2012), PFOS exposures were associated with decreased fecundability ratios (FRs), indicative of longer time to pregnancy, in studies by Fei et al. (2009) [FR = 0.74 (95% CI: 0.58–0.93) and in studies by Jørgensen et al. (2014) [FR = 0.90; 95% CI: 0.76–1.07]. Whitworth et al. (2012) data suggested that reverse causality may explain their observation of subfecundity odds of 2.1 (95% CI: 1.2–3.8) for the highest PFOS quartile among parous females, but a reduced odds among nulliparous females (OR = 0.7; 95% CI: 0.4–1.3). A recent analysis of the pooled DNBC study samples found limited evidence of reverse causality with an overall FR of 0.83 (95% CI: 0.72–0.97) for PFOS exposures, as well as comparable ratios for parous (0.86; 95% CI: 0.70–1.06) and nulliparous (0.78; 95% CI: 0.63–0.97) females (Bach et al. 2015). The same authors reported an increased infertility OR of 1.75 (95% CI: 1.21–2.53) and OR for parous (OR = 1.51; 95% CI: 0.86–2.65) and nulliparous (OR = 1.83; 95% CI: 1.10–3.04) females. Although there remains some concern over the possibility of reverse causation explaining some previous study results, these collective findings indicate a consistent association with fertility and fecundity measures and PFOS exposures.

3.1.1.5 Thyroid Effect Studies

Occupational Populations. In the cross-sectional study described above for production workers, thyroid hormone (TH) levels were also measured in male (n = 215) and female (n = 48) volunteers working at the Decatur, Alabama plant and male (n = 206) and female (n = 49) volunteers working at the Antwerp, Belgium plant (Olsen et al. 2001a). The mean PFOS level in all employees from the Decatur and Antwerp plants was 1.40 μg/mL (range: 0.11–10.06 μg/mL) and 0.96 μg/mL (range: 0.04–6.24 μg/mL), respectively. No significant associations were found for quartile of PFOS level and thyroid-stimulating hormone (TSH), serum thyroxine (T4), free thyroxine (FT4), triiodothyronine (T3), and thyroid hormone binding ratio.

General Population. The relationship between exposure to polyhalogenated compounds, including PFOS, and thyroid hormone homeostasis was examined in a cross-sectional study of the adult Inuit population of Nunavik, Quebec, Canada (Dallaire et al. 2009). Those using medication for thyroid disease and pregnant females were not included in the study.

Concentrations of TSH, FT4, total triiodothyronine (TT3), and thyroxine-binding globulin (TBG) were measured in 623 individuals. Participants were given a survey to indicate smoking status, frequency of alcohol consumption, medications taken, and dietary fish consumption. The study detected PFOS in 100% of individuals, with a mean plasma PFOS concentration of 0.018 μg/mL (95% CI: 0.017–0.019 μg/mL). PFOS was negatively associated with circulating levels of TSH, TT3, and TBG and positively associated with FT4. The results suggest that human thyroid hormone levels could be affected by PFOS exposure. However, because the majority of individuals were reported by the authors to have normal thyroid gland function and the thyroid hormone levels were in the normal range, it is uncertain that these relationships are connected to thyroid disease or are a reflection of hormone variability in the human population.

NHANES data from three independent cross-sectional cycles (1999–2000; 2003–2004, and 2005–2006) were analyzed by Melzer et al. (2010) to estimate associations between serum PFOA and PFOS concentrations and thyroid disease in the general U.S. population. Overall, a total of 3,966 individuals ≥ 20 years of age (1,900 males and 2,066 females) were included. Of these, 292 females and 69 males reported thyroid disease. Overall mean PFOS levels were 0.025 μg/mL for males and 0.019 μg/mL for females. The data showed that males with PFOS levels in the highest quartile ≥ 0.037 μg/mL were more likely to report currently treated thyroid disease than males with PFOS levels in the lowest two quartiles combined, ≤ 0.026 μg/mL (OR = 2.68; 95% CI: 1.03–6.98; p = 0.043). Females had lower levels of PFOS than males and higher prevalence of thyroid disease, but serum PFOS concentration was not significantly associated with treated thyroid disease. With PFOA, the opposite was found, with females in the highest quartile, but not males, more likely to report thyroid disease. Further studies measuring thyroid hormone levels in a larger sample population could clarify whether pathology, changes in exposure, or altered pharmacokinetics can explain the association. Thyroid hormone levels were not reported by Melzer et al. (2010).

Another study of 1,181 members of NHANES for survey years 2007–2008 and 2009–2010 examined the association between serum PFOS levels (and 12 other PFASs) and thyroid hormone levels (Wen et al. 2013). Multivariable linear regression models were used with serum thyroid measures as the dependent variable and individual natural log-transformed PFAS concentration as a predictor along with confounders. The geometric mean serum PFOS level was 0.0142 μg/mL. No associations between PFOS level and thyroid hormones were found in males and females. However in 23 individuals defined as subclinical hypothyroid (TSH above normal range), a 1-unit increase in natural log-PFOS was positively associated with hypothyroidism (OR = 3.03; 95% CI: 1.14–8.07 in females; OR = 1.98; 95% CI: 1.19–3.28 for males; both $p < 0.05$).

Webster et al. (2015) also used NHANES 2007–2008 data from 1,525 adults to explore the contribution of PFOS exposure to those with risk factors for thyroid disease, low iodide status and/or high thyroid peroxidase antibody (TPOAb). Webster et al. 2015 saw that people with both elevated TPOAb and low iodide (those at risk for thyroid insufficiency) were more susceptible to PFOS associated disruption of thyroid hormone concentrations than were people without these two risk factors.

Bloom et al. (2010) examined the potential association between serum concentrations of eight polyhalogenated compounds, including PFOS, and human thyroid function. Levels of TSH and FT4 were measured in a subsample of participants in the cross-sectional New York State Angler Cohort Study (27 males and 4 females). A survey was conducted to determine smoking status, history of thyroid disease, medications used, and dietary fish consumption. None of the participants reported a thyroid condition or the use of thyroid medication. PFOS occurred at a

high concentration compared to the other PFASs measured with a mean concentration of 0.0196 μg/mL (95% CI: 0.0163–0.0235). The results indicated no significant association between PFOS serum concentration (or PFOA) and thyroid hormone levels, potentially due to the study's small sample size.

The relationship between thyroid biomarkers and serum levels of PFOS, PFOA, and other persistent organic pollutants was investigated in older adults (Shrestha et al. 2015). Levels of TSH, FT4, T4, and T3 were measured in 51 males and 36 females with a mean age of 63.6 years. None of the participants had thyroid disease or were taking thyroid medication. Covariates in the analysis included age, sex, education level, polychlorinated biphenyl (PCB) and PBDE exposure, smoking status, and alcohol consumption. The mean PFOS serum level was 0.0366 ± 0.023 μg/mL for all participants. In both unadjusted and adjusted models, PFOS was significantly ($p < 0.05$ or 0.01) and positively associated with FT4 and T4; a possible dose-response was not evaluated in this small sample.

The potential relationship between PFOS exposure and thyroid disease was investigated by Pirali et al. (2009) in a sample of 28 patients undergoing thyroid surgery (22 benign and 6 malignant) and a control group of 7 patients with no evidence of thyroid disease. PFOS was detected in thyroid tissue in 100% of the 8 males and 20 females with thyroid disease, with a median PFOS concentration of 5.3 ng/g, and no significant difference in levels between benign and malignant patients. The median PFOS concentration (4.4 ng/g) in the healthy glands of the control group was similar to that found in the diseased thyroid samples indicating that there was no association between PFOS concentration and thyroid disease.

A cross-sectional study of 903 pregnant females evaluated the association between plasma PFOS levels and plasma TSH (Wang et al. 2013). Twelve other perfluoroalkyl substances were also quantified and evaluated. The females were a cohort of the Norwegian Mother and Child Cohort Study, and the blood samples were drawn at approximately week 18 of gestation. The median PFOS concentration was 0.013 μg/mL with an interquartile range of 0.010–0.017 μg/mL. A trend was observed for increasing TSH across PFOS quartiles, with females in the third and fourth quartiles having significantly higher TSH levels compared with the first quartile. After adjustment, each 0.001 μg/mL increase in PFOS concentration was associated with a 0.8% (95% CI: 0.1%–1.6%) rise in TSH. The odds ratio of having an abnormally high TSH, however, was not increased. The plasma levels of other perfluoroalkyl substances were not related to TSH levels.

Expanding on the above study, Berg et al. (2015) investigated the association between a number of perfluoroalkyl substances, including PFOS, and TSH, T3, T4, free triiodothyronine (FT3), and FT4. A subset of 375 females on the Norwegian Mother and Child Cohort Study with blood samples at about gestational week 18 and at 3 days and 6 weeks after delivery were included. Seven compounds were detected in > 80% of the blood samples with PFOS present in the greatest concentration. The median PFOS level was 0.00803 μg/mL and the females were assigned to quartiles based on the first blood sample at week 18 of gestation. After adjustment for covariates (parity, age, thyroxin binding capacity, BMI), TSH was positively associated with PFOS. Females in the highest quartile had significantly higher mean TSH at all three time points compared to females in the first quartile. No associations were found between PFOS and the other thyroid hormone levels.

Maternal and umbilical cord blood concentrations of a number of fluorinated organic compounds, including PFOS, were determined in 15 females (17–37 years of age) and their

newborns at Sapporo Toho Hospitals in Hokkaido, Japan from February 2003 to July 2003 (Inoue et al. 2004). PFOS was detected in 100% of the maternal and cord blood samples, with maternal blood PFOS ranging from 0.0049 to 0.0176 μg/mL, and cord blood PFOS ranging from 0.0016 to 0.0053 μg/mL. TSH and FT4 levels in the infants between days 4 and 7 of age were not related to cord blood PFOS concentration in this small study.

Chan et al. (2011) used blood from 974 serum samples collected in 2005–2006 from females in Canada (mean age 31.3 years) at 15–20 weeks gestation and measured thyroid hormones, FT4 and the level of PFAS to determine whether PFAS levels were associated with hypothyroxinemia. From the samples, there were 96 identified as *cases* of hypothyroxinemia and 175 identified as controls. The cases had normal TSH concentrations and free T4 concentrations in the lowest 10^{th} percentile (≤ 8.8 pmol/L). The controls had normal TSH concentrations and free T4 concentrations between the 50^{th} and 90^{th} percentiles (12–14.1 pmol/L). The geometric mean for PFOS was 0.0074 μg/mL. The mean free T4 levels were 7.7 pmol/L in the cases and 12.9 in the controls. The mean TSH concentrations were 0.69 milli-Units/L in the cases and 1.13 in the controls. Analysis by conditional logistic regression indicated that the concentration of PFOS (or PFOA) was not significantly associated with hypothyroxinemia. For PFOS, the odds ratio for association of hypothyroxinemia with exposure to PFOS was 0.88 with a 95% CI of 0.63–1.24.

A similar study of 152 Canadian females evaluated maternal serum PFOS levels (and PFHxS, PFNA, PFOA) for associations with thyroid hormone levels during the early second trimester of pregnancy, weeks 15–18 (Webster et al. 2014). Mixed effects linear models were used to examine associations between PFOS levels and FT4, total T4, and TSH; associations were made for all females and separately for females with high levels of thyroid peroxidase antibody, a marker of autoimmune hypothyroidism. Median serum PFOS was 0.0048 μg/mL. No associations were found between levels of PFOS (or PFOA and PFHxS), and thyroid hormone levels in females with normal antibody levels. PFNA was positively associated with TSH. Clinically elevated thyroid peroxidase antibody levels were found in 14 (9%) of the study population. In the females with high antibody levels, PFOS, PFNA, and PFOA were strongly and positively associated with TSH. An IQR increase in maternal PFOS concentrations was associated with a 69% increase in maternal TSH compared to the median TSH level. PFNA and PFOA concentrations were associated with 46% and 54% increases, respectively, in maternal TSH.

Numerous epidemiologic studies have evaluated thyroid hormone levels, thyroid disease, or both in association with serum PFOS concentrations (Table 3-6). These epidemiologic studies provide limited support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and they include large studies of representative samples of the general U.S. adult population (Melzer et al. 2010; Wen et al. 2013). These highly powered studies reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease but not thyroid hormone status. Melzer et al. (2010) studied thyroid disease with medication and Wen et al. (2013) studied subclinical thyroid disease. In studies of pregnant females, PFOS was associated with increased TSH levels (Berg et al. 2015; Wang et al. 2013; Webster et al. 2014). Thyroid function can be affected by iodide sufficiency and by autoimmune disease. Pregnant females testing positive for the anti-thyroid peroxidase (TPO) biomarker showed a positive association with PFOS and TSH (Webster et al. 2014). An association with PFOS and TSH and T3 was found in a subset of the NHANES population with both low iodide status and positive anti-TPO antibodies (Webster et al. 2015). These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for thyroid insufficiency.

Table 3-6. Summary of Epidemiology Studies of PFOS and Thyroid Effects

Study	PFOS level (µg/mL)	TSH	T3	T4
Olsen et al. 2001a n = 263 Decatur, AL plant n = 255 Antwerp, Belgium plant	Decatur plant: 1.4 Antwerp plant: 0.96	No effect observed.	No effect observed.	No effects observed.
Dallaire et al. 2009 Canada n = 623 (adult Inuit population) Adjusted for sex, age, BMI, education, lipids and smoking	0.018	Adjusted Beta = −0.102 (p ≤ 0.05)	Adjusted Beta = −0.017 (p ≤ 0.05)	Adjusted Beta = 0.014 (p≤0.05)
Melzer et al. 2010 n = 3,966 adults, ≥ 20 yrs old NHANES (1999–2000; 2003–2004 and 2005–2006)	0.025 (men) 0.019 (women) Men (µg/mL) Q1: 0.0003–0.018 Q2: 0.0182–0.0255 Q3: 0.0256–0.0367 Q4: 0.0368–0.435 Similar cut-points in women	Self-Reported on thyroid disease, with medication use (fully adjusted); OR (95% CI), p-value Men Q1: 1 (referent) Q2: 0.43 (0.17, 1.08), p = 0.073 Q3: 0.95 (0.34, 2.70), p = 0.926 Q4: 1.89 (0.72, 4.93), p = 0.190 Q4 vs Q1&2: 2.68 (1.03, 6.98), p = 0.043 Women Q1: 1 (referent) Q2: 1.05 (0.55, 2.00), p = 0.89 Q3: 0.81 (0.44, 1.51), p = 0.496 Q4: 1.31 (0.72, 2.36), p = 0.269 Q4 vs Q1&2: 1.27 (0.82, 1.97)		
Wen et al. 2013 United States, NHANES 2007–2008, 2009–2010 n = 1,181 adults, aged ≥ 20 yrs Linear regression, adjusted, with sampling weights	0.0142	Subclinical hypothyroidism (fully adjusted); OR (95% CI), p-value Men 1.98 (1.19, 3.28), p < 0.05 Women 3.03 (1.14, 8.07) No associations between serum PFOS and thyroid hormones.		
Webster et al. 2015 n = 1,525 adults NHANES (2007–2008) Results are on those with high TPOAb and low iodine-n = 26	Geometric mean: 0.014	% difference in serum thyroid hormones for each IQ ratio increase in PFOS (95% CI), p-value (n = 26) 17.1 (6.6, 28.7), p < 0.05	% difference in serum thyroid hormones for each IQ ratio increase in PFOS (95% CI), p-value (n = 26) 4.7 (3.9,5.5), p < 0.05	% difference in serum thyroid hormones for each IQ ratio increase in PFOS (95% CI), p-value (n = 26) −4.4 (−7.6,−1.1), p < 0.05
Bloom et al. 2010 n = 31 adults, subset of New York Angler Cohort study	0.0196	Log-PFOS and log-TSH, (95% CI), p-value Beta = 0.04 (−0.52,0.59), p = 0.90	NM	Log-PFOS and log-FT4, (95% CI), p-value Beta = 0.03 (−0.17, 0.10), p = 0.62
Shrestha et al. 2015 n = 87 adults (mean age of 64) United States	Geometric mean: 0.036	Log-PFOS and log-TSH, (95% CI), p-value Beta = 0.129 (−0.02, 0.28), p= 0.09	Log-PFOS and log-T3, (95% CI), p-value Beta = 2.631 (−2.25, 7.51), p= 0.29	Log-PFOS and log-FT4, (95% CI), p-value Beta = 0.054 (0.002, 0.11), p = 0.04 Log-PFOS and log-T4, (95% CI), p-value Beta = 0.766 (0.33, 1.21), p = 0.001
Pirali et al. 2009 n = 28 patients undergoing thyroid surgery n = 7 control group	5.3 ng/g thyroid tissue No association with PFOS concentration and thyroid disease	NM	NM	NM

Study	PFOS level (µg/mL)	TSH	T3	T4
Wang et al. 2013 n = 903 women Norway (from case-control study of subfecundity in the Norwegian Mother and Child Cohort Study; cases and controls combined) Blood sample (mean 18 weeks pregnancy)	Median: 0.013	PFOS and ln-TSH (95% CI) Beta= 0.012 (0.005, 0.019)	NM	NM
Berg et al. 2015 n = 375 women in the Norwegian Mother and Child Cohort Study Blood samples at week 18, and 3 days/6 weeks post-delivery	Median: 0.00803 (µg/mL) Q1: 0.0003–0.0057 Q2: 0.0058–0.008 Q3: 0.0081–0.011 Q4: 0.0111–0.0359	PFOS and ln-TSH mLU/L (95% CI), p-value Q1: 1 (referent) Q2: 0.18 (0.06, 0.31), p = 0.11 Q3: 0.26 (0.13, 0.40), p = 0.03 Q4: 0.35 (0.21, 0.50), p = 0.00	No association	No association
Inoue et al. 2004 n = 15 women (17–37 yrs old) Japan	0.0016–0.0053 (cord blood) 0.0049–0.0176 (maternal blood)	No correlation between PFOS and TSH	NM	No correlation between PFOS and free T4
Chan et al. 2011 n = 96 identified as cases of hypothyroxinemia n = 175 controls Canada (2005–2006)	Geometric mean: 0.0074	Association of hypothyroxinemia with PFOS exposure, OR (95% CI), adjusted OR = 0.88 (0.63, 1.24)	NM	No association
Webster et al. 2014 n = 152 women Canada Blood samples taken during weeks 15–18 of pregnancy	Median: 0.0048	Beta per IQR PFOS and TSH, (95% CI, p-value) Normal TPOAb 0.07 (−0.06, 0.2), p = 0.28 High TPOAb 0.9 (0.2, 2), p = 0.02 [IQR PFOS = 0.0033 µg/mL]	NM	Beta per IQR PFOS and free T4, (95% CI), p-value Normal TPOAb 0.05 (−0.1, 0.2), p = 0.58 High TPOAb −0.7 (−2, 0.3), p = 0.18 [IQR PFOS = 0.0033 µg/mL]

In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohorts. Studies of thyroid disease and thyroid hormone concentrations in children and pregnant females found mixed effects. TSH was the indicator most frequently associated with PFOS in studies of pregnant females. In cross-sectional studies where thyroid hormones were measured in association with serum PFOS, increased TSH was associated with PFOS exposure in the most cases (Berg et al. 2015; Wang et al. 2013; Webster et al. 2014), but this association was null in a smaller study with 15 participants (Inoue et al. 2004).

A case-control study of hypothyroxinemia (normal TSH and low free T4) in pregnant females (Chan et al. 2011), did not show associations of disease with PFOS exposure; in most other thyroid diseases, T4 and its compensatory TSH co-vary. In children from the C8 cohort, increasing PFOS was associated with increased T4 in children aged 1 to 17 years (Lopez-Espinosa et al. 2011); PFOS was not associated with hypothyroidism. A small South Korean study examined correlations between maternal PFASs during pregnancy and fetal thyroid

hormones in cord blood (Kim et al. 2011). PFOS was associated with increased fetal TSH and with decreased fetal T3 (Kim et al. 2011). Studies of pregnant females show associations between TSH and PFOS, and studies in children show mixed results.

3.1.1.6 Immunotoxicity

Immune suppression

Immune function, and specifically immune system suppression, can affect numerous health outcomes, including risk of common infectious diseases (e.g., colds, flu, otitis media) and some types of cancer. The World Health Organization (WHO) guidelines for immunotoxicity risk assessment recommend measures of vaccine response as a measure of immune effects, with potentially important public health implications (WHO 2012).

Okada et al. (2012) investigated the relationship between maternal PFOS concentration (and PFOA) and otitis media (and allergic conditions), as well as cord blood Immunoglobulin E (IgE) levels during the first 18 months of life. The prospective birth cohort was based on infants delivered at the Sapporo Toho Hospital in Sapporo, Hokkaido, Japan between July 2002 and October 2005. PFOS levels were measured in maternal serum taken after the second trimester (n = 343) and total IgE concentration was measured in cord blood (n = 231) at the time of delivery. Infectious diseases and infant allergies were assessed through a self-administered questionnaire in mothers at 18 months post-delivery. Polynomial regression analyses, adjusted for potential confounders, were performed on log-transformed data. Mean maternal PFOS concentration was 0.0056 μg/mL and cord blood IgE level was 0.62 international units (IU)/mL. No significant associations were observed between maternal PFOS levels (or PFOA) and cord blood IgE levels or incidence of otitis media, wheeze, food allergy, or eczema in infants at 18 months of age.

The population from the DNBC studies evaluated by Fei et al. (2010b) was used to determine whether prenatal exposure to PFOS caused an increased risk of infectious diseases leading to hospitalization in early childhood. Information was collected by telephone interview. No clear pattern was identified when results were stratified by child's age at the time of hospitalization for an infectious disease and the level of PFASs in the maternal blood, although effect modification by sex was indicated (i.e., associations were seen in girls but not in boys). Hospitalizations among girls increased with higher prenatal PFOS concentration (incidence rate ratio [IRR] for trend across PFOS quartiles = 1.18, 95% CI: 1.03–1.36). Mean maternal plasma levels were 0.0353 μg/mL, with a range of 0.0064–0.107 μg/mL.

Two other studies, described below, examined reported history of colds and gastroenteritis in children (up to age 3 years) (Granum et al. 2013) or colds and flu in adults (Looker et al. 2014). Neither study reported associations with PFOS concentration.

Three studies have examined response to one or more vaccine (e.g., measured by antibody titer) in relation to higher exposure to PFOS in children (Grandjean et al. 2012; Granum et al. 2013) or adults (Looker et al. 2014); the latter study was conducted in the high-exposure C8 community population (Table 3-7).

Antibody responses to diphtheria and tetanus toxoids following childhood vaccinations were assessed in context of exposure to perfluorinated compounds (Grandjean et al. 2012). The prospective study included a birth cohort of 587 singleton births during 1999–2001 from the National Hospital in the Faroe Islands. Serum antibody concentrations were measured in children at age 5 years prebooster, approximately 4 weeks after the booster, and at age 7 years. Prenatal exposures to perfluorinated compounds were assessed by analysis of serum collected from the mother during week 32 of pregnancy (geometric mean 0.0273 μg/mL; IQR 0.0232–0.0331); postnatal exposure was assessed from serum collected from the child at 5 years of age (geometric mean 0.0167 μg/mL; IQR 0.0135–0.0211). Multiple regression analyses with covariate adjustments were used to estimate the percent difference in specific antibody concentrations per 2-fold increase in PFOS concentration in both maternal and 5-year serum.

Maternal PFOS serum concentration was inversely associated with antidiphtheria antibody concentration (−39%) at age 5 before booster. In addition, an association of antibody concentrations at age 7 was found with serum PFOS concentrations at age 5. A 2-fold increase in PFOS was associated with a difference in diphtheria antibody of −28% (95% CI: −46% to −3%). Additionally at ages 5 and 7, a small percentage of children had antibody concentrations below the clinically protective level of 0.1 IU/mL. At age 5, the odds ratios of antibody concentrations falling below this level for diphtheria were 2.48 (95% CI: 1.55–3.97) compared with maternal and 1.60 (95% CI: 1.10–2.34) compared with age 5 serum PFOS concentrations. For age 7 antibody levels associated with age 5 PFOS serum concentrations, odds ratios for inadequate antibody concentration were 2.38 (95% CI: 0.89–6.35) for diphtheria and 2.61 (95% CI: 0.77–8.92) for tetanus. Models were adjusted for maternal serum PCB concentration. Similar associations were also observed with PFOA concentrations.

The effects of prenatal exposure to perfluorinated compounds on vaccination responses and clinical health outcomes in early childhood were investigated in a subcohort of the Norwegian Mother and Child Cohort Study (Granum et al. 2013). A total of 56 mother-child pairs, for whom both maternal blood samples at delivery and blood samples from the children at 3 years of age, were evaluated. Antibody titers specific to measles, rubella, tetanus, and influenza were measured as these vaccines are part of the Norwegian Childhood Vaccination Program. Serum IgE levels were also measured. Mean maternal plasma PFOS concentration was 0.0056 μg/mL at delivery; the PFOA level was 0.0011 μg/mL and PFNA and PFHxS were below the limit of quantitation. PFOS levels in the children were not measured. A slight, but significant, inverse relationship between maternal PFOS level and anti-rubella antibodies in children at 3 years was found ($\beta = -.08$ [95% CI: −0.14 to −0.02]). No associations were found with PFOS or any perfluorinated compound and antibody levels to the other vaccines.

A cohort of 411 adult members of the C8 Health Project was evaluated in 2010 to determine whether there was an association between serum PFOS levels and antibody response following vaccination with an inactivated trivalent influenza vaccine (Looker et al. 2014). A prevaccination serum sample was collected at the time of vaccination and a post-vaccination serum sample was collected 21 ± 3 days later. The geometric mean serum PFOS level was 0.0083 μg/mL (95% CI: 0.0077–0.0091), and participants were divided into quartiles for analyses. Vaccine response, as measured by geometric mean antibody titer rise, was not affected by PFOS exposure.

Table 3-7. Summary of Epidemiology Studies of PFOS and Immune Suppression (Infectious Disease and Vaccine Response)

Reference and Study Details	PFOS level	Results
General Population: Children		
Okada et al. 2012 Japan, birth cohort study, July 2002–October 2005 enrollment; follow-up to 18 months; n = 343 Log-transformed PFOS in blood after second trimester Logistic regression adjusting for maternal age, maternal educational level, parity, infant gender, breast-feeding period, environmental tobacco smoke at 18 months, day care attendance, period of blood sampling.	Mean 0.0056 µg/mL	Incidence otitis media 17.8% (n = 61) OR (95% CI) n Overall 1.40 (0.33, 6.00) n = 343 Males 1.38 (0.18, 10.60) n = 169 Females 1.43 (0.17, 12.30) n = 174
Fei et al. 2010b Denmark, birth cohort study, 1996–2002, follow-up through 2008; Number hospitalizations 219 girls, 358 boys Maternal blood sample median 8 weeks gestation Poisson regression adjusting for parity, maternal age, pre-pregnancy BMI, breastfeeding, smoking during pregnancy, socio-occupational status, home density, child's age, gender of child, sibling age difference, gestational age at blood draw, birth year, and birth season.	Mean 0.0353 µg/mL Quartiles Q1 0.0064 – 0.026 Q2 0.0261 – 0.0333 Q3 0.0334 – 0.0432 Q4 ≥ 0.433	Adjusted IRR for hospitalization for infectious diseases by gender, IRR (95% CI) n Overall Q1 1.0 n = 147 Q2 0.93 (0.71, 1.21) n = 142 Q3 0.90 (0.68, 1.18) n = 136 Q4 1.00 (0.76, 1.32) n = 152 Trend 1.00 (0.91, 1.09) Girls Q1 1.0 n = 39 Q2 1.14 (0.73, 1.791) n = 48 Q3 1.61 (1.05, 2.47) n = 67 Q4 1.59 (1.02, 2.49) n = 65 Trend 1.18 (1.03, 1.36) Boys Q1 1.0 n = 108 Q2 0.80 (0.57, 1.13) n = 94 Q3 0.61 (0.42, 0.89) n = 69 Q4 0.77 (0.54, 1.12) n = 87 Trend 0.90 (0.80, 1.02)
Grandjean et al. 2012 Faroe Islands Birth cohort, follow-up to age 7 yrs n = 587 Age 5 pre-booster (e.g., tetanus, diphtheria) and 4 weeks after booster and age 7 PFOS in 3rd trimester blood sample and in child (age 5) Linear regression, adjusted for sex, age, birth weight, maternal smoking, breastfeeding, and PCBs [and time since booster for post-booster analysis]	Geometric mean Maternal sample 0.027 µg/mL Child's sample 0.0167 µg/mL	Log PFOS and Log antibody Beta (95% CI) [% change in antibody titer per 2-fold increase in PFOS] Maternal PFOS — Tetanus — Diptheria Pre-booster −10.1 (−31.9, 18.7) −38.6 (−54.7, −16.9) Post-booster −2.3 (−28.6, 33.6) −20.6 (−37.5, 0.9) Year 7 35.3 (−3.9, 90.6) −19.7 (−41.8, 10.7) Year 7 (adjusted for age 5) 33.1 (1.5, 74.6) −10.0 (−32.6, 20.0) Child's PFOS — Tetanus — Diptheria Pre-booster −11.9 (−30.0, 10.9) −16.0 (−34.9, 8.3) Post-booster −28.5 (−45.5, −6.1) −15.5 (−31.5, 4.3) Year 7 −23.8 (−44.3, 4.2) −27.6 (−45.8, −3.3) Year 7 (adjusted for age 5) −11.4 (−30.5, 12.8) −20.6 (−38.2, 2.1) Similar results seen with PFOA

Reference and Study Details	PFOS level	Results
Granum et al. 2013 Norway Birth cohort, Norwegian Mother and Child Cohort Study n = 56 with maternal blood at delivery and child blood samples at 3 yrs Linear regression, considered potential confounders	Mean 0.0056 µg/mL	Beta (95% CI) (p-value), PFOS and antibody titer Rubella −0.08 (−0.14, −0.02) (p = 0.007) Measles −0.05 (−0.10, 0.01) (p = 0.09) Tetanus −0.002 (−0.03, 0.02) (p = 0.87) Hib −0.16 (−1.02, 0.70) (p = 0.71) Similar results for other PFASs
General Population: Adults		
Looker et al. 2014 C8 Health Project, West Virginia 2005–2005 enrollment and baseline blood sample and questionnaires; 2010 follow-up n = 411 with pre-vaccination blood sample – flu vaccination – 21 day post vaccination blood sample Linear regression: antibody titer rise Logistic regression: seroconversion and seroprotection Considered possible confounders, retained in final model: age, gender, mobility (# addresses), and history of previous influenza vaccination	Geometric mean 0.0083 µg/mL Q1: 0.001–0.0058 Q2: 0.0059–0.0092 Q3: 0.0093–0.0145 Q4: 0.0147–0.0423	Percentage positive) OR (95% CI), by influenza strain: Seroconversion (4-fold increase in antibody titer) / Seroproection (antibody titer 1:40 following vaccine) Influenza B (62%) (66%) PFOS continuous 1.17 (0.63, 2.17) 0.85 (0.44, 1.64) Q1 1.0 (referent) 1.0 (referent) Q2 0.72 (0.39, 1.33) 0.67 (0.35, 1.25) Q3 0.81 (0.42, 1.53) 0.82 (0.42, 1.59) Q4 0.87 (0.43, 1.74) 0.73 (0.36, 1.47) A/H1Na (84%) (96%) PFOS continuous 1.10 (0.51, 2.37) 0.93 (0.23, 3.71) Q1 1.0 (referent) 1.0 (referent) Q2 0.97 (0.44, 2.14) 0.55 (0.13, 2.37) Q3 0.78 (0.35, 1.75) 1.81 (0.32, 10.22) Q4 0.94 (0.38, 2.31) 1.26 (0.24, 6.61) A/H3N2 (65%) (84%) PFOS continuous 1.17 (0.63, 2.15) 0.63 (0.26, 1.49) Q1 1.0 (referent) 1.0 (referent) Q2 1.08 (0.59, 1.97) 0.85 (0.38, 1.88) Q3 1.10 (0.59, 2.06) 1.09 (0.47, 2.56) Q4 1.41 (0.72, 2.78) 0.56 (0.24, 1.28)

Asthma

Humblet et al. (2014) evaluated a cohort from NHANES to investigate children's PFAS serum levels, including PFOS, and their association with asthma-related outcomes. Sera were analyzed for 12 PFAS with focus on PFOA, PFOS, PFHxS, and PFNA. A total of 1,877 children 12–19 years old with at least one serum sample available were included. Asthma and related outcomes were self-reported. Median serum PFOS levels were 0.017 µg/mL for those ever having asthma and 0.0168 µg/mL for children without asthma. In the multivariable adjusted model, a doubling of PFOS level was inversely associated with the odds of ever having asthma (OR = 0.88, 95% CI: 0.74–1.04), but statistical significance was not attained. PFOA was significantly associated with asthma and no associations were found between the other PFASs and outcome.

The association between serum levels of perfluorinated compounds and childhood asthma was investigated by Dong et al. (2013). The cross-sectional study included a total of 231 children aged 10–15 years with physician-diagnosed asthma and 225 age-matched non-asthmatic controls. Between 2009 and 2010, asthmatic children were recruited from two hospitals in Northern Taiwan, while the controls were part of a cohort population in seven public schools in Northern Taiwan. Serum was collected for measurement of ten perfluorinated compounds,

absolute eosinophil counts, total IgE, and eosinophilic cationic protein. A questionnaire was administered to asthmatic children to assess asthma control and to calculate an asthma severity score (including frequency of attacks, use of medicine, and hospitalization) during the previous 4 weeks. Associations of perfluorinated compound quartiles with concentrations of immunological markers and asthma outcomes were estimated using multivariable regression models.

Nine of ten perfluorinated compounds were detectable in ≥ 84.4% of all children with levels generally higher in asthmatic children compared with non-asthmatics. Serum concentrations of PFOS in asthmatic and non-asthmatic children were 0.0455 ± 0.0373 and 0.0334 ± 0.0264 μg/mL, respectively; similar levels were measured for perfluorotetradecanoic acid with much lower concentrations of the remaining six perfluorinated carboxylated and two sulfonates sulfonates. The adjusted odds ratios for asthma association with the highest versus lowest quartile levels were significantly elevated for seven of the PFAS compounds. For PFOS, the odds ratio was 2.63 (95% CI: 1.48–4.69). In asthmatic children, absolute eosinophil counts, total IgE, and eosinophilic cationic protein concentration were positively associated with PFOS levels with a significant monotonic trend with increasing serum concentration. None of these biomarkers was significantly associated with PFOS levels in non-asthmatic children. Serum PFOS levels, as well as three other compounds, were significantly associated with higher asthma severity scores.

A summary of the studies that examined the relationship between PFOS serum levels and markers of immunotoxicity in humans is presented in Table 3-7. A few studies have evaluated associations with measures indicating immunosuppression. Two studies reported decreases in response to one or more vaccines in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing maternal serum PFOS levels during pregnancy or at 5 years of age (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella and mumps antibody concentrations in relation to serum PFOS concentration were found among 12–19 year old children in the NHANES, particularly among seropositive children (Stein et al. 2015). A third study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means < 0.02 μg/ml), the associations with PFOS were also seen with other correlated PFAS, complicating conclusions specifically for PFOS. No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010b; Okada et al. 2012), although an elevation in risk of hospitalizations for an infectious disease was found among girls suggesting an effect at the higher maternal serum levels measured in the Danish population (mean maternal plasma levels were 0.0353 μg/mL).

With regard to other immune dysfunction, serum PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 0.017 μg/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma 0.0339 and 0.0289 μg/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations also were found for other PFASs. Among asthmatics, serum PFOS was also associated with higher severity scores, serum total IgE, absolute eosinophil counts and eosinophilic cationic protein levels.

3.1.1.7 Other Effects

Metabolic syndrome is a combination of medical disorders and risk factors that increase the risk of developing cardiovascular disease and diabetes. Lin et al. (2009) investigated the association between serum PFOS (plus three other PFASs) and glucose homeostasis and metabolic syndrome in adolescents (12–20 years) and adults (> 20 years) by analyzing the 1999–2000 and 2003–2004 NHANES data. The National Cholesterol Education Program Adult Treatment Panel III guidelines were used to define adult metabolic syndrome and the modified guidelines were used to define adolescent metabolic syndrome. The study population included 1,443 subjects (474 adolescents, 969 adults) at least 12 years of age who had a morning examination and triglyceride measurement. There were 266 male and 208 female adolescents and 475 male and 493 female adults. Multiple linear regression and logistic regression models were used to analyze the data. Covariates included age, sex, race, smoking status, alcohol consumption, and household income. Log-transformed PFOS concentration was 3.11 ng/mL and 3.19 ng/mL for adolescents and adults, respectively. In adults, serum PFOS concentration was associated with increased β-cell function (β coefficient 0.15, $p < 0.01$). Serum PFOS concentration was not associated with metabolic syndrome, glucose concentration, homeostasis model of insulin resistance, or insulin levels in adults or adolescents.

3.1.1.8 Summary and conclusions from the human epidemiology studies

Numerous epidemiology studies have been conducted evaluating occupational PFOS exposure and environmental PFOS exposure including a large community highly-exposed to PFOA (the C8 Health Project) and background exposures in the general population in several countries. Occupational and general populations have evaluated the association of PFOS exposure to a variety of health endpoints. Health outcomes assessed include blood lipid and clinical chemistry profiles, thyroid effects, immune function, reproductive effects, pregnancy-related outcomes, fetal growth and developmental outcomes, and cancer.

Serum Lipids. Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration (Table 3-1). These studies provide support for an association between PFOS and small increases in total cholesterol. Hypercholesterolemia, which is clinically defined as cholesterol > 240 mg/dL, was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009). Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003b). Evidence for associations between other serum lipids and PFOS is mixed, including HDL cholesterol, LDL, VLDL, and non-HDL cholesterol, as well as triglycerides. The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults, but some studies exist on children and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the UK, to Scandinavian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design. Also studied were populations with known elevated exposure to other environmental chemicals including PFOA in the C8 population and PBDEs and other persistent organic chemicals in the Inuit population.

Liver. Cross-sectional and longitudinal studies evaluated PFOS and liver enzymes in adults. Lin et al. (2010) looked at data from the NHANES, which is representative of the U.S. national population, and Gallo et al. (2012) reported an analysis of data from the C8 Health Project, reflective of a highly-exposed community. Both studies saw a slight positive association between

serum PFOS levels and increased serum ALT values. The association between PFOS levels and increased serum GGT levels was less defined and overall did not appear to be affected. Total or direct bilirubin showed no association with PFOS in either study. In the Gallo et al. (2012) study, the cross-sectional design and self-reported lifestyle characteristics are limitations to the study, and while both studies showed a trend, it was not large in magnitude.

Kidney. Shankar et al. (2011) and Watkins et al. (2013) analyzed sub-sets or the entire population for an association between PFOS serum levels and either kidney disease or biomarkers that may be associated with kidney function. Shankar et al. (2011) used NHANES data and showed a positive association between increasing levels of PFOS and chronic kidney disease, as defined as an eGFR of < 60 mL/min/1.73 m^2. The odds ratio for chronic kidney disease at > 0.030 µg/mL of PFOS was 1.82 (95% CI: 1.01–3.27), and while the possibility of reverse causality could not be excluded, the association between PFOS and eGFR when examined in those with and without chronic kidney disease supports an effect. Watkins et al. (2013) evaluated C8 Health Project children to look at PFOS levels and kidney function in children, as defined as decreased eGFR, and found a dose-related trend: the decrease was 1.10 mL/min/1.73 m^2 (95% CI: −1.66 to −0.53). Geiger et al. (2014b) found no association in children between serum PFOS levels and hypertension. Steenland et al. (2010) evaluated C8 Health Project adults and found a positive association between PFOS serum levels and an increase in uric acid with odds ratios increasing from 1.02 to 1.26 with each decile. Overall, studies do suggest an association between chronic kidney disease, as defined by estimated glomerular filtration rate; however, reverse causality cannot be excluded.

Fertility, Pregnancy, and Birth Outcomes. Fetal growth retardation was examined through measures including mean birth weight, LBW, and small for gestational age. Mean birth weight examined as a continuous outcome was the most commonly examined endpoint for epidemiology studies of serum/cord PFOS exposures. Although three studies were null (Fei et al. 2008b; Hamm et al. 2010; Monroy et al. 2008), birth weight deficits ranging 29–149 grams were detected in five studies (Apelberg et al. 2007; Chen et al. 2015; Darrow et al. 2013; Maisonet et al. 2012; Washino et al. 2009). Larger reductions (69–149 grams) were noted in three of these studies (Apelberg et al. 2007; Chen et al. 2015; Washino et al. 2009) based on per unit increases in serum/cord PFOS exposures, while the lone categorical data showed an exposure-response deficit in mean birth weight up to 140 grams across the PFOS tertiles (Maisonet et al. 2012). Two (Chen et al. 2015; Whitworth et al. 2012) out of four (Fei et al. 2007; Hamm et al. 2009) studies of SGA and serum/cord PFOS exposures showed some suggestion of increased ORs (range: 1.3–2.3), while three (Chen et al. 2012; Fei et al. 2007; Stein et al. 2009) out of four (Darrow et al. 2014) studies of LBW showed increased risks (OR range: 1.5–4.8). Although a few of these studies showed some suggestion of dose-response relationships across different fetal growth measures (Fei et al. 2007; Maisonet et al. 2012; Stein et al. 2009), study limitations, including the potential for exposure misclassification, likely precluded the ability to adequately examine the exposure-response pattern.

Recent data also indicate an association between low maternal GFR and infant birth weight, supporting GFR as a confounder in epidemiology studies (Morken et al. 2014; Verner et al. 2015). In such cases the increased serum PFOS could be the result of the developmental milestone rather than a cause. However, while a proportion of the association between prenatal PFOS and birth weight may be confounded by low maternal GFR, a direct effect of PFOS on neonatal weight cannot be entirely dismissed based on the available data.

A small set of studies observed an association with gestational diabetes (Zhang et al. 2015, preconception serum PFOS), pre-eclampsia (Stein et al. 2009) and pregnancy-induced hypertension (Darrow et al. 2013) in populations with serum PFOS concentrations of 0.012–0.017 μg/mL. Zhang et al. (2015) and Darrow et al. (2013) used a prospective assessment of adverse pregnancy outcomes in relation to PFASs which addresses some of the limitations in the available cross-sectional studies. Associations with these outcomes and serum PFOA also were observed.

Although there was some suggestion of an association between PFOS exposures and semen quality parameters in a few studies (Joensen et al. 2009; Toft et al. 2012), most studies were largely null (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Vested et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013). For example, morphologically abnormal sperm associated with PFOS were detected in three (Buck Louis et al. 2015; Joensen et al. 2009; Toft et al. 2012) out of eight (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013) studies.

Small increased odds of infertility was found for PFOS exposures in studies by Jørgensen et al. (2014) [OR = 1.39; 95% CI: 0.93–2.07] and Vélez et al. (2015) [OR = 1.14; 95% CI: 0.98–1.34]. Although one study was null (Vestergaard et al. 2012), PFOS exposures associated with decreased FRs, indicative of longer time to pregnancy, were noted in studies by Fei et al. (2009) [FR = 0.74 (95% CI: 0.58–0.93) and in studies by Jørgensen et al. (2014) [FR = 0.90; 95% CI: 0.76–1.07]. Whitworth et al. (2012) data suggested that reverse causality may explain their observation of subfecundity odds of 2.1 (95% CI: 1.2–3.8) for the highest PFOS quartile among parous females, but a reduced odds among nulliparous females (OR = 0.7; 95% CI: 0.4–1.3). A recent analysis of the pooled DNBC study samples did not find strong evidence of differences by parity status with an overall fecundability ratio of 0.83 (95% CI: 0.72–0.97) for PFOS exposures, as well as comparable ratios for parous (0.86; 95% CI: 0.70–1.06) and nulliparous (0.78; 95% : 0.63–0.97) females (Bach et al. 2015). The same authors reported an increased infertility OR of 1.75 (95% CI: 1.21–2.53) and OR for parous (OR = 1.51; 95% CI: 0.86–2.65) and nulliparous (OR = 1.83; 95% CI: 1.10–3.04) females. Although there remains some concern over the possibility of reverse causation explaining some previous study results, these collective findings indicate a consistent association with fertility and fecundity measures and PFOS exposures.

Thyroid. Numerous epidemiologic studies have evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS concentrations. These epidemiologic studies provide limited support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. adult population (Melzer et al. 2010; Wen et al. 2013). These highly powered studies reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease but not thyroid hormone status. Melzer et al. (2010) studied thyroid disease with medication and Wen et al. (2013) studied subclinical thyroid disease. Thyroid function can be affected by iodide sufficiency and by autoimmune disease. People testing positive for the anti-TPO biomarker showed associations with PFOS and TSH or T4 (Webster et al. 2014); this association was stronger in people with both low iodide status and positive anti-TPO antibodies (Webster et al. 2015). These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for thyroid insufficiency. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohorts.

Immune Function. A few studies have evaluated associations with measures indicating immunosuppression. Two studies reported decreases in response to one or more vaccines in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing prenatal serum PFOS levels or at 5 years of age (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella and mumps antibody concentrations in relation to serum PFOS concentration were found among 12–19 year old children in the NHANES, particularly among seropositive children (Stein et al. 2015). A third study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means < 0.02 μg/ml), the associations with PFOS were also seen with other correlated PFASs, complicating conclusions specifically for PFOS.

No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010b; Okada et al. 2012), although an elevation in risk of hospitalizations for an infectious disease was found among girls suggesting an effect at the higher maternal serum levels measured in the Danish population (mean maternal plasma levels were 0.0353 μg/mL). With regard to other immune dysfunction, serum PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 0.017 μg/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma 0.0339 μg/mL and 0.0289 μg/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations also were found for other PFASs. Among asthmatics, serum PFOS was also associated with higher severity scores, serum total IgE, absolute eosinophil counts and eosinophilic cationic protein levels.

3.1.2 Carcinogenicity Studies

Occupational Exposure. Several analyses of various health outcomes have occurred on cohorts of workers at the 3M Decatur, Alabama plant (Alexander et al. 2003; Alexander and Olsen 2007; Mandel and Johnson 1995). Cause-specific mortality was examined in a cohort of 2,083 workers employed for at least 1 year among workers grouped into three PFOS exposure categories: non-exposed, low exposed, and high exposed. Exposure classifications were determined using PFOS serum concentrations measured in a subset of workers linked to specific jobs and work histories. Cumulative exposures were also estimated by applying a weight to each of the exposure categories and multiplying by the number of years of employment for that job for each individual. The geometric mean serum PFOS levels were 0.941 μg/mL for chemical plant employees and 0.136 μg/mL for non-exposed workers. Results of these studies are summarized in Table 3-8.

A total of 145 deaths were identified with 65 of them in high-exposure jobs. Standardized mortality ratios (SMRs) were calculated using the state of Alabama reference data and when analyzing the entire cohort, SMRs were not elevated for most of the cancer types and for non-malignant causes. SMRs that were above 1 included cancer of the esophagus, liver, breast, urinary organs, bladder, and skin. However, the number of cases was very small (1–3), resulting in wide confidence intervals. The SMRs for these causes (except breast cancer) were also elevated when the cohort was limited to the 65 employees ever employed in a high exposure job. The SMR for bladder cancer was 4.81 (95% CI: 0.99–14.06). Three male employees in the cohort died of bladder cancer (0.62 expected). All were employed at the Decatur plant for > 20 years and had worked in high exposure jobs for at least 5 years. The SMR for bladder

cancer for workers who were ever employed in a high exposure job was 12.77 (0.23 expected, CI: 2.63–37.35). When the data were analyzed for workers with > 5 years of employment in a high exposure job, the SMR was 24.49. This effect remained when the data were analyzed using county death rates.

While the three deaths from bladder cancer were greater than the expected number observed in the general population, the small number of deaths (especially for females in all categories) precludes a definitive conclusion regarding an association with PFOS exposure. In addition, six death certificates were not obtained, and smoking status was not known for the cohort increasing the uncertainty with regard to the estimated risk.

Based on these results, another study of this cohort was conducted to evaluate bladder cancer incidence (Alexander and Olsen 2007). Cancer deaths were ascertained from death certificates and via questionnaire for bladder cancer cases, year of diagnosis, and smoking history. Eleven bladder cancer cases were identified: five deaths and six incident cases. Only two of the six self-reported cases were confirmed with medical records. Five of the six incident cases had a history of cigarette smoking. Standardized incidence ratios (SIR) were estimated for the three exposure categories described for the mortality study and compared to U.S. cancer rates. SIRs were 0.61, 2.26, and 1.74 for the nonexposed, ever low, and ever high exposure categories, respectively. Rate ratios by cumulative exposure index were increased in the higher categories (5 to < 10 and ≥ 10) when using either the U.S. population rates or an internal referent population, however the number of cases were few and confidence intervals were wide including the null. These results, while suggestive of an elevated risk of bladder cancer, were not conclusive.

Grice et al. (2007) evaluated associations between PFOS exposure at the 3M Decatur, Alabama plant and various malignant or benign tumors reported by the same study group evaluated by Alexander and Olsen (2007). Current and past employees at the plant answered questionnaires (n = 1,400; 1,137 male and 263 female) about diagnosis of cancers or non-cancerous conditions. Data were analyzed by PFOS exposure category: unexposed (< 0.29 μg/mL), low (0.39–0.89 μg/mL), or high exposure (1.30–1.97 μg/mL) and by categories of estimated cumulative exposure using the same weighted approach described in the previous studies of this cohort. Prostate, melanoma, and colon cancer were the most frequently reported malignancies. When cumulative exposure measures were analyzed, elevated odds ratios were reported for both colon and prostate cancer, however, they did not reach statistical significance. Length of follow-up may not have been adequate to detect cancer incidence in this cohort as approximately one-third of the participants had worked < 5 years in their jobs, and only 41.7% were employed ≥ 20 years.

C8 Health Project Community. Members of the C8 Health Project, 47,151 cancer-free adults and 203 cases, were evaluated for an association between serum PFOS levels and incidence of colorectal cancers (Innes et al. 2014). This cross-sectional study compared serum PFOS (and PFOA) levels at enrollment with diagnosis of primary colorectal cancer. Serum PFOS levels ranged from < 0.0005 to 0.759 μg/mL, with an average of 0.0234 μg/mL. A concentration-related inverse relationship was found between PFOS level and diagnosis of colorectal cancer with OR = 0.24 (95% CI 0.16, 0.37; highest to lowest quartile, p for trend < 0.00001). An inverse association was also found between PFOA and colorectal cancer.

General Population. A subset of females enrolled in the DNBC was evaluated for an association between plasma PFOS levels (as well as 15 other perfluoroalkylated substances) measured during pregnancy and risk of breast cancer during a follow-up period of 10–15 years

(Bonefeld-Jørgensen et al. 2014). A total of 250 females diagnosed with breast cancer were matched for age and parity with 233 controls. The mean PFOS level in the controls was 0.0306 μg/mL while levels in the cases were divided into quintiles ranging from < 0.0204 up to > 0.0391 μg/mL. No association was found between PFOS levels and breast cancer risk in logistic regression models adjusted for age at blood draw, BMI before pregnancy, gravidity, use of oral contraceptives, age at menarche, smoking, alcohol consumption, maternal education and physical activity. A weak positive Relative Risk (1.04; 95% CI: 0.99–1.08) was found only with perfluorooctane-sulfonamide.

These same researchers had previously observed a borderline significant positive association with PFOS levels and breast cancer (adjusted OR = 1.03, 95% CI: 1.001–1.07) in a small cohort from Greenland (Bonefeld-Jørgensen et al. 2011). Logistic regression models were adjusted for age, BMI, total number of full-term pregnancies, breastfeeding, menopausal status, and serum cotinine, but the unadjusted results that included the entire study group were not different. Median serum PFOS levels were 0.0456 μg/mL (range: 0.0116–0.124 μg/mL) among 31 breast cancer patients and 0.0219 μg/mL (range: 0.0015–0.172 μg/mL) among 98 controls. A weak positive odds ratio of 1.03 (95% CI: 1.00–1.05) was also found for the sum of perfluorosulfonated compounds which included PFOS along with perfluorohexane sulfonate and perfluorooctane sulfonamide.

Eriksen et al. (2009) examined the association between plasma PFOS concentration and the risk of cancer in the general Danish population. The study population was chosen from individuals (50–65 years of age) who had enrolled in the prospective Danish cohort Diet, Cancer, and Health study between December 1, 1993 and May 31, 1997. The Danish Cancer Registry and Danish Pathology Data Bank were used to identify cancer patients diagnosed between December 1, 1993, and July 1, 2006. The cancer patients (n = 1,240) consisted of 1,111 males and 129 females whose median age was 59 years having prostate cancer (n = 713), bladder cancer (n = 332), pancreatic cancer (n = 128), and liver cancer (n = 67). The individuals (n = 772) in the subcohort comparison group were randomly chosen from the cohort study and consisted of 680 males and 92 females whose median age was 56 years. The participants answered a questionnaire upon enrollment in the cohort study, and data on known confounders were obtained from the questionnaires. The plasma PFOS concentrations, based on blood samples provided at enrollment (1993–1997) for cancer patients were as follows: males 0.0351 μg/mL, females 0.0321 μg/mL, prostate cancer 0.0368 μg/mL, bladder cancer 0.0323 μg/mL, pancreatic cancer 0.0327 μg/mL, and liver cancer 0.0310 μg/mL. The plasma PFOS concentrations for the subcohort comparison group were 0.0350, 0.0293, and 0.0343 μg/mL for the males, females, and combined, respectively. Incidence rate ratios, crude and adjusted for confounders, did not indicate an association between plasma PFOS concentration and bladder, pancreatic, or liver cancer in models adjusting for potential confounders. For prostate cancer, increased odds ratios 30% above the comparison group for quartiles 2 through 4 were observed, but there was no increasing trend in the analysis using PFOS concentration as a continuous variable. The plasma PFOS levels in the population were lower than those observed in occupational cohorts.

Hardell et al. (2014) investigated an association between prostate cancer and levels of PFAS in whole blood. Patients with newly diagnosed prostate cancer (n = 201) had median PFOS levels of 0.009 μg/mL, while the control group (n = 186) had a median level of 0.0083 μg/mL. PFOS levels, which were measured 1–3 years after cancer diagnosis, were not associated with higher risks of prostate cancer in logistic regression models adjusted for age, BMI, and year of

blood sampling, or when analyzed according to Gleason score (pathology grade) and prostate-specific antigen. A significantly higher risk for prostate cancer was found for a group with PFOS levels above the median and a first-degree relative with prostate cancer indicating a potential genetic risk factor.

A small study found no differences in blood PFOS levels between cancer and non-cancer patients; the types of cancer in the patients were not defined. Vassiliadou et al. (2010) found median serum PFOS concentrations among 40 cancer patients (0.0113 μg/mL, males; 0.008 μg/mL, females) were similar to two control groups (0.0105 and 0.0137 μg/mL, males; 0.007 and 0.0085 μg/mL, females).

Results of the cancer epidemiology studies in the highly exposed and general populations are summarized in Table 3-8.

Table 3-8. Summary of PFOS Epidemiology Studies of Cancer

Reference and Study Details	Analysis Group	Relative Risk Estimates
Occupational Exposure Studies		
Alexander et al. 2003 Fluorochemical production, Decatur, Alabama Film plant and chemical plant employees (current, retired and former), n = 2,083, follow-up through 1998 83% male, median age 50.9 yrs at follow-up, median 13.2 yrs of employment Mortality Comparisons by exposure group classified using matrix of work history (1961–1997) and job-specific serum PFOS concentration: No exposure, low and high potential workplace exposure; Cumulative exposure level based on exposure category weight (1,3, or 10) and years spent in specific jobs	Mortality through 1998 All (Alabama referent) Non-exposed jobs (0.11–0.29 μg/ml) Low exposure jobs (0.39–0.89 μg/ml) High exposure jobs (1.30–1.97 μg/ml) All (Alabama referent) Non-exposed jobs (0.11–0.29 μg/ml) Low exposure jobs (0.39–0.89 μg/ml) High exposure jobs (1.30–1.97 μg/ml)	Liver Cancer SMR (95% CI) 1.61 (0.20, 5.82) (n = 2) No cases 3.94 (0.10, 21.88) (n = 1) 2.00 (0.05, 11.01) (n = 1) Bladder Cancer SMR (95% CI) 4.81 (0.99, 14.06)(n = 3) No cases No cases 12.77 (2.63, 37.35) (n = 3)
Alexander and Olsen 2007; Grice et al. 2007) Fluorochemical production, Decatur, Alabama Film plant and chemical plant employees, n = 1,400 of 2,083 who completed questionnaire in 2002 and 188 decedents since mortality analysis. 495 declined; participation 73.9% of eligible, 43,739 person-years of follow-up. 81.2% male, Incidence (via questionnaire) with confirmation by physician for some	Incidence through 2002 All (U.S. population referent) Non-exposed jobs (0.11–0.29 μg/ml) Low exposure jobs (0.39–0.89 μg/ml) High exposure jobs (1.30–1.97 μg/ml) Ever low or high Low or high (≥ 1 yr) Non-exposed jobs Ever low or high Low or high (≥ 1 yr) High (> 1 yr)	Bladder cancer (2 of 6 reported confirmed; 5 deaths) SIR (95% CI) (n cases) 1.28 (0.64, 2.29) (n = 11) 0.61 (0.07, 2.19) (n = 2) 2.26 (0.91, 4.67) (n = 7) 1.74 (0.64, 3.79) (n = 6) 1.7 (0.77, 3.22) (n = 9) 1.31 (0.48, 2.85) (n = 6) Colon cancer (12 of 22 reported confirmed) OR, 95% CI, (n cases) 1.0 (n = 8) 1.21 (0.51, 2.87) (n = 15) 1.37 (0.57, 3.30) (n = 14) 1.69 (0.68, 4.17) (n = 7)

Reference and Study Details	Analysis Group	Relative Risk Estimates
	Non-exposed jobs Ever low or high Low or high (≥ 1 yr) High (> 1 yr)	Prostate cancer (22 of 29 reported confirmed) OR (95% CI) (n cases) 1.0 (n = 10) 1.34 (0.62, 2.91) (n = 19) 1.36 (0.61, 3.02) (n = 16) 1.08 (0.44, 2.69) (n = 9)
General Population Studies		
Bonefeld-Jørgensen et al. 2014 Denmark; case-control study nested in prospective cohort; DNBC, 1996–2002, follow-up to 2010. 250 women with breast cancer identified using cancer registry (mean age at blood draw 30.4 yr) and 233 controls (mean age at blood draw 29.6 yr), frequency matched on age and parity, selected at random from cohort at baseline. PFOS (and other perfluorochemicals) in blood drawn between gestation weeks 6 and 14.	Mean serum PFOS in controls 0.031 µg/mL; correlation PFOS and PFOA 0.69 Continuous PFOS Quintiles < 0.02 0.02–0.025 0.025–0.030 0.030–0.039 > 0.039	Breast Cancer Adjusted RR (95% CI) (n cases) 0.99 (0.98, 1.01) (n = 221) 1.0 (n = 42) 1.51 (0.081, 2.71) (n = 52) 1.51 (0.82, 2.84) (n = 49) 1.13 (0.59, 2.04) (n = 43) 0.90 (0.47, 1.7) (n = 35)
Hardell et al. 2014 Denmark; case-control study Prostate cancer cases from hospital admissions, 2007–2011 (n = 201, participation 79%, median age 67 yr); population-based controls matched on age geographical location (n = 186, participation 54%); Blood sampling for perfluorinated alkyl acids 2007–2011	Median blood PFOS in cases 0.009 µg/mL, controls 0.0083 µg/mL	Prostate Cancer Adjusted RR (95% CI) (n cases) 1.0 (0.60, 1.5) (n = 109)
Bonefeld-Jørgensen et al. 2011 Greenland, case-control study Inuit women with breast cancer registered at hospital (n = 31, 80% of all cases) in 2000–2003 (median age 50 yr). Age and district-matched (frequency) controls selected from cross-sectional biomonitoring study (n = 115, median age 54 yr)	Median serum PFOS (range) Cases: 0.0456 µg/mL (0.0116−0.124) Controls: 0.0219 µg/mL (0.0015–0.172)	Breast Cancer OR (95% CI), p-value, (n cases/n controls) Unadjusted 1.01 (1.003, 1.02), p = 0.02, (98 cases/31 controls) Adjusted 1.03 (1.001, 1.07), p = 0.05, (69 cases/9 controls)
Eriksen et al. 2009 Denmark Diet, Cancer and Health Study; enrolled December 1, 1993–May 31, 1997; cancer diagnoses between December 1, 1993–July 1, 2006. 1,240 cancer cases (1,111 male, 129 female), median age 59 years compared to 772 participants selected at random from cohort, median age 56 years. Analysis using Cox proportional hazards model stratified by sex (IRR)	Plasma PFOS concentrations at enrollment; range: 0.001–0.131 µg/mL. Quartiles PFOS Q1 Q2 Q3 Q4 Trend per 10 ng/mL increase Q1 Q2 Q3 Q4 Trend per 10 ng/mL increase Q1 Q2 Q3 Q4 Trend per 10 ng/mL increase	 IRR (95% CI) Bladder Cancer (n = 332) 1.0 0.76 (0.50, 1.16) 0.93 (0.61, 1.41) 0.70 (0.446, 1.07) 0.93 (0.83, 1.03) Liver Cancer (67) 1.0 0.62 (0.29, 1.33) 0.72 (0.33, 1.56) 0.59 (0.27, 1.27) 0.97 (0.79, 1.19) Prostate Cancer (n = 713) 1.0 1.35 (0.97, 1.87) 1.31 (0.94, 1.82) 1.38 (0.99, 1.93) 1.05 (0.97, 1.14)

3.1.2.1 Summary and Conclusions from the Human Cancer Epidemiology Studies

A small number of epidemiology studies of PFOS exposure and cancer risk are available. While these studies do report elevated risk of bladder and prostate cancers, limitations in design and analysis preclude the ability to make definitive conclusions. While an elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study (Alexander et al. 2003), a subsequent study to ascertain cancer incidence in the cohort observed elevated but statistically insignificant incidence ratios that were 1.7- to 2-fold higher among workers with higher cumulative exposure (Alexander and Olsen 2007). The risk estimates lacked precision because the number of cases was small. Smoking prevalence was higher in the bladder cancer cases, but the analysis did not control for smoking because data were missing for deceased workers, and therefore positive confounding by smoking is a possibility. Mean PFOS serum levels were 0.941 μg/mL. No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment of 0.001–0.1305 μg/mL (Eriksen et al. 2009).

Elevated odds ratios for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009). However, the confidence intervals included the null, and no association was reported by another case-control study in Denmark (Hardell et al. 2014). A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (0.0015–0.172 μg/mL) reported an association of low magnitude that could not be separated from other perfluorsulfonated acids, and the association was not confirmed in a Danish population (Bonefeld-Jørgensen et al. 2011, 2014). Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis, and the impact of this potential exposure misclassification on the estimated risks is unknown (Bonefeld-Jørgensen et al. 2011; Hardell et al. 2014). No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

3.2 Animal Studies

Acute and short-term studies in rats and mice provide data on lethality, systemic toxicity, neurotoxicity, and mode of action. Subchronic studies in monkeys and rats found decreased body weight, increased liver weight accompanied by microscopic lesions, and decreased serum cholesterol. The most prominent microscopic lesion of the liver in both monkeys and rats was centrilobular hepatocellular hypertrophy. In a chronic bioassay, rats had decreased body weight, increased liver weight with microscopic lesions, and an increased incidence of hepatocellular adenomas. Effects on development and reproduction were found in both rats and mice, including increased neonatal mortality. Other developmental and reproductive toxicity effects included decreased gestation length, lower birth weight, and developmental delays. Postnatal effects of gestational and lactational exposure included evidence of developmental neurotoxicity, changes in thyroid and reproductive hormones, altered lipid and glucose metabolism, and decreased immune function. Each of these studies is described in detail below, and a tabular summary of the animal studies is provided in Appendix C, Table C-2.

3.2.1 Acute Toxicity

The few available acute toxicity studies of PFOS indicate a lethal dose for 50% (statistical median) of animals (LD_{50}) of 251 mg/kg and an LC_{50} of 5.2 ppm in rats (Dean et al. 1978; Rusch et al. 1979). PFOS caused no irritation in a dermal irritation study although limited study details were available (OECD 2002). An eye irritation study was also conducted but few details were provided on effects observed (OECD 2002).

Oral Exposure

Dean et al. (1978) exposed 5 CD rats/sex/dose by gavage to a single dose of 0, 100, 215, 464, or 1,000 mg/kg of PFOS suspended in a 20% acetone/80% corn oil mixture. Rats were observed for abnormal signs for 4 hours after exposure and then daily for up to 14 days. All rats died in the 464 and 1,000 mg/kg group, and 3 of 10 rats died in the 215 mg/kg group. Clinical signs observed included hypoactivity, decreased limb tone, and ataxia. Necropsy results indicated stomach distension, lung congestion, and irritation of the glandular mucosa. Based on the findings, the acute oral LD_{50} was 233 mg/kg in males, 271 mg/kg in females, and 251 mg/kg combined.

Male Wistar rats and male ICR mice (n = 2–3 per group) were administered a single oral dose of PFOS at 0, 125, 250, or 500 mg/kg and monitored for any neurological signs (Sato et al. 2009). Animals of both species treated with ≥ 250 mg/kg had decreased body weight or delay of body weight gain during the 14 days post-exposure. One of three rats in the 250 mg/kg group and both rats in the 500 mg/kg group died. One mouse in each dose group died. No neurological signs were observed. No histopathological changes were observed in the neuronal or glial cells of the cerebrum and cerebellum in rats killed 24 hours after exposure. In these same rats, the highest concentration of PFOS was in the liver and the lowest was in the brain. Rats administered 250 mg/kg bw did not show any differences in the levels of catecholamines (norepinephrine, dopamine, and serotonin) or amino acids (glutamic acid, glycine, and gamma-aminobutyric acid [GABA]) when compared to the controls at 24 and 48 hours post-exposure.

Inhalation Exposure

Rusch et al. (1979) exposed Sprague-Dawley rats (5/sex/dose) to PFOS dust (in air) at concentrations of 0, 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09, or 45.97 mg/L for 1 hour. Rats were observed for abnormal signs prior to exposure, every 15-min during exposure, at removal from the chamber, hourly for 4 hours after exposure, and then daily for up to 14 days. The 45.97 mg/L group was not used in determining the LC_{50} as this portion of the study was terminated on day 2 due to high mortality; the 13.9 mg/L group was also not part of the calculation as this group was terminated early due to mechanical problems. All rats in the 24.09 mg/L group died by day 6. Mortality for the other groups was 0%, 10%, 20%, 80%, and 80% in the 1.89, 2.86, 4.88, 6.49, and 7.05 mg/L groups, respectively. Clinical signs observed included emaciation, red material around the nose or other nasal discharges, dry rales, breathing disturbances, and general poor condition. Necropsy results indicated discoloration of the liver and lung. Based on the findings, the acute inhalation LC_{50} was 5.2 mg/L (ppm).

Dermal/Ocular Exposure

The only dermal and ocular irritation PFOS studies were performed by Biesemeier and Harris (1974) and were summarized in OECD (2002) with few details. In the dermal study, six albino rabbits were treated by placing 0.5 grams of the test material on their intact or abraded backs and covered. Erythema and edema were scored after 24 and 72 hours. The primary

irritation score was zero indicating no irritation or edema. No information was provided on the guidelines followed, sex of the animals, and the vehicle used.

In the ocular study, six albino New Zealand White rabbits, fitted with Elizabethan collars, were treated with one tenth of a gram of the test substance instilled in one eye; the other eye was used as the untreated control. Reaction to the test material was recorded at 1, 24, 48, and 72 hours after treatment; however, the scale criteria were not presented or referenced. Scores were maximal at 1 hour and 24 hours after treatment, then decreased over the rest of the study. The raw data were not provided in the OECD (2002) report.

3.2.2 Short-Term Studies

Short-term oral toxicity studies in rats and mice included data on lethality, body weight, liver weight, and histopathology, as well as serum lipids. Body weight was decreased and liver weight increased at > 2 mg/kg/day in rats. Higher doses resulted in hepatocyte hypertrophy and decreased cholesterol in rats and mice. Mechanistic studies in mice indicate changes suggestive of hepatic hyperlipidemia or fatty liver disease.

Oral Exposure

Rat. Forty to seventy Sprague-Dawley Crl:CD (SD) IGS BR rats/sex/dose were administered PFOS in the diet at concentrations of 0, 0.5, 2.0, 5.0, or 20 ppm as part of a long term chronic cancer bioassay (Seacat et al. 2003). Five animals per dose group were sacrificed for interim necropsies at 4 weeks. Doses were equivalent to 0, 0.05, 0.18, 0.37, and 1.51 mg/kg in males and 0, 0.05, 0.22, 0.47, and 1.77 mg/kg in females. Animals were observed twice daily for mortality and moribundity, with a clinical exam performed weekly. Body weight and food consumption data were recorded weekly. Food efficiency was determined, and mean daily intake of PFOS, cumulative dose, and percentage of dose were identified in the liver and sera. Blood and urine were obtained from 10 animals/sex/dose during week 4 for clinical chemistry, hematology, and urinalysis evaluation. A thorough necropsy was performed on five animals/ sex/dose at the end of 4 weeks of treatment and liver samples were collected for palmitoyl CoA oxidase (PCoAO) activity, liver weight, cell proliferation index (PI), and PFOS concentration analysis. Microscopic analysis of tissues was performed on the control and high-dose animals. Analysis of PFOS in the liver and sera were determined by HPLC/MS/MS and results were considered quantitative to ± 30%.

A summary of findings in the study is provided in Table 3-9. For the animals treated for 4 weeks, terminal body weight in the 20 ppm animals was decreased, although not statistically-significant. Absolute liver weight was not affected, but relative liver weight was increased in the high dose males and females; the increase was significant only for males. Food consumption and food efficiency were decreased only in the 20 ppm females. No treatment-related effects were observed on hematology or urinalysis; male rats treated with 20 ppm had significant decreases in serum glucose. Analysis of PCoAO activity was weakly increased (< 2-fold) when compared to controls in the 20 ppm dose group males in one laboratory and similar to controls in another laboratory analysis. The 20 ppm (1.5 mg/kg/day) dose group was a LOAEL for males following a 4 week exposure.

Table 3-9. Mean (± SD) Values for Select Parameters in Rats Treated for 4 Weeks

Parameter	PFOS (mg/kg/day)				
Males					
	0	**0.05**	**0.18**	**0.37**	**1.51**
Body wt (g)	323 ± 34	315 ± 16	303 ± 25	309 ± 19	296 ± 21
Liver/body wt (%)	3.6 ± 0.2	4.1 ± 0.4	3.9 ± 0.2	3.5 ± 0.3	4.4* ± 0.3
PCNA LI (%)	0.042 ± 0.024	0.038 ± 0.014	0.069 ± 0.028	0.043 ± 0.025	0.065 ± 0.029
Glucose (mg/dL)	97 ± 11	97 ± 5	91 ± 11	94 ± 9	84* ± 5
AST (IU/L)	122 ± 26	146 ± 29	104 ± 23	114 ± 17	131 ± 20
PCoAO (IU/g)	9.0 ± 2.2	9.0 ± 2.3	7.0 ± 4.0	8.0 ± 0.8	6.0 ± 1.4
Females					
	0	**0.05**	**0.22**	**0.47**	**1.77**
Body wt (g)	213 ± 21	192 ± 11	202 ±15	206 ± 29	193 ± 17
Liver/body wt (%)	3.8 ± 0.2	3.7 ± 0.2	3.8 ± 0.2	3.7 ± 0.4	4.1 ± 0.3
PCNA LI (%)	0.53 ± 0.032	0.055 ± 0.015	0.059 ± 0.013	0.097 ± 0.036	0.183 ± 0.085
Glucose (mg/dL)	114 ± 7	11 ± 7[a]	113 ± 18	109 ± 11	107 ± 8
AST (IU/L)	123 ± 28	120 ± 37	101 ± 12	112 ± 24	92 ± 16
PCoAO (IU/g)	5.0 ± 1.5	6.0 ± 1.1	3.0 ± 1.7	2.0** ± 1.1	4.0 ± 1.1

Source: Data from Seacat et al. 2003
Notes: [a] Reviewer suspects this is a typo and should be 111 mg/dL as it was not marked significant and is not in the text.
*Statistically-significant from controls, $p < 0.05$
PCNA LI = proliferating cell nuclear antigen labeling index
IU = international unit

Curran et al. (2008) conducted two 28-day studies in groups of 15 Sprague-Dawley rats/sex/dose. In both studies, the animals were administered 0, 2, 20, 50, or 100 mg PFOS/kg diet which was equivalent to 0, 0.14, 1.33, 3.21, or 6.34 mg PFOS/kg body weight/day, respectively, in males and 0, 0.15, 1.43, 3.73, or 7.58 mg/kg body weight/day, respectively, in females. In the first study (Study 1), rats were assessed for changes in clinical chemistry, hematology, histopathology, and gene expression. In Study 2, blood pressure, erythrocyte deformability and liver fatty acid composition were assessed. Tissues were also analyzed for PFOS residues by LC/MS/MS. Tissue residue results showed a dose-dependent increase with most of the PFOS identified in the liver; values for the PFOS residue levels are reported in section 2.2, Distribution.

There were no treatment-related differences observed in hematology and urinalysis parameters. Statistically-significant ($p \leq 0.05$) decreases in body weight and food consumption were observed in the males and females administered ≥ 50 mg PFOS/kg diet. Food consumption was also statistically decreased in males during week 3 of treatment in the 20 mg PFOS/kg diet group. No differences in blood pressure measurements were observed across the groups. Deformability index values in red blood cells over a range of shear stress levels were significantly lower in both males and females exposed to 100 mg PFOS/kg diet, relative to controls.

Absolute and relative liver weights were statistically-significantly increased in the male and female rats at ≥ 20 mg PFOS/kg diet. Relative liver weight was also statistically increased in the 2 mg PFOS/kg diet females. Histopathological changes were observed in the liver of the males treated with ≥ 50 mg PFOS/kg diet and included hepatocyte hypertrophy and an apparent increase in cytoplasmic homogeneity. Increased hepatocyte hypertrophy and cytoplasmic homogeneity in the females was seen at ≥ 50 mg PFOS/kg diet.

Both males and females showed a significant increase in expression of the gene for peroxisomal acyl-coenzyme A oxidase at concentrations ≥ 50 mg PFOS/kg diet. Cytochrome P-450 4A22 (CYP4A22) expression was increased 4%–15% greater than controls in the males in the ≥ 20 mg/kg diet groups and 3%–7% greater in the females administered ≥ 50 mg PFOS/kg diet. Liver fatty acid profiles showed increased total monounsaturated fatty acid levels and decreased total polyunsaturated fatty acids. A total of 67 fatty acid profiles were examined. The authors stated that the profile changes were similar to those induced by weak peroxisome proliferators.

At the high doses, the serum levels of conjugated bilirubin and total bilirubin were increased significantly. Serum cholesterol was significantly decreased for males and females at ≥ 50 mg PFOS/kg diet. Serum T4 and T3 levels were also decreased in males and females, with T4 levels being statistically-significantly decreased at ≥ 20 mg PFOS/kg diet, when compared to the control levels. Significant differences as observed in this study are provided in Table 3-10.

Table 3-10. Mean (± SD) Values for Select Parameters in Rats Treated for 28 Days

Parameter	PFOS (mg/kg diet)				
	0	2	20	50	100
Males					
Final body wt (g)	415.1 ± 40.1	412.3 ± 32.0	386.2 ± 25.9	363.7* ± 25.7	327.0* ± 21.6
Liver wt (g)	17.7 ± 2.7	17.1 ± 2.8	18.4 ± 3.2	20.8* ± 1.5	21.7* ± 2.3
Liver/body wt (%)	4.24 ± 0.41	4.13 ± 0.48	4.75* ± 0.67	5.73* ± 0.21	6.64* ± 0.41
Thyroid wt (g)	0.021± 0.004	0.022 ± 0.005	0.020 ± 0.004	0.020 ± 0.003	0.021 ± 0.055
Conjugated bilirubin (µmol/L)	0.57 ± 0.18	0.65 ± 0.22	0.62 ± 0.19	0.75 ± 0.27	2.13* ± 0.44
Total bilirubin (µmol/L)	2.75 ± 0.63	2.75 ± 0.89	2.47 ± 0.82	2.55 ± 0.91	4.01* ± 0.87
Cholesterol (mmol/L)	2.54 ± 0.63	2.46 ± 0.55	2.06 ± 0.43	1.63* ± 0.31	0.31* ± 0.18
Triglycerides (mmol/L)	1.74 ± 0.93	1.92 ± 0.78	1.77 ± 0.57	1.00* ± 0.42	0.20* ± 0.08
T4 (nmol/L)	80.94 ± 11.83	66.97 ± 14.75	14.36* ± 4.18	12.88* ± 2.67	13.29* ± 2.59
T3 (nmol/L)	1.60 ± 0.33	1.81 ± 0.19	1.36 ± 0.26	1.29 ± 0.26	1.21* ± 0.23
Females					
Final body wt (g)	247.2 ± 27.5	251.2 ± 13.1	245.9 ± 10.5	217.6* ± 15.1	197.6* ± 10.4
Liver wt (g)	9.1 ± 1.5	10.2 ± 1.2	11.0* ± 1.2	11.2* ± 1.2	12.2* ± 1.4
Liver/body wt (%)	3.64 ± 0.38	4.06* ± 0.39	4.45* ± 0.40	5.12* ± 0.38	6.24* ± 0.67
Thyroid wt (g)	0.016 ± 0.003	0.017 ± 0.004	0.018 ± 0.003	0.017 ± 0.003	0.018 ± 0.005
Conjugated bilirubin (µmol/L)	0.52 ± 0.14	0.47 ± 0.14	0.49 ± 0.17	0.85* ± 0.18	2.60* ± 0.73
Total bilirubin (µmol/L)	2.00 ± 0.75	1.67 ± 0.43	1.51 ± 0.54	2.20 ± 0.43	4.69* ± 1.04
Cholesterol (mmol/L)	2.06 ± 0.36	2.02 ± 0.51	1.66 ± 0.28	1.37* ± 0.24	0.52* ± 0.16
Triglycerides (mmol/L)	0.99 ± 0.46	1.68 ± 0.99	1.11 ± 0.70	0.65 ± 0.30	0.37* ± 0.30
T4 (nmol/L)	37.71 ± 15.41	32.39 ± 10.40	19.62* ± 2.49	15.05* ± 1.99	16.40* ± 4.61
T3 (nmol/L)	1.83 ± 0.17	1.72 ± 0.14	1.75 ± 0.27	1.41* ± 0.22	1.27* ± 0.20

Source: Data from Tables 2-3 and 6-7 in Curran et al. 2008
Note: *Statistically-significant from controls, $p < 0.05$ or $p \leq 0.05$

The LOAEL was the 20 mg/kg dietary level (males: 1.33 mg PFOS/kg/day; females: 1.43 mg PFOS/kg/day) for a significant increase in absolute (females) and relative (males and females) liver weights and significant decrease in serum T4 (males and females). The NOAEL was the 2 mg/kg diet level (0.14–0.15 mg PFOS/kg/day).

Ten three-month old male Sprague-Dawley rats/group were administered 0 (Milli-Q water only), 5, or 20 mg/kg/day PFOS by oral gavage for 28 days (Cui et al. 2009). Rats were sacrificed after exposure, and blood and tissue samples were obtained. All rats (10/10) administered 20 mg/kg/day of PFOS died by study day 26. At necropsy, rats had bleeding around the eye socket and nose and yellow staining in the urogenital region. Prior to death, rats displayed significant weight loss and a decrease in food consumption when compared to controls. Rats administered 5 mg/kg/day also had a significant decrease in body weight when compared to controls at the study termination. Viscera indices were calculated including the hepatosomatic index (HSI), renal-somatic index (RSI), and gonad-somatic index to evaluate the hyperplasia, swelling and/or atrophy of the organs, and all three indices were statistically-significantly increased in all of the treated groups. The increases in the HSI and RSI showed a dose dependency. Rats administered 20 mg/kg/day had swelling and discoloration of the liver, with hepatocyte hypertrophy and cytoplasmic vacuolation observed on histopathological exam. Rats administered 20 mg/kg/day had congestion and thickened walls in the lungs with the pulmonary congestion also observed in the 5 mg/kg rats. Based on the results, a LOAEL of 5 mg/kg/day in rats was identified based on a significant decrease in body weight, dose-related effects in the liver and pulmonary congestion. A NOAEL could not be identified.

Mouse. The variability in the serum lipid profiles in humans suggests that response to PFOS exposure could be impacted by individual physiological differences and that environmental factors such as diet might contribute to intraspecies variability in response. L. Wang et al. (2014) reported on the differences in response of male BALB/c mice (4–5 weeks old) administered PFOS (0, 5, or 20 mg/kg) for 14 days while concurrently given diets that varied in fat [regular fat (RF) versus high fat (HF) content]. The high fat diet contained 10% more lard and 3% more cholesterol than the regular fat diet. Liver and serum responses were evaluated after a 14 day exposure period. The data were for the endpoints monitored were presented graphically.

Following PFOS exposure, there was an increase in liver fat content in both groups and a decrease in liver glycogen in rats on both diets. For the mice on the regular fat diet, the addition of PFOS led to a significant increase in liver fat content (an approximately two-fold increase). For the mice on the high fat diet, the addition of PFOS caused a slight a slight and nonsignificant increase in the liver fat content.

The fat content of the diet alone was associated with significantly higher serum levels of glucose, HDL cholesterol, LDL cholesterol, total cholesterol, and triglycerides. The differences were significant for glucose, albumin, and total cholesterol ($p < 0.01$). For glucose, cholesterol, HDL, and LDL, the serum levels declined as the dose of PFOS increased; for triglycerides the levels increased at a dose of 5 mg PFOS/kg/day and decreased at 20 mg PFOS/kg/day. PPARα expression at the end of 14 day PFOS treatment increased for the RF group, but it decreased for the HF groups (significant for the high dose).

The authors examined the expression of several genes involved with lipid metabolism (CPT1A and CYP7A1). CPT1A plays a role in transport of fatty acid into the mitochondria for beta oxidation, and CYP7A1 is involved with the transformation of cholesterol into bile acids. The high fat diet alone increased the expression of both genes. On the RF diet, the exposure to PFOS was associated with a significant dose-related increase in CPT1A expression, whereas for the high fat diet plus PFOS there was a significant decrease in expression. For CYP7A1 expression there was no significant impact of PFOS with the RF diet, whereas with the high fat diet there was a highly significant decrease in expression with PFOS. The study demonstrates a clear influence of diet alone on the liver and lipid profile of the treated mice, combined with

some dose-related differences in the responses to PFOS exposure. The data support a possible role for PFOS in inhibiting pathways for cholesterol metabolism and export of liver lipids and identify some PFOS associated liver responses that are independent of PPARα activation.

A 21-day study by Wan et al. (2012) examined mechanistic aspects related to the role of PFOS in leading to hepatic steatosis in male CD-1 mice (4/dose). Animals were given PFOS in corn oil by gavage at doses of 0, 1, 5, or 10 mg/kg/day with sacrifice after 3, 7, 14, or 21 days. Liver weights were significantly ($p < 0.05$) increased for the highest two dose groups across the duration of the study and only at day 7 for the 1 mg/kg/day dose. The size of the liver was significantly increased ($p < 0.0003$) at 5 and 10 mg/kg/day and a yellowish coloration of the tissues was visually apparent. Histologically there was microvesicular steatosis on day 14 and macrovesicular steatosis on day 21 at 10 mg/kg/day. The level of liver triglycerides was significantly ($p < 0.001$) increased compared to control for the 5 and 10 mg/kg/day dose groups.

The Wan et al. (2012) study included a series of mechanistic components to investigate the mode of action for the effects observed. Both mRNA and protein expression for fatty acid translocase and lipoprotein lipase were significantly increased for the 10 mg/kg/day dose. Levels of mRNA in adipose tissue from the fat pad were not increased for either enzyme. Export of liver lipids appeared to decrease, leading to lower serum LDL/VLDL levels on days 14 and 21. The change correlated with increased liver weight and decreased expression of liver apolipoprotein B-100 (apob). By day 21, apob expression was significantly decreased ($p < 0.001$) even in the low dose group. Formation of hepatic VLDLs requires apob; the VLDLs are carriers of liver triglycerides and other lipids from liver to serum.

The authors also examined total hepatic β oxidation, peroxisomal β oxidation, and mitochondrial β oxidation using d^{31} palmitic acid. The results of this assay indicated that the PFOS was primarily responsible for a decrease in mitochondrial β oxidation as monitored on day 14. While total and peroxisomal β oxidation were slightly, but significantly, increased ($p < 0.01$) at 10 mg/kg/day, mitochondrial β oxidation was markedly decreased ($p < 0.05$ or 0.01) in all dose groups. Transcripts for mRNA for peroxisomal acyl-CoA oxidase, Cyp 4a14, and acyl-Co A dehydrogenase were significantly increased in the 5 and 10 mg/kg/day dose groups, suggesting breakdown of long chain fatty acids by peroxisomes. Increases in peroxisomal oxidation in the absence of increased mitochondrial beta oxidation can lead to accumulation of fatty acids in the liver (steatosis). The LOAEL identified for this study is 5 mg/kg/day. At 1 mg/kg/day there was increased liver weight in the absence of histopathological correlates. The 1 mg/kg/day dose is accordingly a NOAEL. The authors concluded that the hepatic changes observed in mice were similar to those associated with nonalcoholic fatty liver disease in humans and were not totally a reflection of PPARα activation.

Bijland et al. (2011) examined the molecular biology for the hepatic hyperlipidemia in APOE*3-Leiden.CETP mice, a strain that exhibits human-like lipoprotein metabolism. The experimental animals were fed a western-type diet containing 0.25% cholesterol, 1% corn oil, and 14% bovine fat for 4 weeks with or without 3 mg PFOS/kg/day. The diet contained 0.25% cholesterol, 1% corn oil, and 14% bovine fat. Plasma samples were collected via tail vein bleeding and analyzed for a variety of lipid related endpoints including TC, triglycerides, VLDL, and HDL. Following terminal sacrifice, the liver, heart, perigonadal fat, spleen, and skeletal femoralis muscle were collected for analysis. Fecal samples were collected for measurement of bile acids and neutral sterols.

Significant decreases in triglycerides (−50%), total cholesterol (−60%), HDL (−74%), and non-HDL (−60%) were found in mice given PFOS compared with controls. VLDL was also significantly less than that of controls, but the level was only presented graphically. Radiolabeled VLDL-like emulsion particles showed the plasma half-life of VLDL was reduced by 52% in PFOS treated mice compared with controls accompanied by significantly increased uptake by liver, heart, and muscle. VLDL production by the liver was markedly decreased (−87%) in treated animals. Liver weight and hepatic triglyceride content were significantly greater ($p < 0.0001$) and perigonadal fat pad weight was significantly less ($p < 0.05$) in PFOS treated mice compared to those of controls. Thus, PFOS was found to decrease hepatic VLDL production leading to increased retention of triglycerides (steatosis) and hepatomegaly. As a consequence, there was a decrease in plasma-free fatty acids and glycerol and the mass of perigonadal fat pad. Neutral sterols in the feces were not altered, but the presence of bile acids was decreased by 50%. Hepatic clearance of VLDL and HDL cholesterol were decreased primarily because of impaired hepatic production and clearance of these lipoprotein complexes.

Compared with the controls, PFOS treated animals had 3,986 differentially expressed genes. Impacted hepatic genes involved with lipid metabolism included those involved with VLDL metabolism, fatty acid uptake and transport, fatty acid oxidation, and triglyceride synthesis. Overall, the genes upregulated (1- to 2-fold) were those involved with fatty acid uptake and transport and catabolism; triglyceride synthesis; cholesterol storage; and VLDL synthesis. Genes involved with HDL synthesis, maturation, clearance, and bile acid formation and secretion were downregulated (1-fold for most genes to almost 4-fold for genes involved in secretion). These changes are consistent with increased hepatic hyperlipidemia, decrease in bile acid secretion, and serum hypolipidemia. Many of the genes activated are associated with the nuclear pregnane X receptor (PXR) to a greater extent than PPARα. Lipoprotein lipase activity and mRNA expressions were increased in the liver. This enzyme facilitates removal of TGs from serum LDLs, as well as uptake into the liver and other organs as free fatty acids and glycerol. Lipoprotein lipase activity in the liver is relatively low compared to that of peripheral tissues.

3.2.3 Subchronic Studies

Three monkey studies of oral PFOS exposure (two with rhesus- and one with cynomolgus-strains) and two rat subchronic studies are available. The study with cynomolgus monkeys was a GLP study. There are no subchronic studies by dermal or inhalation routes of exposure with PFOS. In monkeys, clinical signs of toxicity were observed at 0.5 mg/kg/day, while lower body weight, increased liver weight with hepatocellular hypertrophy, and decreased serum cholesterol occurred at 0.75 mg/kg/day. Rats given 1.3–1.6 mg/kg/day had increased liver weight with hepatocyte hypertrophy and decreased cholesterol.

Oral Exposure

Monkey. Two monkey studies were performed with rhesus monkeys (Goldenthal et al. 1978a and 1979). In the first study, 2 monkeys/sex/dose were administered 0, 10, 30, 100, or 300 mg/kg/day of PFOS in distilled water by gavage. The study was terminated on day 20 as all of the 300 mg/kg treated monkeys died beginning on day 4; deaths were also observed at all lower doses, but whether it was one or both of the animals was not stated. Clinical signs of toxicity were observed in all groups and included decreased activity, emesis, body stiffening, general body trembling, twitching, weakness, and convulsions. At necropsy, several of the

100 and 300 mg/kg/day monkeys had a yellowish-brown discoloration of the liver although there were no microscopic lesions. A NOAEL or LOAEL was not determined for this study.

In the second study, 2 rhesus monkeys/sex/dose were administered 0, 0.5, 1.5, or 4.5 mg/kg/day of PFOS in distilled water by gavage for 90 days. All monkeys in the 4.5 mg/kg/day group died or were euthanized *in extremis* by week 7 and exhibited decreased body weight, signs of gastrointestinal tract toxicity (anorexia, emesis, black stool), decreased activity, and marked to severe rigidity and had a significant decrease in serum cholesterol. Histopathology of the 4.5 mg/kg/day monkeys showed diffuse lipid depletion in the adrenals (4/4), diffuse atrophy of the pancreatic exocrine cells (3/4) and moderate diffuse atrophy of the serous alveolar cells (3/4). All monkeys in the 0.5 and 1.5 mg/kg/day treated groups survived, but they exhibited occasional diarrhea, soft stools, and anorexia. These clinical signs showed a dose-related increase, and 1/4 of the 1.5 mg/kg/day monkeys had low serum cholesterol. Body weight was decreased in males and females at 1.5 mg/kg/day. There were no treatment-related effects observed in any of the 0.5 or 1.5 mg/kg/day monkeys at necropsy. Based on the findings, the LOAEL was 0.5 mg/kg/day, and the NOAEL could not be determined.

Seacat et al. (2002) administered 0, 0.03, 0.15, or 0.75 mg/kg/day of potassium PFOS orally in a capsule by intragastric intubation to 6 young-adult to adult cynomolgus monkeys/sex/dose, except for the 0.03 mg/kg/day group (4 monkeys/sex), daily for 26 weeks (182 days) in a GLP study. Two monkeys per sex in the control, 0.15, and 0.75 mg/kg/day groups were monitored for 1 year post-exposure for reversible or delayed toxic effects. Monkeys were observed twice daily for mortality, morbidity, clinical signs, and qualitative food consumption. Body weights were recorded pre-dosing and weekly thereafter, and ophthalmic examinations were performed pre- and post-treatment. PFOS levels were determined in serum and liver tissue and hematology and clinical chemistry were performed. Urine and fecal analyses were done and full histopathology performed at the scheduled sacrifice. Liver samples were also obtained for hepatic peroxisome proliferation determination and immunohistochemistry was performed by PCNA to look for cell proliferation. Selected results are shown in Table 3-11.

Two of the 0.75 mg/kg/day males died; one died on day 155 and one was found moribund and was sacrificed on day 179. The monkey that died had pulmonary necrosis and severe acute recurrences of pulmonary inflammation as its cause of death. The specific cause of the moribund condition was not established, however, the clinical chemistry results were suggestive of hyperkalemia. Overall mean body weight gain was significantly ($p \leq 0.05$) less in the 0.75 mg/kg/day males and females (lost 8 ± 8% and 4 ± 5%, respectively) after the treatment when compared to controls (gained 14 ± 11% and 5 ± 5%, respectively). Mean absolute and relative (to body weight) liver weight was increased significantly in the 0.75 mg/kg/day males and females.

Males and females at 0.75 mg/kg/day had lower total serum cholesterol beginning on day 91 (27%–68% [males] and 33%–49% [females] lower than controls) and lower high density lipoprotein cholesterol beginning on day 153 (72%–79% and 61%–68% lower than controls) when compared to the control values. This effect was reversible, however, as the total cholesterol levels were similar to controls by week 5 during recovery and the total high density lipoprotein cholesterol was similar to controls by week 9. Estradiol values were lower at 0.75 mg/kg in males and females on day 182; however, the data were highly variable and the study authors stated that the change was not well understood. Total triiodothyronine (T3) values were significantly decreased and TSH was increased on day 182 in the high-dose monkeys, but a true dose-response was not observed and the monkeys had no indication of clinical hypothyroidism (TSH values within reference range, no hyperlipidemia, and no thyroid gland histopathological lesions).

Table 3-11. Mean (± SD) Values for Select Parameters in Monkeys Treated for 182 Days

Parameter	PFOS (mg/kg/day)			
Males				
	0	**0.03**	**0.15**	**0.75**
Body wt (g)	3.7 ± 0.7	3.9 ± 0.6	3.3 ± 0.3	3.2 ± 0.8
Body wt change (%)	14 ± 11	16 ± 8	8 ± 7	−8 ± 8*
Liver wt (g)	54.9 ± 8.1	62.1 ± 5.3	57.3 ± 5.5	85.3 ± 38.4
Liver/body wt (%)	1.6 ± 0.2	1.7 ± 0.3	1.8 ± 0.1	2.7 ± 0.3*
Cholesterol (mg/dL)	152 ± 28	110 ±17**	147 ± 24	48 ± 19**
HDL (mg/dL)	63 ± 11	42 ± 4**	48 ± 14	13 ± 5**
Total T3 (ng/dL)	146 ± 19.8	145 ± 18.0	129 ± 4.8	76 ± 22**
TSH (µU/mL)	0.55 ± 0.44	0.56 ± 0.10	1.38 ± 0.78	1.43 ± 0.25*
Estradiol (pg/mL)	23.0 ± 11.5	24.1 ± 14.2	23.2 ± 7.4	0.8 ± 1.0**
Females				
	0	**0.03**	**0.15**	**0.75**
Body wt (g)	3.0 ± 0.4	3.2 ± 0.7	3.1 ± 0.5	2.8 ± 0.4
Body wt change (%)	5 ± 5	6 ± 7	4 ± 5	−4 ± 5
Liver wt (g)	51.1 ± 9.4	56.8 ± 12.6	57.0 ± 3.1	75.3 ± 13.3*
Liver/body wt (%)	1.8 ± 0.2	1.9 ± 0.0	2.1 ± 0.2	2.9 ± 0.3*
Cholesterol (mg/dL)	160 ± 47	122 ± 22	129 ± 22	82 ± 15**
HDL (mg/dL)	56 ± 16	42 ± 9	36 ± 12**	21 ± 7**
Total T3 (ng/dL)	148 ± 21.6	139 ± 11.5	116 ± 16.8	99 ± 16.8*
TSH (µU/mL)	1.02 ± 0.69	2.01 ± 2.09	1.33 ± 1.13	1.86 ± 1.29
Estradiol (pg/mL)	148.5 ± 110.1	125.2 ± 101.2	70.6 ± 62.7	39.9 ± 33.6

Source: Data from Seacat et al. 2002
Notes: *Statistically-significant from controls: *$p < 0.05$
** Statistically-significant from controls: $p < 0.01$.

Hepatic peroxisome proliferation was measured by PCoAO activity and was increased significantly in the 0.75 mg/kg/day females; however, the increase was not dose-related and it was < two-fold. There were no treatment-related effects on cell proliferation in the liver, pancreas, or testes when analyzed by proliferating cell nuclear antigen immunohistochemistry cell labeling index. Two high dose males and one high-dose female had mottled livers on gross examination at sacrifice; this was also observed in the high-dose male that died during the study. All females and 3/4 males at the high-dose had centrilobular or diffuse hepatocellular hypertrophy.

Serum and liver samples collected during the study were analyzed for PFOS and animals showed a dose-dependent increase in concentrations. Values decreased with recovery but never returned to control levels. There was not any gender difference in the amount of PFOS identified in the sera or liver. Based on the decreased body weight gain, decreased serum cholesterol, increased absolute and relative liver weight and histopathological lesions in the liver, the LOAEL in male and female monkeys treated with potassium PFOS was 0.75 mg/kg/day and the NOAEL was 0.15 mg/kg/day. Serum concentrations associated with no adverse effect (0.15 mg/kg/day) were 82.6 µg/mL in males and 66.8 µg/mL in females. Serum concentrations associated with adverse effects (0.75 mg/kg/day) were 173 µg/mL in males and 171 µg/mL in females.

Rat. Goldenthal et al. (1978b) administered 0, 30, 100, 300, 1,000, or 3,000 ppm of PFOS in the diet to five CD rats/sex/group for 90 days. Dietary levels were equivalent to 0, 2, 6, 18, 60, and 200 mg/kg/day, respectively. All rats at ≥ 300 ppm died starting on day 7 after exhibiting emaciation, convulsions, hunched back, increased sensitivity to stimuli, reduced activity, and red material around the nose/mouth At 100 ppm body weights were decreased (~ 16.5%), as was food consumption, when compared to controls. Relative liver weight and relative/absolute liver weight was significantly increased in the 100 ppm males and females, respectively. Both sexes had significant increases in relative kidney weight at 100 ppm. Three males and 2 females from the 100 ppm group died. All rats survived at 30 ppm, but there was a significant decrease in food consumption (males) and significant increase in absolute and relative liver weight (females). All treated animals had very slight to slight cytoplasmic hypertrophy of hepatocytes in the liver. Based on the significant decrease in food consumption and increase in absolute and relative liver weight, the LOAEL was 30 ppm (2 mg/kg/day) and the NOAEL could not be determined.

Seacat et al. (2003) also performed an interim sacrifice for five Sprague-Dawley Crl:CD (SD) IGS BR rats/sex/dose at the end of 14 weeks as part of the long-term cancer bioassay. The animals were administered PFOS in the diet at concentrations of 0, 0.5, 2.0, 5.0, or 20 ppm. Doses were equivalent to 0, 0.03, 0.13, 0.34, and 1.33 mg/kg in males and 0, 0.04, 0.15, 0.40, and 1.56 mg/kg in females, respectively for those sacrificed at 14 weeks. Animals were observed twice daily for mortality and moribundity with a clinical exam performed weekly. Body weight and food consumption data were recorded weekly. Other parameters recorded were food efficiency, mean daily intake of PFOS, and cumulative/percentage of dose in the liver and sera. Blood and urine were obtained from 10 animals/sex/dose during week 14 for clinical chemistry, hematology, and urinalysis evaluation. A thorough necropsy was performed at the end of 14 weeks of treatment for 5 animals/sex/dose, and liver samples were collected for PCoAO activity, cell PI, and PFOS concentration analysis. Microscopic analysis of tissues was performed on the control and high-dose animals. Analysis of PFOS in the liver and sera were determined by HPLC/MS/MS, and results were considered quantitative to ± 30%.

No effects were observed on body weight, food efficiency, urinalysis evaluation, or peroxisome proliferation (hepatic PCoAO was unchanged) at 14 weeks. All significant changes, when compared to controls, were observed in the highest dose group. Food consumption was decreased. Absolute and relative (to body weight) liver weights were increased significantly in the males and males/females, respectively. All hematology parameters were similar to controls. Clinical chemistry parameters that were significantly affected, compared to controls, included decreased serum cholesterol (males), increased alanine aminotransferase [ALT] (males), and increased urea nitrogen (males/females). Select data are provided in Table 3-12.

Histopathological changes were not observed in the kidney; however, centrilobular hepatocyte hypertrophy and mid-zonal to centrilobular vacuolization were observed in the livers of the males and females. Based on the findings, the LOAEL for male and female rats administered PFOS in the diet for up to 14 weeks was 20 ppm (1.33 mg/kg in males and 1.56 mg/kg in females), and the NOAEL was 5 ppm (0.34 mg/kg in males and 0.40 mg/kg in females).

Table 3-12. Mean (± SD) Values for Select Parameters in Rats Treated for 14 Weeks

Parameter	PFOS (mg/kg/day)				
Males					
	0	**0.03**	**0.13**	**0.34**	**1.33**
Body wt (g)	496 ± 56	481 ± 51	434 ± 31	424 ± 44	470 ± 40
Liver wt (g)	15.5 ± 1.1	15.5 ± 2.7	14.0 ± 1.4	18.8 ± 3.0	20.3* ± 2.2
Liver/body wt (%)	3.2 ± 0.3	3.2 ± 0.2	3.2 ± 0.2	3.6 ± 0.3	4.3* ± 0.4
Seg. neutrophils (10^3/µL)	1.1 ± 0.4	1.3 ± 0.3	1.2 ± 0.3	1.2 ± 0.4	1.6* ± 0.4
Glucose (mg/dL)	102 ± 6.2	106 ± 11	91 ± 14	99 ± 9	95 ± 10
Cholesterol (mg/dL)	63 ± 13	53 ± 17	51 ± 15	57 ± 7	37* ± 13
ALT (IU/L)	36 ± 7	41 ± 6	41 ± 5	44 ± 14	65* ± 53
Urea nitrogen (mg/dL)	13 ± 2	14 ± 2	13 ± 2	14 ± 1	16* ± 2
PCoAO (IU/g)	4.6 ± 1.3	4.8 ± 3.3	5.4 ± 3.0	1.8 ± 1.8	5.4 ± 1.9
Females					
	0	**0.04**	**0.15**	**0.40**	**1.56**
Body wt (g)	284 ± 39	298 ± 41	266 ± 16	247 ± 18	249 ± 26
Liver wt (g)	9.3 ± 1.6	9.2 ± 1.3	8.4 ± 0.7	8.7 ± 1.0	10.6 ± 0.7
Liver/body wt (%)	3.3 ± 0.2	3.1 ± 0.1	3.2 ± 0.3	3.5 ± 0.3	4.3* ± 0.4
Seg. neutrophils (10^3/µL)	1.0 ± 0.5	1.0 ± 0.5	0.7 ± 0.2	0.9 ± 0.6	1.0 ± 0.6
Glucose (mg/dL)	106 ± 12	106 ± 9	108 ± 6	95* ± 8	99 ± 7
Cholesterol (mg/dL)	75 ± 15	88 ± 27	87 ± 24	70 ± 13	66 ± 14
ALT (IU/L)	34 ± 2.4	36 ± 9	37 ± 18	34 ± 5	39 ± 18
Urea nitrogen (mg/dL)	12 ± 2	13 ± 2	13 ± 2	14 ± 3	17* ± 2
PCoAO (IU/g)	1.8 ± 1.6	3.0 ± 2.6	1.0 ± 0.8	1.6 ± 2.6	5.0 ± 2.9

Source: Data from Table 1 in Seacat et al. 2003
Note: *Statistically-significant from controls, $p < 0.05$

3.2.4 Neurotoxicity

Available *in vivo* and *in vitro* studies focused on mechanistic endpoints to a greater extent neurobehavioral indications of neurotoxicity. Effects observed included altered levels of excitatory amino acids in the brain, changes in neurotransmitter levels and increases in miniature post-synaptic currents along with inward calcium currents. One study found effects on learning and memory in mice at approximately 2 mg/kg/day.

In vivo

Rat. Yang et al. (2009) determined the effect of PFOS on excitatory amino acids (EAAs) and glutamine synthetase (GS) in the rat central nervous system. Adult male Wistar rats (5/group) were administered a single dose of 0, 12.5, 25, or 50 mg/kg bw PFOS by oral gavage. The animals were sacrificed 5 days after administration. The EAAs analyzed in brain tissue were glutamate (Glu), aspartate, glycine, and GABA.

Rats in the 12.5, 25, and 50 mg/kg groups had significantly ($p < 0.05$) decreased body weights, by 15%, 22%, and 27%, respectively, compared to controls. Among the EAAs, the Glu content was significantly decreased in the hippocampus at the high dose (decrease of 77% compared to controls; $p < 0.05$); no other significant differences were recorded. In the cortex, Glu was the only excitatory amino acid (EAA) affected with significant decreases at 25 (decrease of 33% compared to controls) and 50 (decrease of 47 compared to controls) mg/kg. GS activity was

significantly increased in the hippocampus at 25 and 50 mg/kg bw. The study had a LOAEL of 12.5 mg/kg/day in rats based on the decreased body weight.

Mouse. Groups of 15 adult C57BL6 mice (8 weeks old; number of each sex not specified) were administered PFOS at doses of 0, 0.43, 2.15, or 10.75 mg/kg/day by gavage for three months (Long et al. 2013). Learning and memory were assessed in the Morris water maze. The apoptosis profile of hippocampal cells, as well as the levels of glutamate, GABA, dopamine, 3,4-dihydrophenylacetic acid (DOPAC), and homovanillic acid (HVA) were evaluated. In the water maze trial, animals in the mid- and high-dose groups exhibited a significantly longer latency to escape and spent significantly less time in the target quadrant. A significant increase in the percentage of apoptotic cells was observed in the hippocampus of the mid- and high-dose animals. Neurotransmitter levels were affected only in the high-dose group as based on decreased dopamine and DOPAC levels plus increased glutamate levels. HVA and GABA levels were unchanged by PFOS treatment.

Differential protein expression at the high dose included down-regulation of Mib1 protein (an E3 ubiquitin-protein ligase), Herc5 (hect domain and RLD 5 isoform 2), and Tyro3 (TYRO3 protein tyrosine kinase 3). Succinate dehydrogenase flavoprotein subunit (SDHA), Gzma (Isoform HF1 of Granzyme A precursor), Plau (Urokinase-type plasminogen activator precursor), and Lig4 (DNA ligase 4) were upregulated. The 0.43 mg/kg/day dose group was the NOAEL, and the 2.15 mg/kg/day dose group the LOAEL based on water maze performance.

In vitro. Slotkin et al. (2008) evaluated 10–250 μmol PFOS, PFOA, perfluorooctane sulfamide (PFOSA), and perfluorobutane sulfonate (PFBS) *in vitro* in differentiated and undifferentiated PC12 cells, a neurotypic cell line. The study evaluated the following endpoints as indications of effects:

- Inhibition of DNA synthesis.
- Deficits in cell numbers and growth.
- Oxidative stress.
- Cell viability.
- Shifts in differentiation toward or away from the dopamine and acetylcholine (ACh) neurotransmitter phenotypes.

No effects on cell size, cell number, or neurocyte outgrowth were observed. PFOS decreased cell viability at 250 μmol and promoted differentiation into the ACh phenotype at the expense of the DA phenotype. The study suggests that the mechanisms for the observed effects in the neurotypic cell lines are not the same for the individual perfluoroalkyl acids tested. The rank order for the adverse effects measured *in vitro* was as follows: PFOSA > PFOS > PFBS = PFOA.

Liao et al. (2009) assessed the effect of varying chain lengths of the perfluorinated compounds on cultured Sprague-Dawley rat hippocampal neurons. Spontaneous miniature post-synaptic currents (mPSCs) were recorded in gap-free mode from hippocampal neurons at 8–15 days *in vitro*. The compounds were tested at 100 μmol and included a variety of perfluorinated compounds including PFOS. Testing showed the frequency of mPSCs increased in proportion to the increase in carbon chain length. PFOS had a statistically-significant ($p < 0.001$) increase in the mPSCs when compared to the four carbon PFBS. Inward calcium currents (I_{Ca}) were recorded in the presence or absence of the individual compounds with a ramp depolarization pulse. Voltage values were recorded and plotted versus the corresponding I_{Ca} every 5 mV and the resulting current-voltage relationship curve established. All three sulfonic compounds increased

the I_{Ca}. The longer the chain length the greater was the effect. PFOS caused the greatest increase in IC_a (% increase not provided).

In the same study, the chronic effects of perfluorinated compounds (50 μmol) on neuronal development were evaluated by measuring neurite outgrowth and branching. Among the sulfonic compounds, only PFOS statistically suppressed the length of neurites ($p < 0.001$; 25% below that of controls) and sum length of neurites per neuron ($p < 0.001$; 31% below that of controls). The study suggested that the effects of perfluorinated sulfonates on neurons were greater than the perfluorinated carboxylates. The study authors hypothesized that this reflects the fact that PFOS was more likely to be incorporated into the lipid bilayer of cell membranes. This is consistent with the results from a study by Matyszewska et al. (2008) who found that PFOS incorporation into a model biological membrane was superior to PFOA and that it caused a change in membrane fluidity and thickness depending on the amount incorporated.

3.2.5 Developmental/Reproductive Toxicity

Rats and mice were found to be affected in developmental/reproductive studies with orally-administered PFOS. Prenatal exposure of rats to PFOS caused an increase in neonatal mortality when dams were given doses ≥ 1 mg/kg/day and lowered pup body weight occurred at maternal doses of 0.4 mg/kg/day. Neonatal death was shown to be a direct effect of PFOS on the lung surfactant. Other developmental and reproductive toxicity effects included decreased gestation length and developmental delays. Higher doses resulted in fetal sternal defects and cleft palate in both rats and mice.

Many specialized developmental studies have also been conducted with PFOS to assess long-term effects in offspring (see section 3.2.6). Postnatal effects of gestational and lactational exposure included evidence of developmental neurotoxicity, changes in thyroid and reproductive hormones, altered lipid and glucose metabolism, and decreased immune function.

Reproductive Effects

Rat. A two-generation reproductive study was conducted in Crl:CD(SD)IGS VAF rats with five groups of 35 rats/sex/group administered 0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day of PFOS by gavage for 6 weeks prior to and during mating (Luebker et al. 2005b). Treatment in males continued through the cohabitation interval, and females were treated throughout gestation, parturition, and lactation.

F0 Generation: Parental animals (F0) were observed twice daily for clinical signs, and body weight and food consumption monitored. Two sets of females in each dose group were treated and had Caesarean-sections (C-sections) performed on GD 10; others delivered naturally and were killed on LD 21. Typical reproductive parameters were monitored in the females. The F0 male rats were sacrificed and necropsied after the cohabitation interval, with the testes, epididymides, prostate, and seminal vesicles weighed. All livers from adults were removed, weighed, and examined. Blood samples were collected from five male rats at sacrifice and five female rats on LD 21 for pharmacokinetic analysis; livers of pups from the litters of these five dams were also collected for analysis.

In the F0 generation male rats, mortality, clinical signs, and mating/fertility parameters were unaffected. During pre-mating, decreases in terminal body weight, body weight gain, and food consumption occurred at 1.6 and 3.2 mg/kg/day in males. The only effect on weight of the

organs evaluated was a significant reduction in the absolute weight of the seminal vesicles (with fluid) and prostate in males administered 3.2 mg/kg/day. In the F0 generation female rats, there were no deaths and no effects on the reproductive parameters measured in both dams sacrificed on GD 10 and those allowed to deliver naturally. The F0 dams administered ≥ 0.4 mg/kg/day had localized alopecia during pre-mating, gestation, and lactation, and a decrease in body weight and food consumption.

F1 Generation: The F1 generation pup viability was significantly reduced at 1.6 and 3.2 mg/kg/day, therefore only the 0.1 and 0.4 mg/kg/day dose groups were carried into the second generation. Twenty-five F1 rats/sex/dose were administered 0, 0.1, or 0.4 mg/kg/day of PFOS by oral gavage beginning at weaning on post-natal day (PND) 22 and continuing until sacrifice. One rat/sex/litter was tested in a passive avoidance paradigm at 24 days of age and one rat/sex/litter was evaluated in a water-filled M-maze on PND 70. On PND 28, females were evaluated for vaginal patency and on PND 34 males were examined for preputial separation. On PND 90, rats were assigned within each dose group to cohabitation, and once confirmed pregnant, the females were housed individually. The F1 generation male rats were sacrificed after mating, necropsied, and evaluated as described in the F0 generation. All F1 generation females were allowed to deliver and were sacrificed and necropsied on LD 21.

Mortality occurred in the F1 offspring of dams administered 1.6 or 3.2 mg/kg/day. At 1.6 mg/kg/day, over 26% of the pups were found dead between LDs 2 and 4. At 3.2 mg/kg/day, 45% of the pups were found dead on LD 1, with 100% dead by LD 2. The dams dosed with 3.2 mg/kg/day also had a significant increase in stillborn pups and the viability index was 0% at 3.2 mg/kg/day and 66% at 1.6 mg/kg/day. The lactation index was 94.6% at 1.6 mg/kg/day. At 3.2 mg/kg/day, there were significant decreases in gestation length and number of implantation sites, and reductions in litter size. Statistically-significant decreases in pup body weight were also observed at the two highest doses. Additional adverse effects in pups at 3.2 mg/kg/day included impacts on lactation (i.e., high number [~ 75%] of pups not nursing and not having milk present in the stomach), an increased incidence of stillborn pups, and a high incidence of maternal cannibalization of the pups.

In the F1 generation offspring, pups administered 3.2 mg/kg/day could only be evaluated on LD 1 due to the high mortality. All viable pups from the 1.6 mg/kg/day group had significantly ($p < 0.05$ or 0.01) delayed eye opening, pinna unfolding, surface righting, and air righting during lactation. No delays were observed in rats administered doses ≤ 0.4 mg/kg/day. Sexual maturation was not affected in the 0.1 and 0.4 mg/kg/day groups after weaning. The results from the passive avoidance (beginning at 24 days of age) and water maze tests (beginning at 70 days of age) for neurobehavioral effects showed no dose-related effects on learning and memory.

F2 Generation: F1 parental animals displayed no clinical signs or mortality. Food consumption was transiently decreased in F1 males, but it was not affected in F1 females. Reproductive performance was unaffected in the F1 dams.

All F2 generation pups were sacrificed, necropsied, and examined on LD 21 as previously described for the F1 generation pups. In the F2 generation pups, decreases in mean pup body weights were observed at 0.1 mg/kg/day on LDs 4 and 7, but mean pup body weights were similar to controls by LD 14. The pups in the 0.4 mg/kg/day group displayed significant decreases in body weight on LDs 7–14; after LD 21, body weights remained lower than controls, but were not statistically-significant. No other treatment-related effects were observed.

Based on the decreases in body weight gain and food consumption, the LOAEL for both the F0 male and female rats was 0.4 mg/kg/day and the NOAEL was 0.1 mg/kg/day. For the F1 rats, the NOAEL was 0.4 mg/kg/day and the LOAEL was not identified. For the F1 offspring, the LOAEL was 1.6 mg/kg/day based on the significant decrease in the pup viability, pup weight, and survival; the NOAEL was 0.4 mg/kg/day. In the F2 generation offspring, the LOAEL was 0.4 mg/kg/day, based on the significant decreases in mean pup body weight; the NOAEL was 0.1 mg/kg/day.

Because of the significant reductions in pup viability observed at 1.6 and 3.2 mg/kg/day, a cross-fostering study was conducted as a means of determining whether the effects observed in pups were a result of *in utero* exposure to PFOS or as a result of exposure during lactation (Luebker et al. 2005b). Twenty five female Sprague-Dawley rats/group were administered 0 or 1.6 mg/kg/day PFOS in 0.5% Tween-80 by gavage, beginning 42 days prior to mating with untreated males, and continuing throughout gestation until LD 21. Parental females were observed twice daily for viability and clinical observations were recorded. Maternal body weight and food consumption were recorded. All maternal rats were sacrificed on LD 22 and gross necropsy was performed; the number and distribution of implantation sites were recorded. After parturition, litters were immediately removed from their respective dams and placed with either a control- or PFOS-treated dam for rearing. This cross-fostering procedure resulted in four groups as follows:

- Control dams with litters from control dams (negative control).
- Control dams with litters from PFOS-treated dams (*in utero* exposure only).
- PFOS-treated dams with litters from control dams (post-natal exposure only).
- PFOS-treated dams with litters from PFOS-treated dams (both *in utero* and post-natal exposure).

There were no mortality or clinical signs associated with treatment in the dams. Mean maternal body weight gain and food consumption at 1.6 mg/kg/day was reduced compared to controls during premating and continuing throughout gestation, but not lactation. Significant reductions in gestation length, the average number of implantation sites, total litter size (live and dead), and live litter size were observed for treated dams.

Live litter sizes were comparable between treated and control groups following cross-fostering. However, on LDs 2–4, approximately 19% of the pups in the group exposed gestationally and lactationally were either found dead or presumed cannibalized compared to 1.6% for the negative control. For pups only exposed prenatally, mortality was 9% compared to 1.1% for those exposed during lactation only. Reductions in pup body weights on LD 1 were observed in groups exposed both gestationally and lactationally and in those with gestational exposure only. On LDs 4–21, pup body weights were reduced in all exposed groups when compared to the negative control ($p < 0.05$ or 0.01). The greatest deficit in body weight compared to controls was the group exposed during both gestation and lactation.

Sex ratios and the lactation index were comparable among all groups. Electron microscopic examination of the livers revealed an increase in the number of peroxisomes in pups from treated dams. No significant differences in pup lung histopathology were observed between the negative control group and the treated animals.

Serum PFOS concentrations in untreated dams ranged from below the limit of detection (0.05 µg/mL) to 5.34 µg/mL. Serum PFOS concentrations in the pups from the negative controls

were below the limit of detection. Serum PFOS concentrations in the pups from treated dams, fostered with untreated dams (*in utero* exposures) ranged 47.6–59.2 μg/mL. Serum PFOS concentrations of treated dams ranged 59.2–157 μg/mL. Serum PFOS concentrations in the pups from untreated dams, fostered with treated dams (lactational exposure), ranged from below the limit of detection to 35.7 μg/mL. Serum PFOS concentrations in the pups from treated dams, fostered with treated dams (*in utero* plus lactational exposures), ranged 79.5–96.9 μg/mL. These data indicate that exposure to PFOS can occur both *in utero* and via milk from treated dams (3M Environmental Laboratory 1999). The accuracy of quantitation for the analyses was ± 30%.

In conclusion, pups from control dams that were cross-fostered with PFOS-treated dams (lactational exposure only) had the same low mortality rate (1.1%) as pups from control dams cross-fostered with control dams (1.6%; negative control). Mortality rates in the remaining two groups (gestational exposures and gestational plus lactational exposures) were much higher at 9% and 19%, respectively. Although the study is limited, the data to indicate that reduced pup survival is mainly a result of *in utero* exposure to PFOS and that post-natal exposure via milk in conjunction with *in utero* exposure increases the risk of mortality. In contrast, when the pups were nursed by dams that had been exposed there was no significant effect on pup viability even though the dams continued to receive PFOS during the period of lactation.

The dose-response curve for neonatal mortality in rat pups born to PFOS exposed dams and the associated biochemical and pharmacokinetic parameters were investigated in a companion study (Luebker et al. 2005a). At 6 weeks prior to mating, female Crl:CD(SD)IGS VAF/Plus rats were administered 0, 0.4, 0.8, 1.0, 1.2, 1.6, or 2.0 mg PFOS/kg bw/day by oral gavage. Dosing continued during the mating interval and through GD 20 for dams assigned to C-section which included eight dams in the control, 1.6, and 2.0 mg/kg/day groups, but none from the other dose groups. Another group (~ 20 dams per dose group) was allowed to deliver and nurse their pups through LD 4. These dams and their pups were sacrificed on LD 5.

The dams in the C-section group were examined for the number of corpora lutea, number of implantation sites, live/dead fetuses, and early/late resorptions. Maternal liver weights were determined and the maternal organs examined by gross necropsy. Fetuses were pooled by litter and mean weight recorded. For the dams that were allowed to deliver, reproductive and fetal parameters (Table 3-13) were measured and recorded. Biochemical parameters investigated in the dams and litters included: serum lipids, glucose, mevalonic acid, thyroid hormones (TT4 and FT4, TT3, and FT3, and TSH), milk cholesterol, and liver lipids. Mevalonic acid was included as it is a biomarker of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity. Some chemicals that are inhibitors of this enzyme are known to cause developmental effects in rats.

No mortality occurred and no effects were observed in reproductive parameters (corpora lutea, implantations, fetuses/litter) in those dams receiving C-sections. Overall absolute body weights of the dams were reduced slightly (5%–7% of that for the controls) in the 1.6 and 2.0 mg/kg/day group dams during gestation; the changes, although slight, were statistically-significant. Body weight change was significantly reduced ($p < 0.05$ or 0.01) during premating at 2 mg/kg/day and during lactation at ≥ 0.8 mg/kg/day. Food consumption showed a decreasing trend with increasing dose during pre-mating, gestation and lactation. For dams allowed to deliver, the fertility index, implantations per delivered litter, gestation index, live births, and delivered pups/litter were similar between treated and control dams. Based on the decreased body weight gain, the LOAEL for the F0 dams was 0.8 mg/kg/day and the NOAEL was 0.4 mg/kg/day.

Table 3-13. Fertility and Litter Observations in Dams Administered 0 to 2.0 mg PFOS/kg/day

	0.0	0.4	0.8	1.0	1.2	1.6	2.0
Fertility index[a] (%)	96.4	100.0	89.5	95.0	94.7	92.6	96.4
Implantations per delivered litter	14.7 ± 2.3	16.2 ± 1.8	15.1 ± 2.2	15.9 ± 2.0	15.3 ± 2.5	14.3 ± 2.1	14.4 ± 1.9
Gestation length (days)	22.9 ± 0.3	22.6 ± 0.5	22.5 ± 0.5*	22.4 ± 0.6**	22.3 ± 0.5**	22.0 ± 0.0**	22.2 ± 0.4**
Gestation index[b] (%)	100	100	100	100	100	100	100
Delivered pups/litter	13.9 ± 2.6	15.0 ± 2.3	14.5 ± 2.3	15.1 ± 2.3	14.0 ± 2.9	13.6 ± 2.8	13.3 ± 2.5
Live births (%)	98.1	97.0	99.2	99.3	99.6	98.3	99.6
Dams with all pups dying on LDs 1–5	0	0	0	1	0	4	14**
Viability index[c] (%)	97.3	97.6	93.1	88.8	81.7	49.3**	17.1**

Source: Data from Luebker et al. 2005a
Notes: [a] Number of dams pregnant/number of dams mated x 100
[b] Number of dams with live offspring/number of pregnant dams x 100
[c] Number of live pups on day 5 postpartum/number of live births x 100
*Statistically-significant at $p \leq 0.05$
** Statistically-significant at $p \leq 0.01$

In the group sacrificed on LD 5, a significant decrease in gestation length was observed at doses ≥ 0.8 mg/kg. Offspring viability was decreased starting at 0.8 mg/kg and was statistically-significant at 1.6 and 2.0 mg/kg. The viability indices were 97.3%, 97.6%, 93.1%, 88.8%, 81.7%, 49.3%, and 17.1% at 0, 0.4, 0.8, 1.0, 1.2, 1.6, and 2.0 mg/kg, respectively (Table 3-13). Lipids, glucose utilization, and thyroid hormones were similar or slightly different for treated animals compared to controls. In all treated groups, pup body weight at birth on PND 5 was significantly less than that of controls. In one male and one female pup at 2.0 mg/kg/day, the heart and thyroid were collected and examined microscopically. No lesions were found when compared to the controls. The LOAEL for the F1 generation was 0.4 mg/kg/day based on decreased body weight and a NOAEL was not identified.

Several benchmark dose (BMD) estimates (BMD_5 and benchmark dose for the lower 95th percentile confidence bound [$BMDL_5$]) were presented in the study. They were as follows:

- Effect on gestation length: BMD_5 = 0.45 mg/kg/day, $BMDL_5$ = 0.31 mg/kg/day.
- Birth weight effect: BMD_5 = 0.63 mg/kg/day, $BMDL_5$ = 0.39 mg/kg/day.
- Decreased pup weight (day 5): BMD_5 = 0.39 mg/kg/day, $BMDL_5$ = 0.27 mg/kg/day.
- Pup weight gain (day 5): BMD_5 = 0.41 mg/kg/day, $BMDL_5$ = 0.28 mg/kg/day.
- Decreased survival of pups to day 6: BMD_5 = 1.06 mg/kg/day, $BMDL_5$ = 0.89 mg/kg/day.

The impact of PFOS exposure on the hypothalamic-pituitary-testicular axis in groups of 19 adult male rats was studied by López-Doval et al. (2014) following dosing at levels of 0, 0.5, 1, 3, or 6 mg/kg/day by gavage for 28 days. Serum LH, FSH, and testosterone were measured in all animals. The histology of the hypothalamus, pituitary gland, and testes were examined by light microscopy and by electron microscopy (two animals/dose group using each method). Noradrenaline concentration in the anterior and medial hypothalamus and median eminence and GnRH in the whole hypothalamus were also determined in five animals/dose group each. For the

remaining five animals/dose group, GnRH gene expression in the hypothalamus and LH and FSH gene expression in the pituitary gland were assayed.

The pituitary gonadotrophic cells examined using an electron microscope showed structural abnormalities in all exposed animals, although under light microscopy, the cells at the lowest exposure levels appeared normal. At doses ≥ 3 mg/kg/day the most active gonadotrophic cells were classified as inactive based on the lack of homogeneous endoplasmic reticulum and a well-developed Golgi complex. Many cells in the process of degeneration were observed. The hypothalamus appeared to be normal at the two lowest doses, but not for doses ≥ 3 mg/kg/day at which basophilia, vacuolation, and irregular nuclear borders were seen. Histological abnormalities (edema around seminiferous tubules and malformed spermatids) in the testes were seen at doses ≥ 1 mg/kg/day. Gene expression for LH and FSH were increased compared to controls at the two lowest doses, with subsequent decreases at the higher doses. Serum LH and testosterone were significantly decreased and FSH was significantly increased at all doses. Gene expression for GnRH was significantly decreased compared to controls at all doses, while GnRH levels in the hypothalamus were increased at the high dose. The results are consistent with inhibition of the reproductive hypothalamus-pituitary-testicular axis at doses of 0.5 mg/kg/day and above. The 0.5 mg/kg/day was the LOAEL based on significantly decreased LH and testosterone concentration and increased FSH concentration. The authors stated that the various biochemical changes observed are linked and could be due to PFOS antiandrogenic and/or estrogenic properties as has been proposed by other researchers.

Developmental Studies

Rat. Thibodeaux et al. (2003) administered 0, 1, 2, 3, 5, or 10 mg/kg PFOS in 0.5% Tween-20 daily by gavage during gestational days (GDs) 2–20 to groups of 9–16 pregnant Sprague-Dawley rats. Maternal weight gain, food and water consumption, and serum clinical chemistries were monitored and recorded. Rats were euthanized on GD 21 and uterine contents examined. At sacrifice, PFOS levels were measured in the serum and maternal and fetal livers.

Maternal body weight, food consumption and water consumption were significantly decreased ($p < 0.0001$) in a dose-dependent manner at ≥ 2 mg/kg; these data were presented graphically. A dose-dependent increase in the serum PFOS concentration was observed with liver concentrations approximately four times higher than serum at each dose. Liver weight was not affected in the treated rats. Serum chemistry showed significant decreases in cholesterol (decrease of 14% compared to controls) and triglycerides (decrease of 34% compared to controls) at 10 mg/kg. Serum thyroxine (T4) and T3 were significantly decreased in all treated rats when compared to controls, however, a feedback response on TSH was not observed. The number of implantations or live fetuses at term was not affected by treatment. There was a decrease in fetal weight, and birth defects such as cleft palate, ventricular septal defect, and enlargement of the right atrium were observed at 10 mg/kg, but the litter incidence rates were not given. Benchmark dose estimates provided for different parameters were as follows:

- Maternal weight reduction BMD_5 = 0.22 mg/kg and $BMDL_5$ = 0.15 mg/kg (polynomial model).
- T4 effects on GD 7 BMD_5 = 0.23 mg/kg and $BMDL_5$ = 0.05 mg/kg (Hill model).
- Fetal sternal defects BMD_5 = 0.31 mg/kg and $BMDL_5$ = 0.12 mg/kg (logistic model).
- Fetal cleft palate BMD_5 = 8.85 mg/kg and $BMDL_5$ = 3.33 mg/kg (logistic model).

Lau et al. (2003) conducted a companion study to the one by Thibodeaux et al. (2003) in order to examine the post-natal impact of *in utero* exposure to PFOS. Sprague-Dawley rats were administered 0, 1, 2, 3, 5, or 10 mg/kg/day PFOS in 0.5% Tween-20 by gavage on GDs 2–21. On GD 22, dams were monitored for signs of parturition. The day after parturition was designated PND 1. The number of pups per litter, number of live pups in the litter and body weight were monitored. All pups were weaned on PND 21 and separated by gender. Additional pregnant rats were dosed in the same manner to 0, 1, 2, 3, or 5 mg/kg/day of PFOS, and four pups from each litter were sacrificed within 2–4 hours after birth and used to determine blood and liver PFOS concentrations and thyroid hormone analysis. The other pups were maintained in the study and used for serum collection and thyroid hormone analysis and as the subjects for the neurobehavioral tests.

In dams administered 10 mg/kg/day, the neonates became pale, inactive, and moribund within 30–60 minutes of birth and all died. In 5 mg/kg/day dams, the neonates became moribund after 8–12 hours, with 95% dying within the first 24 hours. A 50% fetal mortality was observed in dams administered 3 mg/kg/day. Pups from dams treated with 2 mg/kg/day still had significant increases in mortality, but those from dams administered 1 mg/kg/day were similar to controls (these data were presented graphically). No differences were observed in liver weight in the neonates. Pup body weight was significantly decreased in dams administered ≥ 2 mg/kg/day. A significant ($p < 0.05$) delay in eye opening was observed at the same dose in the pups, but no differences in onset of puberty were observed at that dose. On PND 2, serum levels of both total T4 and free T4 were decreased significantly in all the treated groups, but total T4 recovered to levels similar to those of controls by weaning. No changes were observed in serum T3 or TSH. The thyroid hormone data were presented graphically. Choline acetyltransferase activity in the prefrontal lobe, which is sensitive to thyroid status, was slightly reduced in rat pups, but activity in the hippocampus was not. T-maze testing did not demonstrate any learning deficiencies. Based on the findings, the developmental LOAEL is 2 mg/kg/day PFOS for mortality, decreased body weight, and a significant 1-day delay in eye opening; the NOAEL is 1 mg/kg/day. The authors calculated a $BMDL_5$ for a 6 day survival of 7.02 mg/kg/day.

Because of the high number of fetal deaths, a sub-study was performed with newborns from the 5 mg/kg/day PFOS group wherein they were cross-fostered with control dams immediately after parturition. Survival was monitored for 3 days. Cross-fostering the pups from PFOS-treated rats (5 mg/kg/day) with control dams did not increase their survival. Conversely, all control pups fostered by PFOS treated dams survived, supporting the Luebker et al. (2005a) observations.

Grasty et al. (2003) exposed pregnant rats to 25 mg/kg/day by gavage for four consecutive days during critical windows of development (GDs 2–5, 6–9, 10–13, 14–17, or 17–20) or at 25 or 50 mg/kg/day on GDs 19–20. Litter size at birth was unaffected, but pup weight was decreased in dams exposed for each of the 4 day intervals. Neonates died after dosing in all the gestation time periods tested and the number of deaths increased as the time of dosing moved closer to the end of gestation period. Mortality was 100% when administered on GD 17–20. Most deaths occurred within 24 hours; all pups had died by PND 4.

In the dams treated only on GDs 19–20, survival of the pups was 98%, 66%, and 3% in the control, 25, and 50 mg/kg/day groups on PND 5, respectively. Histological examination of the lungs showed differences in the level of maturation between the control and treated pups.

Grasty et al. (2005) performed a study with a comparable design to their 2003 study in order to determine whether delayed lung surfactant maturation was responsible for neonatal deaths.

Dams were given 25 or 50 mg/kg/day on GDs 19–20 and offspring evaluated on GD 21 or PND 0 immediately after birth. The newborns had normal pulmonary surfactant profiles. Morphometric measurements of the histological lung sections of newborns showed significantly ($p < 0.05$) increased proportion of solid tissue and decreased proportion of small airway space at both doses. Co-treatment of dams with dexamethasone or trans-retinol palmitate as rescue agents did not improve survival of newborns. These agents are used therapeutically to promote lung maturation and surfactant production.

While lung surfactant maturation did not appear to be the cause of death in the Grasty et al. (2003) study, some data support effects of PFOS on lung surfactants. Xie et al. (2007, 2010a, 2010b) found that PFOS interacts with dipalmitoylphosphatidylcholine, a major lung surfactant. As discussed in the distribution section, Borg et al. (2010) found that radiolabeled PFOS was localized in the perinatal lung on GD 18 after it was administered to the dams on GD 16. In these same pups, the PFOS levels in the lungs were three-fold higher than what was in the maternal blood on PND 1.

Chen et al. (2012) administered 0, 0.1, or 2.0 mg/kg/day PFOS in 0.05% Tween 80 in deionized water by gavage to 10 pregnant Sprague-Dawley rats/group on GDs 1–21. After parturition (PND 0), pups were counted and weighed, and 2 male and 2 female pups/litter were randomly selected for sacrifice and serum and lung collection. Six offspring/litter were kept until PND 21 when they were sacrificed for serum and lung collection. Lung tissue was assessed for markers of oxidative stress and cytoplasmic protein and examined histologically. The serum and lungs were also analyzed for PFOS concentration. Three additional groups of 10 rats/dose were treated as described above and the number of deaths/litter recorded until PND 4.

Body weight of the pups was decreased and postnatal pup mortality (by PND 3) was increased significantly ($p < 0.05$ and 0.01, respectively) at 2.0 mg/kg/day, when compared to the control litters. No treatment-related findings were observed at 0.1 mg/kg/day. Postnatal pup mortality in the control, 0.1, and 2.0 mg/kg/day groups on PND 3 was approximately 4%, 3%, and 23%, respectively. On PND 0, PFOS concentrations in the pup serum (μg/mL) were approximately 2 times greater than that found in the pup lung (μg/g) at both 0.1 and 0.2 mg/kg/day. PFOS concentrations decreased in both the serum and lungs on PND 21, but they were still greater compared to serum. PFOS was not detected in control pups at either timepoint.

Histopathological changes observed in pup lungs at 2.0 mg/kg/day on PND 0 included marked alveolar hemorrhage, thickened interalveolar septum, and focal lung consolidation. On PND 21, the lungs also had alveolar hemorrhage, thickened septum, and inflammatory cell infiltration. Numerous apoptotic cells were observed. No abnormalities were observed on examination of the control rats or the pups from dams receiving 0.1 mg/kg/day.

An increase in biomarkers associated with oxidative stress was found in pups from the 2.0mg/kg/day dams. The levels of malondialdehyde (MDA) were 473% and 305% of controls on PND 0 and 21, respectively, and glutathione levels and superoxide dismutase (SOD) activity decreased at both time-points compared to controls. Cytochrome *c* release from the inner mitochondrial membrane and increased caspase −3, −8, and −9 are biomarkers for apoptotic cell death. Each of these factors was significantly increased above that for controls at 2.0 mg/kg/day on both PNDs 0 and 21. No changes were observed in the pups from dams receiving 0.1 mg/kg/day. The NOAEL for histopathological lesions in the lung, oxidative stress, and apoptosis was 0.1 mg/kg/day with a LOAEL of 2 mg/kg/day.

Ye et al. (2012) administered 0, 5, or 20 mg PFOS/kg/day by gavage in 0.5% Tween-20 to Sprague-Dawley rats on GDs 12–18. Animals were sacrificed on GD 18.5 and the lungs analyzed for histological lesions and gene expression profiles. Maternal treatment with PFOS did not result in any apparent microscopic changes in the fetal lung. However, gene expression profiling showed a dose-dependent upregulated expression of 21 genes at 5 mg/kg/day and of 43 genes at 20 mg/kg/day. The genes included five PPARα target genes, four of which are involved in lipid metabolism; the remaining upregulated genes were involved in significant cytoskeletal, extracellular matrix remodeling, and transport and secretion of proteins.

Lv et al. (2013) investigated the impact of gestational and lactational exposure to PFOS on glucose and lipid homeostasis in offspring. Groups of 6 pregnant SPF Wistar rats were given doses of 0, 0.5, or 1.5 mg/kg/day dissolved in 0.5% Tween 20 from GD 0 to PND 20. After birth, pups were sexed, randomly selected and cross-fostered to insure there were equal pups per litter (5 male and 5 female). Pup weights were determined on PNDs 0, 5, 10, 15, and 21. Serum and liver samples were also collected at PND 0 and 21 from an unspecified number of pups. The remaining pups were maintained for 19 weeks after weaning before final sacrifice. Blood samples were collected at 10 and 15 weeks after weaning and examined for fasting serum triglycerides, total cholesterol, and fasting blood glucose. A glucose tolerance test was administered after a 16-hour overnight fast. The adult pups were sacrificed at 22 weeks of age for collection of total liver RNA with analysis for hepatic transcription factor SREBP-1c (sterol regulatory element binding protein 1c) as a reflection of lipogenesis linked to glucose. Other parameters evaluated included serum insulin, leptin, and adiponectin, and gonadal fat weight, pancreatic beta cell area, fat accumulation in the liver as monitored through oil red and hematoxylin and eosin staining.

Body weight of pups from treated dams was significantly reduced ($p < 0.05$) at birth, throughout lactation, and persisted until week 8 post-weaning. A dose-related increase in glucose intolerance was observed at 10 weeks post-weaning in pups from treated dams with statistical significance attained at 1.5 mg/kg/day. At 15 weeks, pups from the 0.5 mg/kg/day dams had significantly increased glucose intolerance, while that for high-dose pups was increased but did not attain statistical significance. Fasting glucose levels and serum glycosylated serum protein concentrations were similar between pups from treated and control dams at 10 and 15 weeks post-weaning. At 18 weeks after weaning, pups from dams given 1.5 mg/kg/day had significant increases in serum insulin, insulin resistance index, and serum leptin. Serum adiponectin was significantly decreased in pups from both treated groups compared with that of controls. At sacrifice, pups from both treated groups had a significant increase in epigonadal fat pad weight, and fat accumulation was observed in the liver of high-dose animals. The lowest dose tested (0.5 mg/kg/day) was a LOAEL for a significant decrease in birth weight that persisted until week 8 of the post-lactation period, a significant increase of the epigonadal fat pad weight at 19 weeks after weaning, impaired glucose tolerance at 15 weeks after weaning, and decreased serum adiponectin.

Mouse. As described for rats, a two-part developmental study with PFOS was performed in mice by Thibodeaux et al. (2003) and Lau et al. (2003). In the first study, groups of 20–29 CD-1 mice were administered 0, 1, 5, 10, 15, or 20 mg/kg/day PFOS during GDs 1–17 (Thibodeaux et al. 2003). Maternal weight gain, food and water consumption, and serum clinical chemistries were monitored and recorded. Mice were euthanized on GD 18. Parameters as described for the rat were also measured in the mice.

Maternal body weight gain was significantly decreased at 20 mg/kg/day. Food and water consumption were not affected by treatment. Increases in serum PFOS were comparable to the rat. PFOS treatment increased ($p < 0.05$) the liver weight in a dose-dependent manner in the mice. T4 was decreased in a dose-dependent manner on GD 6 with statistical significance ($p < 0.05$) attained for the 20 mg/kg/day group; levels of T3 and TSH were not affected by treatment. A significant increase in post-implantation loss was observed in animals administered 20 mg/kg/day, and reduced fetal weight ($p < 0.05$) was observed from dams in the 10 and 15 mg/kg/day groups. Birth defects such as cleft palate, ventricular septal defect, and enlargement of the right atrium were observed at doses ≥ 10 mg/kg.

In the second part of the developmental study, the post-natal effects of *in utero* exposure to PFOS were evaluated in the mouse (Lau et al. 2003). CD-1 mice were administered 0, 1, 5, 10, 15 or 20 mg/kg/day of PFOS in 0.5% Tween-20 by gavage on GDs 1–17.

Most mouse pups from dams administered 15 or 20 mg/kg/day did not survive for 24 hours after birth. Fifty percent mortality was observed at 10 mg/kg/day. Survival of pups in the 1 and 5 mg/kg/day treated dams was similar to controls. A significant ($p < 0.0001$) increase in absolute liver weight was observed at ≥ 5 mg/kg/day. A significant delay in eye opening was observed at ≥ 5 mg/kg/day. No dose- or treatment-related effects were observed on T4, T3, and TSH levels in the pups. The LOAEL for this study in mice was 5 mg/kg/day and the NOAEL was 1 mg/kg/day. The authors calculated a $BLDL_5$ for survival at 6 days of 3.88 mg/kg/day.

Ten pregnant ICR mice/group were administered 0, 1, 10, or 20 mg/kg of PFOS daily by gavage from GD 1 to GD 17 or 18 (Yahia et al. 2008). Five dams/group were sacrificed on GD 18 for fetal external and skeletal effects and histological examination of the maternal liver, kidneys, lungs and brain; the other five were left to give birth. Body weight, food consumption, and water consumption were monitored in the dams. In the dams sacrificed on GD 18, the gravid uterus was removed and the number of live/dead fetuses, fetal body weight, and number of resorptions were recorded. Four pups/litter were sacrificed immediately after birth for examination of their lungs.

All dams survived and exhibited no clinical signs. A statistically-significant ($p < 0.05$ or $p < 0.01$) decrease in body weight was observed in the dams administered 20 mg/kg/day beginning on GD 10. Water consumption was increased. Maternal absolute liver weight increased in a dose-dependent manner, significantly in the 10 (59%) and 20 (60%) mg/kg/day groups.

All neonates in the 20 mg/kg/day dose group were born pale, weak, and inactive, and all died within a few hours of birth. At 10 mg/kg/day, 45% of those born died within 24 hours. Survival of the 1 mg/kg/day group was similar to that of controls. Neonatal weight was significantly decreased at 10 and 20 mg/kg/day. In the fetuses from dams treated with 20 mg/kg/day, there were large numbers of cleft palates (98.56%), sternal defects (100%), delayed ossification of phalanges (57.23%), wavy ribs (84.09%), spina bifida occulta (100%), and curved fetus (68.47%). Similar defects were observed in the fetuses from dams treated with 10 mg/kg/day except at a lower incidence. Results from this study are summarized in Table 3-14.

Histopathological exam showed that all fetuses examined on GD 18 from dams treated with 20 mg/kg were alive and had normal lung structures but mild to severe intracranial dilatation of the blood vessels. Neonates from the 20 mg/kg treated dams had fetal lung atelectasis (partial or complete collapse of the lung or a lobe of the lung) with reduction of alveolar space and

intracranial blood vessel dilatation when examined histopathologically. Three neonates from each of the five dams treated with 10 mg/kg were examined, and 27% had slight lung atelectasis and 87% had mild to severe dilatation of the brain blood vessel. Based on the significant increase in liver organ weight, the maternal LOAEL was 10 mg/kg/day and the NOAEL was 1 mg/kg/day. Based on the abnormalities observed in the fetuses and decreased survival rate, the developmental LOAEL was 10 mg/kg/day and the NOAEL was 1 mg/kg/day.

Table 3-14. Effects Observed in the Mice Administered PFOS from GD 0 to GD 17/18

Effects	Control	1 mg/kg	10 mg/kg	20 mg/kg
Number of dams	5	5	5	5
Total # of fetuses	80	76	79	71
Live fetuses (%)	98.75 ± 1.25	98.88 ± 1.12	96.85 ± 1.97	90.06 ± 3.02*
Body weight of fetuses (g)	1.49 ± 0.01	1.46 ± 0.01	1.41 ± 0.01**	1.10 ± 0.02**
# of fetuses examined	60	44	68	60
Cleft palate (%)	0	1.96 ± 1.96	23.36 ± 8.27**	98.56 ± 1.44**
Sternal defects (%)	0	15.77 ± 0.99**	52.44 ± 2.79**	100**
Delayed ossification of phalanges (%)	0	1.96 ± 1.96	4.34 ± 1.80	57.23 ± 9.60**
Wavy ribs (%)	0	0	7.31 ± 0.34*	84.09 ± 2.56**
Curved fetus (%)	3.55 ± 2.11	4.94 ± 2.47	33.38 ± 8.47**	68.47 ± 6.71**
Spina bifida occulta (%)	0	1.96 ± 1.96	23.13 ± 3.94**	100**
Survival rate at PND 4 (%)	98.18 ± 1.82	100	55.20 ± 18.98*	0**

Source: Data from Tables 2-3 in Yahia et al. 2008
Notes: *Statistically-significant difference between control and treated groups, $p < 0.05$
** Statistically-significant difference between control and treated groups, $p < 0.01$

The effects of developmental PFOS exposure during gestation and lactation on glucose metabolism in adult CD-1 mice were studied by Wan et al. (2014b). The effects observed are consistent with those in Wistar rats (Lv et al. 2013) discussed above. The dams were exposed to doses of 0, 0.3, or 3 mg/kg/day dissolved in dimethyl sulfoxide (DMSO) and then in corn oil from GD 3 to sacrifice on PND 21. The final concentration of DMSO was < 0.05% throughout gestation and lactation. At PND 21, all dams and 2 pups per litter were sacrificed. The remaining pups were randomly divided into two groups that were fed with either a standard diet or a high fat diet until PND 63. Dams had increased liver weight at 3 mg/kg/day but no differences in fasting serum glucose or insulin levels.

There were no significant differences in pup weights at PND 21 although liver weights were increased significantly ($p < 0.05$) at the highest dose for both the male and female pups. Both sexes also had significant changes in genes regulating lipids and glucose at the highest dose. Expression of CYP4A14, lipoprotein lipase, fatty acids translocase, the hepatic insulin receptor, and insulin-like growth factor-1 receptor were significantly increased ($p < 0.05$) in males and females from high-dose dams. The genes for prolactin receptor and insulin-like growth factor-1 were significantly decreased ($p < 0.05$) in males and females at 3 mg/kg/day.

When evaluated at PND 63, liver weight in the pups was significantly increased at the high dose in males, but not females. In the animals on the standard diet, fasting serum glucose was significantly ($p < 0.05$) higher for males and females at both doses, but fasting serum insulin attained statistical significance only for the animals in the highest dose group. There were no significant differences in oral glucose tolerance. The HOMA-IR index was increased significantly for the high-dose group receiving the standard diet.

The results from the glucose tolerance test (fasting blood glucose levels and blood glucose levels over 2 hours following oral glucose challenge) became statistically-significant ($p < 0.05$) at the high dose in both sexes fed high fat diets on PND 63. Fasting serum insulin was significantly increased ($p < 0.05$) at 3 mg/kg/day in males and females on both diets, with the effects more pronounced in mice on the high fat diet than in mice on the standard diet. The HOMA-IR index was significantly increased ($p < 0.01$) at both doses for males and females on the high fat diet.

3.2.6 Specialized Developmental/Reproductive Studies

Hormonal Disruption

Rat. Yu et al. (2009a) fed pregnant adult Wistar rats (n = 20/group) a control diet or a diet containing 3.2 mg PFOS/kg feed. Doses to the dams were not calculated, and body weight and feed consumption data were not presented. Treatment continued for both groups throughout gestation and lactation. Dams were allowed to deliver, and on the day of delivery (PND 0) samples were collected from two control litters and two PFOS treated litters. The remaining litters were cross-fostered within 12 hours of birth to make the following groups:

- Litters from control dams fostered by control dams (CC, unexposed control; n = 8).
- Litters from treated dams fostered by control dams (TC, prenatal exposure; n = 8).
- Litters from control dams fostered by treated dams (CT, post-natal exposure; n = 8).
- Litters from treated dams fostered by treated dams (TT, prenatal + postnatal exposure; n = 10).

The pups were weaned on PND 21 and then fed the same diet as the foster dam. Pups were weighed and sacrificed on PNDs 0, 7, 14, 21, or 35. Serum thyroid hormone analysis was performed and included total thyroxine (T4), total triiodothyronine (T3), reverse T3 (rT3), and hepatic expression of genes involved in thyroid hormone (TH) transport, metabolism, and receptors. The genes associated with thyroid metabolism included type 1 deiodinase (DI01) and uridine diphosphoglucuronosyl transferase 1A1 and 1A6 (UGT1A1 and UGT1A6). Those associated with thyroid hormone transport included transthyretin (TTR). The genes for the thyroid hormone receptors α and β (TRα and TRβ) were also studied.

No mortality or clinical signs were observed in the dams. Body weight in offspring from PFOS treated groups did not differ significantly from controls. Liver weights in pups from the pre- and postnatal exposure (group TT) were significantly increased on PNDs 21 and 35. As observed in other studies, levels of PFOS in the dams and offspring were higher in the liver when compared to the serum. The levels of PFOS in both the serum and liver increased with time in the pups exposed postnatally (group CT) but decreased with time in those exposed only prenatally (group TC). The levels increased in those in the TT group. These results indicate that PFOS can be transferred by the placenta and through lactation.

The total T3 and rT3 were not affected by PFOS treatment of the pups. Compared to controls, pups in all treated groups had significant ($p < 0.05$ or 0.01) decreases in total T4 on PNDs 21 and 35, with the response in the CT and TT groups larger than that of the TC group. On PNDs 21 and 35, T4 levels were 71%–75% and 63%–64% of controls for the CT and TT groups, respectively, compared with 80%–81% of control for the TC group on both days. Pups in the TT group (exposed pre- and postnatally) had T4 levels that were significantly lower than the controls at PND 14. For gene expression, no statistically-significant differences were observed between

litters born to control dams or litters born to treated dams on PND 0. The only significant finding in gene expression at the other sacrifice time-points was a significant ($p < 0.01$) increase (1.5 times greater than the controls) in TTR on PND 21 in the pups that had been treated both in the prenatal and postnatal interval. Lactational exposure appears to be an important contributor to the observed thyroid effects given that the serum PFOS levels were higher, and T4 levels lower, in the CT group than in the TC group.

The effects of PFOS on testosterone production by fetal Leydig cells were investigated following prenatal exposures (Zhao et al. 2014). Pregnant Sprague-Dawley rats (n = 4) were administered PFOS by gavage at doses of 0, 5, or 20 mg/kg/day on GDs 11–19; controls received the 0.05% Tween 20 vehicle. Dams were killed on GD 20 and the male pups removed, weighed, and measured for length and anogenital distance. The fetal testes were removed for analysis of testosterone production, fetal Leydig cell numbers, ultrastructure, and gene and protein expression levels. Dams given 20 mg/kg/day had significantly lower body weight and serum cholesterol levels on GD 20. Male fetuses had significantly lower body weight at 5 and 20 mg/kg/day. At 20 mg/kg/day there were significant differences in body length, anogenital distance, and testes weight; all measures were lower than those for controls.

Testicular mRNA levels of growth factors (*Kitl*), cholesterol transporters (*Scarb1 and Star*), steroidogenic enzymes (*Cyp11a1, Cyp17a, and Hsd3b1*), junction protein (*Trmp2*), and LH receptor (*Lhcgr*) were significantly reduced in fetuses from dams given 20 mg/kg/day. Fetuses from high-dose dams also had significantly lower testicular testosterone levels, enzyme activity, and protein levels for 3β-hydrosteroid dehydrogenase and 17α-hydroxylase/20-lyase. Liver cholesterol and testes HDL-cholesterol levels were reduced in fetuses from high dose dams. Histologically, the number of fetal Leydig cells was reduced and showed a decreased number of lipid droplets and features of apoptosis at 20 mg/kg/day. The 5 mg/kg/day dose was a LOAEL for effects on male fetal body weight.

Developmental Neurotoxicity

Rat. Twenty five female Sprague-Dawley rats/group were administered 0, 0.1, 0.3, or 1.0 mg/kg/day of potassium PFOS by gavage from GD 0 through PND 20 (Butenhoff et al. 2009). An additional 10 mated females/group were used to collect additional blood and tissue samples. Offspring were monitored through PND 72 for growth, maturation, motor activity, learning and memory, acoustic startle reflex, and brain weight.

There were no treatment-related effects on the pregnancy rates, gestation length, number of implantation sites, number of pups born, sex ratio, birth to PND 4 survival, PND 4–21 survival, pup body weights through PND 72, and gross internal findings. Maternal body weight and body weight gain during gestation were comparable between the treated and control groups. On LDs 1–4, dams in the 1.0 mg/kg/day group had slightly, but not significantly, lower weight gain and food consumption than those of controls resulting in significantly lower ($p < 0.05$ or 0.01) absolute body weight throughout lactation. Food consumption was transiently decreased ($p < 0.05$ or 0.01) on GDs 6–9 for the 0.3 mg/kg/day group and on GDs 6–12 for the 1.0 mg/kg/day group. These findings in the treated dams are not considered to be treatment-related or adverse. Based on results, the maternal toxicity NOAEL was 1.0 mg/kg/day and the LOAEL could not be determined.

No treatment related effects were observed on functional observational battery assessments performed on PNDs 4, 11, 21, 35, 45, and 60. Male offspring from dams administered 0.3 and

1.0 mg/kg/day had statistically-significant ($p < 0.05$) increases in motor activity on PND 17, but this was not observed on PND 13, 21 or 61. No effect on habituation was observed in the 0.1 and 0.3 mg/kg/day males or in the 1.0 mg/kg/day females. On PND 17, males at 1.0 mg/kg/day showed a lack of habituation as evidenced by significantly ($p < 0.05$) increased activity counts for the sequential time intervals of 16–30, 31–45, and 46–60 minutes. The normal habituation response is for motor activity to be highest when the animals are first exposed to a new environment and to decline during later exposures to the same environment as they have learned what to expect. There were no effects in males or females on acoustic startle reflexes or in the Biel swimming maze trials. Mean absolute and relative (to body weight) brain weight and brain measurements (length, width) were similar between the control and treated animals. Based on the increased motor activity observed reflecting decreased habituation, the LOAEL for developmental neurotoxicity in male rats was 1.0 mg/kg/day and the NOAEL was 0.3 mg/kg/day.

Y. Wang et al. (2015) examined the effects of PFOS on spatial learning and memory following pre- and post-natal exposure. Pregnant Wistar rats were administered PFOS in the drinking water at 0, 5, or 15 mg/L beginning on GD 1 and continuing through lactation. Doses to the animals were not calculated, and body weight and water consumption data were not presented. Doses were estimated as 0, 0.8, or 2.4 mg/kg/day using subchronic values for female Wistar rats from USEPA (1988). Maternal serum levels in the treated groups were 25.7 and 99.3 μg/mL, respectively, on PND 7 and 64.3 and 207.7 μg/mL, respectively, on PND 35. On PND 1 pups were cross-fostered to establish groups for unexposed controls, only prenatal exposure, only post-natal exposure, and continuous exposure. After weaning, pups were given the same treated or control water as their foster dam. Three pups per group were sacrificed on PNDs 7 and 35 for measurement of protein and RNA levels in the hippocampus. On PND 35, 8–10 pups per group were tested in the Morris Water Maze which consisted of one day of visible platform tests, seven days of hidden platform tests, and a probe trial 24 hours after the last hidden platform test.

Offspring survival on PND 1 was significantly reduced from high-dose dams before cross-fostering; survival on PND 5 was not given. On water maze testing day 1, swimming speed and the time to reach the visible platform were similar between all treated and control groups. Thereafter, escape latency was significantly increased for all treated groups on one or more testing days. The most pronounced and significant effect was in pups exposed prenatally from dams given 15 mg/L and cross-fostered to control dams. Similar trends were observed for escape distance. During the probe trial for memory testing, pups continuously exposed pre- and post-natally to 15 mg/L spent less time in the target quadrant than the unexposed controls but statistical significance was not achieved as consistently as that for the group exposed only during gestation. Protein levels of growth-associated protein-43, neural cell adhesion molecule 1, nerve growth factor, and brain-derived neurotrophic factor were significantly decreased in the hippocampus on PND 35, especially in pups exposed prenatally to 15 mg/L and cross-fostered to control dams.

Ten pregnant Sprague-Dawley rats/group were administered 0, 0.1, 0.6, or 2.0 mg/kg/day of PFOS in 0.5% Tween 80 by oral gavage from GD 2 to GD 21 (Zeng et al. 2011). On GD 21, dams were monitored for parturition and the day of delivery was designated PND 0. On PND 0, five pups/litter were sacrificed and the trunk blood, cortex, and hippocampus were collected for examination. Astrocyte activation markers, glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B, which are associated with morphological changes inside the cell,

were evaluated with immunohistochemistry. The other pups were randomly redistributed to dams within the dosage groups and allowed to nurse until PND 21, when they were sacrificed with the same tissues collected as described for PND 0. PFOS concentration in the hippocampus, cortex, and serum increased in a dose-dependent manner, but overall was lower in all tissues on PND 21 than on PND 0.

The number of GFAP positive cells was significantly increased in the hippocampus and cortex of offspring from treated dams on PND 21. The protein levels of GFAP in PND 21 offspring were also increased in the hippocampus and cortex on Western Blot tests. The S100 calcium binding protein B was increased in the offspring's hippocampus and cortex on PND 21 in those from dams treated with 0.6 and 2.0 mg/kg/day.

In other tests, PFOS increased the mRNA expression of two inflammatory cytokines, interleukin 1 beta (IL-1β) and tumor necrosis factor-α(TNF) The expression of IL-1β and TNF-α was significantly increased compared to controls in all treated offspring in the hippocampus on PND 0 and in those from dams administered ≥ 0.6 mg/kg on PND 21. In the cortex, IL-1β and TNF-α were only significantly increased in the 0.6 mg/kg group and 2.0 mg/kg group, respectively, on PND 0. On PND 21 in the cortex, IL-1β was increased at ≥ 0.6 mg/kg and TNF-α was increased in the high dose group.

To determine the mechanisms leading to the inflammatory effect after PFOS exposure, mRNA levels of three pro-inflammatory transcription factors in both brain tissues were examined. The greatest increase was observed in the hippocampus on with a significant increase in activation protein-1 (AP-1) in all dose groups and an increase in nuclear factor-κB (NF-κB) and cAMP response element-binding protein at ≥ 0.6 mg/kg groups at PND 0. Two synaptic proteins, synapsin 1 (Syn 1) and synaptophysin (Syp) were also affected; Syn 1 was decreased with PFOS exposure primarily in the hippocampus. Syp was decreased in the hippocampus, but increased in the cortex.

Mouse. Fuentes et al. (2007) treated 8–10 pregnant Charles River CD-1 mice/group to 0 or 6 mg/kg/day of PFOS dissolved in 0.5% Tween-20 daily by gavage on gestation days (GDs) 12–18. After treatment, mice were either left alone or restrained (immobilized) three times per day for 30 minutes to induce maternal stress. Maternal body weight and food and water consumption were monitored. At birth, the length of gestation, number of live/dead pups, and sex/weight of pups were recorded.

During the post-natal period, the body weight of the pups was recorded, landmarks for development were monitored, and neuromotor maturation tests (i.e., surface righting reflex, forelimb grip strength) were conducted. At 3 months of age, the pups were tested in open-field and rotarod tests to further assess development. The PFOS treatment had no effect on maternal body weight or food/water consumption. On PNDs 4 and 8, pups from dams treated with 6 mg/kg of PFOS had reduced body weight, as well as delayed ($p < 0.05$) eye opening, pinna detachment, and surface righting reflex. Female pups from dams exposed to 6 mg/kg of PFOS and stressed by immobilization exhibited reduced open-field activity. No differences in activity were observed for male pups and rotarod performance was not affected in any group by PFOS alone or combined with maternal stress.

Ten-day old male neonatal Naval Medical Research Institute (NMRI) mice (4–7/group) were exposed once to 0, 0.75, or 11.3 mg/kg bw of PFOS by oral gavage (Johansson et al. 2008). Spontaneous behavior (locomotion, rearing, and total activity) and habituation were examined in

the mice at 2 and 4 months old. Behavior was tested in an automated device equipped with horizontal infrared beams. Motor activity was measured during a 60-minute period divided into three 20-minute sessions. Locomotion, rearing, and total activity were recorded.

No effects were observed on body weight. At 2 months old, mice exposed to 0.75 and 11.3 mg/kg bw of PFOS exhibited significant ($p \leq 0.01$) decreases in locomotion, rearing, and total activity during the first 20 minutes compared to controls. After 60 minutes, activity was significantly increased in the 11.3 mg/kg bw dose group when compared to controls. The expected habituation response is for the highest activity pattern to occur in the first 20-minute period not the last period. The same trend was observed at 4 months in the mice exposed to 11.3 mg/kg bw. At 4 months the responses in the 0.75 mg/kg bw dose group were similar to the controls. Overall, a single PFOS treatment on PND 10 affected habituation even up to 4 months of age for mice in the high dose group (11.3 mg/kg/day). The LOAEL was 0.75 mg/kg based on decreased locomotion, rearing, and total activity in 2 month old mice.

Johansson et al. (2009) administered a single oral dose of 0 (3 litters) or 11.3 mg/kg (four litters) to NMRI male mice (10 days old). The exact number of male mice in each litter was not provided. Sacrifice occurred 24 hours after treatment and the brain was dissected. The cerebral cortex and hippocampus were homogenized to determine if PFOS affected the protein levels of calcium/calmodulin-dependent protein kinase II (CaMKII), growth-associated protein-43 (GAP-43), synaptophysin, and tau, which are all proteins involved in neuronal survival, growth, and synaptogenesis change during the *brain growth spurt*.

There were no clinical signs of acute toxicity, and no treatment-related body weight differences. The CaMKII and GAP-43 protein levels in the hippocampus were both increased in the PFOS treated males; levels were increased 57% ($p < 0.001$) and 22% ($p < 0.01$), respectively, when compared to controls. Protein values in the cerebral cortex were similar between the control and treated mice. Synaptophysin protein levels were increased significantly ($p < 0.001$; 48%) in the hippocampus and ($p < 0.01$; 59%) in the cerebral cortex of the treated mice. The tau protein levels in the cerebral cortex were increased significantly ($p < 0.05$; 80%) in treated animals compared to controls. Overall, the study indicates that a one-time treatment with 11.3 mg//kg PFOS had a significant effect on the neuronal proteins evaluated.

Tissue and Metabolic effects

Zeng et al. (2014) examined cardiac mitochondria mediated apoptosis in weaned rats exposed by way of their dams (10 per dose group) to 0, 0.1, 0.6, or 2 mg/kg/day in 0.05% Tween 80 by gavage on GDs 2–21. The pups were sacrificed at the end of the lactation period. Trunk blood and the heart were recovered. Apoptotic cells in the heart tissue from six animals per dose group were measured using a Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assay by an individual pathologist blinded to the exposure group. The apoptosis index was recorded as percent apoptotic cells per 1,000 cells in the same section. PFOS exposure was associated with a dose dependent increase in the percentage of TUNEL positive nuclei ($p < 0.05$). The 0.6 mg/kg/day dose was the LOAEL and the 0.1 mg/kg/day dose the NOAEL. The researchers found that biomarkers for apoptosis were supportive of the TUNEL results. The expression of BCL2-associated X protein and cytochrome c were upregulated and bcl-2 downregulated. The concentration of caspase 9 was significantly increased above the control levels at all doses and caspase 3 levels were significantly increased for all but the lowest dose level.

3.2.7 Chronic Toxicity

Only a single chronic exposure study in animals is available (Thomford 2002/Butenhoff et al. 2012). It is the long term component of the Seacat et al. (2002) subchronic study reported in section 3.2.3. Sprague-Dawley Crl:CD (SD)IGS BR, rats (n = 40–70) were dosed using a PFOS containing diet for up to 105 weeks. Five per sex per dose group were sacrificed at 4 and 14 weeks as described earlier. Treatment resulted in decreased body weight, with increased liver weight with hepatocellular hypertrophy. A satellite group of animals received 20 ppm of the PFOS containing diet for 52 weeks, followed by the control diet until sacrifice at week 106.

The animals received dietary levels of 0, 0.5, 2, 5, or 20 ppm PFOS as the potassium salt. Corresponding PFOS doses were 0, 0.024, 0.098, 0.24, and 0.984 mg/kg/day, respectively, for males and 0, 0.029, 0.120, 0.299, 1.251 mg/kg/day, respectively, for females. Five animals/sex in the treated groups were sacrificed during week 53 and liver samples were obtained for mitochondrial activity, hepatocellular proliferation rate, and determination of palmitoyl-CoA oxidase activity; liver weight was recorded. The results from the 4-week and 14-week sacrifices (Seacat et al. 2002) from this study are provided in sections 3.2.2 and 3.2.3, respectively. Serum samples were collected at weeks 27 and 53 from 10 rats/sex/dose group and were examined for clinical effects associated with systemic toxicity; liver samples were obtained during and at the end of the study for determination of PFOS concentration. Data on chronic effects were not reported for the recovery group. The concentration of PFOS in serum was measured at weeks 4, 14, and 105. In males the serum levels decreased between week 14 and 105 by 50% for all but the 0.5 ppm group where the decrease in serum concentration was larger. A serum measurement was available at 53 weeks for the high dose males and was comparable to the value at 14 weeks. In females serum levels serum levels remained relatively constant at 14 and 105 weeks. In both males and females the concentrations in the liver were lower at 105 weeks than they were at 14 weeks.

The clinical serum observations for ALT at 53 weeks were consistent with those at 14 weeks in demonstrating significant ($p < 0.05$) increases for the high dose males but not females. At week 27, ALT was increased for high-dose males, but did not attain statistical significance. For males at 53 weeks in the 0, 0.5, 2, 5, and 20 ppm groups, ALT values were 54 ± 66, 62 ± 52, 40 ± 7.5, 44 ± 8.3, and 83 ± 84 IU/L, respectively. The large SDs were the result of high values in one animal in each of the control and 0.5 ppm groups and two animals in the 20 ppm group. Thus, some animals may be more sensitive to liver damage as a result of exposure than others. AST levels were not increased for either sex. Serum blood urea nitrogen (BUN) was significantly ($p \leq 0.05$) increased at 20 ppm for males and females at weeks 14, 27, and 53 and in 5 ppm males and females at 27 and 53 weeks. The males in the 2 ppm group also had a significant ($p \leq 0.05$) increase in BUN at 53 weeks. These data were presented graphically in Butenhoff et al. (2012).

Nonneoplastic lesions in the liver are shown in Table 3-15. At sacrifice, males at 2 ppm had a significant ($p < 0.05$) increase in hepatocellular centrilobular hypertrophy. In the males and females at 5 and 20 ppm, there were significant ($p < 0.05$) increases in centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules (females only), and centrilobular hepatocytic vacuolation (males only). At the high dose, there was a significant increase in the number of animals with single cell hepatic necrosis in both males and females at 53 weeks. Necrosis in the recovery animals was comparable to the controls.

Table 3-15. Incidence of Nonneoplastic Liver Lesions in Rats (Number Affected/Total Number)

Lesion	0 ppm 0 mg/kg/day (d)	0.5 ppm 0.024 mg/kg/d	2.0 ppm 0.098 mg/kg/d	5.0 ppm 0.242 mg/kg/d	20 ppm 0.984 mg/kg/d
Males					
Centrilobular hypertrophy	0/65	2/55	4/55*	22/55**	42/65**
Eosinophilic granules	0/65	0/55	0/55	0/55	14/65*
Vacuolation	3/65	3/55	6/55	13/55**	19/65**
Single cell necrosis	5/65	4/55	6/55	5/55	14/65*
Females					
	0 mg/kg/d	**0.029 mg/kg/d**	**0.120 mg/kg/d**	**0.299 mg/kg/d**	**1.251 mg/kg/d**
Centrilobular hypertrophy	2/65	1/55	4/55	16/55**	52/65**
Eosinophilic granules	0/65	0/55	0/55	7/55**	36/65**
Single cell necrosis	7/65	6/55	6/55	6/55	15/65*

Source: Data from Thomford 2002/Butenhoff et al. 2012
Notes: *Significantly increased over control: $p < 0.05$
** Significantly increased over control: $p < 0.01$.

No effects were observed on hepatic palmitoyl-CoA oxidase activity or increases in proliferative cell nuclear antigen (PCNA) at weeks 4 and 14 or bromodeoxyuridine at week 53. PFOS was identified in the liver and serum samples of the treated animals and trace amounts were identified in the control animals. The LOAEL at termination for male rats was 2 ppm (0.098 mg/kg/day) and for female rats was 5 ppm (0.299 mg/kg/day) based on the liver histopathology. The NOAEL for the males was 0.5 ppm (0.024 mg/kg/day) and 2 ppm (0.120 mg/kg/day) for females. Additional details from the study in regard to carcinogenicity are provided in section 3.2.8.

Survival was not affected by PFOS administration. Males and females administered 20 ppm had statistically-significantly decreased mean body weight compared to controls during weeks 9–37 and 3–101, respectively, but was similar to controls by week 105. The females at 20 ppm had decreased food consumption during weeks 2–44. At the week 14 and 53 sacrifices, absolute and relative liver weights were significantly increased at 20 ppm in males and relative liver weight was increased at 20 ppm in females. At week 53, liver weight data were given only for the control and 20 ppm groups such that a dose-response could not be evaluated.

3.2.8 Carcinogenicity

Rat. Tumor data were collected as part of the chronic study (Thomford 2002/Butenhoff et al. 2012) described above. The tumor results are provided in Table 3-16. A significant positive trend ($p = 0.0276$) was noted in the incidence of hepatocellular adenoma in male rats. This was associated with a significant increase ($p < 0.0456$) in the high-dose group (7/60, 11.7%) over the control (0/60, 0%). No hepatocellular tumors were observed in the recovery group exposed for 52 weeks and sacrificed at 106 weeks. Liver tumors were observed in males at all doses (0%, 6%, 6%, 2%, and 11.7%). In females, significant positive trends were observed in the incidences of hepatocellular adenoma ($p = 0.0153$) and combined hepatocellular adenoma and carcinoma ($p = 0.0057$) at sacrifice. Here too, the response was not linear to dose with sequential values of 0%, 2%, 2%, 2%, and 8.3%. These cases were associated with significant increases in the high-dose group 5/60 ($p = 0.0386$; 8.3%) for adenomas and 6/60 ($p = 0.0204$; 10%) for combined adenomas and carcinomas compared to the control. The female recovery group had 2/20 liver adenomas (5%) and no carcinomas. The presence of increased levels of ALT in the males of the

high dose group at 14, 27, and 53 weeks supports hepatic tissue damage with compensatory repair as a probable a possible mode of action (MOA) for the liver tumors. In all cases the SDs about the means are broad suggesting that some animals could be less resilient than others to the liver effects.

Table 3-16. Tumor Incidence (%)

Tumors	0 ppm 0 mg/kg/d	0.5 ppm 0.024 mg/kg/d	2.0 ppm 0.098 mg/kg/d	5.0 ppm 0.242 mg/kg/d	20 ppm 0.984 mg/kg/d	20 ppm recovery 1.144 mg/kg/day
Males						
Liver						
hepatocellular adenoma+	0 (0/60)	6.0 (3/50)	6.0 (3/50)	2.0 (1/50)	11.7* (7/60)	0 (0/40)
Thyroid						
follicular cell adenoma	5.0 (3/60)	10.2 (5/49)	8.0 (4/50)	8.2 (4/49)	6.8 (4/59)	23.1* (9/39)
follicular cell carcinoma	5.0 (3/60)	2.0 (1/49)	2.0 (1/50)	4.1 (2/49)	1.7 (1/59)	2.6 (1/39)
combined	10.0 (6/60)	12.2 (6/49)	10.0 (5/50)	10.2 (5/49)	8.5 (5/59)	25.6 (10/39)
Females						
	0 mg/kg/d	**0.029 mg/kg/d**	**0.120 mg/kg/d**	**0.299 mg/kg/d**	**1.251 mg/kg/d**	**1.385 mg/kg/d**
Liver						
hepatocellular adenoma+	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	8.3* (5/60)	5.0 (2/40)
hepatocellular carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	0 (0/50)	1.7 (1/60)	0 (0/40)
combined+	0 (0/60)	2.0 (1/50)	2.0 (1/49)	2.0 (1/50)	10.0* (6/60)	5.0 (2/40)
Thyroid						
follicular cell adenoma	0 (0/60)	0 (0/50)	0 (0/49)	4.0 (2/50)	1.7 (1/60)	2.5 (1/40)
follicular cell carcinoma	0 (0/60)	0 (0/50)	0 (0/49)	2.0 (1/50)	0 (0/60)	0 (0/40)
follicular cell combined	0 (0/60)	0 (0/50)	0 (0/49)	6.0* (3/50)	1.7 (1/60)	2.5 (1/40)
C-cell adenomas	20.0 (12/60)	12.0 (6/50)	12.2 (6/49)	16.0 (8/50)	8.3* (5/60)	15.0 (6/40)
C-cell Carcinomas	0 (0/60)	2.0 (1/50)	0 (0/49)	0 (0/50)	0 (0/60)	2.5 (1/40)
C-cell combined	20.0 (12/60)	14.0 (7/50)	12.2 (6/49)	16.0 (8/50)	8.3* (5/60)	17.5 (7/40)
Mammary						
Fibroma/Adenoma	33.3 (20/60)	54.0* (27/50)	39.6 (19/48)	48.0 (24/50)	18* (11/60)	37.5 (15/40)
Adenoma	11.7 (7/60)	12.0 (6/50)	10.4 (5/48)	14.0 (7/50)	6.7 (4/60)	10.0 (4/40)
Combined adenomas	38.3 (23/60)	60.0* (30/50)	45.8 (22/48)	52.0 (26/50)	25.0* (15/60)	40.0 (16/40)
carcinoma	18.3 (11/60)	24.0 (12/50)	31.3 (15/48)	22.0 (11/50)	23.3 (14/60)	10.0 (4/40)

Source: Data from Thomford 2002/Butenhoff et al. 2012.
Notes: +Significant positive trend.
* Significantly increased over the control: $p < 0.05$
** Significantly increased over the control: $p < 0.01$.

There were cases of thyroid follicular cell adenomas and carcinomas in both the male and female rats but no pattern of dose-response or significant increases compared to controls. The incidence of thyroid follicular cell adenomas in the male recovery group was increased significantly ($p = 0.028$) over controls (23.1% vs 5%). The incidence of combined thyroid follicular cell adenoma and carcinoma in the recovery group males (10/39, 25.6%) did not attain statistical significance compared to that of the control group (6/60, 10%). The males that were continually dosed for 105 weeks had a much lower adenoma incidence than the recovery group (6.8% versus 23.1%). In no case were thyroid tumors determined to be a cause of death.

In females, there was a significant increase ($p = 0.0471$) for combined thyroid follicular cell adenoma and carcinoma in the mid-high (5.0 ppm) group (3/50, 6%) compared to the control group (0/60, 0%). The incidence data for thyroid follicular tumors lacked dose-response. C-cell thyroid adenomas had a higher incidence than the follicular cell tumors in female rats. The

highest incidence was in the control group (20%); there was a lack of dose-response across groups (8%–18%). As was the case with the combined adenomas and carcinomas, the C-cell tumors were not identified as a cause of death.

There was a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma (25%–60%), but the incidence lacked dose-response for all tumor classifications. Significant (p = 0.0318) increases combined mammary fibroadenoma/adenoma (30/50, 60%; p = 0.0318) were observed in the low–dose (0.5 ppm) group compared to the respective controls but there was a lack of dose response with the high dose group having a lower incidence (25%) than the controls (38%). Mammary gland carcinomas also lacked dose-response and had a relatively comparable incidence across dose groups including the controls.

Mouse. The mouse model C57BL/6J-*Min*/+ for intestinal neoplasia was used to study the obesogenic and tumorigenesis effects of PFOS following *in utero* exposure (Ngo et al. 2014). The C57BL/6J-*Apc*$^{Min/+}$ mouse has a heterozygote mutation in the tumor suppressor gene adenomatous polyposis coli (Apc), and is therefore a sensitive model in which to test whether chemicals can affect intestinal tumorigenesis. Wild-type females (Apc$^{+/+}$), mated to heterozygous males (Apc$^{Min/+}$), were given 0, 0.01, 0.1, or 3 mg/kg/day by gavage on GDs 1–17 and allowed to litter naturally. Offspring with ApcMin/+ genotype were terminated at 11 weeks of age for study of intestinal tumorigenesis and obesogenic effect while wild-type (Apc+/+) offspring were sacrificed at 20 weeks to assess any obesogenic effect at an older age. In the treated groups, whole litter loss occurred in 6/16, 10/28, and 7/14 dams, respectively, compared with 2/22 controls; the timing of loss, late, or early gestation, was not stated. No clinical signs of toxicity were observed during dosing and maternal body weight was similar between treated and control groups. For offspring of either genotype, terminal body weight, liver and spleen weights, and plasma glucose were not affected by *in utero* exposure. PFOS did not increase intestinal tumorigenesis in susceptible, Apc$^{Min/+}$, offspring.

3.3 Other Key Data

3.3.1 Mutagenicity and Genotoxicity

Results of genotoxicity testing with PFOS are summarized in Tables 3-17 and 3-18. PFOS was tested for mutation in the Ames Salmonella/Microsome plate test and in the D4 strain of *Saccharomyces cerevisiae* (Litton Bionetics, Inc. 1979). It was also tested in a *Salmonella-Escherichia coli*/Mammalian-microsome reverse mutation assay with and without metabolic activation (Mecchi 1999), in an *in vitro* assay for chromosomal aberrations in human whole blood lymphocytes with and without metabolic activation (Murli 1999), and in an unscheduled DNA synthesis assay in rat liver primary cell cultures (Cifone 1999). In all these assays, PFOS was negative. In an *in vivo* mouse micronucleus assay, PFOS did not induce any micronuclei in the bone marrow of Crl:CD-1 BR mice (Murli 1996). A 50% w/w solution of the diethanolammonium salt of PFOS in water (T-2247 CoC) was also tested to determine whether induction of gene mutation in five strains of *S. typhimurium* and in *S. cerevisiae* strain D3 would take place with and without metabolic activation (Simmon 1978). The results were negative.

Governini et al. (2015) collected semen samples from 59 healthy-nonsmoking patients attending a Center for Couple Sterility at the University in Siena, Italy. The subjects were divided into those that were normozoospermic (13) and those that were oligoasthenoterato-

zoospermic (46). PFOS was present in 25% of the seminal plasma samples and 84% of the serum samples. Conversely PFOA was present in 75% of the seminal plasma samples and only 16% of the blood samples. Sperm were evaluated for the presence of aneuploidy and diploidy, and sperm DNA was evaluated for fragmentation using the TUNEL assay. The frequencies of aneuploidy and diploidy were significantly greater in the PFAS positive samples than in the PFC negative samples ($p < 0.001$ and $p < 0.05$, respectively) suggesting the possibility for errors in cell division. The levels of fragmented chromatin were significantly increased ($p < 0.001$) for the PFC positive group compared with the PFAS negative group.

Table 3-17. Genotoxicity of PFOS *in vitro*

Species (test system)	End-point	With activation	Without activation	Reference
Salmonella strains and D4 strain of *Saccharomyces cerevisiae*	Gene mutation	negative	negative	Litton Bionetics, Inc. 1979
Salmonella strains and *Escherichia coli* WP2*uvr*	Gene mutation	negative	negative	Mecchi 1999
5 strains of *S. typhimurium* and *S. cerevisiae* strain D3	Gene mutation	negative	negative	Simmon 1978
Human lymphocytes	Chromosome aberrations	negative	negative	Murli 1999
Hepatocytes from Fisher 344 male rats	DNA synthesis		negative	Cifone 1999

Table 3-18. Genotoxicity of PFOS *in vivo*

Species (test system)	End-point	Results	Reference
Crl:CD-1 BR mice	Presence of micronuclei in bone marrow	negative	Murli 1996

3.3.2 Protein binding

The ability of PFOS to bind to serum proteins for distribution is discussed in section 2.2. PFC protein binding can also impact cellular function in cases where the proteins in question are transporters (serum albumin and fatty acid binding protein), enzymes (lysine decarboxylase), or membrane receptors such as members of the PPAR family and thyroid hormone receptors. The mechanistic studies of the membrane receptors are described in section 3.3.4.

Ren et al. (2015) examined the relative binding affinities of 16 perfluoroalkyl compounds for the human thyroid hormone receptor's α ligand binding domain (TRα-LBD) using a fluorescence competitive binding assay. Solutions of 1μmol TRα-LBD were prepared in DMSO. Changes in TRα-LBD tryptophan fluorescence after binding to 10 μmol T3 in the absence or presence of the PFAS was used to determine the binding properties of the PFAS. IC_{50} values were calculated by linear extrapolation between two responses located in the vicinity of a 50% inhibition level. All the PFAS had a lower affinity for the receptor than T3, but the binding affinity of PFOS was greater than that for PFOA and the other sulfonates tested. The IC_{50} value for PFOS was 16 μmol, compared with 0.3 μmol for T3.

Lysine decarboxylase is a key enzyme involved in the production of cadaverine from the amino acid lysine. S. Wang et al. (2014) studied the impact of a series of 16 PFAS on the activity and conformation of this enzyme because of its involvement in growth and development. The interaction assays were carried out *in vitro* using a fluorescent probe to measure enzyme activity. The impact of a PFAS on enzyme activity caused a decrease in fluorescence that represented enzyme inhibition. Varying the PFAS concentrations provided the data for determining inhibition constants for each compound tested. Members of the sulfonate family were stronger inhibitors than the carboxylic acids, and enzyme inhibition increased as did the length of the carbon chain. Only the 4, 6, and 8 carbon members of the sulfonate family were tested.

Circular dichroism was used as a tool for determining changes in enzyme conformation in the presence of the tested PFAS (S. Wang et al. 2014). PFOS caused a greater change in enzyme conformation than PFOA. Cellular cadaverine production was decreased indicating the potential for PFOS to alter metabolism by way of enzyme inhibition as a consequence of its protein binding properties. To date there has been scant investigation of PFOS or other PFASs as enzyme inhibitors.

An *in vitro* study of the impact of PFOS (and other PFASs) on the conformation of several proteins (BSA, ovalbumin, and β-galactosidase) in solution found that the denaturing effect of the PFAS depended on the amino acid composition and conformation of the protein as well as the individual PFAS (Ospinal-Jiménez and Pozzo 2012). The PFOS concentration (1 millimole [mmol]) was higher than one would expect *in vivo* because the study was designed to examine denaturing potential.

Enzymes targeted by PFOS can vary. Molecular docking analysis of PFOS's potential to bind with and change the activity of enzymes along metabolic pathways associated with its critical effects could provide important insights related to toxicity. The importance of the S. Wang et al. (2014) and Ospinal-Jiménez and Pozzo (2012) studies are the evidence they produced showing that the protein binding properties of a PFAS can impact the conformation, thereby possibly changing activity.

3.3.3 Immunotoxicity

Human–in vitro. In a pilot study, Brieger et al. (2011) examined the effects of PFOS on human leukocytes. Peripheral blood mononuclear cells (PBMC) were obtained from 11 voluntary donors (n = 6 females, 5 males). The mean plasma concentrations of PFOS were 0.004, 0.0028, and 0.0055 μg/mL for all, female, and male volunteers, respectively. PBMCs were incubated with varying concentrations of PFOS followed by assays for cell viability, proliferation, and natural killer (NK) cell activity. The human promyelocytic leukemia cell line, HL-60, was also used in cell viability and monocyte differentiation assays. The various components of the assays employed and the results are identified as follows:

1. In the cell viability assay, the PBMCs and HL-60 cells were incubated with 0–125 μg/mL of PFOS for 24 hours. Viability was determined after incubation by measuring neutral red uptake. No significant reduction of viability was observed up to 125 μg/mL; however, the highest concentration for PFOS could not be evaluated due to limited solubility. Therefore, 100 μg/mL was the highest concentration used thereafter.
2. In the proliferation assay, the PBMCs were incubated with 0, 1, 10, or 100 μg/mL of PFOS for 24 hours; labeled with 6-carboxyfluorescein succinimidyl ester (CFSE); stimulated with concanavalinA, a T-cell mitogen (ConA, 5 μg/mL to half of all samples);

and incubated for an additional 72 hours. Proliferation was slightly increased at 100 μg/mL and slightly reduced with the presence of ConA, but neither effect was statistically-significant.

3. For the NK cell assays, PBMCs were incubated with 0, 1, 10, or 100 μg/mL of PFOS for 24 hours followed by incubation for 3 hours with K562 target cells (12.5:1 ratio) labeled with CFSE. K562 cells are a chronic myelogenous leukemia cell line known to be susceptible to NK cell induced cytotoxicity. PFOS significantly ($p < 0.001$) reduced NK cell cytotoxicity to K562 cells by 32% at 100 μg/mL.
4. In the monocyte differentiation assay, HL-60 cells were incubated with 0, 1, 10, or 100 μg/mL of PFOS for 72 hours. Half of each sample was stimulated with 25 nmol calcitrol, 1α,25-dehydroxyvitamin D3 ($1,25D_3$) 24 hours into the incubation period. Expression of CD11b and CD14 were measured as markers of differentiation. In the presence of $1,25D_3$, PFOS had no significant effect on the percentage of HL-60 cells expressing CD11b and CD14. No differences in monocyte differentiation were observed in the absence of $1,25D_3$.
5. Whole blood was incubated with 0–100 μg/mL of PFOS in the presence or absence of 25 μg/mL phytohemagglutinin (PHA), a T-cell cytokine secretion stimulator, for 48 hours. Lipopolysaccharide (LPS, 0 or 250 ng/mL), a monocyte stimulator, was added to whole blood incubated with 0.1–100 μg/mL of PFOS either 4 or 24 hours prior to the end of the 48 hour incubation period. Release of the cytokines TNF-α and IL-6 from T-cells or monocytes was quantified. Cytokine release from T-cells was not affected by PFOS. PFOS significantly ($p < 0.001$) reduced the release of the pro-inflammatory cytokine TNF-α after monocyte LPS stimulation. The authors also looked at the correlation between basal PFOS concentration of the blood donor and cytokine release. A significant association was observed between PFOS concentration and the release of LPS-induced IL-6 by peripheral monocytes.

This study suggests some effects on immunity in humans; however the sample size used is small and the concentrations at which effects were observed are much higher than the levels of PFOS in human blood samples.

Midgett et al. (2014) examined the effects on IL-2 production using stimulated cultured human Jurkat cells and CD4+ T cells recovered from 11 healthy volunteers. Both cell types were stimulated with PHA/phorbal myristate acetate (PMA) or anti-CD3 to produce IL-2 and incubated with 0–100 μg PFOS/mL; separate experiments were conducted with human Jurkat cells in the presence or absence of a PPAR antagonist. Cell viability was not affected in either cell type up to and including the highest concentration of PFOS. In the human Jurkat cells stimulated with PHA/PMA a concentration of 10 μg/mL was a NOEL and 50 μg/mL a LOEL for inhibition of IL-2 production in the absence and presence of a PPARα inhibitor. In the presence of anti-CD3, the NOEL was 1 μg/mL and the LOEL 5 μg/mL. In primary human CD4+ T cells stimulated with PHA/PMA, the NOEL was the 10 μg/mL concentration and the LOEL 100 μg/mL for inhibition of IL-2 production. A decrease in T cell IL-2 production is a characteristic associated with autoimmune disorders, suggesting that this population could be sensitive to PFOS exposures. However, the authors caution that the results from the in vitro studies do not reflect any potential decrease in circulating PFOS as the result of protein binding to albumin or other serum proteins. In this study the observed IL-2 effects in the Jurkat cells were demonstrated to be independent of PPARα activation as the inhibition was similar with and without the PPAR antagonist.

Mouse. Qazi et al. (2009a) administered diets containing 0, 0.001%, 0.005% , 0.02% (40 mg/kg bw/day), 0.05% (100 mg/kg bw/day), 0.1%, 0.25%, 0.5%, or 1% PFOS and 0.02% PFOA for 10 days to 4–6 six male (6–8 weeks old) C57Bl/6 mice/group. Doses for all dietary levels were not presented by the study authors. PFOS and PFOA were dissolved in 20 mL of acetone prior to being mixed with the chow and then dried to allow the odor of the acetone to dissipate prior to administration. At the end of 10 days, mice were bled for analysis of PFOA and PFOS, and then killed. Weights were obtained for the thymus, spleen, liver, and epididymal fat. The number of thymocytes and splenocytes were measured and checked for viability. Histology was also performed on the thymus and spleen.

The mice treated with dietary concentrations of > 0.02% (~ 40 mg PFOS/kg bw/day) PFOS exhibited pronounced weight loss (> 20%), a decrease in food consumption (> 40%), and lethargy and were withdrawn from the experiment after 5 days of exposure. The author stated that this was not due to taste aversion since it is also observed when PFOS is administered intraperitoneally or subcutaneously. The background levels of PFOS and PFOA were both similar in the control mice; however, after administration of 0.02% in the diet, the serum level of PFOS was approximately twice that of PFOA. Only the animals treated with 0.02% PFOS had a significant decrease in total body weight and in the wet weights of the thymus, spleen, and epididymal fat pads compared to the controls. However, all three doses resulted in a significant increase ($p < 0.05$ or 0.01) in liver weight, compared to controls. Similar findings slightly more pronounced were observed in mice administered PFOA. The mice administered 0.02% of PFOS demonstrated a marked decrease in the total number of thymocytes (84% of controls) and splenocytes (43% of controls), and they had thymocytes and splenocytes that were reduced in size. Finally, in the mice administered 0.02% PFOS or PFOA, the thymic cortex was small and devoid of cells and the cortical/medullary junction was not distinguishable. No obvious histological differences in the spleen of the mice administered any dose of PFOA or PFOS were observed.

Qazi et al. (2009b) also performed a study to see if exposure to PFOS influenced the cells of the innate immune system. Four male C57Bl/6 mice per dose were exposed to rat chow supplemented with 0%, 0.001%, or 0.02% PFOS for 10 consecutive days. A second, similar study was performed to determine if the PFAS exposure influenced innate immune response to bacterial LPS. Mice were exposed to PFOS as described above. On day 10, some mice were injected intravenously with 0.1 mL sterile saline containing 300 µg LPS (*E. coli*), while others received vehicle only. In the first study, mice were bled directly after the 10 day exposure and in the second study mice were bled 2 hours after administration of LPS. The spleen, thymus, epididymal fat, liver, and peritoneal and bone marrow cells were collected.

No effects were observed in any of the mice exposed to 0.001% PFOS. Exposure to 0.02% PFOS caused an increase in liver weight and a decrease in the weight of other organs and overall body weight. Food consumption in these mice was also decreased 25% when compared to control mice. The total intake of PFOS over the 10 days was approximately 6 mg (0.6 mg/kg/day), and the total concentration of PFOS in the serum was 340 ± 16 µg/mL (ppm). The overall total number of white blood cells and lymphocytes were decreased while the neutrophil counts were similar to controls. The number of macrophages in the bone marrow was increased but not those of the peritoneum and spleen. Cells isolated from the peritoneal cavity and bone marrow, but not spleen, of mice exposed to the high level of PFOS had enhanced levels of the pro-inflammatory cytokines, TNF-α, and IL-6 in response to stimulation by LPS. The levels of these cytokines in the serum were not elevated. This study indicates that PFOS can have

an effect on the innate immune responses in mice following a 10-day exposure to about 0.6 mg/kg/day.

In Qazi et al. (2010), male C57BL/6 (H-2^b) mice (n = 7) were administered PFOS in the diet at 0.005% (w/w) for 10 days to determine the effect on the histology and immune status of the liver. There was no effect on body weight, food intake, thymus, spleen or fat pad mass, serum levels of ALT or AST, hematocrit, hemoglobin, or the numbers of thymocytes and splenocytes. However, the liver mass was increased 1.6-fold when compared to untreated controls, and hypertrophic hepatocytes surrounded the central vein. No necrosis was noted. Total serum cholesterol was decreased and there was a moderate increase in serum ALP. At the end of the study, the total mean serum PFOS concentration for four mice was 125.8 μg/mL. PFOS increased only one type of intrahepatic immune cells ($TER119^+$). The treated mice also had lower levels of the hepatic cytokines, TNF-α, IFN-γ, and IL-4, when compared to the control mice and an increase in hepatic erythropoietin. The IgM response of the intrahepatic B and T cells was normal.

Peden-Adams et al. (2008) gave PFOS in Milli-Q water containing 0.5% Tween 20, daily by gavage for 28 days to five adult male and female $B6C3F_1$ mice/group. Equivalent daily PFOS doses to the seven dose groups were 0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, and 0.166 mg/kg/day, respectively. Animals were euthanized at the end of treatment. Various immune parameters, including lymphocytic proliferation, NK cell activity, lysozyme activity, antigen specific IgM production, lymphocyte immunophenotypes, and serum PFOS concentrations were determined after exposure.

Survival, behavior, body weight, spleen, thymus, kidney, gonad and liver weights, and lymphocytic proliferation were not affected by treatment. Lysozyme activity increased significantly in females, but not males, at 0.0033 and 0.166 mg/kg/day, respectively compared to the control group; however, the response as not dose-related. NK cell activity was increased significantly ($p \leq 0.05$) 2- to 2.5-fold in males at 0.017, 0.033, and 0.166 mg/kg/day, but was not affected in any of the females. Splenic T-cell immunophenotypes were slightly affected in females, but they were significantly altered in males treated with ≥ 0.0033 mg/kg/day. In both genders, thymus cell populations were less sensitive to PFOS. Male thymic T-cell subpopulations were not affected with PFOS treatment and in females were increased only at 0.033 and 0.166 mg/kg/day.

Because IgM suppression can result from effects on both T- and B-cells, antibody production was measured in response to sheep red blood cells (SRBC) (T-dependent) and a trinitrophenyl (TNP) LPS conjugate (T-independent). The SRBC plaque-forming response was suppressed and demonstrated a dose-response in males beginning at 0.0017 mg/kg/day and in females at 0.017 mg/kg/day. In males it was suppressed by 52%–78% and females by 50%–74%. For evaluation of T-independent (TI) responses, an additional group of female mice was treated with 0 or 0.334 mg/kg/day of PFOS orally for 21 days and challenged with a TI antigen TNP-LPS conjugate. Serum TNP-specific IgM titers were decreased after the TNP-LPS challenge with serum levels of TNP-specific IgM significantly suppressed by 62% compared with controls. Based on the IgM suppression observed in both the T-cell independent and dependent tests, humoral immune effects can be attributed to B-cells, rather than T-cells. Serum levels of PFOS were similar between males and females. Based on the results the LOAEL in mice is 0.0017 mg/kg/day in males and 0.017 mg/kg/day in females. The NOAELs are 0.00017 mg/kg/day in males and 0.0033 mg/kg/day in females.

Potassium PFOS suspensions were made with deionized water with 2% Tween 80 and administered orally by gavage at doses of 0, 5, 20, or 40 mg/kg bw to twelve male (8–10 weeks old) C57BL/6 mice/group daily for 7 days (Zheng et al. 2009). Food consumption and body weight were measured daily for 7 days. Mice were bled on the eighth day (24 hours after the last treatment) and subsequently sacrificed. The blood was analyzed for corticosterone and PFOS concentration. Spleen, thymus, liver, and kidneys were collected and weighed, and the spleen and thymus were processed into suspensions to look at functional immune endpoints and T-cell immunophenotype determinations.

Starting on about day 3, mean body weights were significantly decreased compared to the controls for the 20 and 40 mg/kg bw/day doses. However, food consumption decreased with treatment. At the end of treatment, the body weight, splenic, and thymic weights were all decreased at 20 and 40 mg/kg bw/day, compared to the controls. Liver weight was increased by 34%, 79%, and 117% over controls at 5, 20, and 40 mg/kg bw/day, respectively. A dose-dependent increase in PFOS was observed in the serum samples; levels in the controls were below the limit of detection. Serum corticosterone levels increased significantly in mice treated with doses ≥ 20 mg/kg/day. Splenic and thymic cellularity were significantly decreased ($p \leq 0.05$) at 20 and 40 mg/kg bw/day; cellularity in the spleen and thymus in the mice administered 40 mg/kg/day was decreased by 51% and 61%, respectively, compared to the control mice. To determine population changes in functional cell types of spleen and thymic lymphocytes, CD4/CD8 marker analysis was performed. Significant decreases in CD4+ and CD8+ cells were observed in both the spleen and thymus in the mice administered ≥ 20 mg/kg/day PFOS.

A lactate-dehydrogenase release assay was performed to determine NK cell activity. The average NK-cell activity was decreased at 20 and 40 mg/kg/day compared to control mice, 18.04 ± 1.42 and 13.08 ± 1.11, respectively compared to 50.33 ± 4.08 in controls. No numeric data were provided for the 5 mg/kg/day group. Treatment in all groups of mice resulted in a significant suppression of the plaque-forming cell response after 7 days of treatment; results were 63%, 77%, and 86% that of controls at 5, 20, and 40 mg/kg bw/day, respectively. Based on the increase in liver weight and the suppression of the plaque-forming cell response, the LOAEL was 5 mg/kg/day in mice and the NOAEL could not be determined.

In order to observe chronic effects of immunotoxicity, adult male C57BL/6 mice (10/group) were administered 0, 0.008, 0.083, 0.417, 0.833, and 2.083 mg/kg/day PFOS with 2% Tween 80 in de-ionized water daily by gavage for 60 days (Dong et al. 2009). Parameters similar to those described above for Zheng et al. (2009) were measured.

At sacrifice, mice treated with ≥ 0.417 mg/kg/day had significantly lower body weight compared to the control mice, as well as significant decreases in spleen, thymus and kidney weight. Food consumption in the study was decreased in mice at 0.833 and 2.083 mg/kg/day. Liver weight was increased significantly in all dose groups compared to controls, 5.17 ± 0.12 g (control), 5.21 ± 0.17 g, 5.78 ± 0.13 g, 6.67 ± 0.11 g, 8.17 ± 0.21 g, and 11.47 ± 0.12 g, respectively. Serum corticosterone was decreased in mice at the two higher doses. As in the shorter-term study, thymic and splenic cellularity was decreased in a dose-dependent trend, with significant decreases observed in mice receiving ≥ 0.417 mg/kg/day. The CD4/CD8 marker analysis performed on splenic and thymic lymphocytes demonstrated that the numbers of T cell and B cell CD4/CD8 subpopulations were decreased starting at 0.417 mg PFOS/kg/day. Splenic NK cell activity was increased significantly compared to controls (31.14 ± 1.93%) in the mice at 0.083 mg/kg/day (45.43 ± 4.74%) with significant marked decreases at 0.833 mg/kg/day

(20.28 ± 2.51%) and 2.083 mg/kg/day (15.67 ± 1.52%). The SRBC-specific IgM plaque forming cell response showed a dose-related decrease with statistical significance at 0.083 mg/kg/day and higher. Based on the findings in the 60 day study, the NOAEL was 0.008 mg/kg/day and the LOAEL was 0.083 mg/kg/day. The serum concentration at the LOAEL was 7.132 mg/L.

Keil et al. (2008) treated pregnant C57BL/6N females (bred with male C3H/HeJ mice) with PFOS to evaluate developmental immunity in their inbred $B6C3F_1$ offspring. The females (10–12/group) were administered 0, 0.1, 1, or 5 mg/kg of PFOS in 0.5% Tween-20 by gavage daily on gestation days (GDs) 1–17. Pups remained with the dam for approximately 3 weeks with immunotoxicity evaluations performed at 4 and 8 weeks. Body weight was recorded for dams during the study and pups after delivery. Organ weights (spleen, liver, thymus and uterus) from the pups were recorded at sacrifice. Only litters with 6 to 9 pups were retained for the immunotoxicity studies. One male and one female were selected from the retained litters (total of 6 male and 6 female pups) for testing of the immunotoxicity parameters; positive controls were included for each assay.

NK cell activity was not altered in any pups at 4 weeks old. At 8 weeks, however, NK cell activity was suppressed in males treated with 1 and 5 mg/kg/day (42.5% and 32.1% decreases compared to controls, respectively) and in females at 5 mg/kg/day (35.1%, compared to controls). The positive control for NK cell activity produced the appropriate response. The plaque-forming cell response for SRBC IgM production by B cells was only assessed at 8 weeks and was significantly suppressed in the 5 mg/kg/day males (53%); no effect was observed in the females. The only significant differences in lymphocyte immunophenotypes was a 21% decrease in absolute numbers of B220+ cells in 4-week-old females in the 5 mg/kg/day group compared to controls; this effect was not observed at 8 weeks. The other significant change was a 25% decrease in CD3+ and 28% decrease in CD4+ thymocytes at 5 mg/kg/day in males at the 8-week evaluation. Functional responses (nitrite production) to LPS and interferon-gamma by peritoneal macrophages were not affected with treatment in the 8-week-old mice (not evaluated at 4 weeks). Based on the changes in the immunotoxicity parameters evaluated, the LOAEL in mice is 1 mg/kg/day in males and 5 mg/kg/day in females. The NOAEL is 0.1 mg/kg/day in males and 1 mg/kg/day in females.

Guruge et al. (2009) administered 0, 5, or 25 µg/kg PFOS (0, 0.005, or 0.025 mg/kg, respectively) in 30 female $B6C3F_1$ mice/group for 21 days and then exposed them intranasally to 100 plaque forming units (pfu; in 30 µL of phosphate buffered saline) influenza A virus suspension. Mice were observed for 20 days past inoculation. Concentrations of PFOS in the plasma, spleen, thymus, and lung all showed a dose-dependent increase; however, there was not a significant change in body or organ weights (spleen, thymus, liver, kidney, and lung) of the treated mice compared to the controls. Survival rate was significantly decreased in the mice at 25 µg/kg PFOS after viral exposure. Survival rate in the mice on day 20 was 46% in the controls and 17% in the high-dose group.

The four studies in mice discussed above examined NK cell activity and SRBC response. The results from those studies are summarized in Table 3-19. Three of the studies showed effects on SRBC response, NK cell activity, or both at the same dose that caused increased liver weight. Based on the limited evidence, neither response appeared more sensitive than the other. The NK cell activity was enhanced at very low PFOS doses, while it was depressed at higher doses. The animal studies indicate that females are less susceptible to impacts on NK cell activity and the SRBC response than males.

Table 3-19. Summary of SRBC and NK Cell Findings in Mice after PFOS Exposure

Study	Strain	Duration Days	SRBC		NK Cell activity		Increased Liver wt.
			NOAEL mg/kg/day	LOAEL mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	LOAEL mg/kg/day
Dong et al. (2009)	C57BL/6 (M)	60	0.008	0.083 (↓)	0.008	0.083 (↑) 0.833 (↓)	0.083
Keil et al. (2008)	B6C3F$_1$ (M, F pups)	GDs 1–17 Dams only*	1 (M) 5 (F)	5 (↓M) -	- 1 (F)	1 (↓M) 5 (↓F)	5 (M at 4 wks only)
Peden-Adams et al. (2008)	B6C3F1 (M, F)	28	0.00017 (M) 0.0033 (F)	0.0017(↓M) 0.017 (↓M)	0.0033 (M) 0.166 (F)	0.017 (↑M)	None
Zheng et al. (2009)	C56BL/6 (M)	7		5 (↓)	5	20 (↓)	5

Notes: Direct dosing of the dams did not continue during the lactation period. The immune system response was evaluated in pups at 4 and 8 weeks. Effects were seen at 8 weeks but not at 4 weeks.
The direct ion of the arrow indicates if the change from control was an increase or a decrease.
M = male; F = female

3.3.4 Physiological or Mechanistic Studies of Noncancer Effects

Hormone Disruption

Martin et al. (2007) administered 10 mg PFOS/kg to adult male Sprague-Dawley rats (n = 5) for 1, 3, or 5 days by oral gavage and determined the impact of PFOS on hormone levels. Blood was collected via cardiac puncture, and the serum was analyzed for cholesterol, testosterone, free and total T4, and total T3. RNA extracted from the livers was used for gene expression profiling, genomic signatures, and pathway analyses to determine a mechanism of toxicity.

Following a 1-day, 3-day, and 5-day dose, a significant decrease ($p < 0.05$) was observed in total T4 (~ decrease of 47–80%) and free T4 (~ decrease of 60–82%). The total T3 was only significantly deceased after day 5 (decrease of ~ 23%). Serum cholesterol was significantly decreased ($p < 0.05$) after dosing for 3 and 5 days. Serum testosterone was similar to controls at all timepoints. PFOS treatment caused hepatomegaly, hepatocellular hypertrophy, and macrovesicular steatosis. Genes associated with the thyroid hormone release and synthesis pathway included type 3 deiodinase DIO3, which catalyzes the inactivation of T3 and type 1 deiodinase DIO1, which deiodinates prohormone T4 to bioactivate T3. Treatment with PFOS caused significant ($p < 0.05$) DIO1 repression and *Dio3* induction only on day 5.

Chang et al. (2007) investigated whether the decrease of FT4 often observed in animals upon PFOS exposure was due to competition for carrier protein binding interference. The study used equilibrium dialysis radioimmunoassay (ED-RIA) for FT4 measurements in *in vitro* and *in vivo* protocols. PFOS did not decrease serum total thyroxine (TT4) or FT4 at concentrations up to 200 µmol *in vitro*. Female rats administered three daily 5 mg/kg oral doses of PFOS also had no changes to serum TSH and FT4 when checked by ED-RIA. However, FT4 was significantly decreased in the animals when measured with two analog methods, chemiluminescence immunoassay and simple RIA. The authors suggested that further testing for thyroid hormone parameters should use a reference method such as ED-RIA for determining serum FT4 as analog methods may falsely appear to decrease free thyroid hormones.

Chang et al. (2008) investigated whether PFOS competed with thyroxine for serum binding proteins in rats. Three different experimental designs were employed. In the first part, five to fifteen female Sprague-Dawley rats/group were given either a single oral dose of vehicle (0.5% Tween 20 in distilled water; three groups) or 15 mg potassium PFOS/kg bw (three groups) suspended in vehicle. Rats were killed at 2, 6, and 24 hours post-dosing, and blood samples were obtained. Serum FT4, total thyroxine (TT4), triiodothyronine (TT3), reverse triiodothyronine (rT3), and thyrotropin were measured at each timepoint. TSH was measured only at the 6 and 24 hour timepoints. PFOS concentrations in the blood and liver were also measured along with hepatic transcripts for UDP-glucuronosyltransferase 1A (UGT1A) (involved in glucuronidation and T4 turnover) and malic enzyme (ME). ME activity is an indicator for tissue response to thyroid hormone.

Serum TT4 decreased significantly ($p < 0.05$) compared to controls after 2 hours (decrease of 24%), 6 hours (decrease of 38%), and 24 hours (decrease of 53%). The TT3 and rT3 only decreased significantly at the 24-hour time-point, while FT4 was increased significantly at 2 and 6 hours (68% and 90% over control, respectively) before becoming similar to that of controls at the 24-hour time-point. Serum levels of PFOS were significantly ($p < 0.05$) higher than controls at all time-points (control: < LOQ; treated: 37.28, 66.90, and 61.58 µg/mL at 2, 6, and 24 hours, respectively). A similar trend was observed with the concentration of PFOS in the liver (control: < LOQ; treated: 30.60, 44.84, 45.00 µg/g at 2, 6, and 24 hours, respectively). The ME and UGT1A mRNA transcripts were significantly increased ($p < 0.05$) only at the 2 hour time-point, compared to controls, and the ME activity was increased significantly only at the 24-hour sampling.

In the second part of the study, Sprague-Dawley rats were injected intravenously with either 9.3 µCi (females; n = 5/group) or 11 µCi (males; n = 4/group) of ^{125}I-T4 followed by a single oral dose of either vehicle or 15 mg potassium PFOS/kg bw. Urine and feces were collected for 24 hours after administration to determine the ^{125}I elimination. At the end of the 24 hours, the animals were killed and liver and serum samples collected. Serum TT4 concentration was decreased by 55% in the PFOS treated males and females compared to controls. There was also a decrease in serum ^{125}I in the treated males. Liver ^{125}I radioactivity decreased by 40% and 30% in males and females, respectively, but the urine and feces ^{125}I radioactivity increased, with the males exhibiting the most activity. This indicates a loss of thyroid hormones and increased turnover.

In the last part of the study, adult male Sprague-Dawley rats (4–6/group) were administered either vehicle only by gavage, 3 mg/kg bw of potassium PFOS suspended in vehicle by gavage, 10 µg/mL (10 ppm) propylthiouracil (PTU) in drinking water, or 10 ppm PTU in drinking water + 3 mg PFOS/kg bw for 7 consecutive days. PTU is an inhibitor of thyroid hormone synthesis. On days 1, 3, 7, and 8, TT4, TT3, and TSH were monitored and on day 8, the pituitaries were removed and placed in static culture to assess thyrotropin releasing hormone- (TRH)-mediated release of TSH. During the days of dosing with PFOS, TSH levels did not increase, but TT4 and TT3 were decreased. Pituitary response to TRH-mediated TSH release was not affected or lessened after the PFOS-only administration.

Results suggest that oral PFOS administration results in a transiently increased tissue availability of thyroid hormones, increased turnover of T4, and a reduction in TT4, but PFOS administration does not induce a typical hypothyroid state or alter the hypothalamic-pituitary-thyroid axis.

In the study by Curran et al. (2008) (see section 3.2.2 of this document) where Sprague-Dawley rats (15/sex/group) were administered 0, 2, 20, 50, or 100 mg PFOS/kg diet for 28 days, T4 and T3 levels were decreased. T4 levels were statistically-significantly decreased at ≥ 20 mg PFOS/kg diet, when compared to the control levels, in both males and females. T3 levels were decreased significantly at ≥ 50 mg/kg diet in the females and 100 mg/kg diet in the males. There were no treatment-related changes observed with absolute thyroid weight.

Yu et al. (2009a) fed adult pregnant Wistar rats (n = 20/group) a control diet or a diet containing 3.2 mg PFOS/kg feed. Treatment continued for both groups throughout gestation and lactation. Dams were allowed to deliver naturally and on the day of delivery (PND 0), samples were collected from two control litters and two PFOS treated litters. Litters were cross-fostered to help determine whether PFOS had more effect when administered prenatally, postnatally, or both. The total T3 and rT3 were not affected with PFOS treatment in the pups. Pups in all groups, except the controls, had significant ($p < 0.05$ or 0.01) decreases in total T4 on PNDs 21 and 35. Pups exposed pre- and postnatally were also significantly T4-deficient at PND 14.

Male Sprague-Dawley rats (8–10/group) were administered 0, 1.7, 5.0, or 15.0 mg/L PFOS in drinking water for 91 days (Yu et al. 2009b). At the end of exposure, serum was collected and analyzed for total thyroxine (T4), FT4, total triiodothyronine (T3), and TSH. Liver and thyroid organ weights were obtained as well. Also measured were messenger RNA (mRNA) levels for two isoforms of uridine diphosphoglucuronosyl transferase (UGT1A6 and UGT1A1) and DIO1 in liver; sodium iodide symporter (NIS), TSH receptor (TSHR), and DIO1 in thyroid; and activity of thyroid peroxidase (TPO).

No treatment-related effects were observed on body weight or thyroid absolute and relative weight. Absolute and relative (to body weight) liver weights were increased significantly ($p < 0.05$ or 0.01) in the rats administered 5 and 15 mg/L. Levels of the thyroid hormone activity measured are in Table 3-20 and show that total T4 decreased in a significant dose-dependent manner in the treated rats. Serum FT4 was only decreased at 5 mg/L, total T3 was only increased at 1.7 mg/L, and there was no effect on TSH.

Table 3-20. Thyroid Hormone Levels in PFOS Treated Rats

Dose administered mg/L	Total T3 (µg/L)	Total T4 (µg/L)	Free T4 (pmol/L)	TSH (IU/L)	PFOS (mg/L)
0	0.29 ± 0.04	40.9 ± 1.8	19.0 ± 1.3	0.72 ± 0.30	< LOQ
1.7	0.48* ± 0.08	23.9** ± 1.3	16.7 ± 1.4	0.67 ± 0.27	5.0 ± 0.3
5.0	0.23 ± 0.05	16.4** ± 5.4	12.6* ± 1.5	1.12 ± 0.34	33.6 ± 2.1
15.0	0.23 ± 0.03	8.5**± 1.6	17.3 ± 1.1	1.62 ± 0.67	88.2 ± 4.2

Source: Data from Table 3 in Yu et al. 2009b
Notes: *statistically-significant at $p < 0.05$
** statistically-significant at $p < 0.01$
LOQ = limit of quantification

Hepatic UGT1A6 was not affected with treatment, but hepatic UGT1A1 mRNA expression was upregulated in the rats treated with 5 and 15 mg/L. Exposure to PFOS at ≥ 5 mg/L also lowered DIO1 mRNA in the liver when compared to controls. The DIO1 levels in the thyroid increased in these same treatment groups by 1.8- and 2.9-fold, respectively, compared to controls. PFOS treatment had no effect on NIS, TSHR, or TPO activity.

Six female Wistar rats/dose were administered 0, 0.2, 1.0, or 3.0 mg/kg of PFOS by oral gavage daily for 5 consecutive days (Yu et al. 2011). Groups of six were also administered propylthiouracil at 10 mg/kg or PTU (10 mg/kg) + PFOS (3.0 mg/kg) in the same manner. Serum and bile were evaluated for total T4 (TT4), TT3, transthyretin, and thyroglobulin. Serum TT4 and TT3 both decreased significantly at 1.0 and 3.0 mg/kg for the TT4 (~ 63.7% and 58.9% of controls) and 3.0 mg/kg for the TT3 (~ 62.9% of the control value). The values in bile were not affected and were similar to controls. Serum transthyretin and thyroglobulin were also similar to controls. As stated earlier (section 2.2.1), Yu et al. (2011) found that liver OATp2 was increased significantly (143% compared to controls) in rats at 3.0 mg/kg, indicating that this transporter may be involved in hepatic T4 uptake and could potentially lead to the decrease observed in serum TT3 and TT4. Relative liver weight and absolute and relative thyroid weight were all increased significantly with treatment of PFOS, PTU, and PFOS + PTU. In the thyroid, PTU had the most effect followed by the PFOS/PTU mixture and then the PFOS alone. In the liver, PFOS alone had the most effect.

Ren et al. (2015) examined the comparative agonist and antagonist properties of the PFCs as revealed using a T3 cell proliferation assay in GH2 cancer cells. Antagonist activity was measured using cell proliferation response in the presence of 0.2 nmol T3 and the PFAS. PFOS had the strongest potency as an agonist among the PFAS compounds tested but was still less potent than T3. PFOS also upregulated three thyroid hormone response genes and downregulated another three, one of those being the fatty acid binding protein gene in tadpoles. Molecular docking analysis was used to examine the mode of interaction between the PFOS and the TRα-ligand binding domain protein. PFOS and T3 both hydrogen bonded with Arg-228, with the PFOS sulfonate functional group facing into the pocket and the perfluorinated carbon chain oriented towards the exterior of the pocket.

Kjeldsen and Bonefeld-Jørgensen (2013) conducted an *in vitro* study in an attempt to elucidate the mechanisms by which PFAS, including PFOS, affect the estrogen receptor (ER) and androgen receptor (AR) transactivity, as well as aromatase activity. Estrogenic and antiestrogenic activities were assessed using the stably transfected MVLN cell line carrying an estrogen response element luciferase reporter vector. Androgenic and antiandrogenic activities were assessed using the Chinese hamster ovary cell line CHO-K1 transiently co-transfected with an MMTV-LUC reporter vector and an AR expression plasmid pSVAR0. Effects on aromatase activity were assessed using the human choriocarcinoma JEG-3 cell line. PFOS had no effect on aromatase activity, but it was cytotoxic at $\geq 1 \times 10^{-4}$ M.

In the ER transactivation assay, PFOS was cytotoxic to MVLN cells at concentrations $\geq 6 \times 10^{-5}$ M. The half maximal effective concentration (EC_{50}) for PFOS was 2.9×10^{-5} M compared with 4.8×10^{-11} M for 17β-estradiol (E2). Co-exposure of cells with E2 and PFOS enhanced the E2-induced ER response at the highest non-cytotoxic PFOS concentration. No evidence of antagonism was observed.

In the AR transactivation assay, PFOS was cytotoxic to CHO-K1 cells at concentrations $\geq 1 \times 10^{-4}$ M. PFOS did not act as an agonist, however, it elicited a significant ($p < 0.05$) inhibiting effect (76%) on AR function at a relative high test concentration of 5×10^{-5} M. Co-exposure of cells with dihydrotestosterone and PFOS elicited a significant ($p < 0.05$) concentration-dependent antagonistic effect on DHT-induced AR transactivity; the IC_{50} was 4.7×10^{-6} M.

PPAR activity

Studies have been conducted in order to determine if PFOS activates PPARs. The PPARs are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. These factors can alter gene expression in response to endogenous and exogenous ligands and are associated with lipid metabolism, energy homeostasis, and cell differentiation. The three types, PPARα, β/δ, or γ, are encoded by different genes, expressed in many tissues, and have specific roles during development as well as in the adult (Takacs and Abbott 2007).

In vitro. Shipley et al. (2004) tested PFOS to determine whether it activated human or mouse PPARα in a COS-1 cell-based luciferase reporter *trans*-activation assay. The COS-1 is a fibroblast-like cell line derived from monkey kidney. Concentrations at 8, 16, 32, 64, 125, 250, 500, and 1000 μmol were tested. The COS-1 cells were transfected with either a mouse or human PPARα expression plasmid along with the reporter plasmid, pHD(x3)luc, which has three PPAR binding sites that are linked to a minimal promoter controlling the gene for Firefly luciferase. Cells were also cotransfected with a plasmid encoding *Renilla* luciferase to serve as a control. A positive control, WY-14,643, was also used. In the experiments, PFOS activated both human and mouse PPARα. The highest PFOS-activation was 4- to 6-fold and was similar to that obtained with the positive control. The average EC_{50} was 13 μmol in the mouse and 15 μmol in the human PPARα.

Both PFOS and PFOA were tested to determine whether they could activate PPARs in a transient transfection cell assay (Takacs and Abbott 2007). The Cos-1 cells were cultured in Dulbecco's Minimal Essential Medium (DMEM) with fetal bovine serum in 96-well plates and transfected with mouse or human PPARα, β/δ, or γ reporter plasmids. Transfected cells were then exposed to PFOS (1–250 μmol), positive controls (known agonists and antagonists), or negative controls (DMEM, 0.1% water and 0.1% dimethyl sulfoxide). The positive control agonists and antagonists were WY-14,643 and MK-886, respectively, for PPARα, and troglitazone and GW9662, respectively, for PPARγ. Only the agonist L165,041 was used for PPARβ/δ. After treatment for 24 hours, activity was measured using the Luciferase reporter assay. WY-14,643 was used for mouse and human PPARα, and it exhibited 15- and 1-fold increase, respectively over the luciferase response of the negative controls. L165,041 was the agonist for mouse and human PPARβ/δ. It exhibited 28- and 13-fold increases in the luciferase response, respectively, compared to the negative controls. Finally, troglitazone, the agonist for mouse and human PPARγ, increased the luciferase response 3- and 2-fold over the negative controls, respectively. The antagonists showed appropriate inhibitory responses with maximum inhibition of agonist activity of 90% and 60% for mouse and human PPAR α, respectively, and 47% and 45% for mouse and human PPARγ.

In this study, PFOS activated the mouse PPARα with a significant ($p < 0.01$) 1.5-fold increase in activity at 120 μmol PFOS, compared to the negative control. PFOS did not significantly increase activity in the human PPARα construct. PFOS activated the mouse PPARβ/δ but not the human PPARβ/δ construct. It did not activate the mouse or human PPARγ construct. Table 3-21 shows summary data. The authors concluded that PFOA activated PPARα more than PFOS and that the mouse was more responsive than the human. PFOA and PFOS both activated mouse but not human PPARβ/δ, and neither chemical activated human or mouse PPARγ.

Table 3-21. Summary of PFAS Transactivation of Mouse and Human PPARα, β/δ, and γ

PPAR isoform	PFAS	Mouse LOEC[a]	Human LOEC[a]
α	PFOA PFOS	10 µmol 120 µmol	30 µmol NA[b]
β/δ	PFOA PFOS	40 µmol 20 µmol	NA NA
γ	PFOA PFOS	NA NA	NA NA

Source: Data from Table 1 in Takacs and Abbott 2007
Notes: [a] LOEC = lowest observed effect concentration; lowest concentration (µmol) at which there was a significant difference compared to the negative control ($p < 0.05$)
[c] NA = not activated

Wolf et al. (2008) tested PFAS, including PFOS, to determine whether mouse and human PPARα activity could be induced in transiently transfected COS-1 cell assays. COS-1 cells were transfected with either a mouse or human PPAR-α receptor-luciferase reporter plasmid and after 24-hours were exposed to either negative controls (water or 0.1% DMSO), a positive control (WY-14,643), or PFOS at 1–250 µmol. At the end of 24-hours of exposure, the luciferase activity was measured. The no observed effect concentration (NOEC) for PFOS was 60 µmol in the mouse; the LOEC was 90 µmol (48.4 µg/mL), and the C_{20max} was 94 µmol. The corresponding values for humans were: NOEC = 20 µmol, LOEC = 30 µmol (16.2 µg/mL), and C_{20max} = 262 µmol.

Wolf et al. (2012) incubated transfected cells with PFAS at concentrations of 0.5 to 100 µmol, vehicle (water or 0.1% DMSO as negative control), or with 10 µmol WY-14,643 (positive control). Assays were performed with three identical plates per compound per species, with nine concentrations per plate and eight wells per concentration. Cell viability was assessed using the Cell Titer Blue cell viability kit and read in a fluorometer. The positive and negative controls had the expected results. All PFAS significantly induced human and mouse PPARα. The study also provided the C_{20max}, which was the concentration at which a PFAS produced 20% of the maximal response elicited by the most active PFAS. For PFOS, this was 94 µmol in mouse PPARα and 262 µmol in human PPARα. For comparison, PFOA was 6 µmol and 7 µmol, respectively.

Several studies have suggested that PFOS may target PPARγ and influence metabolism via pathways under its control. L. Zhang et al. (2014) examined the direct binding properties of PFOS and other PFASs using the ligand binding domain of human PPARγ. Interactions between transfected B1.21(DE3) *E. coli* supported derivation of IC_{50} values for the different PFAS examined. The IC_{50} values were derived using a fluorescence displacement method and comparing the results from the tested chemicals with those of decanoic and octanoic acid. The PFAS binding increased with carbon chain length (C4 to C11). The authors also examined the PFAS binding to the PPARγ ligand binding domain. For compounds with fewer than 11 carbons there was a correlation between binding and chain length. The authors interpreted this as an indication that hydrophobic interactions between the amino acids of the binding domain and the PFAS are responsible for the stability of the complex. PFOA and PFOS induced receptor activation to a similar extent, 2.8 and 2.5 times greater than the control, respectively. The authors concluded that PFASs induce disruption of lipid homeostasis and inflammation by the PPARγ pathway as well as the PPARα pathway. Among the three members of the sulfonate family tested (4, 6, and 8 carbons), PFOS displayed the strongest activation potency.

In vivo

Rats. Martin et al. (2007) administered PFOS to male Sprague-Dawley rats by oral gavage at doses of 0 or 10 mg/kg/day for 1, 3, or 5 consecutive days. Clinical chemistry, hematology, histopathology, and gene expression profiling of the livers from three rats/group were performed. Body weight was not affected with treatment, but relative liver weight increased after 5 days of treatment. PFOS exhibited peroxisome proliferator-activated receptor alpha agonist-like effects on genes associated with fatty acid homeostasis. Exposure also caused down-regulation of cholesterol biosynthesis genes. PFOS caused significant DIO1 repression and *Dio3* induction on day 5 of exposure, which corresponded to decreases of T3 only on day 5 and total and free T4 decreases. DIO1 deiodinates thyroxine (T4) to bioactivate T3 and *Dio3* catalyzes the inactivation of T3. PFOS was poorly correlated with peroxisome proliferators in the global gene expression patterns and indicated weak matches with hepatotoxicity related signatures and weak correlation to PPARα agonist treatment. Expression of HMG-CoA reductase was significantly upregulated, and cholesterol biosynthesis was downregulated in a manner suggesting a mechanism distinct from the statins. The authors suggested a link between PFOS, PPAR, and thyroid hormone homeostasis based on work by Miller et al. (2001) who observed decreased serum T4 and T3 levels and increased hepatic proliferation following exposure to peroxisome proliferators. They also noted that PFOS exhibited similarities to compounds that induce xenobiotic metabolizing enzymes through PPARγ and constitutive androstane receptor (CAR).

Wang et al. (2010) dosed albino Wistar female rats with 3.2 mg PFOS/kg diet from GD 1 to weaning (PND 21). Pups were allowed access to the treated feed until PND 35. To determine if prenatal or lactational exposure had more effect on altering gene expression, pups were divided into cross fostering groups on PND 2. These groups are listed below:

- Pups born to treated dams fostered by control dams.
- Pups born to control dams fostered by treated dams.
- Pups born to control dams fostered by other control dams.
- Pups born to treated dams fostered by other treated dams.

Gene expression changes were examined on PNDs 1, 7, and 35. Significant effects were observed on genes involved in neuroactive ligand-receptor interaction, calcium signaling pathways, cell communication, the cell cycle, and peroxisome proliferator-activated receptor (PPAR) signaling. Transthyretin (TTR) which is a serum and cerebrospinal fluid carrier of thyroxine (T4) was decreased after PND 1. Based on analysis of PFOS in the serum, the half-life of PFOS in the neonates was approximately 14 days, and overall, prenatal exposure altered gene expression more than lactational exposure.

In a 4-week study in rats, the hepatic effects of PFOS, WY-14,648 and phenobarbital (PB) were compared (Elcombe et al. 2012). Groups of 30 male Sprague-Dawley rats were administered either 20 ppm PFOS, 100 ppm PFOS, 50 ppm WY-14,648, or 500 ppm PB in the diet *ad libitum* for either 1, 7, or 28 days. Control animals received only diet *ad libitum* for the duration of the study. Ten animals from each group were sacrificed on days 2, 8, and 29 for evaluation of liver weights, peroxisome proliferation, enzyme induction, cell proliferation, apoptosis, and other clinical and pathological parameters. The study showed that PFOS exhibits the combined effects of WY-14,643 and PB, behaving as a combined peroxisome proliferator and *phenobarbital-like* enzyme inducer. The data suggested that PFOS may activate not only PPARα, but also CAR and PXR.

Mice. To assess PPAR involvement in developmental effects of PFOS, male and female 129S1/Svlm wild-type and PPARα knockout (KO) mice were bred and pregnancy confirmed (Abbott et al. 2009). The females (n = 8–20 dams/group) were administered either vehicle (0.5% Tween-20) or PFOS by gavage on GDs 15–18; the wild-type mice were administered 4.5, 6.5, 8.5, or 10.5 mg/kg/day PFOS and the KO mice, 8.5 or 10.5 mg/kg/day. Dams and pups were observed daily, and pups were weighed on PNDs 1 and 15. Eye opening was recorded on PNDs 12–15. Dams and pups were killed on PND 15, and body and liver weights were recorded and serum collected.

Reproductive parameters measured included maternal body weight, maternal body weight gain, implantation sites, total number of pups at birth, and the percent litter loss from implantation to birth. Pup body weight and pup body weight gain were not affected with treatment in either the KO or wild-type mice. PFOS exposure had no effect on absolute or relative liver weight in any of the dams. In both strains of pups, PFOS exposure at 10.5 mg/kg/day caused a significant increase in relative liver weight (sexes were combined). Survival of the pups was affected with treatment. Most post-natal deaths occurred between PNDs 1 and 2. Survival of the wild-type pups was significantly ($p < 0.001$) decreased and was 65% ± 10 (n = 16), 45% ± 14 (n = 8), 55% ± 6 (n = 7), 43% ± 9 (n = 20), and 26% ± 9 (n = 17) in the control, 4.5, 6.5, 8.5, and 10.5 mg/kg/day groups, respectively. Survival of the KO pups was significantly ($p < 0.001$) decreased and was 84% ± 9 (n = 12), 56% ± 12 (n = 13), and 62% ± 8 (n = 14) in the control, 8.5, and 10.5 mg/kg/day groups, respectively.

Post-natal development was also affected in the wild-type and KO pups. On PND 13, 44% of the control pups and none in the 8.5 mg/kg/day wild-type group had experienced their eye opening. In the KO mice, open eyes were reported in 23% of the 10.5 mg/kg pups and 59% of the controls on PND 14. All serum samples (pups and adults) showed a linear relationship between the amount of PFOS administered and the amount found in the serum, with levels in treated groups being significantly increased compared to the controls. As the results from the wild-type and KO pups were similar, the author concluded that PFOS-induced neonatal lethality and delayed eye opening were not dependent on the PPARα activation.

In another mechanistic developmental study, a PFOS solution with 0.5% Tween-20 was administered to timed-pregnant CD-1 mice by oral gavage at 0, 5, or 10 mg/kg/day for GD 1–17 (Rosen et al. 2009). Five dams per group were euthanized at *term*, and three fetuses per litter were collected for preparation of total RNA from liver and lung. Additional liver and lungs were collected from two more fetuses/litter for histological examination.

Treatment with PFOS had no effect on body weight, general appearance, or litter size. Hematoxylin and eosin stained sections from treated and control fetal livers showed eosinophilic granules characteristic of peroxisome proliferation in the PFOS treated dose groups. At 5 mg/kg/day, 753 fully annotated genes were altered in the fetal liver. In the fetal liver, PFOS upregulated a number of markers for fatty acid metabolism, xenobiotic metabolism, peroxisome biogenesis, cholesterol biosynthesis, bile acid biosynthesis, and glucose and glycogen metabolism. In the fetal lungs, up-regulation only occurred in a limited group of genes including: Cyp4a14, enoyl-Coenzyme A hydratase (Ehhadh), and fatty acid binding protein 1 (FABP1).

Qazi et al. (2009a) tested the effects of 0, 0.005%, or 0.02% PFOS on wild-type and PPARα-null 129/Sv mice. Dietary administration of 0.02% PFOS for 10 days resulted in a significant increase in liver weight and a reduction in the weight of the spleen in both the wild-type and null mice; the thymus and epididymal fat pad weights were both decreased in the wild-type mice

only. The wild-type mice administered 0.02% PFOS in the diet had a pronounced decrease in the total number of thymocytes (by 84%) and splenocytes (by 43%), as well as a decrease in the size of all subpopulations of thymocytes and splenocytes. In the knock-out mice, the reduction in the total number of thymocytes and subpopulations was partially or almost totally attenuated; effects on splenocytes were mostly eliminated. There were no effects in the wild-type or knock-out mice administered 0.005%. The study indicated that the immunomodulation was partially dependent on PPARα activation.

Changes in gene expression were examined in wild-type and PPARα-null mice administered PFOS by gavage at 0, 3, or 10 mg/kg/day for 7 days (Rosen et al. 2010). At sacrifice, liver tissues were processed for histopathology and total RNA; microarray analysis was conducted using Affymetrix GeneChip 430_2 mouse genome arrays. Liver weight was increased at 10 mg/kg/day in both wild-type and null mice. Overall gene expression showed dose-related changes in wild-type mice, while the number of transcripts influenced by PFOS in null mice was similar across the dose groups. This finding suggests that there are PPARα-independent effects in null mice that also occur in wild-type mice. Thus, some of the liver effects in the wild-type animals are not necessarily a reflection of PPARα activation.

In wild-type mice, PFOS altered the expression of PPARα-regulated genes including those involved in lipid metabolism, peroxisome biogenesis, proteasome activation, and inflammatory response. Altered PPARα-independent genes included those associated with xenobiotic metabolism in both wild-type and null mice. PFOS caused induction of a constitutive androstane receptor (CAR) inducible gene, *Cyp2b10*, indicating the likelihood that PFOS also activates CAR. In null mice, changes induced by PFOS included up-regulation of genes in the cholesterol biosynthesis pathway and modest down-regulation of genes associated with oxidative phosphorylation and ribosome biogenesis (Figure 3-1). Unique in null mice, PFOS upregulated *Cyp7a1*, an important gene related to bile acid/cholesterol homeostasis. The results support those from other studies that indicate PFOS exposure results in metabolic changes both linked to, and independent of, PPAR-α.

The variability in the serum lipid profiles in humans suggests that response to PFOS exposure could be impacted by individual physiological differences and that environmental factors such as diet could contribute to intraspecies differences in response. L. Wang et al. (2014) reported on the differences in response of male BALB/c mice (4–5 weeks old) administered PFOS (0, 5, or 20 mg/kg) for 14 days while concurrently given diets that varied in dietary fat [regular fat (RF) versus high fat (HF)] content. Following PFOS exposure, there was an increase in liver fat content in both groups and a decrease in liver glycogen. However, the increase in fat content was more pronounced with the RF mice than in the mice on the HF diet. This study is described in more detail in section 3.2.2.

The fat content of the diet alone was associated with significantly higher serum levels of glucose, HDL cholesterol, LDL cholesterol, and total cholesterol. For glucose, cholesterol, HDL, and LDL the levels declined as the dose of PFOS increased. In the case of triglycerides, the levels increased with 5 mg/kg/day PFOS and decreased at 20 mg/kg/day. PPARα expression at the end of the 14-day PFOS treatment increased for the RF group but decreased for the HF groups (significant for the high dose).

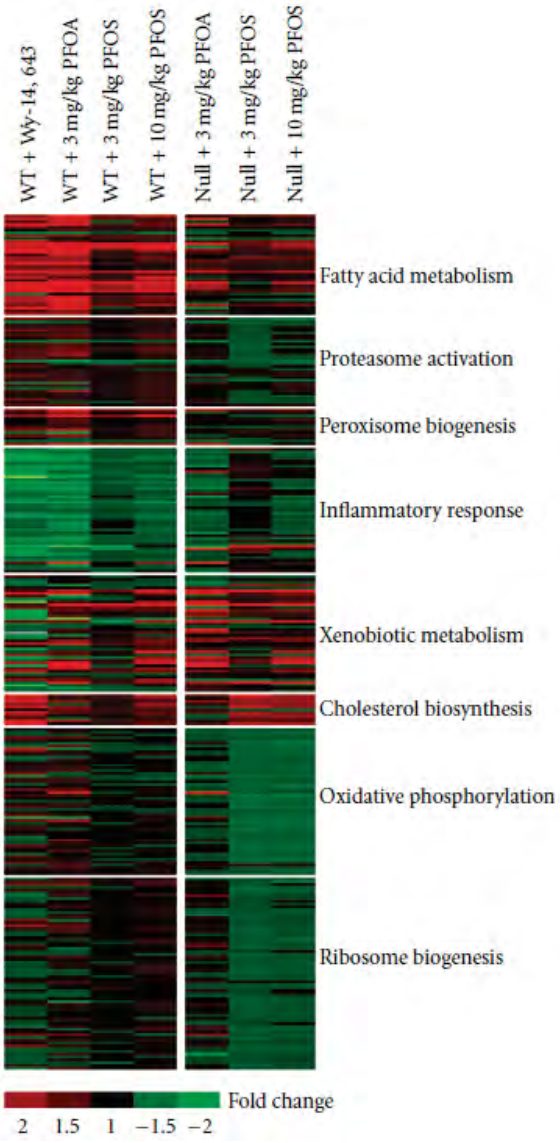


Figure 3-1. Functional Categories of Genes Modified by PFOS in Wild-Type and Null Mice

The high fat diet alone increased the expression of CPT1A and CYP7A1 genes involved with lipid metabolism. On the RF diet, the exposure to PFOS was associated with a significant dose-related increase in CTP1A expression, whereas for the high fat diet plus PFOS there was a significant decrease in expression. For CYP7A1 expression there was no significant impact of PFOS with the RF diet, whereas with the high fat diet there was a highly significant decrease in expression with PFOS.

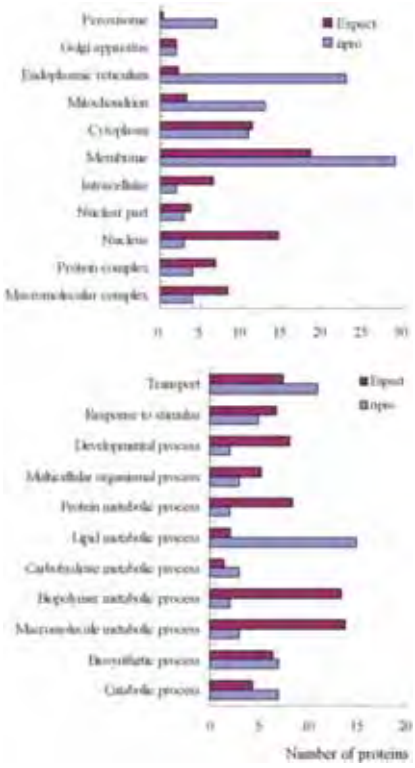
The L. Wang et al. (2014) study demonstrates a clear influence of diet alone on the liver and lipid profile that was combined with some dose-related differences in the responses to PFOS exposure. The data support a possible role for PFOS in inhibiting pathways for metabolism and export of liver lipids and identify some PFOS associated liver responses that are independent of PPARα activation.

Tan et al. (2012) conducted a dose-response study of hepatic proteonomic responses following exposure of male Kunming mice (5/dose group) to PFOS at levels of 0.1, 1.5, or 5 mg/kg/day by interperitoneal (ip) injection for 7 days. Twenty-four hours after the last dose,

the animals were sacrificed and the livers harvested, weighed, and preserved in liquid nitrogen. Body weight was recorded at study initiation and immediately before sacrifice.

Liver tissues were pooled for each dose group and homogenized for proteonomic analysis. The liver proteins were extracted and grouped using iTRAQ labeling guidelines, digested with trypsin, and labeled with iTRAQ reagent. The iTRAQ proteonomic analysis is a novel, MS-based approach for the relative quantification of proteins. It relies on the derivatization of primary amino groups in intact proteins using isobaric tags for relative and absolute quantitation (Wiese et al. 2007). The tryptic peptides were separated using reverse phase liquid chromatography, identified following LC/MS/MS analysis, and correlated to intact proteins based on peptide structures.

Treatment with PFOS caused a slight deficit in body weight for the high dose group and a significant dose-related increase in liver weight for the two highest dose groups. The iTRAQ process identified 1,502 unique proteins; 71 showed a greater than 1.5-fold change in expression. Sixty-two proteins showed increased expression, and nine showed decreased expression. Figure 3-2 illustrates the impact of the PFOS exposure on identified proteins as associated with subcompartments within the liver cells compared to the proteins in the reference data base. Enrichment was greatest for peroxisomes and endoplasmic reticulum, mitochondrial, and cell membrane proteins. Relative to biochemical processes, Figure 3-2 shows that the majority of enriched proteins were involved with lipid metabolism, transport, biosynthetic processes, catabolic processes, and carbohydrate metabolic processes.



Top: cellular component; Bottom: biological process
npro: the number of proteins belongs to one category in the proteome database
Expect: the number of proteins having an ontology annotation in the reference database.

Figure 3-2. Function Distribution and Category Enrichment Analysis of the Differential Proteins

Sixteen proteins were identified that showed dose-response for the increase in expression. Four of these were related to peroxisomal beta-oxidation, four were related to CYP-450 aromatase activity, and three had transferase activity including GSTmu3 and GSTmu6. A GTP binding protein (GTP sar-1b) also displayed a dose-related response. One of the remaining four proteins exhibiting dose-response, cysteine sulfinic acid decarboxylase, is the rate limiting enzyme in taurine production and has been proposed as a biomarker for hepatocarcinogenesis.

In the developmental study by Butenhoff et al. (2009), mRNA transcript data for the control and 1.0 mg/kg/day dose group (GD 20 dams and fetuses and PND 21 male pups) was obtained by quantitative reverse transcription polymerase chain reaction. Results for this part of the study were reported by Chang et al. (2009). Statistically-significant changes included:

- Increased Cyp2b2 levels in dams and male pups (2.8-fold and 1.8-fold, respectively) than in controls on GD 20 and PND 21.
- Higher mean acyl CoA (ACoA) and Cyp4a1 levels in male pups (1.5-fold and 2.1-fold, respectively) than those of controls.
- Lower mean Cyp7a1 (3.5-fold) than that for controls.

These results suggest induction of PPARα as well as hepatic CAR. Transcripts possibly related to thyroid status were all similar between the treated dams and pups and the controls.

Oxidative Damage

Liu et al. (2009) conducted a study of KM mice in which 3–6 pups/sex/group were administered one subcutaneous injection of 0 or 50 mg PFOS/kg bw on PNDs 7, 14, 21, 28, or 35. The study was done in an attempt to determine the effects of treatment on the oxidation-antioxidation system by measuring MDA content, SOD activity, and total antioxidation capability (T-AOC). Animals were sacrificed 24 hours post-treatment, blood was collected, and liver and brain were removed and weighed.

No treatment-related effects were observed on body weight. Relative liver weight was significantly increased ($p < 0.01$ or $p < 0.05$) when compared to controls in both males and females at most time-points. The levels of MDA in the brain and liver and SOD activity were similar between treated mice and the controls at most time-points. On PNDs 7 and 21 in the treated males, brain SOD activity was significantly lower when compared to controls by 19% and 13%, respectively. Liver SOD activity was lower (decrease of 19%) in the treated females on PND 14 when compared to controls. Male brain T-AOC was decreased at all stages of post-natal development but only significantly at PND 21 (decrease of 15%). Male liver T-AOC was decreased significantly at PND 7 (decrease of 25%) and 14 (decrease of 27%). Female brain T-AOC had no significant differences from controls and the liver T-AOC was decreased only at PND 21 (decrease of 15%). The study also demonstrated that distribution increased in the liver and lessened in the blood and brain with postnatal development in both the males and females. On PND 7, PFOS concentrations were 11.78%, 5.04%, and 14.84% in the male mouse blood, brain, and liver, respectively. On PND 28, the PFOS concentrations were 9.89%, 0.85%, and 63.39% in the male mouse blood, brain, and liver, respectively. PFOS brain levels were about 5-fold higher on PND 7 than they were on PND 28. A similar trend was observed in the females. The study suggested that oxidative damage from PFOS can occur, is more prominent in the younger neonates, and is slightly more pronounced in the males.

Gap Junctional Intercellular Communication (GJIC)

Gap junctions are found in the cell's plasma membrane and formed by proteins that connect to form an intercellular connection that allows a direct exchange of chemicals from the interior of one cell to that of adjacent cells without passage into the extracellular space. The GJIC is considered to be essential in maintaining healthy cells and thus disruptions are thought to cause abnormal cell growth, including tumor formation. They also appear to be linked to some neurological, reproductive or endocrine abnormalities.

Hu et al. (2002) tested PFOS exposure *in vitro* and *in vivo* to determine whether disruption to the GJIC could possibly be a mechanism for the effects observed with PFOS exposure. The study exposed a rat epithelial cell line (WB-F344) and a dolphin kidney epithelial cell line (CDK) to PFOS at concentrations of 0, 3.1, 6.25, 12.5, 50, 100, or 160 μmol for 30 minutes. GJIC effects were measured using the scrape loading dye technique. PFOS inhibited GJIC rapidly in a dose-dependent method starting at 12.5 μmol, but it was reversible once exposure ended. Additionally, 4 to 6 Sprague-Dawley rats/sex/group were exposed to 0 or 5 mg/kg PFOS by gavage for either 3 or 21 days. GJIC was significantly reduced in the liver tissue after 3 days of exposure. Inhibition also occurred in rats exposed for 21 days, but it was comparable to that seen after 3 days. No differences were observed between the male and female rats.

Wan et al. (2014a) isolated and cultured Sertoli cells from testes of 20-day old rats to examine PFOS's effects on blood testes barrier function. By day 3 the cultures had established a functional tight junction barrier. Gap junction communication was assayed by means of fluorescence recovery using a photo bleaching assay that measured the ability of a fluorescent dye to move from one cell to another in the presence or absence of PFOS (20 μmol; a 3-hour exposure) in a 120 second period. Cells treated with PFOS displayed significantly lower fluorescence recovery than the control cells in the absence of cytotoxicity. The assays were performed in triplicate. The authors identified this as a matter of concern because it represents diminished function of the blood testes barrier in coordinating an intercellular junction necessary for intercellular communication during spermatogenesis. The authors also examined other characteristics of the blood testes barrier finding effects of PFOS on the cytoskeleton manifest in the form of shortened F-actin filaments.

Mitochondrial Function

Starkov and Wallace (2002) isolated mitochondria from the livers of adult male Sprague-Dawley rats and used them to measure mitochondrial membrane potential and oxygen consumption when exposed to PFOS. PFOS appeared to be a weak mitochondrial toxicant. At higher concentrations, PFOS caused a small increase in resting respiration rate and slightly decreased the membrane potential. The observed effects were attributed to a slight increase in nonselective permeability of the mitochondria membranes caused by the surface-active property of the compound.

Wallace et al. (2013) also examined the impact of 16 different PFASs on mitochondrial respiration rate and oxidative phosphorylation as measured *in vitro* using isolated rat liver mitochondria. Inhibition was determined through the reduction in oxygen consumption in response to the addition of ADP to isolated mitochondria. PFOS displayed a 20–30 times more potent inhibitory effect among the other sulfonates evaluated (PFBS and PFHxS). PFOS was three times more potent than PFOA. The inhibition mode of action seemed to vary across different PFAS families. In the case of PFOS, its impact on membrane fluidity appeared to be

responsible for the observed respiratory inhibition. The authors' proposed mode of action for this effect from PFOS is consistent with the findings of Matyszewska et al. (2008) that PFOS increased the membrane fluidity and thickness of a model biological membrane in a manner similar to that resulting from cholesterol insertion into a lipid by-layer.

3.3.5 Structure-Activity Relationship

In vitro. Bjork and Wallace (2009) performed a study to see whether the PPARα agonism was relevant in human cell lines and whether effects differed with various chain lengths. Primary rat and human hepatocytes and HepG2/C3A hepatoma cells were exposed to 25 µmol PFAS for 24 hours to determine the structure-activity relationships across various chain lengths. The concentration used was the maximum concentration that did not lead to cell injury in any of the cell lines. The PFAS tested included perfluorinated carboxylic acids with carbon chain lengths of 2 to 8 and perfluorinated sulfonic acids with chain lengths of 4 to 8.

The PFAS stimulated mRNA expression of either acyl CoA oxidase (Acox) or acyl CoA thioesterase (Cte-rats or Acot 1-humans) only in rat hepatocytes and within both series and transcripts; the degree of stimulation of gene expression increased with increasing carbon number. Maximum stimulation of Acox gene expression was 3-fold over control for PFOS; maximum stimulation for Cte/Acot 1 gene expression was 4-fold for PFOS. PFOS did not cause any significant stimulation of Acox or Cte/Acot 1 gene expression in human hepatocytes. The Cyp4a11 gene was not expressed or stimulated by any of the PFASs in HepG2/C3A cells. However, this gene expression was stimulated by PFAS exposure in both rat and human hepatocytes with the perfluorocarboxylates indicating a chain-length dependent structure activity relationship. Maximum gene expression stimulation was in the longer carbon PFAS, but the variability was large with little statistical difference between the 6 and 8 carbon molecules. Study results suggested that the PPARα related gene expression by PFAS was induced in primary rat hepatocytes, increased with carbon chain length, and appeared to be greater in the carboxylic acids (such as PFOA) when compared to the sulfonates (such as PFOS). There was no induction of peroxisome-related fatty acid oxidation gene expression (Acox and Cte/Acot 1) in either primary or transformed human liver cells in culture. This suggests that the PPARα mediated peroxisome proliferation observed in rodent liver may not be relevant as an indicator of human risk.

3.3.6 ToxCast Assays

The Toxicity Forecaster (ToxCast) database is a large high throughput screening compilation of public *in vitro* and *in vivo* assays on over 9,000 chemicals. PFOS was tested in 1,087 assays and was active in 175. Assay activation defines the occurrence of the molecular event within the assay (cytotoxicity, induction, binding, and so forth.) with the concentration resulting in 50% activity, AC_{50}, used for comparison to other assays. Assays with < 50% reported efficacy or over-fitting issues are not included in the results discussed. Some of the data from the ToxCast assays such as the interactions with PPAR and CAR support the experimental data for PFOS and PFOA. In cases where effects were only observed at concentrations greater than those causing cytotoxicity, attributing the outcome to PFOS rather than the cytotoxicity is less certain.

Cytotoxicity. Of the active assays, 20 were examining endpoints based on cytotoxicity. Most cell types offered at least one cytotoxicity AC_{50} for comparison to other *in vitro* assays. If no cytotoxicity AC_{50} was reported for a specific cell type, the minimum *in vitro* cytotoxicity

endpoint for PFOS was used for comparison. The lowest PFOS induced AC_{50} recorded in the ToxCast database is 5.34 μM in the assay for induction of tumor protein 53 using liver cells and the highest AC_{50} recorded is 172.02 μM for measuring tumor protein in intestinal cells (SD = 45.15; standard error = 9.9).

Endocrine Disruption. Four different estrogen receptor (ESR) assays reported activation following PFOS treatment, all of which were Estrogen Receptor 1- (ESR1-) related. Estrogen and its receptors are essential for sexual development and reproductive function, but they also play a role in other tissues, such as bone. Estrogen receptors are also involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis (NCBI 2016). Two assays of the same cell type were related to ESR1 induction with the lowest AC_{50}, 18.06 μM. This is lower than the cytotoxicity AC_{50} reported for the cell type used, 23.76 μM, and is indicative of ESR1 induction. In a different ESR1 assay, antagonism was recorded at an AC50 of 83.57 μM, a value higher than the cytotoxicity AC50 for that cell type, 66.31 μM. PFOS induced the estrogen DNA binding site with an AC_{50} of 87.42 μM. There was no cell-specific reference cytotoxicity value for comparison. The ToxCast assays suggest that PFOS has the ability to induce ESR1.

PFOS antagonized the androgen receptor (AR) at 12.57 μM in human cells and 4.27 μM in rats. Although there is no direct cellular cytotoxicity value to compare, PFOS rat AR antagonism occurred at lower concentrations than the minimum cytotoxicity value (5.34 μM). This implies that PFOS reacts with the AR receptor in the rat and perhaps the human. The progesterone receptor (PR) was also antagonized within the same human cell type, and had a higher AC_{50}, 22.2 μM, than the minimum cytotoxicity AC_{50}. Thyroid receptor (TR) antagonism AC_{50}, 91.24 μM, was also higher than its respective cell specific cytotoxicity AC_{50} (33.323 μM). This signifies that assay activation (i.e., positive results) might have occurred due to cytotoxicity rather than PR, TR, or human AR antagonism. However, PFOS-induced ESR1 and antagonized rat AR was observed.

Immunotoxicity. PFOS activated a variety of genes related to immunotoxicity in the ToxCast database. These genes include: chemokine ligand (CXCL) 10, CXCL8, collagen type II alpha (COL3A), interleukin-1 alpha (IL-1α), plasminogen activator (PLA), plasminogen activator urokinase (PLAUR), vascular cell adhesion molecule (VCAM1), and the TNF receptor subfamily gene CD40 (CD40). All of the immunological assays were performed by the vendor BioSeek. The vendor did not have a cytotoxicity AC_{50} for every cell type utilized and used only two cytotoxicity AC_{50} values for comparison. Genes that had lower AC_{50} values than cell or BioSeek specific cytotoxicity AC_{50} were: CD40, PLAUR, PLA, VCAM1, and CXCL8. Given the limited cytotoxicity reference values it is difficult to determine if all gene activity can be attributed to PFOS. For PLAUR and VCAM1, AC_{50} results were lower than their cell specific cytotoxicity AC_{50} and have stronger translational potential. VCAM1 and PLAUR play a role in inducing chronic inflammation and vascularization *in vivo* (Kleinstreuer et al. 2014). This implies PFOS may play a role in inducing chronic inflammation and/or vascularization, both of which are important for the development of rheumatoid arthritis (Khansari et al. 2009).

Neurotoxicity (*in vitro*). PFOS activated five different neurological receptor families with seven different receptor types in cell-based assays. The receptors activated were: 5-hydroxytryptamine receptor (5HT) 5a, 6, and 7, the adenosine A2a receptor (ADORA2), the adrenoceptor alpha 2C (ADRA2C), and beta 1 (ADRB1), as well as the dopamine receptor D4 (DRD4). Cell-specific cytotoxicity AC_{50} values for reference were lacking for all of the *in vitro* assays; only ADORA2 had an activation AC_{50} lower than the lowest PFOS cytotoxicity AC_{50} of 5.34 μM. Therefore, it

is difficult to draw any conclusions on the potential neurotoxicity of PFOS using the ToxCast data.

Fish Toxicity *(in vivo*). Oregon State University conducted a large number of toxicity studies using a zebrafish model. PFOS gave positive results in nine assays. Positive effects were recorded for limb malformations 5 days after a 1-time exposure during embryonic development. Other assays with positive results were those for Axis Malformation, Jaw Malformation, Pericaradiac Edema, Snout Malformation, Touch Response, Trunk Malformation, Yolk Sac Edema, and Mortality. Mortality had the lowest reported AC_{50} at 0.54 μmol. The results provide strong evidence for PFOS developmental toxicity in fish and suggest a potential for human developmental human toxicity.

PPAR/PXR/RAR Receptors. PFOS activated PPARs, PXR, constitutive adrostane receptor (CAR), and retinoic acid receptor (RAR) in assays conducted under the ToxCast program. PFOS induced the DNA sequences for PPAR alpha (PPARα), peroxisome proliferator hormone response elements (PPRE), and PPAR gamma (PPARγ) and antagonized the PPARγ receptor. The only PPAR assay AC_{50} that was above the cell-specific cytotoxicity AC_{50} was PPARγ antagonism at 5.91 μM. However, it is possible that cytotoxicity occurs due to PPAR induction or that the PPAR antagonism contributes to cytotoxicity. PFOS induced DNA sequences for PXR, AC_{50} 9.42 μM, at concentrations lower than the cell-specific cytotoxicity AC_{50}. CAR and RAR alpha antagonism were also observed but not at levels below the cell specific cytotoxicity values of 17.57 μM and 28.45 μM respectively. PPAR, PXR, CAR, and RAR pathways are all nuclear receptors that can form heterodimers with one another to induce translation of linked genes. Some of these genes are important for development, reproduction, waste degradation, and even induction of cytotoxicity. Therefore, PFOS induction of these assays are consistent with the experimental data on PPAR and CAR receptor activation.

Cytochrome P450s Activation. Cytochrome P450 (CYP) enzyme bindings were also examined within the ToxCast database in order to understand any metabolic potential for a chemical. Though PFOS is not known to be metabolically active, it showed activation in four acceptable CYP assays: CYP2C18, CYP2C19, and CYP2C9 in human cells, and CYP2C11 in rat. All of the CYP assays had activation at concentrations lower than the lowest cytotoxic AC_{50}. The CYP2C class is known to be involved in the metabolism of xenobiotics including drugs, such as the anti-seizure medication diazepam, the beta blocker propranolol, and the selective serotonergic reuptake inhibitor citalopram. Though there is no evidence of metabolism of PFOS by these CYPs, it is possibly acting as a competitive or allosteric inhibitor for other substrates. This, coupled with PFOS's high affinity for albumin, could significantly alter the pharmacokinetics of various necessary and habitual pharmaceuticals.

3.4 Hazard Characterization

3.4.1 Synthesis and Evaluation of Major Noncancer Effects

3.4.1.1 Liver Effects, Cholesterol, and Uric Acid

Good correlation between serum and hepatic levels of PFOS has been shown for human samples (Kärrman et al. 2010; Olsen et al. 2003a). However, no consistent adverse effects on the liver were found in epidemiology studies. Biomonitoring studies performed at the 3M Decatur, Alabama plant (Olsen et al. 2001a, 2001b, 2003b) identified occasional differences in hepatic

clinical chemistry values but no associated hepatic disease and or hepatic carcinogenicity was reported.

Multiple epidemiologic studies have evaluated serum lipid status in association with PFOS concentration. These studies provide support for an association between PFOS and small increases in total cholesterol. Hypercholesterolemia, which is clinically defined as cholesterol greater than 240 mg/dL, was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009). PFOS levels in these studies were 0.0084 μg/mL and 0.022 μg/mL, respectively. Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003b), with much higher PFOS serum levels of up to 1.40 μg/mL. Evidence for associations between other serum lipids and PFOS is mixed, with some studies showing an association with measurements of concurrent HDL and/or LDL and others failing to measure the serum lipoprotien complexes. The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults, but some studies exist on children and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the UK, to Scandanivian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design.

There are several characteristics of HDLs that explain the association of increased serum concentration of HDL with PFOS or PFOA levels. HDLs accept cholesterol from other serum lipoprotein complexes and bring it to the liver for degradation and conversion to bile salts (Montgomery et al. 1990). Competition between PFOS and bile salts for biliary transport could result in impeded removal of HDL lipids from serum and increase both HDL cholesterol and total cholesterol. The liver is the only tissue that can rid the body of excess cholesterol by secreting it in bile for removal with the feces (Montgomery et al. 1990). In addition, HDLs have the highest ratio of protein to lipid (50:50) among the serum lipoprotein complexes (Montgomery et al. 1990). Binding of PFOS to HDL protein could impede the HDL interaction with liver tissue receptors resulting in increased serum levels of HDL. LDLs contain 21% protein. LDL uptake by tissues is mediated by binding of the LDL apo-B-100 protein and by a receptor independent route (Montgomery et al. 1990). Thus, conformational changes in the lipoprotein proteins as a result of PFOS binding can also impact serum LDL levels.

PFOS, when absorbed, is primarily found in the liver tissue. In monkeys, rats, and mice, PFOS levels in the liver showed a dose-dependent increase that was consistently greater than serum levels (Curran et al. 2008; Goldenthal et al. 1978a; Liu et al. 2009; Seacat et al. 2002; Thomford 2002/Butenhoff et al. 2012). Chang et al. (2009) identified PFOS levels in the liver of rat offspring as early as GD 20, and Stein et al. (2012) measured PFOS in human amniotic fluid supporting placental transfer.

In experimental studies, increased absolute liver weight was observed in monkeys exposed to 0.75 mg/kg/day for 182 days (Seacat et al. 2002), in rats at ≥ 1.33 mg/kg/day for 14 weeks (Curran et al. 2008; Seacat et al. 2003), and in rats at ≥ 0.77 mg/kg/day for 53 weeks (Thomford 2002/Butenhoff et al. 2012). Microscopic lesions of the liver were observed in rats and monkeys. Lesions were found in rats at 1.33–1.56 mg/kg/day after 14 weeks (Seacat et al. 2003), in rats at 0.098–0.299 mg/kg/day after 104 weeks (Thomford 2002/Butenhoff et al. 2012), and in monkeys at 0.75 mg/kg/day after 53 weeks (Seacat et al. 2002). Liver lesions were similar in both species and included centrilobular hypertrophy and vacuolation after the subchronic and chronic exposures with eosinophilic granules also observed after chronic duration. Single cell necrosis

was also found in rats at 0.984–1.251 mg/kg/day after 104 weeks (Thomford 2002/Butenhoff et al. 2012; Table 3-15). In these studies, no evidence of peroxisome proliferation was found in either species.

Hepatomegaly and increased liver weight alone are not considered adverse in cases where a chemical such as PFOA causes stimulation of PPAR-α, CAR, and/or PXR cellular receptors (Hall et al. 2012). However, when accompanied by necrosis (Thomford 2002/Butenhoff et al. 2012) and/or fatty acid steatosis (Bijland et al. 2011; Wan et al. 2012), liver weight increases are considered adverse.

In contrast with humans, rats, mice, and monkeys displayed a decrease in cholesterol levels and high density lipoprotein cholesterol following PFOS administration in short and long term studies (Curran et al. 2008; Seacat et al. 2003; L. Wang et al. 2014) when compared to the controls. Male rats had decreased serum cholesterol after 14 weeks at a dose of about 1.4 mg/kg/day (Curran et al. 2008). Wan et al. (2012) found evidence for hepatic macrovesicular steatosis in mice given ≥ 5 mg/kg/day that was not entirely due to PPARα activation. Steatosis was exacerbated when PFOS exposure was combined with a high fat diet.

As discussed above in section 3.3.4, mice administered PFOS showed differential expression of genes or proteins mainly involved in lipid metabolism, transport, biosynthetic processes, and response to stimulus (Tan et al. 2012; L. Wang et al. 2014) and in genes involved in cholesterol biosynthesis and xenobiotic metabolism (Rosen et al. 2010). More specific investigations into the genes involved in lipoprotein metabolism were conducted by Bijland et al. (2011) as described below. In addition, the nuclear hormone receptors CAR and PXR have been shown to be activated in mice (Bijland et al. 2011; Rosen et al. 2010) and rats (Elcombe et al. 2012). Taken together, these studies consistently show an effect on expression of genes involved in lipid metabolism and cholesterol transport and biosynthesis following *in vivo* PFOS exposure.

To further examine PFOS-specific effects on lipid metabolism, Bijland et al. (2011) examined the molecular biology of hepatic hyperlipidemia in APOE*3-Leiden.CETP mice, a strain that exhibits human-like lipoprotein metabolism. Details of the experimental procedure were given in section 3.2.2. Animals fed 3 mg/kg/day for 4 weeks had decreased hepatic VLDL production leading to increased retention of triglycerides and hepatomegaly, with concomitant decreased hepatic clearance of VLDL and HDL cholesterol. Fecal bile acid content was decreased by 50%.

Overall the genes upregulated were those involved with fatty acid uptake, transport, and catabolism; triglyceride synthesis; cholesterol ester synthesis; and VLDL synthesis and secretion. Genes involved with HDL synthesis, maturation, clearance, and bile acid formation were downregulated. Lipoprotein lipase activity and mRNA expression, both normally low in the liver, were increased.

Many of the genes activated are associated with the nuclear PXR receptor to a greater extent than PPARα. Lipoprotein lipase activity facilitates removal of TGs from serum LDLs, and uptake into the liver and other organs as free fatty acids and glycerol.

No animal studies were identified that examined serum uric acid levels following PFOS exposures.

3.4.1.2 Developmental/Reproductive Toxicity

PFOS has been detected in amniotic fluid samples indicating that the chemical crosses the placenta. The median ratio of maternal serum:amniotic fluid concentration was 25.5:1, and PFOS was rarely detected in amniotic fluid until the serum concentration reached at least 0.0055 μg/mL (Stein et al. 2012). Studies evaluating the reproductive and developmental health in humans exposed to PFOS have been performed in both occupational settings and in the general population.

Although not entirely consistent, the set of studies evaluating fetal growth retardation suggest an association of prenatal serum PFOS with deficits in mean birth weight and with LBW. Although three studies were null (Fei et al. 2008a; Hamm et al. 2010; Monroy et al. 2008), birth weight deficits ranging from 29 to 149 grams were detected in 5 studies (Apelberg et al. 2007; Chen et al. 2015; Darrow et al. 2013; Maisonet et al. 2012; Washino et al. 2009). In these studies, PFOS serum levels ranged from 0.005 to 0.0132 μg/mL. Three (Chen et al. 2012; Fei et al. 2007; Stein et al. 2009) out of four (Darrow et al. 2014) studies of LBW showed increased risks (OR range: 1.5–4.8). Studies have questioned whether low maternal GFR is a positive confounder in epidemiology studies of birth weight and PFAS (Morken et al. 2014; Verner et al. 2015). The Verner et al. (2015) comparison between a meta-analysis and PBPK simulations suggests that the some but not all of the association reported between PFOS and birth weight could be attributable to low GFR.

A small set of studies observed an association with gestational diabetes (Zhang et al. 2015, preconception serum PFOS), pre-eclampsia (Stein et al. 2009), and pregnancy-induced hypertension (Darrow et al. 2013) in populations with serum PFOS concentrations of 0.012–0.017 μg/mL. Zhang et al. (2015), and Darrow et al. (2013) used a prospective assessment of adverse pregnancy outcomes in relation to PFAS which addresses some of the limitations the available cross-sectional studies. Associations with these outcomes and serum PFOA also were observed.

There also is generally consistent evidence of associations of serum PFOS with decreased fertility and fecundity (Bach et al. 2015; Fei et al. 2009; Jørgensen et al. 2014; Vélez et al. 2015); there was one null study (Vestergaard et al. 2012). While a concern over the possibility of reverse causation explaining observed associations has been raised (Whitworth et al. 2012), the collective findings, particularly from a more recent study (Bach et al. 2015), support a consistent association with fertility and fecundity measures and PFOS exposures. Although there was some suggestion of an association between PFOS exposures and semen quality parameters in a few studies (Joensen et al. 2009; Toft et al. 2012), most studies were largely null (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013).

No animal studies were identified that suggested effects on fertility in males or females. However, López-Doval et al. (2014) found structural effects on the hypothalamic-pituitary axis in adult male rats after exposure to PFOS. There were histopathological lesions of the testes at doses ≥ 1 mg/kg/day and only a few active gonadotrophic cells at doses ≥ 3 mg/kg/day. The lowest dose tested, 0.5 mg/kg/day, was accompanied by decreased LH and testosterone levels and increased FSH levels.

Increased pup mortality was observed when rat dams were treated only during gestation as part of developmental toxicity studies (Chen et al. 2012; Lau et al. 2003; Thibodeaux et al. 2003). Chen et al. (2012) found increased mortality, decreased body weight, and histopathological changes in the lungs (alveolar hemorrhage, thickened interalveolar septum) in rat offspring from dams treated with 2.0 mg/kg/day from GD 1 to 21. No effects were observed in those administered 0.1 mg/kg/day. Developmental delays were found in rat offspring at a lower dose than that affecting survival (1 mg/kg/day; Butenhoff et al. 2009) and in mice at a slightly higher dose (5 mg/kg/day; Lau et al. 2003; Thibodeaux et al. 2003).

Rat dams were treated with PFOS for 63 or 84 days in a one- or two-generation reproductive study, respectively (Luebker et al. 2005a, 2005b). No changes in maternal liver weight were observed on either protocol. The most sensitive endpoint was decreased pup body weight, with reduced survival also observed at higher maternal doses. A NOAEL for pup body weight effects is 0.1 mg/kg/day in the two-generation study (Luebker et al. 2005b); this dose was not tested in the one-generation study (Luebker et al. 2005a) where the LOAEL was 0.4 mg/kg/day for decreased pup body weight, decreased maternal body weight, and decreased gestation length. A 0.4 mg/kg/day dose was a LOAEL in the both the one and two generation studies. The dose associated with a significant decrease in pup survival for the two generation study was 1.6 mg/kg/day and the dose for a decreased viability index was 0.8 mg/kg/day ($BMDL_5$ = 0.89 mg/kg/day) in the one-generation study.

To help characterize the mechanism of PFOS induced neonatal mortality, Grasty et al. (2003) examined critical windows of exposure by treating rats with a high dose of PFOS (25 mg/kg/day) for a 4-day period during various stages of pregnancy. Mortality was highest when treatment occurred on GDs 17–20, identifying late gestation as the sensitive window for neonatal death. In a subsequent experiment, exposure to 50 mg/kg/day of PFOS on GDs 19 and 20 alone was sufficient to produce almost 100% mortality to pups at birth.

Studies by Grasty et al. (2003, 2005) and Chen et al. (2012) describe significant histological and morphometric differences in the lungs between control and PFOS-exposed newborn pups, suggesting that lung maturation and pulmonary surfactant interactions are potential MOAs. Changes in lung morphology were noted in rat pups, but prenatal exposure to PFOS did not affect lung phospholipids or alter the expression of marker genes for alveolar differentiation associated with lung maturation (Grasty et al. 2005). Chen et al. (2012) found that PFOS caused oxidative stress and cell apoptosis in the lungs of offspring from mothers treated with 2.0 mg/kg/day during GDs 1–21.

Currently, the leading hypothesis for the MOA of PFOS-induced neonatal mortality is that PFOS interacts directly with components of natural lung surfactants (Grasty et al. 2005; Xie et al. 2010a, 2010b). PFOS interacts with the major phosphatidylcholine components of pulmonary surfactants and cell membranes and, therefore, has the potential to alter the dynamic properties of lung surfactant (Xie et al. 2010a). PFOS partitions into phospholipid membranes to increase membrane fluidity in several cell types (Xie et al. 2010b). This high tendency of PFOS to partition into phosphatidylcholine lipid bilayers is consistent with its resemblance to medium chain fatty acids and may be responsible for interfering with the normal physiological function of pulmonary surfactant.

Prenatal PFOS exposures appear to influence hormones during gestation, as well as in neonate and adult animals. Zhao et al. (2014) examined the testes from male Sprague-Dawley rat fetuses on GD 20 following maternal exposure to 0, 5, or 20 mg/kg/day on GDs 11–19. Fetal

Leydig cells were found to be reduced in number with evidence of apoptosis. Levels of testosterone were reduced along with the levels of key enzymes or mRNA for proteins involved with testosterone production.

Studies have examined the impact of gestational and lactational exposures on the pups as adults (Lv et al. 2013 rats; Wan et al. 2014b, mice). In both cases early life exposure through maternal treatment with PFOS was associated with abnormal glucose control in the mature offspring. In both cases, serum glucose was significantly higher in the adult animals exposed during gestation and lactation than in controls and there was evidence of insulin resistance. The LOAEL was 0.5 mg/kg/day in the Lv et al. (2013) study and 3 mg/kg/day for pups fed a diet with normal fat levels (Wan et al. 2014b). In the Wan et al. (2014b) study, the NOAEL was 0.3 mg/kg/day. When accompanied by a high fat diet, 0.3 mg/kg/day was a LOAEL for increased insulin resistance.

3.4.1.3 Immunotoxicity

A few studies have evaluated associations with measures indicating immunosuppression. Two studies reported decreases in response to one or more vaccines (diphtheria, rubella) in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing maternal serum PFOS levels (maternal levels ranging from 0.0056 to 0.027 μg/mL) during pregnancy or in the children at 5 years of age (mean child 0.0167 μg/mL) (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella and mumps antibody concentrations in relation to serum PFOS concentration were found among 12–19 year old children in the NHANES, particularly among seropositive children (Stein et al. 2015). A third study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means < 0.02 μg/mL), the associations with PFOS were also seen with other correlated PFAS, complicating conclusions specifically for PFOS.

No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010b; Okada et al. 2012), although there might be effect modification by sex. With regard to other immune dysfunction, serum PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 0.017 μg/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma 0.0339 and 0.0289 μg/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations also were found for other PFASs. Among asthmatics, serum PFOS was also associated with higher severity scores, serum total IgE, absolute eosinophil counts, and eosinophilic cationic protein levels.

Other data on the immunotoxicity of PFOS in humans are limited to *in vitro* studies using cells recovered from human blood (PBMCs; Brieger et al. 2011 or CD4+ T cells; Midgett et al. 2014). In both cases the concentration of PFOS with a demonstrated significant effect was 100 μg/mL, and the concentration that lacked any effects was 10 μg/mL. A significant ($p < 0.001$) decrease in TNFα and a nonsignificant trend towards increasing IL-6 release from stimulated monocytes were seen, but no effects were measured on stimulated T cells (Brieger et al. 2011). T cell IL-2 production was decreased in the Midgett et al. (2014) study.

Studies in mice examined NK cell activity and SRBC response following oral dosing with PFOS. Three of four studies showed effects on SRBC response and/or NK cell activity at the same dose that caused increased liver weight (Dong et al. 2009; Keil et al. 2008; Zheng et al. 2009). Based on the limited evidence, neither response appeared more sensitive than the other. The animal studies indicate that females are less susceptible to impacts on NK cell activity and the SRBC response than males.

The NK cell activity was enhanced at very low PFOS doses, while it was depressed at higher doses. Peden-Adams et al. (2008) and Dong et al. (2009) showed increased NK cell activity in male mice following exposure to 0.0017 mg/kg/day and 0.083 mg/kg/day, respectively. The increased activity in Dong et al. (2009) correlated with a PFOS serum level of approximately 7.1 µg/mL. In the Dong et al. (2009) study, the NK cell activity was significantly decreased at a higher dose of 0.833 mg/kg/day, demonstrating a U-shaped response to dose. Doses ≥ 1 mg/kg/day resulted in decreased NK cell activity in offspring of dams treated during gestation (Keil et al. 2008) and in adult male mice (Zheng et al. 2009).

In the Peden-Adams et al. (2008) study, IgM suppression occurred after 28 days of treatment with 0.0017 mg/kg/day although there were not any overt signs of toxicity. Further investigation found that the IgM suppression was observed in both the T-cell independent and dependent tests making the humoral immune effects caused by B-cells. Other studies also showed a suppression of the SRBC response at higher doses of PFOS (Dong et al. 2009; Keil et al. 2008; Zheng et al. 2009). Guruge et al. (2009) found a decrease in survival in mice exposed to 0.025 mg/kg of PFOS after exposure to influenza A virus.

Qazi et al. (2009a) reported that approximately 40 mg/kg/day in the diet for 10 days in wild-type and PPARα-null 129/Sv knock-out mice caused a pronounced decrease in the total number of thymocytes and splenocytes, as well as a decrease in size of the those present in wild-type mice. Knock-out mice had a reduction in the total number of thymocytes that was less than that seen in the wild-type mice. Effects on splenocytes were mostly eliminated in knock-out mice. The study, thus, indicated that the immunomodulation was partially dependent on PPARα. Mechanisms that could cause these effects other than PPAR activation are not known. At the same dose, Qazi et al. (2009b) did not find elevated levels in serum or spleen of TNF-α and IL-6 in response to stimulation by LPS in C57Bl/6 mice, but levels were increased in the cells from the peritoneal cavity and bone marrow

3.4.1.4 Neurotoxicity

Developmental neurotoxicity and adult neurotoxicity studies have been conducted in rats and mice. Mechanistic studies have examined effects on excitatory amino acids and gene profiles following PFOS exposures.

Butenhoff et al. (2009) found significantly increased motor activity and decreased habituation of male offspring at one time point (PND 17) following gestational and lactational dosing of dams with 1.0 mg/kg/day. No effects were found on learning and memory with the Biel swimming maze. Luebker et al. (2005b) found no effects on passive avoidance behavior or water maze learning and memory in F1 offspring at a daily dose of 0.4 mg/kg/day. Y. Wang et al. (2015) used water maze testing on offspring from treated dams who were cross-fostered with either control or treated dams, and continued on the treatment of their lactational dam. Escape latency was significantly increased for all treated groups on one or more testing days with the most pronounced effect in pups exposed prenatally from dams given 15 mg/L drinking water and

cross-fostered to control dams. Y. Wang et al. (2015) did not provide data on water intake or body weight data. A drinking water concentration of 5 mg/L was a NOAEL, and a concentration of 15 mg/L was a LOAEL for offspring. Estimated adult doses are 0.8 and 2.4 mg/kg/day, respectively, using the subchronic USEPA (1988) conventions for water intake and body weight. Long et al. (2013) found a significantly longer latency to escape, with significantly less time spent in the target quadrant in the Morris water maze test for learning and memory at a dose of 2.5 mg/kg/day in 8-week-old C57BL6 mice. The NOAEL for these effects was 0.43 mg/kg/day.

Effects were observed on excitatory amino acids in the central nervous systems of rats when administered 25 mg/kg/day of PFOS one time (Yang et al. 2009). Wang et al. (2010) found that pre-natal exposure to 3.2 mg/kg/day of PFOS in the feed had some effect on gene expression involved in neuroactive ligand-receptor interaction, calcium signaling pathways and PPAR signaling. Zeng et al. (2011) also found PFOS administered to pregnant rats as low as 0.1 mg/kg from GD 2 to 21 caused significant increases of PFOS in the brain (hippocampus and cortex) of the offspring, with effects on inflammatory markers and transcription factors. Two-month-old mice exposed to 0.75 mg/kg of PFOS when they were 10 days old (Johansson et al. 2008) displayed abnormal habituation responses in motor activity testing. Cultured hippocampal neurite growth and branching were suppressed by exposure to 50 μmol PFOS. The authors hypothesized that this was a consequence of PFOS incorporation into the neuronal lipid bilayer membrane (Liao et al. 2009). The effect of PFOS was greater than that of PFOA. PFOS was the only member of the sulfonate family to exhibit this effect.

3.4.1.5 Thyroid Effects

Numerous epidemiologic studies have evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS concentrations. These epidemiologic studies provide limited support for an association between PFOS exposure and incidence or prevalence of thyroid disease, and include large studies of representative samples of the general U.S. adult population (Melzer et al. 2010; Wen et al. 2013). These highly powered studies reported associations between PFOS exposure (serum PFOS concentrations) and thyroid disease but not thyroid hormone status. Melzer et al. (2010) studied thyroid disease with medication (PFOS level of 0.025 μg/mL in males and 0.019 μg/mL in females) and Wen et al. (2013) studied subclinical thyroid disease (mean serum 0.0142 μg/mL). Thyroid function can be affected by iodide sufficiency and by autoimmune disease. People testing positive for the anti-TPO biomarker for autoimmune thyroid disease showed associations with PFOS (0.0048 μg/mL) and TSH or T4 (Webster et al. 2014); this association was stronger in people with both low iodide status and positive anti-TPO antibodies, with a PFOS level of 0.014 μg/mL (Webster et al. 2015). These studies used anti-TPO antibody levels as an indication of stress to the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for iodine deficiency than those receiving adequate dietary iodine. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohorts.

Several animal models have described changes in thyroid hormone levels after administration of PFOS. In contrast to the human epidemiology studies, the most consistent finding in animals treated with PFOS was a decrease in T4 with slight, or no, changes in T3. Any changes found in T3 and T4 levels failed to activate the hypothalamic-pituitary-thyroid (HPT) feedback mechanism to produce significant elevations of serum TSH.

Rats treated orally with PFOS for 1–5 days had significant decreases in total T4 at doses of 10 and 15 mg/kg, but not at 5 mg/kg (Chang et al. 2007, 2008; Martin et al. 2007). With treatment for 7 days, total T4 was decreased at 1 and 3 mg/kg (Yu et al. 2011).

In Cynomolgus monkeys treated with 0.03, 0.15, or 0.75 mg/kg/day of PFOS for 26 weeks, Seacat et al. (2002) saw significant reductions of total triiodothyronine (T3) (~ 50%), and a less consistent effect in total thyroxine (TT4, females only). This was more pronounced at the end of exposure period in the high-dose group but neither a dose-response nor evidence of hypothyroidism was observed. TSH levels were variable during the study, but increased 2-fold in the high-dose males at the end of exposure.

Exposure of pregnant rats to PFOS at 1 mg/kg/day, which corresponded to maternal serum concentrations of 14–26 μg/mL, resulted in decreases in T4 and T3 in dams (Thibodeaux et al. 2003) and decreased T4 in pups (Lau et al. 2003). No effect was observed on serum TSH. In contrast, no effects were found on thyroid hormones in either dams or pups when females were treated prior to mating and through LD 4 (Luebker et al. 2005a). Histological and morphometric evaluations of the fetal and neonatal thyroid glands indicated normal number and size distribution of follicles, and normal follicular epithelial cell heights and colloid areas (Chang et al. 2009).

In addition to the evaluation of PFOS's effects on serum TT4, several studies have examined the levels of circulating FT4 (Lau et al. 2003; Luebker et al. 2005a; Thibodeaux et al. 2003; Yu et al. 2011). In these studies, FT4 was reduced after PFOS administration when measured by analog radioimmunoassays (RIA). However, when the FT4 was measured by an equilibrium dialysis step prior to the standard RIA (ED-RIA), FT4 levels in the PFOS-treated rats were comparable to those of controls (Luebker et al. 2005a).

Mechanisms underlying the PFOS-induced alterations in thyroid hormones are still under active investigation, but do not likely involve altered *de novo* biosynthesis of the hormones or compromised integrity of the HPT axis. Yu et al. (2009b) reported no significant effects of PFOS on the sodium iodide symporter gene expression (for iodide uptake) or thyroid peroxidase activity in the thyroid gland. Chang et al. (2008) showed that release of TSH from the pituitary in response to *ex vivo* TRH stimulation was not altered by PFOS exposure.

Weiss et al. (2009) demonstrated that perfluorinated chemicals (including PFOS) are capable of competing with T4 and displacing the hormone from binding to the human thyroid hormone transport protein transthyretin (TTR). In fact, PFOS ranks the second highest in binding potency among all perfluorinated compounds examined, although its TTR binding potency is only one-fifteenth of that for T4. Similarly, Ren et al. (2015) demonstrated that PFOS bound to the ligand binding domain of the human thyroid hormone receptor, although with a much lower affinity than T3.

Several possibilities might account for the differential findings of thyroid hormone disruption between animal models and human biomonitoring data. First, decreased T3 or T4 was observed in adult monkeys and rodents only when serum PFOS reached the 70–90 μg/mL range. Pregnant rats and neonatal rats appeared to be more sensitive, exhibiting TT4 depression when serum PFOS reached about 20 and 40 μg/mL, respectively. However, serum PFOS in general populations of humans is estimated to be 0.018–0.037 μg/mL, about three orders of magnitude lower than the effective body burden for thyroid hormone disruption in animal models. Secondly, TBG (rather than TTR as in rodents) is the major thyroid hormone transporter in

humans. Although PFOS can bind to human TTR and effectively displaces T4 as illustrated in the rat model, its binding affinity to TBG is unknown. PFOS has been shown to have much a lower binding affinity for both TTR and the thyroid hormone receptor than do T4 and T3, respectively (Ren et al. 2015; Weiss et al. 2009).

3.4.2 Synthesis and Evaluation of Carcinogenic Effects

The small set of epidemiology studies of PFOS exposure do not suggest that there is an association with cancer, but the breadth and scope of the studies are not adequate to make definitive conclusions. While an elevated risk of bladder cancer mortality was associated with PFOS exposure in an occupational study (Alexander et al. 2003), a subsequent study to ascertain cancer incidence in the cohort observed elevated but statistically insignificant incidence ratios that were 1.7- to 2-fold higher among exposed workers (Alexander and Olsen 2007). Mean PFOS serum levels were 0.941 μg/mL. No elevated bladder cancer risk was observed in a nested case-control study in a Danish cohort with plasma PFOS concentrations at enrollment ranging 0.001–0.1305 μg/mL (Eriksen et al. 2009).

Elevated odds ratios for prostate cancer were reported for the occupational cohort examined by Alexander and Olsen (2007) and the Danish population-based cohort examined by Eriksen et al. (2009), however the confidence intervals included the null, and no association was reported by another case-control study in Denmark (Hardell et al. 2014). A case-control study of breast cancer among Inuit females in Greenland with similar serum PFOS levels to those of the Danish population (0.0015–0.172 μg/mL) reported an association of low magnitude that could not be separated from other perfluorsulfonated acids, and the association was not confirmed in a Danish population (Bonefeld-Jørgensen et al. 2011, 2014). Some studies evaluated associations with serum PFOS concentration at the time of cancer diagnosis and the impact of this potential exposure misclassification on the estimated risks is unknown (Bonefeld-Jørgensen et al. 2011; Hardell et al. 2014). No associations were adjusted for other perfluorinated chemicals in serum in any of the occupational and population-based studies.

The only chronic toxicity/carcinogenicity study in animals was a rat study (Thomford 2002/Butenhoff et al. 2012). Increased incidence of hepatocellular adenomas in the male (12% at the high dose) and female rats (8% at the high dose) and combined adenomas/carcinomas in the females (10% at the high dose) were observed, but they did not display a clear dose-related response. In males but not females the serum ALT levels were increased at 14, 27, and 53 weeks. At 105 weeks there was an increase in eosinophilic clear cell foci, and cystic hepatocellular degeneration in males given 2, 5, and 20 ppm PFOS. Low levels of single cell necrosis in all dose groups (males and females) were identified; the increase compared to controls was significant at the high dose in males and females (Table 3-15).

Thyroid tumors (adenomas and carcinomas) were seen in males receiving 0, 0.5, 2, 5, or 20 ppm and in females receiving 5 or 20 ppm in their diet. The tumor (adenomas + carcinomas) prevalence for males was consistent across dose groups. In males the incidence of thyroid tumors was significantly elevated only in the high-dose, recovery group males exposed for 52 weeks (10/39) but not in the animals receiving the same dose at 105 weeks. There were very few follicular cell acenomas/carcinomas in the females (5 total) with no dose-response. The most frequent thyroid tumor type in the females was C-cell adenomas, but the highest incidence was that for the controls and there was a lack of dose response among the exposed groups. C-cell adenomas were not observed in males (Thomford 2002/Butenhoff et al. 2012).

There was a high background incidence in mammary gland tumors in the female rats, primarily combined fibroma adenoma and adenoma, but the incidence lacked dose-response for all tumor classifications. Mammary gland carcinomas also lacked dose-response and had a relatively comparable incidence across dose groups including the controls (Thomford 2002/Butenhoff et al. 2012).

All genotoxicity studies including an Ames test, mammalian-microsome reverse mutation assay, an *in vitro* assay for chromosomal aberrations, an unscheduled DNA synthesis assay, and mouse micronucleus assay were negative.

3.4.3 Mode of Action and Implications in Cancer Assessment

Short-term genotoxicity assays suggested that PFOS is not a DNA-reactive compound. The results from five *in vitro* studies (Cifone 1999; Litton Bionetics, Inc. 1979; Mecchi 1999; Murli 1999; Simmon 1978) were negative, as was the result from an *in vivo* bone marrow micronucleus assay (Murli 1996).

Induction of peroxisome proliferation has been suggested as the mode of action for an increasing number of non-genotoxic carcinogens that induce liver tumors upon chronic administration to rats, mice, or both (Ashby et al. 1994; Rao and Reddy 1996). The liver-expressed peroxisome PPARα regulates the transcription of genes involved in peroxisome proliferation, cell cycle control, apoptosis, and lipid metabolism. The data for PFOS illustrate the ability of PFOS to activate PPARα (Martin et al. 2007; Shipley et al. 2004; Wolf et al. 2008, 2012). However, data are generally lacking for increased cell proliferation. No increase in hepatic cell proliferation was detected in the subchronic study (Seacat et al. 2003) or the cancer bioassay (Thomford 2002/Butenhoff et al. 2012); limited necrosis was observed across all doses and significantly ($p < 0.05$) increased for the 20 ppm males and females. In addition, no subchronic or longer term studies revealed evidence of preneoplastic foci in the liver. Liu et al. (2009) studied biomarkers for oxidative stress in the liver and brain in KD mice. Levels of MDA did not differ between controls and exposed animals; SOD activity was lower than that observed in the controls.

Other possible MOAs for carcinogenicity have been explored, including mitochondrial biogenetics and GJIC. While PFOS was shown to be a weak toxicant to isolated mitochondria (Starkov and Wallace 2002), it inhibited GJIC in a dose-dependent manner in two cell lines and in liver tissue from rats exposed orally (Hu et al. 2002). These are not clearly defined MOAs, and their importance relative to PFOS exposure is not certain. Ngo et al. (2014) used the mouse model C57BL/6J –Min/+ for intestinal neoplasia to determine effects following *in utero* exposure. Maternal treatment with PFOS at doses up to 0.3 mg/kg/day during gestation did not result in an increase of intestinal tumors in either wildtype or susceptible offspring up to 20 weeks of age.

3.4.4 Weight of Evidence Evaluation for Carcinogenicity

Under the EPA *Guidelines for Carcinogen Risk Assessment* (USEPA 2005a) there is *suggestive evidence of carcinogenic potential* of PFOS in humans. A single chronic cancer bioassay in animals is available for PFOS. Although liver adenomas were significantly increased in males and females at the highest dose and a positive trend was observed ($p = 0.03$), a dose-response pattern was not observed. In males the incidence of thyroid follicular tumors was

elevated only in the high-dose, recovery group exposed for 52 weeks, where it was about 3 times greater than the incidence in rats given the same dose for 104 weeks. As was the case for the liver tumors, the thyroid adenoma data did not show a direct response to dose. Based on the available evidence, the data are inadequate to support a PPARα-linked MOA for the liver and thyroid adenomas observed by Thomford (2002)/Butenhoff et al. (2012) in the chronic 2 year bioassay in Sprague-Dawley Crl:CD(SD)IGS BR rats.

3.4.5 Potentially Sensitive Populations

In humans, single blood samplings of different populations within the United States do not support major gender differences in half-life or sensitivity to PFOS. Gender differences could not be determined by those exposed by occupational exposure, as the majority of those tested were males. Serum monitoring among the NHANES populations (2004–2008) found significantly ($p < 0.05$) higher PFOS levels in males (0.020 μg/L) than females (0.014 μg/L). However, this difference is more likely to be related to exposures than to sensitivity.

Evidence from animal studies does not suggest major differences between genders in the amount of PFOS identified in the serum and liver tissue of animals or in the toxicity. In the monkey studies and most developmental rat studies, there do not appear to be any differences between the males and females after administration of PFOS. However, in the chronic/carcinogenicity study in rats, the male rats do appear to be slightly more sensitive to liver toxicity. In animal studies of immunological effects, the response to NK cell suppression occurred at a lower dose in males than in females (Peden-Adams et al. 2008).

Animal studies clearly show that developmental exposure of rats or mice to PFOS administered during gestation results in rapid, dose-dependent effects on neonatal survival (Lau et al. 2003; Luebker et al. 2005a, 2005b). Additional long term effects on postnatal growth and delays in developmental landmarks (eye opening, pinna unfolding, surface righting, air righting) occur in surviving rat pups. The mechanistic cause of this developmental toxicity is unknown, but investigations of several potential modes of action are summarized here. Generally, there is a lack of consistency among the epidemiology studies regarding potential associations between PFOS levels during pregnancy and developmental birth outcomes. Some studies indicate a potential impact on birth weight, but this finding is not consistent across studies.

The animal data on LBW receive support from the epidemiolgy (Apelberg et al. 2007; Chen et al. 2015; Darrow et al. 2013; Maisonet et al. 2012; Washino et al. 2009). For humans with low GFR (females with pregnancy-induced hypertension or preeclampsia in late pregnancy), the impact on body weight is likely due to a combination of the low GFR and the serum PFOS (Verner et al. 2015). Low GFR in pregnant females will tend to cause an increase in serum PFOS compared to individuals with a normal GFR. Females with hypertension during pregnancy could have an increased risk for having a LBW baby.

The fat content of the diet appears to be an important variable that influences the effects from PFOS exposures. Elevated total cholesterol, HDL, and sometimes triglycerides are effects seen in a number of the human epidemiology studies. However, none of the studies evaluated appeared to control for fat content in the typical diet of the subjects. Martin et al. (2007), Bijland et al. (2011), and Wan et al. (2012) found hepatic steatosis in PFOS-treated animals. Liver fat increased with both a high fat diet alone and with a high fat diet plus PFOS (Wan et al. 2012). In the same study, significant increases in the expression of fatty acid translocase and lipoprotein lipase was observed at the 10 mg/kg/day PFOS dose. Mobilization of liver lipids appeared to

decrease following the PFOS exposure leading to lower serum LDL/VLDL levels; VLDLs are carriers of liver triglycerides and other lipids from liver to serum.

To help characterize the mechanism of PFOS induced neonatal mortality, Grasty et al. (2003) examined critical windows of exposure by treating rats with a high dose of PFOS (25 mg/kg/day) for a 4-day period during various stages of pregnancy. Neonatal mortality occurred after all treatment periods, but the incidence of neonatal death increased when exposure occurred later in gestation. Mortality was highest when treatment occurred on gestation days (GDs) 17–20, identifying late gestation as a critical exposure window for increasing the risk of neonatal survival. The effects of PFOS at this stage of development could be related to an impact of PFOS on lung surfactants leading to respiratory distress syndrome. Both Luebker et al. (2005a) and Lau et al. (2003) identified pup mortality as adverse effects of gestational PFOS exposures.

4. DOSE-RESPONSE ASSESSMENT

A Reference Dose (RfD) or Reference Concentration (RfC) is used as a benchmark for the prevention of long-term toxic effects other than carcinogenicity. RfD/RfC determination assumes that thresholds exist for toxic effects, such as cellular necrosis, significant body or organ weight changes, blood disorders, and so on. The RfD is expressed in terms of milligrams per kilogram per day (mg/kg/day), and the RfC is expressed in milligrams per cubic meter (mg/m^3). The RfD and RfC are estimates (with uncertainties spanning perhaps an order of magnitude) of the daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

4.1 Dose-Response for Noncancer Effects

4.1.1 RfD Determination

Human Data. In humans, data have been obtained from studies evaluating both occupational and general population exposure scenarios. Some studies monitored similar populations over time to determine whether or not a trend was present. Pathways of exposure in the general population appear to be from drinking water, food (especially fish/seafood), and some environmental exposures (e.g., carpets, house dust). In general, PFOS levels in the serum of the general population have decreased since production was stopped in the United States.

Multiple epidemiology studies evaluated serum lipid status in association with PFOS concentration. These studies provide support for an association between PFOS and small increases in total cholesterol. Hypercholesterolemia, (clinically defined as cholesterol > 240 mg/dL) was associated with PFOS exposure in a Canadian cohort (Fisher et al. 2013) and in the C8 cohort (Steenland et al. 2009); PFOS levels in these studies were 0.0084 µg/mL and 0.022 µg/mL, respectively. Cross-sectional occupational studies demonstrated an association between PFOS and total cholesterol (Olsen et al. 2001a, 2001b, 2003b), with much higher PFOS serum levels of up to 1.40 µg/mL. Evidence for associations between PFOS and other serum lipids including HDL cholesterol, LDL, VLDL, non-HDL cholesterol, and triglycerides is mixed.

The studies on serum lipids in association with PFOS serum concentrations are largely cross-sectional in nature and were largely conducted in adults. Some studies exist on children and pregnant females. The location of these cohorts varied from the U.S. population including NHANES volunteers, to the Avon cohort in the UK, to and Scandanivian countries. Limitations to these studies include the frequently high correlation between PFOA and PFOS exposure; not all studies control for PFOA in study design.

Studies that evaluated thyroid hormone levels and/or thyroid disease in association with serum PFOS concentrations include large representative samples of the general U.S. adult population and provide limited support for an association between PFOS exposure and the incidence or prevalence of thyroid disease. PFOS levels in Melzer et al. (2010) were 0.025 µg/mL in males and 0.019 µg/mL in females, and in Wen et al. (2013) they were 0.0142 µg/mL. Pregnant females testing positive for the anti-TPO biomarker for autoimmune thyroid disease showed a positive association with PFOS (0.0048 µg/mL) and TSH (Webster et al. 2014). In a second study, Webster et al. (2015) found an association with PFOS (0.014 µg/mL) and TSH and T3 in a subset of the NHANES population with both low iodide status and positive anti-TPO antibodies. Anti-TPO antibody levels are an indication of stress to

the thyroid system, not a disease state. Thus, the association between PFOS and altered thyroid hormone levels is stronger in people at risk for thyroid insufficiency or disease. In people without diagnosed thyroid disease or without biomarkers of thyroid disease, thyroid hormones (TSH, T3, or T4) show mixed effects across cohorts.

A few studies evaluated associations with measures of immunosuppression. Two studies reported decreases in response to one or more vaccines (diphtheria, rubella) in children aged 3, 5, and 7 years (e.g., measured by antibody titer) in relation to increasing maternal serum PFOS levels (ranging 0.0056–0.027 μg/mL) during pregnancy or at 5 years of age (Grandjean et al. 2012; Granum et al. 2013). Decreased rubella and mumps antibody concentrations in relation to serum PFOS concentration were found among 12–19 year old children in the NHANES, particularly among seropositive children (Stein et al. 2015). A study of adults found no associations with antibody response to influenza vaccine (Looker et al. 2014). In the three studies examining exposures in the background range among children (i.e., general population exposures, geometric means < 0.02 μg/ml), the associations with PFOS were also correlated with other PFASs, complicating conclusions as they applied to PFOS.

No clear associations were reported between prenatal PFOS exposure and incidence of infectious disease among children (Fei et al. 2010b; Okada et al. 2012), although an elevation in risk of hospitalizations for infectious disease was found among girls, suggesting effect modification by sex. PFOS levels were not associated with risk of ever having had asthma among children in the NHANES with median levels of 0.017 μg/mL (Humblet et al. 2014). A study among children in Taiwan with higher serum PFOS concentrations (median with and without asthma 0.0339 and 0.0289 μg/mL, respectively) found higher odds ratios for physician-diagnosed asthma with increasing serum PFOS quartile (Dong et al. 2013). Associations with other PFASs were also positive. Among asthmatics, serum PFOS was associated with higher severity scores, serum total IgE, absolute eosinophil counts, and eosinophilic cationic protein levels.

The set of studies evaluating fetal growth retardation suggest an association of prenatal serum PFOS with deficits in mean birth weight and with LBW, however it is not entirely consistent. Birth weight deficits ranging from 29 to 149 grams were detected in five studies (Apelberg et al. 2007; Chen et al. 2015; Darrow et al. 2013; Maisonet et al. 2012; Washino et al. 2009). In these studies, PFOS serum levels ranged from 0.005 to 0.0132 μg/mL. Three (Chen et al. 2012; Fei et al. 2007; Stein et al. 2009) out of four (Darrow et al. 2014) studies of LBW showed increased risks (OR range: 1.5–4.8). Studies have questioned whether low maternal GFR is a confounder in epidemiology studies of birth weight and PFOS (Morken et al. 2014; Verner et al. 2015). The Verner et al. (2015) study compared the results from a meta-analysis of the epidemiology data with PBPK simulations and concluded that the some, but not all, of the association reported between PFOS and birth weight is attributable to low GFR. Thus, the interpretation of the observed associations is unclear.

A small set of studies observed an association with gestational diabetes (Zhang et al. 2015, preconception serum PFOS), pre-eclampsia (Stein et al. 2009), and pregnancy-induced hypertension (Darrow et al. 2013) in populations with serum PFOS concentrations of 0.012 – 0.017 μg/mL. Zhang et al. (2015) and Darrow et al. (2013) used a prospective assessment of adverse pregnancy outcomes in relation to serum PFAS thereby avoiding some of the limitations of the available cross-sectional studies. Associations with serum PFOA and adverse pregnancy outcome were identified.

There is consistent evidence of associations of serum PFOS with decreased fertility and fecundity (Bach et al. 2015; Fei et al. 2009; Jørgensen et al. 2014; Vélez et al. 2015). While a concern over the possibility of reverse causation explaining observed associations has been raised (Whitworth et al. 2012), the collective findings, particularly from a more recent study (Bach et al. 2015), support a consistent association with fertility and fecundity measures and PFOS exposures. Although there was some suggestion of an association between PFOS exposures and semen quality parameters in a few studies (Joensen et al. 2009; Toft et al. 2012), most studies were largely null (Buck Louis et al. 2015; Ding et al. 2013; Joensen et al. 2013; Raymer et al. 2012; Specht et al. 2012; Vested et al. 2013).

Animal Data. Adequate studies were available for short-term, subchronic, chronic, developmental, and reproductive parameters in rats, mice, and primates. Subchronic, chronic, and reproductive toxicity animal studies, all with exposure duration greater than 60 days, have been summarized in Table 4-1. Shorter duration studies that focused on immunotoxicity endpoints and developmental toxicity studies are summarized in Table 4-2. Although the exposure durations are shorter in developmental studies, they are important in quantification of dose-response because the exposures occur during critical windows of development and are often symptomatic of effects that can occur later in life. It is noted, however, that in some of these studies, steady states of PFOS might not have been achieved due to the long half-life of PFOS in animal models (see discussion of steady state in section 4.1.1.1)

Seacat et al. (2002) treated monkeys with PFOS for up to 6 months and found increased liver weight and centrilobular or diffuse hepatocellular hypertrophy at 0.75 mg/kg/day, but no clear evidence of peroxisomal or cell proliferation. Hepatic peroxisome proliferation, measured by PCoAO activity, was increased significantly in the females at 0.75 mg/kg/day; however, the magnitude was less than the 2-fold increase typically indicating biological significance and PPARα activation. There were no treatment-related effects on cell proliferation in the liver, pancreas, or testes; survival was decreased among the males. At the dose with no effects observed (0.15 mg/kg/day), the serum concentration was 83 μg/mL in males and 67 μg/mL in females. At the effect level (0.75 mg/kg/day), the serum concentrations were 173 μg/mL in males and 171 μg/mL in females, about twice those for the no-effect serum level despite a 5-fold increase in dose.

Microscopic lesions of the liver were observed at doses of 1.33 mg/kg/day in males and 1.56 mg/kg/day in females after 14 weeks (Seacat et al. 2003) and at 0.098 mg/kg/day in males and 0.299 mg/kg/day in females after 105 weeks (Thomford 2002/Butenhoff et al. 2012). Liver lesions included centrilobular hypertrophy and vacuolation after the subchronic and chronic exposures with eosinophilic granules observed after 104 weeks. No evidence of peroxisome proliferation was found during either phase of the study. Mean no effect levels in males and females were 0.34 mg/kg/day and 0.40 mg/kg/day, respectively, after 14 weeks and 0.024 mg/kg/day and 0.120 mg/kg/day, respectively, after 104 weeks.

Rat dams were treated with PFOS for 63 or 84 days in one- and two-generation reproductive studies, respectively (Luebker et al. 2005a, 2005b). No changes in maternal liver weight were observed with either protocol. The most sensitive endpoint was decreased pup body weight at 0.4 mg/kg/day in both the one- and two-generation study. A NOAEL for pup body weight effects was 0.1 mg/kg/day in the two-generation study; the one-generation study (Luebker et al. 2005a) lacked a NOAEL, as pup body weight was impacted at the lowest dose tested (0.4 mg/kg/day).

Table 4-1. NOAEL/LOAEL and Effects for Longer-Term Duration Studies of PFOS

Species	Study Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical Effect(s)	Reference
Monkey	90 days	ND	0.5	diarrhea, anorexia	Goldenthal et al. 1979
Monkey	182 days (6 months)	0.15	0.75	↓ survival, body wt gain ↑ liver wt; hepatocyte hypertrophy, ↓T3 and ↑TSH	Seacat et al. 2002
Rat	90 days	ND	2.0	↑ liver wt hepatocyte hypertrophy	Goldenthal et al. 1978b
Rat	98 days (14 weeks)	0.40 (F) 0.34 (M)	1.56 (F) 1.33 (M)	↑ liver wt ↓ cholesterol (M) ↑ ALT (M), ↑BUN (M/F) ↑ liver hypertrophy hepatic centrilobular vacuolization	Seacat et al. 2003
Rat	2 generation (84 days; 12 weeks)	0.1	0.4	↓ adult body wt gain ↓ pup body wt	Luebker et al. 2005b
Rat	1 generation (females only) (63 days)	0.4	0.8	↓ maternal wt gain ↓ gestation length ↓ pup survival	Luebker et al. 2005a
Rat	1 generation (females only) (63 days)	ND	0.4	↓ pup body weight	Luebker et al. 2005a
Rat	728 days (104 weeks; 2 yrs)	0.120 (F) 0.024 (M)	0.299 (F) 0.098 (M)	Cystic degeneration, centrilobular vacuolation (M) and centrilobular eosinophilic granules (F); ↑hepatic necrosis centrilobular vacuolation at higher doses	Thomford 2002/Butenhoff et al. 2012
Mouse	60 days	0.008	0.083	↑ liver wt ↑ splenic NK cell activity; ↓ SRBC response	Dong et al. 2009
Mouse	90 days	0.43	2.15	Impaired spatial learning and memory	Long et al. 2013

Notes: ND = not determined
BUN = blood urea nitrogen
M = male; F = female

Offspring survival was affected in a dose-related manner in the one-generation study, with a biologically important decrease in viability index attained at 0.8 mg/kg/day and statistical significance reached at 1.6 mg/kg/day (Luebker et al. 2005a). In the two generation study (Luebker et al. 2005b), F1 offspring viability was markedly impacted at a dose of 1.6 mg/kg/day, resulting in discontinuation of that dose for production of the F2 generation

Some effects on thyroid-related parameters were noted in animals, but there did not appear to be any increase in hypothyroid or hyperthyroid disorders. In the Seacat et al. monkey study (2002), trends for reduced total triiodothyronine (T3) and increased TSH (males only) were observed and reached statistical significance for T3 in males and females. In the case of TSH, the decrease was significant only for males. The trend in females lacked clear dose-response. There was no evidence of hypothyroidism. PFOS-induced alterations of thyroid hormones were also seen studies on adult rats (Martin et al. 2007; Thibodeaux et al. 2003; Yu et al. 2009b, 2011);

however, most reductions involved circulating TT4, instead of T3. In most animal studies, however, the changes in T3 and TT4 failed to activate the HPT feedback mechanism to produce significant elevations of serum TSH.

Across the range of longer-term studies, the lowest LOAEL is 0.098 mg/kg/day for histopathological changes in the liver of male Sprague-Dawley rats following a 104-week (2-year) exposure (Thomford 2002/Butenhoff et al. 2012). Histological changes observed included centrilobular eosinophilic granules, centrilobular vacuolation, and centrilobular hypertrophy with single cell necrosis at a higher dose. Significant increases in absolute and relative liver weights were not observed. The LOAEL for comparable effects in females was about 3 times higher. After 14 weeks, Seacat et al. (2003) reported increased absolute and relative liver weights in male and absolute liver weight in female Sprague-Dawley rats, accompanied by centrilobular hypertrophy and decreased cholesterol levels at a dose of 1.33 mg/kg/day for the males and 1.56 mg/kg/day for the females. An increase in serum ALT at the same dose is suggestive of liver damage, but these data were highly variable and did not notably progress in the Thomford 2002/Butenhoff et al. 2012 study at 27 and 53 weeks. In monkeys, decreased survival, increased relative liver weight, and decreased cholesterol were seen at a LOAEL of 0.75 mg/kg/day administered for 6 months (Seacat et al. 2002).

In the Dong et al. (2009) study, an increase in splenic NK cell activity, a decrease in the SRBC response, and increased liver weight were seen in male mice after 60 days of treatment with 0.083 mg/kg/day; resulting PFOS serum concentrations were approximately 7.1 mg/L. At a 10-fold higher dose, NK response was decreased and indicative of a U-shaped response to dose. No other studies of an immunological endpoint with a comparable exposure duration were identified.

The most severe of the effects observed in the longer-term studies was the decrease pup survival in the one-generation study by Luebker et al. (2005a) in rats at a LOAEL of 0.8 mg/kg/day, a dose not evaluated in the two-generation study. The LOAEL for the less serious effect of decreased pup body weight was 0.4 mg/kg/day in the one- and two-generation studies.

The short-term and developmental exposure studies compiled in Table 4-2 below support the concern for low dose-effects on pup body weight and survival. The majority of the short-term, dose-response studies of PFOS were designed to examine developmental end-points.

Similar to the decreased offspring survival described in the one-generation reproductive toxicity study (Luebker et al. 2005a), increased pup mortality was observed when rat dams were treated only during gestation as part of developmental toxicity studies (Chen et al. 2012; Lau et al. 2003; Thibodeaux et al. 2003). Chen et al. (2012) found increased mortality, decreased body weight, and histopathological changes in the lungs (alveolar hemorrhage, thickened interalveolar septum) in rat offspring from dams treated with 2.0 mg/kg/day from GD 1 to 21. No effects were observed in those administered 0.1 mg/kg/day. Data from Borg et al. (2010) demonstrated significantly increased levels of fetal and neonatal PFOS concentrations in the lung between GD 18 and PND 1 compared with their dams, providing a possible link to the changes observed by Chen et al. (2012). Thibodeaux et al. (2003) and Lau et al. (2003) both found decreased maternal and pup weight, but no effects on maternal liver weight, when dams were dosed at 2 mg/kg/day from GD 2 to 20.

Table 4-2. NOAEL/LOAEL Data for Short-Term Oral Studies of PFOS

Species	Study Duration	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Critical Effect(s)	Reference
Rat	28 days	ND (F) 0.14 (M)	0.15 (F) 1.33 (M)	↑ relative liver wt (M/F), ↓T4 (M/F)	Curran et al. 2008
Rat	GDs 1–21	0.1	2.0	↓ pup survival histopathological changes to lungs (pups)	Chen et al. 2012
Rat	GD 0 to PND 20	-	0.5	↓ body weight impaired glucose tolerance	Lv et al. 2013
Rat	GDs 11–19	-	5	↓body weight, ↓ fetal Leydig cells, and ↓testosterone	Zhao et al. 2014
Rat	GDs 2–20	1.0	2.0	↓ dam and pup body weight ↓ pup survival	Thibodeaux et al. 2003; Lau et al. 2003
Rat	GD 0–PND 20	0.3	1.0	↑ motor activity and decreased habituation in male pups	Butenhoff et al. 2009
Rat	GDs 0–20	0.8	2.5	↑water maze escape distance and escapre latency	Y. Wang et al. 2015
Rat	GD 0–LD 21	0.8	2.5	↑water maze escape distance and escapre latency	Y. Wang et al. 2015
Mouse	GDs 1–17	1.0	5.0	↑ liver wt, dams and pups; delayed eye opening	Thibodeaux et al. 2003; Lau et al. 2003
Mouse	GD 3–PND 21 (dams) (offspring evaluated on PND 63)	0.3	3.0	↑ liver weight, increased insulin resistance	Wan et al. 2014a
Mouse	21 days	1	5	↑liver weight hepatic steatosis	Wan et al. 2012
Mouse	28 days	0.00017 (M) 0.0033 (F)	0.0017 (M) 0.017 (F)	↓ SRBC plaque-forming cell response	Peden-Adams et al. 2008
Mouse	GDs 1–17	(M) 1 (F)	1 (M) 5 (F)	↓ NK cell activity at postnatal week 8	Keil et al. 2008

Note: M = male; F = female

In the standard developmental neurotoxicity study by Butenhoff et al. (2009), male offspring showed increased motor activity and decreased habituation on PND 17 following a maternal dose of 1 mg/kg/day; no effects on body weight were reported. In Y. Wang et al. (2015), the NOAEL for learning and memory as reflected in Morris water maze results for rats exposed during gestation and gestation/lactation was 0.8 mg/kg/day and the LOAEL was 2.4 mg/kg/day. In the longer-term 90-day study by Long et al. (2013), the NOAEL for effects on learning and memory was 0.43 mg/kg/day with a LOAEL of 2.12 in mice first exposed at 8 weeks. Evaluating postnatal effects of *in utero* exposure in the mouse, Lau et al. (2003) reported increased liver weight and delayed eye opening in offspring from dams treated with 5 mg/kg/day.

The studies by Lv et al. (2013) in rats and Wan et al. (2014b) in mice provide evidence for long lasting impacts on blood glucose control in adult animals exposed to PFOS gestationally and lactationally. In both studies, dams were exposed throughout gestation and lactation, but the offspring were not directly treated. In the Lv et al. (2013) study, the animals were evaluated at 22 weeks of age and in the Wan et al. (2014b) study animals were evaluated at 63 days of age

(9 weeks). In both cases, the rats exposed during gestation had signs of insulin resistance, resulting in elevated serum glucose levels.

Peden-Adams et al. (2008) identified immunotoxicity in male mice exposed to 0.0017 mg/kg/day. IgM production was suppressed after 28 days of treatment although no overt signs of toxicity were observed at any dose. In the Keil et al. (2008) study, crossbred mice exposed during gestation had decreased NK cell activity in males and females at postnatal week 8. The SRBC IgM response was suppressed in males at a higher dose (5 mg/kg/day), but not in females. The 52%–78% decrease in the SRBC plaque-forming cell response in male mice in the study by Peden-Adams et al. (2008) with a LOAEL of 0.0017 mg/kg/day and an NOAEL of 0.00017 mg/kg/day is the only effect at a LOAEL less than that in male rats (0.072 mg/kg/day) from the Thomford (2002)/Butenhoff et al. (2012) chronic study. The number of animals per dose group utilized by Peden-Adams et al. (2008) was small (n = 5). The SRBC response suppression in male pups (n = 6) from the Keil et al. (2008) developmental exposure was higher at 5 mg/kg/d; females showed no response. The longer duration study by Dong et al. (2009) also had a higher LOAEL at 0.083 mg/kg/day for SRBC suppression and increased liver weight.

Decreased NK cell activity occurred at a lower dose than the SRBC response in the Keil et al. (2008) study, at a higher dose in the Peden-Adams et al. (2008) study, and at the same dose in the Dong et al. (2009) study. The NK cell activity was enhanced at very low PFOS doses, while it was depressed at higher doses. These differences highlight the need for additional research to confirm the NOAEL and LOAEL for the immunological endpoints. In all three studies with the low dose responses, males responded at lower doses than females.

Studies in mice examined NK cell activity and SRBC response. Three of four studies showed effects on SRBC response, NK cell activity, or both at the same dose that caused increased liver weight (0.083 mg/kg/day, Dong et al. 2009; 5 mg/kg/day, Keil et al. 2008; Zheng et al. 2009). The extremely low-dose effects found in Peden-Adams et al. (2008) with a LOAEL for SRBC response of 0.0017 mg/kg/day after 28 days are not supported by the LOAEL of 0.083 mg/kg/day for a dosing duration of 60 days from Dong et al. (2009).

Taken together, the lower antibody titers associated with PFOS levels in humans and the consistent suppression of SRBC response in animals indicates a concern for adverse effects on the immune system. However, lack of human dosing information and lack of low-dose confirmation of effects in animals for the short-duration study precludes the use of these immunotoxicity data in setting the RfD.

4.1.1.1 Pharmacokinetic Model

Among the studies summarized in Tables 4-1 and 4-2, a number reported low-dose adverse effects and had data on measured serum concentrations that made them suitable for pharmacokinetic modeling in order predict a time-integrated average serum concentration for the exposure duration and experimental doses. Because of the complexities of the pharmacokinetic differences between animals and humans and across animal species, the average serum values are a superior point of departure (POD) for RfD derivation, rather than the external doses in the studies. Generally, it was assumed that animals were observed at the end of dosing. The published Wambaugh et al. (2013) model described in section 2.5.1 was applied to the selected studies. The use of the animal data and the available PK model allows for the incorporation of species differences in saturable renal resorption, dosing duration, and serum measurements for

doses administered to determine human equivalent doses based on average serum concentration and clearance.

The results for studies in the rats are summarized in Table 4-3. For the Butenhoff et al. (2009) study two different AUCs were calculated—gestational only (for the male offspring endpoint) and gestational plus 20 days postnatal (for the maternal endpoint). This separation of the two exposures neglects lactational transfer of compound, which was not modeled.

The predicted results from studies in mice and the monkey are provided in Tables 4-4 and 4-5, respectively. The Lau et al. (2003) data on mice are representative of the impact of PFOS on developmental endpoints. Although the duration of this study is relatively short at 19 days, the average serum levels associated with the observed effects on pup body weight and developmental milestones merit consideration. The Seacat et al. (2002) study on monkeys is a long term (6 month), multiple dose study of systemic toxicity in which the LOAEL for effects on liver weight, liver histopathology, cholesterol, body weight gain, T3, and TSH was accompanied by early death in two of six monkeys.

The AUC for the LOAEL or NOAEL of each data set can be used to determine an average serum concentration by dividing it by the duration of the study in days with adjustment for the number of hours in a day. The average serum concentration given in Table 4-6 for the LOAEL or NOAEL was determined through numeric simulation. Averaging the serum concentrations for the duration of exposure is important because of the variability in the times of exposure across the studies (17–182 days).

Average serum concentration has the advantage of normalizing across the exposure durations to generate a uniform metric for internal dose in situations where the dosing durations varied and serum measurements were taken immediately prior to sacrifice. The averaged serum concentration is a hybrid of the AUC and the maximum serum concentration. As applied to the database for PFOS, average serum concentration appears to be a stable reflection of internal dosimetry.

Table 4-6 provides dosing duration and the predicted average serum concentration from each of the modeled studies. Internal doses associated with developmental toxicity were 19.9–25 µg/mL for reduced pup body weight (Luebker et al. 2005a, 2005b), 34.6 µg/mL for changes in motor activity (Butenhoff et al. 2009), and 35.1–39.7 µg/mL for pup survival (Lau et al. 2003; Luebker et al. 2005a). In comparison, internal doses associated with increased liver weight were 64.6–157 µg/mL (Seacat et al. 2002, 2003). Thus, the internal doses associated with the developmental and liver effect levels (LOAELs) differ by less than an order of magnitude (19.9–157 µg/mL), while the corresponding AUC values (Tables 4-3 through 4-5) differ by more than an order of magnitude (30,100 µg/mL*h–684,000 µg/mL*h).

Table 4-3. Predicted Final Serum Concentration and Time Integrated Serum Concentration (AUC) for Different Treatments of Rat

Study	Species / Strain	Study Duration	Oral Doses mg/kg/day	Measured Serum Concentration µg/mL	Species / Strain Used for Prediction	Predicted Final Serum Concentration µg/mL	Predicted AUC µg/mL*h
Seacat et al. 2003	Male Rat/ Crl:CD(SD) IGS BR	98 Days	0.03	4.04 (0.80)	Male Rat/Sprague-Dawley	2.29 (0.0888)	3,430 (108)
			0.13	17.1 (1.22)		9.94 (0.386)	14,900 (480)
			0.34	43.9 (4.9)		25.9 (0.976)	38,900 (1,230)
			1.33	148 (14)		101 (3.94)	152,000 (4,860)
Seacat et al. 2003	Female Rat/ Crl:CD(SD) IGS BR	98 Days	0.04	6.96 (0.99)	Female Rat/Sprague-Dawley	4.86 (0.0978)	6,620 (143)
			0.15	27.3 (2.3)		18.2 (0.364)	24,800 (561)
			0.40	64.4 (5.5)		48.3 (1.07)	65,800 (1,500)
			1.56	223 (22)		187 (7.98)	256,000 (7,500)
Butenhoff et al. 2009 and Chang et al. 2009	Rat/Sprague-Dawley	Gestation (22 Days)	0.1	1.722 (0.068)	Female Rat/Sprague-Dawley	3.7 (0.121)	1,060 (37.7)
			0.3	6.245 (0.096)		11.1 (0.367)	3,180 (114)
			1	26.630 (3.943)		37.1 (1.2)	10,600 (376)
Butenhoff et al. 2009 and Chang et al. 2009	Rat/Sprague-Dawley	Gestation (21 Days) + Postnatal (20 Days)	0.1	3.159 (0.081)	Female Rat/Sprague-Dawley	6.36 (0.167)	3,410 (105)
			0.3	8.981 (0.275)		19.1 (0.512)	10,300 (323)
			1	30.480 (1.294)		63.5 (1.67)	34,100 (1,040)
Thibodeaux et al. 2003 and Lau et al. 2003	Rat/Sprague-Dawley	GDs 2–20 (19 days)	1	19.69[a]	Female Rat/Sprague-Dawley	32.4 (1.05)	8,020 (279)
			2	44.33[a]		64.8 (2.23)	16,000 (594)
			3	70.62[a]		97 (3.26)	24,000 (866)
			5	79.39[a]		162 (5.61)	40,100 (1,430)
			10	189.4[a]		321 (15)	79,800 (3,070)
Luebker et al. 2005b	Rat/Crl:CD (SD)IGS VAF/Plus	6 wks prior to mating through gestation and lactation (84 Days)	0.1	4.52 (1.15)	Female Rat/Sprague-Dawley	11 (0.226)	12,600 (312)
			0.4	26.2 (16.1)		43.8 (0.882)	50,400 (1,180)
			1.6	136 (86.5)		174 (5.73)	201,000 (5,250)
			3.2	155 (39.3)		342 (24.5)	398,000 (17,700)
Luebker et al. 2005a	Rat/Crl:CD (SD)IGS VAF/Plus	6 wks prior to mating through gestation (63 Days)	0.4	NT	Female Rat/Sprague-Dawley	35.7 (0.765)	30,100 (794)
			0.8	NT		71.3 (1.65)	60,100 (1,640)
			1.0	NT		88.9 (2.25)	75,000 (2,060)
			1.2	NT		107 (2.91)	90,000 (2,600)
			1.6	NT		142 (4.13)	120,000 (3,400)
			2.0	NT		177 (6.38)	150,000 (4,530)

Notes: Numbers in parentheses indicate SD
GD = gestation day; NT = not tested
[a] Thibodeaux et al. (2003) data available only in a graph in the published paper; the values for the model obtained from author.

Table 4-4. Predicted Final Serum Concentration and Time Integrated Serum Concentration (AUC) for the Mouse

Study	Species / Strain	Study Duration And Type	Administered Doses mg/kg/day	Measured Final Serum Concentration µg/mL	Species / Strain Used for Prediction	Predicted Final Serum Concentration µg/mL	Predicted AUC µg/mL*h
Lau et al. 2003	Female Mouse/CD-1	GDs 1–17 (17 days)	1	NT	Female Mouse / CD1	54.8 (1.78)	13,500 (460)
			5	NT		195 (38.4)	57,700 (5,220)
			10	NT		259 (103)	88,900 (19,700)
			15	NT		289 (158)	106,000 (35,000)
			20	NT		312 (217)	118,000 (50,300)

Notes: Numbers in parentheses indicate SD
GD = gestation day; NT = not tested

Table 4-5. Predicted Final Serum Concentration and Time Integrated Serum Concentration (AUC) for the Monkey

Study	Species / Strain	Study Duration And Type	Administered Doses mg/kg/day	Measured Final Serum Concentration µg/mL	Species / Strain Used for Prediction	Predicted Final Serum Concentration µg/mL	Predicted AUC µg/mL*h
Seacat et al. 2002	Monkey / Cynomol-gus	182 days	0.03	F: 13.2 (1.4) M: 15.8 (1.4)	Monkey / Cynomol-gus	14.3 (0.228)	33,800 (547)
			0.15	F: 66.8 (10.8) M: 82.6 (25.2)		68.8 (0.978)	166,000 (2460)
			0.75	F: 171 (22) M: 173 (37)		225 (6.28)	684,000 (10,700)

Notes: Numbers in parentheses indicate SD
M = male; F = female

Table 4-6. Average Serum Concentrations for the Duration of Dosing

Study	Dosing duration days	NOAEL mg/kg/day	NOAEL (Av serum µg/mL)[a]	LOAEL mg/kg/day	LOAEL (Av serum µg/mL)[a]
Seacat et al. 2002 monkey: ↑liver weight + histopathology; ↓body weight; ↓T3; ↑TSH	182	0.15	38 (0.564)	0.75	157 (2.45)
Seacat et al. 2003 male rat: ↑liver weight, centrilobular vacuolization,↑ ALT, ↑BUN	98	0.34	16.5 (0.522)	1.33	64.6 (2.06)
Luebker et al. 2005b: ↓ rat pup body weight[b]	84	0.1	6.26 (0.155)	0.4	25 (0.583)
Luebker et al. 2005a: ↓ rat pup body weight[b]	63	None	None	0.4	19.9 (0.525)
Luebker et al. 2005a rat: ↓ maternal body weight, gestation length and pup survival[b]	63	0.4	19.9 (0.525)	0.8	39.7 (1.09)
Butenhoff et al. 2009 rat developmental neurotoxictiy: ↑increased motor activity↓ habituation	41	0.3	10.4 (0.328)	1.0	34.6 (1.05)
Lau et al. 2003: ↓rat pup survival; ↓maternal and pup body weight	19	1.0	17.5 (0.609)	2.0	35.1 (1.3)

Notes: [a] Average serum concentrations predicted from PK simulations of dose regimens were performed using species-specific parameter distributions. The number in parentheses is the SD.
[b] Multiple effects are included for the Luebker et al. (2005a, 2005b) studies to distinguish between the effects quantified for dose-response.

The internal doses associated with no adverse effects on developmental and liver endpoints (NOAELs) were very similar with overlapping ranges; the average serum concentrations ranged 6.26–19.9 μg/mL for developmental/neurodevelopmental endpoints (Butenhoff et al. 2009; Lau et al. 2003; Luebker et al. 2005a, 2005b) and 16.5–38 μg/mL for liver weight changes and accompanying liver pathology and changes in serum biochemistry (Seacat et al. 2002, 2003). Despite the similarity in average serum concentrations, the AUC values differ by an order of magnitude (12,600 μg/mL*h–166,000 μg/mL*h). Given the differences in external doses, the projected serum levels are proportionally quite similar. Table 4-6 identifies 6.26 and 10.4 μg/mL as the lowest average serum concentrations associated with a NOAEL for offspring effects; the associated LOAELs were based on decreased pup body weight (Luebker et al. 2005b) and increased motor activity in male pups (Butenhoff et al. 2009). Average serum values for no increases in liver weight, liver histopathology, changes in body weight, and serum biochemistry in monkeys (38 μg/mL; Seacat et al. 2002) and male rats (16.5 μg/mL; Seacat et al. 2003) are very similar to the average no effect serum value in Lau et al. (2003) for decreased pup survival with a shorter averaging time (17.5 μg/mL). Thus, it appears that the NOAELs are consistent across gender, species, and treatment with respect to average serum concentration. Assuming that mode of action and susceptibility to toxicity do not vary and that pharmacokinetics alone explain variation, it is reasonable to expect similar concentrations to cause similar effects in humans.

The Wambaugh et al. (2013) model employed here to generate the average serum concentrations shown in Table 4-6 does not include a gestational or lactational component. However the results are in good agreement with those of Loccisano et al. (2012b) from their gestational and lactational model. Comparison of the average maternal serum concentrations calculated for developmental endpoints (Butenhoff et al. 2009; Lau et al. 2003; Luebker et al. 2005a) with those depicted graphically in Figure 3-7 (from Loccisano et al. 2012b), demonstrates good agreement between the two models. For example the LOAEL of 1 mg/kg/day for developmental neurotoxicity (Butenhoff et al. 2009) yields a calculated average maternal serum of 34.6 μg/mL as seen in Table 4-6, which is very similar to the approximately 25 μg/mL for the dams that can be estimated from the graph (Loccisano et al. 2012b). The slightly higher value calculated from the Wambaugh et al. (2013) model might be due to the longer dosing interval, 41 days, used by Butenhoff et al. (2009), versus GD 20 levels presented graphically by Loccisano et al. (2012b). Fetal PFOS serum concentration on GD 20 was published by Chang et al. (2009), but because the Wambaugh et al. (2013) model predicts maternal values, a direct comparison to the fetal plasma predicted by Loccisano et al. (2012b; Figure 3-7) cannot be made. However, despite the limitations in the fetal data, values generated by the Wambaugh et al. (2013) model can be accepted with reasonable confidence that the predicted AUC values accurately represent maternal levels during gestational and lactational exposures.

The Andersen et al. (2006) model, used to make the predictions in Tables 4-3 through 4-6, calls for numerical simulation in order to make predictions for serum concentrations resulting from a regimen of discrete doses. However, one can predict the steady-state concentration (C_{ss}) resulting from a fixed infusion dose rate (DR, in units of μmol/h):

$$C_{ss} = \frac{DR}{free * Q_{fil}}\left(1 + \frac{T_{max}}{Q_{fil} * k_T + DR}\right)$$

The C_{ss} depends non-linearly on DR. The PFOS studies in Tables 4-1 and 4-2, used discrete, daily doses that can be converted to DR by dividing the daily dose (mg/kg/day) by 24 hours to

give and approximate measure of DR. For each DR and species a range of C_{ss} values can be calculated by using species-specific combinations of parameters from the Bayesian analysis of the available PK data. In Table 4-7, the C_{ss} is compared with the average serum concentration predicted for each of the studies in Table 4-6. The average serum concentration fraction of the C_{ss} for the 182-day Seacat et al. (2002) study in monkeys is approximately 69% of the steady-state concentration. The 19-day average serum concentration from Thibodeaux et al. (2003) is only approximately 9% of C_{ss}, while the average serum concentration for the rest of the modeled studies ranges 17%–50% of C_{ss}.

The shortest duration study in Table 4-7 had a higher administered LOAEL dose than the longest studies (0.75 mg/kg/day for 182 days versus 2.0 mg/kg/day for 19 days). Despite the higher administered dose, the short 19-day study resulted in effects at a lower serum concentration than that for the longest duration of exposure, the one closest to steady state. In fact, the average serum values from the studies that do not approach steady state have lower average serum LOAELs for endpoints of toxicological concern. Thus, the data do not appear to indicate increasing sensitivity as steady-state is approached. If anything, the average serum values appear to be more protective than serum concentrations at steady state.

Table 4-7. Comparison of Average Serum Concentration and Steady-State Concentration

Study	Dosing duration days	LOAEL mg/kg/day	C_{ss} (mg/L) for constant infusion of LOAEL	Average Serum Conc. for Study (mg/L)	Fraction of C_{ss} (Average / C_{ss})
Seacat et al. 2002: monkey: ↑liver weight + histopathology; ↓body weight; ↓T3; ↑TSH	182	0.75	227 (6.95)	157 (2.45)	0.689 (0.0131)
Seacat et al. 2003: male rat: ↑liver weight, centrilobular vacuolization,↑ ALT, ↑BUN	98	1.33	128 (7.9)	64.6 (2.06)	0.504 (0.0211)
Luebker et al. 2005b: ↓ rat pup body weight	84	0.4	83.4 (6.96)	25 (0.583)	0.302 (0.027)
Luebker et al. 2005a: ↓rat pup body weight	63	0.4	83.3 (7.08)	19.9 (0.525)	0.24 (0.0232)
Luebker et al. 2005a: rat pup survival and ↓maternal body weight	63	0.8	163 (15.9)	39.7 (1.09)	0.246 (0.0273)
Butenhoff et al. 2009: ↓rat pup body weight	41	1.0	203 (22.5)	34.6 (1.05)	0.173 (0.0245)
Lau et al. 2003rat: pup survival; ↓maternal and pup body weight	19	2.0	397 (57.6)	35.1 (1.3)	0.0911 (0.0202)

Notes: Average serum concentrations from PK simulations of toxicity study treatment regimens and C_{ss} were both predicted using species-specific parameter distributions. The number in parentheses is the SD.

For human exposure to PFOS one needs to rely on average serum calculations since there is a lack of both the sufficient PK and exposure knowledge to make more complicated estimates. The average serum concentrations of the LOAEL in Table 4-7 range from 19.9 to 157 µg/mL; all are within one order of magnitude. The predicted toxic serum concentrations can be converted into an oral equivalent dose at steady state by recognizing that, at steady state, clearance from the body must equal dose to the body. Clearance can be calculated if the rate of elimination (derived from half-life) and the volume of distribution are both known.

A reliable measure of half-life in humans is available from a retired worker population followed for 5 years. Olsen et al. (2007) calculated the PFOS half-life in this former worker population as 5.4 years (see section 2.5.2). Thompson et al. (2010) give a volume of distribution of 0.23 L/kg bw (see section 2.5.3). These values combined give a clearance of 8.1×10^{-5} L/kg bw/day as determined by the following equation:

$$CL = V_d \times (\ln 2 \div t_{1/2}) = 0.23\ L/kg\ bw \times (0.693 \div 1{,}971\ days) = 0.000081\ L/kg\ bw/day$$

Where:

V_d = 0.23 L/kg
ln 2 = 0.693
$t_{1/2}$ = 1,971 days (5.4 years x 365 days/year = 1,971 days)

These values combined give a clearance of 8.1×10^{-5} L/kg bw/day.

Scaling the derived average concentrations (in μg/mL) for the NOAELs and LOAELs in Table 4-6 gives predicted oral HEDs in mg/kg bw/day for each corresponding serum measurement. The HED values are the predicted human oral exposures necessary to achieve serum concentrations equivalent to the NOAEL or LOAEL in the animal toxicity studies. Note that this scaling uses linear human kinetics in contrast to the non-linear phenomena observed at high doses in animals.

Thus, HED = average serum concentration (in μg/mL) x CL

Where:

Average serum is from model output in Table 4-6
CL = 0.000081 L/kg bw/day

The resulting HED values are shown in Table 4-8. Endpoints considered as critical effects in multiple studies include offspring growth and survival, liver weight changes, liver histopathology, and changes in serum biochemistry indicative of systemic effects. Each study selected for modeling was of high quality and show effects at low doses. In all cases but one (Luebker et al. 2005a) the POD for the analysis was a NOAEL rather than a LOAEL. The developmental effects of reduced pup body weight and survival occurred in the absence of changes in maternal liver weight, indicating that maternal toxicity and PPARα were not confounding variables.

The external dose NOAELs and LOAELs from other studies summarized in Tables 4-1 and 4-2 that lacked serum information are comparable to those in the modeled studies. For example, the NOAEL in the Long et al. (2013) 90-day mouse study for effects on learning and memory is 0.43 mg/kg/day (Table 4-1) compared to the 0.3 mg/kg/day for Butenhoff et al. (2009) in rats and the LOAEL for mice is 2.15 mg/kg/day compared to the value of 1 mg/kg/day for rats. The LOAEL from Luebker et al. (2005a) of 0.4 mg/kg/day for decreased pup body weight is not unlike the 0.5 mg/kg/day observed by Lv et al. (2013) for decreased pup body weight and increased insulin resistance (Table 4-2). The 1.0 mg/kg/day NOAEL and 2.0 mg/kg/day LOAEL for decreased body weight in rat dams and pups combined with decreased pup survival (Lau et al. 2003; Thibodeaux et al. 2003) are quite similar to the corresponding values of 1 and 5 mg/kg/day, respectively, in the study of mice conducted by the same authors (increased maternal liver weight and delayed pup eye opening).

Table 4-8. Human Equivalent Doses Derived from the Modeled Animal Average Serum Values

Study	Dosing duration days	NOAEL mg/kg/d	NOAEL Av serum µg/mL	HED mg/kg/d	LOAEL mg/kg/d	LOAEL Av serum µg/mL	HED mg/kg/d
Seacat et al. 2002 monkey: ↑liver weight + histopathology; ↓body weight; ↓T3; ↑TSH	182	0.15	38	0.0031	0.75	157	0.013
Seacat et al. 2003 male rat: ↑liver weight, centrilobular vacuolization,↑ ALT, ↑BUN	98	0.34	16.5	0.0013	1.33	64.6	0.0052
Luebker et al. 2005b rat: ↓pup body weight	84	0.1	6.26	0.00051	0.4	25	0.002
Luebker et al. 2005a rat: ↓ pup body weight	63	None	None	None	0.4	19.9	0.0016
Luebker et al. 2005a rat: ↓maternal body weight, gestation length and pup survival	63	0.4	19.9	0.0016	0.8	39.7	0.0032
Butenhoff et al. 2009 rat developmental neurotoxictiy: ↑motor activity, ↓habituation	41	0.3	10.4	0.00084	1.0	34.6	0.0028
Lau et al. 2003 rat: ↓ pup survival; maternal and pup body weight	19	1.0	17.5	0.0014	2.0	35.1	0.0028

4.1.1.2 RfD Quantification

Several acceptable PODs can be used in the process of RfD development based on the modeled human equivalent doses (Table 4-9).

All modeled studies identified a NOAEL for PFOS except for the endpoint of offspring growth as measured by body weight in the one-generation study by Luebker et al. (2005a) with a LOAEL of 0.4 mg/kg/day. The same external dose was also a LOAEL for the same effect in the two-generation study by Luebker et al. (2005b), with a NOAEL of 0.1 mg/kg/day, a dose not tested in the one-generation study. The calculated HED values associated with no adverse effects on developmental and liver endpoints (NOAELs) were very similar with a range of 0.00051–0.0031 mg/kg/day.

Two effect-level doses were modeled from the Luebker et al. (2005a) one-generation rat study: (1) the NOAEL for the effects on pup survival (0.4 mg/kg/day), which was the LOAEL for the body weight effect, and (2) the LOAEL (0.8 mg/kg/day) for the pup survival effect to illustrate the importance of the body weight LOAEL in both the one- and two-generation Luebker et al. (2005a, 2005b) studies. In the two-generation study, 1.6 mg/kg/day resulted in the death of > 26% of the pups between LD 2 and 4. Support for the pup survival serum level LOAEL is provided by the Lau et al. (2003) rat study, with a HED for the same end point that is comparable to that in the Luebker et al. (2005b) study (0.0028 mg/L and 0.0032 mg/L, respectively).

Table 4-9. POD Outcomes for the HEDs from the Pharmacokinetic Model Average Serum Values

POD	POD Value mg/kg/day	UF_H	UF_A	UF_L	UF_S	UF_D	UF_{total}	Candidate RfD mg/kg/day
PK-HED (Seacat et al. 2003; rat, NOAEL,↑ ALT, ↑BUN	0.0013	10	3	1	1	1	30	0.00004
PK-HED (Lau et al. 2003; rat, NOAEL ↓pup survival)	0.0014	10	3	1	1	1	30	0.00005
PK-HED (Butenhoff et al. 2009; rat, NOAEL ↑motor activity ↓habituation)	0.00084	10	3	1	1	1	30	0.00003
PK-HED (Luebker et al. 2005b; rat, NOAEL↓pup body wt)	0.00051	10	3	1	1	1	30	0.00002
PK-HED LOAEL (Luebker et al. 2005a; rat, LOAEL↓pup body wt)	0.0016	10	3	3	1	1	100	0.00002
PK-HED (Luebker et al. 2005a; rat, NOAEL ↓pup survival)	0.0016	10	3	1	1	1	30	0.00005

Notes: UF_H: Intra-individual uncertainty factor, UF_A: Interspecies uncertainty factor, UF_S: Subchronic to chronic uncertainty factor, UF_L: LOAEL to NOAEL uncertainty factor, UF_D: incomplete database uncertainty factor, UF_{total}: Total (multiplied) uncertainty factor

The pharmacokinetically-modeled average serum values from the animal studies are restricted to the animal species selected for their low dose response to oral PFOS intakes. However, the modeled average serum values from animals are several orders of magnitude greater than measured values in humans. Thus, extrapolation to humans adds a layer of uncertainty that needs to be accommodated in deriving the RfD.

HED PODs. The PK HEDs derived from Seacat et al. (2003), Lau et al. (2003), Butenhoff et al. (2009), and Luebker et al. (2005a, 2005b) were each examined as the potential basis for the RfD (ph). The Seacat et al. (2002) results for male monkeys were not utilized in the derivation of the RfD because of the premature deaths in two of the six males at the LOAEL. Each of these studies, except one, contained a NOAEL from which the HED could be derived. The outcomes for potential RfD values are similar demonstrating the ability of the model to normalize the animal data across species, gender, and exposure duration.

Uncertainty Factors

An uncertainty factor for intraspecies variability (**UF_H**) of 10 is assigned to account for variability in the responses within the human populations because of both intrinsic (genetic, life stage, health status) and extrinsic (life style) factors that can influence the response to exposure. No information was available relative to variability in the human population that supports a factor other than 10.

An uncertainty factor for interspecies variability (**UF_A**) of three was applied to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). The 3-fold factor is applied to account for toxicodynamic differences between the animals and

humans. The HEDs were derived using average serum values from a model to account for pharmacokinetic differences between animals and humans.

An uncertainty factor for LOAEL to NOAEL extrapolation (**UF_L**) of one was applied to all PODs, except the LOAEL of 0.4 mg/kg/day for effects on pup body weight in the one-generation Luebker et al. (2005a) study. A value of three is assigned for this study based on the fact that the NOAEL for this effect was 0.1 mg/kg/day in the two-generation (Luebker et al. 2005b) study, a dose that was not used in the one-generation study. The LOAEL in the two-generation study was 0.4 mg/kg/day, demonstrating that the difference between a NOAEL and LOAEL for the body weight is not a factor of 10, the default value for NOAEL/LOAEL extrapolation.

An uncertainty factor for extrapolation from a subchronic to a chronic exposure duration (**UF_S**) of one was applied because the PODs are based on average serum concentrations for all studies except Seacat et al. (2013). The studies for developmental endpoints are not adjusted for lifetime exposures because they cover a critical window of exposure with lifetime consequences. The average serum value associated with the developmental (Luebker et al. 2005b) POD is lower than that for any of the other modeled studies including those with systemic effects after longer exposures. It is accordingly more protective of adverse effects than the POD for any of the longer-term studies despite the limited exposure duration. The serum from the Seacat et al. (2013) study was collected at 14 weeks. Some of the animals in the study continued to be dosed for a total of 105 weeks, but the effects observed at the LOAEL did not increase in magnitude. Serum measurements taken before sacrifice were 2-fold higher at 14 weeks in males than they were at 105 weeks. Concentrations of PFOS in the liver were lower at 105 weeks than they were at 14 weeks. The PFOS concentrations in the diet were constant. SDs about the monitored ALT and BUN were broad indicating higher sensitivity is some animals than others. The serum and effects data for the male rats justify the subchronic to chronic adjustment to the study NOAEL for this study.

A database uncertainty factor (**UF_D**) of one was applied to account for deficiencies in the database for PFOS. The epidemiology data provide strong support for the identification of hazards observed following exposure to PFOS in the laboratory animal studies and human relevance. However, uncertainties in the use of the available epidemiology data precluded their use at this time in the quantification of the effect level for derivation of the drinking water health advisory. In animals, comprehensive oral short term, subchronic, and chronic studies in three species and several strains of laboratory animals have been conducted and published in the peer reviewed literature. Additionally, there are several neurotoxicity studies (including developmental neurotoxicity) and several reproductive (including one- and two-generation reproductive toxicity studies) and developmental toxicity studies including assessment of immune effects following developmental exposure.

RfD Selection

Based on the consistency of the response and of the use of the most sensitive endpoint, developmental toxicity, as the critical effect, the RfD of 0.00002 mg/kg/day from Luebker et al. (2005a) is selected as the RfD for PFOS. This RfD is derived from reduced pup body weight in the two-generation study in rats. The POD for the derivation of the RfD for PFOS is the HED of 0.00051 mg/kg/day that corresponds to a NOAEL that represents approximately 30% of steady-state concentration. An UF of 30 (10 UF_H and 3 UF_A) was applied to the HED NOAEL to derive an RfD of 0.00002 mg/kg/day. This is supported by the 0.00002 mg/kg/day value derived from the LOAEL for the same effect in the one-generation Luebker et al. (2005a) study and the

0.00003 mg/kg/day value for neonatal neurodevelopmental effects in the Butenhoff et al. (2009) study.

Low body weights in neonates are a biomarker for developmental deficits and linked to problems often manifest later in life. A study by Lv et al. (2013) that lacked serum data for pharmacokinetic modeling identified 0.5 mg/kg/day as a LOAEL for effects on body weight in Wistar rat pups exposed during gestation, an observation that was accompanied by increased insulin resistance, problems with glucose homeostasis, and hepatic fat accumulation in the pups as adults. A similar effect on glucose homeostasis was observed in CD-1 mice at PND 63 in a study by Wan et al. (2014b), with a dose of 3 mg/kg/day for animals receiving a diet with regular fat content. For animals receiving a high fat diet, the LOAEL was 0.3 mg/kg/day. Support for the neurodevelopmental effects in Butenhoff et al. (2009) at a dose 1 mg/kg/day kg/day is provided by the NOAEL (0.43 mg/kg/day) in the Long et al. (2013) 90-day mouse study for effects on learning and memory.

Use of the developmental toxicity endpoint is directly relevant to human health because *in utero* and lactational exposures have been demonstrated. PFOS has been measured in the blood of newborns (Spliethoff et al. 2008), in human breast milk (Kärrman et al. 2010), and in serum samples from children aged 5–15 years (Dong et al. 2013; Grandjean et al. 2012). A human epidemiology study found no association with maternal PFOS levels and motor or mental development of their children; the mean maternal serum concentration was approximately 0.035 µg/mL (Fei et al. 2008b).

4.1.2 RfC Determination

The only inhalation study available is an acute lethality inhalation study in rats (Rusch et al. 1979); no inhalation data are available in humans. Thus, data are insufficient for the development of an RfC for PFOS.

4.2 Dose-Response for Cancer Effects

Under the EPA (2005a) *Guidelines for Carcinogen Risk Assessment*, when the evidence from the epidemiology studies and the cancer bioassays is *suggestive* for carcinogenicity, a quantitative estimate of risk is generally not performed unless there is a well-conducted study that could serve a useful purpose by providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In the case of PFOS, the existing evidence does not support a strong correlation between the tumor incidence and dose to justify a quantitative assessment.

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Appendix A: Literature Search Strategy Developing the Search

The literature search strategy was planned with input from EPA library services staff. CAS numbers served as the basis for identification of relevant search terms. Trial searches were conducted and results were evaluated to refine the search strategy (e.g., to prevent retrieval of citations unrelated to health and occurrence). The search string was refined to improve the relevancy of the results. All searches were conducted in the PubMed database, which contains peer-reviewed journal abstracts and articles in various biological, medical, public health, and chemical topics. The first search string (as well as future iterations) is presented below.

Every two weeks, a search was run in PubMed and a bibliography of the search results was compiled.

In 2012, the State of New Jersey Department of Environmental Protection (NJDEP) initiated a monthly search in PubMed for emerging literature on perfluorinated chemicals primarily from the carboxylic acid and sulfonate families. These searches were provided to the EPA on a monthly basis. There was a high degree of overlap with the results from the EPA search, thus increasing the confidence in the search strategy.

In 2013, the EPA search strategy was expanded to cover other members of the perfluorocarboxylic acids (C-4 to C-12) and sulfonate families (C-4, C-6, C-8). The search string was altered in June of 2013 to rely more on the search features offered by PubMed.

A change in the PubMed database structure in 2015 required some modification to the search strategy. A search in August 2015 returned more than 4,000 records, a number that was inconsistent with prior searches. The cause was PubMed's lack of recognition of the search term, "**Heptadecafluorooctane-1-sulphonic acid"** and interpreting the term as **"ACID."** The resolution is highlighted in the search strings below.

All search iterations are noted below.

Search Strategy Examples: (Arranged from most recent to oldest).

2015

Search: perfluorooctanoate OR "perfluorooctanoic acid" OR "perfluoroctanoic acid" OR pfoa OR "perfluorinated chemicals" OR "perfluorinated compounds" OR "perfluorinated homologue groups" OR "perfluorinated contaminants" OR "perfluorinated surfactants" OR perfluoroalkyl acids OR "perfluorinated alkylated substances" OR "perfluoroalkylated substances" OR pfba OR "perfluorobutanoic acid" OR perfluorochemicals OR "telomer alcohol" OR "telomer alcohols" OR "fluorotelomer alcohols" OR "polyfluoroalkyl compounds" OR "perfluorooctane sulfonate" OR pfos OR "perfluorooctanesulfonic acid" OR "perfluorooctane sulfonic acid" OR "perfluorooctane sulphonate" OR perfluorooctane sulfonate OR "perfluorooctanyl sulfonate" OR "Heptadecafluorooctane-1-sulphonic" OR "Heptadecafluoro-1-octanesulfonic acid" OR perfluorononanoate OR pfhxa OR "perfluorohexanoic acid" OR "fluorinated surfactants"

Filters: English.
Frequency: Every 2 weeks

September 2013

Search: perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkyl acids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctane sulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants”

Filters: English.
Frequency: Every 2 weeks

June 2013

Search: (PFOA[tw] OR perfluorooctanoic acid[tw] OR 335-67-1[tw] OR PFBA[tw] OR perfluorobutanoate[tw] OR 3794-64-7[tw] OR PFDA[tw] OR perflurordecanoic acid[tw] OR 335-76-2[tw] OR PFHpA[tw] OR perfluoroheptanoic acid[tw] OR 375-85-9[tw] OR PFHxA[tw] OR perfluorohexanoic acid[tw] OR 307-24-4[tw] OR PFNA[tw] OR perfluorononanoic acid[tw] OR 375-95-1[tw] OR PFPtA[tw] OR perfluoropentanoic acid[tw] OR 2706-90-3[tw] OR PFPA[tw] OR pentafluoropropionic acid[tw] OR 422-64-0[tw]) AND (human* [tw] OR mammal*[tw]) NOT (environment* OR ecolog*)

Filters: English.
Frequency: Every 2 weeks

February 2013

Search: perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkyl acids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctane sulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants”

Filters: English.
Frequency: Every 2 weeks

June 2011

Search (perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkylacids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR

“perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctanesulfonate OR “perfluorooctanyl sulfonate” OR “Heptadecafluorooctane-1-sulphonic acid” OR “Heptadecafluoro-1-octanesulfonic acid” OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants” OR 335-67-1 [rn])

Limits: Publication Date [Dates will change for each search], English Language only.

June 2009

Search (perfluorooctanoate OR “perfluorooctanoic acid” OR “perfluoroctanoic acid” OR pfoa OR “perfluorinated chemicals” OR “perfluorinated compounds” OR “perfluorinated homologue groups” OR “perfluorinated contaminants” OR “perfluorinated surfactants” OR perfluoroalkylacids OR “perfluorinated alkylated substances” OR “perfluoroalkylated substances” OR pfba OR “perfluorobutanoic acid” OR perfluorochemicals OR “telomer alcohol” OR “telomer alcohols” OR “fluorotelomer alcohols” OR “polyfluoroalkyl compounds” OR “perfluorooctane sulfonate” OR pfos OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulfonic acid” OR “perfluorooctane sulphonate” OR perfluorooctanesulfonate OR perfluorononanoate OR pfhxa OR “perfluorohexanoic acid” OR “fluorinated surfactants” OR 335-67-1 [rn] OR 1763-23-1 [rn])

Limits: Entrez Date from 2009/04/07 to 2009/04/12.

New Jersey Search Terms

Search: perfluorinated OR perfluorooctanoate OR perfluorononanoate OR perfluorooctanesulfonate OR perfluorooctanesulphonate OR perfluoroalkylated OR perfluoroalkyl OR polyfluoroalkyl OR polyfluorinated OR PFBA OR PFBS OR PFDA OR PFHA OR PFHPA OR PFHXA OR PFHXS OR PFNA OR PFOA OR PFOAs OR PFOS OR PFUNDA OR “perfluorooctanoic acid” OR “perfluoro octanoic acid” OR “perfluorooctane sulfonate” OR “perfluorooctane sulfonic acid” OR “perfluorooctanesulfonic acid” OR “perfluorooctane sulphonate” OR “perfluorooctanyl sulfonate” OR “perfluorobutanoic acid” OR “perfluoroalkyl acids” OR “perfluorononanoic acid” OR “perfluorohexanoic acid” OR “perfluorohexane sulfonate” OR “perfluorohexane sulphonate” OR perfluorobutanoate OR “perfluoro butanoate” OR perfluorohexanoate OR “perfluoro hexanoate”

Filters: 1

Appendix B: Studies Evaluated Since August 2014

The tables that follow identify the papers that were retrieved and reviewed for inclusion following the August 2014 peer review for the draft PFOS Health Effects Support Document. The papers listed include those recommended by the peer reviewers or public commenters, as well as those identified from the literature searches between the completion of the peer review draft and December 2015. Papers included in the final Health Effects Support Document (HESD) are noted and reasons provided for those that were not included in the final document.

The tables for document retrieval and review are followed by updated versions of the summaries of the epidemiology summary tables from the peer reviewed draft as recommended by the peer reviewers. They are a useful tool to facilitate a high level comparison of the study outcomes for each of the epidemiological study groupings.

The criteria utilized in determining the papers that were included in the HESD after the peer review and presented in the Background were the following:

1. The study examines a toxicity endpoint or population that had not been examined by studies already present in the draft assessment.
2. Aspects of the study design, such as the size of the population exposed or quantification approach, make it superior to key studies already included in the draft document.
3. The data contribute substantially to the weight of evidence for any of the toxicity endpoints covered by the draft document.
4. There are elements of the study design that merit its inclusion in the draft assessment based on its contribution to the mode of action or the quantification approach.
5. The study elucidates the mode of action for any toxicity endpoint or toxicokinetic property associated with PFOS exposure.
6. The effects observed differ from those in other studies with comparable protocols.

Table B-1. PFOS Epi Papers—Post Peer Review (Retrieved and Reviewed)

Authors and year	Topic—key words	Status/Notes
Andersen et al. 2013	Postnatal growth	Added PFOA/PFOS
Back et al. 2015	Time to pregnancy	Added PFOA
Barrett et al. 2015	Ovarian hormone	Not Added—No association observed for PFOA; PFOS was not included in the assessment
Berg et al. 2015	Thyroid	Added PFOA/PFOS
Bonefeld-Jørgenson et al. 2014	Breast cancer	Added PFOA/PFOS
Bonefeld-Jørgenson et al. 2011	Breast cancer	Added PFOA/PFOS
Brieger et al. 2011	Immune effects	Already presented in PFOS/PFOA
Buck Louis et al. 2015	Semen quality	Added PFOA/PFOS
Chang et al. 2014	Analysis of human cancer studies	Added PFOA in the cancer weight of evidence section
Chen et al. 2015	Birth weight	Added PFOS
Dankers et al. 2013	Blood-testis barrier	Reviewed,—not added; Study of an assay that used PFOA as one chemical in the test battery
Darrow et al. 2013	Reproductive outcome	Added PFOA/PFOS
Darrow et al. 2014	Miscarriage	Added PFOA/PFOS

Authors and year	Topic—key words	Status/Notes
Donauer et al. 2015	Infant Neurobehavior	Not added—negative for PFOS; No statistical differences in PFOA levels during pregnancy and any neuro endpoint. Better studies.
Eriksen et al. 2013	Total cholesterol—Danish	Added PFOA/PFOS
Fitz-Simon et al. 2013	Serum lipids	Added PFOA/PFOS
Fisher et al. 2013	Plasma lipids	Added PFOA/PFOS
Fletcher et al. 2013	Cholesterol–genes	Added PFOA/PFOS
Fu et al. 2014	Serum lipids in Chinese subjects	Not added: Chinese population, dataset available on U.S. population. More branched chain isomers found among the people in China.
Geiger et al. 2014a	Lipids/children	Added PFOA/PFOS
Geiger et al. 2014b	Hypertension/children	Added PFOA/PFOS
Ghisari et al. 2014	Breast cancer—Inuit	Not added; same population as Bonefeld-Jørgensen et al. 2014; this study focuses on gene polymorphisms
Governini et al. 2015	DNA effects in sperm	Added PFOA/PFOS
Grandjean and Clapp 2015	Health Risks	Not added; the primary studies are already included in the documents.
Granum et al. 2013	Immune children	Added PFOA/PFOS
Hardell et al. 2014	Prostate cancer	Added PFOA/PFOS
Høyer et al. 2015a	Human weight	Added PFOA/PFOS
Høyer et al. 2015b	Behavior motor development	Added PFOA/PFOS
Humblet et al. 2014	Asthma	Added PFOA/PFOS
Jain 2014	NHANES	Added PFOA/PFOS
Innes et al. 2014	Colorectal cancer	Added PFOA/PFOS
Joensen et al. 2013	Sperm	Added PFOA/PFOS
Kerger et al. 2011	Cholesterol C8	Added; demographics for cholesterol and PFOS in summary section of epi studies
Kjeldsen and Bonefeld-Jørgensen 2013	Sex hormones	Covered multiple PFAS *in vitro* no impact on weight of evidence
Kristensen et al. 2013	Prenatal female repro	Added PFOA/PFOS
Liew et al. 2014	Cerebral palsy children	Added PFOA/PFOS
Looker et al. 2014	Immune	Added PFOA/PFOS
López-Doval et al. 2014	Male repro	Added PFOS
Maisonet et al. 2015	Gestational diabetes	Added PFOA/PFOS
Maisonet et al. 2012	Birth weight	Added PFOA/PFOS
Mørck et al. 2015	PFAS levels in children	Not added; No significant impact
Okada et al. 2014	Allergy children	Added PFOS
Osuna et al. 2014	Antibodies PFOS PFOA	Not added; focus more on methylHg and PCB than PFAS; only n = 38 as preliminary study
Roth and Wilks 2014	Neurodevelopmental	Not added; no significant impact
Shrestha et al. 2015	Thyroid	Added PFOA/PFOS
Starling et al. 2014	Plasma lipids	Added PFOA/PFOS
Steenland et al. 2015	Workers	Added PFOA

Authors and year	Topic—key words	Status/Notes
Stein et al. 2009	Pregnancy	Added PFOA
Taylor et al. 2014	Menopause	Added PFOA/PFOS
Vanden Heuvel 2013	Serum lipids	Not added; is a rebuttal of Fletcher et al. 2013 conclusions. No significant impact
Vassiliadou et al. 2010	PFOS in cancer vs non-cancer patients	Added PFOA/PFOS
Vélez et al. 2015	Fertility	Added PFOA/PFOS
Verner et al. 2015	Fetal growth GFR	Added PFOA/PFOS
Verner and Longnecker 2015	Menstruation/excretion	Added PFOS
Vested et al. 2013	Semen quality and hormones	Added PFOS/PFOS
Vesterinen et al. 2014	Fetal Growth GFR	Added PFOA/PFOS
Wang et al. 2013	Thyroid	Added PFOA/PFOS
Watkins et al. 2013	Kidney function	Added PFOA/PFOS
Webster et al. 2014	Maternal thyroid	Added PFOA/PFOS
Webster et al. 2015	Thyroid—iodine statue	Added PFOS
Wen et al. 2013	Thyroid	Added PFOA/PFOS
Yeung et al. 2013	Liver cancer	Added PFOA/PFOS
Zhang et al. 2015	Gestational diabetes	Added PFOA/PFOS

Table B-2. PFOA Post Peer Review Animal Toxicity Studies

Authors and year	Topic	Action notes
Bjork et al. 2011	Nuclear receptor activation	In vitro, mechanistic findings comparable to studies already included
Corsini et al. 2014	Immune data review	Not added; no significant impact
Corsini et al. 2012	Immune in vitro data review	Not added; no significant impact
Dewitt et al. 2015	Immunotoxicity	Added PFOA
Fenton 2015	Repro editorial	Not added
Filgo et al. 2015	Liver tumors in females developmentally exposed	Added PFOA
Hall et al. 2012	PPARα and cancer	Cited in synthesis. Paper on adversity of liver hypertrophy PFOA/ PFOS
Koustas et al. 2014	Fetal growth (animal studies) navigation guide	Added PFOA
Liu et al. 2015	Testes	Added PFOA
Long et al. 2013	Neurotoxicity adult PFOS	Added PFOS
Lu et al. 2015	Testes	Added PFOA
Ngo et al. 2014	Tumors mice Min/+ PFOS	Added PFOS
Post et al. 2012	Review paper	Not added. Key studies included in the document; no significant impact
Quist et al. 2015	Liver histopathology/high fat diet post weaning exposure	Added PFOA
Rigden et al. 2015	Acute liver effects	Added PFOA
Shabalina et al. 2015	Brown fat uncoupling protein 1	Not added. Mechanistic; no significant impact
Sheng et al. 2016	Binding to liver fatty acid binding protein	Not added; no significant impact, topic covered by other papers

Authors and year	Topic	Action notes
Tan et al. 2012	Gene activation	Added PFOA/PFOS
Tan et al. 2013	Gene activation dietary fat	Added PFOA
Tucker et al. 2015	Mammary gland	Added PFOA
Wallace et al. 2013	Mitochodrial respiration	Not added. No significant impact, topic covered by other papers
Wan et al. 2014b	Glucose metabolism	Added PFOS
Wan et al. 2012	Hepatic steatosis	Added PFOS
Wan et al. 2014a	Sertoli cells	Added PFOS
F. Wang et al. 2015	MiRNA liver PFOS early life	Not added; no significant impact
S. Wang et al. 2014	Lysine decarboxylase	Added PFOA/PFOS
L. Wang et al. 2014	Inhibition of LDL	Added PFOS
Y. Wang et al. 2015	Special learning and memory	Added PFOS
Yan et al. 2015	Glucose homeostasis	Not added. Dose-response in Wan (2014b) presented (more robust). Single dose for whole animal
Yu et al. 2015	Thyroid PFOS isomers	Added PFOS
Zeng et al. 2014	Mitochondrial mediated apoptosis of the heart	Added PFOS
L. Zhang et al. 2013	Fatty acid binding protein	Added PFOA/PFOS
Y. Zhang et al. 2013	Biological half-life	Added PFOA/PFOS
W. Zhang et al. 2014	Breast cancer cell invasion—mechanistic	Not added; *in vitro*, no significant impact
Zhao et al. 2014	Testosterone reduction in Leydig cells PFOS	Added PFOS

Table B-3. Toxicokinetics: Post Peer Review

Authors and year	Topic	Action Notes
D'Alessandro et al. 2013	Serum albumin	Added PFOS
Augustine et al. 2005	Transporter expression testes	Not added background paper on testes transporters –no relationship to PFOA PFOA or any PFAS
Beesoon et al. 2011	Isomer profile	Added PFOA
Beesoon and Martin 2015	Albumin binding	Added PFOA
Cui et al. 2010	Excretion subchronic	Added PFOA/PFOS
Fàbrega et al. 2014	PK model	Added PFOA/PFOS
Kerstner-Wood et al. 2003	Plasma protein binding	Added—PFOA/PFOS
Klaassen and Aleksunes 2010	Transporter paper—Provided diagram of kidney transporters	Added PFOA
Loccisano et al. 2013	PK model—Human	Added PFOA/PFOS
Mondal et al. 2014	Breast milk	Added PFOS/PFOA
Ospinal-Jimenez and Pozzo 2012	Protein denaturation	Added PFOS
Pérez et al. 2013	Human tissue levels	New PFOA/PFOS
Ren et al. 2015	Thyroid hormone receptor binding (*in vitro*)	Added PFOA/PFOS
Rigden et al. 2015	Liver and excretion	Added PFOA
Shabalina et al. 2015	Brown fat	Not added; No information on MOA for body weight effects in the animal or human studies
Slitt et al. 2007	Transporter expression PFOA	Not added. Reported on transporters during extrahepatic cholestasis. No data on PFOA and PFOS. No significant impact.
Tucker et al. 2015	Menstruation-excretory route	Added PFOA
Verner and Longnecker 2015	Excretion PFOS	Added PFOS
Wambaugh et al. 2013	PK model	Added PFOA/PFOS
Wong et al. 2014	Menstrual blood as excretory route	Added PFOA/PFOS
T. Zhang et al. 2014	Excretion general population and pregnancy	Added PFOA/PFOS
L. Zhang et al. 2014	PPAR gamma	Added PFOS
Y. Zhang et al. 2013	Excretion, half-life	Added PFOA/PFOS
T. Zhang et al. 2013	Maternal transfer	Added PFOA/PFOS

Table B-4. Association of Serum PFOS with Serum Lipids and Uric Acid

Reference	Study type	n	Mean or median serum PFOS (µg/mL)	TC	VLDL	LDL	HDL	Non-HDL	TG	UA
Occupational Populations										
Olsen et al. 2001a, 2003b	Cross-sectional	263 (Decatur) 255 (Antwerp)	1.40 0.96	↑	NM	NM	↔	NM	↑	NM
Olsen et al. 2001b, 2003b	Longitudinal; ~5 years	175 (Decatur and Antwerp combined for analysis)	2.62 (baseline) 1.67 (follow-up) (Decatur) 1.87 (baseline) 1.16 (follow-up) (Antwerp)	↔	NM	NM	↔	NM	↔	NM
General Populations										
Steenland et al. 2009	Cross-sectional (C8)	46,294	0.022	↑	NM	↑	↔	NM	↑	NM
Steenland et al. 2010	Cross-sectional (C8)	54,951	0.023	NM	NM	NM	NM	NM	NM	↑
Frisbee et al. 2010	Cross-sectional (C8, children)	12,476	0.023	↑	NM	↑	↑	NM	↔	NM
Fitz-Simon et al. 2013	Longitudinal; 4.4 years (C8)	521	0.023 (baseline) 0.011 (follow-up)	↔	NM	↔	↔	NM	↔	NM
Nelson et al. 2010	Cross-sectional (NHANES)	860	0.025	↑	NM	↔	↔	↑	NM	NM
Lin et al. 2009	Cross-sectional (NHANES)	3,685	0.0031 (12–< 20 yrs) 0.0032 (≥ 20 yrs)	NM	NM	NM	↑	NM	↔	NM
Maisonet et al. 2015	Longitudinal; prenatal and aged 7 and 15 years	111 (age 7 years) 88 (age 15 years)	0.022	↔	NM	↔	↔	NM	↔	NM
Timmermann et al. 2014	Cross-sectional (children 8–10 years)	499	0.0412	↔	NM	↔	↔	NM	↔ (normal wt) ↑ (overweight)	NM
Château-Degat et al. 2010	Cross-sectional	723	0.019	↔	NM	↔	↑	NM	↔	NM
Eriksen et al. 2013	Cross-sectional	663	0.036	↑	NM	NM	NM	NM	NM	NM

Reference	Study type	n	Mean or median serum PFOS (µg/mL)	TC	VLDL	LDL	HDL	Non-HDL	TG	UA
Starling et al. 2014	Cross-sectional (maternal at 14–26 weeks gestation)	891	0.013	↑	NM	↑	↑	NM	↔	NM
Fisher et al. 2013	Cross-sectional	2,700	0.0084	↔	NM	↔	↔	NM	NM	NM

Notes: ↑ = positive association; ↓= negative association; ↔ = no association; TC = total cholesterol; VLDL= very low density lipoprotein; LDL= low density lipoprotein; non-HDL= TC(VLDL,IDL, LDL)-HDL; HDL= high density lipoprotein; TG = triglycerides; UA = uric acid; NM = not measured

Table B-5. Association of Serum PFOS with Reproductive and Developmental Outcomes

Study	Study type	n	Mean or median serum PFOS (µg/mL)	Outcome	Measures at birth	Growth/ Development	Fecundity/ Fertility
Occupational Populations							
Grice et al. 2007	Survey	263	Not measured; exposure categorized by job	↔ (any adverse)	NM	NM	NM
General Populations—Measures at Birth							
Fei et al. 2007, 2008a, 2010a	Cross-sectional	1,400	0.033–0.039 (first trimester)	↔ (gestation length) ↓ (length of breastfeeding)	↔ (weight) ↔ (size) ↔ (Apgar score)	NM	NM
Monroy et al. 2008	Cross-sectional	101	0.018 (maternal at 24–28 weeks) 0.016 (maternal at delivery) 0.0072 (umbilical cord blood)	↔ (gestation length)	↔ (weight)	NM	NM
Washino et al. 2009	Cross-sectional	428	0.0056 (maternal)	NM	↑ (low weight females only)	NM	NM
Hamm et al. 2009	Cross-sectional	252	0.009 (maternal)	↔ (gestation length)	↔ (weight)	NM	NM
Stein et al. 2009	Cross-sectional (C8)	5,262	0.014	↔ (miscarriage)	↑ (low weight)	NM	NM
Darrow et al. 2013, 2014	Cross-sectional (C8)	1330	0.016–0.017	↔ (preterm) ↑ (miscarriage)	↔ (low weight) ↑ (birth weight decreased)	NM	↑ (hypertension)
Apelberg et al. 2007	Cross-sectional	293	0.005 (cord blood)	↔ (gestational age)	↓ (weight, head circumference, ponderal index)	NM	NM
General Populations—Measures of Postnatal Growth							
Fei et al. 2008b	Cross-sectional	1,400	0.033–0.039 (first trimester)	NM	NM	↔ (at 6 months) ↑ (at 18 months; sitting up later)	MN
Liew et al. 2014	Cross-sectional	156 cases 550 controls	0.026–0.029 (first trimester)	NM	NM	↑ (cerebral palsy in boys)	NM

Study	Study type	n	Mean or median serum PFOS (µg/mL)	Outcome	Measures at birth	Growth/ Development	Fecundity/ Fertility
Andersen et al. 2010	Cross-sectional	1,010	0.0334 (first trimester)	NM	↓ (birth weight in girls)	↓ (weight and BMI at 12 months in boys)	NM
Andersen et al. 2013	Cross-sectional	811 (children at age 7 years)	0.033–0.039 (first trimester)	NM	NM	↔ (height, weight, waist measurement, risk of overweight)	NM
Fei and Olsen 2011	Cross-sectional	787 (behavior) 537 (coordination)	0.036 (first trimester)	NM	NM	↔ (behavior and coordination at 7 years)	NM
Høyer et al. 2015b	Cross-sectional	1,106	0.01 (maternal)	NM	NM	↔ (motor skills, hyperactivity)	NM
Hoffman et al. 2010	Cross-sectional (NHANES)	571 (children)	0.023	NM	NM	↑ (ADHD)	NM
Høyer et al. 2015a	Cross-sectional	1,022 (children)	0.005–0.0202 (maternal)	NM	NM	↔ (overweight) ↑ (waist-to-height ratio)	NM
Lopez-Espinosa et al. 2011	Cross-sectional (C8)	3,076 boys 2,931 girls	0.0098–0.036	NM	NM	↑ (delayed puberty)	NM
Kristensen et al. 2013; Vested et al. 2013	Cross-sectional	343 women 169 men (~ 20 years)	0.0211–0.0212 (maternal)	NM	NM	NM	↔ (measures of reproductive function)
Christensen et al. 2011	Cross-sectional	448 girls	0.019–0.02 (maternal)	NM	NM	↔ (age at menarche)	NM
Halldorsson et al. 2012	Cross-sectional	665	0.0285 (maternal)	NM	NM	↑ (overweight in females at 20 years)	NM
General Populations—Male and Female Fertility							
Zhang et al. 2015	Cross-sectional	258	0.012–0.0131 (preconception)	↑ (gestational diabetes)	NM	NM	NM
Vélez et al. 2015	Cross-sectional	1,743	0.005	NM	NM	NM	↔ (time to pregnancy) ↔ (infertility)
Fei et al. 2009	Cross-sectional	1,400	0.033–0.039 (first trimester)	NM	NM	NM	↑ (time to pregnancy) ↑ (infertility)

Study	Study type	n	Mean or median serum PFOS (µg/mL)	Outcome	Measures at birth	Growth/ Development	Fecundity/ Fertility
Knox et al. 2011	Cross-sectional (C8)	25,957	0.018	NM	NM	NM	↑ (early menopause)
Joensen et al. 2009 (PFOA/PFOS combined)	Cross-sectional	105	0.025	NM	NM	NM	↑ (lower number normal sperm) ↔ (testosterone)
Joensen et al. 2013	Cross-sectional	247	0.0085	NM	NM	NM	↔ (semen parameters) ↓ (testosterone)
Buck Louis et al. 2014	Cross-sectional	462	0.017–0.021	NM	NM	NM	↑ (lower % sperm with coiled tail) (total of six PFAS associated with changes in sperm quality)

Notes: ↑ = positive association; ↓= negative association; ↔ = no association; NM = Not Measured

Table B-6. Association of PFOS Level with the Prevalence of Thyroid Disease and Thyroid Hormone Levels

Study	Study type	Population (n)	Mean serum PFOS (µg/mL)	Thyroid Disease	TSH	T3	T4
Olsen et al. 2001a	Cross-sectional	Adult workers (263 Decatur) (255 Antwerp)	 1.4 0.96	NM	↔	↔	↔
Dallaire et al. 2009	Cross-sectional	Adults (623)	0.018	↔	↓	↓	↑
Bloom et al. 2010	Cross-sectional	Adults (31)	0.0196	↔	↔	NM	↔
Melzer et al. 2010	Cross-sectional	Adult (NHANES; 3,966)	0.025 (men) 0.019 (women)	↔ (women) ↑ (men)	NM	NM	NM
Shrestha et al. 2015	Cross-sectional	Adults (51 men, 36 women)	0.036	↔	↔	↔	↑
Pirali et al. 2009	Cross-sectional	Adults (28)	5.3 ng/g thyroid tissue	↔	NM	NM	NM
Wang et al. 2013	Cross-sectional	Women at gestation week 18 (Norwegian Mother/Child Cohort; 903)	0.0128	NM	↑	NM	NM
Berg et al. 2015	Cross-sectional	Women at gestation week 18, day 3 and week 6 after delivery (Norwegian Mother/Child Cohort; 375)	0.00803	NM	↑	↔	↔
Inoue et al. 2004	Cross-sectional	Newborns (15)	0.0016–0.0053 (cord blood)	NM	↔	NM	↔
Chan et al. 2011	Cross-sectional	Women at gestation week 15–20 (974)	0.0074	↔	↔	NM	↔
Webster et al. 2014	Cross-sectional	152 women at gestation week 15–18	0.0017	NM	↔	↔	↔

Notes: ↑= positive association; ↓= negative association; ↔= no association; NM = Not Monitored

Table B-7. Association of Serum PFOS with Markers of Immunotoxicity

Study	Study type	Population (n)	Mean or median serum PFOS (µg/mL)	Disease prevalence in children	Vaccine response
Okada et al. 2012	Prospective cohort	Maternal, third trimester (343)	0.0056	↔	NM
Fei et al. 2010b	Cross-sectional	Maternal, first trimester (1,400)	0.0353	↔	NM
Grandjean et al. 2012	Prospective cohort	Maternal at gestation week 32 (587)	0.0273 (maternal)	NM	↓ (antibody titer in child at age 5 yrs)
Grandjean et al. 2012	Prospective cohort	Children age 5 years (587)	0.0167 (child at age 5 years)	NM	↓ (antibody titer in child at age 7 yrs)
Granum et al. 2013	Prospective cohort	Women at delivery (56)	0.0056 (maternal)	↔	↓ (antibody titer in child at age 3 years)
Humblet et al. 2014	Cross-sectional	Children at 12–19 years (1,877)	0.017 (asthmatics) 0.0168 (non-asthmatics)	↔ for asthma	NM
Dong et al. 2013	Cross-sectional	Children age 10–15 years (231 asthmatics and 225 controls)	0.0455 (asthmatics) 0.0334 (non-asthmatics)	↑ for asthma	NM
Looker et al. 2014	Cross-sectional	Adults (411)	0.0083	NM	↔

Notes: ↑= positive association; ↓= negative association; ↔= no association; NM = Not Measured

Appendix C: Summary of Data

Table C-1. PFOS Toxicokinetic Information

Species	Dose	Route of exposure	Effects observed	PFOS in liver (µg/g)		PFOS in blood (µg/mL)		Reference
				M	F	M	F	
Human	NA	Unknown	↑ TC; ↑ TG	NS		0.96–1.40		Olsen et al. 2001b, 2003b
Human	NA	Unknown	None observed on cholesterol	NS		1.16–2.62		Olsen et al. 2001b, 2003b
Human	NA	Drinking water	↑ TC; ↑ TG; ↑ LDL; ↑ UA	NS	NS	0.022–0.023		Steenland et al. 2009, 2010
Human	NA	Drinking water	↑ TC; ↑ LDL; ↑ HDL	NS	NS	0.023		Frisbee et al. 2010
Human	NA	Drinking water	None observed on cholesterol	NS	NS	0.011–0.023		Fitz-Simon et al. 2013
Human	NA	Unknown	↑ TC; ↑ non-HDL	NS	NS	0.025		Nelson et al. 2010
Human	NA	Unknown	↑ HDL	NS	NS	0.019		Château-Degat et al. 2010
Human	NA	Unknown	↑ TC	NS	NS	0.036		Eriksen et al. 2013
Human	NA	Unknown	↑ TC; ↑ LDL; ↑ HDL	NS	NS	0.013		Starling et al. 2014
Human	NA	Unknown	None observed on cholesterol	NS	NS	0.0084		Fisher et al. 2013
Human	NA	Unknown/ drinking water	Developmental delays	NS	NS	NS	0.0098–0.039	Fei et al. 2008b; Lopez-Espinosa et al. 2011
Human	NA	Unknown/ drinking water	LBW	NS	NS	NS	0.0056–0.016	Washino et al. 2009; Stein et al. 2009; Darrow et al. 2013
Human	NA	Unknown	None on birth outcome; birth weight and length; growth to 7 years	NS	NS	NS	0.009–0.039	Fei et al. 2007, 2008a; Monroy et al. 2008; Hamm et al. 2009; Andersen et al. 2013
Human	NA	Unknown	↑ time to pregnancy	NS	NS	NS	0.033–0.039	Fei et al. 2009

Species	Dose	Route of exposure	Effects observed	PFOS in liver (µg/g)		PFOS in blood (µg/mL)		Reference
				M	F	M	F	
Human	NA	Unknown	Effects on sperm numbers and morphology	NS	NS	0.017–0.025	NS	Joensen et al. 2009; Buck Louis et al. 2014
Human	NA	Unknown	None on semen parameters	NS	NS	0.0085	NS	Joensen et al. 2013
Human	NA	Unknown	None on thyroid hormones	NS	NS	0.96–1.4		Olsen et al. 2001b; 2003b
Human	NA	Unknown	↓TSH, T3; ↑T4	NS	NS	0.018		Dallaire et al. 2009
Human	NA	Unknown	None on thyroid hormones	NS	NS	0.0196		Bloom et al. 2010
Human	NA	Unknown	↑ incidence of thyroid disease (men only)	NS	NS	0.025	0.019	Melzer et al. 2010
Human	NA	Unknown	↑T4	NS	NS	0.036		Shrestha et al. 2015
Human	NA	Unknown	↑TSH	NS	NS	NS	0.008–0.0128 (gestation wk 18)	Wang et al. 2013; Berg et al. 2015
Human	NA	Unknown	None on thyroid hormones	NS	NS	NS	0.0074 (gestation wk 15–20)	Chan et al. 2011
Human	NA	Unknown	None on diseases in children	NS	NS	NS	0.0056 (maternal, third trimester)	Okada et al. 2012
Human	NA	Unknown	None on diseases in children	NS	NS	NS	0.0353 (maternal, first trimester)	Fei et al. 2010b
Human	NA	Unknown	↓ antibody titer in children	NS	NS	NS	0.0273 (maternal, gestation wk 32)	Grandjean et al. 2012
						0.0167 (child age 5 years)		
Human	NA	Unknown	↓ antibody titer in children	NS	NS	NS	0.0056 (maternal at delivery)	Granum et al. 2013
Human	NA	Unknown	↑asthma	NS	NS	0.0455 (asthmatic children)		Dong et al. 2013

Species	Dose	Route of exposure	Effects observed	PFOS in liver (µg/g)		PFOS in blood (µg/mL)		Reference
				M	F	M	F	
Human	NA	Unknown	None on vaccine response	NS	NS	0.0083		Looker et al. 2014
Monkey	0.15 mg/kg/day for 26 weeks with 52 week recovery	capsule	None observed	NS	NS	(serum) wk 1: 4.60 wk 26: 82.6 wk 35: 84.5 wk 79: 19.1	(serum) wk 1: 3.71 wk 26: 66.8 wk 35: 74.7 wk 79: 21.4	Seacat et al. 2002
Monkey	0.75 mg/kg/day for 26 weeks with 52 week recovery	capsule	↑ liver wt ↓ cholesterol and body wt	NS	NS	(serum) wk 1: 21.0 wk 26: 173 wk 35: 181 wk 79: 41.1	(serum) wk 1: 20.4 wk 26: 171 wk 35: 171 wk 79: 41.4	Seacat et al. 2002
Rat	0.018–0.023 mg/kg/day for 104 weeks	diet	None observed	wk 0: 11.0 wk 10: 23.8 wk 105: 7.83	wk 0: 8.71 wk 10: 19.2 wk 105: 12.9	(serum) wk 0: 0.91 wk 14: 4.04 wk 105: 1.31	(serum) wk 0: 1.61 wk 14: 6.96 wk 105: 4.35	Thomford 2002
Rat	0.184–0.247 mg/kg/day for 104 weeks	diet	↑ liver histopath. lesions	wk 0: 47.6 wk 10: 358 wk 105: 70.5	wk 0: 83.0 wk 10: 370 wk 105: 131	(serum) wk 0: 7.57 wk 14: 43.9 wk 105: 22.5	(serum) wk 0: 12.6 wk 14: 64.4 wk 105: 75.0	Thomford 2002
Rat	0.765–1.10 mg/kg/day for 104 weeks	diet	↑ body and liver wt ↑ hepatocellular adenoma	wk 0: 282 wk 10: 568 wk 105: 189	wk 0: 373 wk 10: 635 wk 105: 381	(serum) wk 0: 41.8 wk 14: 148 wk 105: 69.3	(serum) wk 0: 54.0 wk 14: 223 wk 105: 233	Thomford 2002
Rat (male only)	5 mg/kg for 28 days	oral gavage	↓ body wt	345	NS	(whole blood) 72.0	NS	Cui et al. 2009
Rat (male only)	20 mg/kg for 28 days	oral gavage	10/10 died (day 26) hepatic hypertrophy	648	NS	(whole blood) NS	NS	Cui et al. 2009

Species	Dose	Route of exposure	Effects observed	PFOS in liver (µg/g)		PFOS in blood (µg/mL)		Reference
				M	F	M	F	
Rat	0.4 mg/kg 42 days prior to cohabitation through GD 21	oral gavage	↓ maternal and pup body wt	NS	GD 21: dams = 107 fetuses = 30.6	NS	(serum) GD 1: 40.7 GD 7: 40.9 GD 21: dams = 26.2 fetuses = 34.3	Luebker et al. 2005b
Rat	1.6 mg/kg 42 days prior to cohabitation through GD 21	oral gavage	↓ maternal and pup body wt ↓ pup survival	NS	GD 21: dams = 388 fetuses = 86.5	NS	(serum) GD 1: 160 GD 7: 154 GD 21: dams = 136 fetuses = 101	Luebker et al. 2005b
Rat	3.2 mg/kg 42 days prior to cohabitation through GD 21	oral gavage	100% pup mortality by PND 2	NS	GD 21: dams = 610 fetuses = 230	NS	(serum) GD 1: 318 GD 7: 306 GD 21: dams = 155 fetuses = 164	Luebker et al. 2005b
Rat	0.1 mg/kg GD 0 to PND 20	oral gavage	None observed in dams or offspring	PND 21: Offspring = 5.98 PND 72: Offspring = 0.98	GD 20: Dams = 8.35 Offspring = 3.21 PND 21: Dams = NS Offspring = 5.28 PND 72: Dams = NS Offspring = 0.80	(serum) PND 21: Offspring = 1.73 PND 72: Offspring = 0.04	(serum) GD 20: Dams = 1.72 Offspring = 3.91 PND 21: Dams = 3.16 Offspring = 1.77 PND 72: Dams = NS Offspring = 0.21	Butenhoff et al. 2009; Chang et al. 2009

Species	Dose	Route of exposure	Effects observed	PFOS in liver (µg/g)		PFOS in blood (µg/mL)		Reference
				M	F	M	F	
Rat	1.0 mg/kg GD 0 to PND 20	oral gavage	↑ motor activity and ↓ habituation in male offspring	PND 21: Offspring = 44.89 PND 72: Offspring = 7.17	GD 20: Dams = 48.88 Offspring = 20.03 PND 21: Dams = NS Offspring = 41.23 PND 72: Dams = NS Offspring = 7.2	(serum) PND 21: Offspring = 18.61 PND 72: Offspring = 0.56	(serum) GD 20: Dams = 26.63 Offspring = 31.46 PND 21: Dams = 30.48 Offspring = 18.01 PND 72: Dams = NS Offspring = 1.99	Butenhoff et al. 2009; Chang et al. 2009
Rat	1.0 mg/kg/day GDs 2–20	oral gavage	none	NS	NS	NS	19.69	Thibodeaux et al. 2003; Lau et al. 2003
Rat	2.0 mg/kg/day GDs 2–20	oral gavage	↓ dam and pup wt; ↓pup survival	NS	NS	NS	44.33	Thibodeaux et al. 2003; Lau et al. 2003

Note: NS = no sample obtained or recorded

Table C-2. Summary of Animal Studies with Exposure to PFOS

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	20 days	monkey	0, 10, 30, 100, or 300 mg/kg/day 2 monkeys/sex/dose	NOAEL= NA LOAEL= 10 mg/kg/day from deaths at all doses	Goldenthal et al. 1978a
oral gavage	90 days	monkey	0, 0.5, 1.5, or 4.5 mg/kg/day 2 monkeys/sex/dose	NOAEL= NA LOAEL= 0.5 mg/kg/day based on diarrhea and anorexia	Goldenthal et al. 1979
oral (capsule)	182 days	monkey	0, 0.03, 0.15, or 0.75 mg/kg/day 4–6 monkeys/sex/dose	NOAEL= 0.15 mg/kg/day LOAEL= 0.75 mg/kg/day from ↓ body weight, ↑liver wt and ↓cholesterol	Seacat et al. 2002
oral gavage	single dose	rat	0, 100, 215, 464, or 1,000 mg/kg 5 rats/sex/dose	LD50 = 251 mg/kg (combined)	Dean et al. 1978
oral gavage	single dose	rat	0, 12.5, 25, or 50 mg/kg 5 male rats/dose	NOAEL= NA LOAEL= 12.5 mg/kg based on ↓ body weight	Yang et al. 2009
oral gavage	single dose thyroid hormone activity	rat	0 or 15 mg/kg 5/15 female rats/group	Total thyroxine (TT4)-significant ↓ at 2, 6 and 24 hrs Triiodothyronine (TT3) and reverse triiodothyronine (rT3)-significant ↓ at 24 hrs Free thyroxine-significant ↑ at 2 and 6 hrs; normal at 24 hrs	Chang et al. 2008
inhalation	1 hour	rat	0, 1.89, 2.86, 4.88, 6.49, 7.05, 13.9, 24.09, or 45.97 ppm 5 rats/sex/dose	LC50 = 5.2 ppm	Rusch et al. 1979
oral (in diet)	28 days	rat	0, 0.05, 0.18, 0.37, or 1.51 mg/kg/day—males 0, 0.05, 0.22, 0.47, or 1.77 mg/kg/day—females (0, 0.5, 2, 5, or 20 ppm) 5 rats/sex/dose	NOAEL = 0.37 mg/kg/day in males and 0.47 mg/kg/day in females LOAEL = 1.51 mg/kg/day in males and 1.77 mg/kg/day in females, based on ↓ body wt (M/F) and ↓ food consumption (F)	Seacat et al. 2003
oral (in diet)	28 days	rat	0.14, 1.33, 3.21, 6.34 mg/kg/day—males 0.15, 1.43, 3.73, 7.58 mg/kg/day—females (0, 2, 20, 50, or 100 mg/kg diet) 15 rats/sex/dose	NOAEL = 0.14 mg /kg/day in males and NA in females LOAEL = 1.33 mg /kg/day in males and 0.15 mg/kg/day in females based on ↑ relative liver wt	Curran et al. 2008

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	28 days	rat	0, 5, or 20 mg/kg/day 10 males/dose	NOAEL= NA LOAEL= 5 mg/kg/day based on ↓ body wt and lung congestion	Cui et al. 2009
oral gavage	28 days	rat	0, 0.5, 1, 3, or 6 mg/kg/day 19 males/dose	NOAEL = NA LOAEL = 0.5 mg/kg/day based on ↓ LH and testosterone and ↑ FSH	López-Doval et al. 2014
oral (in diet)	90 days	rat	0, 2, 6, 18, 60, or 200 mg/kg/day 5 rats/sex/dose	NOAEL= NA LOAEL= 2 mg/kg/day, from ↑ liver wt, ↓ food consumption	Goldenthal et al. 1978b
oral (in diet)	98 days	rat	0, 0.5, 2.0, 5.0, or 20 ppm 0, 0.03, 0.13, 0.34 or 1.33 mg/kg/day-males 0, 0.04, 0.15, 0.40 or 1.56 mg/kg/day-females 5 rats/sex/dose	NOAEL = 0.34 mg/kg/day in males and 0.40 mg/kg/day in females LOAEL = 1.33 mg/kg/day in males and 1.56 mg/kg/day in females, based on ↑ liver wt (M) and ↑ relative liver wt (M/F)	Seacat et al. 2003
oral gavage	GD 0 to PND 20[a] developmental neurotoxicity study	rat	0, 0.1, 0.3, or 1.0 mg/kg/day 25 females/dose	Maternal NOAEL= 1 mg/kg/day LOAEL= NA Developmental NOAEL= 0.3 mg/kg/day LOAEL= 1 mg/kg/day based on ↑ motor activity	Butenhoff et al. 2009
oral gavage	GDs 2–20	rat	0, 1, 2, 3, 5, or 10 mg/kg	Maternal NOAEL= 1 mg/kg LOAEL= 2 mg/kg based on ↓ body wt Developmental NOAEL= 1 mg/kg LOAEL= 2 mg/kg based on ↓ survival $BMDL_5$ corresponding to maternal dose for survival of rat pups at PND 8 was 0.58 mg/kg	Thibodeaux et al. 2003 and Lau et al. 2003
oral gavage	GDs 2–21	rat	0, 0.1, 0.6, or 2.0 mg/kg	Offspring NOAEL = cannot be determined LOAEL= 0.1 mg/kg based on changes in the cortex and hippocampus (astrocyte activation markers, pro-inflammatory transcription factors)	Zeng et al. 2011

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	GDs 2–21	rat	0, 0.1, 0.6, or 2 mg/kg/day	Offspring on PND 21 NOAEL = 0.1 mg/kg/day LOAEL = 0.6 mg/kg/day based on increased apoptosis in heart cells	Zeng et al. 2014
oral gavage	GDs 1–21	rat	0, 0.1, or 2.0 mg/kg/day	Offspring NOAEL= 0.1 mg/kg/day LOAEL = 2.0 mg/kg/day based on histopathological changes in lungs, ↓ body wt and ↑ mortality	Chen et al. 2012
oral gavage	GD 0–PND 20	rat	0, 0.5, or 1.5 mg/kg/day 6 dams/dose	NOAEL = NA LOAEL = 0.5 mg/kg/day based on ↓ offspring body wt, impaired glucose tolerance	Lv et al. 2013
oral gavage	GDs 11–19	rat	0, 5, or 20 mg/kg/day 4 dams/dose	NOAEL = NA LOAEL = 5 mg/kg/day based on ↓ offspring body wt	Zhao et al. 2014
oral gavage	6 wks prior to mating and Males—22 days Females—through gestation, parturition and lactation reproductive study	rat	0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day 35 rats/sex/dose	F0 (M/F) parents NOAEL= 0.1 mg/kg/day LOAEL= 0.4 mg/kg/day based on ↓ bwt gain/food consumption F1 (M/F) parents NOAEL = 0.4 mg/kg LOAEL = NA, higher dose not tested F1 offspring NOAEL= 0.4 mg/kg/day LOAEL= 1.6 mg/kg/day based on ↓ viability, body wt F2 offspring NOAEL= 0.1 mg/kg/day LOAEL= 0.4 mg/kg/day based on ↓ body wt	Luebker et al. 2005b

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	6 wks prior to mating and continued through mating, gestation and LD 4 reproductive study	rat	0, 0.4, 0.8. 1.0, 1.2, 1.6 and 2.0 mg/kg/day 20–28 dams/dose	F0 dams NOAEL= 0.4 mg/kg/day LOAEL= 0.8 mg/kg/day based on ↓ bwt gain F1 offspring NOAEL= not identified LOAEL= 0.4 mg/kg/day based on ↓ pup body weight $BMDL_5$ estimates for decreased gestation length was 0.31 and viability was 0.89 mg/kg/day	Luebker et al. 2005a
oral (diet)	104 weeks	rat	0, 0.024, 0.098, 0.242, or 0.984 mg/kg/day—males 0, 0.029, 0.120, 0.299, or 1.251 mg/kg/day—females 0, 0.5, 2, 5, or 20 ppm 40–70 males and females	Males NOAEL= 0.024 mg/kg/day Males LOAEL= 0.098 mg/kg/day based on liver histopathology Females NOAEL = 0.120 mg/kg/day Females LOAEL = 0.299 mg/kg/day based on liver histopathology Suggestive of carcinogenicity	Thomford 2002/ Butenhoff et al. 2012
oral gavage	1 time on PND 10 developmental neurotoxicity	mouse	0, 0.75, or 11.3 mg/kg 4–7 males/group	Mice at both concentrations showed ↓ activity and ↑ neuroprotein levels in the hippocampus	Johansson et al. 2008, 2009
oral gavage	7 days immunotoxicity study	mouse	0, 5, 20, or 40 mg/kg 12 male mice/dose	NOAEL= NA LOAEL= 5 mg/kg based on ↑ liver wt and suppression of the plaque forming cell response	Zheng et al. 2009
oral gavage	GDs 1–17 developmental immunotoxicity	mouse	0, 0.1, 1, or 5 mg/kg 10–12 female mice/dose	Males NOAEL = 0.1 mg/kg Males LOAEL = 1 mg/kg based on ↓NK cell activity Females NOAEL = 1 mg/kg Females LOAEL = 5 mg/kg based on ↓NK cell activity	Keil et al. 2008

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	GDs 1–17	mouse	0, 1, 5, 10, 15, or 20 mg/kg	Maternal NOAEL= 1 mg/kg LOAEL= 5 mg/kg based on ↑ liver wt Developmental NOAEL= 1 mg/kg LOAEL= 5 mg/kg based on ↑ liver wt, delayed eye opening $BMDL_5$ corresponding to maternal dose for survival of mouse pups at PND 6 was 3.88 mg/kg	Thibodeaux et al. 2003; Lau et al. 2003
oral gavage	GDs 12–18 developmental	mouse	0 or 6 mg/kg/day 8–10 mice/dose	Maternal NOAEL= 6 mg/kg/day LOAEL= NA Developmental NOAEL= NA LOAEL= 6 mg/kg/day based on ↓ body wt	Fuentes et al. 2007
oral gavage	GDs 1–17/18 developmental	mouse	0, 1, 10, or 20 mg/kg/day 10 mice/dose	Maternal NOAEL = 1 mg/kg/day LOAEL= 10 mg/kg/day, based on ↑ liver organ wt. Developmental NOAEL= 1 mg/kg/day LOAEL= 10 mg/kg/day, based on fetal abnormalities and ↓survival	Yahia et al. 2008
oral gavage	14 days With regular or high fat diet	mouse	0, 5, or 20 mg/kg/day 16 males/dose/diet	NOAEL = NA LOAEL = 5 mg/kg/day based on wt loss on high fat diet	L. Wang et al. 2014
oral gavage	3–21 days	mouse	0, 1, 5, or 10 mg/kg/day 4 males/dose	NOAEL = 1 mg/kg/day LOAEL = 5 mg/kg/day based on ↑ liver wt, changes in oxidation biochemical parameters	Wan et al. 2012
oral gavage	GD 0–PND 21	mouse	0, 0.3, 3 mg/kg/day 6 dams/dose	NOAEL = 0.3 mg/kg/day LOAEL = 3 mg/kg/day based on ↑ liver wt in dams and male offspring, ↑ fasting serum insulin in males	Wan et al. 2014b

Method of exposure	Length of study	Species	Concentration	Results	Reference
oral gavage	28 days immunotoxicity	mouse	0, 0.00017, 0.0017, 0.0033, 0.017, 0.033, or 0.166 mg/kg 5 mice/dose	Males NOAEL= 0.00017 mg/kg Males LOAEL= 0.0017 mg/kg based on ↓ plaque forming cell response Females NOAEL= 0.0033 mg/kg Females LOAEL= 0.017 mg/kg based on ↓ plaque forming cell response	Peden-Adams et al. 2008
oral gavage	60 days immunotoxicity	mouse	0, 0.008, 0.083, 0.417, 0.833, or 2.083 mg/kg 10 male mice/group	NOAEL = 0.008 mg/kg/day LOAEL = 0.083 mg/kg based on ↑ splenic NK cell activity and ↑ liver weight	Dong et al. 2009
oral gavage	90 days neurotoxicity	mouse	0, 0.43, 2.15, or 10.75 mg/kg/day 15/group, sex not specified	NOAEL = 0.43 mg/kg/day LOAEL = 2.15 mg/kg/day based on changes in water maze and histopath. in hippocampus	Long et al. 2013
dermal	single dose	rabbit	0.5 g* (no data on gender)	No irritation	Biesemeier and Harris 1974
ocular	single dose	rabbit	0.5 g* (no data on gender)	Exact score not provided except maximal score at 1 and 24 hrs	Biesemeier and Harris 1974

Notes: *Exact dose not provided; NA= not applicable; could not be determined
[a] GD = gestation day and PND = post natal day
M = male; F = female

Attachment

1D

5

Toxicological Profile for Perfluoroalkyls

Released May 2021

Last Updated March 2020

ATSDR
U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

DISCLAIMER

Use of trade names is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry, the Public Health Service, or the U.S. Department of Health and Human Services.

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance due to associated acute, intermediate, and chronic exposures;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, intermediate, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed through September 2018. New studies were added in 2019 following public comment, and NHANES data were updated. Staff from the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

Patrick N. Breysse, Ph.D., CIH
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*Legislative Background

The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "…effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "…establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

VERSION HISTORY

Date	Description
May 2021	Final toxicological profile released
March 2020	Toxicological profile last updated
June 2018	Draft for public comment toxicological profile released
August 2015	Draft for public comment toxicological profile released
May 2009	Draft for public comment toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

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CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

This toxicological profile on perfluoroalkyls discusses information on 10 perfluoroalkyls that have been measured in the serum collected from a representative U.S. population 12 years of age and older in the National Health and Nutrition Examination Survey (NHANES) 2003–2004 (Calafat et al. 2007b), as well as 2 compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile include:

Compound	Acronym	CAS Registry Number
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoDA	307-55-1
Perfluorobutane sulfonic acid	PFBS	375-73-5
Perfluorohexane sulfonic acid	PFHxS	355-46-4
Perfluorooctane sulfonic acid	PFOS	1763-23-1
Perfluorooctane sulfonamide	FOSA	754-91-6

Perfluoroalkyls can exist in several ionic forms, most commonly as the anionic form or acidic form. In the environment, perfluoroalkyls are found in the anionic form (ITRC 2017). The names for the anionic and acidic forms (e.g., perfluorooctanoate and perfluorooctanoic acid) are often used interchangeably even though there are differences in physical and chemical properties and behavior in the environment, and the same acronym is used for both forms (e.g., PFOA). ATSDR has opted to the utilize the same terminology as NHANES (i.e., the acidic form names).

The term “perfluoroalkyls” used throughout the toxicological profile is referring to at least one of these 12 compounds and the information may not be applicable to other perfluoroalkyl compounds.

1.1 OVERVIEW AND U.S. EXPOSURES

The perfluoroalkyls discussed in this profile primarily consist of perfluorinated aliphatic carboxylic acids (PFCAs) and perfluorinated aliphatic sulfonic acids (PFSAs). These substances have been used extensively in surface coating and protectant formulations due to their unique surfactant properties (Kissa

2001; Schultz et al. 2003). Major applications have included protectants for paper and cardboard packaging products, carpets, leather products, and textiles that enhance water, grease, and soil repellency (3M 1999; Hekster et al. 2003; Kissa 2001; Schultz et al. 2003), and in firefighting foams (Schultz et al. 2003). Perfluoroalkyls such as PFOA have also been used as processing aids in the manufacture of fluoropolymers such as nonstick coatings on cookware (DuPont 2008; EPA 2008a).

Perfluoroalkyls are human-made substances that do not occur naturally in the environment. The perfluoroalkyl substances discussed in this profile, especially PFOS and PFOA, have been detected in air, water, and soil in and around fluorochemical facilities. However, these industrial releases have been declining since eight companies began voluntarily phasing out the production and use of several perfluoroalkyls in the early 2000s (3M 2007b, 2008a, 2008b; Barton et al. 2007; Davis et al. 2007; DuPont 2008; EPA 2007a, 2008a, 2016a). PFOA and PFOS may still be produced domestically, imported, and used by companies not participating in the PFOA Stewardship program. Under the Toxic Substances Control Act (TSCA), EPA has proposed a significant new use rule (SNUR) for long-chain perfluoroalkyl carboxylate (LCPFAC) chemical substances and sulfonates to ensure that the manufacture, import, or processing of LCPFAC chemical substances for any discontinued uses cannot begin without EPA review. EPA essentially excluded the use or import of all LCPFAC chemical substances by proposing a SNUR for LCPFACs and sulfonates (EPA 2015). Data are becoming more available regarding current releases of shorter-chain perfluoroalkyls (perfluorinated carboxylic acids with six or fewer carbons and perfluorosulfonic acids with five or fewer carbons) that are now being used in surface treatment products or perfluoropolyethers that are used as a replacement for PFOA in emulsion polymerization. Environmental fate and toxicity research of newer replacement substances is ongoing (De Silva et al. 2016; Gomis et al. 2018; Kabore et al. 2018).

In the environment, some of the perfluoroalkyls discussed in this profile can also be formed from environmental degradation of precursor compounds released during the manufacture and use of consumer products containing perfluoroalkyls (D'eon and Mabury 2007; D'eon et al. 2009; Martin et al. 2006; Prevedouros et al. 2006). Under the PFOA Stewardship Program with the U.S. Environmental Protection Agency (EPA), eight major fluoropolymer producers have phased out PFOA, precursor substances that can degrade to long-chain perfluoroalkyls such as PFOA, and higher homologues from emissions and products (EPA 2008a, 2016a).

1. RELEVANCE TO PUBLIC HEALTH

Due to the strength of the carbon-fluorine bonds, perfluoroalkyls are very stable in the environment and are resistant to biodegradation, photooxidation, direct photolysis, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The perfluoroalkyl carboxylic acids and sulfonic acids have very low volatility due to their ionic nature (Kissa 2001; Prevedouros et al. 2006; SPARC 2008). As a group, perfluoroalkyls are persistent in soil and water (3M 2000; Prevedouros et al. 2006). Perfluoroalkyls are mobile in soil and leach into groundwater (Davis et al. 2007). Volatile fluorotelomer alcohols may be broken down into substances like PFOA, and atmospheric deposition can lead to contamination of soils and leaching into groundwater away from point sources. Perfluoroalkyls have been detected in many parts of the world, including oceans and the Arctic, indicating that long-range transport is possible (Armitage et al. 2006; Barber et al. 2007; Prevedouros et al. 2006; Wania 2007; Wei et al. 2007a; Yamashita et al. 2005, 2008).

Perfluoroalkyls have been detected in all environmental media including air, surface water, groundwater (including drinking water), soil, and food. Human exposure may occur from all of these media. Contaminated drinking water led to increased levels of exposure to PFOA, PFOS, and other perfluoroalkyls for some populations residing near fluoropolymer manufacturing facilities (ATSDR 2008; Emmett et al. 2006a; Steenland et al. 2009b). Median PFOA serum levels (measured in 2005–2006) of 45,276 non-occupationally exposed individuals residing in southeastern Ohio and West Virginia who were exposed to PFOA via contaminated drinking water (Shin et al. 2011b) were approximately 6 times greater than the median serum PFOA concentration in a representative sample of the U.S. general population (2005–2006 NHANES data; CDC 2018). Serum levels of PFOA and PFOS in the general population of the United States have sharply declined in recent years as U.S. production of these substances ceased (CDC 2019). For example, the geometric mean concentrations of PFOA and PFOS in the general population were 5.2 and 30.4 ng/mL (ppb), respectively, in 1999–2000; in 2015–2016, PFOA declined by 70% to 1.56 ng/mL and PFOS declined 84% to 4.72 ng/mL (CDC 2018, 2019).

Based on environmental measurements and theoretical models, one study has proposed that the major exposure pathways for PFOS for the general population in Europe and North America are food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets (Trudel et al. 2008). For PFOA, major exposure pathways were proposed to be oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. This includes exposure to 8:2 fluorotelomer alcohol in food packaging and air, which can be broken down into PFOA. PFOS and PFOA exposure pathways are proposed to be similar for children except that exposure from hand-to-mouth transfer from treated carpets is expected to

be much greater in children. Based on these exposure pathways, adult uptake doses estimated for high-exposure scenarios were approximately 30 and 47 ng/kg/day for PFOS and PFOA, respectively (Trudel et al. 2008). PFOS and PFOA doses estimated for children under the age of 12 under high exposure scenarios were 101–219 and 65.2–128 ng/kg/day, respectively. Since PFOA and PFOS are no longer produced or used in the United States, current exposure levels may be lower than those predicted by Trudel et al. (2008). A study by Vestergren and Cousins (2009) evaluated potential exposure to perfluorocarboxylate homologues for different populations and also concluded that dietary intake was the primary background exposure pathway for the general population, while inhalation of indoor air was the main exposure pathway for occupationally exposed individuals with estimated intakes >150 ng/kg/day. Although not well studied, the available absorption data (Fasano et al. 2005; Franko et al. 2012) suggest that dermal contact may also contribute to the overall perfluoroalkyl body burden.

Perfluoroalkyls have been detected in human breast milk and umbilical cord blood. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples from women living in Massachusetts (samples were collected in 2004) were 0.617 and 0.161 ng/mL, respectively (Tao et al. 2008b). Maximum concentrations of other perfluoroalkyls were <0.06 ng/mL. In most umbilical cord samples collected in 2004–2005 in Maryland, the maximum concentrations of PFOS and PFOA were 34.8 and 7.1 ng/mL, respectively (Apelberg et al. 2007a, 2007b). Other perfluoroalkyls have been detected less frequently.

1.2 SUMMARY OF HEALTH EFFECTS

The toxicity of PFOA and PFOS has been evaluated in a large number of studies of humans and laboratory animals; less toxicity data are available for other perfluoroalkyls. However, comparison of the toxicity of perfluoroalkyls across species is problematic due to differences in elimination half-lives, lack of adequate mechanistic data, species differences in the mechanism of toxicity for some endpoints, and differences in measurement of exposure levels between epidemiological and experimental studies. Table 1-1 lists half-lives for PFOA, PFOS, PFHxS, PFNA, PFBS, and PFBA for human, nonhuman primates, rats, and mice to illustrate some of the species differences. For example, for PFOA, the estimated elimination half-life is measured in years in humans and in hours in female rats.

Table 1-1. Summary of Estimated Elimination Half-lives for Select Perfluoroalkyls[a]

	Humans	Nonhuman primates	Rats	Mice
PFOA	2.1–10.1 years	20.1–32.6 days	Males: 44–322 hours Females: 1.9–16.2 hours	
PFOS	3.3–27 years	110–170 days	179–1,968 hours	731–1,027 hours
PFHxS	4.7–35 years	87–141 days	Males: 382–688 hours Females: 1.03–41.28 hours	597–643 hours
PFNA	2.5–4.3 years		Males: 710–1,128 hours Females: 33.6–58.6 hours	619.2– 1,653 hours
PFBS	665 hours	8.0–95.2 hours	2.1–7.42 hours	
PFBA	72–81 hours	40.3–41.0 hours	1.03–9.22 hours	2.79–13.34 hours

[a]See Table 3-5 for additional information and citations.

The mechanisms of toxicity of perfluoroalkyls have not been fully elucidated. There is strong evidence that many of the adverse effects observed in laboratory animals involve the activation of peroxisome proliferator-activated receptor-α (PPARα), which can mediate a broad range of biological responses (Issemann and Green 1990). There are species differences in the activation of PPARα; rats and mice are the most sensitive species and guinea pigs, nonhuman primates, and humans are less responsive. Although humans are less responsive to PPARα agonists, they do have functional PPARα. This may explain some of the species differences in perfluoroalkyl toxicity. PPARα-dependent mechanisms have been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, decreased pup survival, and some immune effects. However, there is evidence that PPARα-independent mechanisms are also involved in PFOA and PFOS toxicity, including liver and immune toxicity; it is not known if species differences exist for these mechanisms. In general, epidemiological studies use serum perfluoroalkyl levels as a biomarker of exposure, which contrasts with experimental studies that utilize dose, expressed in mg/kg body weight/day units, or air concentrations as the dose metric. Although physiologically based pharmacokinetic (PBPK) models have been developed for rodents and humans, these models are not sufficient to allow for direct comparisons between administered doses in laboratory animals and serum concentrations in humans.

***Effects in Humans*.** Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. A large number of epidemiological studies have evaluated possible associations between perfluoroalkyl exposure and a wide range of adverse health outcomes. However, most of the studies have focused on PFOA and/or PFOS; fewer studies have evaluated a smaller number of potential health outcomes for the remaining 10 perfluoroalkyls included in

this toxicological profile. Most of the epidemiological studies lack exposure monitoring data, and there is a potential for multiple routes of exposure (inhalation and oral); however, most of the studies used serum perfluoroalkyl level as a biomarker of exposure. The three primary sources of this information are occupational exposure studies, studies of communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and studies of populations exposed to background levels of perfluoroalkyls (referred to as general population studies). In the studies examined, workers have the highest potential exposure to a specific perfluoroalkyl, followed by the highly-exposed residents such as residents in the Mid-Ohio Valley who have elevated levels of PFOA and background levels of other perfluoroalkyls, and then the general population. In one study of workers at the Washington Works facility in West Virginia, the arithmetic mean serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the arithmetic mean PFOA level in highly-exposed residents (without occupational exposure) near this facility was 423 ng/mL in 2004–2005 (Emmett et al. 2006a). By comparison, the arithmetic mean concentration of PFOA in the U.S. population was 4.91 ng/mL in 2005–2006 (calculated by ATSDR from NHANES data reported in CDC 2013). Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. Based on a number of factors (described in Section 2.1), the available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- Pregnancy-induced hypertension/pre-eclampsia (PFOA, PFOS)
- Increases in serum hepatic enzymes, particularly alanine aminotransferase (ALT), and decreases in serum bilirubin levels (PFOA, PFOS, PFHxS)
- Increases in serum lipids, particularly total cholesterol and low-density lipoprotein (LDL) cholesterol (PFOA, PFOS, PFNA, PFDA)
- Decreased antibody response to vaccines (PFOA, PFOS, PFHxS, PFDA)
- Small (<20-g or 0.7-ounce decrease in birth weight per 1 ng/mL increase in either PFOA or PFOS blood level) decreases in birth weight (PFOA, PFOS)

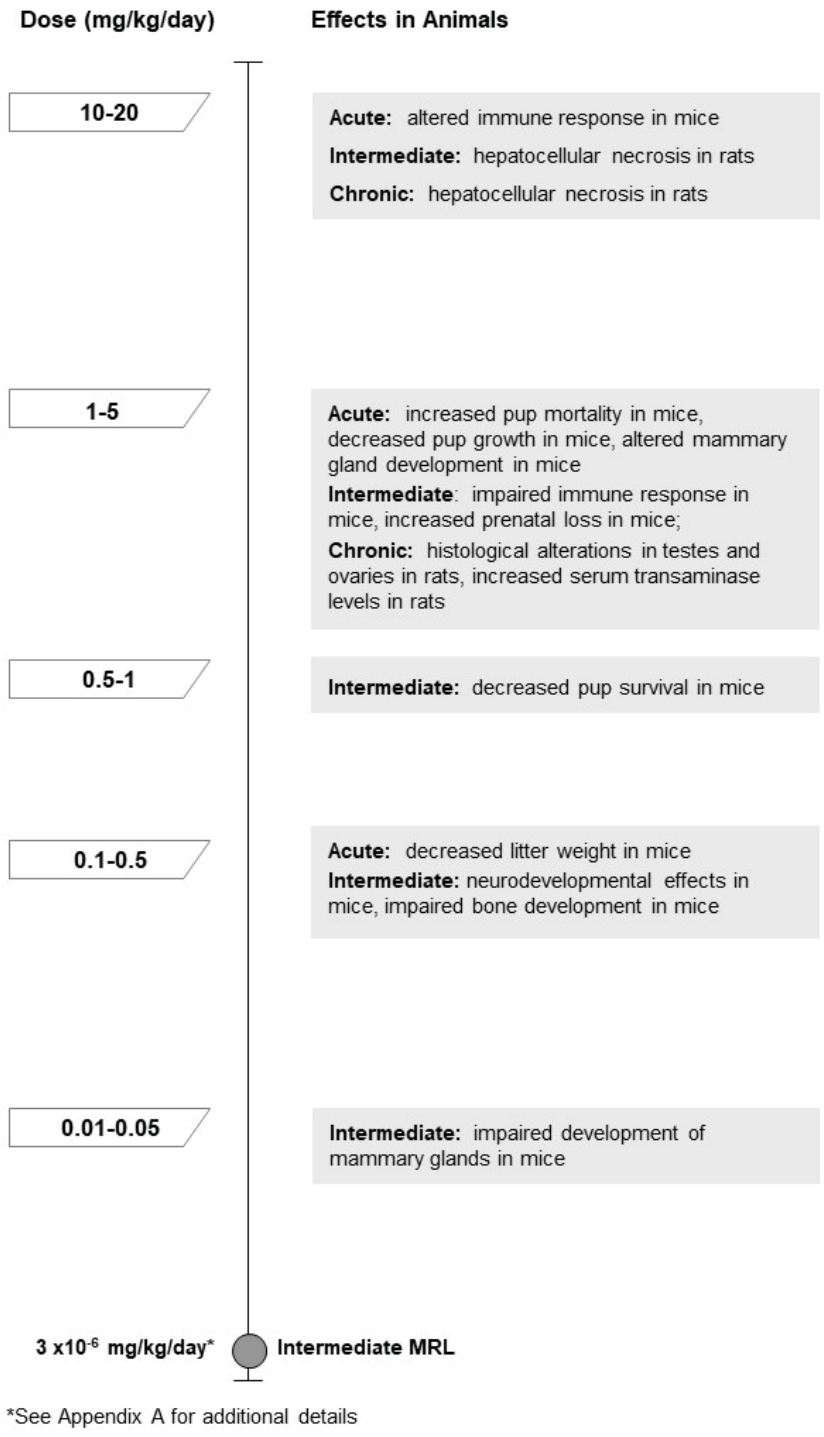
The International Agency for Research on Cancer (IARC 2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B), and EPA (2016e, 2016f) concluded that there was suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. Increases in testicular and kidney cancer have been observed in highly exposed humans.

There is also some suggestive evidence for associations between perfluoroalkyls and additional health outcomes; there is less certainty in these associations due to inconsistencies across studies and/or a smaller number of studies examining a specific outcome. These health outcomes include osteoarthritis in women under 50 years of age (PFOA, PFOS) and decreased antibody response to vaccines (PFNA, PFUnA, PFDoDA). Additionally, associations between serum PFOA and PFOS and decreases in glomerular filtration rate and increases in serum uric acid levels and between serum PFOA, PFOS, PFHxS, and PFNA and increased risk of early menopause have been observed; these effects may be due to reverse causation, where the effect (disease) causes the change in serum perfluoroalkyl levels (exposure).

***Effects in Laboratory Animals*.** Most of the information regarding the effects of perfluoroalkyls in animals is derived from oral studies; considerably less information is available from inhalation and dermal exposure studies. PFOA and PFOS are the most studied perfluoroalkyls, with considerably less data for the other compounds. Of the 233 animal studies reviewed in this toxicological profile, 42% examined PFOA, 31% examined PFOS, and 27% examined other perfluoroalkyls (8 studies on PFHxS, 17 studies on PFNA, 1 study on PFUnA, 5 studies on PFBS, 6 studies on PFBA, 9 studies on PFDA, 8 studies on PFDoDA, 1 study on FOSA, and 8 studies on PFHxA). The primary effects observed in rats and mice exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity (see Figures 1-1, 1-2, and 1-3); not all of these effects have been observed or examined for all perfluoroalkyls. Based on limited data, the toxicity of perfluoroalkyls does not appear to be specific to the route of administration. It should be noted that, for the most part, adverse health effects in studies in animals have been associated with exposure concentrations or doses that resulted in blood levels of perfluoroalkyls that were significantly higher than those reported in perfluoroalkyl workers or in the general population. Furthermore, there are profound differences in the toxicokinetics of perfluoroalkyls between humans and experimental animals. The elimination $t_{1/2}$ of PFOA is approximately 4 years in humans compared with days or hours in rodents. These factors, plus issues related to the mode of action of perfluoroalkyls (see below), make it somewhat difficult at this time to determine the true relevance of some effects reported in animal studies to human health.

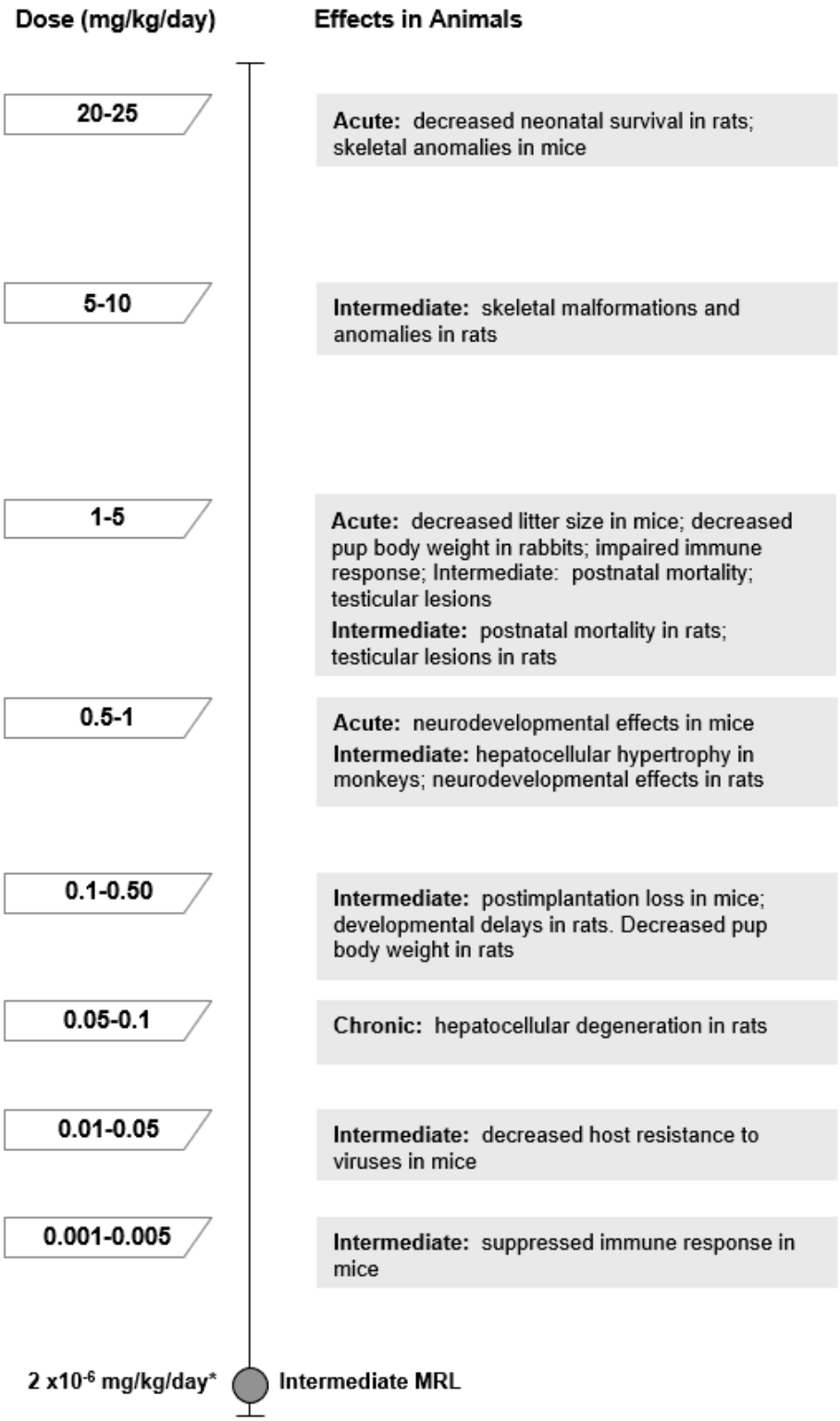
Liver Effects. Many studies have described morphological and biochemical alterations in the liver from rodents following acute and longer-term oral exposure to PFOA. Some of the effects observed in rats include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels (e.g., Butenhoff et al. 2004b; Liu et al. 1996; Pastoor et al. 1987; Yang et al. 2001; see Section 2.9 for a complete list of citations). The observed hepatomegaly and hypertrophy are likely due

Figure 1-1. Health Effects Found in Animals Following Oral Exposure to PFOA



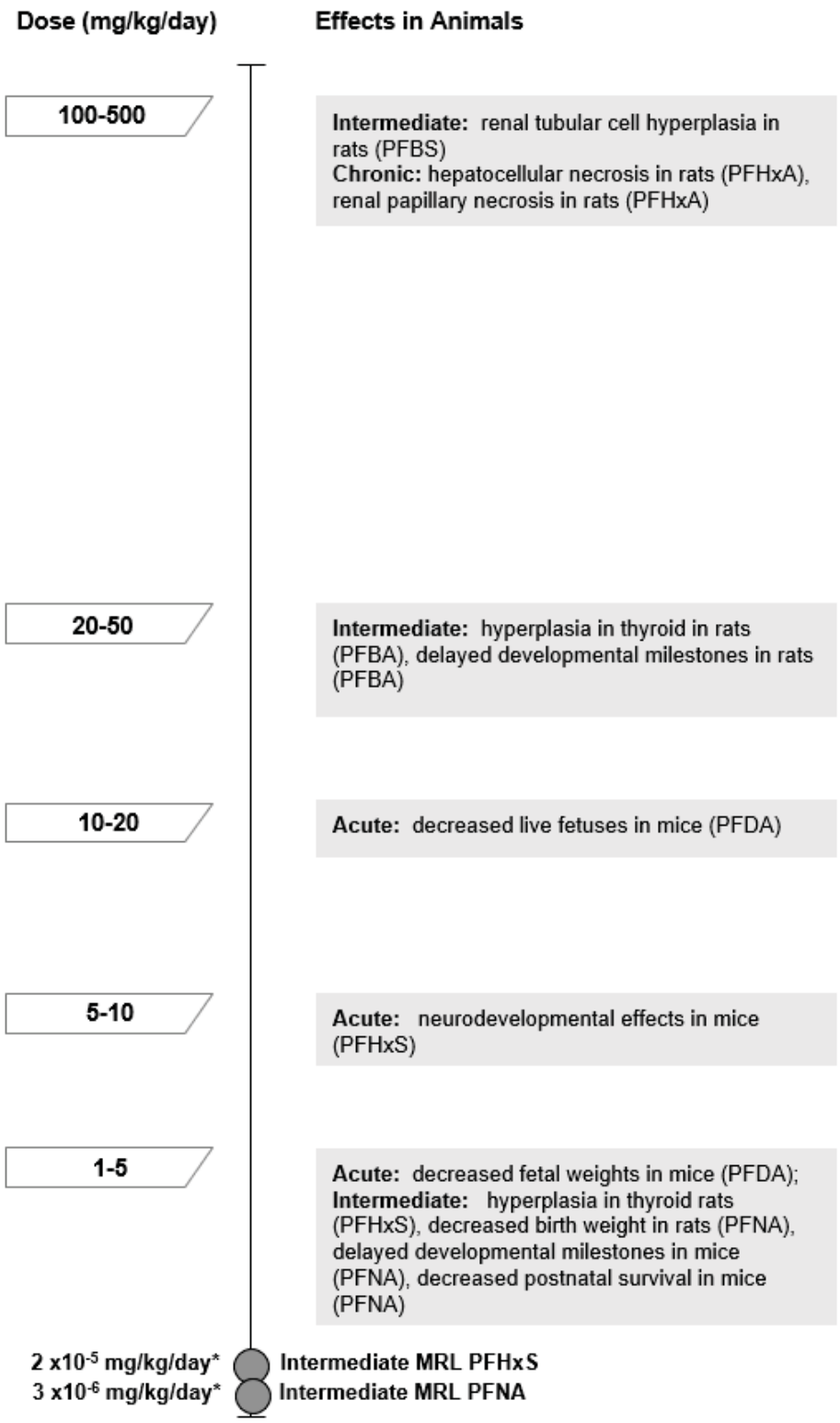
*See Appendix A for additional details

Figure 1-2. Health Effects Found in Animals Following Oral Exposure to PFOS



*See Appendix A for additional details

Figure 1-3. Health Effects Found in Animals Following Oral Exposure to Other Perfluoroalkyls



*See Appendix A for additional details

to expansion of the smooth endoplasmic reticulum and proliferation of peroxisomes, as confirmed by increased activity of biochemical markers and light and electron microscopy (Pastoor et al. 1987). It is important to note also that there appear to be different sensitivities for different endpoints. For example, in male rats dosed with PFOA for 14 days, absolute liver weight and fatty acid β-oxidation activity were significantly increased at 2 mg/kg/day, whereas hepatic microsomal concentration of total cytochrome P450 was significantly increased at 20 mg/kg/day (Liu et al. 1996). In general, longer-term studies with PFOA have shown that the hepatic effects are reversible once dosing ceases and that recovery tends to parallel the decline in blood levels of PFOA (Perkins et al. 2004). Studies in mice have provided similar results. However, studies in PPARα-null mice suggest that hepatomegaly may also be due to a PPARα-independent process in mice (Yang et al. 2002b), since PFOA induced hepatomegaly to the same extent in wild-type mice and PPARα-null mice, but failed to increase acyl-CoA oxidase activity in PPARα-null mice. PFOA exposure also resulted in increases in absolute liver weight in monkeys treated with ≥3 mg/kg/day for 26 weeks, an effect that was partly associated with significant mitochondrial proliferation, but not peroxisome proliferation (Butenhoff et al. 2002).

Similar to PFOA, PFOS exposure results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels in rodents (e.g., Elcombe et al. 2012a, 2012b; Era et al. 2009; Seacat et al. 2003; Thibodeaux et al. 2003). PFOS induced an increase in absolute liver weight, a decrease in serum cholesterol, and hepatocellular hypertrophy and lipid vacuolation in monkeys in a 26-week study (Seacat et al. 2002). Not unexpectedly, there was no evidence of peroxisome proliferation and no increase in hepatic palmitoyl-CoA oxidase, consistent with the fact that monkeys (and humans) seem to be refractory to peroxisome proliferative responses (Cattley et al. 1998; Klaunig et al. 2003).

Studies with other perfluoroalkyls have shown that, in general, liver weight and parameters of fatty acid β-oxidation are more severely affected as the carbon length increases up to about a 10-carbon chain length (Butenhoff et al. 2009a, 2012a; Goecke-Flora and Reo 1996; Goecke et al. 1992; Kudo et al. 2000, 2006; Permadi et al. 1992, 1993; van Otterdijk 2007a, 2007b). Significant peroxisome activity seems to require a carbon length >7 (Goecke-Flora and Reo 1996; Goecke et al. 1992), but increases over control levels have been reported with a four-carbon chain length (Permadi et al. 1993; Wolf et al. 2008a). In an *in vitro* study in COS-1 cells transfected with mouse PPARα, PFOA had the lowest effective concentration needed for PPARα activation followed by PFNA and PFDA, PFHxA, and PFBA (Wolf et al. 2008a). This pattern was not found for the sulfonates; the lowest effective concentration was for PFHxS followed by PFOS and PFBS. Wolf et al. (2008a) also found that carboxylate perfluoroalkyls activated PPARα at lower concentrations than the sulfonate perfluoroalkyls. In COS-1 cells transfected

with human PPARα, PFNA had the lowest effective concentration followed by PFOA PFHxA, PFHxS, PFOS, PFBS, PFBA, and PFDA (Wolf et al. 2008a). Studies have shown that the differential activity is also related to differential accumulation of the perfluoroalkyls in the liver (Kudo and Kawashima 2003; Kudo et al. 2000, 2006). Hydrophobicity, which increases as carbon length increases, seems to favor biliary enterohepatic recirculation, resulting in a more protracted toxicity (Goecke-Flora and Reo 1996). As discussed in greater detail in Section 2.9, the increases in liver weight and hepatocellular hypertrophy observed in the rat and mouse studies were considered rodent-specific adaptive responses and were not considered relevant to humans. However, other liver effects including biliary effects and hepatocellular necrosis were considered relevant to humans.

Developmental Effects. PFOA and PFOS have induced developmental effects in rodents. Most studies with PFOA have been conducted in mice. Specific effects reported include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, altered bone development, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (Abbott et al. 2007; Albrecht et al. 2013; Cheng et al. 2013; Johansson et al. 2008; Koskela et al. 2016; Lau et al. 2006; Macon et al. 2011; Ngo et al. 2014; Onishchenko et al. 2011; Sobolewski et al. 2014; White et al. 2007, 2009, 2011; Wolf et al. 2007; Yahia et al. 2010). These effects occurred generally in the absence of overt maternal toxicity. Some of these effects, such as reduced pup survival from birth to weaning, have been observed in mice treated with as low as 0.6 mg/kg/day PFOA on gestation days (GDs) 1–17 (Abbott et al. 2007). This dose level resulted in mean serum PFOA concentrations of 5,200 and 3,800 ng/mL in dams and pups, respectively, on postnatal day (PND) 22. A cross-fostering study in mice showed that *in utero*, lactation only, and *in utero* and lactation exposure resulted in significant decreases in postnatal growth (Wolf et al. 2007). Alterations in spontaneous behavior were reported in 2- or 4-month-old male mice that were administered a single gavage dose of PFOA at the age of 10 days (Johansson et al. 2008). Increases in motor activity were also observed following *in utero* exposure to PFOA (Cheng et al. 2013; Onishchenko et al. 2011). Gestational exposure resulted in altered bone morphology and bone mineral density in the mature offspring (Koskela et al. 2016). Delays in ossification were found in another gestational exposure study in mice (Lau et al. 2006). A cross-fostering study showed that the delays in mammary gland development were observed following *in utero* exposure and following lactation-only exposure (White et al. 2009); however, the results of a 2-generation study showed that the delayed development did not appear to affect lactational support (White et al. 2011). No fetal toxicity or teratogenicity was reported in offspring of rabbits exposed to up to 50 mg/kg/day PFOA on GDs 6–18 (Gortner et al. 1982), suggesting that rabbits are less susceptible than mice to the developmental effects of PFOA, although comparing administered doses is probably not very informative due to toxicokinetic differences between species.

There were significant increases in body weight gain in mice aged 10–40 weeks that were exposed to low levels of PFOA (0.01–0.3 mg/kg/day) on GDs 1–17 (Hines et al. 2009). Increases in serum insulin and leptin levels were also observed, but there was no change in serum glucose or the response to a glucose challenge. A comparison of the effects of *in utero* exposure (GDs 1–17) to adult exposure (17 days at age 8 weeks) demonstrated that *in utero* exposure resulted in higher body weights, white fat weight, and brown fat weight at age 18 months (Hines et al. 2009).

Studies conducted with wild-type and PPARα knockout mice showed that PPARα was required for PFOA-induced postnatal lethality and that the expression of one copy of the gene was sufficient to mediate this effect (Abbott et al. 2007). Strain or PPARα expression did not affect serum PFOA levels. The mechanism of reduced postnatal viability has not been elucidated. Alterations in gene expression in both fetal liver and lung have been reported following exposure of mice to PFOA during pregnancy (Rosen et al. 2007). In the liver, PFOA altered the expression of genes linked to fatty acid catabolism, lipid transport, ketogenesis, glucose metabolism, lipoprotein metabolism, cholesterol biosynthesis, steroid metabolism, bile acid biosynthesis, phospholipid metabolism, retinol metabolism, proteosome activation, and inflammation. In the lung, transcriptional-related changes were predominantly associated with fatty acid catabolism. Although decreased pup survival appears to be linked to PPARα expression, there are insufficient data to determine whether other developmental effects observed in rats and mice are PPARα-independent.

PFOS significantly decreased birth weight and survival in neonatal rats exposed *in utero* (Chen et al. 2012b; Lau et al. 2003; Xia et al. 2011), and cross-fostering exposed pups with unexposed dams failed to improve survival rates (Lau et al. 2003). PFOS serum levels of pups at birth associated with significant decreased survival were approximately ≥70,000 ng/mL. In contrast to PFOA, the results of a study in wild-type and PPARα-null mice suggest that the decrease in pup survival was not dependent on PPARα activation (Abbott et al. 2009). Dosing rats late during gestation (GDs 17–20) caused significantly more lethality than dosing early (GDs 2–5) (Grasty et al. 2003). Since pups had difficulty breathing within minutes of birth and their lungs showed evidence of delayed lung maturation and other histological alterations (Grasty et al. 2003, 2005; Yahia et al. 2008), the possibility that this caused the early death has been suggested. Other effects included decreases in birth weight or pup body weight, delays in eye opening, cleft palate, and neurodevelopmental alterations (Butenhoff et al. 2009b; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Lau et al. 2003; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; Wang et al. 2015c; Yahia et al. 2008). Alterations in spontaneous motor activity were observed in mice. A decrease in activity was observed

when mice were placed in a novel environment (Fuentes et al. 2007a; Onishchenko et al. 2011); another study found a decrease in motor activity followed by increased activity (Johansson et al. 2009). Evaluation of immunological parameters in 8-week-old pups from mice exposed to PFOS during gestation showed reduced natural killer (NK) cell activity, suppressed IgM response to immunization, and alterations in splenic and thymic lymphocyte subpopulations (Keil et al. 2008).

Similar to PFOA and PFOS, increases in fetal mortality were observed in mice exposed to PFDA on GDs 6–15 (Harris and Birnbaum 1989) and decreases in litter size and pup survival were observed in mice exposed to PFNA (Wolf et al. 2010). In contrast, gestational exposure to PFBA, PFBS, or PFHxS did not result in alterations in pup survival or pup body weight (Das et al. 2008; Hoberman and York 2003; Lieder et al. 2009b). Decreases in spontaneous activity followed by an increase in activity were observed in mice exposed to PFHxS on PND 10 (Viberg et al. 2013); no alterations were observed in mice similarly exposed to PFDA (Johansson et al. 2008).

Immunological Effects. A number of studies have examined the immunotoxicity of perfluoroalkyls in rats and mice; these data suggest that mice are considerably more sensitive than rats. PFOA- and PFOS-induced immunological alterations in adult mice are characterized by thymus and spleen atrophy, alterations in thymic and splenic lymphocyte phenotypes, and impaired response to T-dependent antigens (DeWitt et al. 2008, 2009; Dong et al. 2009; Guruge et al. 2009; Lefebvre et al. 2008; Loveless et al. 2008; Qazi et al. 2012; Yang et al. 2000, 2002a; Zheng et al. 2009). The lowest lowest-observed-adverse-effect level (LOAEL) for immune effects in mice exposed to PFOA was 3.75 mg/kg/day administered for 15 days; this dosing level resulted in a mean PFOA serum level of 75,000 ng/mL (DeWitt et al. 2008). For PFOS, several studies identified LOAELs of 0.02–0.8 mg/kg/day (Dong et al. 2009, 2011; Zheng et al. 2009) and one study identified a LOAEL of 0.00166 mg/kg/day for suppressed response to a T-dependent antigen (Peden-Adams et al. 2008). PFOA applied to the skin of mice increased serum IgE levels following a challenge with ovalbumin relative to mice treated with ovalbumin alone, which led the investigators to suggest that PFOA may increase the IgE response to environmental allergens (Fairley et al. 2007). More limited data are available for other perfluoroalkyls. Thymic and/or splenic alterations were observed in rats and mice administered ≥1 mg/kg/day PFNA (Fang et al. 2008, 2009, 2010). No histological alterations were observed in rodents exposed to PFHxS (Butenhoff et al. 2009a), PFDA (Harris et al. 1989), PFBS (3M 2001), or PFBA (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

Cancer Effects. PFOA, as many other PPARα agonists, induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). An increase in hepatocellular adenomas was also observed in rats chronically exposed to PFOS (Butenhoff et al. 2012b). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα activation. Although Leydig cell tumors are also commonly induced by peroxisome proliferating agents, the mode of action by which these tumors are induced by PFOA, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced decreases in circulating testosterone levels, leading to increased production of gonadotropin releasing hormone and circulating luteinizing hormone (LH), which promotes Leydig cell proliferation. Reduced testosterone levels may occur through decreased biosynthesis, or via the conversion of testosterone to estradiol via the enzyme aromatase, both of which may be related to PPARα activation (EPA 2016h). However, the data supporting a PPARα-dependent mode of action for Leydig cell tumors is not sufficiently established to rule out human relevance (EPA 2016h). Likewise, the mechanism of PFOA-induced pancreatic acinar cell tumors may include a PPARα-dependent component, but the mechanism has not been fully elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Support for this mode of action is limited to information demonstrating increased biliary excretion of PFOA in wild-type and PPARα null mice (Minata et al. 2010) and data showing altered expression of bile acid transporters (OATPs and MRPs) in exposed laboratory animals (Cheng and Klassen 2008a; Maher et al. 2008). The limitations in available data on the mode of action for pancreatic tumor development preclude a conclusion regarding the human relevance of PFOA-induced pancreatic tumors (EPA 2016h).

1.3 MINIMAL RISK LEVELS (MRLs)

ATSDR develops MRLs as screening tools to help identify chemicals that may be of concern. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to determine areas and populations potentially at risk for health effects from exposure to a particular substance. Exposure above the MRLs does not mean that health problems will occur. Instead, it may act

as a signal to health assessors to look more closely at a particular site where exposures may be identified. MRLs do not define regulatory or action levels for ATSDR.

ATSDR uses the point of departure (POD)/uncertainty factor approach to derive MRLs. Potential PODs are no-observed-adverse-effect levels (NOAELs), LOAELs, or the lower limit of the benchmark dose (BMDL). MRLs are set below levels that, based on current information, might cause adverse health effects in the people most sensitive to such substance-induced effects. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys) are not used as a basis for establishing MRLs. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. ATSDR does not extrapolate across exposure durations to derive MRLs for durations with limited databases.

Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals. ATSDR utilizes uncertainty factors to account for uncertainties associated with: (1) extrapolating from a LOAEL to a NOAEL; (2) extrapolating from animals to humans; and (3) to account for human variability. Default values of 10 are used for each of these categories of uncertainty factors; a value of 1 can be used if complete certainty exists for a particular uncertainty factor category. A partial uncertainty factor of 3 can be used when chemical-specific data decreases the uncertainty. On a case-by-case basis, ATSDR also utilizes modifying factors to account for MRL-specific database deficiencies.

Oral MRLs have been derived for several perfluoroalkyls. A summary of the MRLs derived for perfluoroalkyls is presented in Table 1-2 and detailed discussions of MRLs are provided in Appendix A. The database was not considered adequate for derivation of inhalation MRLs. Though inhalation data are available for PFOA and PFNA, these studies examined a limited number of endpoints and the data are not adequate for identifying the most sensitive targets of toxicity or establishing dose-response relationships. No inhalation data are available for other perfluoroalkyls.

Table 1-2. Overview of Minimal Risk Levels Derived for Perfluoroalkyls

	Inhalation MRLs			Oral MRLs		
Compound	Acute	Intermediate	Chronic	Acute	Intermediate	Chronic
PFOA	X[a]	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-3)	X
PFOS	X	X	X	X	$2x10^{-6}$ mg/kg/day (Table 1-4)	X
PFHxS	X	X	X	X	$2x10^{-5}$ mg/kg/day (Table 1-5)	X
PFNA	X	X	X	X	$3x10^{-6}$ mg/kg/day (Table 1-6)	X
PFDA	X	X	X	X	X	X
PFUnA	X	X	X	X	X	X
PFHpA	X	X	X	X	X	X
PFBS	X	X	X	X	X	X
PFBA	X	X	X	X	X	X
PFDoDA	X	X	X	X	X	X
PFHxA	X	X	X	X	X	X
FOSA	X	X	X	X	X	X

[a]X indicates that no MRL was derived.

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The oral databases were considered adequate for derivation of intermediate-duration oral MRLs for PFOA, PFOS, PFHxS, and PFNA based on laboratory animal data. The databases were not considered adequate for derivation of MRLs for the other perfluoroalkyls. Hepatic, immune, and developmental endpoints were the most sensitive targets in laboratory animals exposed to PFOA (see Figure 1-4) and PFOS (see Figure 1-5), respectively. The most sensitive targets were hepatic and thyroid endpoints for PFHxS and body weight and developmental endpoints for PFNA. As discussed in Section 1.2, toxicokinetic and mechanistic differences exist between humans and laboratory animals, in particular differences in elimination rates and the relevance of effects associated with activation of PPARα. The uncertainties in the relevance of animal data for developing screening levels are decreased by focusing on health outcomes also reported in epidemiological studies or involving PPARα-independent mechanisms of action and estimating a POD using serum perfluoroalkyl concentrations. The MRL values for PFOA, PFOS, PFHxS, and PFNA are summarized in Tables 1-3, 1-4, 1-5, and 1-6 and discussed in greater detail in Appendix A.

Figure 1-4. Summary of Sensitive Targets of PFOA – Oral

Developmental endpoints are the most sensitive target of PFOA.
Numbers in circles are the lowest LOAELs for all health effects in animals.

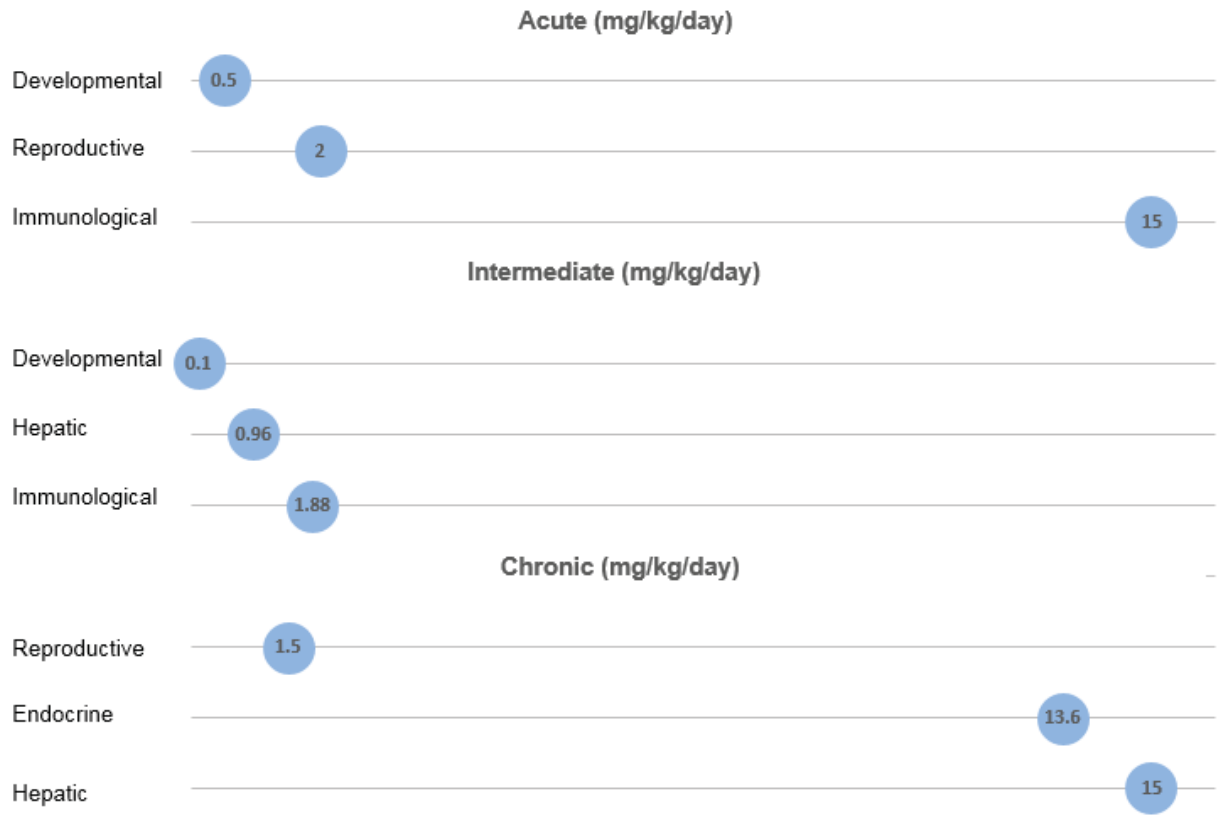


Figure 1-5. Summary of Sensitive Targets of PFOS – Oral

The immune system and developing organism are the most sensitive targets of PFOS.
Numbers in circles are the lowest LOAELs for all health effects in animals.

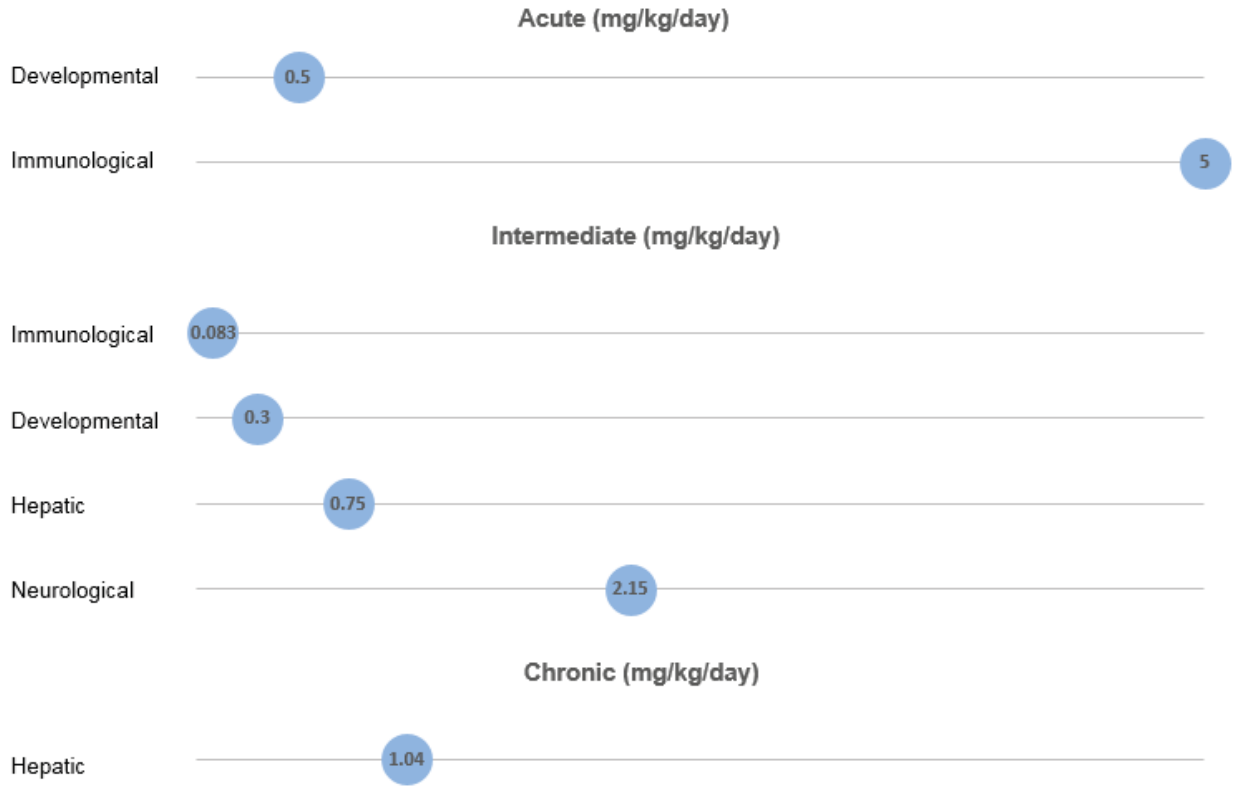


Table 1-3. Minimal Risk Levels (MRLs) for PFOA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Skeletal effects in mice	0.000821 ($LOAEL_{HED}$)	300	Koskela et al. 2016
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; PFOA = perfluorooctanoic acid

Table 1-4. Minimal Risk Levels (MRLs) for PFOS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-6}$	Delayed eye opening and decreased pup weight in rats	0.000515 ($NOAEL_{HED}$)[b]	30 10	Luebker et al. 2005a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid

Table 1-5. Minimal Risk Levels (MRLs) for PFHxS[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$2x10^{-5}$	Thyroid follicular epithelial hypertrophy/ hyperplasia in rats	0.0047 ($NOAEL_{HED}$)	30 10	Butenhoff et al. 2009a
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFHxS = perfluorohexane sulfonic acid

Table 1-6. Minimal Risk Levels (MRLs) for PFNA[a]

Exposure duration	MRL	Critical effect	Point of departure	Uncertainty and modifying factors	Reference
Inhalation exposure					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	Inadequate intermediate-duration study (exposure 15–364 days)				
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				
Oral exposure (mg/kg/day)					
Acute	Inadequate acute-duration study (exposure ≤14 days)				
Intermediate	$3x10^{-6}$	Decreased body weight and developmental delays in mice	0.001 ($NOAEL_{HED}$)	30 10	Das et al. 2015
Chronic	Inadequate chronic-duration study (exposure ≥365 days)				

[a]See Appendix A for additional information.

HED = human equivalent dose; NOAEL = no-observed-adverse-effect level; PFNOA = perfluorononanoic acid

CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of perfluoroalkyls. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

This document discusses information on perfluoroalkyls that have been measured in the serum collected from a representative U.S. population ≥12 years of age in the 2003–2004 NHANES (Calafat et al. 2007b), as well as two compounds (PFBA and PFHxA) that have been identified in other monitoring studies. More recent NHANES monitoring studies have not evaluated additional perfluoroalkyl compounds (CDC 2019). The perfluoroalkyl compounds discussed in the profile are listed below. They are discussed in the profile in following order, based on the abundance of epidemiological data:

Perfluorooctanoic acid (PFOA, CAS Registry Number 335-67-1)
Perfluorooctane sulfonic acid (PFOS, CAS Registry Number 1763-23-1)
Perfluorohexane sulfonic acid (PFHxS, CAS Registry Number 355-46-4)
Perfluorononanoic acid (PFNA, CAS Registry Number 375-95-1)
Perfluorodecanoic acid (PFDA, CAS Registry Number 335-76-2)
Perfluoroundecanoic acid (PFUnA, CAS Registry Number 2058-94-8)
Perfluoroheptanoic acid (PFHpA, CAS Registry Number 375-85-9)
Perfluorobutane sulfonic acid (PFBS, CAS Registry Number 375-73-5)
Perfluorobutanoic acid (PFBA, CAS Registry Number 375-22-4)
Perfluorododecanoic acid (PFDoDA, CAS Registry Number 307-55-1)
Perfluorohexanoic acid (PFHxA, CAS Registry Number 307-24-4)
Perfluorooctane sulfonamide (FOSA, CAS Registry Number 754-91-6)

The term perfluoroalkyls used throughout the profile is referring to at least one of these 12 compounds and may not be applicable to other perfluoroalkyl compounds.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figures 2-1, 2-2, 2-3, 2-4, and 2-5 provide an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to perfluoroalkyls, but may not be inclusive of the entire body of literature. ATSDR's approach for assessing study quality and weight-of-evidence evaluation is described in the Agency's Guidance for the Preparation of Toxicological Profile document (https://www.atsdr.cdc.gov/toxprofiles/guidance/profile_development_guidance.pdf).

Summaries of the epidemiological studies, including details on the study design and results, are presented in tables in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*; briefer summaries of the studies are presented in summary tables for each endpoint. For studies in which the population was divided into perfluoroalkyl exposure categories, such as quartiles, the risk ratio reported in the summary table is for the lowest exposure category with a statistically significant association; risk ratios for higher exposure categories are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls* tables. In general, associations were also found for higher exposure categories.

Summaries of experimental studies are separated by exposure route and are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6. The inhalation data for PFOA and other perfluoroalkyls are presented in Tables 2-1 and 2-2, respectively. A large number of experimental studies have evaluated the oral toxicity of PFOA and PFOS, the results of these studies are presented in Tables 2-3 and 2-4, respectively. Lesser amounts of data are available for the remaining 10 perfluoroalkyl compounds; the study results for these compounds are presented in Table 2-5. Table 2-5 is divided by exposure duration and by compound. The dermal data for PFOA is presented in Table 2-6. In addition, the NOAEL and LOAEL values from inhalation and oral studies are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. The points in the figures showing NOAELs or LOAELs reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant

dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints (ATSDR 2003). ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

The discussion of the available data for each health effect is divided into several subsections. Each health effect section begins with an overview, which contains a brief discussion of the available data and conclusions that can be drawn from the data. Compound-specific discussions follow the overview; the perfluoroalkyls are discussed in the following order: PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, and FOSA. It is noted that for most health effects, there are no data for a number of the perfluoroalkyls. The health effect endpoints examined in epidemiological and experimental data for each perfluoroalkyl is summarized in Figures 2-1 and 2-2, respectively. The compound-specific discussions are further divided into Epidemiological Studies and Laboratory Animal Studies; for data-rich endpoints, a compound-specific summary is also included. Each perfluoroalkyl is treated separately in this chapter. Although there is some evidence of similar health outcomes for some compounds, there is evidence of qualitative and mechanistic differences (Peters and Gonzalez 2011).

The health effects of perfluoroalkyls have been evaluated in a large number of epidemiological and animal studies; the literature search framework for identifying these studies is discussed in Appendix B. As illustrated in Figures 2-3, 2-4, and 2-5, most of the health effects data come from epidemiological studies. For PFOA, PFOS, and other perfluoroalkyls, 74, 76, and 70%, respectively, of the health effect studies were in humans; it is noted that most epidemiological studies examined more than one perfluoroalkyl. More than half (52%) of the epidemiological studies were cross-sectional studies, 29% were prospective studies, and the remainder were retrospective, case-control, cohort, or longitudinal studies. Three population categories were examined in epidemiological studies: workers at facilities involved in the production or use of perfluoroalkyls (most of the studies involved workers at two U.S. facilities and typically involved higher than background exposure to PFOA and PFOS), communities living near a PFOA manufacturing facility with high levels of PFOA in the drinking water (almost all of

the studies involved residents living near a PFOA production facility in West Virginia; elevated PFOA exposure and background exposure to other perfluoroalkyls), and populations exposed to background levels of perfluoroalkyls (referred to as general population studies). Most of the studies of communities living near perfluoroalkyl manufacturing facilities are part of the C8 Health Project and C8 Health Study (C8 is a synonym for PFOA). The C8 Health Project was a population study of Ohio and West Virginia residents living near the DuPont Washington Works facility in West Virginia and was funded by DuPont as part of a class action settlement agreement. The Washington Works facility began using PFOA in 1951 and peak use was in the late 1990s. At the time of enrollment (2005–2006), blood samples were collected from over 69,000 participants who lived, worked, or attended school in six contaminated water districts surrounding the facility for at least 12 months between 1950 and December 2004 (Frisbee et al. 2009); the six water districts were Little Hocking Water Association, Tuppers Plains Chester Water District, Village of Pomeroy, Lubeck Public Service District, Mason County Public Service District, or private water sources within these areas. The participants ranged in age from 1.5 to >100 years, with an average age of 39.1 years.

Serum perfluoroalkyl levels were used as the biomarker of exposure in almost all of the epidemiological studies since most of the studies did not provide external exposure levels. The highest levels of serum PFOA were found among workers, followed by the community members, and then the general population. One study of PFOA workers in 2004–2005 reported an average serum PFOA level of 1,000 ng/mL (Sakr et al. 2007a). A study of community members living near this same facility reported a mean serum PFOA level of 423 ng/mL in 2004–2005. In the United States, the mean geometric mean serum PFOA level in 2005–2006 was 3.92 ng/mL (CDC 2018). In a study of two PFOS facilities, mean serum PFOS levels in workers were 960–1,400 ng/mL in 2000 (Olsen et al. 2003a); the geometric mean serum PFOS levels in the U.S. general population in 1999–2000 was 30.4 ng/mL (CDC 2018). Bach et al. (2015b) investigated whether transport of blood samples under ambient temperature conditions and processing delays impact serum perfluoroalkyl concentrations. Using the conditions of the Danish National Birth Cohort study, Bach et al. (2015a) found relative differences between serum samples that were transported with processing delays and those processed immediately of 1% (winter sampling) to 3% (summer sampling) for PFOA, -29–2% for PFOS, 12–11% for PFHxS, -5–3% for PFNA, -39–0% for PFDA, -77 to -7% for PFUnA, and 38–17% for PFHpA. This discrepancy has not been verified for other Danish National Birth Cohort studies or for other studies.

Most of the epidemiological studies provided a single serum perfluoroalkyl concentration, which has been shown to be a reliable biomarker of recent exposure; however, it does not provide information on

historical exposure. The lack of historical exposure data is a particular limitation of the occupational and community population studies where past exposures were typically higher than current exposures.

Another limitation of the epidemiological studies involves co-exposure to multiple perfluoroalkyls. A number of the epidemiological studies have found strong correlations between serum levels of different perfluoroalkyls. *In vitro* studies (Carr et al. 2013; Wolf et al. 2014) have shown that at lower concentrations, binary pairs of perfluoroalkyls demonstrate concentration and response additivity, but deviate from additivity at higher concentrations (Wolf et al. 2014). These possible interactions (or dose additivity) complicate the interpretation of the epidemiological data.

Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies are cross-sectional in design and do not establish causality. ATSDR evaluated the available epidemiological data to assess whether the preponderance of the evidence suggested a possible association between perfluoroalkyl exposure and a particular health effect. This approach took into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide evidence for an association, it does not always imply that the observed effect is biologically relevant because the magnitude of the change may be within the normal limits or not indicative of an adverse health outcome. Plausibility depends primarily on experimental toxicology studies that establish a plausible biological mechanism for the observed effects. ATSDR's toxicological profile development guidance (https://www.atsdr.cdc.gov/toxprofiledocs/additional_resources.html/#Profile_Development) describes in detail the weight-of-evidence approach that includes quality assessment of every study included in the profile.

The available epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes; however, cause-and-effect relationships have not been established for these outcomes:

- **Hepatic effects.** Increases in serum enzymes and decreases in serum bilirubin, observed in studies of PFOA, PFOS, and PFHxS, are suggestive of liver alterations. In addition, the results of epidemiological studies of PFOA, PFOS, PFNA, and PFDA suggest an association between perfluoroalkyl exposure and increases in serum lipid levels, particularly total cholesterol and LDL cholesterol; see Section 2.9 for detailed discussion and citations.

- **Cardiovascular effects.** There is suggestive epidemiological evidence for an association between serum PFOA and PFOS and pregnancy-induced hypertension and/or pre-eclampsia; see Section 2.5 for detailed discussion and citations.

- **Immune effects.** Evidence is suggestive of an association between serum PFOA, PFOS, PFHxS, and PFDA levels and decreased antibody responses to vaccines; there is also limited evidence for PFNA, PFUnA, and PFDoDA; see Section 2.14 for detailed discussion and citations.

- **Developmental effects.** Evidence is suggestive of an association between serum PFOA and PFOS and small decreases in birth weight; the decrease in birth weight is <20 g (0.7 ounces) per 1 ng/mL increase in blood PFOA or PFOS level; see Section 2.17 for detailed discussion and citations.

As presented in Figures 2-3, 2-4, and 2-5, most of the available literature on the health effects of perfluoroalkyls in laboratory animals was conducted in oral studies, with a few inhalation and dermal exposure studies identified. The most commonly examined endpoints were liver, body weight, developmental, reproductive, and immunological.

The results of the animal studies suggest the following:

- **Hepatic effects.** Evidence from acute, intermediate, and/or chronic oral studies in rats, mice, and monkeys indicates that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBA, PFBS, PFDoDA, and PFHpA toxicity. The effects include increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. These effects were considered specific to rodents and were not considered relevant to humans. Some degenerative and necrotic effects that are likely relevant to humans have also been observed for PFOA, PFOS, and PFHpA. See Section 2.9 for detailed discussion and citations.

- **Immune effects.** Evidence from acute and intermediate oral studies in mice indicates that immune endpoints are sensitive targets of PFOA and PFOS toxicity. The most commonly reported effect was an impaired response to antigens. No alteration in antigen response was observed in the one study of PFNA. Immune function has not been tested for the other perfluoroalkyls examined in this profile. See Section 2.14 for detailed discussion and citations.

- **Reproductive effects.** Impaired mammary gland development has been observed in mice orally exposed to PFOA. In general, studies of PFOA and PFOS have not found alterations in fertility. See Section 2.16 for detailed discussion and citations.

- **Developmental effects.** Evidence from acute and intermediate oral studies in rats and/or mice indicates that developmental endpoints are targets of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, and PFBA toxicity. The developmental effects include decreases in pup body weight, decreases in pup survival, and alterations in locomotor activity. See Section 2.17 for detailed discussion and citations.

Figure 2-1. Health Effect Endpoints Examined in Epidemiological Studies

Health Effect Endpoint	Perfluoroalkyl											
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
Body weight	•	•	•	•	•	•				•		•
Respiratory	•											
Cardiovascular	•	•	•	•	•	•	•	•	•	•	•	•
Gastrointestinal		•										
Hematological	•	•										
Musculoskeletal	•	•	•	•								
Hepatic	•	•	•	•	•	•	•	•	•	•		
Renal	•	•	•	•	•			•		•	•	
Dermal												
Ocular												
Endocrine	•	•	•	•	•	•				•		
Immunological	•	•	•	•	•	•	•	•		•	•	•
Neurological	•	•	•	•								
Reproductive	•	•	•	•	•	•		•		•	•	•
Developmental	•	•	•	•	•	•	•		•	•		•
Other noncancer	•	•	•	•	•	•	•					•
Cancer	•	•	•	•	•	•	•			•		•

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

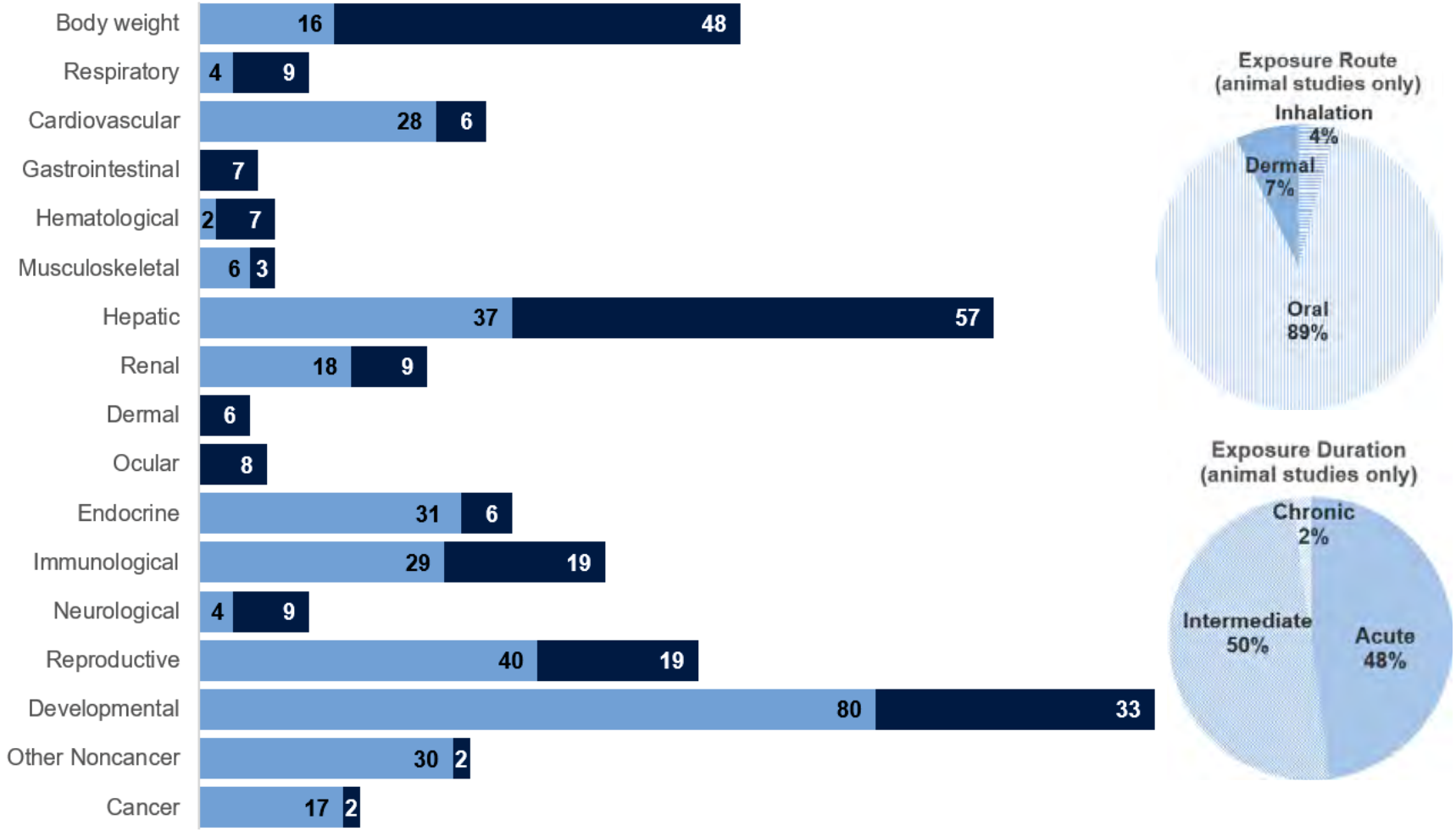
Figure 2-2. Health Effect Endpoints Examined in Laboratory Animal Studies

Health Effect Endpoint	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFBA	PFDoDA	PFHxA	FOSA
	Perfluoroalkyl											
Body weight	•	•	•	•	•	•		•	•	•	•	•
Respiratory	•	•	•	•	•			•	•		•	
Cardiovascular	•	•	•		•			•	•	•	•	
Gastrointestinal	•	•	•		•			•	•	•	•	
Hematological	•	•	•		•	•		•	•	•	•	
Musculoskeletal	•	•	•					•	•		•	
Hepatic	•	•	•	•	•	•		•	•	•	•	•
Renal	•	•	•		•	•		•	•	•	•	
Dermal	•	•							•			
Ocular	•	•						•	•		•	
Endocrine	•	•	•		•			•	•	•	•	
Immunological	•	•	•	•	•			•	•		•	
Neurological	•	•	•		•			•	•	•	•	
Reproductive	•	•	•	•				•	•	•	•	
Developmental	•	•	•	•	•	•		•	•	•	•	
Other noncancer	•			•								
Cancer	•	•										

FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Figure 2-3. Overview of the Number of Studies Examining PFOA Health Effects*

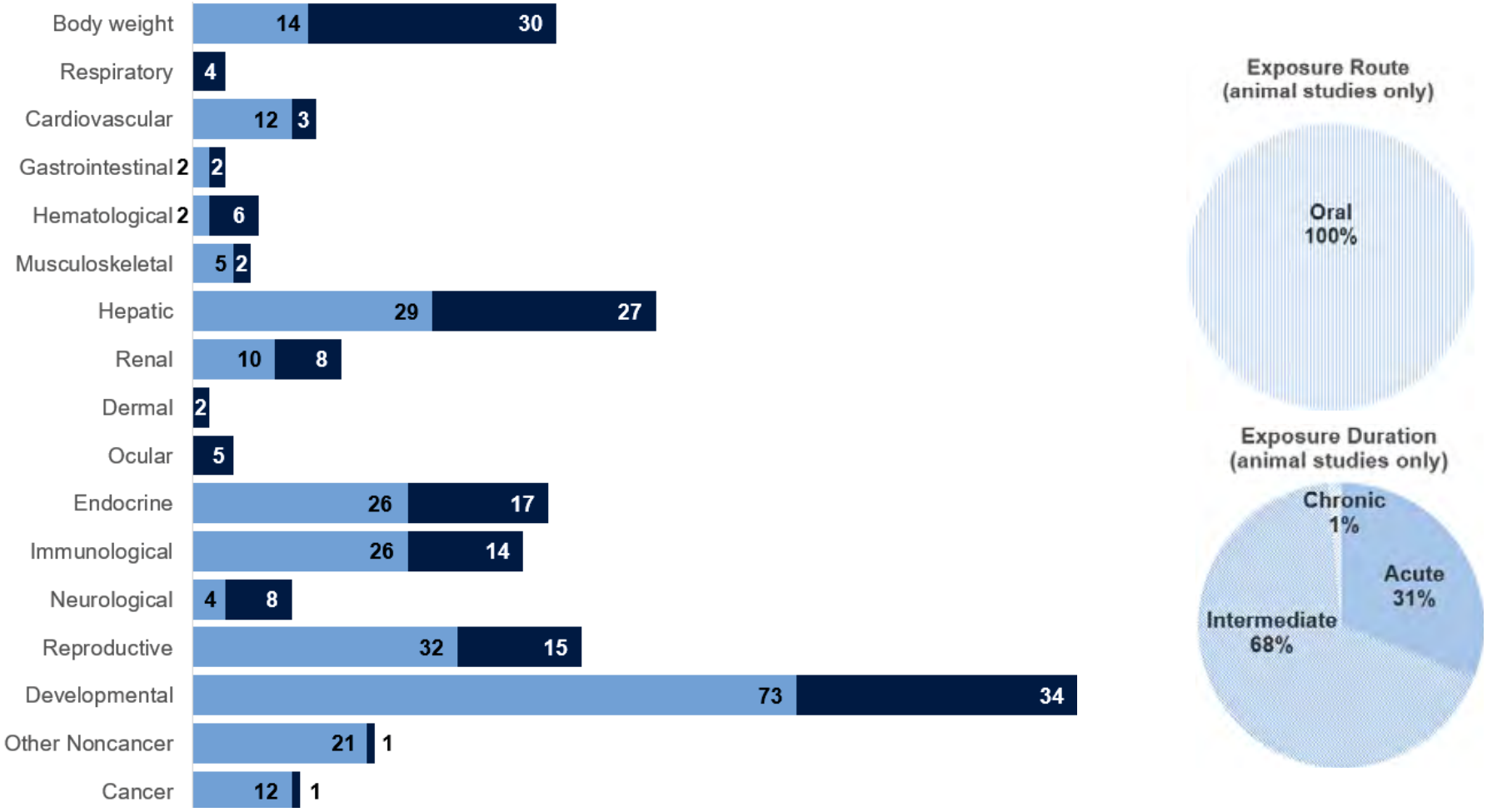
Developmental, hepatic, and body weight effects of PFOA were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)



*Includes studies discussed in Chapter 2. A total of 363 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple endpoints.

Figure 2-4. Overview of the Number of Studies Examining PFOS Health Effects*

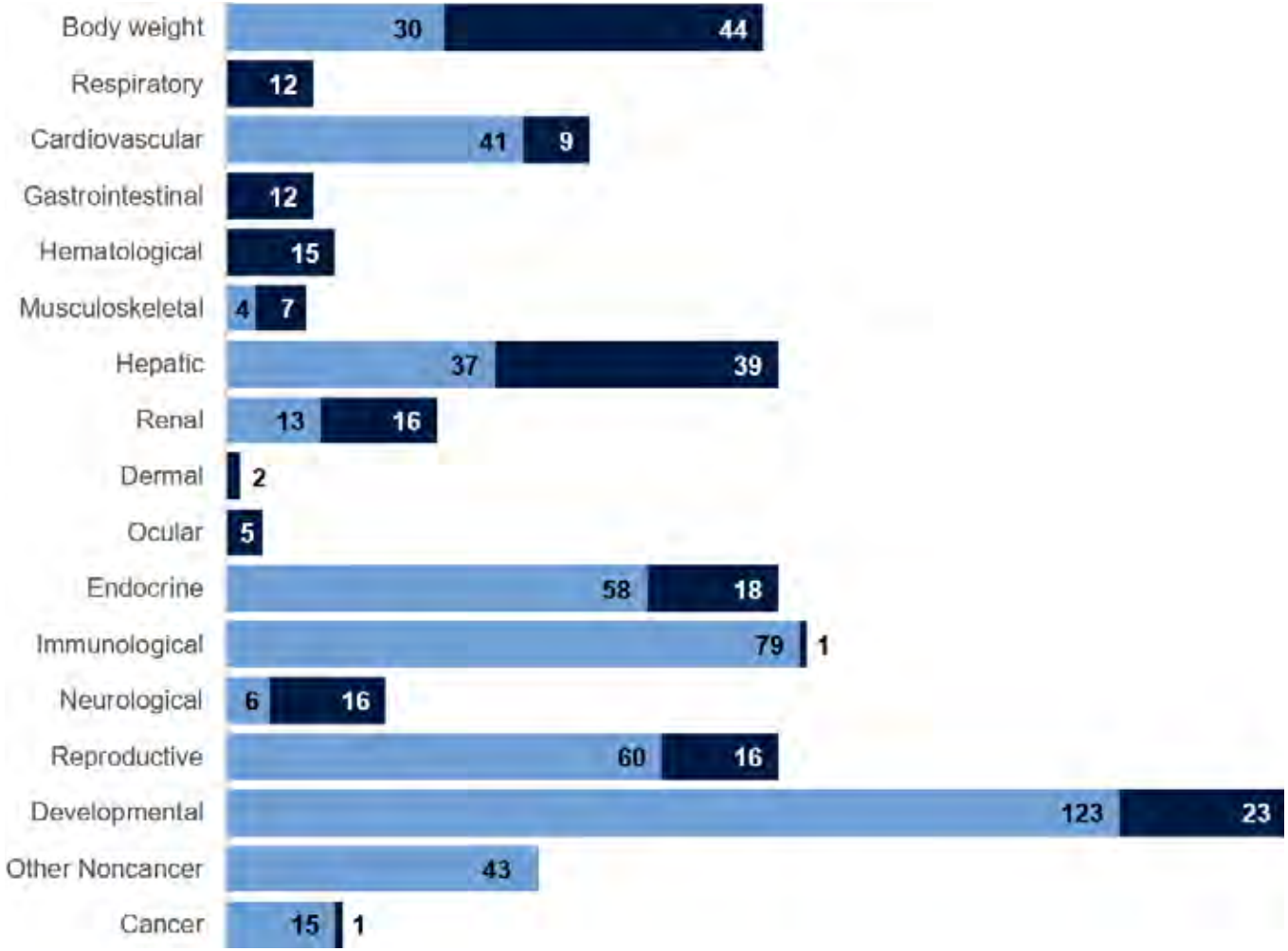
Developmental, hepatic, and reproductive effects of PFOS were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)

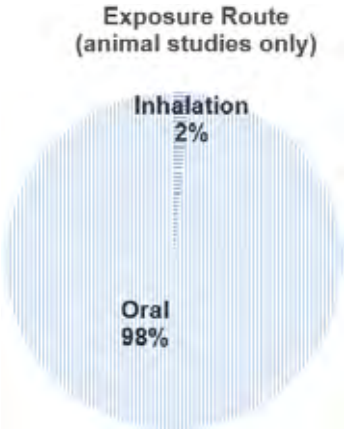


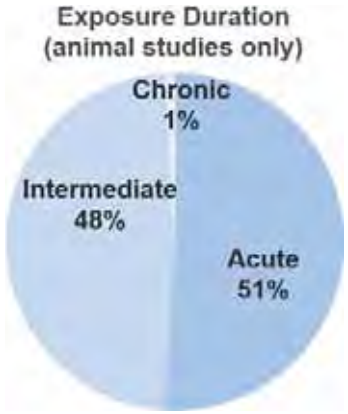
*Includes studies discussed in Chapter 2. A total of 301 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. In this figure, the number of human studies is referring to the number of publications; most human studies examined multiple perfluoroalkyls.

Figure 2-5. Overview of the Number of Studies Examining Other Perfluoroalkyls Health Effects*

Developmental, hepatic, and body weight effects of other perfluoroalkyls were the most widely examined potential toxicity outcomes
More studies evaluated health effects in **humans** than **animals** (counts represent studies examining endpoint)







*Includes studies discussed in Chapter 2. A total of 213 studies (including those finding no effect) have examined toxicity; most animal studies examined multiple endpoints. Most human studies examined multiple perfluoroalkyls; within each publication, the results for each perfluoroalkyl is counted as a study.

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
1	Rat (albino) 5 M,F	1 hour (NS)	18,600	CS, BW, GN, HP	Resp		18,600		Red nasal discharge; dry rales
					Ocular		18,600		Red material around the eyes; lacrimation
					Neuro		18,600		Excessive salivation
Griffith and Long 1980									
APFO									
2	Rat (CD) 36 M	4 hours	380, 810, 830, 2,200, 4,800, 5,700	CS, HE, BI, GN, HP	Death			980 M	LC_{50}
					Bd wt			380 M	Weight loss for 1–2 days after exposure (data not shown)
					Resp			380 M	Pulmonary edema
					Gastro		380 M		Stomach irritation
					Hepatic	810			Liver enlargement at 810 mg/m^3; no histological alterations
					Ocular	380 M		810 M	Corneal opacity and corrosion
Kennedy et al. 1986									
APFO									
3	Rat (CD) 24 M	2 weeks 6 hours/day 5 days/week	0, 1, 7.6, 84	CS, HE, BI, GN, HP	Bd wt	7.6	84		7% lower body weight on exposure day 5
					Resp	84			
					Cardio	84			
					Gastro	84			
					Hemato	84			
					Musc/skel	84			
					Hepatic	84			Increased absolute and relative liver weight; hepatocellular hypertrophy at 7.6 mg/m^3
					Renal	84			
					Dermal	84			
					Ocular	84			
					Endocr	84			
					Neuro	84			No histological alterations
					Repro	84			No histological alterations
Kennedy et al. 1986									
APFO									

Table 2-1. Levels of Significant Exposure to PFOA – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
4	Rat (Sprague-Dawley) 12 F	GDs 6–15 6 hours/day	0, 0.1, 1, 10, 25	MX, DX, OW, CS, HP	Death			25	3/12 deaths on GDs 12, 13, and 17
					Bd wt	1	10		12% decrease weight gain on GDs 6–15
					Hepatic	25			18% increase absolute liver weight at 25 mg/m^3
					Develop	10	25		10% decreased neonatal body weight on PND 1

Staples et al. 1984
APFO

[a]The number corresponds to entries in Figure 2-6.

APFO = ammonium perfluorooctanoate (ammonia salt of PFOA); BI = biochemical changes; BW or Bd wt = body weight; F = female(s); Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; Endocr = endocrine; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory

Figure 2-6. Levels of Significant Exposure to PFOA – Inhalation
Acute (≤14 days)

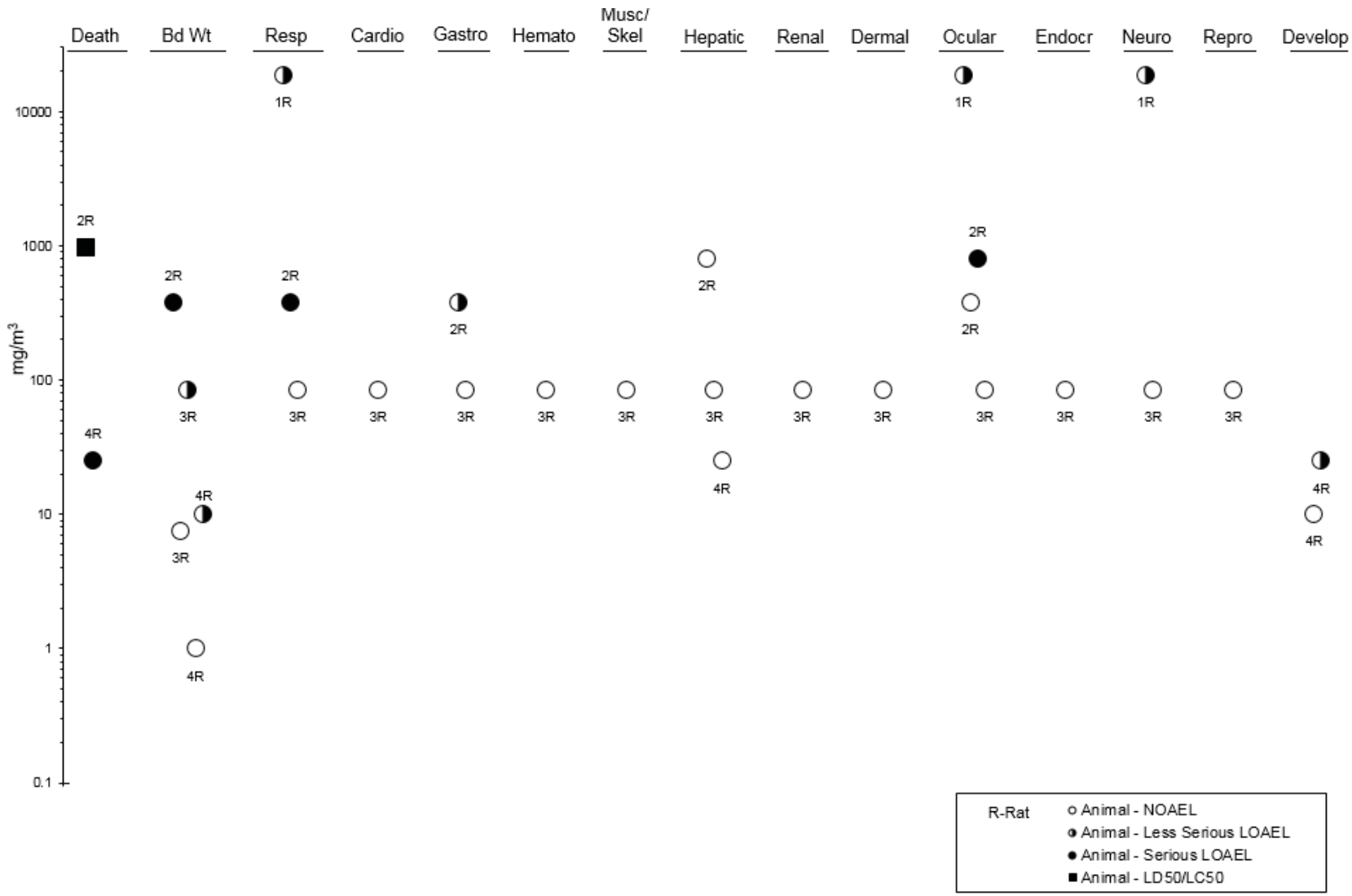


Table 2-2. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/m^3)	Parameters monitored	Endpoint	NOAEL (mg/m^3)	Less serious LOAEL (mg/m^3)	Serious LOAEL (mg/m^3)	Effect
ACUTE EXPOSURE									
PFNA									
1	Rat (CD) 6 M	4 hours	67–4,600	LE	Death			820	14-day LC_{50}
Kinney et al. 1989									
Exposure was nose-only.									
2	Rat (CD) 10 M	4 hours	0, 67, 590	BW, OW	Bd wt	67	590		Reduced 18% 5 days after exposure
					Resp	67	590		Lung noise; labored breathing during and after exposure
					Hepatic	67			28% increase in absolute liver weight 5 days after exposure to ≥67 mg/m^3
Kinney et al. 1989									
Exposure was nose-only.									

[a]The number corresponds to entries in Figure 2-7.

BW or Bd wt = body weight; LC_{50} = lethal concentration, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no-observed-adverse-effect level; OW = organ weight; PFNA = perfluorononanoic acid; Resp = respiratory

Figure 2-7. Levels of Significant Exposure to Other Perfluoroalkyls – Inhalation
Acute (≤14 days)

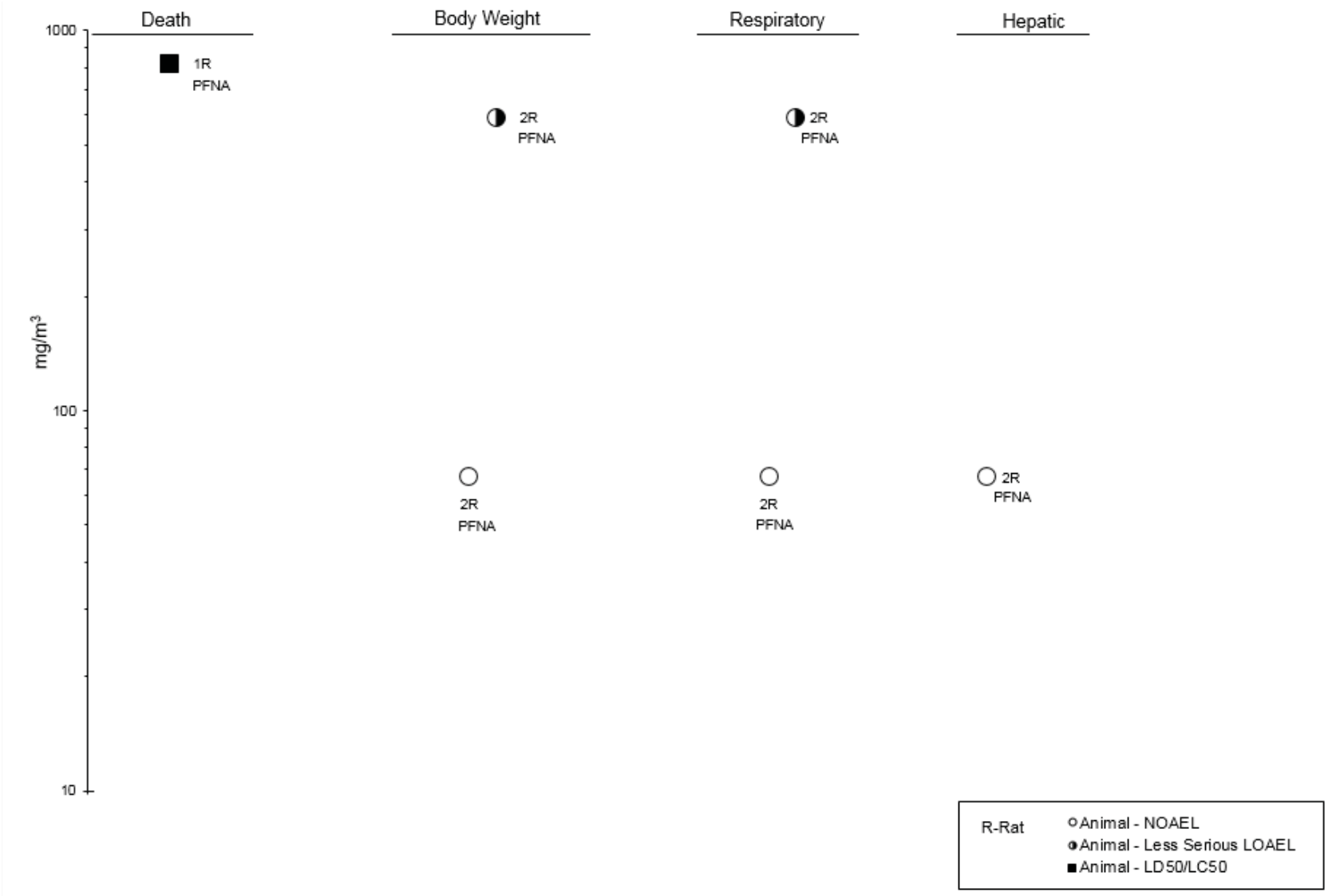


Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (Rhesus) 10 M,F	2 weeks (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			100	Unspecified number out of four died on week 2
Griffith and Long 1980									
APFO									
2	Rat (CD) 10 M	14 days (GW)	0, 25	BI, OW	Repro		25		184% increase in serum estradiol
Biegel et al. 1995									
APFO									
3	Rat (CD) 75 M	14 days (GW)	0, 1, 10, 25, 50	BW, OW, BI, HP	Bd wt	10	25		14% reduction in final body weight
					Hepatic	50			46% increase in relative liver weight at ≥10 mg/kg/day
					Repro	1	10		63% increase in serum estradiol
Cook et al. 1992									
APFO									
4	Rat (Sprague-Dawley) 18 M	1 or 7 days (F)	0, 18, 23		Hepatic	23			Increased liver weight, decreased serum cholesterol, triglyceride, hepatocellular hypertrophy at ≥18 mg/kg/day
Elcombe et al. 2010									
APFO									
5	Rat (albino) 25 M,F	Once (GO)	100, 215, 464, 1,000, 2,150		Death			680 M 430 F	LD_{50} LD_{50}
Griffith and Long 1980									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (albino) 40 M,F	28 days (F)	M: 0, 3, 10, 30, 10, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400		Death			1,000 M 1,130 F	5/5 males and 5/5 females died before end of 1st week of study
Griffith and Long 1980									
APFO									
7	Rat (Wistar) 8 M	7 days ad lib (F)	0, 16	BW, OW, BI, EA	Bd wt	16			
					Hepatic	16			66% increase in absolute liver weight
Haughom and Spydevold 1992									
APFO									
8	Rat (Sprague-Dawley) 3 M	14 days (F)	0, 20	OW, EA	Hepatic	20			45% increase in relative liver weight
Ikeda et al. 1985									
PFOA									
9	Rat (Sprague-Dawley) 16 M	14 days (GW)	0, 0.5, 5, 50	BW, OW, CS, HE, BI	Bd wt	50			
					Hepatic	50			2-fold increased mean relative liver weight at 50 mg/kg/day
					Immuno	50			No alterations in spleen weight or splenocyte phenotype
Iwai and Yamashita 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
10	Rat (Wistar) 5–12 M	Once (GO)	0, 50	BW, FX	BW	50			
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
PFOA									
11	Rat (Wistar) 30 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5	BW, OW, EA, HP	Bd wt	38			
					Hepatic	9.5			Significant increase in absolute and relative liver weight at ≥4.7 mg/kg/day
Kawashima et al. 1995									
PFOA									
12	Rat (SD-IGS BR) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	1	3		24% decrease in overall body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									
13	Rat (CD) 15 M	14 days (G)	0, 0.2, 2, 20, 40	BW, OW, EA	Bd wt	2	20		14% lower final body weight
					Hepatic	40			34% increase in absolute and relative liver weight at ≥2 mg/kg/day
					Repro	0.2	2		2-fold increase in serum estradiol
Liu et al. 1996									
APFO									
14	Rat (Sprague-Dawley) 24 M	1, 3, 7 days (GW)	0, 50	BW, BI, EA, HP	Bd wt		50		17% weight loss
					Hepatic	50			2-fold increase in relative and absolute liver weight
Pastoor et al. 1987									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
15	Rat (Sprague-Dawley) 12 or 25 F	GDs 6–15 (GO)	0, 100	DX, MX, BW	Bd wt			100	33% reduced maternal body weight gain
					Develop	100			No alterations in fetal body weight or teratology
Staples et al. 1984									
APFO									
16	Mouse (Kunming) 12 F	GDs 1–7 or 13 (GW)	0, 2.5, 5, 10	BC, OW, RX	Repro		2.5		Decreases in progesterone levels at ≥5 mg/kg in GD 13 group; increased serum estradiol level at 10 mg/kg/day on GD 7; decreases in number of corpora lutea at ≥2.5 mg/kg/day in GD 1–7 groups and at ≥5 mg/kg/day in GD 13 groups
					Develop	5	10		Increased resorbed embryos at 10 mg/kg/day in GD 13 group
Chen et al. 2017b									
PFOA									
17	Mouse (SV129 wild type) 4 M	7 days (G)	0, 10	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
PFOA									
18	Mouse (C57BL/6N) 6 F	10 days (W)	0, 3.75, 7.5, 15	FX	Immuno	7.5	15		Altered response to sRBC
DeWitt et al. 2009									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
19	Mouse (CD) 40 M,F	28 days (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800		Death			180 M 195 F	5/5 died before 2nd week of study 5/5 died before 2nd week of study
Griffith and Long 1980									
APFO									
20	Mouse (C57BL/6N) 16 F	GDs 6–17 (DW)	0, 0.5, 1		Bd wt	1			
					Develop		0.5		7–10% decrease in litter weight on PND 2
Hu et al. 2010									
APFO									
21	Mouse (CD-1) 10 M	Once (G)	0, 0.58, 8.70	CS, OF, DX	Develop		0.58		Decreased spontaneous activity and altered response to cholinergic stimulant
Johansson et al. 2008									
APFO									
10-day-old mice were administered a single dose of PFOA; neurodevelopmental testing was conducted when the pups were 2 or 4 months of age									
22	Mouse (CD-1) 5 M,F	14 days (F)	0, 5.3, 54, 537	OW	Hepatic	537			123–155% increase in absolute liver weight in 14 days at ≥5.3 mg/kg/day
Kennedy 1987									
APFO									
23	Mouse (CD-1) 10 M	14 days (GW)	0, 0.3, 1, 3, 10, 30,	BW, OW, BC	Bd wt	3		10	6–12% decreased in body weight gain
					Hepatic	30			Decreased serum cholesterol levels at ≥0.3 mg/kg/day
Loveless et al. 2006									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
24	Mouse (C57BL/6N) 3 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 10 days of treatment
					Hepatic	390			74% increase in absolute liver weight at ≥78 mg/kg/day
Permadi et al. 1992									
PFOA									
25	Mouse (C57BL/6N) 4 M	2–10 days (F)	0, 78, 390	BW, OW, EA	Bd wt			78	25% body weight loss after 5 days of treatment
					Hepatic	390			74% increase in absolute liver weight in 5 days at ≥78 mg/kg/day
Permadi et al. 1993									
PFOA									
26	Mouse (C57BL/6) 4 M	10 days (F)	0, 2	BW, FI, BC, HP	Bd wt	2			
					Hemato	2			
					Hepatic	2			68% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	2			
Qazi et al. 2010a									
PFOA									
27	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		36% decrease in body weight gain
					Hepatic	20			32% increase in relative liver weight
					Immuno		20		Inhibition of T-and B-lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Mouse (CD-1) 14 F	GDs 8–17 GDs 1217 (GW)	0, 5	DX, GN	Develop		5		Altered mammary gland development in female pups; reduced pup weight on PND 20
White et al. 2007									
APFO									
29	Mouse (CD-1) 56 F	GDs 8–17 (GW)	0, 5		Bd wt	5			
					Hepatic	5			40–120% increased relative liver weight in lactating dams on PNDs 1–10
					Repro		5		Immature mammary gland morphology in lactating dams on PNDs 1–10
					Develop		5		Delayed mammary gland development (30–60%) in female pups on PNDs 1–10
White et al. 2009									
APFO									
30	Mouse (CD-1) 12–14 F	GDs 7–17 GDs 10–17 GDs 13–17 GDs 15–17	0, 5		Develop		5		Delayed mammary gland development (31–47%) in female pups on PNDs 22–32 and at 18 months
White et al. 2009									
APFO									
31	Mouse (CD-1) 6–14 F	GDs 7–17, GDs 10–17 GDs 13–17 GDs 15–17 (GW)	0, 5, 20	DX, MX, BW, OW	Bd wt	20			No alterations in dams dosed on GDs 15–17
					Hepatic	20			Increase in relative liver weight in dams dosed on GDs 13–17, 10–17, or 7–17 at ≥5 mg/kg/day
					Develop		5		Reduced pup body weight at weaning, 43% in males and 35% in females
Wolf et al. 2007									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Mouse (C57BL/6N) 8 M	7 days (F)	0, 24	CS, BW, OW, BI	Bd wt		24		>10% reduced final body weight
					Hepatic	24			2-fold increase in absolute liver weight
Xie et al. 2003									
PFOA									
33	Mouse (C57BL/6N) 8 M	10 days (F)	0, 30	BW, OW, BI, CS	Bd wt		30		17% decrease in final body weight
					Hepatic	30			>90% increase in absolute and relative liver weight
					Immuno			30	86% reduction in absolute thymus weight; 30% reduction in absolute spleen weight
Yang et al. 2000									
PFOA									
34	Mouse (C57BL/6N) 8 M	10 days (F)	0, 1, 3.5, 11.5, 23, 58	CS, BW, OW, OF, BI	Hepatic	1			35% increase in absolute liver weight at ≥1 mg/kg/day
					Immuno		11.5		40–50% decrease in spleen and thymus weights
Yang et al. 2001									
PFOA									
35	Mouse (C57BL/6N) 8–12 M	7 days (F)	0, 24	CS, BW, BI, OF	Immuno		24		Decreased humoral response to immunization with horse red blood cells
Yang et al. 2002a									
PFOA									
36	Mouse (C57BL/6N) 16 M	7 days ad lib (F)	0, 33	BW, OW, BI, OF	Bd wt		33		14% decreased mean body weight
					Hepatic	33			86% increase in absolute liver weight
					Immuno		33		40% reduction in spleen weight and 79% reduction in thymus weight
Yang et al. 2002b									
PFOA									
Experiments with PPARα-null mice suggested PPARα-dependent and -independent immune effects									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rabbit (New Zealand) 18 F	GDs 6–18 1 time/day (GW)	0, 1.5, 5, 50	CS, MX, DX, BW	Develop	50			
Gortner et al. 1982									
PFOA									
INTERMEDIATE EXPOSURE									
38	Monkey (Cynomolgus) 4–6 M	26 weeks 1 time/day (C)	0, 3, 10, 30/20	BC, BW, CS, EA, FI, GN, HE, HP, LE, OP, OW, UR	Bd wt	10	20		12% decrease by week 10
					Resp	20			
					Cardio	20			
					Gastro	20			
					Hemato	20			
					Musc/skel	20			
					Hepatic		3		36% increase in absolute liver weight at ≥3 mg/kg/day; increased serum triglyceride levels at 30/20 mg/kg/day
					Renal	20			
					Dermal	20			
					Ocular	20			
					Endocr	3	10		Significant decrease in serum TT4 (27–35%) and FT4 (30–38%)
					Immuno	20			No histological alterations
					Neuro	20			No histological alterations
					Repro	20			No histological alterations
Butenhoff et al. 2002									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
39	Monkey (Rhesus) 10 M,F	90 days 1 time/day (G)	0, 3, 10, 30, 100	LE, CS, HE, BI, GN, HP	Death			30	One male and two females died during weeks 7–12
					Bd wt	10		30	33% body weight loss by week 6
					Cardio	10			
					Gastro	10	30		Emesis
					Hemato	30			
					Hepatic	10			
					Renal	10			
					Immuno	10	30		Atrophy of lymphoid follicles in spleen and lymph nodes
					Neuro	10	30		Hypoactivity and prostration
					Repro	100			No histological alterations in testes or ovaries
Griffith and Long 1980									
APFO									
40	Monkey (Cynomolgus) 8 M	30 day 1 time/day (C)	0, 2, 20	CS, BW, FI, BI, HE, EA, GN, HP	Bd wt	20			
					Hemato	20			
					Hepatic	20			
					Endocr	20			No alterations in serum levels of thyroid hormones and TSH and histopathology of adrenals
					Repro	20			No alterations in serum estradiol, estriol, or histopathology of the testes
Thomford 2001									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Rat (CD) 156 M	1 year ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduced weight gain
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Significant increase in serum estradiol at 1, 3, 6, 9, and 12 months (~100–180%); prolactin was decreased at all time points, but not always significantly
Biegel et al. 2001									
APFO									
42	Rat (Sprague-Dawley) 30 M,F	70–90 days 1 time/day (GW)	0, 1, 3, 10, 30	MX, DX, OF, BW, GN, HP	Bd wt	3	10		>11% reduced body weight
					Hepatic	1	3		Increased absolute and relative liver weight in P-generation (36%) and F1-generation (30%) males; hepatocellular hypertrophy and less commonly necrosis in F1 males (incidence not reported)
					Renal		3		Increased absolute and relative kidney weight in P-generation (14%) and F1-generation (11%) males
					Endocr	10	30		Vacuolation of zona glomerulosa of adrenal gland
					Repro	30			No alteration in reproductive performance in P0 or F1 generation
					Develop	10		30	Increased number of dead pups on PNDs 6–8
Butenhoff et al. 2004b									
APFO									
43	Rat (Wistar) 5 F	GD 1 to PND 21 ad lib (W)	0, 1.6		Develop		1.6		17–18% reduced motor coordination and increased locomotor activity in pups on PNDs 34–35
Cheng et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	OW, HP	Resp		5		
					Hepatic		5		Cytoplasmic vacuolization, necrosis, hypertrophy, increased liver weight at ≥5 mg/kg/day; fatty degeneration, angiectasis and congestion in the hepatic sinusoid or central vein at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009									
PFOA									
45	Rat (Sprague-Dawley) 10 M	28 days (F)	0, 18		Hepatic		18		Increased liver weight (43% on day 29), decreased serum cholesterol (39% on day 29) and triglyceride (73% on day 29), hepatocellular hypertrophy and hyperplasia
Elcombe et al. 2010									
APFO									
46	Rat (CD) 40 M,F	28 days ad lib (F)	M: 0, 3, 10, 30, 100, 300, 1,000, 3,000; F: 0, 3.4, 11.3, 34, 113, 340, 1,130, 3,400	BW, FI, HP	Bd wt	10 M	30 M	100 M	30 mg/kg/day: 11% reduction in final body weight; 100 mg/kg/day: 33% reduction in final body weight
					Hepatic	3 M			Hepatocyte hypertrophy
Griffith and Long 1980									
APFO									
47	Rat (CD) 30 M,F	90 days ad lib (F)	M: 0, 1, 3, 10, 30, 100; F: 0, 1.1, 3.4, 11, 34, 110	CS, BW, FI, HE BI, GN, HP, OW	Bd wt	30 M 110 F	100 M		33% reduction in final mean body weight
					Resp	100 F			
					Cardio	110 F			
					Gastro	110 F			
					Musc/skel	110 F			
					Hepatic	100 M			Hepatocyte hypertrophy; 50% increase in absolute liver weight
					Renal	110 F			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Dermal	110 F			
					Ocular	110 F			
					Endocr	110 F			
					Immuno	110 F			
					Neuro	110 F			No histological alterations
					Repro	100 M 110 F			No histological alterations
Griffith and Long 1980									
APFO									
48	Rat (CD) 10 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96	9.6		10% decrease in final body weight
					Hemato	29			
					Hepatic	0.29	29		34% decrease in serum triglyceride levels, minimal hepatocellular hypertrophy at ≥0.29 mg/kg/day; minimal focal necrosis at 29 mg/kg/day
					Immuno	29			
Loveless et al. 2008									
APFO									
49	Rat (CD) 55 M	13 weeks ad lib (F)	0, 0.06, 0.64, 1.94, 6.5	CS, BW, FI, OW, GN, HP	Bd wt	6.5			
					Resp	6.5			
					Hepatic	6.5			Minimal to moderate hepatocellular hypertrophy at ≥0.64 mg/kg/day
					Neuro	6.5			No histological alterations
					Repro	6.5			No histological alterations
Perkins et al. 2004									
APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
50	Mouse (129S1/SvlmJ WT) 17 F	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1, 3, 5, 10, 20	MX, DX, BW	Bd wt	10 F			
					Hepatic	20			Increased absolute and relative liver weight of dams on PND 22 at ≥1 mg/kg/day
					Develop	0.3		0.6	Significantly reduced pup survival (46%) from birth to weaning
Abbott et al. 2007									
APFO									
Body weight NOAEL is for changes during pregnancy.									
51	Mouse (wild-type Sv/129) 5–6 F	GDs 1–17 1 time/day (GW)	0, 3		Hepatic	3			28% increased liver weight, hepatocellular hypertrophy with increased peroxisomes
					Develop			3	31.5% reduced pups per litter on PND 20
Albrecht et al. 2013									
PFOA									
52	Mouse (C57BL/6N) 8 F	15 days ad lib (W)	0, 0.94, 1.88, 3.75, 7.5, 15, 30	BW, OW, OF	Bd wt	7.5		15	Weight loss (~5%)
					Immuno	1.88	3.75		Reduced sRBC-specific response to IgM antibody titers
DeWitt et al. 2008									
APFO									
53	Mouse (wild-type C57BL/6-Tac and PPARα knockout) 8 F	15 days (W)	0, 7.5, 30	BW, OW, BC	Bd wt		30		14–20% decrease in body weight in wild-type mice
					Immuno	7.5	30		16 and 14% reduction in sRBC-specific antibody response in wild-type and PPARα knockout mice, respectively; 29.8% decrease in relative spleen weight in wild-type mice
DeWitt et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Mouse (C57BL/6N) 8 F	15 days (W)	0, 0.94, 1.88, 3.75, 7.5	BW, OW, BC	Bd wt	7.5			
					Immuno	0.94	1.88		Decrease in DNP-specific IgM antibody responses; decreases in relative spleen (17%) and thymus weights (14%) at 7.5 mg/kg/day
DeWitt et al. 2016									
PFOA									
55	Mouse (C57BL/6N) 8 F	10, 13, 15 days (W)	0, 3.75, 7.5	BC	Bd wt	7.5			
DeWitt et al. 2016									
PFOA									
56	Mouse (CD-1) 6–14 dams; 21–37 F offspring	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 5	HP	Hepatic	0.3	1		Increase in severity of chronic inflammation at ≥1 mg/kg/day; Ito cell hypertrophy and centrilobular hepatocellular hypertrophy at 5 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Hines et al. 2009									
57	Mouse (129/Sv WT) 3–7 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 0.6, 1	HP	Hepatic	1			Increased severity of centrilobular hepatocyte hypertrophy at ≥0.3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									
58	Mouse (129/Sv PPARα-knockout) 5–9 dams; 6–10 F offspring	GDs 1–17 (GW)	0, 0.1, 0.3, 1, 3	HP	Hepatic	1	3		Centrilobular hepatocyte hypertrophy and bile duct hyperplasia at 3 mg/kg/day
Filgo et al. 2015a, 2015b									
PFOA									
Animals exposed *in utero* on GDs 1–17 and examined at 18 months of age; the offspring were also examined by Abbott et al. 2007									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
59	Mouse (CD-1) NS F	GDs 1–17 (GW)	0, 0.1, 0.3, 1	NX	Develop	0.3	1		Increased ambulatory activity and decreased methamphetamine-induced activity
Goulding et al. 2017									
PFOA									
60	Mouse (CD) 40 M,F	28 days ad lib (F)	M: 0, 5.4, 18.0, 54, 180, 540, 1,800, 5,400; F: 0, 5.8, 19.5, 58, 195, 580, 1,950, 5,800	BW, FI, HP	Death			54 M 58 F	4/5 died before end of 4th week 5/5 died before 4th week of study
					Bd wt		5.4 M	5.8 F	Males: final body weight 20% lower than controls; females: final body weight 25% lower than controls
					Hepatic	18			3-fold or greater increased absolute and relative liver weight and hepatocellular hypertrophy at ≥5.4 mg/kg/day
Griffith and Long 1980									
APFO									
61	Mouse (CD-1) 5–14F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1, 3, 5	BW FX	Develop		5 F		Decreased birth weight (approximately 8%) and body weight at weaning (24%)
Hines et al. 2009									
APFO									
62	Mouse (CD-1) 5 M,F	21 days ad lib (F)	0, 0.0018, 0.0054, 0.018, 0.054, 0.18, 0.54, 1.8, 5.4	OW	Hepatic	5.4			39–41% increase in absolute liver weight at ≥5.4 mg/kg/day
Kennedy 1987									
APFO									
63	Mouse (C57BL/6) 10 F	GDs 1–21 (F)	0.3	BW, DX	Develop		0.3[b]		Altered femur and tibial bone morphology, decreased tibial mineral density
Koskela et al. 2016									
PFOA									
Offspring were examined at 13 and 17 months of age									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
64	Mouse (CD-1) 9–45 M	GDs 1–17 1 time/day (GW)	0, 1, 3, 5, 10, 20, 40	MX, DX, BW, OW	Bd wt	5		10	32% reduced weight gain during pregnancy
					Hepatic	1			38% increase in absolute liver weight at ≥1 mg/kg/day
					Develop		1	5	Reduced ossification of proximal phalanges and advanced preputial separation at ≥1 mg/kg/day; 20% decrease in pup body weight on PND 23 at ≥3 mg/kg/day; increased number of dams with full litter resorptions, decrease in neonatal survival, tail and limb defects, delay in eye opening, and delay in first estrus at ≥5 mg/kg/day
Lau et al. 2006									
APFO									
65	Mouse (CD) 20 M	28 days (G)	0, 0.29, 0.96, 9.6, 29		Bd wt	0.96		9.6	Weight loss (86% of controls)
					Hepatic	0.29	0.96		Mild hepatocellular hypertrophy at ≥0.29, moderate to severe hypertrophy and single cell necrosis at ≥0.96 mg/kg/day; decreased serum cholesterol (31%) and triglyceride (53%) at ≥9.6 mg/kg/day
					Immuno	0.96 M	9.6 M		Decreased response to sRBC, decreased number of splenic and thymic lymphocytes
Loveless et al. 2008									
APFO									
66	Mouse (CD-1) 13 F	GDs 1–17 (G)	0, 0.3, 1.0, 3.0	DX	Develop		0.3		Impaired development of mammary glands in offspring
Macon et al. 2011									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
67	Mouse (CD-1) 13 F	GDs 10–17 (G)	0, 0.01, 0.1, 1.0	DX	Develop		0.01		Developmental delays in mammary gland development
Macon et al. 2011									
PFOA									
68	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOA									
69	Mouse (C57BL/6/Bk1) 6 M, 10 F	GDs 1–21 ad lib (F)	0, 0.3		Develop		0.3		Altered exploratory behavior in adult offspring (increased in males and decreased in females); increased global activity in males
Onishchenko et al. 2011									
PFOA									
70	Mouse (CD-1) 17–21 F dams; 4–6 M,F pups	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1	BI, BW, HP, OF, OW	Hepatic		0.01		Increased severity of hepatocellular hypertrophy at PND 91 and periportal inflammation on PND 21 at ≥0.01 mg/kg/day (incidence was not reported); decreased serum total cholesterol, LDL, and HDL levels in high-fat fasted animals on PND 91 at ≥0.3 mg/kg/day
Quist et al. 2015a, 2015b									
PFOA									
Subgroup of female offspring were fed a high-fat diet (50% calories from diet) for 6 weeks									
71	Mouse (C57BL/6) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			66–67% increase in relative liver weight in males; increased plasma cholesterol levels in males (35%) and females (70%)
Rebholz et al. 2016									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
72	Mouse (BALB/c) 6 M,F	6 weeks (F)	0.55	BC, BW, FI, OW	Hepatic	0.55			54–65% increase in relative liver weight; 20% increase in plasma cholesterol in males
Rebholz et al. 2016									
PFOA									
73	Mouse (C57BL/6) 6 F	GD 7 to PND 21 (F)	0, 0.1	BH, BW	Develop		0.1		Increased horizontal and ambulatory locomotor activity and decreased resting time in males; decrease in novel object recognition in males and females
Sobolewski et al. 2014									
PFOA									
74	Mouse (ICR) 10 M	21 days ad lib (W)	0, 0.5, 2.6, 18, 47	BW, OW, GN, HP	Bd wt	2.6		18	17% decrease in weight gain
					Hepatic	18	47		27% increase in relative liver weight at ≥0.5 mg/kg/day; increases in ALT at ≥2.6 mg/kg/day; hepatocytomegaly at 18 mg/kg/day; necrosis at 47 mg/kg/day
					Renal	47			
Son et al. 2008									
APFO									
75	Mouse (ICR) 10 M	21 days (W)	0, 0.49, 2.64, 17.63, 47.21	FX HP	Immuno		47.21		Marked hyperplasia in spleen white pulp and thymic atrophy
Son et al. 2009									
PFOA									
76	Mouse (C57BL/6N) 7–8 M	3 weeks (F)	0, 5	HP	Hepatic		5		Hepatocellular hypertrophy and degeneration
Tan et al. 2013									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
77	Mouse (CD-1) 4–12 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop		0.01		Developmental delays in the mammary glands on PNDs 35 (26%) and 56 (30%)
Tucker et al. 2015 PFOA									
78	Mouse (C57BL/6) 2–6 F	GDs 1–17 (GW)	0, 0.01, 0.1, 0.3, 1.0	BW, DX, OW	Develop	0.1	0.3		Developmental delays in the mammary glands on PNDs 21 (38%) and 61 (25%)
Tucker et al. 2015 PFOA									
79	Mouse (CD-1) 14–16 F	GDs 1–17 GDs 8–17 1 time/day GDs 12–17 (GW)	0, 5	MX, DX, GN, HP	Repro		5		Delayed mammary gland differentiation
White et al. 2007 APFO									
80	Mouse (CD-1) 14 F	GDs 1–17 1 time/day (GW)	0, 5	DX, GN	Develop			5	Increased prenatal loss; 40% reduced neonatal body weight on PNDs 5 and 10
White et al. 2007 APFO									
81	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5		Develop		3		Delayed mammary gland development in female pups on PNDs 22–63 and at 18 months
White et al. 2009 APFO									
82	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 1, 5		Repro		1		Delayed mammary gland lactational differentiation in dams on PND 22
					Develop	1		5	323% increased prenatal loss, 16.7% decreased live fetuses, 24.3% decreased neonatal survival
White et al. 2011 APFO									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
83	Mouse (CD-1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.0024, 1.0024		Repro		0.0024		Delayed mammary gland lactational differentiation in dams on PND 22
		GD 7 to PND 22 (W); 3-generation study			Develop		0.0024		Delayed mammary gland development in female pups on PNDs 22–63
White et al. 2011									
APFO									
84	Mouse (CD-1) 28–48 F	GDs 1–17 1 time/day (GW)	0, 3, 5	CS, BW, MX, DX, OW	Bd wt	5			
					Hepatic	5			Significant increase in relative and absolute maternal liver weight on PND 22 at ≥3 mg/kg/day
					Develop		3	5	3 mg/kg/day: reduced weight gain through lactation (14.8% in males and 20.6% in females); delayed eye opening and hair growth at ≥3 mg/kg/day; decreased pup survival from birth to weaning at 5 mg/kg/day
Wolf et al. 2007									
APFO									
85	Mouse (ICR) 5–10 F	GDs 0–17 or GDs 0–18 (GW)	0, 1, 5, 10	BW, FI, WI, OW, HP, DX	Hepatic	1	10		35% increased maternal relative liver weight with hepatocellular hypertrophy at ≥1 mg/kg/day; single cell necrosis and mild calcification at 10 mg/kg/day
					Renal	1			35% increased maternal relative kidney weight with renal hypertrophy
					Develop	1		5	14% increased neonatal mortality, 9.5% reduced fetal body weight
Yahia et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
86	Mouse (BALB/c) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		13% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop		1		Delay in vaginal opening at 1 mg/kg and mammary gland growth inhibition at 5 and 10 mg/kg
Yang et al. 2009									
PFOA									
87	Mouse (C57BL/6)) 5 F	5 days/week 4 weeks starting at PND 21 (GW)	0, 1, 5, 10	BW, OW, HP	Bd wt	5	10		10% decrease in body weight gain
					Hepatic	10			Increases in liver weight and hepatocellular hypertrophy at ≥1 mg/kg
					Develop	1	5		Delay in vaginal opening at 5 mg/kg and mammary gland growth stimulation a 5 mg/kg and inhibition at 10 mg/kg
Yang et al. 2009									
PFOA									
88	Mouse (C57BL/6)) 2–5 F	5 days/week 4 weeks (GW)	0, 5	BC	Repro		5		Increased progesterone levels
Zhao et al. 2010									
PFOA									
89	Mouse (PPARα knockout) NR F	5 days/week 4 weeks (GW)	0, 5	HP	Develop		5		Mammary gland growth stimulation
Zhao et al. 2010									
PFOA									

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
CHRONIC EXPOSURE									
90	Rat (CD) 156 M	2 years ad lib (F)	0, 13.6	CS, BW, FI, OW, GN, HP	Bd wt		13.6		>10% reduction in weight gain most of the study
					Hepatic	13.6			Increased relative liver weight
					Repro		13.6		Increased incidence of Leydig cell hyperplasia; elevated serum LH at 18 months
					Other noncancer		13.6		Increased incidence of acinar cell hyperplasia in pancreas
					Cancer			13.6	CEL: testicular Leydig cell adenomas and pancreatic acinar cell adenomas
Biegel et al. 2001									
APFO									
91	Rat (Sprague-Dawley) 50–65 M, 50–65 F	2 years ad lib (F)	0, 1.5, 15	CS, FI, BW, OW, HE, BI, GN, HP	Bd wt	1.5 F	15 F		10.3% lower terminal body weight
					Resp	15			
					Cardio	15			
					Gastro	15			
					Hemato	15			
					Hepatic	1.5	15		Increased serum ALT and AST at ≥1.5 mg/kg/day; hepatocellular hypertrophy at 15 mg/kg/day; hepatocellular necrosis at 15 mg/kg/day only at 1 year
					Renal	15			
					Ocular	15			
					Endocr	15			
					Immuno	15			
					Neuro	15			

Table 2-3. Levels of Significant Exposure to PFOA – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Repro	1.5 M 15 F	15 M		Vascular mineralization in the testes
					Other noncancer		1.5 M		Inflammation of the salivary gland
					Cancer			15	CEL: testicular Leydig cell adenomas

3M 1983; Butenhoff et al. 2012c

APFO

[a]The number corresponds to entries in Figure 2-8.
[b]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 8.29 µg/mL at the LOAEL dose and an empirical clearance model to estimate a HED. The $LOAEL_{HED}$ of 0.000821 mg/kg/day was divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability).

ad lib = *ad libitum*; ALT = alanine aminotransferase; APFO = ammonium perfluorooctanoate (ammonium salt of PFOA); AST = aspartate aminotransferase; BC = biochemistry; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DW = drinking water; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FT4 = free thyroxine; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LDL = low-density lipoprotein; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NX = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFOA = perfluorooctanoic acid; PND = postnatal day; PPARα = peroxisome proliferator-activated receptor-α; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; UR = urinalysis; W = water; WI = water intake

Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Acute (≤14 days)

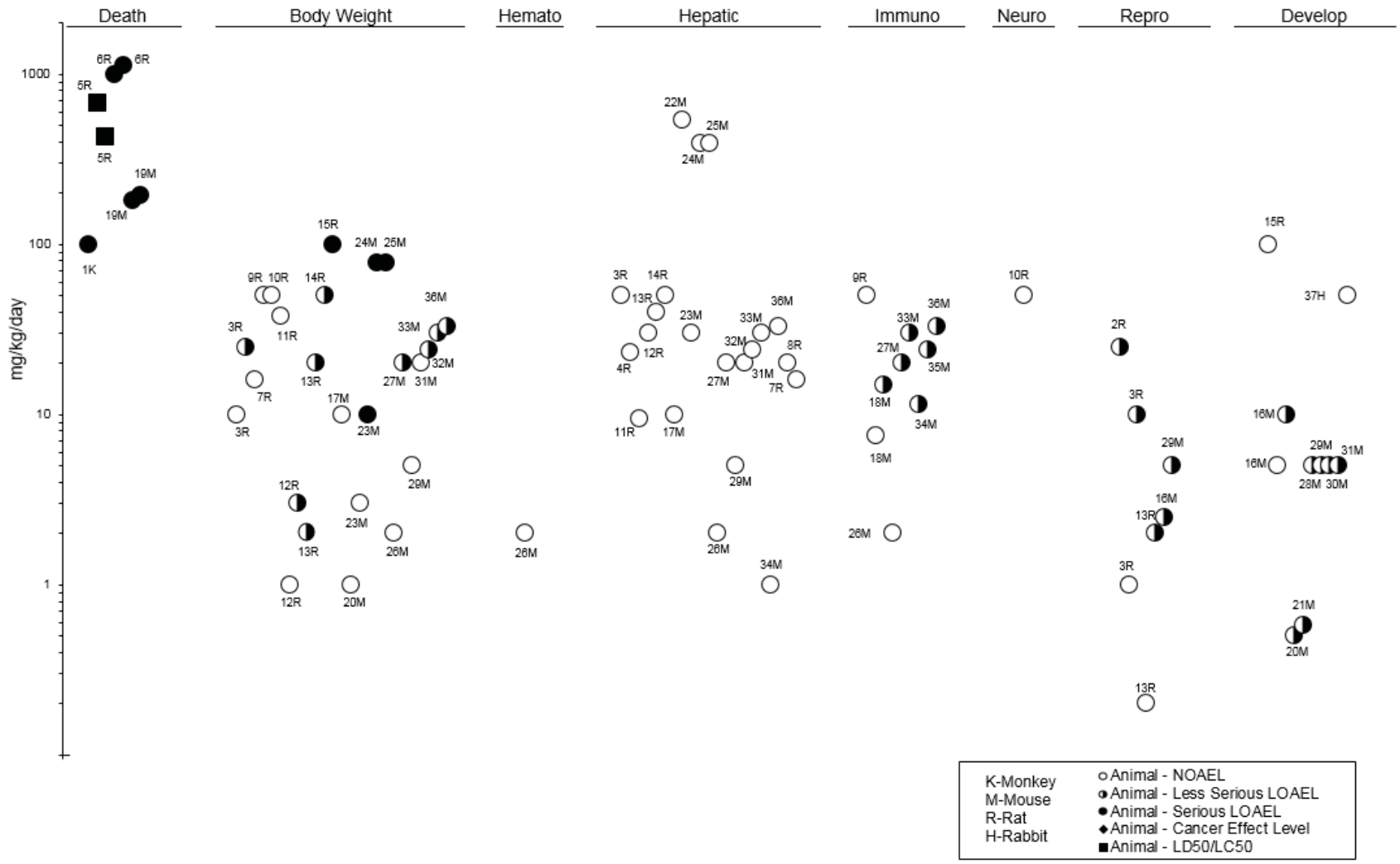


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

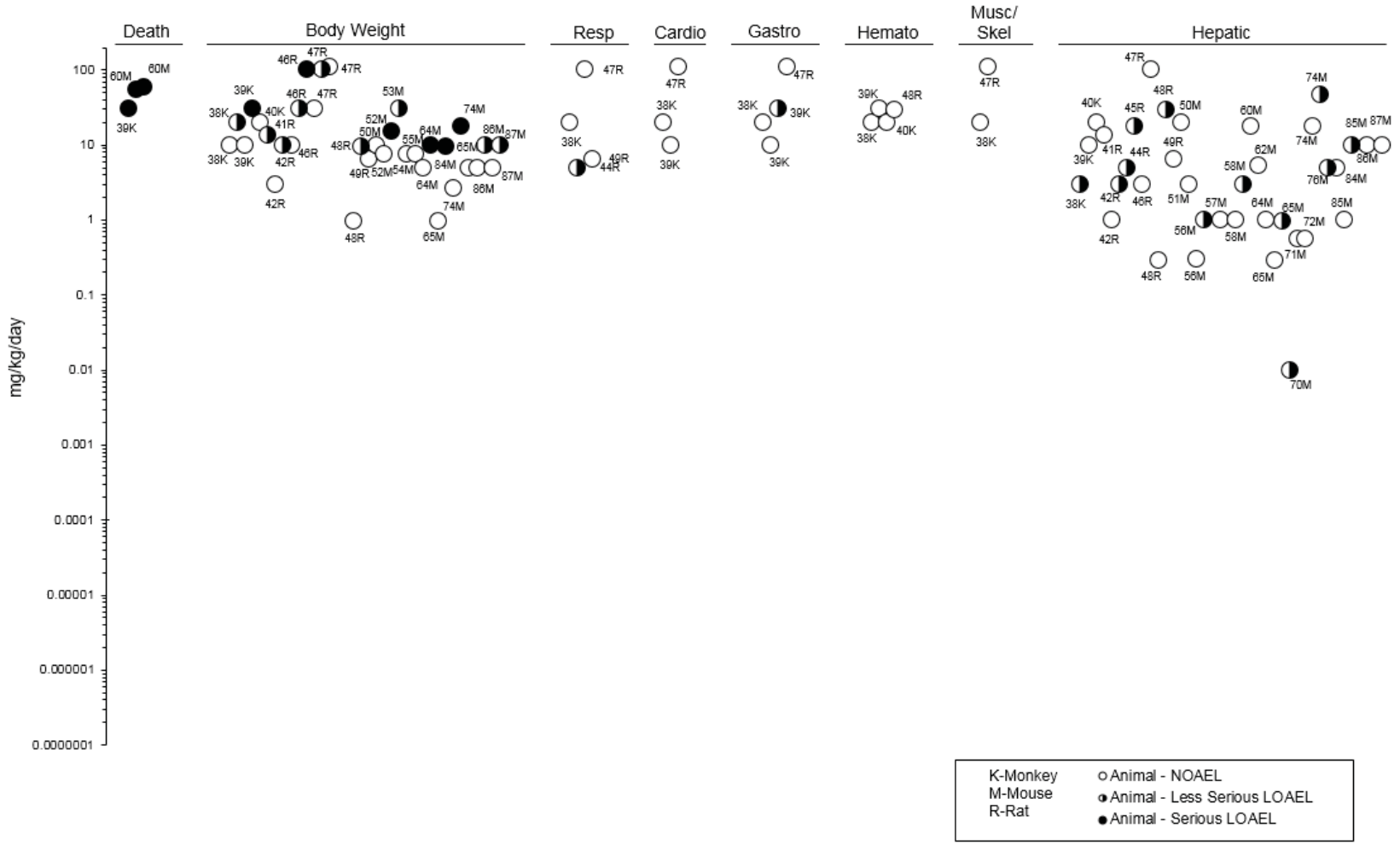


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Intermediate (15–364 days)

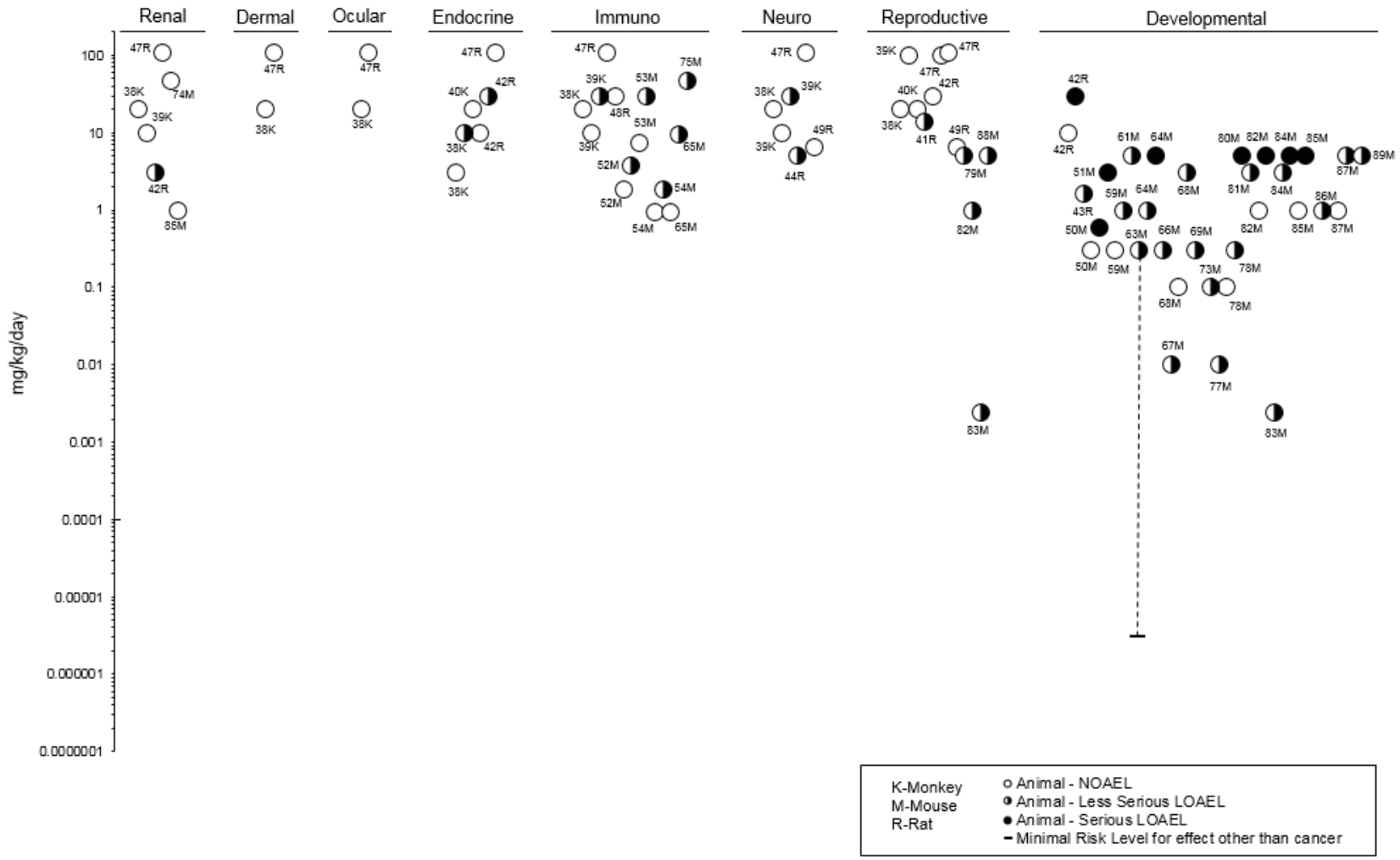


Figure 2-8. Levels of Significant Exposure to PFOA – Oral
Chronic (≥365 days)

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
1	Monkey (cynomolgus) 4–6M, 4–6F	Once or 3 times in 315 days (G)	0, 9, 13.3 (M), 14 (F)	BW, BC	Bd wt	13.3 M 14 F			
					Hepatic		13.3 M 14 F		Decrease in HDL cholesterol; values still within normal range
					Renal	13.3 M 14 F			
					Endocr		13.3 M 14 F		Decreased serum T4; values still within normal range
Chang et al. 2017									
PFOS potassium salt									
2	Rat (Sprague-Dawley) 5–15 F	Once (GW)	0, 15	BI, OF	Endocr		15		Transient decrease in serum TT4 (24, 38, and 53% after 2, 6, and 24 hours, respectively)
Chang et al. 2008b									
PFOS potassium salt									
3	Rat (Sprague-Dawley) 30 M	1 day (F)	0, 1.97, 10.3	HP	Hepatic	10.3			
					Endocr	10.3			
Elcombe et al. 2012a									
PFOS potassium salt									
4	Rat (Sprague-Dawley) 30 M	7 day (F)	0, 0, 1.72, 8.17	HP	Hepatic	8.17			Decreased serum cholesterol (38%) and triglyceride (55%) levels at 8.17 mg/kg/day
					Endocr	8.17			
Elcombe et al. 2012a									
PFOS potassium salt									
5	Rat (Sprague-Dawley) 10 M	7 day (F)	0, 1.79, 8.96	HP	Hepatic	8.96			Hepatocellular hypertrophy, increased liver weight, decreased serum cholesterol at ≥1.79 mg/kg/day
					Endocr	8.96			
Elcombe et al. 2012b									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
6	Rat (Sprague-Dawley) NS F	2 day GDs 19–20 1 time/day (GW)	0, 25, 50	BW, MX, DX	Develop			25	Decreased neonatal survival (82% of controls on PND 1)
Grasty et al. 2003									
PFOS potassium salt									
7	Rat (Sprague-Dawley) NS F	4 days GDs 2–5, 6–9, 10–13, 14–17, or 17–20 (GW)	0, 25	MX, DX	Bd wt			25	Weight loss during treatment when treated on GDs 2–5 (22%) or 6–9 (17%)
					Develop			25	Decreased neonatal survival (90% survival on GDs 2–5; 30% survival on GDs 17–20)
Grasty et al. 2003									
PFOS potassium salt									
8	Rat (Sprague-Dawley) NS F	2 days GDs 19–20 1 time/day (G)	0, 25, 50		Develop			25	Increased neonatal mortality
Grasty et al. 2005									
PFOS potassium salt									
9	Rat (Wistar) 8 M	7 days ad lib (F)	0, 15	BW, OW, BI, EA	Hepatic	15			40% increase in absolute liver weight
Haughom and Spydevold 1992									
PFOS potassium salt									
10	Mouse (wild-type 129S1/Svlm) 8–20 F	GDs 15–18 1 time/day (GW)	0, 4.5, 6.5, 8.5, 10.5	DX	Develop			4.5	31% reduced percentage of live pups per litter on PND 15
Abbott et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
11	Mouse (ICR) 5–7 F	GDs 11–15 1 time/day (GW)	0, 50	BW, OW, DX	Hepatic	50			103% increased maternal relative liver weight
					Develop		50		6.1% increased cleft palate and 12.7% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
12	Mouse (CD-1) 10–11 F	GDs 6–18 1 time/day (GW)	0, 1.5, 3, 6	MX, DX, BW, CS, OW	Bd wt	6			
					Hepatic	6			21% increase in absolute liver weight at ≥3 mg/kg/day
					Endocr	6			No alterations in serum T3 or T4 levels
					Develop	6			
Fuentes et al. 2006									
PFOS potassium salt									
13	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	CS, BW, BH, MX, DX	Bd wt	6			
					Develop		6		Reduced body weight of pups on PNDs 4 and 8
Fuentes et al. 2007b									
PFOS potassium salt									
14	Mouse (CD-1) 8–10 F	GDs 12–18 (GW)	0, 6	DX	Develop		6		Decreased distance traveled in open field test at 3 months of age
Fuentes et al. 2007a									
PFOS potassium salt									
15	Mouse (NMRI) 12 M pups	Single dose (GO)	0, 11.3	BH, BW, OF	Develop		11.3		Altered spontaneous behavior (≤60, 87.5, or 60% changes in total activity, rearing, and locomotion)
Hallgren et al. 2015									
PFOS									
16	Mouse (CD-1) 10 M pups	Once (G)	0, 0.75, 11.3	CS, OF, DX	Develop		0.75 M		24% decreased total spontaneous activity at 2 months of age; no significant alteration at 4 months
Johansson et al. 2008									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
17	Mouse (CD-1) 10 F	GDs 11–16 (G)	0, 0.5, 2.0, 8.0	BC, BW, DX, HP	Bd wt		8.0		21% reduction in maternal body weight gain on GDs 14–17
					Repro		0.5		Decreases in mean fetal placental weight and placental capacity
					Develop		0.5	2.0	Post-implantation losses at ≥0.5 mg/kg/day; 24 and 35% reduction fetal body weight and 31 and 52% reduction in the number of live fetuses at 2.0 and 8.0 mg/kg/day
Lee et al. 2015a									
PFOS									
18	Mouse (C57BL/6) 4M	10 days (F)	0, 6	BW FI BC HP	Bd wt	6			
					Hemato	6			
					Hepatic	6			41% increase in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels
					Immuno	6			
Qazi et al. 2010a									
PFOS									
19	Mouse (BALB/c) 5 F	7 days (GW)	20	BW, OW, BC	Bd wt		20		41% decreased body weight gain
					Hepatic	20			59% increase in relative liver weight
					Immuno		20		Inhibition of T lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA
Vetvicka and Vetvickova 2013									
PFOS									
20	Mouse (CD-1) 4 M	14 days 1 time/day (GO)	0, 1, 5, 10		Bd wt	10			
					Hepatic	10			~70% increased absolute liver weight at ≥5 mg/kg/day
					Repro	10			
Wan et al. 2011									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
21	Mouse (C57BL/6) 10 M	1 day (GO)	0, 300, 400, 500, 600, 700	BW, CS, HP, LE	Death			579	LD_{50}
Xing et al. 2016									
PFOS									
22	Mouse (C57BL/6N) 12 M	7 days (G)	0, 5, 20, 40	FX	Immuno		5		Impaired response to T-cell mitogens; suppressed response to sRBC
Zheng et al. 2009									
PFOS									
23	Rabbit (New Zealand) 22 F	GDs 6–20 1 time/day (GW)	0, 0.1, 1.0, 2.5, 3.75	MX, DX, BW, CS	Bd wt	0.1		1 F	21% decreased mean maternal body weight gain on GDs 7–21; no effect on food consumption
					Develop	1	2.5	3.75	Decreased fetal body weight; 10% at 2.5 mg/kg/day and 24% at 3.75 mg/kg/day; 10/22 does aborted between GD 22 and 28 at 3.75 mg/kg/day
Case et al. 2001									
PFOS potassium salt									
INTERMEDIATE									
24	Monkey (Cynomolgus) 4–6 M, 4–6 F	26 weeks 1 time/day (C)	0, 0.03, 0.15, 0.75	CS, BW, OW, HE, BI, GN, HP	Bd wt	0.15 M 0.75 F	0.75 M		13.5% reduction in final body weight
					Resp	0.75			
					Cardio	0.75			
					Gastro	0.75			
					Hemato	0.75			
					Musc/skel	0.75			
					Hepatic	0.15	0.75		47–55% increased absolute liver weight; 50–60% decreased serum cholesterol; hepatocellular hypertrophy, mild bile stasis, and lipid vacuolation at 0.75 mg/kg/day
					Renal	0.75			
					Dermal	0.75			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Ocular	0.75			
					Endocr	0.15	0.75		Increased TSH and decreased total T3
					Immuno	0.75			
					Neuro	0.75			No histological alterations
					Repro	0.15	0.75 M		Significant decrease in serum estradiol on days 62 (48%), 91 (42%), and 182 (96%); no histological alterations
Seacat et al. 2002									
PFOS potassium salt									
25	Monkey (Cynomolgus) 6 M,F	4 weeks 1 time/day (C)	0, 0.02, 2	CS, BW, FC, HE, BI, GN, HP, EA	Bd wt	2			
					Resp	2			
					Hemato	2			
					Hepatic	2			
					Renal	2			
					Ocular	2			
					Endocr	2			
					Immuno	2			No histological alteration
					Repro	2			No histological alterations
Thomford 2002a									
PFOS potassium salt									
26	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Repro	1			
					Develop	0.3	1		~30% increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17
Butenhoff et al. 2009b									
PFOS potassium salt									
27	Rat (Sprague-Dawley) 25 F	GD 0 to PND 20 1 time/day (GW)	0, 0.1, 0.3, 1		Develop		1		2.1-fold increased fetal thyroid cell proliferation on GD 20
Chang et al. 2009									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
28	Rat (Sprague-Dawley) 10 F	GDs 1–21 1 time/day (GW)	0, 0.1, 2		Develop	0.1		2	~5-fold increased postnatal mortality and severe lung histopathology in pups
Chen et al. 2012b PFOS									
29	Rat (Sprague-Dawley) 10 M	Daily 28 days (G)	0, 5, 20	HP	Death			20	100% by day 26
					Resp		5		Pulmonary congestion
					Hepatic		5		Hepatocellular hypertrophy and focal necrosis at ≥5 mg/kg/day; fatty degeneration at 20 mg/kg/day
					Neuro		5		Cachexia and lethargy
Cui et al. 2009 PFOS									
30	Rat (Sprague-Dawley) 15M, 15F	Daily 28 days (F)	M: 0, 0.13, 1.23, 2.98, 5.89 F: 0, 0.14, 1.33, 3.47, 7.01		Cardio	5.89 M			
					Hemato	3.47 F	7.01 F		Decreased red blood cells (8.9%), hemoglobin (10%), hematocrit (8.8%)
					Hepatic	5.89 M			Increased relative liver weight; hepatocellular hypertrophy in males at 5.89 mg/kg/day
					Renal	5.89 M			
					Endocr	0.14	1.23		Decreased T4 level (82% in males; 48% in females)
Curran et al. 2008 PFOS potassium salt									
31	Rat (Sprague-Dawley) 30 M	28 days (F)	0, 1.54, 7.34	HP	Hepatic	7.34			Hepatocellular hypertrophy and decreased serum cholesterol levels at ≥1.54 mg/kg/day
					Endocr	7.34			
Elcombe et al. 2012a PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
32	Rat (Wistar) 5–6 M	Daily 28 days (G)	0, 0.12, 0.5, 2.0, 8.5	FX	Neuro	2	8.5		Tonic convulsions in response to stimuli
Kawamoto et al. 2011									
PFOS									
33	Rat (Sprague-Dawley) NS	GDs 2–21 (GW)	0, 1, 2, 3, 5, 10		Develop	1		2	Reduced serum T4 in pups at 1 mg/kg/day; approximately 60% survival at weaning versus 80% in controls at 2 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
34	Rat (Sprague-Dawley) 15M, 15F	28 days (F)	M: 0, 0.14, 1.33, 3.21, 6.34; F: 0, 0.15, 1.43, 3.73, 7.58	OW, BW	Bd wt Hepatic Immuno	1.33 M 6.34 M 6.34 M	3.21 M		12% decrease in terminal body weight Increased relative liver weight at ≥0.14 mg/kg/day
Lefebvre et al. 2008									
PFOS									
35	Rat (Sprague-Dawley) 10 dams; 12–13 pups	GDs 12–18 (GO)	0, 5, and 20	BC, BW, DX, OW	Bd wt Develop	5 5	20 20		30% reduction in body weight of dams 13% reduction in body weight of male pups
Li et al. 2016									
PFOS									
36	Rat (Sprague-Dawley) 5M	21 days (G)	0, 5, 10	BW, BC, OW, HP	Bd wt Repro	10	5		Delayed maturation of testicular Leydig cells, decreased seminal vesicle weight, decreased epididymal sperm count, decreased serum testosterone levels
Li et al. 2018									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
37	Rat (Sprague-Dawley) 19 M	28 days (GO)	0, 0.5, 1.0, 3.0, 6.0	BC, BW, HP, OF, OW	Neuro	0.5	1		Degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day; dense chromatin, condensed ribosomes, loss of morphology in the hypothalamus at ≥3.0 mg/kg/day
					Repro	0.5	1		Loss/degeneration of spermatozoids, marked edema in the testes
Lopez-Doval et al. 2014									
PFOS									
38	Rat (Sprague-Dawley) 35 M,F	84 days (6 weeks prior to mating GD 0 to PND 21) 1 time/day (GW)	0, 0.1, 0.4, 1.6, 3.2	MX, DX, BW, OW, OF, GN, HP, FC	Bd wt	1.6	3.2		>10% reduction in body weight
					Repro	3.2			No alterations in mating and fertility parameters
					Develop	0.1[b]	0.4	1.6	Delayed eye opening and transient decrease in F2 pup body weight (13%) on LDs 7–14 at ≥0.4 mg/kg/day; decreased pup survival to postpartum day 21 at ≥1.6 mg/kg/day
Luebker et al. 2005a									
PFOS potassium salt									
39	Rat (Sprague-Dawley) 50 F	90 day 1 time/day	0, 1.6	MX, DX, BW, OW, OF, GN		Develop		1.6	Increased pup mortality during PNDs 1–4
Luebker et al. 2005a									
PFOS potassium salt									
Cross-foster study									
40	Rat (Sprague-Dawley) 20 F	62–67 days 42 days prior to mating through GD 20 or PND 4 (G)	0, 0.4, 0.8, 1, 1.2, 1.6, 2	MX, DX, BW, CS, BI	Bd wt	1.6	2		22% reduction in body weight gain during premating; food consumption reduced 5.8%
					Hepatic	2			16% reduction in serum total cholesterol on PND 5 at ≥0.4 mg/kg/day; increased liver weight in dams at ≥0.8 mg/kg/day
					Endocr		0.4		46% reduction in total T4 on PND 5
					Repro	2			No alteration in fertility

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop		0.4	1.6	>10% decrease in mean pup weight per litter on PND 5 at ≥0.4 mg/kg/day; ~50% decrease mean pup survival per litter on PND 5 at ≥1.6 mg/kg/day
Luebker et al. 2005b									
PFOS potassium salt									
41	Rat (Sprague-Dawley) 10 M	25 days (GW)	0, 0.5, 1.0, 3.0 and 6.0	OF, OW	Endocr		0.5		Decreases in serum corticosterone (~58%) and ACTH levels (~11%), decrease in corticotrophin releasing hormone levels in hypothalamus (~8%); decrease in relative adrenal weight (~43%),
Pereiro et al. 2014									
PFOS									
42	Mouse (C57) 12 M	5 weeks (GO)	0, 0.5, 10	BW, OW, HP, RX	Bd wt	0.5	10		17% decrease in body weight
					Repro	0.5	10		Decreases in sperm concentration, serum testosterone levels; vacuolation in testicular spermatogonia, spermatocyte, and Leydig cells
Qu et al. 2016									
PFOS									
43	Rat (Sprague-Dawley) 21 dams; 10–12 M,F pups/litter	GDs 2–6 (G)	0, 18.75	BW, OF, OW	Bd wt		18.75		Reduced body weight in dams; approximately 98% on GD 8 and 33% on GD 20
					Develop		18.75		Decreased birth weight in females only (approximately 11%); increased systolic blood pressure in male offspring at 7 and 52 weeks and in female offspring at 37 and 65 weeks; reduced nephron endowment
Rogers et al. 2014									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
44	Rat (Sprague-Dawley) 7 M	28 days (GW)	0, 3.0, and 6.0	BI, BW, OW	Bd wt	6			
					Endocr		3		Decreased serum prolactin (~78%) and estradiol concentrations (~18%)
Salgado et al. 2015 PFOS									
45	Rat (Sprague-Dawley) 25 M,F	4 weeks ad lib (F)	M: 0, 0.05, 0.18, 0.37, 1.51; F: 0, 0.05, 0.22, 0.47, 1.77	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.77 F			
					Hemato	1.77 F			
					Hepatic	1.77 F			
					Renal	1.77 F			
					Ocular	1.77 F			
					Immuno	1.77 F			No histological alterations
					Neuro	1.77 F			No histological alterations
					Repro	1.51 M 1.77 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									
46	Rat (Sprague-Dawley) 25 M,F	14 weeks ad lib (F)	M:0, 0.03, 0.13, 0.34, 1.33; F:0, 0.04, 0.15, 0.4, 1.56	CS, CO, OW, HE, BI, GN, HP	Bd wt	1.56 F			
					Hemato	0.34 M	1.33 M		45% increase in non-segmented neutrophils
					Hepatic	1.33 M			Increased absolute and relative liver weight; increased serum ALT; hepatocyte hypertrophy and vacuolation at 1.33/1.55 mg/kg/day
					Renal	1.56 F			
					Ocular	1.56 F			
					Endocr	1.56 F			
					Immuno	1.56 F			No histological alterations
					Neuro	1.56 F			No histological alterations
					Repro	1.33 M 1.56 F			No histological alterations
Seacat et al. 2003 PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
47	Rat (Sprague-Dawley) 25–50 F	GDs 2–20 (GW)	0, 1, 2, 3, 5, 10	MX, DX, BW, FI, WI, OW, BI	Bd wt	1	2		Decreased mean body weight gain, 10% at 2 mg/kg/day and 33% at 5 mg/kg/day
					Hepatic	10			
					Endocr		1		Reduced total and free T4 and T3
					Develop		10		Increased incidences of cleft palate
Thibodeaux et al. 2003									
PFOS potassium salt									
48	Rat (Wistar) 10 or 15 dams; 6–10 M,F pups	GD 1 to PND 1, PNDs 1–7 or 35, or GD 1 to PND 7 or 35	0, 0.8, 2.4	DX	Develop		0.8		Decreased spatial learning ability in prenatally or postnatally exposed offspring at ≥0.8 mg/kg/day and in offspring exposure pre- and postnatally at 2.4 mg/kg/day; decreased memory ability in offspring exposure pre- and postnatally at 2.4 mg/kg/day
Wang et al. 2015c									
PFOS									
49	Rat (Sprague-Dawley) 10 F	GDs 2–21 1 time/day (GW)	0, 0.1, 0.6, 2		Develop	0.6		2	5-fold increased neonatal mortality on PNDs 1–3
Xia et al. 2011									
PFOS									
50	Rat (Sprague-Dawley) 8–10 M	91 days (W)	0, 0.27, 0.79, 2.37	BC	Endocr		0.27 M		42% decrease in total T4 levels
					Develop		3.2 F		19–36% reduced serum T4 levels in pups on PNDs 21–35 after gestation- and/or postnatal-only exposure
Yu et al. 2009a									
PFOS potassium salt									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 3	BW, FI, BC, OW	Bd wt	3			
					Hepatic	3			Decreased plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
PFOS									
52	Mouse (C57BL/6N) 10 M	60 days (G)	0, 0.00833, 0.08333, 0.41667, 0.83333, 2.0833	FX	Immuno	0.0083	0.0833		Impaired response to sRBC
Dong et al. 2009									
PFOS potassium salt									
53	Mouse (C57BL/6N) 12 M	60 days (G)	0, 0.00833, 0.0167, 0.0833, 0.4167, 0.8333	FX	Immuno	0.0167	0.0833		Impaired response to sRBC
Dong et al. 2011									
PFOS potassium salt									
54	Mouse (ICR) 6–8 F	GDs 1–17 1 time/day (GW)	0, 20		Bd wt		20		35% reduced maternal body weight
					Develop		20		89% increased cleft palate, 25% reduced body weight in fetuses
Era et al. 2009									
PFOS potassium salt									
55	Mouse (CD-1) 10 M	4 weeks (GW)	0, 3, 6	CS, BW, BH, MX, DX	Develop		3		Impaired retention of the task in the water maze test
Fuentes et al. 2007c									
PFOS potassium salt									
56	Mouse (B6C3F1) 30 F	21 days (G)	0, 0.005, 0.025		Immuno	0.005	0.025		Decreased host resistance to influenza virus
Guruge et al. 2009									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
57	Mouse (B6C3F1) 10–12 F	GDs 1–17 1 time/day (GW)	0, 0.1, 1, 5	MX, DX, BW, CS, OF	Develop	0.1	1		42.5% reduced NK cell activity in male pups at 8 weeks of age
Keil et al. 2008									
PFOS potassium salt									
58	Mouse (CD-1) 21–22 F	GDs 1–17 1 time/day (GW)	0, 1, 5, 10, 15, 20	DX, OW, BI	Develop		1	10	1 mg/kg/day: delayed eye opening at ≥1 mg/kg/day, ~ 0% postnatal survival at weaning versus 90% in controls at 10 mg/kg/day
Lau et al. 2003									
PFOS potassium salt									
59	Mouse (CD-1) 4 dams; 8 fetuses	GDs 1–17 (GO)	3	BC, EA, OF	Hepatic	3			No alteration in maternal hepatic lipid levels
					Develop		3		Significant increase in cholesterol levels in fetal livers
Lee et al. 2015b									
PFOS									
60	Mouse (C57BL/6) 15 M,F	Daily 3 months (G)	0, 0.43, 2.15, 10.75	FX	Neuro	0.43	2.15		Impaired spatial learning and memory
Long et al. 2013									
PFOS									
61	Mouse (BALB/c) 28 M	3 weeks	0, 2.5, 5 and 10	BW, OF, OW	Bd wt		10 M		~15% reduction in body weight during the recovery period
					Immuno	2.5 M	5 M		~36% decrease in spleen index during recovery; ~15% inhibition in Con A-induced T-cell proliferation during treatment; 32% increase in CD3+ cells after recovery; ~60% increase in CD3+CD8+ cells and ~56% increase in CD3+CD4+ cells on week 2; 15% inhibition in Con A-induced T-cell proliferation during recovery
Lv et al. 2015									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Mouse (C57BL/6J-Apc+/+) 20–21 F	GDs 1–17 (GW)	0, 0.01, 0.1, 3.0	BC, BW, FI, HE, OW	Develop	0.1	3		Decrease in number of successful births
Ngo et al. 2014									
PFOS									
63	Mouse (C57BL/6/Bk1) 6 F	GDs 1–21 ad lib	0, 0.3		Develop		0.3		Decreased locomotion, muscle strength, and motor coordination in adult offspring
Onishchenko et al. 2011									
PFOS, potassium salt									
64	Mouse (B6C3F1) 5M, 5F	28 days (G)	0, 0.000166, 0.00166, 0.00331, 0.0166, 0.0331, 0.166	OW, FX	Immuno	0.000166 M	0.00166 M		Suppressed response to sRBC (~60%)
Peden-Adams et al. 2008									
PFOS potassium salt									
65	Mouse (B6C3F1) 5 M	28 days (F)	0.20	NS	Bd wt		0.2		21% reduction in body weight
					Immuno	0.2			No alterations in thymic lymphocyte phenotypes, response to sRBC, or IgM antibodies to LPS
Qazi et al. 2010b									
PFOS									
66	Mouse (CD-1) 5 F	GDs 1–17 1 time/day (GW)	0, 5, 10		Develop	5			Peroxisome proliferation in fetal liver at ≥5 mg/kg/day
Rosen et al. 2009									
PFOS potassium salt									
67	Mouse (CD-1) 60–80 F	GDs 1–17 (GW)	0, 1, 5, 10, 15, 20	BW, OW, BI, DX	Bd wt	20			
					Hepatic	20			Increase in absolute and relative liver weight and decreased serum triglycerides at ≥5 mg/kg/day
					Endocr	15	20		Decreased total T4 on GD 6

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Develop	1	5	20	Increased incidences of sternal defects at ≥5 mg/kg/day; reduced percentage of live fetuses (9%) at 20 mg/kg/day
Thibodeaux et al. 2003									
PFOS potassium salt									
68	Mouse (CD-1) 4 M	21 days 1 time/day (GO)	0, 1, 5, 10	BW, BI, OW, HP	Bd wt	5	10		~15% reduced body weight
					Hepatic	10			Increased absolute liver weight at ≥5 mg/kg/day
					Repro	5	10		~17% reduced serum testosterone, ~38% reduced epididymal sperm count
Wan et al. 2011									
PFOS potassium salt									
69	Mouse (CD-1) 6 F (dams)	GD 3 to PND 21 or GD 3 to PND 63 (GO)	0, 0.3, 3	BW, OF, OW	Hepatic	3			~24% increase in relative liver weight in dams at 3 mg/kg/day
					Develop	3			Increase in relative liver weight at 3 mg/kg/day in male and female pups on PND 21 (~20–32%), in male STD-fed adults (~11%), and in male HDF-fed adults (~33%)
					Develop		0.3		In PND 63 offspring fed a high fat diet, increased serum glucose levels at ≥0.3 mg/kg/day in females (~40%) and 3 mg/kg/day in males (~8%); increased serum insulin in males (~109%) and females (~85%); increased response to oral glucose tolerance test, increased HOMA-IR index, and 33% increased relative liver weight at 3 mg/kg/day
					Other noncancer	3 F			No significant alteration in fasting serum insulin or glucose levels; significant increase in HOMA-IR index at 0.3 and 3 mg/kg/day
Wan et al. 2014b									
PFOS									

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
70	Mouse (C57BL/6) 10 M	30 days (GO)	0, 2.5, 5, 10	BW, HP, OF, OW	Bd wt		10		31% reduction in body weight (correlated with 68% reduction in feed consumption)
					Hepatic		2.5		Increased liver weight (35%) and serum AST(~12%) and GGT levels (~98%) at ≥2.5 mg/kg/day; increases in ALT (~45%) and ALP (~36%) at ≥5 mg/kg/day; cytoplasmic vacuolation, focal or flake-like necrosis, and hepatocellular hypertrophy observed, but no incidence data provided
					Renal	10			
Xing et al. 2016									
PFOS									
71	Mouse (ICR) 5 F	GDs 0–17 GDs 0–18 (GW)	0, 1, 10, 20		Hepatic	20			60% increased absolute liver weight at ≥10 mg/kg/day
					Develop		1	20	GDs 0–17: 15.8% increased sternal defects in fetuses at ≥1 mg/kg/day; 8.8% decrease in number of live fetuses at 20 mg/kg/day
					Develop			10	GDs 0–18: decreased survival (55.2%) at 10 mg/kg/day on PND 4, decreased neonatal BW, intracranial blood vessel dilatation, lung atelectasis
Yahia et al. 2008									
PFOS potassium salt									
CHRONIC EXPOSURE									
72	Rat (Sprague-Dawley) 70 M,F	104 weeks ad lib (F)	0, 0.025, 0.10, 0.25, 1.04	CS, BW, FC, GN, HP, BI	Bd wt	0.25 F	1.04 F		14% reduction in final body weight
					Resp	1.04			
					Cardio	1.04			
					Gastro	1.04			
					Hemato	1.04			
					Musc/skel	1.04			

Table 2-4. Levels of Significant Exposure to PFOS – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	0.25 M	1.04 M		Hepatocellular hypertrophy at ≥0.1 mg/kg/day; single cell necrosis and cystic degeneration at 1.04 mg/kg/day
					Renal	1.04			
					Dermal	1.04			
					Ocular	1.04			
					Endocr	1.04			
					Immuno	1.04			No histological alterations
					Neuro	1.04			No histological alterations
					Repro	1.04			No histological alterations
Butenhoff et al. 2012b; Thomford 2002b									
PFOS potassium salt									

[a]The number corresponds to entries in Figure 2-9.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day based on the predicted TWA serum PFOA level of 29.7 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.000515 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

ad lib = *ad libitum*; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BW or Bd wt = body weight; C = capsule; Cardio = cardiovascular; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FX = fetal toxicity; FI = food intake; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GGT = gamma-glutamyl transferase; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HED = human equivalent dose; HOMA IR = Homeostatic Model Assessment of Insulin Resistance; HP = histopathology; Immuno = immunotoxicological; LD = lactation day; LD50 = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; LPS = lipopolysaccharide; M = male(s); MRL = Minimal Risk Level; Musc/skel = musculoskeletal; MX = maternal toxicity; Neuro = neurological; NK = natural killer; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; sRBC = sheep red blood cell; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid-stimulating hormone; TT4 = total thyroxine; TWA = time-weighted average; W = water

Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Acute (≤14 days)

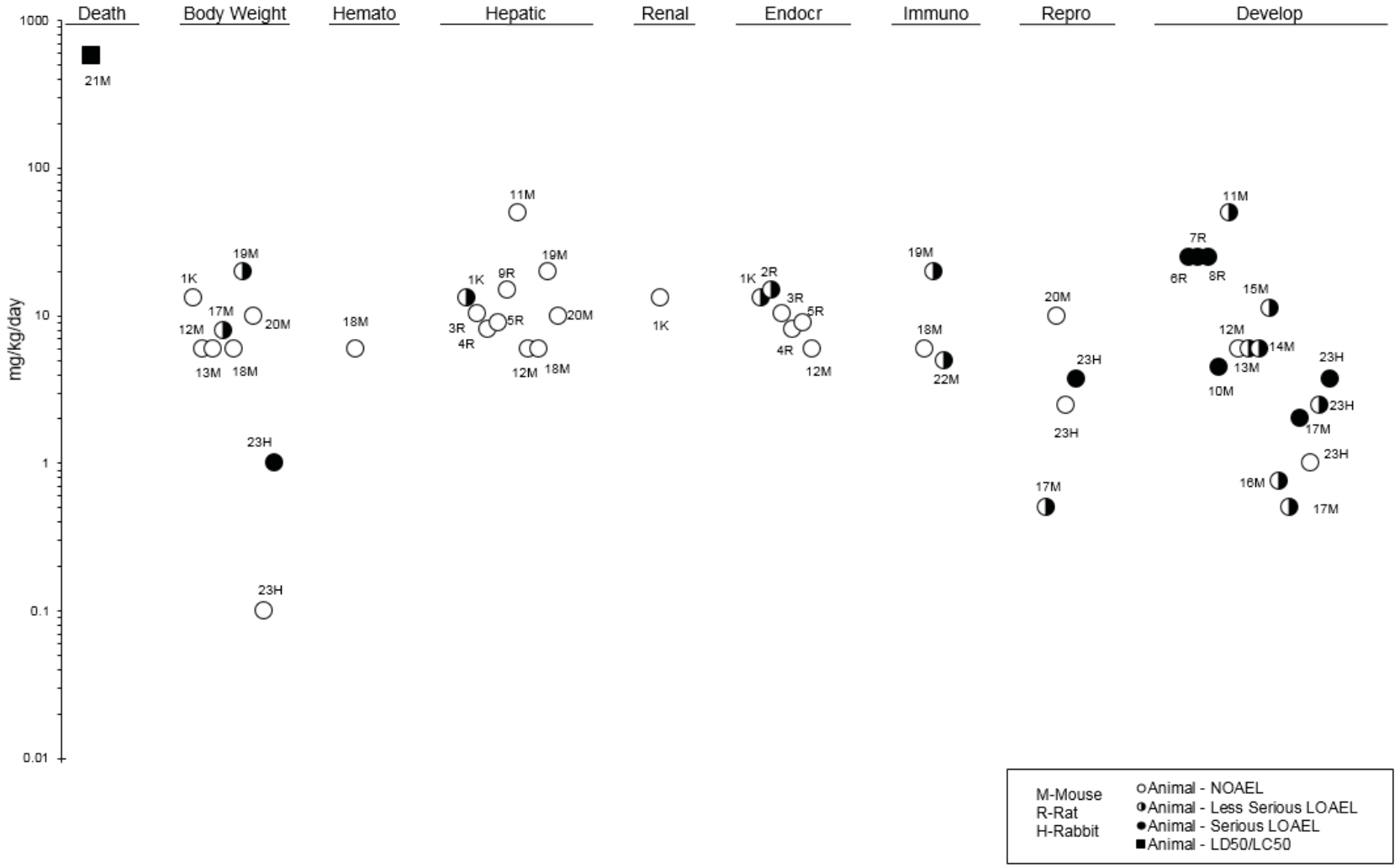


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

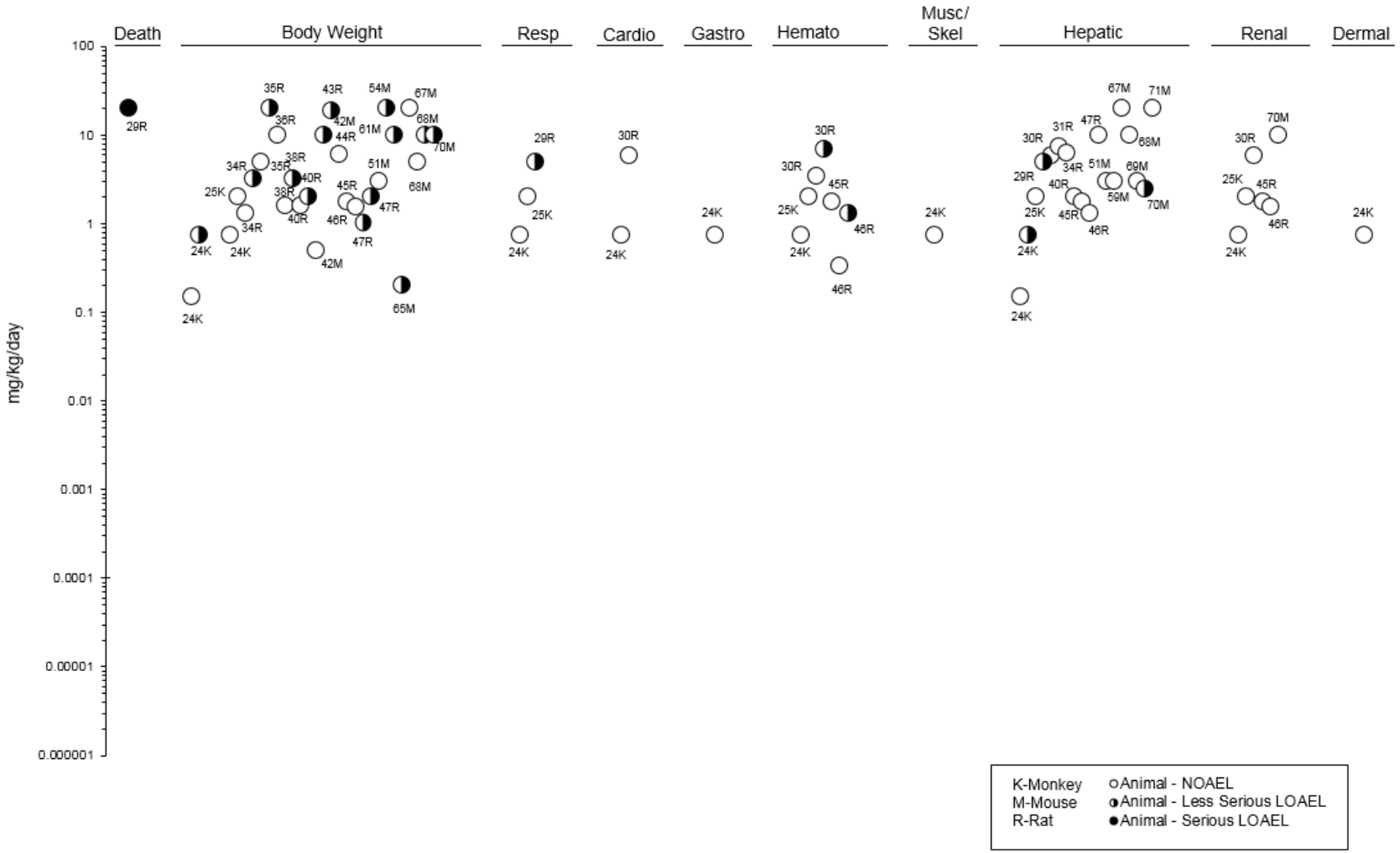


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Intermediate (15–364 days)

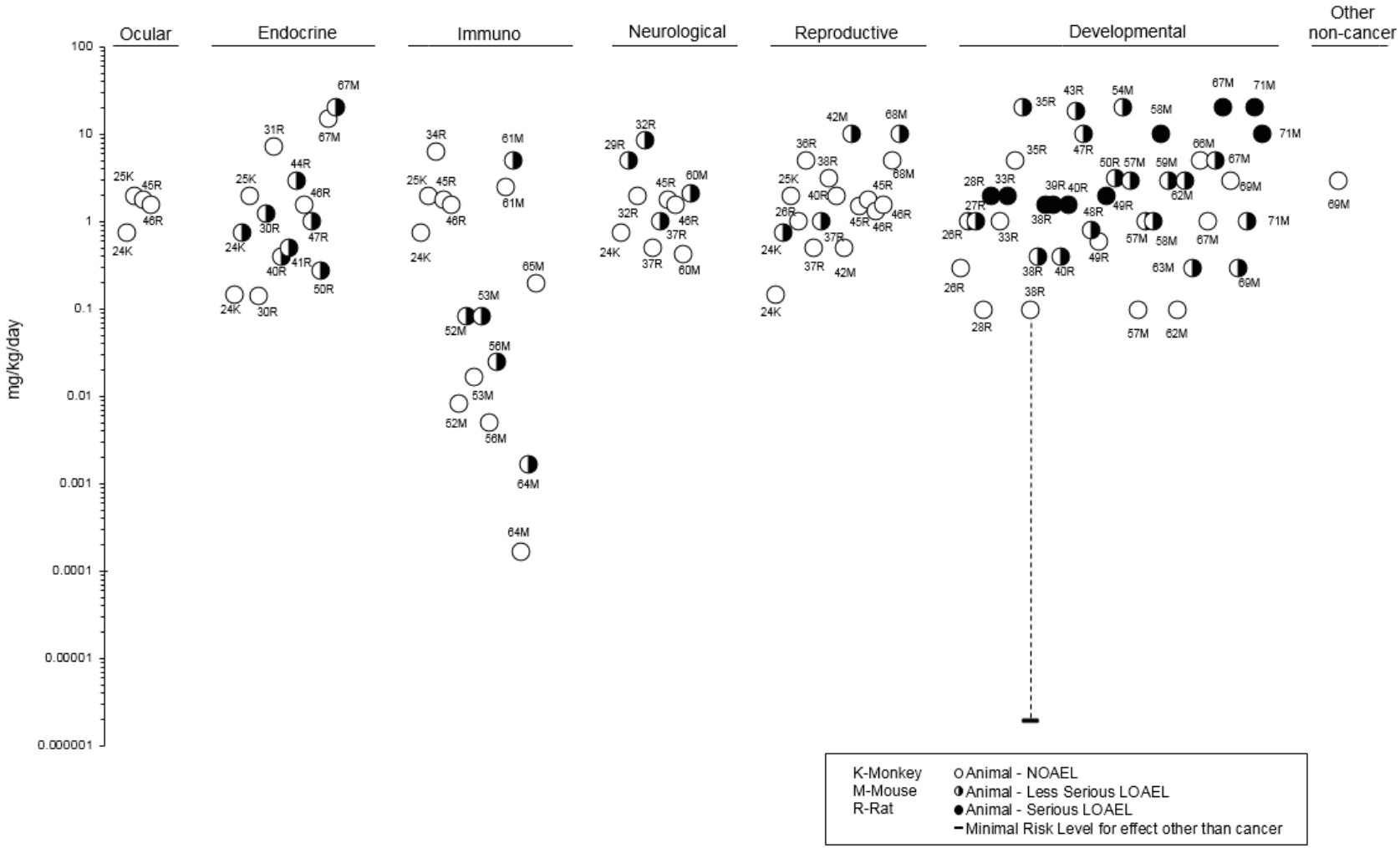


Figure 2-9. Levels of Significant Exposure to PFOS – Oral
Chronic (≥365 days)

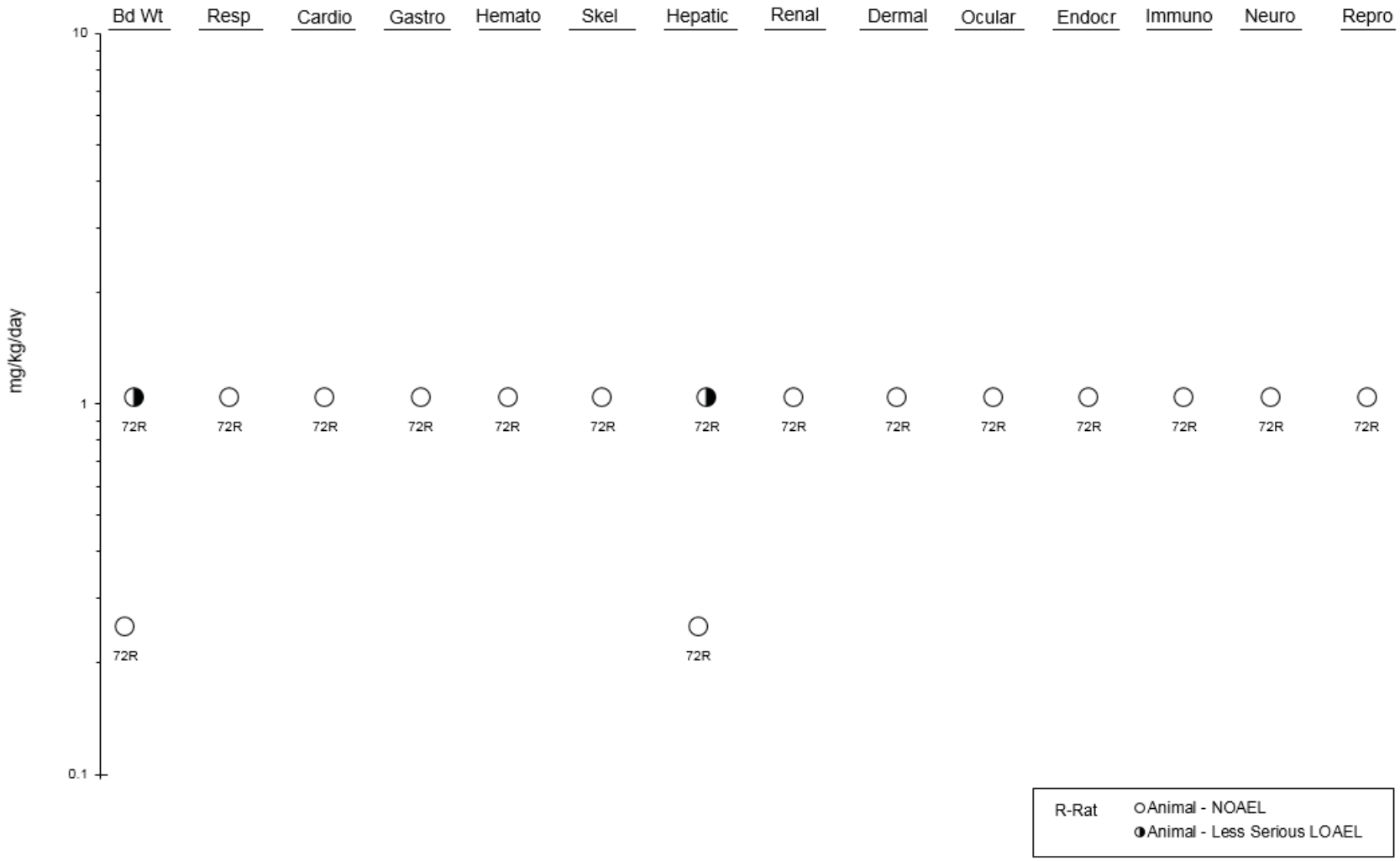


Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE									
PFHxS									
1	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFHxS	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
2	Mouse (NMRI) 14–18 M,F	PND 10 Once (GO)	0, 0.61, 6.1, 9.2 PFHxS		Develop	6.1	9.2		Altered spontaneous behavior and habituation in adults exposed as neonates
Viberg et al. 2013									
PFNA									
3	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, BW, OW	Bd wt	1	3		Decreases in body weight at 3 and 5 mg/kg/day (18 and 39%)
					Immuno		1		24% increase in relative thymus weight at 1 mg/kg/day; 20% decrease in thymus weight at 3 or 5 mg/kg/day, increases in thymic cortex:medulla ratios, alterations in cytokine levels at ≥3 mg/kg/day
Fang et al. 2009									
4	Rat (Sprague-Dawley) 10 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, OW	Immuno	3	5		Decreased relative spleen weight and increases in cytokine levels
Fang et al. 2010									
5	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	BC	Hepatic	5			Decreased HDL levels at ≥1 mg/kg/day
					Other noncancer (glucose)	0.2	1		Increased serum glucose levels (1.11-fold at 1 mg/kg/day and 1.16-fold at 5 mg/kg/day)
Fang et al. 2012a									
6	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 0.2, 1, 5 PFNA	HP	Hepatic	5			Hepatocellular vacuolation and lipid accumulation at 5 mg/kg/day
Fang et al. 2012b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
7	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	BC, HP	Repro	3	5		85.4% decrease in serum testosterone and 105% increase in estradiol levels at 5 mg/kg/day; atrophy of seminiferous tubule epithelium
Feng et al. 2009									
8	Rat (Sprague-Dawley) 6 M	14 days (GW)	0, 1, 3, 5 PFNA	HP	Repro	3	5		Large vacuoles between testicular Sertoli cells and spermatogonia
Feng et al. 2010									
9	Rat (Wistar)	14 days (GO) 8 or 10M	0, 0.0125, 0.25, 5 PFNA	BI, BW, OW, GN, HP	Bd wt		5		Decreased body weight; magnitude of effect was not reported
					Endocr		5		Decreased androstenedione and testosterone concentrations (data not shown)
Hadrup et al. 2016									
10	Mouse (SV129 WT and PPARα null) 4 M	7 days (G)	0, 10 PFNA	BW, HP	Bd wt	10			
					Hepatic	10			Hepatocellular hypertrophy, steatosis, and increased hepatic triglyceride levels
Das et al. 2017									
11	Mouse (BALB/c) 6 M	14 days (G)	0, 1, 3, 5 PFNA	FX	Immuno		1		Decreases in the percentages of F4/80+ and CD49b+ cells in the spleen; no alteration in the response of splenic lymphocytes to ConA at ≤5 mg/kg/day
Fang et al. 2008									
12	Mouse (CD-1) 5 M,F	14 days (F)	0, 0.5, 1.8, 5.3, 54, 537 PFNA	LE, OW	Death			54	100% mortality before day 14
					Hepatic	5.3			50–70% increase in absolute liver weight at ≥0.5 mg/kg/day
Kennedy 1987									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
13	Mouse (BALB/c) 8 M	14 days (G)	0, 0.2, 1, 5 PFNA	BW, OW, BC	Bd wt	1	5		~25% decrease in body weight
					Hepatic	1	5		Increases in liver weight (~160%) and increases in hepatic triglyceride (~66%) and cholesterol levels (~26%) at ≥0.2 mg/kg/day; decreases in serum triglyceride (~67%) and cholesterol levels (~32%) and increases in serum ALT (~900%) and AST (~280%) levels at 5 mg/kg/day
Wang et al. 2015a									
PFDA									
14	Rat (Wistar) 5–12 M	Once (GO)	0, 50 PFDA	BW, FX	BW			50	8% weight loss was observed 10-days post-exposure
					Neuro	50			No alteration in performance on novel object recognition test
Kawabata et al. 2017									
15	Rat (Wistar) 25 M	1 week (F)	0, 1.2, 2.4, 4.7, 9.5 PFDA	BE, OW, EA	Bd wt	4.7		9.5	~32% weight loss
					Hepatic	9.5			Increases in liver weight at ≥2.4 mg/kg/day; increases in hepatic cholesterol at 9.5 mg/kg/day
Kawashima et al. 1995									
16	Mouse (C57BL/6N) 4 F	Once (GO)	0, 40, 80, 100, 120, 160 PFDA	OW, EA	Hepatic	160			Increase in hepatic lipids and liver weight 2 days post-exposure at 40 mg/kg/day
Brewster and Birnbaum 1989									
17	Mouse (C57BL/6N) 12–14 F	GDs 6–15 (GO)	0, 0.03, 0.1, 0.3, 1, 3, 6.4, 12.8 PFDA	MX, DX, BW, OW	Bd wt	3	6.4	12.8	No weight gain at 6.4 mg/kg/day and weight loss at 12.8 mg/kg/day (net change of -2.4)
					Develop	0.3	1	12.8	18–22% decreases in fetal weight per litter at ≥1 mg/kg/day; decreases in live fetuses per litter at 12.8 mg/kg/day (4.6 versus 7.2 in controls)
Harris and Birnbaum 1989									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
18	Mouse (C57BL/6N) 10 F	Once (GO)	0, 20, 40, 80, 160, 320 PFDA	BW, OW, GN, HP	Death			120	LD_{50} in 30-day observation period
					Bd wt	40	80		12% decreased body weight 30 days post-exposure
					Cardio	80			No histological alterations in the heart 30 days post-exposure; decreased relative heart weight at 80 mg/kg/day
					Hepatic	80			Increases in liver weight and pancellular hypertrophy at ≥20 mg/kg/day 30 days post-exposure
					Renal	80			No histological alterations 30 days post-exposure
					Endocr	40	80		2-fold increase in T3 and 4-fold increase in T4 levels 30 days post-exposure
					Immuno	40	80	160	28% decrease in relative spleen weight at 80 mg/kg/day; atrophy and lymphoid depletion in thymus and spleen at 160 mg/kg/day
Harris et al. 1989									
19	Mouse (CD-1) 10 M	Once on PND 10 (G)	0, 0.72, 10.8 PFDA	CS, DX	Develop	10.8			No alteration in spontaneous activity or habituation at 2–4 months of age
Johansson et al. 2008									
20	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFDA	BW, OW, EA	Bd wt			78	33% weight loss
					Hepatic	78			36% increase in liver weight
Permadi et al. 1992, 1993									
PFBA									
21	Rat (Sprague-Dawley) 3 M,F	5 days 1 time/day (GW)	0, 18, 58, 184 PFBA	CS, BW, OW, HE, BI, GN, HP	Bd wt	184			
					Resp	184			
					Cardio	184			
					Gastro	184			
					Hemato	184			
					Musc/skel	184			
					Hepatic	184			
					Renal	184			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Endocr	184			
					Immuno	184			No histological alterations
					Neuro	184			No histological alterations
					Repro	184			No histological alterations
3M 2007a									
22	Rat (Sprague-Dawley) 3 M	14 days ad lib (F)	0, 20 PFBA	OW, EA	Hepatic	20			Biochemical and ultrastructural evidence of peroxisome proliferation
Ikeda et al. 1985									
23	Mouse (C57BL/6N) 4 M	10 days (F)	0, 78 PFBA	BW, OW, EA	Bd wt	78			
					Hepatic	78			63% increase in absolute liver weight
Permadi et al. 1992, 1993									
PFDoDA									
24	Rat (Wistar) 5–12 M	Once (GO)	0, 5, 20, 50 PFDoDA	BW, FX	BW		50		44% decrease in body weight gain (measured 10-days post-exposure)
					Neuro		50		Impaired performance on novel object recognition test
Kawabata et al. 2017									
25	Rat (Wistar) 10 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in open field activity
Kawabata et al. 2017									
26	Rat (Wistar) 8 M	Once (GO)	0, 50 PFDoDA	FX	Neuro	50			No alterations in tests of working memory or depressive behavior
Kawabata et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
27	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	BW, OW, BI	Bd wt	1	5		25% reduction in final body weight
					Hepatic	10			35% increase in total serum cholesterol at 10 mg/kg/day
					Repro	1	5		Decreased serum testosterone (38%) and estradiol (~38%), and ultrastructural alterations in testes at ≥5 mg/kg/day; decreased testicular weight at 10 mg/kg/day (22%)
Shi et al. 2007									
28	Rat (Sprague-Dawley) 10 M	14 days 1 time/day (GW)	0, 1, 5, 10 PFDoDA	OW, BI, HP	Hepatic	10			Increased liver weight, increased hepatic triglyceride and cholesterol levels at ≥5 mg/kg/day; increased serum triglyceride levels at 10 mg/kg/day
Zhang et al. 2008									
FOSA									
29	Rat (Sprague-Dawley) 15 M	Once (G)	0, 5 FOSA	BW, OW	Bd wt	5			
					Hepatic	5			No alterations in liver weight
Seacat and Luebker 2000									
PFHxA									
30	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 100, 350, 500 PFHxA	CS, DX	Develop	100	350		12.5% decrease in birth weight and delayed eye opening at ≥350 mg/kg/day; increased pup mortality (PND 0–3) and decreased pup survival at 500 mg/kg/day
Iwai and Hoberman 2014									
31	Mouse (CD1) 20 F	GDs 6–18 (GW)	0, 7, 35, 175 PFHxA	CS, DX	Develop	35	175		Increase in stillborn pups and 12.5% decrease in birth weight
Iwai and Hoberman 2014									
32	Rat (Crl:CD (SD)) NS F	Once (GW)	175, 550, 1,550, 5,000 NaPFHx	LE, CS, BW	Death			1,750	1/4 died at 1,750 mg/kg/day; 3/3 died at 5,000 mg/kg/day
					Neuro			175	Abnormal gait, salivation, ataxia, lethargy
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
INTERMEDIATE EXPOSURE									
PFHxS									
33	Rat (Sprague-Dawley) 15 M,F	42–56 days 1 time/day (GW)	0, 0.3, 1, 3, 10 PFHxS	CS, BW, MX, DX, OW, OF, BI, HE, GN, HP, FI	Bd wt	10			
					Resp	10			
					Cardio	10			
					Gastro	10			
					Hemato	10 F	0.3 M		6% increase in prothrombin time in males at ≥0.3 mg/kg/day; decreases in hemoglobin (4%), hematocrit (3%), and RBC levels (2.4%) in males at ≥3 mg/kg/day
					Hepatic	10			Increased liver weight; centrilobular hepatocellular hypertrophy in males at ≥3 mg/kg/day
					Renal	3 M	10 M		31% increase in BUN in males
					Endocr	1[b] M 10 F	3 M		Hypertrophy and hyperplasia of thyroid follicular cells in males
					Immuno	10			No histological alterations
					Neuro	10			No histological alterations
					Repro	10			No histological alterations or effects on fertility
					Develop	10 F			
Butenhoff et al. 2009a (results also reported in Hoberman and York 2003)									
34	Rat (Wistar) 8 F	GDs 7–22 (GO)	0, 25, 45 PFHxS	CS, BW, BC, DX, OW	Bd wt	45			
					Endocr		25		60% decreases in serum T4 levels in dams
					Develop		25		40% decrease in serum T4 levels in pups on PND 16
Ramhøj et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
35	Rat (Wistar) 16–20F	GDs 7–22 (GO)	0, 0.05, 5, 25 PFHxS	CS, BW, BC, DX, OW	Bd wt	25			
					Endocr	0.05	5		20–30% decreases in serum T4 levels in dams
					Develop	0.05	5		20% decrease in serum T4 levels in pups on PND 16/17
Ramhøj et al. 2018									
36	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 6 PFHxS	BW, FI, BC, OW	Bd wt	6			
					Hepatic	6			Decreased plasma triglyceride, cholesterol, non-HDL cholesterol, and HDL cholesterol levels; increased hepatic triglyceride levels, increased liver weight
Bijland et al. 2011									
37	Mouse (CD-1) 30 M, 30 F	M: 42 days F: 14 days premating, mating, gestation, lactation (G)	0, 0.3, 1, 3 PFHxS	CS, BW, FI, HE, BC, GN, HP, FX, RX, DX	Bd wt	3			
					Resp	3			
					Cardio	3			
					Gastro	3			
					Hemato	3			
					Musc/skel	3			
					Hepatic	1	3		Single cell necrosis and microvascular fatty changes at 3 mg/kg/day; centrilobular hepatocellular hypertrophy at ≥0.3 mg/kg/day in F0 mice and at 3 mg/kg/day in F1 mice
					Renal	3			
					Endocr	3			
					Neuro	**3**			
					Repro	3			
					Develop	0.3	1		Decrease in number of pups per litter with no change in pup to implantation site ratio
Chang et al. 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFNA									
38	Rat (Sprague-Dawley) 18 dams; 10–12 M,F pups/litter	GDs 1–20 (GW)	5 PFNA	BW, OF, OW	Bd wt		5		Reduced maternal body weight; approximately 33% on GD 7 and 10% on GD 20
					Develop		5		Decreased birth weight in female pups only (approximately 11%); increased systolic blood pressure in 10-week-old male and female offspring; reduced nephron endowment
Rogers et al. 2014									
39	Mouse (CD-1) 8–10 F	GDs 1–17 (GW)	0, 1, 3, 5, 10 PFNA	BC, DX, FX, MX, OW	Bd wt			10	43% maternal weight loss at GD 13
					Hepatic	10			Increases in absolute and relative liver weights in dams on GD 17 and on post-weaning day 28 at ≥1 mg/kg/day
					Develop	1[c]	3	5	Delayed postnatal development [eye opening, preputial separation and vaginal opening] and decreased body weight gain persisting in males up to PND 287 at ≥3 mg/kg/day; decreased postnatal survival between PND 2 and 10 with 80% mortality at ≥5 mg/kg/day; full litter resorptions at 10 mg/kg/day
Das et al. 2015									
40	Mouse (Parkes) 14 M	90 days (GW)	0, 0.2, 0.5 PFNA	BW, OW, BC HP, RX	Bd wt	0.5			
					Hepatic	0.5			33% decrease in serum cholesterol
					Repro	0.2	0.5		Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size
Singh and Singh 2018									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
41	Mouse (129S1/ Svlm) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increase in dam liver weight at ≥0.83 mg/kg/day
					Develop	0.83	1.1		Decreased number of live births (36 and 31%) and pup survival at 1.1 and 2.0 mg/kg/day, but not 1.5 mg/kg/day; decreased number of live pups per litter and decreased pup body weight gain in females at 2 mg/kg/day; increased pup liver weight at ≥0.83 mg/kg/day
Wolf et al. 2010									
42	Mouse (PPARα knockout) 8 F	GDs 1–18 (G)	0, 0.83, 1.1, 1.5, 2.0 PFNA	BW, OW, DX	Bd wt	2.0			No alterations in maternal body weight or gestational weight gain
					Hepatic	2.0			Increases in liver weight at ≥1.5 mg/kg/day non-pregnant adults, but not in the dams
					Develop	2.0			No alterations in the number of live pups per litter, birth weight, pup survival, day of eye opening, or pup body weight gain; no increases in pup liver weight were observed
Wolf et al. 2010									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDA									
43	Rat (Sprague-Dawley) 8 F	28 days	0, 0.125, 0.25, 0.5, 1, 2 PFDA	BW, HE, OW, HP, IX	Bd wt	0.5	1		Decreased body weight gain (21%)
					Resp	0.5			
					Gastro	0.5			
					Hemato	0.125	0.25		Decreased MCH and MCHC
					Hepatic	0.25	0.5		Single cell necrosis
					Hepatic	0.5			
					Renal	0.5			
					Endocr	0.5			
					Immuno	0.125	0.25		Decreased phagocytosis by fixed tissue macrophages in the liver
Frawley et al. 2018									
44	Mouse (B6C3F1/N) 8 F	1 time/week 4 weeks	0, 0.325, 0.625, 1.25, 2.5, 5 PFDA	BW, HE, OW, HP, IX	Bd wt	5			
					Resp	5			
					Gastro	5			
					Hemato	5			
					Hepatic	5			
					Renal	5			
					Endocr	5			
					Immuno	0.625	1.25		Decreases in splenic T-cells, T-cell subsets, and macrophages
Frawley et al. 2018									
PFUnA									
45	Rat (Crl:CD[SD]) 12 M,F (main); 5 M,F (other)	41–46 days (GO)	0, 0.1, 0.3, 1.0 PFUnA	BH, BW, CS, HP, OF, OW, UR	Bd wt	0.3	1.0		Decreased body weight (~10%) in males during exposure and recovery and in satellite females during dosing (~23% on day 40) and recovery (~10%)
					Hemato	0.3	1.0		Main study males: decreased MCV (5%), MCH (5%), APTT (25%), and fibrinogen (33%) and increased platelet counts (7%); satellite males: increased WBC (52%) and decreased APTT (16%) and fibrinogen (19%); main study females: increased MCV (10%) and MCH (10%) and decreased fibrinogen (32%)

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	1.0			Increased absolute and relative liver weight and centrilobular hypertrophy at 1.0 mg/kg/day
					Renal	0.3	1.0		Increased BUN (61%) and ALP (140%); decreased total protein (11%) and albumin (7%) in main group males
					Develop	0.3 F	1.0 F		Decreased body weight in pups on PNDs 0 and 4 (13–19% in males and 12–16% in females)
Takahashi et al. 2014									
PFBS									
46	Rat (Sprague-Dawley)	28 days 1 time/day (GO)	0, 100, 300, 900 PFBS	CS, BW, FI, HE, BI, GN, HP, OF	Bd wt	900			
					Resp	900			
					Cardio	900			
					Gastro	900			
					Hemato	900			
					Musc/Skel	900			
					Hepatic	900			Increased absolute and relative liver weight at 900 mg/kg/day
					Renal	900			
					Ocular	900			
					Endocr	900			
					Immuno	900			No histological alterations
					Neuro	900			No histological alterations
					Repro	900			No histological alterations
3M 2001									
47	Rat (Sprague-Dawley) 10 NS	90 days (G)	0, 60, 200, 600 PFBS	LT, BW, OW, GN, HP, BC, CS, BI, BH, HE	Resp	600			
					Cardio	600			
					Gastro	200	600		Necrosis of individual squamous cells in forestomach and hyperplasia and hyperkeratosis of limiting ridge
					Hemato	60 M	200 M		Decreased hemoglobin (4.9%) and hematocrit (5.2%)
					Musc/skel	600			

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Hepatic	600			
					Renal	200	600		Hyperplasia of medullary and papillary tubules and medullary ducts and focal papillary edema
					Endocr	600			
					Neuro	600			
Lieder et al. 2009a (data also reported in York 2003b)									
48	Rat (Sprague-Dawley)	P0: starting 70 days prior to mating; F1: starting at weaning (G)	0, 30, 100, 300, 1,000 PFBS	BW, OW, FI, GN, HP, FX, MX, DX, TG	Bd wt	1,000			
					Hepatic	1,000			Increased liver weight in males at ≥300 mg/kg/day
					Renal	100	300		Medullary/papillary tubular and ductal hyperplasia in P0 and F1
					Repro	1,000			
					Develop	1,000			
Lieder et al. 2009b (data also reported in York 2003c)									
49	Mouse (E3L CETP) 6–8 M	4–6 weeks (F)	0, 30 PFBS	BW, FI, BC, OW	Bd wt	30			
					Hepatic	30			Decreased plasma triglyceride levels
Bijland et al. 2011									
50	Mouse (ICR) 30 F	GDs 1–20 (G)	0, 50, 200, 500 PFBS	BW, BC, OW, DX	Bd wt	500			
					Endocr	50	200		Decreased maternal total thyroxine, free thyroxine, and total triiodothyronine and increased TSH
					Develop	50	200		Decreases in pup body weight; delays in eye opening, vaginal opening, and first estrous; decreases in ovarian follicles; decreases in uterine endometrial and myometrial thickness; alterations in reproductive hormone levels; decreases in total T4 and total T3 and increases in TSH
Feng et al. 2017									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
51	Rat (Crl:CD(SD) 25 F	GDs 6–20 (GW)	0, 100, 300, 1,000 PFBS	BW FI DX	Bd wt	300	1,000		31% decrease in maternal body weight gain
					Develop	300	1,000		Decreases in fetal body weight (9%) and delays in hindlimb ossification
York 2002									
52	Rat (Crl:CD(SD) 8 F	GDs 6–20 (GW)	0, 100, 300, 1,000, 2,000 PFBS	BW FI DX	Bd wt	1,000	2,000		12% decrease in maternal body weight
					Develop	1,000	2,000		12–13% decrease in fetal body weights
York 2003a									
PFBA									
53	Rat (Sprague-Dawley) 10 M,F	28 days 1 time/day	0, 6, 30, 150 PFBA	CS, BW, OW, FI, BI, HE, GN, HP, OF	Bd wt	150			
					Resp	150			
					Cardio	150			
					Gastro	150			
					Hemato	150			
					Musc/skel	150			
					Hepatic	150			Increased absolute and relative liver weight and decreased serum cholesterol in males at ≥30 mg/kg/day; hepatocellular hypertrophy in males at 150 mg/kg/day
					Renal	150			
					Dermal	150			
					Ocular	150			
					Endocr	6 M	30 M		Hyperplasia/hypertrophy of follicular epithelium of the thyroid
					Immuno	150			No histological alterations
					Neuro	30 M	150 M		Delayed pupillary reflex
					Repro	150			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007a									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
54	Rat (Sprague-Dawley) 10 M,F	90 days 1 time/day	0, 1.2, 6, 30 PFBA	CS, BW, FI, OW, BI, HE, GN, HP	Bd wt	30			
					Resp	30			
					Cardio	30			
					Gastro	30			
					Hemato	6	30 M		Reduced erythrocyte counts (3.8%), hemoglobin (5.7%), and hematocrit (4.5%)
					Musc/skel	30			
					Hepatic	30			Diffuse panlobular hepatocyte hypertrophy at 30 mg/kg/day in males
					Renal	30			
					Dermal	30			
					Ocular	30			
					Endocr	6 M	30 M		Hypertrophy/hyperplasia of follicular epithelium of the thyroid gland
					Immuno	30			No histological alterations
					Neuro	30			No histological alterations
					Repro	30			No histological alterations
Butenhoff et al. 2012a; van Otterdijk 2007b									
55	Mouse (CD-1)	18 days GDs 1–17 1 time/day (GW)	0, 35, 175, 350 PFBA	BW, MX, DX	Hepatic	350			Significant increase in absolute and relative liver weight at ≥175 mg/kg/day
					Develop		35		Eye opening delayed approximately 1 day
Das et al. 2008									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
PFDoDA									
56	Rat (Crl:CD (SD)) 12 M, 17 F	42–47 days (G)	0, 0.1, 0.5, 2.5 PFDoDA	CS, BW, BC, HE, OW, HP, NX, RX, DX	Death			2.5 F	58% mortality
					Bd wt	0.5	2.5		Decreased body weight gain (20–30%)
					Cardio	2.5			
					Gastro	2.5			
					Hemato	0.5	2.5		Decreased mean corpuscular volume and reticulocytes and increased mean corpuscular hemoglobin concentration in males
					Hepatic	0.5 F	2.5 F		Single cell necrosis in females; hepatocellular hypertrophy in males and females at 2.5 mg/kg/day; bile duct proliferation at 2.5 mg/kg/day in females in recovery group
					Renal	2.5			
					Endocr	0.5	2.5		Pancreas: edema in the interstitium in females and decrease in zymogen granules in males; thymus: atrophy of the cortex in females; adrenals: atrophy of the cortex in males
					Neuro	0.5	2.5		Decreased forelimb grip in males and motor activity in females during recovery period
					Repro	0.5	2.5 F		Hemorrhage at the implantation site; continuous diestrus in nonmated rats
					Develop	0.5	2.5		Decreases in pup body weight (only one litter had live pups)
Kato et al. 2015									
57	Rat (Sprague-Dawley) 8 F	28 days PNDs 24–72 (GW)	0, 0.5, 1.5, 3 PFDoDA		Endocr	1	3		40% reduced serum estradiol in pubertal females
Shi et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
58	Rat (Sprague-Dawley) 6 M	110 days GW	0, 0.02. 0.05, 0.2, 0.5 PFDoDA	BW, BI, OW, HP	Bd wt	0.5			
					Repro	0.05	0.2		Decreased serum testosterone (44%)
Shi et al. 2009a									
PFHxA									
59	Rat (Crl:CD (SD)) 10 M, 10 F	90 days (GW)	0, 10, 50, 200 PFHxA	CS, BW, FI, HE, BC, UR, OP, NX, OW, GN, HP	Bd wt	200			
					Resp	200			
					Cardio	200			
					Gastro	200			
					Hemato	50	200		Slight decreases in RBC, hemoglobin, and hematocrit and increases in reticulocytes
					Musc/skel	200			
					Hepatic	200			Centrilobular hepatocellular hypertrophy at 200 mg/kg/day
					Renal	200			
					Ocular	200			
					Endo	200			
					Neuro	200			
					Repro	200			
Chengelis et al. 2009b									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
60	Rat (CRL:CD(SD)) 10–15 M, 10–15 F	32–44 days (GW)	0, 50, 150, 315 (TWA dose), 450 (4 days) **PFHxA**	CS, BW, FI, HE, BC, RX, DX, OW, GN, HP	Death			450	8/30 deaths (includes rats sacrificed *in extremis*) during first 4 days of exposure
					Bd wt	315			
					Gastro	315			Stomach erosions/ulceration in dying or sacrificed *in extremis* rats administered 450 mg/kg/day for 4 days
					Hemato	50 M 315 F	150 M		Decreased hemoglobin levels; increased reticulocytes at 315 mg/kg/day
					Hepatic	315			Increased relative liver weight at ≥150 mg/kg/day; hepatocellular hyperplasia and decreased serum cholesterol observed at 315 mg/kg/day
					Renal	315			
					Immuno	150 F 315 M	315 F		Thymic atrophy in 3/9 females
					Repro	315			
					Develop	315			
Kirkpatrick 2005									
61	Rat (CRL:CD (SD)) 30 M, 30 F	92–93 days (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, OP, BC, HE, UR, OW, GN, HP, NX	Bd wt	200 M	500 M		19% decrease in body weight gain
					Resp	20	100		Degeneration/atrophy of nasal olfactory epithelium at ≥100 mg/kg/day and respiratory metaplasia at 500 mg/kg/day
					Hemato	100	500		Decreased RBC, hemoglobin, and hematocrit; increased reticulocytes; erythroid hypertrophy in bone marrow and extramedullary hematopoiesis in spleen
					Hepatic	500			Hepatocellular hypertrophy in males at ≥100 mg/kg/day and females at 500 mg/kg/day
					Ocular	500			
					Endocr	100 F	500 F		Thyroid follicular epithelial hypertrophy
					Neuro	500			
Loveless et al. 2009									

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
62	Rat (CRL:CD (SD) 20M, 20F	110–126 days; 70 days prior to mating and during mating gestation, lactation (GW)	0, 20, 100, 500 NaPFHx	CS, BW, FI, RX, DX	Bd wt	20 M 100 F	100 M 500 F		Decreased weight gain (12%); decreased maternal weight gain during GDs 0–7 and increased maternal weight gain during lactation
					Repro	500			
					Develop	100	500		Decreased pup body weight (17–18%) during lactation period
Loveless et al. 2009									
63	Rat (CRL:CD (SD) 22 F	GDs 1–20 (GW)	0, 20, 100, 500 NaPFHx	BW, FI, DX	Bd wt	100	500		Decreased maternal weight gain (19%)
					Develop	100	500		Decreased fetal weight (10%)
Loveless et al. 2009									
CHRONIC EXPOSURE									
PFHxA									
64	Rat (Sprague-Dawley) 60 or 70 M,F	104 weeks (GW)	M: 0, 2.5, 15, 100 F: 0, 5, 30, 200 PFHxA	BC, BW, CS, GN, HP, LE, OP, OW, UR	Death			200 F	36, 43, 33, and 22% survival rate in females at 0, 5, 30, and 200 mg/kg/day, respectively
					Bd wt	100 M 200 F			
					Hemato	100 M	200 F		8.1% reduction in mean RBC count and 5.2% reduction in hemoglobin at 51 weeks; 23.6 and 53.6% increase in reticulocyte counts at weeks 25 and 51, respectively
					Hepatic	100 M 30F	200 F		Males: 42% decrease in triglycerides, 19% decrease in free fatty acids in males at 100 mg/kg/day; hepatocellular necrosis; 66% increase in triglycerides, 44% decrease in non-HDL cholesterol in females at 200 mg/kg/day

Table 2-5. Levels of Significant Exposure to Other Perfluoroalkyls – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Renal	100 M	200 F		Mild renal tubular degeneration and mild to severe papillary necrosis; increased mean urine volume (109%) and reduced specific gravity (0.96%)
					Neuro	100 M 200 F			
Klaunig et al. 2015									

[a]The number corresponds to entries in Figure 2-10.
[b]Used to derive an intermediate-duration oral MRL of $2x10^{-5}$ mg/kg/day for PFHxS based on a measured serum PFHxS level of 89.12 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.0047 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.
[c]Used to derive an intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day for PFNA based on a measured serum PFNA level of 8.91 µg/mL at the NOAEL dose and an empirical clearance model to estimate a HED. The $NOAEL_{HED}$ of 0.001 mg/kg/day was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) and a modifying factor of 10 for database deficiencies.

ad lib = *ad libitum*; ALT = alanine aminotransferase; APTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BC = biochemistry; BH = behavioral; BI = biochemical changes; BUN = blood urea nitrogen; BW or Bd wt = body weight; Cardio = cardiovascular; CI = confidence interval; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; (F) = feed; F = female(s); FI = food intake; FOSA = perfluorooctane sulfonamide; FX = fetal toxicity; G = gavage; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GO = gavage in oil vehicle; GW = gavage in water vehicle; HDL = high-density lipoprotein; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; IX = immunotoxicity; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; Musc/skel = musculoskeletal; MX = maternal toxicity; NaPFHx = sodium perfluorohexanoate; Neuro = neurological; NOAEL = no observed-adverse-effect level; NS = not specified; NS = neurotoxicity; OF = organ function; OP = ophthalmology; OW = organ weight; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid; PPARα = peroxisome proliferator-activated receptor-α; RBC = red blood cell; Repro = reproductive; Resp = respiratory; RX = reproductive toxicity; T3 = triiodothyronine; T4 = thyroxine; TG = teratogenicity; TSH =thyroid stimulating hormone; TWA = time-weighted average; UR = urinalysis

Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Acute (≤14 days)

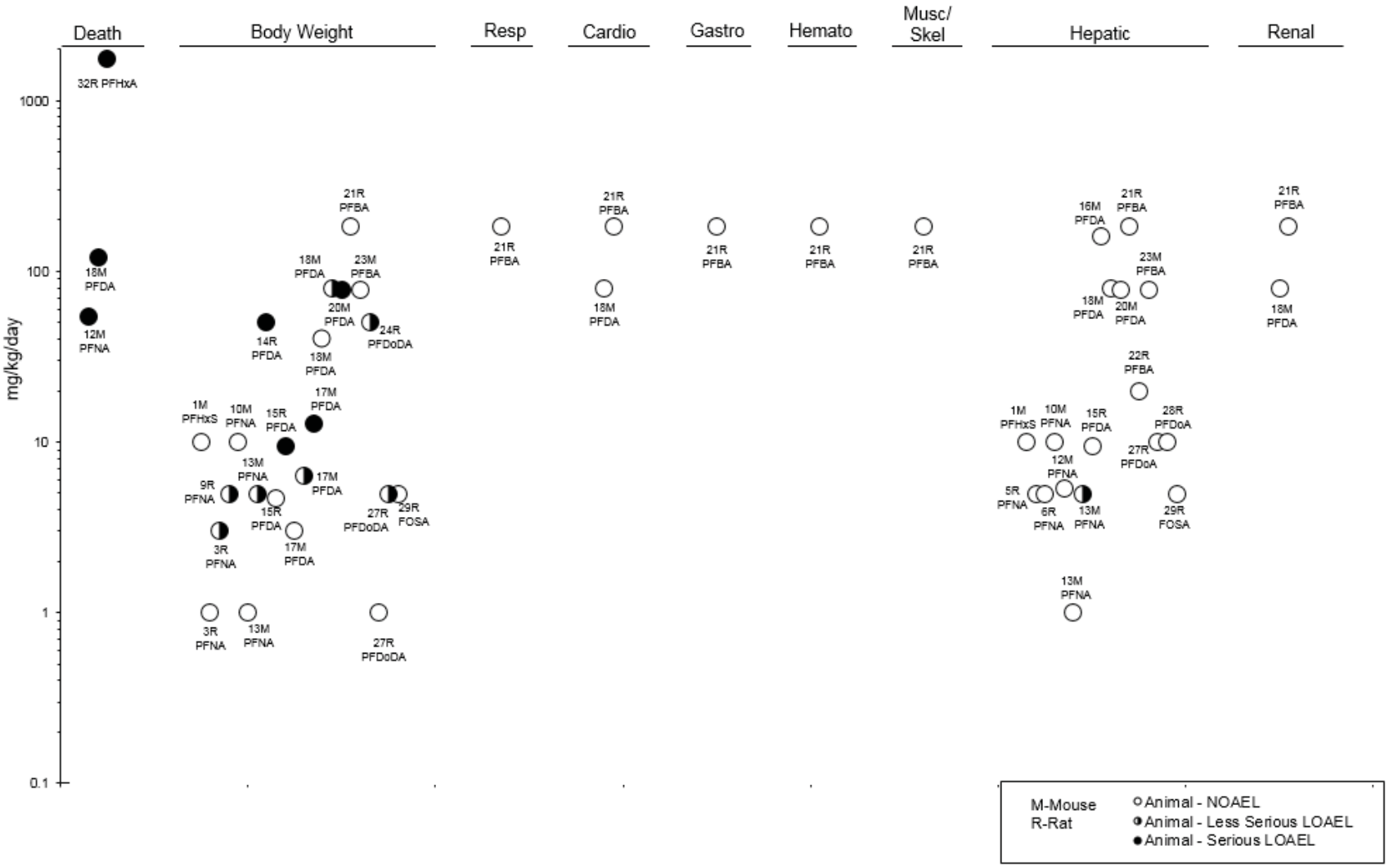


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Acute (≤14 days)

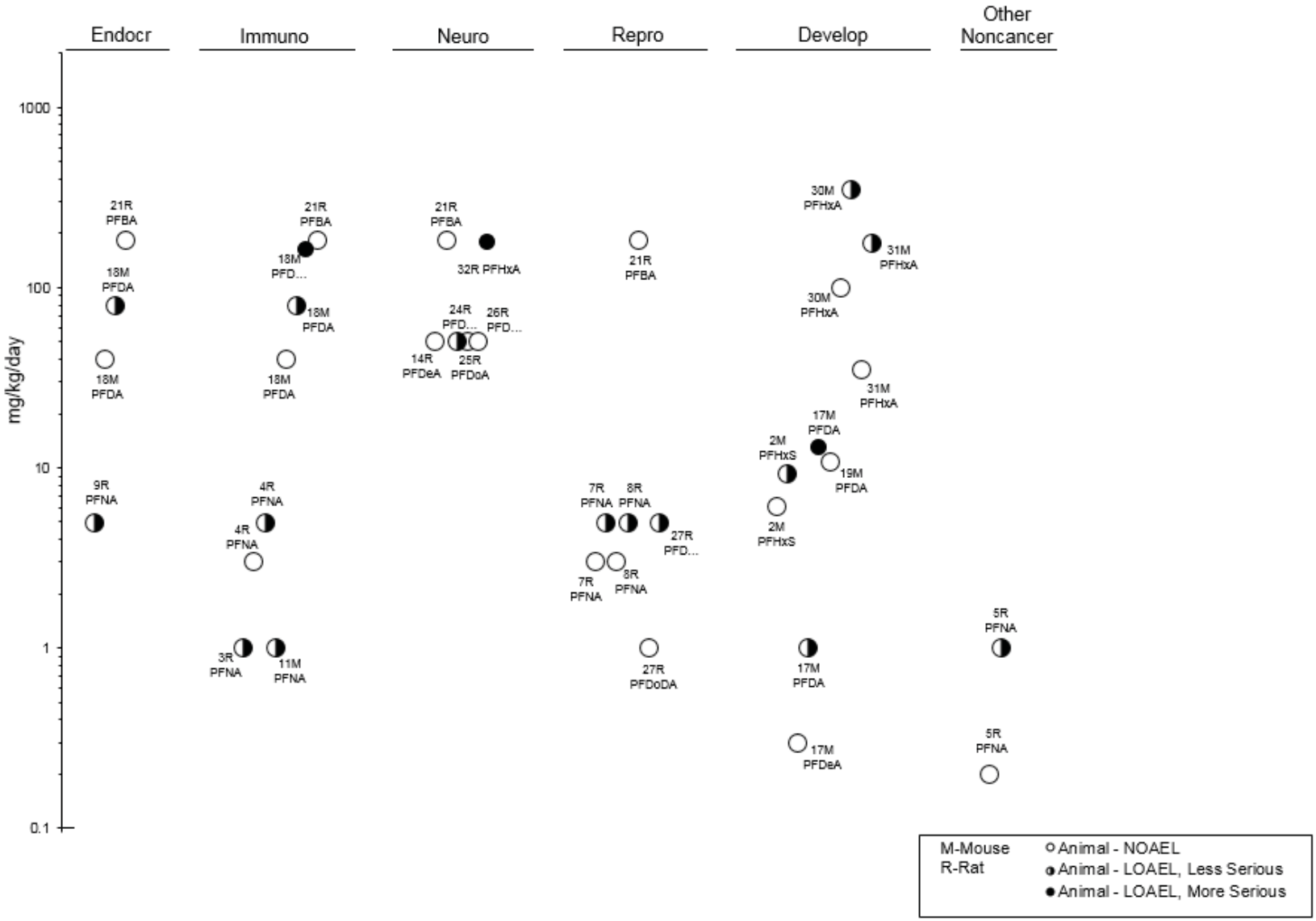


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

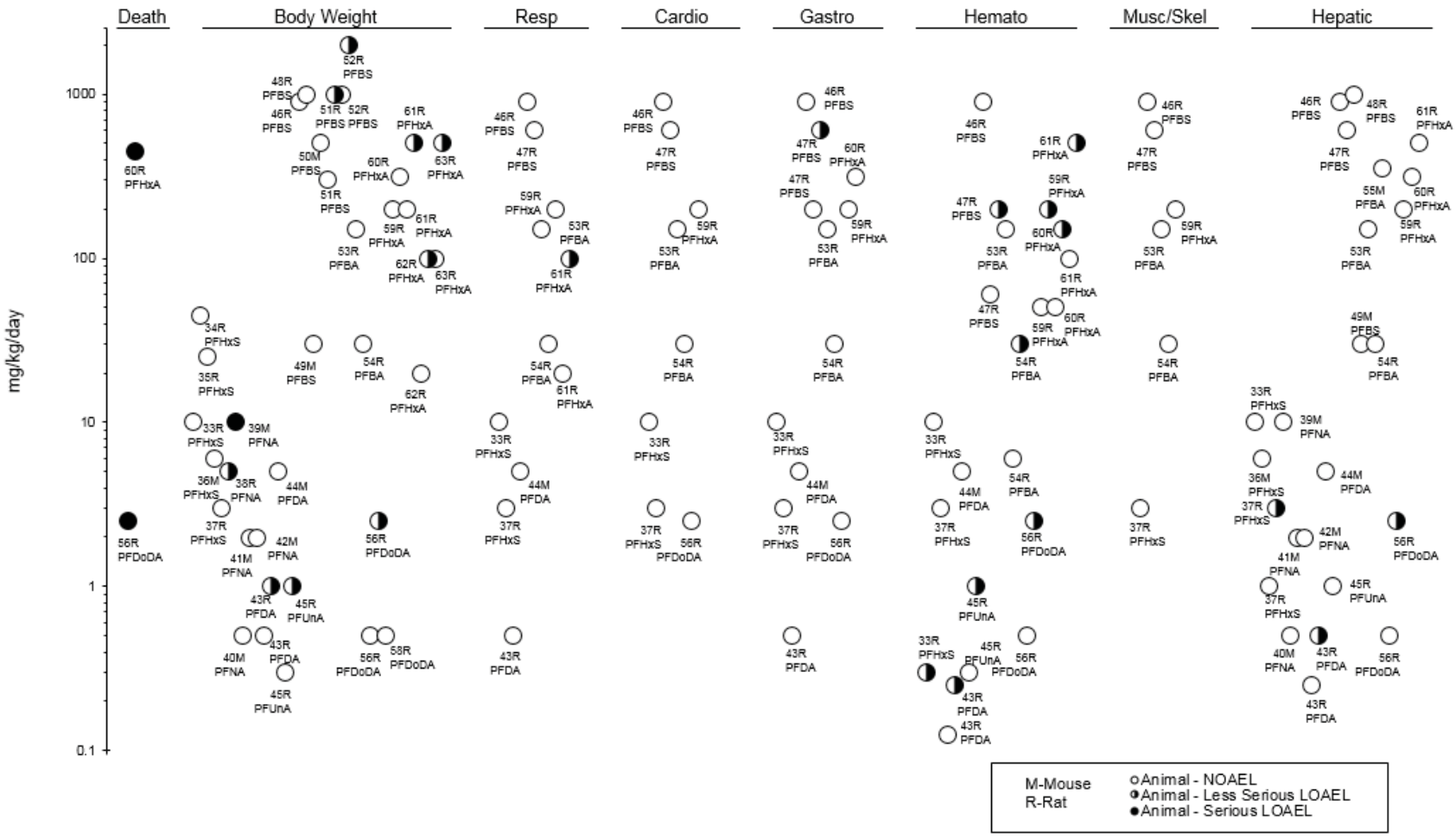


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Intermediate (15–364 days)

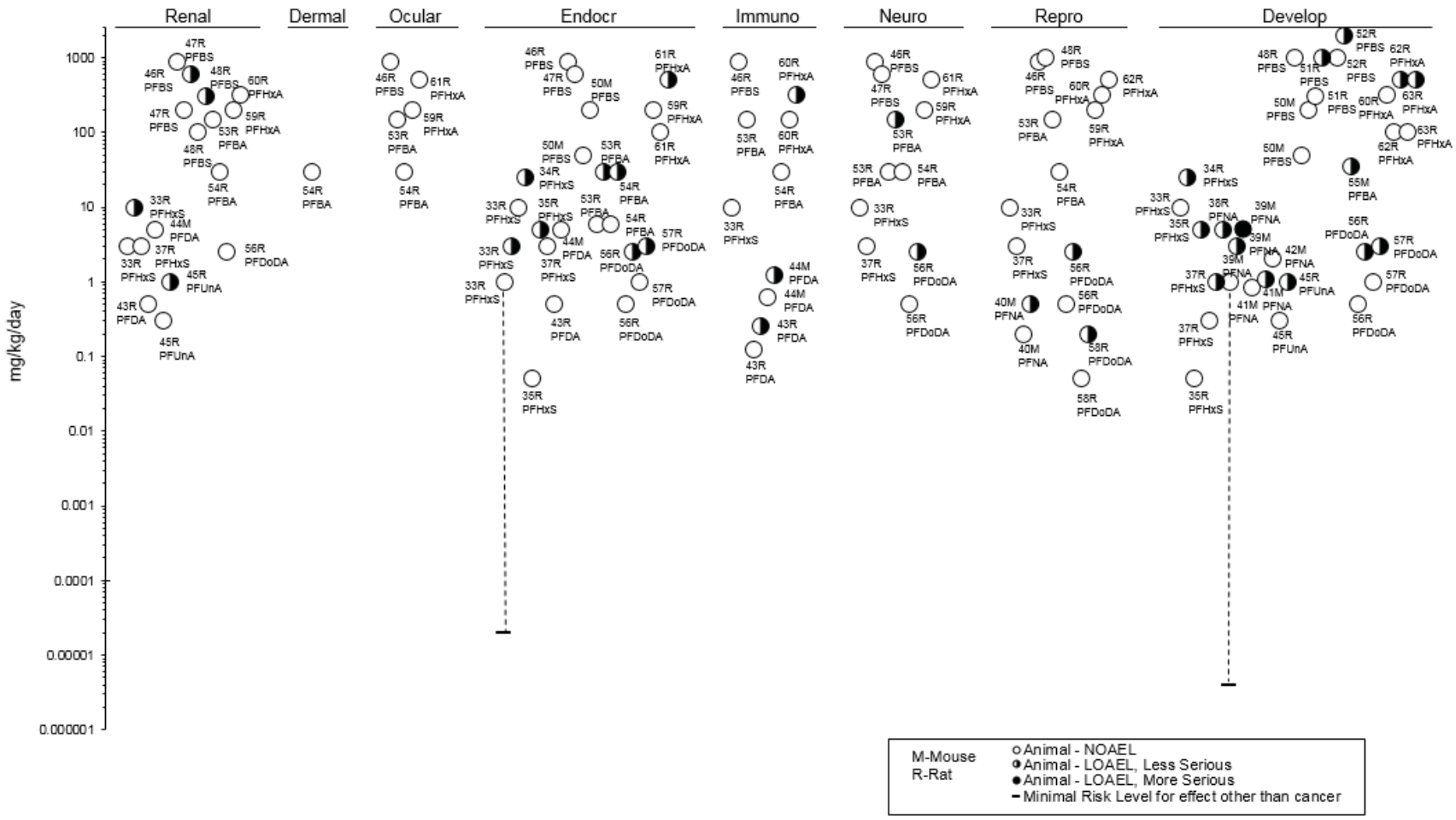


Figure 2-10. Levels of Significant Exposure to Other Perfluoroalkyls – Oral
Chronic (≥365 days)

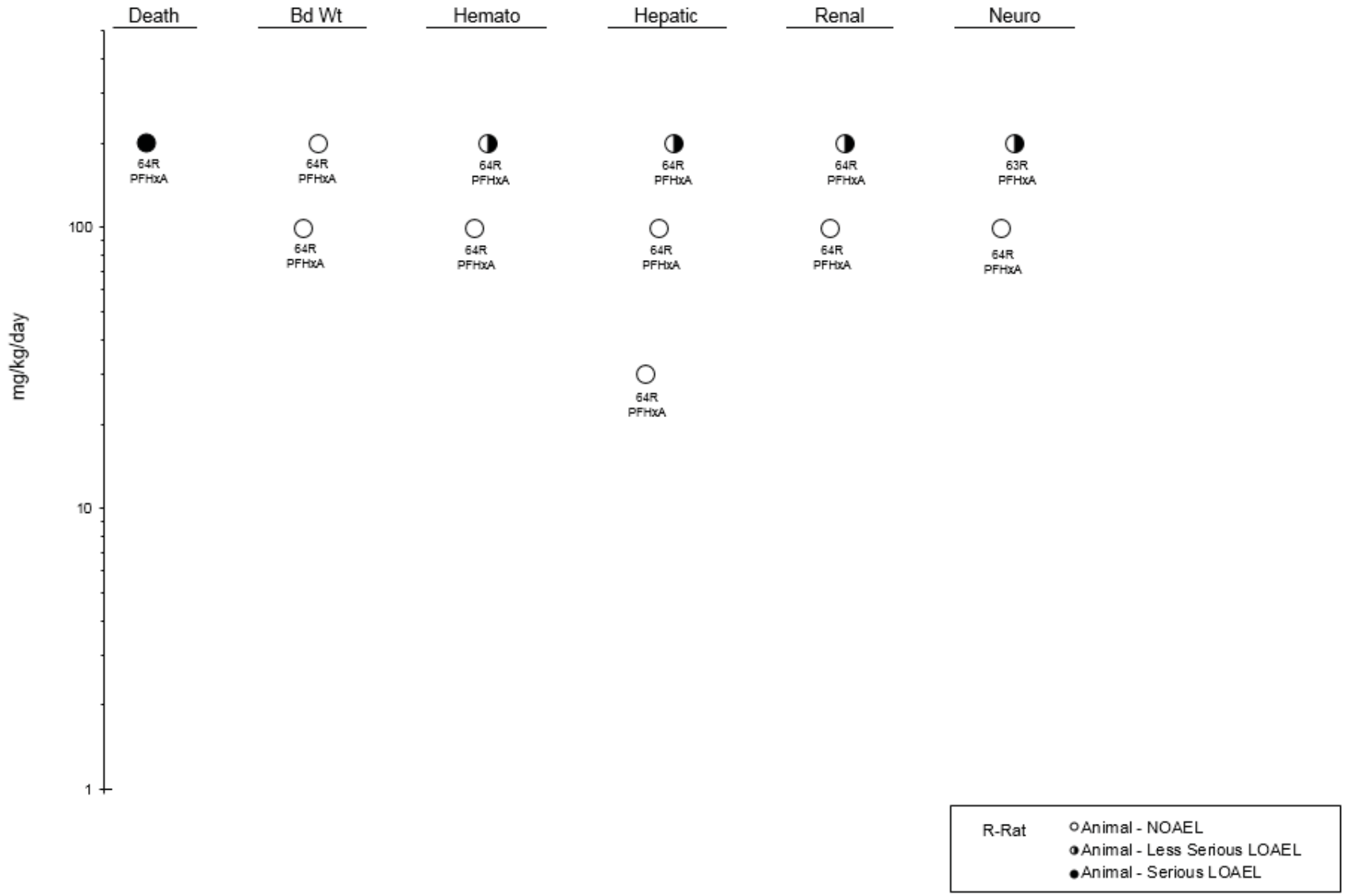


Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
ACUTE EXPOSURE								
Rat (CD) 15 M,F	Once	3,000, 5,000, 7,500 mg/kg	CS, LE	Death			7,000 M	14-day LD_{50}
				Bd wt			3,000 M F	5–7% transient weight loss
				Dermal	3,000 M F	5,000 M F		Mild skin irritation
Kennedy 1985								
APFO								
LD_{50} in females was >7,500 mg/kg								
Rat (CD) 60 M	2 weeks 6 hours/day 5 days/week	0, 20, 200, 2,000 mg/kg/day	CS, BW, HE, BI, GN, HP	Bd wt	20 M		200 M	14% weight loss
				Resp	2,000 M			
				Cardio	2,000 M			
				Gastro	2,000 M			
				Hemato	2,000 M			
				Hepatic		20 M		Foci of coagulative necrosis
				Renal	2,000 M			
				Dermal	20 M	200 M	2,000 M	Skin irritation; acute necrotizing dermatitis
				Ocular	2,000 M			
				Endocr	2,000 M			
				Immuno	2,000 M			
				Neuro	2,000 M			
				Repro	2,000 M			
Kennedy 1985								
APFO								
The immunological NOAEL is for histopathology of the spleen, thymus, and lymph nodes. The neurological NOAEL is for histopathology of the brain. The reproductive NOAEL is for histopathology of the testes.								
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 0.25, 2.5, 6.25, 12.5, 25, 50 mg/kg/day	BW, OW, OF	Bd wt	50 F			
				Hepatic	2.5 F	6.25 F		52% increase in absolute liver weight
Fairley et al. 2007								
PFOA								

Table 2-6. Levels of Significant Exposure to PFOA – Dermal

Species (strain) No./group	Exposure parameters	Doses	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
Mouse (BALB/c) 35 F	4 days 1 time/day	0, 12.5, 18.8, 25, 50 mg/kg/day	BW, OW, OF	Immuno	12.5	18.8		Increased serum IgE following ovalbumin challenge
Fairley et al. 2007								
PFOA								
Rabbit (albino) 6 NS	Once (NS)	100 mg	CS	Ocular		100		Moderate eye irritation
Griffith and Long 1980								
APFO								
Rabbit (albino) 6 NS	24 hours (NS)	500 mg	CS	Dermal	500			
Griffith and Long 1980								
APFO								
Rabbit (New Zealand) 17 M	Once	1,500, 3,000, 5,000, 7,500 mg/kg	CS, LE	Death		4,300		14-day LD_{50}
Kennedy 1985								
APFO								

APFO = ammonium perfluorooctanoate; BI = biochemical changes; BW or Bd wt = body weight; Cardio = cardiovascular; CS = clinical signs; Endocr = endocrine; F = female(s); Gastro = gastrointestinal; GN = gross necropsy; HE or Hemato = hematological; HP = histopathology; Immuno = immunotoxicological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); Neuro = neurological; NOAEL = no-observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; PFOA = perfluorooctanoic acid; Repro = reproductive; Resp = respiratory

2.2 DEATH

Overview. There are limited data regarding the lethality of perfluoroalkyls in humans; the available data primarily come from cohort mortality studies in workers; data were only available for PFOA and PFOS. These studies did not find increases in deaths from all causes associated with PFOA and PFOS, although some increases in disease-specific mortalities were observed. Laboratory animal studies have measured LC_{50} and LD_{50} values and reported deaths following inhalation, oral, or dermal exposure to perfluoroalkyls. Increases in mortality have also been observed in repeated-exposure studies. These data are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFHxS, PFUnA, PFHpA, PFBS, PFBA, or FOSA.

PFOA

Epidemiological Studies. Five occupational exposure studies at two PFOA manufacturing facilities have examined the possible associations between PFOA exposure and increases in mortality from all causes and have not found associations (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). Some increases in disease-specific mortality have been observed; these data are discussed in subsequent sections of this chapter (Sections 2.5, 2.8, 2.10, 2.18, and 2.19).

Laboratory Animal Studies. Limited data are available regarding death in animals following inhalation exposure to perfluoroalkyls. Exposure of male and female rats to 18,600 mg/m^3 ammonium perfluorooctanoate (APFO) dusts for 1 hour did not result in deaths during exposure or during a 14-day observation period (Griffith and Long 1980); APFO is the ammonium salt of PFOA. An LC_{50} of 980 mg/m^3 was reported in male CD rats exposed head-only to APFO dusts for 4 hours (Kennedy et al. 1986). Deaths occurred at all exposure levels (380–5,700 mg/m^3) and all deaths occurred within 48 hours of exposure. Rats dying during exposure had hyperinflated lungs. A similar LC_{50} value of 820 mg/m^3 was calculated for male CD rats exposed nose-only to APFO dusts for 4 hours (Kinney et al. 1989). Unlike the Kennedy et al. (1986) study, one death was observed at 590 mg/m^3 and no deaths occurred at 620 mg/m^3. In a developmental study with APFO, whole-body exposure of 12 pregnant rats to 25 mg/m^3, 6 hours/day during GDs 6–15 resulted in three deaths on GDs 12, 13, and 17 compared with no deaths in groups exposed to $\leq$10 mg/m^3 (Staples et al. 1984). The cause of death was not reported.

Oral LD_{50} values of 680 and 430 mg/kg were reported for male and female albino rats, respectively, administered single gavage doses of APFO and observed for 14 days (Griffith and Long 1980); all animals at the highest dose of 2,150 mg/kg died on day 1. Nonlethal signs observed included ptosis, piloerection, hypoactivity, decreased limb tone, ataxia, and corneal opacity. All signs were intermittent and there was no apparent dose-response relationship. In a 28-day dietary study with APFO in rats, all rats (males and females) in groups receiving approximately 1,000–1,130 mg/kg/day APFO died before the end of the first week (Griffith and Long 1980). In a similar study in mice, all mice receiving doses of approximately 180–195 mg/kg/day died before the second week of the study (Griffith and Long 1980). In this study, doses of approximately 54–58 mg/kg/day APFO were lethal to 4/5 male and 5/5 female mice before the 4^{th} week of the study.

In a 90-day gavage study, treatment of Rhesus monkeys with 100 mg/kg/day APFO by gavage resulted in the death of an unspecified number of animals (group size was 10/sex) on week 2 (Griffith and Long 1980). Doses of approximately 30 mg/kg/day were lethal to one male and two females during weeks 7–12. All animals that died in the 30 and 100 mg/kg/day groups had anorexia, emesis, black stool, pale face and gums, swollen face and eyes, hypoactivity, and prostration. Microscopic examination of tissues showed marked diffuse lipid depletion in the adrenals, slight to moderate hypocellularity of the bone marrow, moderate atrophy of the lymphoid follicles of the spleen, and moderate atrophy of the lymphoid follicles of the lymph nodes. No deaths occurred at 10 mg/kg/day. Deaths were also reported in intermediate-duration studies in Cynomolgus monkeys (Butenhoff et al. 2002). One monkey exposed to 30/20 mg/kg/day PFOA (12 days of exposure to 30 mg/kg/day, 10 days with no exposure, 23 weeks of exposure to 20 mg/kg/day) was sacrificed in moribund condition; the animal had a body weight loss of 12.5%, was notably hypoactive, and was cold to the touch (Butenhoff et al. 2002). The investigators noted that the death was likely due to the high toxicity of the 30 mg/kg/day dose. It is unclear if these deaths were compound-related; one monkey had pulmonary necrosis with a severe acute recurrence of pulmonary inflammation and the cause of morbidity for the second monkey was likely hyperkalemia. Neither effect was observed in the surviving animals.

The dermal LD_{50} values for APFO were 7,000 mg/kg in male CD rats and >7,500 mg/kg in female rats (Kennedy 1985). The protocol consisted of application of PFOA (as an aqueous paste) to a clipped area of the skin, which immediately was covered with gauze pads and wrapped with rubber sheeting around the trunk. The contact period was 24 hours, at which time the application site was washed with water and the rats were observed for clinical signs for 14 days. Using the same protocol, the dermal LD_{50} in male rabbits was 4,300 mg/kg (Kennedy 1985). Rabbits treated with 1,500 mg/kg showed skin irritation with

formation of a large crusty area at the application site. No deaths occurred at 1,500 mg/kg. Rabbits treated with 3,000 mg/kg were lethargic and a single death occurred 7 days after treatment. At 5,000 mg/kg, deaths occurred in 3–4 days. These rabbits also showed nasal discharge, pallor, diarrhea, weakness, severe weight loss, and severe skin irritation along with areas of necrosis.

PFOS

Epidemiological Studies. One occupational exposure study evaluated the potential of PFOS to increase lethality; the study did not find increases in deaths from all causes in workers at a PFOS manufacturing facility (Alexander et al. 2003). Alterations in disease-specific mortality are discussed in subsequent sections of this chapter.

Laboratory Animal Studies. Unpublished information summarized by the Organization for Economic Co-operation and Development (OECD) (2002) indicates that an LC_{50} of 5,200 mg/m^3 was calculated for PFOS in male and female Sprague-Dawley rats exposed to airborne concentrations of PFOS dusts from 1,890 to 45,970 mg/m^3 for 1 hour. All rats exposed to 24,090 mg/m^3 died by day 6.

Unpublished information summarized by OECD (2002) indicate that LD_{50} values of 233 and 271 mg/kg were calculated for male and female CD rats, respectively, following administration by gavage of single doses of up to 1,000 mg/kg of powdered PFOS suspended in an acetone/oil mixture and observed for 14 days. All rats (5/sex/dose group) dosed with ≥464 mg/kg PFOS died before the end of the study. The signs most frequently observed were hypoactivity, decreased limb tone, and ataxia. Gross necropsy showed stomach distension and signs of irritation of the glandular mucosa, and lung congestion. OECD (2002) also reported that a different study estimated that the acute oral LD_{50} for PFOS by gavage in water in Sherman-Wistar albino rats was >50 and <1,500 mg/kg. An oral LD_{50} value of 579 mg/kg/day was reported for male C57/BL/6 mice administered single gavage doses of PFOS and observed for 14 days (Xing et al. 2016). Mortality occurred within 3 hours of dosing, and moribund mice displayed signs of neurotoxicity (abdominal breathing, hind limb spasticity, tics, and urinary incontinence).

In a 26-week study, 2/6 male Cynomolgus monkeys administered 0.75 mg/kg/day PFOS via a capsule died or were sacrificed due to morbidity (Seacat et al. 2002). The cause of death in one monkey was pulmonary inflammation; the cause of morbidity in the second monkey was not determined, but the animal did have hyperkalemia.

PFNA

Laboratory Animal Studies. A LC_{50} of 820 mg/m^3 was identified in rats exposed to airborne PFNA for 4 hours (Kinney et al. 1989). In a 14-day dietary exposure study, all mice administered approximately 54 mg/kg/day PFNA died before the study period ended; no deaths occurred at 5.3 mg/kg/day (Kennedy 1987).

PFDA

Laboratory Animal Studies. An LD_{50} of 120 mg/kg was estimated for PFDA in female C57BL/6N mice administered single doses between 20 and 320 mg/kg/day PFDA by gavage in corn oil and observed for 30 days (Harris et al. 1989). All mice receiving 160 or 320 mg/kg were dead by 14 days; no mice died at ≤80 mg/kg PFDA. Early death was associated with mural thrombosis in the left ventricle of the heart. Without providing any details, George and Andersen (1986) reported that the 30-day oral LD_{50} for PFDA in male Fischer-344 rats was 57 mg/kg.

PFDoDA

Laboratory Animal Studies. Increases in mortality were observed in pregnant rats administered 2.5 mg/kg/day for 14 days prior to mating and throughout gestation; 4/12 dams between GD 18 and 22 and another 3 dams were sacrificed during the period due to morbidity (Kato et al. 2015). No deaths were observed in males or nonpregnant females exposed to 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. In a single exposure gavage study, deaths occurred in rats administered 1,750 or 5,000 mg/kg sodium perfluorohexanoate (NaPFHx) (Loveless et al. 2009). Decreased survival was observed in female Sprague-Dawley rats administered 200 mg/kg/day PFHxA via gavage in a 104-week study (Klaunig et al. 2015). There was no significant effect on survival rates of males. Mortality and morbidity were observed in male and female rats administered 450 mg/kg/day PFHxA via gavage for 4 days (Kirkpatrick 2005). The cause of death was determined to be renal papillary necrosis and/or stomach erosion/ulceration.

2.3 BODY WEIGHT

Overview. Epidemiological studies have examined the possible associations between *in utero* and/or early life exposure to perfluoroalkyls and body weight, body mass index (BMI; measure of body fat based on body weight and height), etc. Other studies have examined possible associations between serum perfluoroalkyl levels in older children or adults and body weight, adiposity markers, and the risk of being overweight or obese. The results of the epidemiological studies are summarized in Table 2-7, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 1. No epidemiological data were available for PFHpA, PFBS, PFBA, PFDoDA, or PFHxA. Animal studies have evaluated changes in body weight, including maternal body weight, in response to inhalation, oral, or dermal exposure to perfluoroalkyls; these data are summarized in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies examining body weight were identified for PFHpA.

Overall, the evidence from epidemiological studies does not suggest an association between *in utero* and/or early life exposure to perfluoroalkyls and alterations in growth (body weight or length), body composition (e.g., BMI), or the risk of being overweight or obese in children for PFOA, PFOS, PFHxS, or PFNA. Conclusions cannot be drawn for PFDA, PFUnA, PFDoDA, or FOSA because of the small number of studies (less than 5 studies for each compound) examining potential body weight endpoints. A small number of studies examined potential associations between PFOA and body weight effects in adults and only one study examined PFOS, PFHxS, PFNA, and PFDA associations; these data were considered inadequate for assessing potential associations in adults.

Studies in laboratory animals exposed to PFOA, PFOS, PFNA, PFDA, PFUnA, PFDoDA, or PFHxA have consistently shown decreases in body weight or decreases in body weight gain. Studies with PFOA suggest that the decrease in body weight gain does not appear to be associated with alterations in food consumption and the mechanism may involve PPARα as studies in PPARα null have not found decreases in body weight gain. The small number of studies examining PFHxS, PFBS, PFBA, and FOSA have not reported decreases in body weight; although decreases in maternal body weight gain were observed for PFBS.

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Barry et al. 2014 Community (C8) (n=8,764 20–40-year-olds)	164.6 and 194.3 ng/mL (estimated early life [first 3 years] median PFOA)	Overweight or obesity at age 20–40 years	OR 0.9 (0.7–1.1), males OR 0.9 (0.7–1.1), females
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	2.43 ng/mL (maternal geometric mean serum PFOA)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	5.21 ng/mL (maternal median PFOA)	Body weight (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		BMI (age 5 and 12 months)	**Inverse association (p<0.05)*, boys NS (p>0.05), girls**
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	5.25 ng/mL (maternal median PFOA)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	5.3 ng/mL (maternal median PFOA)	Changes in BMI scores between 2 and 8 years of age	**Association (p=0.03)***
		Overweight/obesity risk	RR 1.54 (0.77–3.07), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	1.59 ng/mL (mean cord serum PFOA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.16)
		Head circumference at 19 months	NS (p=0.94)
de Cock et al. 2014 General population (n=89 infants aged 1–11 months)	0.9402 ng/mL (cord blood mean PFOA)	Weight	NS (p=0.350)
		Height	NS (p=0.045)
		BMI	NS (p=0.813)
		Head circumference	NS (p=0.774)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Halldorsson et al. 2012 General population (n=665 20-year-olds)	3.7 and 5.8 ng/mL (maternal median PFOA and 4th quartile median)	BMI	**Association (p=0.001)*, females**
		Waist circumference	**Association (p=0.006)*, females**
		Overweight risk	**RR 3.1 (1.4–6.9)*, females 4th quartile**
		High waist circumference	**RR 3.0 (1.3–6.8)*, females 4th quartile**
Hartman et al. 2017 General population (n=359 girls aged 9 years)	3.7 ng/mL (maternal median PFOA)	Total body fatness	NS (p=0.20)
		Trunk fatness	NS (p=0.05)
		BMI	NS (p=0.05)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	2.2–5.1 and 1.1–9.8 ng/mL (maternal 3rd tertile PFOA for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 1.23 (0.87–1.74), 3rd tertile RR 0.78 (0.47–1.29), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.18 (0.80–1.74), 3rd tertile RR 1.11 (0.48–2.57), 3rd tertile
Karlsen et al. 2017 General population (n=444 children)	1.37 ng/mL (maternal geometric mean serum PFOA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.92–1.4)
		Risk of being overweight 5 years	**RR 1.50 (1.01–2.24, p<0.05)***
Karlsen et al. 2017 General population (n=444 children aged 5 years)	2.22 ng/mL (child geometric mean serum PFOA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.68 (0.38–1.22)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.90–1.13)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	4.5 ng/mL (median serum PFOA)	Weight loss	NS (p=0.73, trend)
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.78, trend) **Association (p=0.007, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 NS (p=0.48, trend) **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean serum PFOA)	Weight gain until 6 months of age	β 0.04 (-0.04–0.12)
		BMI at 4 years of age	β 0.04 (-0.04–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.13)
		Waist circumference at 4 years of age	β 0.00 (-0.09–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.11–0.06)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in early childhood group)	BMI	β 0.09 (-0.02–0.19),
		Waist circumference	**β 0.31 (0.04–0.57)*, boys and girls** **β 0.50 (0.06–0.93)*, boys only** β 0.14 (-0.18–0.47), girls only
		Risk of being overweight	RRR 1.05 (0.87–1.26)
		Risk of being obese	RRR 1.03 (0.80–1.32)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	5.6 ng/mL (maternal median plasma PFOA in late childhood group)	BMI	β 0.13 (-0.10–0.35)
		Total fat mass index	β 0.13 (0.02–0.29)
		Waist circumference	β 0.20 (-0.39–0.80)
		Risk of being overweight	RRR 1.02 (0.88–1.29)
		Risk of being obese	RRR 1.10 (0.88–1.37)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	9.3 ng/mL (mean PFOA)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOA increase
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Growth during childhood	NS (p>0.05)
PFOS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (maternal geometric mean serum PFOS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
Andersen et al. 2010 General population (n=1,010 infants)	33.8 ng/mL (maternal median PFOS)	Body weight (age 5 months)	NS (p>0.05), boys NS (p>0.05), girls
		Body weight (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		BMI (age 5 months)	NS, boys NS (p>0.05), girls
		BMI (age 12 months)	**Inverse association (p<0.05)*, boys** NS (p>0.05), girls
		Height (age 5 and 12 months)	NS (p>0.05), boys NS (p>0.05), girls
Andersen et al. 2013 General population (n=811 children aged 7 years)	33.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.05)
		Waist circumference	NS (p>0.05)
Braun et al. 2016a, 2016b General population (n=204 children)	13 ng/mL (maternal median PFOS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.08 (0.59–1.95), 3rd tertile

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 infants)	1.43 ng/mL (mean cord serum PFOS)	Body weight at 19 months	NS (p=0.72)
		Length at 19 months	NS (p=0.91)
		Head circumference at 19 months	NS (p=0.63)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	21.5 and 5.8 ng/mL (maternal median PFOS)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Høyer et al. 2015b General population (n=1,122 children aged 5–9 years; n=531 for Greenland cohort and n=491 for Ukraine cohort)	23.9–87.3 and 5.9–18.1 ng/mL (maternal 3rd tertile PFOS for Greenland and Ukraine cohorts)	Overweight Greenland cohort Ukraine cohort	 RR 0.84 (0.61–1.14), 3rd tertile RR 0.89 (0.57–1.37), 3rd tertile
		Waist-to-height ratio >0.5 Greenland cohort Ukraine cohort	 RR 1.22 (0.86–1.74), 3rd tertile RR 1.44 (0.62–3.31), 3rd tertile
Hartman et al. 2017 General population (n=359 girls aged 9 years)	19.7 ng/mL (maternal median PFOS)	Total body fatness	NS (p=0.12)
		Trunk fatness	**Inverse association (p=0.02)**
		BMI	**Inverse association (p=0.03)***
Karlsen et al. 2017 General population (n=444 children)	8.04 ng/mL (maternal geometric mean serum PFOS)	BMI score, 18 months	**Association (p<0.05)**
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	**RR 1.29 (1.01–1.64)***
		Risk of being overweight 5 years	RR 1.01 (0.58–1.75)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	4.68 ng/mL (child geometric mean serum PFOS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.68 (0.36–1.29)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Risk of being overweight	OR 1.00 (0.90–1.07)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	24.5 ng/mL (median serum PFOS)	Weight loss	NS (p=0.27, trend).
		Weight regain Males Females	**Association (p=0.009, trend)*** NS (p=0.34, trend) **Association (p=0.001, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p<0.001, trend)***
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (median maternal PFOS)	Body weight at 20 months (adjusted for birth weight)	**Significant trend (p<0.0001) when adjusted for birth weight and height**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean serum PFOS)	Weight gain until 6 months of age	β -0.02 (-0.11–0.07)
		BMI at 4 years of age	β 0.04 (-0.05–0.13)
		BMI at 7 years of age	β 0.03 (-0.08–0.14)
		Waist circumference at 4 years of age	β -0.03 (-0.13–0.07)
		Waist circumference at 7 years of age	β 0.00 (-0.09–0.09)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.8 ng/mL (maternal median plasma PFOS in early childhood group)	BMI	**β 0.04 (0.05–0.12)*, boys and girls** β 0.02 (-0.11–0.15), boys only β 0.04 (-0.08–0.16), girls only
		Waist circumference	β 0.05 (-0.17–0.27)
		Risk of being overweight	RRR 1.07 (0.92–1.24)
		Risk of being obese	RRR 0.97 (0.76–1.23)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	24.7 ng/mL (maternal median plasma PFOS in late childhood group)	BMI	β 0.16 (-0.04–0.36)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.34 (-0.19–0.87),
		Risk of being overweight	RRR 1.15 (0.95–1.40)
		Risk of being obese	RRR 1.12 (0.99–1.47)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2014 General population (n=499 8–10-year-old children)	41.5 ng/mL (mean PFOS)	Adiposity markers	NS (p>0.05), per 10 ng/mL PFOS increase
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (maternal geometric mean serum PFHxS)	Body weight at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body weight (longitudinal analysis)	**β -5.270 (-9.591 to -0.950)***
		Body length at 1, 4, 6, and 12 months of age	NS (p>0.05)
		Body length (longitudinal analysis)	**β 4.516 (1.368–7.664)***
Braun et al. 2016a, 2016b General population (n=204 children)	1.4 ng/mL (maternal median PFHxS)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.48 (0.75–2.96), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.16 ng/mL (mean cord serum PFHxS)	Body weight at 19 months	NS (p=0.96)
		Length at 19 months	NS (p=0.31)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	1.6 ng/mL (maternal median PFHxS)	Total body fatness	NS (p=0.47)
		Trunk fatness	NS (p=0.77)
		BMI	NS (p=0.37)
Karlsen et al. 2017 General population (n=444 children)	0.19 ng/mL (maternal geometric mean serum PFHxS)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.12 (0.97–1.30)
		Risk of being overweight 5 years	RR 1.11 (0.77–1.59)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.34 ng/mL (child geometric mean serum PFHxS)	BMI score	NS (p>0.05)
		Risk of being overweight	RR 0.73 (0.44–1.23)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Risk of being overweight	OR 1.04 (0.97–1.11)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	3.6 ng/mL (median serum PFHxS)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.49, trend) NS (p=0.17 trend) **Association (p=0.009, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.04, trend)*** **Association (p=0.02, trend)***
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Body weight at 20 months	NS (p=0.4375 for trend)
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFHxS)	Weight gain until 6 months of age	β -0.06 (-0.15–0.02)
		BMI at 4 years of age	β -0.02 (-0.10–0.07)
		BMI at 7 years of age	β -0.04 (-0.14–0.06)
		Waist circumference at 4 years of age	B -0.04 (0.14–0.15)
		Waist circumference at 7 years of age	NS β -0.04 (-0.12–0.04)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.4 ng/mL (maternal median plasma PFHxS in early childhood group)	BMI	β 0.01 (-0.05–0.06)
		Waist circumference	β 0.03 (-0.10–0.16)
		Subscapular and triceps skinfold thickness	**β 0.16 (0.01–0.31)*, boys and girls** β 0.15 (-0.09–0.38), boys only β 0.18 (-0.03–0.38, girls only
		Risk of being overweight	RRR 1.03 (0.94–1.13)
		Risk of being obese	RRR 1.02 (0.89–1.17)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	2.3 ng/mL (maternal median plasma PFHxS in late childhood group)	BMI	β 0.04 (-0.08–0.17)
		Total fat mass index	β 0.11 (-0.03–0.25)
		Waist circumference	β 0.11 (-0.22–0.43)
		Subscapular to triceps skinfold thickness ratio	β 0.02 (-0.02–0.06), boys and girls β -0.50 (-1.70–0.71), boys only **β 1.61 (0.58–2.65)*, girls only**
		Risk of being overweight	RRR 1.04 (0.92–1.17)
		Risk of being obese	RRR 1.07 (0.94–1.22)
PFNA			
Braun et al. 2016a, 2016b General population (n=204 children)	0.9 ng/mL (maternal median PFNA)	Changes in BMI scores between 2 and 8 years of age	NS (p>0.23)
		Overweight/obesity risk	RR 1.26 (0.64–2.48), 3rd tertile
Cao et al. 2018 General population (n=337 infants)	0.13 ng/mL (mean cord serum PFNA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	NS (p=0.15)
		Head circumference at 19 months	NS (p=0.62)
Halldorsson et al. 2012 General population (n=665 20-year-olds)	0.3 ng/mL (maternal median PFNA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)
Hartman et al. 2017 General population (n=359 girls aged 9 years)	0.5 ng/mL (maternal median PFNA)	Total body fatness	NS (p=0.26)
		Trunk fatness	NS (p=0.97)
		BMI	NS (p=0.68)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children)	0.67 ng/mL (maternal geometric mean serum PFNA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.02 (0.79–1.31)
		Risk of being overweight 5 years	RR 1.15 (0.67–1.98)
Karlsen et al. 2017 General population (n=444 children aged 5 years)	1.12 ng/mL (child geometric mean serum PFNA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	RR 0.67 (0.45–1.00)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Risk of being overweight	OR 1.01 (0.92–1.13)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	1.5 ng/mL (median serum PFNA)	Weight loss	NS (p=0.28, trend).
		Weight regain Males Females	**Association (p=0.01, trend)*** NS (p=0.48 trend) **Association (p=0.006, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p<0.001, trend)*** **Association (p=0.03, trend)***
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean serum PFNA)	Weight gain until 6 months of age	β 0.0 (-0.07–0.09)
		BMI at 4 years of age	β 0.05 (-0.03–0.13)
		BMI at 7 years of age	β 0.06 (-0.04–0.16)
		Waist circumference at 4 years of age	β 0.02 (-0.07–0.10)
		Waist circumference at 7 years of age	β -0.02 (-0.07–0.10)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in early childhood group)	BMI	β 0.02 (-0.07–0.12)
		Waist circumference	β 0.01 (-0.23–0.22),
		Risk of being overweight	RRR 1.12 (0.96–1.30)
		Risk of being obese	RRR 0.97 (0.75–1.27)
Mora et al. 2017 General population (n=1,006 at median age of 3.2 years and n=876 at median age of 7.7 years)	0.6 ng/mL (maternal median plasma PFNA in late childhood group)	BMI	β 0.17 (-0.03–0.36)
		Total fat mass index	β 0.08 (-0.07–0.23)
		Waist circumference	β 0.31 (-0.19–0.82)
		Subscapular and triceps skinfold thickness	**β 0.62 (0.01–1.22)*, boys and girls** β 0.13 (0.74–1.01), boys only **β 1.01 (0.16–1.86)*, girls only**
		Subscapular to triceps skinfold thickness ratio	**β 1.78 (0.57–2.98)*, boys and girls** β 1.23 (-0.58–3.03), boys only **β 2.17 (0.52–3.83)*, girls only**
		Risk of being overweight	RRR 1.06 (0.85–1.32)
		Risk of being obese	RRR 1.21 (0.99–1.47)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Growth during childhood	NS (p>0.05)
PFDA			
Cao et al. 2018 General population (n=337 infants)	0.12 ng/mL (mean cord serum PFDA)	Body weight at 19 months	NS (p=0.57)
		Length at 19 months	NS (p=0.18)
		Head circumference at 19 months	NS (p=0.94)
Karlsen et al. 2017 General population (n=444 children)	0.26 ng/mL (maternal geometric mean serum PFDA)	BMI score, 18 months	NS (p>0.05)
		BMI score, 5 years	NS (p>0.05)
		Risk of being overweight 18 months	RR 1.14 (0.91–1.43)
		Risk of being overweight 5 years	RR 1.02 (0.61–1.70)

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karlsen et al. 2017 General population (n=444 children aged 5 years)	0.33 ng/mL (child geometric mean serum PFDA)	BMI score	**Inverse association (p<0.05)***
		Risk of being overweight	**RR 0.64 (0.46–0.90)***
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Risk of being overweight	OR 0.98 (0.93–1.03)
Liu et al. 2018a General population (n=621 adults in weight loss clinical trial)	0.37 ng/mL (median serum PFDA)	Weight loss	NS (p=0.45, trend).
		Weight regain Males Females	NS (p=0.16, trend) NS (p=0.75 trend) **Association (p=0.03, trend)***
		Resting metabolic rate Weight loss period Weight regain period	 **Association (p=0.01, trend)*** **Association (p=0.05, trend)***
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFUnA			
Cao et al. 2018 General population (n=337 infants)	0.06–0.11 ng/mL (2nd quartile cord serum PFUnA)	Body weight at 19 months	NS (p=0.88)
		Length at 19 months	**β 1.19 (-0.68–3.07, p<0.05)*, 2nd quartile**
		Head circumference at 19 months	NS (p=0.60)
Koshy et al. 2017 General population (World Trade Center Health Registry, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCH group and comparison group)	Risk of being overweight	**OR 0.95 (0.91–0.99)***

Table 2-7. Body Weight Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
PFDoDA			
Cao et al. 2018 General population (n=337 infants)	0.04 ng/mL (mean cord serum PFDoDA)	Body weight at 19 months	NS (p=0.74)
		Head circumference at 19 months	NS (p=0.97)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Growth during childhood Weight Height	 **Inverse association (p<0.05)*, girls** **Inverse association (p<0.05)*, girls**
FOSA			
Halldorsson et al. 2012 General population (n=665 20-year-olds)	1.1 ng/mL (maternal median FOSA)	BMI	NS (p>0.56)
		Waist circumference	NS (p>0.56)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 1 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; WTCHR = World Trade Center Health Registry

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PFOA

Epidemiological Studies. Mixed results were found in studies of monitoring infant growth from 1 to 12 months of age. Andersen et al. (2010) found an inverse association between maternal serum PFOA and body weight and BMI in male infants at 5 and 12 months of age; no associations were found in girls. Other studies of infants less than 19 months of age did not find associations between maternal serum PFOA (Alkhalawi et al. 2016; Manzano-Salgado et al. 2017b) or cord blood PFOA (Cao et al. 2018; de Cock et al. 2014) levels and weight, length, head circumference, or BMI. One study of children (Braun et al. 2016a) found an association between changes in BMI scores between ages 2 and 8 years and maternal PFOA levels; however, there was no increase in the risk of being overweight or obese. Another study of young children (median age 3.2 years) found an association between maternal PFOA and waist circumference (Mora et al. 2017); when the children were segregated by sex, the association was only found in boys. This study did not find associations between maternal PFOA and waist circumference when the children were older (median age 7.7 years). Other studies in children (2–11 years of age) found no associations between maternal PFOA or cord blood PFOA and growth during childhood (Wang et al. 2016), risk of being overweight or obese (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Mora et al. 2017), waist circumference (Manzano-Salgado et al. 2017b), BMI (Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or risk of having a waist-to-height ratio of >0.5 (Høyer et al. 2015b). In a study of children aged 8–10 years, no associations were found between plasma PFOA levels and markers of adiposity (BMI, skinfold thickness, waist circumference, adiponectin levels, and leptin levels) (Timmermann et al. 2014). Similarly, in a study of children in the World Trade Center Health Registry, no association was found between serum PFOA and risk of being overweight (Koshy et al. 2017). In contrast, a study of 5-year-old children found an inverse association between the child's serum PFOA levels and BMI score, but no association with the risk of being overweight (Karlsen et al. 2017). Overall, the available epidemiological data do not suggest a connection between serum PFOA levels and body weight or risk of being overweight/obese in children.

Two studies in adults have not found associations between PFOA and body weight gain. A general population study of 20-year-old females found associations between maternal PFOA levels and BMI and waist circumferences, and increases in the risk of being overweight and having a high waist circumference (Halldorsson et al. 2012); these associations were not observed in males. No increases in the risk of being overweight or obese were observed in male or female C8 participants (20–40 years of age) when estimated early life PFOA exposure was used as the exposure metric (Barry et al. 2014). In a

study of participants in a weight loss study, no association between weight loss and PFOA levels was found; however, PFOA was associated with weight gain in females, but not males (Liu et al. 2018a). PFOA was also associated with a lower increase in resting metabolic rate in all participants during the weight regain period of the study.

Laboratory Animal Studies. Male rats that survived a 4-hour inhalation exposure to 380 mg/m^3 APFO dusts lost weight for 1–2 days after exposure, but resumed normal weight gain thereafter (Kennedy et al. 1986). Male rats exposed via inhalation intermittently to 84 mg/m^3 APFO dusts for 2 weeks lost approximately 7% of their body weight by day 5 of exposure (250 g at start of study, 237 g on day 5) (Kennedy et al. 1986), but recovered by day 16 after exposure ceased. Nose-only exposure of male CD rats to 590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours resulted in 18 and 36% reductions in body weight 5 and 12 days after exposure, respectively (Kinney et al. 1989). Inhalation exposure to 67 mg/m^3 had no significant effect on body weight. In a developmental study, inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts during GDs 6–15 induced a 37% reduction in maternal body weight gain relative to controls during the exposure period (Staples et al. 1984); in a pair-fed group, the reduction of weight gain during the same period was 61% relative to *ad libitum* controls.

Reductions in body weight or body weight gain are typical, although not particularly sensitive, responses of rodents to oral exposure to perfluoroalkyls. In many cases, this effect is not associated with reduced food intake, and in some cases, exposed animals have shown an increase in relative food consumption (grams of food/grams of body weight) relative to controls. For example, administration of 50 mg/kg/day APFO for 7 days resulted in 17% weight loss; a similar decrease was observed in a pair-fed group (Pastoor et al. 1987). In mice, doses of approximately 25–30 mg/kg/day PFOA in the food for 7 days reduced terminal body weight by >10% relative to controls without a significant reduction in food intake (Xie et al. 2003; Yang et al. 2000, 2002a, 2002b). However, administration of the same dose to PPARα-null mice did not cause a reduction in weight gain, suggesting that the effect on body weight is a specific effect of peroxisome proliferators possibly due to increased fat utilization (Yang et al. 2002b). In general, body weight recovered once treatment ceased.

Intermediate-duration oral studies in rats have also reported reduced body weight gain with doses ≥10 mg/kg/day APFO (Butenhoff et al. 2004b; Griffith and Long 1980). In the former study, mean absolute food consumption was decreased, but mean relative food consumption was increased. In a 2-year bioassay, body weight gain in rats dosed with 15 mg/kg/day PFOA was reduced >10% relative to controls at the 1-year mark and at termination (Biegel et al. 2001). Similar observations have been made

in mice dosed with approximately ≥18 mg/kg/day APFO for 28 days (Griffith and Long 1980), or 10 mg/kg 5 days/week for 4 weeks (Yang et al. 2009), and in pregnant mice dosed with ≥10 mg/kg/day APFO during GDs 1–17 (Lau et al. 2006). A study comparing wild-type mice and PPARα knockout mice (DeWitt et al. 2016) found a decrease in body weight gain in the wild-type mice, but not in the knockout mice. A 90-day and a 26-week study in monkeys also reported significant reductions in body weight gain or weight loss associated with decreased food consumption at dose levels in the range of 20–30 mg/kg/day APFO (Butenhoff et al. 2002; Griffith and Long 1980), but a 4-week study in monkeys dosed with 20 mg/kg/day PFOA did not (Thomford 2001).

Transient weight loss was reported in rats applied 3,000 mg/kg APFO to the shaven skin for 24 hours (Kennedy 1985). In the 2-week study, rats in the 200 and 2,000 mg/kg/day groups lost weight during the treatment period (14 and 24%, respectively, on test day 10), but body weights were comparable to controls after 42 days of recovery. No changes in body weight were reported in mice applied up to 50 mg/kg/day PFOA daily for 4 days on the dorsal surface of the ears (Fairley et al. 2007).

PFOS

Epidemiological Studies. General population studies have evaluated body weight, height, and BMI in infants, children, and adults to assess whether there were associations between growth and maternal serum PFOS levels. Andersen et al. (2010) found that maternal PFOS levels were inversely related to body weight and BMI in 12-month-old male infants; no associations were found in females at 12 months of age or in males and females at 5 months of age. The magnitude of the effect on body weight in the boys was small, 9 g per 1 ng/mL increase in maternal serum PFOS level. Other studies have not found associations between maternal PFOS or cord blood PFOS and body weight, length, or head circumference in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017b). Hartman et al. (2017) also found an inverse association between maternal serum PFOS and trunk body fatness in 9-year-old girls, but no associations with total body fatness or BMI. Karlsen et al. (2017) found associations between maternal PFOS levels and BMI and risk of being overweight at 18 months of age, but not at 5 years of age. Maisonet et al. (2012) found that at 20 months of age, girls whose mothers had serum PFOS levels in the 3rd tertile weighed 438 g more than those in the first tertile. Studies in children (Andersen et al. 2013; Braun et al. 2016a; Høyer et al. 2015b; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017) or young adults (Halldorsson et al. 2012) did not find associations between maternal PFOS levels and BMI, waist circumference, and/or risk of being overweight. No associations between plasma PFOS and markers of adiposity (BMI, skinfold thickness, waist circumference,

adiponectin levels, and leptin levels) were found in a study of children aged 8–10 years (Timmermann et al. 2014). Similarly, a study of 5-year-old children found no association between child serum PFOS levels and BMI score or risk of being overweight (Karlsen et al. 2017). Overall, the epidemiological studies do not suggest a connection between serum PFOS and body weight or the risk of being overweight/obese.

In a study of weight loss programs, PFOS did not influence weight loss, but was associated with greater weight regain in women (Liu et al. 2018a). PFOS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period of the study and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Dietary treatment of rats with 15 mg/kg/day PFOS (only dose level tested) for 7 days did not significantly alter body weight (Haughom and Spydevold 1992). Oral treatment of pregnant rats with 25 mg/kg/day PFOS on GDs 2–5 or 6–9 resulted in maternal weight loss during treatment, whereas treatment on GDs 10–13, 14–17, or 17–20 resulted in significant reductions in maternal weight gain (Grasty et al. 2003). In pregnant mice, oral dosing with up to 6 mg/kg/day PFOS on GDs 6–18 or 12–18 did not significantly affect body weight (Fuentes et al. 2006, 2007b). Decreases in maternal body weight were observed in rats administered 20 mg/kg/day on GDs 12–18 (Li et al. 2016). Pregnant rabbits appeared to be more sensitive as oral doses of 1 mg/kg/day on GDs 6–20 caused a 21% reduction in weight gain during treatment without altering food consumption (Case et al. 2001).

Alterations in body weight have also been observed following intermediate- or chronic-duration exposure. Reductions in body weight gain of >10% have been reported in intermediate-duration studies in rats dosed with ≥2 mg/kg/day PFOS associated with reductions in mean absolute and relative food consumption (Luebker et al. 2005a, 2005b). In a developmental toxicity study, treatment of pregnant rats with ≥2 mg/kg/day PFOS on GDs 2–20 resulted in significant reductions in body weight gain, which were associated with significant reductions in mean absolute food and water consumption (Thibodeaux et al. 2003). In a 4-week study, treatment of Cynomolgus monkeys with up to 2 mg/kg/day, administered via a capsule, did not affect body weight gain (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, produced a 13.5% reduction in final body weight, at which time the mean concentration of PFOS in serum was 172 µg/mL (Seacat et al. 2002). In a 2-year dietary study in rats, final mean body weight of females that received doses of approximately 1.04 mg/kg/day PFOS was 14% lower than controls; this could have been due, in part, to a tendency of decreased food consumption during weeks 28 through 104 of the study (Butenhoff et al. 2012b;

Thomford 2002b). No significant effect (<10% difference with controls) was seen in females dosed with ≤0.25 mg/kg/day PFOS.

PFHxS

Epidemiological Studies. Nine studies have evaluated the influence of *in utero* PFHxS exposure on childhood growth and found no associations between maternal PFHxS levels and body weight in infants <2 years of age (Alkhalawi et al. 2016; Cao et al. 2018; Maisonet et al. 2012; Manzano-Salgado et al. 2017b), body fatness or BMI at 9 years of age (Hartman et al. 2017), BMI or waist circumference at 3 or 7 years of age (Mora et al. 2017), changes in BMI scores between 2 and 8 years of age (Braun et al. 2016a), BMI at 18 months or 5 years of age (Karlsen et al. 2017), BMI at 4 or 7 years of age (Manzano-Salgado et al. 2017b), or the risk of childhood overweight/obesity (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). Similarly, no associations were found between serum PFHxS levels in 5-year-old children and their BMI score or risk of being overweight (Karlsen et al. 2017) or between serum PFHxS and risk of being overweight in children in the World Trade Center Healthy Registry (Koshy et al. 2017). Alkhalawi et al. (2016) found no associations between maternal PFHxS levels and infant body weight or length at 1, 4, 6, or 12 months of age; however, longitudinal analysis of growth during this period showed an inverse association for body weight and an association for length.

In a clinical trial of weight loss programs, PFHxS was not associated with weight loss during the first 6 months of the study, but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFHxS was also associated with greater declines in resting metabolic rate in all participants during the weight loss period and lower increases in resting metabolic rate during the weight regain period.

Laboratory Animal Studies. Administration of PFHxS by gavage for 40–60 days did not significantly affect body weight in rats at ≤10 mg/kg/day PFHxS (Butenhoff et al. 2009a) or mice at ≤3 mg/kg/day (Chang et al. 2018); the mean terminal body weights were within 10% of the body weight of the control group (Butenhoff et al. 2009a).

PFNA

Epidemiological Studies. Several studies have examined the influence of maternal serum PFNA levels on childhood growth. These studies did not find associations between maternal PFNA levels and growth

during childhood (Cao et al. 2018; Wang et al. 2016), BMI (Braun et al. 2016a; Halldorsson et al. 2012; Hartman et al. 2017; Karlsen et al. 2017; Manzano-Salgado et al. 2017b; Mora et al. 2017), body fatness (Hartman et al. 2017), or overweight/obesity risk (Braun et al. 2016a; Karlsen et al. 2017; Mora et al. 2017). However, when the child's serum PFNA levels at age 5 years were used as the exposure biometric, an inverse association was found for BMI, but not for the risk of being overweight (Karlsen et al. 2017). Koshy et al. (2017) found no associations between serum PFNA and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

PFNA was associated with greater weight regains in a study of participants in a 2-year weight loss clinical trial, but was not associated with weight loss during the first 6 months of the study (Liu et al. 2018b). PFNA also affected resting metabolic rate in all participants; it was associated with a greater decline during the weight loss period of the study and a lower increase during the weight regain period.

Laboratory Animal Studies. Decreases in body weight gain have been observed in rats administered ≥3 mg/kg/day for 14 days (Fang et al. 2009, 2010; Hadrup et al. 2016) and in mice administered 5 mg/kg/day for 14 days (Wang et al. 2015a). The NOAEL for body weight effects was 1 mg/kg/day for both species. In intermediate-duration developmental toxicity studies, decreases in body weight were observed at 5 mg/kg/day in rats (Rogers et al. 2014) and weight loss was observed in mice at 10 mg/kg/day (Das et al. 2015). No alterations in maternal weight gain were observed in mice at 2.0 mg/kg/day (Wolf et al. 2010).

PFDA

Epidemiological Studies. Four studies examined the effect of PFDA levels on childhood growth. Cao et al. (2018) did not find associations between cord blood PFDA and body weight, length, or head circumference in 19-month-old infants. Wang et al. (2016) reported decreases in weight and height in girls associated with increasing maternal serum PFDA levels. Inverse associations between serum PFDA levels in 5-year-old children and BMI and the risk of being overweight were reported by Karlsen et al. (2017). When using maternal serum PFDA levels (measured 2 weeks after childbirth) as the biomarker of exposure, no associations were found with BMI or the risk of being overweight in children aged 18 months or 5 years (Karlsen et al. 2017). In a study of children in the World Trade Center Health Registry, no association between serum PFDA and risk of being overweight was found (Koshy et al. 2017).

In a study of adult participants in a 2-year weight loss clinical trial, PFDA was not associated with weight loss but was associated with weight regain in females during the last 18 months of the study (Liu et al. 2018a). PFDA also was associated with greater declines in resting metabolic rate during the weight loss period and lower increases in resting metabolic rate during the weight regain period of the study.

Laboratory Animal Studies. Ten days following administration of a single gavage dose of 50 mg/kg, weight loss was observed in rats (Kawabata et al. 2017). In a 1-week study, exposure to 9.5 mg/kg/day PFDA in the diet resulted in a 32% weight loss in rats (Kawashima et al. 1995); the NOAEL was 4.7 mg/kg/day. Rats administered 1 mg/kg/day PFDA for 28 days exhibited a 21% decrease in body weight gain (Frawley et al. 2018).

Body weight of female C57BL/6N mice administered a single gavage dose of 80 mg/kg PFDA was reduced 12% relative to controls 30 days post dosing (Harris et al. 1989); no significant effect was seen at 40 mg/kg PFDA. In a developmental study, pregnant mice dosed with 6.4 mg/kg/day PFDA on GDs 6–15 gained 92% less weight (adjusted for the weight of the gravid uterus) on GDs 6–18 than controls; mice dosed with 12.8 mg/kg/day lost weight (Harris and Birnbaum 1989). Weight loss was also observed in C57BL/6N mice exposed to 78 mg/kg/day PFDA in the diet for 10 days (Permadi et al. 1992, 1993).

PFUnA

Epidemiological Studies. Cao et al. (2018) found an association between cord blood PFUnA levels and length at 19 months of age, but found no associations for body weight or head circumference. Wang et al. (2016) found an inverse association between maternal serum PFUnA levels and weight and height in girls. Koshy et al. (2017) also found an inverse association between the serum PFUnA levels and the risk of being overweight in children enrolled in the World Trade Center Health Registry.

Laboratory Animal Studies. Decreases in body weight gain (10% in males and 23% in females) were observed in rats exposed to 1.0 mg/kg/day in a 41–46-day developmental toxicity study (Takahashi et al. 2014).

PFBS

Laboratory Animal Studies. No significant alterations in body weight gain were observed in Sprague-Dawley rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001) or in Sprague-

Dawley rats administered ≤1,000 mg/kg/day PFBS via gavage for at least 70 days (Lieder et al. 2009b). Two studies did report decreases in maternal body weight gain in rats administered 1,000 or 2,000 mg/kg/day (York 2002, 2003a).

PFBA

Laboratory Animal Studies. Alterations in body weight do not appear to be a sensitive outcome of PFBA exposure in rats or mice. No alterations in body weight gain were observed in Sprague-Dawley rats administered 184 mg/kg/day PFBA via gavage for 5 days (3M 2007a), C57BL/6 mice exposed to 78 mg/kg/day PFBA in the diet for 10 days (Permadi et al. 1992, 1993), Sprague-Dawley rats administered 150 mg/kg/day PFBA via gavage for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or Sprague-Dawley rats administered 30 mg/kg/day PFBA via gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies. Cao et al. (2018) found no association between cord blood PFDoDA and body weight or head circumference at 19 months of age. In contrast, Wang et al. (2016) found an inverse association between maternal serum PFDoDA levels and growth (weight and height) in girls.

Laboratory Animal Studies. Dosing of Sprague-Dawley rats with 5 mg/kg/day PFDoDA by gavage for 14 days resulted in a 25% reduction in final body weight relative to a control group or 7% loss of body weight compared with the starting body weight (Shi et al. 2007). Decreases in body weight gain (measured 10 days postexposure) were also observed in rats administered a single gavage dose of 50 mg/kg PFDoDA (Kawabata et al. 2017). Gavage administration of 2.5 mg/kg/day for 42 days resulted in approximately 30% decreases in male rats; the decreases in body weight gain persisted during a 14-day recovery period (Kato et al. 2015). An approximately 20% decrease in body weight gain was also observed in pregnant and nonpregnant females similarly exposed to 2.5 mg/kg/day (Kato et al. 2015). The decreases in body weight gain were accompanied by decreases in food intake in males and females. In a longer duration study (110 days), no alterations in body weight gain were observed in rats administered 0.5 mg/kg/day (Shi et al. 2009a).

PFHxA

Laboratory Animal Studies. Gavage administration of up to 315 mg/kg/day did not result in alterations in body weight gain in rats exposed for 32–44 days (Kirkpatrick 2005), 90 days (Chengelis et al. 2009b), 92–93 days (Loveless et al. 2009), or 2 years (Klaunig et al. 2015). A 19% decrease in body weight gain was observed in rats administered 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) and a 19% decrease in maternal body weight gain was observed in rats administered 500 mg/kg/day on GDs 1–20 (Loveless et al. 2009). In contrast to these findings, a 110–126-day study found a 12% decrease in male rats administered 100 mg/kg/day NaPFHx (Loveless et al. 2009).

FOSA

Epidemiological Studies. Halldorsson et al. (2012) did not find associations between maternal serum FOSA levels and BMI or waist circumference in 20-year-olds.

Laboratory Animal Studies. No alterations in body weight were observed in Sprague-Dawley rats following a single gavage dose of 5 mg/kg FOSA in 2% Tween 80 vehicle (Seacat and Luebker 2000).

2.4 RESPIRATORY

Overview. A small number of epidemiological studies have examined the potential of PFOA to damage the respiratory tract; detailed descriptions of these studies are presented in Table 2 in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls.* Epidemiological studies examining respiratory endpoints were not identified for the other perfluoroalkyls. These studies were primarily conducted in PFOA workers or in residents of nearby communities. The possible associations between perfluoroalkyl exposure and asthma are discussed along with other immune effects in Section 2.14. Studies in laboratory animals have examined the potential for perfluoroalkyls to induce histological lesions in the lungs following inhalation (see Tables 2-1 and 2-2) or oral exposure (see Tables 2-3, 2-4, and 2-5). No laboratory animal studies examining potential respiratory tract effects were identified for PFUnA, PFHpA, PFDoDA, or FOSA.

Epidemiological studies examining respiratory effects are only available for PFOA. No alterations in lung function were observed in workers at a PFOA facility but increases in respiratory illnesses were observed in residents living near the PFOA facility.

Inhalation exposure to PFOA, PFOS, or PFNA dusts have resulted in nasal discharge, rales, and/or labored breathing in laboratory animals. Oral exposure studies in laboratory animals have not found consistent evidence of histological alterations for PFOA, PFOS, PFHxS, PFBS, or PFBA. An oral study with PFHxA reported nasal lesions in rats, however, a second study did not find these effects at higher doses.

PFOA

Epidemiological Studies. There are limited data on the potential of PFOA to damage the respiratory tract. Pulmonary function tests and chest roentgenograms conducted on workers potentially exposed to PFOA at the Washington Works fluoropolymers production facility were within normal limits (Sakr et al. 2007b); the serum PFOA levels ranged from 5 to 9,550 ng/mL. Another study of workers at this facility did not find an association between estimated cumulative serum PFOA levels and the risk of chronic obstructive pulmonary disease (Steenland et al. 2015). In contrast, a study of residents living near this facility found an increase in the risk of chronic bronchitis (standard prevalence ratio [SPR] of 3.60, 95% confidence interval [CI] 2.92–4.44) and shortness of breath (SPR 2.05, 95% CI 1.70–2.46) (Anderson-Mahoney et al. 2008); it is noted that results were based on health surveys, and some of the subjects also worked at the facility. Summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 2.

Laboratory Animal Studies. Inhalation exposure of male and female rats to 18,600 mg/m^3 APFO dusts for 1 hour induced a red nasal discharge and dry rales (Griffith and Long 1980). Necropsy conducted 14 days after exposure showed bilateral mottling of the lungs in 8 out of 10 rats. Head-only exposure for 4 hours to 380 mg/m^3 APFO dusts, a concentration that was lethal to some rats, produced pulmonary edema, which disappeared within 1 week of exposure (Kennedy et al. 1986). Examination of the lungs and trachea from rats exposed head-only to up to 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks showed no significant gross or microscopic alterations (Kennedy et al. 1986). Male CD rats exposed nose-only to ≥590 mg/m^3 ammonium perfluorononanoate dusts for 4 hours exhibited lung noise and labored breathing during exposure and throughout a 12-day recovery period (Kinney et al. 1989).

Oral dosing of male and female CD rats with ≤110 mg/kg/day APFO did not induce gross or microscopic changes in the lungs (Griffith and Long 1980; Perkins et al. 2004). Dosing for 2 years with 15 mg/kg/day APFO increased the incidence of lung hemorrhage in males (3M 1983; Butenhoff et al. 2012c). The

incidences were 10/50, 14/50, and 22/50 for groups receiving doses of 0, 1.5, and 15 mg/kg/day, respectively. Pair-wise comparison between controls and high-dose groups revealed a statistically significant difference ($p<0.05$). The investigators suggested that pulmonary lesions were not related to PFOA based on lower incidence of interstitial pneumonia in the 15 mg/kg/day males. In a study in monkeys administered up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks, no signs of respiratory problems were observed during the study and no gross or microscopic alterations in the lungs and trachea were observed at termination (Butenhoff et al. 2002).

No gross or microscopic alterations were found in the lung and trachea from male CD rats following application of up to 2,000 mg/kg/day APFO as an aqueous paste to an area of the shaven back (approximately 15% of the total body surface) 6 hours/day, 5 days/week for 2 weeks (Kennedy 1985).

PFOS

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to concentrations of PFOS dust between 1,890 and 45,970 mg/m^3 for 1 hour induced dry rales and other breathing disturbances.

Dosing of Cynomolgus monkeys with up to 2 mg/kg/day PFOS, administered in a capsule, for 4 weeks had no effect on the gross or microscopic morphology of the lungs (Thomford 2002a). Administration of doses of up to 0.75 mg/kg/day of PFOS (potassium salt) administered via a capsule to Cynomolgus monkeys for 26 weeks did not produce any gross or microscopic alterations in the lungs or the trachea (Seacat et al. 2002). Dosing rats with up to 1.04 mg PFOS/kg/day in the diet for 104 weeks did not induce significant gross or microscopic alterations in the lungs or trachea (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Examination of the respiratory tract of rats administered ≤10 mg/kg/day PFHxS or mice administered ≤3 mg/kg/day by gavage in a reproductive study (40–60 days of dosing) showed no treatment-related effects (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Laboratory Animal Studies. Labored breathing during and after a 4-hour nose-only exposure to 590 mg/m^3 PFNA dust was reported in rats (Kinney et al. 1989).

PFDA

Laboratory Animal Studies. No histological alterations were observed in the respiratory tract of rats administered 0.5 mg/kg/day for 28 days or mice administered 5 mg/kg once a week for 4 weeks (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Administration of PFBS at gavage doses of ≤900 mg/kg/day for 28 days (3M 2001) or 600 mg/kg/day for 90 days (Lieder et al. 2009a) had no significant effect on the gross or microscopic morphology of the lungs or trachea in rats; no increases in nasal lesions were observed in the 90-day study (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses ≤184 mg/kg/day for 5 days (3M 2007a), ≤150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or ≤30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b) did not cause morphological alterations in the respiratory tract.

PFHxA

Laboratory Animal Studies. Degeneration/atrophy of the nasal olfactory epithelium was observed in rats administered via gavage 100 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009); at 500 mg/kg/day, respiratory metaplasia was observed in the nasal cavity. A second study did not report histological alterations in the nasal cavity of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.5 CARDIOVASCULAR

Overview. Epidemiological and laboratory animal studies have evaluated the toxicity of perfluoroalkyls to the cardiovascular system. The epidemiological studies evaluated several cardiovascular outcomes including ischemic heart disease, cerebrovascular disease, stroke, cardiovascular disease, myocardial infarction, hypertension, and pregnancy-induced hypertension. The results of these studies are summarized in Table 2-8, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 3. The available occupational, community, and general population studies have not consistently found increases in the risk of heart disease or stroke that were associated with serum PFOA levels. Considerably less epidemiological data are available for other perfluoroalkyls; general population studies for PFOS, PFHxS, PFNA, PFDA, PFHpA, and PFDoDA have not consistently found increases in the risk of cardiovascular disease, although single studies for PFUnA, PFBS, PFHxA, and FOSA have found associations. Most of the available epidemiological studies did not find an association between serum PFOA and hypertension. A small number of studies (three or less for each compound) have examined potential associations with hypertension for other perfluoroalkyls. These studies found associations (PFBA), no associations (PFHxS, PFDA, PFUnA, PFHpA, PFBS, PFDoDA), or mixed results (PFOS, PFNA).

Several studies have evaluated the possible associations between serum perfluoroalkyls and pregnancy-induced hypertension and pre-eclampsia. Pregnancy-induced hypertension, also referred to as gestational hypertension, is the onset of hypertension after the 20th week of pregnancy. Pre-eclampsia is pregnancy-induced hypertension accompanied by signs of damage to another organ system, such as the kidney or liver; elevated levels of protein in the urine are often present. While the two diseases are distinct, they can be inaccurately reported in studies that relied on self-reporting or use of birth certificates (birth certificates often only have an option for pregnancy-induced hypertension; thus, pre-eclampsia may be reported as pregnancy-induced hypertension). Due to possibility of misreporting, ATSDR has opted to group these two outcomes together. Although mixed results were found in studies of highly exposed community residents, the strongest methodological study (Darrow et al. 2013) found an increased risk of pregnancy-induced hypertension that was associated with serum PFOA levels. Increases in the risk of pregnancy-induced hypertension associated with serum PFOS levels were also found in two community studies. General population studies have not found associations between serum PFHxS or PFDA and pre-eclampsia; one study on PFUnA found an inverse association.

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	5–9,550 ng/mL (PFOA range)	Heart disease deaths	SMR 110 (98–123)
		Cerebrovascular disease deaths	SMR 86 (60–120)
		Ischemic heart disease deaths	SMR 109 (96–124)
Lundin et al. 2009 Occupational (n=3,993)	2,600–5,200 ng/mL (range of definite exposure group)	Heart disease deaths	SMR 0.7 (0.5–1.3)
		Cerebrovascular disease deaths	SMR 1.6 (0.5–3.7)
		Ischemic heart disease deaths	SMR 0.8 (0.5–1.4)
		Cerebrovascular disease risk	**HR 4.6 (1.3–17.0)* workers with definite exposure of ≥6 months HR 2.1 (1.0–4.6)* workers with definite exposure ≥5 years**
Raleigh et al. 2014 Occupational (n=9,027)	Cumulative PFOA exposure	Ischemic heart disease deaths	**SMR 0.84 (0.74–0.95)***
		Cerebrovascular disease	SMR 0.81 (0.61–1.05)
		Ischemic heart disease risk	HR 0.89 (0.66–1.21), 4th quartile
		Cerebrovascular disease risk	HR 0.98 (0.53–1.81), 4th quartile
Sakr et al. 2009 Occupational (n=4,747)	NR	Ischemic heart disease risk	NS (p=0.16 for trend)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA exposure	Coronary artery disease risk	NS (p=0.78 for trend), no lag NS (p=0.75 for trend), 10-year lag
		Hypertension	NS (p=0.95 for trend), no lag NS (p=0.54 for trend), 10-year lag
		Stroke	NS (p=0.35 for trend), no lag NS (p=0.64 for trend), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-years (mean estimated cumulative PFOA exposure)	Ischemic heart disease deaths	SMR 0.93 (0.72–1.19), no lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Cardiovascular disease (self-reported)	**SPR 4.29 (3.47–5.29)***
		Angina (self-reported)	**SPR 8.07 (6.54–9.95)***
		Myocardial infarction	**SPR (1.91 (1.40–2.62)***
		Stroke	**SPR 2.17 (1.47–3.21)***
		Hypertension	SPR 1.18 (0.97–1.43)
Darrow et al. 2013 Community (C8) (n=1,330)	6.9–<11.1 ng/mL (2nd PFOA quintile)	Pregnancy-induced hypertension	**OR 2.39 (1.05–5.46)* (2nd quintile)**
Nolan et al. 2009 Community (C8) (n=1,555 women)	NR	Pregnancy-induced hypertension LHWA residents Partial LHWA residents	 OR 1.2 (0.7–2.0), unadjusted OR 0.8 (0.5–1.4), unadjusted
Savitz et al. 2012a Community (C8) (n=11,737 pregnant women)	6.8–<16.6 ng/mL (2nd PFOA quartile, estimated)	Pre-eclampsia	**OR 1.2 (1.0–1.5)***
Savitz et al. 2012b Community (C8) (n=224 cases of pregnancy-induced hypertension)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.0 (0.7–1.3), 5th quintile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Savitz et al. 2012b Community (C8) (n=4,547 pregnant women)	21.0–717.6 ng/mL (5th PFOA quintile, estimated)	Pregnancy induced hypertension	OR 1.1 (0.8–1.5)
Simpson et al. 2013 Community (C8) (n=28,541; 11% also had occupational exposure)	>178–319 ng/mL (cumulative, estimated 2nd PFOA quintile)	Stroke	**OR 1.39 (1.11–1.76)*, 2nd quintile**
Stein et al. 2009 Community (C8) (n=5.262 pregnant women)	120.6–894.4 ng/mL (4th PFOA quartile)	Pre-eclampsia	OR 0.9 (0.5–1.8)
Winquist and Steenland 2014a Community (C8) (n=28,541; 11% also had occupational exposure)	≥3,579 ng/mL (cumulative, estimated 5th PFOA quintile)	Hypertension	HR 0.98 (0.91–1.06), 5th quintile
		Coronary artery disease	HR 1.07 (0.93–1.23), 5th quintile
Bao et al. 2017 General population (n=1,612 adults)	6.19 ng/mL (median serum PFOA in males and females); 6.59 and 5.08 ng/mL (median serum PFOA in males and females, respectively)	Risk of hypertension	OR 1.12 (0.97–1.30)
		Systolic blood pressure	β-0.06 mm Hg (-1.70–1.59), males **β 2.91 mm Hg (0.10–5.72)*, females** **β 1.69 mm Hg (0.25–3.13)*, combined**
		Diastolic blood pressure	**β 1.48 mm Hg (0.60–2.35)*, males** β 1.34 mm Hg (-0.14–3.05), females **β 2.12 mm Hg (1.33–2.90)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>5.4 ng/mL (4th quartile PFOA)	Hypertension	OR 0.69 (0.41–1.17), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	3.17 ng/mL (median serum PFOA)	Risk of cardiovascular disease	OR 1.25 (0.91–1.70), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	Association (p=0.03)*
		Arterial pulse wave velocity	NS (p=0.39)
Lin et al. 2013a, 2013b General population (n=644)	3.49 ng/mL (median PFOA)	Carotid intima media thickness	NS (p=0.285 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.58)
		Echogenicity of intima media complex	NS (p=0.80), males NS (p=0.25), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	2.32 ng/mL (maternal geometric mean PFOA)	Blood pressure at 4 years of age	β -0.06 (-0.16–0.04)
		Blood pressure at 7 years of age	β -0.02 (-0.11–0.07)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	4.2 and 4.0 ng/mL (median PFOA in cases and controls)	Coronary artery disease	OR 0.88 (0.50–1.55), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	10.39 and 9.47 ng/mL (mean 4th quartile PFOA)	Coronary artery disease, angina, and/or heart attack	OR 1.08 (0.70–1.69, p=0.715), 4th quartile
Min et al. 2012 General population (NHANES) (n=2,208)	4.00 ng/mL (geometric mean PFOA)	Systolic blood pressure	**Association (p=0.0004)***
		Hypertension risk	**OR 1.71 (1.23–2.36)*, 4th quartile**
Shankar et al. 2012 General population (NHANES) (n=1,216)	4.0–5.6 and 4.4–6.1 ng/mL (females and males, 3rd PFOA quartile) >5.6 and >6.1 ng/mL (females and males, 4th PFOA quartile)	Cardiovascular disease	**OR 1.77 (1.04–3.02)*, 3rd quartile**
		Peripheral arterial disease	**OR 1.78 (1.03–3.08)*, 4th quartile**
		Coronary heart disease	**OR 2.24 (1.02–4.94)*, 4th quartile**
		Stroke	**OR 4.26 (1.84–9.89)*, 4th quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014b General population (n=976 pregnant women)	2.78 ng/mL (median PFOA)	Pre-eclampsia	HR 0.89 (0.65–1.22), per ln unit
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330)	12.1–<15.9 ng/mL (3rd PFOS quintile)	Pregnancy-induced hypertension	**OR 2.71 (1.33–5.52)* (3rd quintile)**
Stein et al. 2009 Community (C8) (n=5,262 pregnant women)	23.2–83.4 ng/mL (4th PFOS quartile)	Pre-eclampsia	**OR 1.6 (1.2–2.3)***
Bao et al. 2017 General population (n=1,612 adults)	24.22 ng/mL (median serum PFOS in males and females); 27.39 and 14.05 ng/mL (median serum PFOS in males and females, respectively)	Risk of hypertension	OR 1.08 (0.90–1.29), males **OR 1.63 (1.24–2.13)*, females** **OR 1.24 (1.08–1.44)*, combined**
		Systolic blood pressure	B 1.50 mm Hg (-0.17–3.18), males **β 6.65 mm Hg (4.32–8.99)*, females** **β 4.84 mm Hg (3.55–6.12)*, combined**
		Diastolic blood pressure	β 0.45 mm Hg (-0.47–4.36), males **β 2.86 mm Hg (1.51–4.20)*, females** **β 2.70 mm Hg (1.98–3.42)*, combined**
Geiger et al. 2014a General population (NHANES) (n=1,655 adolescents)	>25.5 ng/mL (4th PFOS quartile)	Hypertension	OR 0.77 (0.37–1.61), 4th quartile
Huang et al. 2018 General population (NHANES, n=10,859 adults)	12.40 ng/mL (median serum PFOS)	Risk of cardiovascular disease	OR 1.25 (0.92–1.69), 4th quartile
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.06)
		Arterial pulse wave velocity	NS (p=0.51)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lin et al. 2013a, 2013b General population (n=644)	8.65 ng/mL (median PFOS)	Carotid intima media thickness	**Association (p<0.001 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.72)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.56), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	5.80 ng/mL (maternal geometric mean PFOS)	Blood pressure at 4 years of age	β 0.00 (-0.09–0.10)
		Blood pressure at 7 years of age	β -0.05 (-0.15–0.06)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	22.8 and 22.0 ng/mL (median PFOS in cases and controls)	Coronary artery disease	OR 1.07 (0.60–1.92), 4th quartile
Melzer et al. 2010 General population (NHANES) (n=3,966 adults)	57.73 and 50.96 ng/mL (mean 4th quartile PFOS)	Coronary artery disease, angina, and/or heart attack	OR 0.91 (0.570–1.64, p=0.745), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	12.87 ng/mL (median PFOS)	Pre-eclampsia	HR 1.13 (0.84–1.52), per ln unit
PFHxS			
Bao et al. 2017 General population (n=1,612 adults)	0.71 ng/mL (median serum PFHxS in males and females)	Risk of hypertension	OR 0.99 (0.95–1.03)
		Systolic blood pressure	β 0.10 mm Hg (-0.30–0.51)
		Diastolic blood pressure	β 0.12 mm Hg (-0.11–0.35)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	1.60 ng/mL (median serum PFHxS)	Risk of cardiovascular disease	OR 0.96 (0.68–1.37), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.69)
		Arterial pulse wave velocity	NS (p=0.89)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.90)
		Echogenicity of intima media complex	NS (p=0.40), males NS (p=0.95), females
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.61 ng/mL (maternal geometric mean PFHxS)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.09)
		Blood pressure at 7 years of age	β 0.04 (-0.04–0.13)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	1.6 ng/mL (median PFHxS in cases and controls)	Coronary artery disease	OR 0.95 (0.54–1.67), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.69 ng/mL (median PFHxS)	Pre-eclampsia	HR 0.91 (0.72–1.14), per ln unit
PFNA			
Bao et al. 2017 General population (n=1,612 adults)	1.96 ng/mL (median serum PFNA in males and females); 2.19 and 1.31 ng/mL (median serum PFNA in males and females, respectively)	Risk of hypertension	OR 1.08 (0.92–1.26), males **OR 1.49 (1.16–1.92)*, females** **OR 1.19 (1.04–1.36)*, combined**
		Systolic blood pressure	B -0.12 mm Hg (-1.62–1.39), males **β 5.70 mm Hg (3.55–7.85)*, females** **β** 3.01 mm Hg (1.79–4.23)***, combined**
		Diastolic blood pressure	**β 0.94 mm Hg (0.12–1.76)*, males** **β 2.74 mm Hg (1.51–3.97)*, females** **β 2.48 mm Hg (1.80–3.16)*, combined**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.98 ng/mL (median serum PFNA)	Risk of cardiovascular disease	OR 1.30 (0.99–1.72), 4th quartile **OR 1.42 (1.07–1.88)*, 4th quartile with adjustment for serum total proteins and eGFR**
		Risk of coronary heart disease	**OR 1.89 (1.29–2.76)*, 4th quartile**
		Risk of heart attack	**OR 1.51 (1.02–2.23)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	**Association (p=0.04)***
		Arterial pulse wave velocity	NS (p=0.14)
Lin et al. 2013a, 2013b General population (n=644)	0.38 ng/mL (median PFNA)	Carotid intima media thickness	**Inverse association (p=0.014 for trend)***
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.76)
		Echogenicity of intima media complex	NS (p=0.66), males **Association (p=0.01)*, females**
Manzano-Salgado et al. 2017b General population (n=1,230 children)	0.66 ng/mL (maternal geometric mean PFNA)	Blood pressure at 4 years of age	β -0.01 (-0.10–0.08)
		Blood pressure at 7 years of age	β 0.00 (-0.08–0.09)
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.5 ng/mL (median PFNA in cases and controls)	Coronary artery disease	OR 0.68 (0.39–1.20), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.54 ng/mL (median PFNA)	Pre-eclampsia	HR 0.90 (0.70–1.16), per ln unit

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Bao et al. 2017 General population (n=1,612 adults)	0.86 ng/mL (median serum PFNA in males and females)	Risk of hypertension	OR 0.96 (0.85–1.09)
		Systolic blood pressure	β -0.19 mm Hg (-1.39–1.02)
		Diastolic blood pressure	**β 0.81 mm Hg (0.08–1.54)*, males** β 0.61 mm Hg (-0.81–2.04), females **β 1.19 mm Hg (0.52–1.37)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFDA)	Risk of cardiovascular disease	OR 1.32 (0.99–1.78), 4th quartile **OR 1.43 (1.06–1.92)*, 4th quartile with adjustment for serum total proteins and eGFR**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.10)
		Arterial pulse wave velocity	NS (p=0.39)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.85)
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=014), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFDA in cases and controls)	Coronary artery disease	OR 0.92 (0.53–1.60), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.10 ng/mL (median PFDA)	Pre-eclampsia	HR 0.88 (0.75–1.04), per ln unit
PFUnA			
Bao et al. 2017 General population (n=1,612 adults)	0.5 ng/mL (median serum PFUnA in males and females)	Risk of hypertension	OR 0.95 (0.90–1.01)
		Systolic blood pressure	β -0.49 mm Hg (-1.04–0.05)
		Diastolic blood pressure	β -0.11 mm Hg (-0.41–0.20)

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFUnA)	Risk of cardiovascular disease	**OR 1.58 (1.17–2.12)*, 2nd quartile**
		Risk of coronary heart disease	**OR 1.57 (1.00–2.46)*, 2nd quartile**
		Risk of angina pectoris	**OR 1.97 (1.09–3.55)*, 3rd quartile**
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group, respectively)	Arterial wall stiffness	NS (p=0.97)
		Arterial pulse wave velocity	NS (p=0.41)
Lin et al. 2013a, 2013b General population (n=644)	6.59 ng/mL (median PFUnA)	Carotid intima media thickness	NS (p=0.953 for trend)
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.96)
		Echogenicity of intima media complex	NS (p=0.09), males NS (p=0.14), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.2 ng/mL (median PFUnA in cases and controls)	Coronary artery disease	OR 0.88 (0.51–1.51), 4th quartile
Starling et al. 2014b General population (n=976 pregnant women)	0.17 ng/mL (median PFUnA)	Pre-eclampsia	**HR 0.78 (0.66–0.92)*, per ln unit**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFHpA in males and females); 0.01 and 0.01 ng/mL (median serum PFHpA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.89–1.16)
		Systolic blood pressure	**β 1.84 mm Hg (0.43–3.25)*, males** β 0.34 mm Hg (-2.47–3.15), females **β 1.50 mm Hg (0.21–2.80)*, combined**
		Diastolic blood pressure	**β 0.79 mm Hg (0.02–1.55)*, males** β 0.14 mm Hg (-1.45–1.73), females β 0.66 mm Hg (0.05–1.40), combined
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.20 ng/mL (median serum PFHpA)	Risk of cardiovascular disease	OR 1.16 (0.71–1.91), 4th quartile
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.78)
		Echogenicity of intima media complex	NS (p=0.53), males NS (p=0.13), females
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.06 and 0.04 ng/mL (median PFHpA in cases and controls)	Coronary artery disease	**OR 2.58 (1.39–4.78)*, 3rd quartile** OR 1.73 (0.94–3.16), 4th quartile
PFBS			
Bao et al. 2017 General population (n=1,612 adults)	0.01 ng/mL (median serum PFBS in males and females)	Risk of hypertension	OR 0.94 (0.78–1.12)
		Systolic blood pressure	β -0.69 mm Hg (-2.49–1.11)
		Diastolic blood pressure	β -0.41 mm Hg (-1.42–0.60)
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum PFBS)	Risk of cardiovascular disease	**OR 1.34 (1.05–1.723)*, 2nd quartile**

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Bao et al. 2017 General population (n=1,612 adults)	0.15 ng/mL (median serum PFBA in males and females); 0.17 and 0.12 ng/mL (median serum PFBA in males and females, respectively)	Risk of hypertension	**OR 1.09 (1.02–1.16)*, males** **OR 1.16 (1.04–1.29)*, females** **OR 1.10 (1.04–1.17)*, combined**
		Systolic blood pressure	**β 0.66 mm Hg (0.03–1.28)*, males** β 0.77 mm Hg (-0.27–1.80), females **β 0.80 mm Hg (0.25–1.34)*, combined**
		Diastolic blood pressure	β 0.09 mm Hg (-0.22–0.40), combined
PFDoDA			
Bao et al. 2017 General population (n=1,612 adults)	0.12 ng/mL (median serum PFDoDA in males and females); 0.17 and 0.12 ng/mL (median serum PFDoDA in males and females, respectively)	Risk of hypertension	OR 1.02 (0.93–1.11)
		Systolic blood pressure	β -0.74 (-1.71–0.22), males **β 1.89 mm Hg (0.21–3.56*, females** β 0.30 mm Hg (-0.56–1.16), combined
		Diastolic blood pressure	β 0.13 mm Hg (-0.40–0.66), males **β 1.02 mm Hg (0.07–1.97)*, females** **β 0.59 mm Hg (0.12–1.07)*, combined**
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.14 ng/mL (median serum PFDoDA)	Risk of cardiovascular disease	**OR 1.53 (1.14–2.04)*, 4th quartile**
		Risk of congestive heart failure	**OR 1.55 (1.07–2.25)*, 3rd quartile**
		Risk of angina pectoris	**OR 1.64 (1.06–2.54)*, 4th quartile**
Mattsson et al. 2015 General population (n=231 cases with CHD, 231 controls)	0.02 ng/mL (median PFDoDA in cases and controls)	Coronary artery disease	OR 0.63 (0.35–1.11), 4th quartile

Table 2-8. Summary of Cardiovascular Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Huang et al. 2018 General population (NHANES, n=10,859 adults)	0.07 ng/mL (median serum FOSA)	Risk of cardiovascular disease	**OR 1.29 (1.01–1.65)*, 2nd quartile**
Lind et al. 2017b General population (n=1,016, 70-year-old adults)	NR	Intima media thickness in common carotid artery	NS (p=0.35), males **Association (p=0.004)*, females** **Association (p=0.01)*, combined**
		Echogenicity of intima media complex	NS (p=0.84), males NS (p=0.78, females

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 3 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CHD = coronary heart disease; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LHWA = Little Hocking Water Authority; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standard prevalence ratio; WTCHR = World Trade Center Health Registry

Examination of the cardiovascular system in laboratory animals primarily consists of inhalation, oral, and dermal studies examining the heart for morphological alterations (see Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies in laboratory animals were identified for PFNA, PFUnA, PFHpA, or FOSA.

The laboratory animal studies did not find increases in the incidence of histological alterations in the heart following exposure to PFOA, PFOS, PFHxS, PFDA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies—Heart Disease. Possible associations between PFOA exposure and increased risk of heart disease have been examined in cohort mortality studies of workers, community members living near a PFOA facility, and the general population. Occupational exposure studies have not found increases in deaths from all heart disease, cerebrovascular disease, or ischemic heart disease when compared to U.S. general populations, state populations, and/or a population of workers at other company facilities (Leonard 2006; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). One occupational exposure study found an increase in the risk of cerebrovascular disease in workers with definite exposure for at least 6 months compared to an internal referent group (Lundin et al. 2009). However, other studies have not found increased risks of ischemic heart disease (Raleigh et al. 2014; Sakr et al. 2009), cerebrovascular disease (Raleigh et al. 2014), or coronary artery disease (Steenland et al. 2015). In another occupational exposure study, the investigators noted that electrocardiograms (EKGs) were within normal limits (Sakr et al. 2007b).

Studies of residents living near the Washington Works facility in West Virginia reported increased risks of self-reported cardiovascular disease (Anderson-Mahoney et al. 2008), angina (Anderson-Mahoney et al. 2008), myocardial infarction (Anderson-Mahoney et al. 2008), and stroke (Anderson-Mahoney et al. 2008; Simpson et al. 2013). It is noted that the Anderson-Mahoney et al. (2008) study did not measure serum PFOA levels; the incidences of self-reported diseases were compared to NHANES rates. Another community study of residents in this area did not find an increased risk of coronary artery disease (Winquist and Steenland 2014a). Seven general population studies have examined possible associations between serum PFOA and heart disease risks. A case-control study did not find increases in the risk of coronary artery disease in subjects with median serum PFOA levels of 4.2 ng/mL (cases) or 4.0 ng/mL (controls) (Mattsson et al. 2015). Utilizing the NHANES data set, Shankar et al. (2012) found increases in the risk of peripheral arterial disease, coronary heart disease, or stroke in participants with serum PFOA levels in the 4th quartile (>5.6 and >6.1 ng/mL in females and males, respectively) and for cardiovascular

disease in participants with serum PFOA levels in the 3rd and 4th quartiles (>4.0 and >4.4 ng/L for females and males, respectively). In contrast, two other NHANES studies did not find associations between serum PFOA and physician-diagnosed coronary artery disease, angina, and/or heart attack (Melzer et al. 2010) or total cardiovascular heart disease (Huang et al. 2018). Two general population studies did not find associations between serum PFOA levels and carotid intima media thickness (Lin et al. 2013a; Lind et al. 2017b). Another study did not find associations with arterial wall stiffness or arterial pulse wave velocity (Koshy et al. 2017).

Epidemiological Studies—Hypertension. Occupational, community, and general population exposure studies have investigated the possible association between PFOA and blood pressure, the risk of hypertension, and the risk of pregnancy-induced hypertension and/or pre-eclampsia. A study by Min et al. (2012) utilizing NHANES data found an increase in hypertension risk among participants with serum PFOA levels in the 4th quartile. Another general population study did not find an association between serum PFOA and the risk of hypertension, but did find associations between serum PFOA and systolic and diastolic blood pressure (Bao et al. 2017). In contrast, no increases in the risk of hypertension were observed in workers at the Washington Works facility (Steenland et al. 2015), adult community members living near this facility (Winquist and Steenland 2014a), or adolescent NHANES participants (Geiger et al. 2014a). Additionally, Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOA levels and blood pressure in children at ages 4 or 7 years. There is some epidemiological evidence suggesting that an elevated uric acid level is a risk factor for hypertension (Johnson et al. 2003; Sündstrom et al. 2005). Several occupational, community, and general population studies have found increases in uric acid levels and increased risks of hyperuricemia; these data are discussed in Section 2.10. Overall, the results of these studies are suggestive of a connection between serum PFOA and increased risk of hyperuricemia.

Several studies have examined the possible associations between PFOA and pregnancy-induced hypertension/pre-eclampsia. Four studies have evaluated the community living near the Washington Works facility using different approaches to assess PFOA exposure. Savitz et al. (2012a, 2012b) used residential history and environmental dispersion of PFOA to estimate serum PFOA levels over time. Stein et al. (2009) used serum PFOA levels measured in 2005–2006 to assess the risk of pre-eclampsia occurring prior to the blood sampling. Darrow et al. (2013) primarily used serum PFOA levels measured in 2005–2006 to assess the association with pregnancy-induced hypertension occurring after the blood samples were collected. Savitz et al. (2012a) found an increased risk of self-reported pre-eclampsia in C8 Health Project participants with elevated PFOA levels and Darrow et al. (2013) found significant

increases in the odds ratios (ORs) for self-reported pregnancy-induced hypertension in women with higher PFOA (≥6.9 ng/mL) levels. A third study of highly exposed residents reported a weak association between serum PFOA and self-reported pre-eclampsia in subjects whose serum PFOA levels were above the median (Stein et al. 2009); however, there was no dose-response gradient. Using birth record data and serum PFOA levels predicted from addresses, Savitz et al. (2012b) found no consistent associations between serum PFOA and the occurrence of pregnancy-induced hypertension in participants in the C8 Health Project. Similarly, Stein et al. (2009) did not find increases in the odds of self-reported pre-eclampsia among C8 Health Project participants categorized by serum PFOA levels. Another study of residents of this area did not find increases in the risk of pregnancy-induced hypertension among residents living in an area where PFOA-contaminated water was supplied by the Little Hocking Water Authority (Nolan et al. 2010). A general population study did not find an association between plasma PFOA and the risk of pre-eclampsia (Starling et al. 2014a).

Laboratory Animal Studies. A small number of laboratory animal studies have evaluated the cardiovascular toxicity of PFOA. These studies focused on potential histological alterations in the heart; none of the available studies evaluated endpoints related to hypertension. No histopathological alterations were seen in the heart from rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Administration of APFO in the diet at doses up to approximately 100–110 mg/kg/day to male and female CD rats or 10 mg/kg/day by gavage to Rhesus monkeys did not cause gross or microscopic alterations in the heart or aorta (Griffith and Long 1980). Similar negative findings were reported in Cynomolgus monkeys administered up to 20 mg/kg/day APFO by capsule for 26 weeks (Butenhoff et al. 2002) and in male and female Sprague-Dawley rats that received doses of up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c). No morphological alterations were seen in the heart from male rats dermally exposed to ≤2,000 mg/kg APFO for 2 weeks (Kennedy 1985).

Summary. Cardiovascular toxicity as assessed by deaths from heart disease, risk of heart disease, and risk of hypertension has been evaluated in workers, community members living near a PFOA facility, and the general population. In general, occupational exposure studies have not found increases in the risks of deaths from heart disease or in the risks of ischemic heart disease, cerebrovascular disease, or coronary disease. Inconsistent results have been found in a small number of studies examining residents living in areas with high PFOA drinking water contamination or the general population. Studies of hypertension have also not found associations between serum PFOA and hypertension risk. However, studies of highly exposed residents provide some suggestive evidence of an association between serum PFOA and

increased risks of pregnancy-induced hypertension/pre-eclampsia. Studies in laboratory animals did not find histological alterations in the heart following acute-, intermediate-, or chronic-duration oral exposure.

PFOS

Epidemiological Studies—Heart Disease. Three studies have evaluated the possible association between PFOS and heart disease. Melzer et al. (2010) did not find an association between serum PFOS and the risk of physician-diagnosed coronary artery disease, angina, and/or heart attack among NHANES participants; Huang et al. (2018) did not find increases in the risk of cardiovascular disease among NHANES participants. In a case-control study (Mattsson et al. 2015), no alterations in the risk of coronary artery disease were observed. Lin et al. (2013a) found an association between serum PFOS levels and carotid intima media thickness in a general population study. When the subjects were divided into subpopulations, associations between PFOS and carotid intima media thickness were found for females, nonsmokers, subjects 12–19 years of age, BMI <24, and those with an apolipoprotein E genotype of E2 carrier or E3/E3. A second study of 70-year-old subjects did not find associations between serum PFOS and the intima media thickness of the common carotid artery (Lind et al. 2017b). Similarly, no alterations in arterial wall stiffness or pulse wave velocity were found in children enrolled in the World Trade Center Health Registry (Koshy et al. 2017).

Epidemiological Studies—Hypertension. An increased risk of hypertension associated with serum PFOS levels were observed in adults; when categorized by sex, the association was only found in females (Bao et al. 2017). The study also found associations for systolic and diastolic blood pressure in males and females combined and in females only. No increases in the risk of hypertension associated with serum PFOS levels were observed in adolescent NHANES participants (Geiger et al. 2014a). Similarly, no associations between maternal serum PFOS levels and blood pressure were found in children at ages 4 and 7 years (Manzano-Salgado 2017b). Two studies found increases in the risk of self-reported pregnancy-induced hypertension (Darrow et al. 2013) or self-reported pre-eclampsia (Stein et al. 2009) associated with serum PFOS levels among C8 participants. No increase in the risk of pre-eclampsia was observed in a general population study (Starling et al. 2014b).

Laboratory Animal Studies. Studies in laboratory animal studies have evaluated the cardiovascular toxicity of PFOS but have not evaluated endpoints related to hypertension. Administration of doses of up to 0.75 mg/kg/day PFOS (potassium salt) via capsule to Cynomolgus monkeys for 26 weeks did not cause any significant gross or microscopic alterations in the heart or aorta (Seacat et al. 2002). Rats that

received up to approximately 1.04 mg/kg/day of PFOS in the diet for 2 years had no significant gross or microscopic changes in the heart (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Eight general population studies examined possible cardiovascular outcomes associated with PFHxS exposure. No increases in the risk of coronary artery disease (Mattsson et al. 2015) or cardiovascular disease (Huang et al. 2018) were found. Serum PFHxS levels were not associated with arterial wall stiffness (Koshy et al. 2017) or carotid artery intima media thickness (Lind et al. 2017b). Studies examining blood pressure have not found associations in adults (Bao et al. 2017) or children (Manzano-Salgado et al. 2017b). Additionally, no association between serum PFHxS and pre-eclampsia were found (Starling et al. 2014b).

Laboratory Animal Studies. Dosing of rats with≤10 mg/kg/day PFHxS or mice with ≤3 mg/kg/day by gavage for 40–60 days did not cause morphological alterations in the heart (Butenhoff et al. 2009a; Chang et al. 2018).

PFNA

Epidemiological Studies. In a general population study, an inverse association between serum PFNA levels and carotid intima media thickness was observed (Lin et al. 2013a). The investigators suggested that this finding may be secondary to an interaction between higher serum PFOS levels and lower serum PFNA levels in the study population. Associations were only found in subjects with serum PFOS higher than the 50th percentile regardless of whether the serum PFNA was higher or lower than the 60th percentile. A second study did not find an association between serum PFNA and intima media thickness (Lind et al. 2017b), but did find an association with the echogenicity of the intima media complex, an indicator of early changes in the carotid artery. Koshy et al. (2017) also found an association between serum PFNA and arterial wall stiffness in children enrolled in the World Trade Center Health Registry. Increased risks of cardiovascular disease, coronary heart disease, and heart attack were found in NHANES participants (Huang et al. 2018). In contrast, another general population study did not find increases in the risk of coronary heart disease (Mattsson et al. 2015). An association between serum PFNA and hypertension risk and systolic and diastolic blood pressure was found in a general population study (Bao et al. 2017). Manzano-Salgado et al. (2017b) did not find associations between maternal

serum PFNA and blood pressure in children aged 4 or 7 years, and Starling et al. (2014b) did not find associations between serum PFNA and pre-eclampsia (Starling et al. 2014b).

PFDA

Epidemiological Studies. In a study of NHANES participants, Huang et al. (2018) found an increased risk of any type of cardiovascular disease among participants with the highest serum PFDA levels when the statistical analyses adjusted for serum total protein levels and estimated glomerular filtration rate; however, no associations were found for specific types of cardiovascular disease. In another general population study, Mattsson et al. (2015) found no association between serum PFDA and the risk of coronary artery disease. Studies examining carotid artery intima media thickness or arterial wall stiffness of the brachial artery did not find associations with serum PFDA levels (Koshy et al. 2017; Lind et al. 2017b). Although Bao et al. (2017) did not find an association between serum PFDA levels and the risk of hypertension or systolic blood pressure levels, associations were found in diastolic blood pressure levels in males only and in males and females combined. No association was found between serum PFDA and pre-eclampsia (Starling et al. 2014b).

Laboratory Animal Studies. Death in female C57BL/6N mice following administration of a single lethal dose of 160 or 320 mg/kg PFDA by gavage was associated with mural thrombosis of the left ventricle of the heart (Harris et al. 1989). Doses ≤80 mg/kg did not cause gross or microscopic alterations in the heart, assessed 30 days after dosing, but 80 mg/kg significantly decreased relative heart weight (Harris et al. 1989).

PFUnA

Epidemiological Studies. Serum PFUnA levels were associated with increased risks of any type of cardiovascular disease, coronary heart disease, and angina pectoris in NHANES participants (Huang et al. 2018). No associations between serum PFUnA levels and the risk of hypertension of systolic or diastolic blood pressure were observed (Bao et al. 2017). Starling et al. (2014b) found an inverse association between serum PFUnA levels and the risk of pre-eclampsia in pregnant women. No associations between serum PFUnA levels and carotid intima artery thickness (Lin et al. 2013a; Lind et al. 2017b) or brachial artery wall stiffness (Koshy et al. 2017) were observed in general population studies. Another general population study (Mattsson et al. 2015) did not find an increase in the risk of coronary artery disease associated with serum PFUnA levels.

PFHpA

Epidemiological Studies. Mattsson et al. (2015) found an increase in the risk of coronary artery disease in individuals with serum PFHpA levels in the 3rd quartile; however, the risk was not increased for those with serum levels in the 4th quartile. A study of NHANES participants did not find an association between the serum PFHpA levels and any type of cardiovascular disease or a specific type of heart disease (Huang et al. 2018). No associations between serum PFHpA and the thickness of the intima media of the common carotid artery were observed in a general population study of 70-year-old adults (Lind et al. 2017b). Bao et al. (2017) did not find an association between serum PFHpA levels and the risk of hypertension; the study did find associations for systolic and diastolic blood pressure levels in males only.

PFBS

Epidemiological Studies. Two general population studies have evaluated the potential associations between serum PFBS and cardiovascular effects. Huang et al. (2018) found increased risks of cardiovascular disease (all types combined) in NHANES participants with serum PFBS levels in the 2nd quartile and higher; however, no associations were found for specific disease types. Bao et al. (2017) did not find associations between serum PFBS levels and the risk of hypertension or systolic or diastolic blood pressure levels among adults.

Laboratory Animal Studies. No morphological alterations were reported in the heart or aorta from rats dosed with ≤900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or ≤600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a).

PFBA

Epidemiological Studies. Only one epidemiological study examined potential cardiovascular health outcomes. Bao et al. (2017) found increases in the risk of hypertension in male and female adults, which was associated with serum PFBA levels. Systolic blood pressure levels were also associated with serum PFBA levels in males and females combined or in males only; no associations were found for diastolic blood pressure.

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in the heart (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. No increase in the risk of coronary heart disease associated with serum PFDoDA levels was found in a general population study (Mattsson et al. 2015). In contrast, Huang et al. (2018) found increased risks of cardiovascular disease (any type), congestive heart failure, or angina pectoris in NHANES participants with higher serum PFDoDA levels. Bao et al. (2017) reported associations between serum PFDoDA levels in systolic and diastolic blood pressure levels among women, but there was no association with the risk of hypertension.

Laboratory Animal Studies. No histological alterations were observed in male rats administered 2.5 mg/kg/day for 42 days (Kato et al. 2015).

PFHxA

Epidemiological Studies. An increased risk of cardiovascular disease (any type) was found in NHANES participants with higher serum PFHxA levels (Huang et al. 2018). A study of 70-year-old adults reported increases in the intima media thickness in the common carotid artery that was associated with serum PFHxA levels (Lind et al. 2017b).

Laboratory Animal Studies. No histological alterations were observed in the heart of rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

FOSA

Epidemiological Studies. Serum FOSA levels were associated with an increased risk of cardiovascular disease (any type) in a study of NHANES participants (Huang et al. 2018). Increases in the intima media thickness in the common carotid artery was associated with serum FOSA levels in a study of 70-year-old men and women (Lind et al. 2017b).

2.6 GASTROINTESTINAL

Overview. Available epidemiological data on the potential of perfluoroalkyls to induce gastrointestinal effects are limited to two studies of workers at a PFOS facility that found mixed results on the possible association between PFOS and colon polyps; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 4. Epidemiological studies examining potential gastrointestinal effects were not identified for the other perfluoroalkyls. Studies examining ulcerative colitis are discussed in Section 2.14, Immunological. Laboratory animal studies have examined the gastrointestinal tract for morphological alterations following inhalation, oral, or dermal exposure to PFOA (Tables 2-1, 2-3, and 2-6), oral exposure to PFOS (Table 2-4), and oral exposure to other perfluoroalkyls (Table 2-5); the NOAELs and LOAELs are presented in Figures 2-6, 2-8, and 2-9. No laboratory animal studies were identified for PFNA, PFUnA, PFHpA, or FOSA. Studies on PFOA and PFBS have reported some signs of gastrointestinal irritation following gavage administration. Most studies did not report histological alterations in the gastrointestinal tract following exposure to PFOA, PFOS, PFHxS, PFDA, PFBA, PFDoDA, or PFHxA.

PFOA

Laboratory Animal Studies. The available data in rats and monkeys do not suggest that the gastrointestinal tract is a sensitive target of toxicity, although two studies did report some signs of irritation. Stomach irritation was reported in male rats exposed head-only to ≥380 mg/m^3 APFO dusts for 4 hours (Kennedy et al. 1986). No histopathological alterations were seen in the stomach, small intestine, or large intestine from male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No significant gross or microscopic alterations of the gastrointestinal tract were observed in male or female rats exposed to approximately 100–110 mg/kg/day APFO through the diet for 90 days (Griffith and Long 1980). Similar observations were reported in male and female rats exposed to 15 mg/kg/day APFO via the diet for 2 years (3M 1983; Butenhoff et al. 2012c). The same investigators also reported that emesis occurred in Rhesus monkeys exposed to lethal doses (30 and 100 mg/kg/day) of APFO by gavage for 90 days (Griffith and Long 1980). In another intermediate-duration study in which Cynomolgus monkeys were exposed to up to 20 mg/kg/day APFO administered via a capsule for

26 weeks, no treatment-related alterations in the gastrointestinal tract were observed at termination (Butenhoff et al. 2002).

Intermittent application of up to 2,000 mg/kg/day APFO to the skin of male rats for up to 2 weeks did not result in gross or microscopic alterations in the gastrointestinal tract (Kennedy 1985).

PFOS

Epidemiological Studies. There are limited data available on the potential of PFOS to induce gastrointestinal damage. A study of current, retired, or former workers employed for at least 1 year at a PFOS-based fluorochemical manufacturing facility in Decatur, Alabama found no association between self-reported incidence of gastric ulcer or colon polyps and having worked in a job with either low (estimated serum PFOS levels of 390–890 ng/mL) or high (estimated PFOS serum levels of 1,300–1,970 ng/mL) exposure to PFOS, as compared to workers with no direct workplace exposure (estimated serum PFOS levels of 110–290 ng/mL) (Grice et al. 2007). A second study of workers at the Decatur facility found an increase in the risk ratio episodes of care for benign colonic polyps in workers with high potential exposure to PFOS (Olsen et al. 2004a).

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that distension of the small intestine was observed in rats exposed to lethal concentrations of airborne PFOS dusts (1,890–45,970 mg/m^3) for 1 hour. Treatment of rats with up to approximately 1.04 mg/kg/day PFOS via the diet for 2 years did not induce morphological alterations in the gastrointestinal tract (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. No morphological alterations were observed in the gastrointestinal tract of rats administered ≤10 mg/kg/day or mice administered ≤3 mg/kg/day PFHxS via gavage for 40–60 days (Butenhoff et al. 2009a; Chang et al. 2018).

PFDA

Laboratory Animal Studies. Administration of 0.5 mg/kg/day PFDA to rats for 28 days or 5 mg/kg to mice for 4 weeks (once/week) did not result in histological alterations in the gastrointestinal tract (Frawley et al. 2018).

PFBS

Laboratory Animal Studies. Necrosis of individual squamous cells and hyperplasia and hyperkeratosis were observed in the limiting ridge of the forestomach of male and female rats administered 600 mg/kg/day PFBS via gavage for 90 days (Lieder et al. 2009a); these lesions were likely due to irritation from the repeated gavage administration with PFBS. In another study, no morphological alterations were observed in the gastrointestinal tract of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA to rats by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days did not cause morphological alterations in the gastrointestinal tract (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Laboratory Animal Studies. No histological alterations were observed in the gastrointestinal tract of male rats receiving gavage administration of 2.5 mg/kg/day for 42 days or in male and female rats administered 42 mg/kg/day for 42 days and allowed to recover for 14 days (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Rat administered 200 mg/kg/day NaPFHx for 90 days did not exhibit histological alterations in the gastrointestinal tract (Chengelis et al. 2009b). Erosions/ulcerations were observed in the glandular or nonglandular stomach of rats receiving gavage doses of 450 mg/kg/day PFHxA for 4 days; all animals exhibiting these lesions died early or were sacrificed *in extremis*

(Kirkpatrick 2005). No gastrointestinal lesions were observed in rats administered a time-weighted average (TWA) dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005).

2.7 HEMATOLOGICAL

Overview. A small number of epidemiological studies have evaluated hematological endpoints in workers exposed to PFOA or PFOS and in a community exposure study; these studies did not find alterations in hematological indices; epidemiological data were not identified for the other perfluoroalkyls. Details of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 5. Laboratory animal studies have evaluated potential alterations in hematological endpoints for a variety of perfluoroalkyls (Tables 2-1, 2-3, 2-4, 2-5, and 2-6). No studies examining hematological endpoints were identified for PFNA, PFHpA, or FOSA. Some laboratory animal studies have reported alterations in hematological indices following exposure to higher doses of PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA.

PFOA

Epidemiological Studies. Information on effects on hematological parameters is available from a study of residents in the Little Hocking water district in southeastern Ohio where there was significant environmental exposure to PFOA via the water supply (Emmett et al. 2006b). No significant correlations between any of the hematology parameters evaluated (including hemoglobin, hematocrit, red blood cell indices, white cell count, and platelet count) and serum PFOA were observed, whether the analysis included all of the individuals as a group or separate analyses were done for adults or children. In an occupational study, the investigators reported no alterations in blood counts in workers, with a range of serum PFOA levels of 5–9,550 ng/mL (Sakr et al. 2007b). A second occupational exposure study found an inverse association between serum fluorine (used as a measure of PFOA exposure) and hemoglobin levels (Gilliland 1992); no alterations in mean corpuscular hemoglobin or volume were found. Although no associations were found for total leukocyte counts, an inverse association with lymphocyte count and association with monocyte counts was found.

Laboratory Animal Studies. No treatment-related hematological alterations were reported in male rats exposed intermittently nose-only to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). The specific parameters evaluated included erythrocyte counts, hemoglobin concentration, hematocrit, and differential leukocyte counts.

No significant hematological alterations were reported in male and female rats orally dosed with approximately 100–110 mg/kg/day APFO in diet for 90 days (Griffith and Long 1980). Similar results were reported in Cynomolgus monkeys treated daily with up to 20 mg/kg/day APFO administered via a capsule (Butenhoff et al. 2002; Thomford 2001) or in Rhesus monkeys dosed daily by gavage with up to 30 mg/kg/day (Griffith and Long 1980). In a 2-year dietary study in rats dosed with 1.5 or 15 mg/kg/day APFO, hematology tests performed at various times during the study showed changes in treated groups consisting of decreases in red blood cell counts, hemoglobin concentration, and hematocrit that were not always dose-related or consistent among sexes and were within acceptable ranges for the rat (3M 1983; Butenhoff et al. 2012c).

Hematology tests (erythrocyte count, hemoglobin concentration, hematocrit, total and differential leukocyte count, and red cell indices) conducted in blood from rats following intermittent dermal exposure to ≤2,000 mg/kg/day APFO for 2 weeks showed inconsistent alterations or changes of unlikely biological significance (Kennedy 1985).

PFOS

Epidemiological Studies. Two occupational exposure studies (Olsen et al. 1998a, 2003a) have examined the potential association between serum PFOS and hematological parameters (including hematocrit, hemoglobin, red blood cells, white blood cells, and platelets) in workers at 3M facilities in Decatur, Alabama and Antwerp, Belgium; mean measured levels of serum PFOS ranged from 800 to 2,440 ng/mL. No consistent alterations in hematological parameters were observed at either facility or at the different measuring time points.

Laboratory Animal Studies. Treatment of male and female rats with approximately 1.5–1.8 mg/kg/day PFOS (potassium salt) in the diet for 4 weeks did not result in significant alterations in hematological parameters (Seacat et al. 2003). Oral dosing with 1.3–1.6 mg/kg/day for 14 weeks resulted in a significant increase (45%) in non-segmented neutrophils (Seacat et al. 2003). The biological significance of this finding was not discussed by the investigators. In a 4-week study, oral administration of up to 2 mg/kg/day PFOS to Cynomolgus monkeys had no effect on hematological parameters (Thomford 2002a). In Cynomolgus monkeys dosed with 0, 0.03, 0.15, or 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks and subjected to comprehensive hematological tests during the study, the only significant effect was a 9% decrease in hemoglobin in 0.75 mg/kg/day males at

termination (Seacat et al. 2002). The investigators considered this a treatment-related effect, but not biologically significant given that the value was within the published range and there was no evidence of blood in the stools. No significant hematological effects were reported in a 2-year study in rats dosed with approximately 1.04 mg/kg/day PFOS in the diet (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Laboratory Animal Studies. Treatment of male rats with doses ≥0.3 mg/kg/day PFHxS by gavage for at least 42 days significantly increased prothrombin time (Butenhoff et al. 2009a). Doses ≥1 mg/kg/day significantly decreased hemoglobin concentration, whereas ≥3 mg/kg/day decreased erythrocyte count and hematocrit; the decrease in hemoglobin (<5%) was not considered adverse at 1 mg/kg/day. Oral treatment of female rats with up to 10 mg/kg/day PFHxS did not significantly alter hematological parameters (Butenhoff et al. 2009a). No alterations in hematological parameters were observed in mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation (Chang et al. 2018).

PFDA

Laboratory Animal Studies. Significant decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed in rats administered 0.25 or 0.5 mg/kg/day for 28 days (Frawley et al. 2018). No other alterations in hematological parameters were observed. Hematological alterations were also not observed in mice receiving once weekly doses of 5 mg/kg PFDA for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant hematological changes (Takahashi et al. 2014). Effects in males included decreased mean corpuscular volume (MCV) (5%), mean corpuscular hemoglobin (MCH) (5%), activated partial thromboplastin time (APTT) (16–25%), and fibrinogen (19–33%), and increased platelet counts (13%) and white blood cells (7%). In females, there were increases in MCV (10%) and MCH (10%) and a decrease in fibrinogen (32%). The NOAEL was 0.3 mg/kg/day.

PFBS

Laboratory Animal Studies. A 90-day exposure to PFBS resulted in significant decreases in hemoglobin and hematocrit levels in males orally administered 200 or 600 mg/kg/day, and a decrease in erythrocyte levels was observed in males administered 600 mg/kg/day; the NOAEL was 60 mg/kg/day (Lieder et al. 2009a). In contrast, no hematological alterations were observed in rats administered 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. Administration of PFBA by gavage to rats in doses of up to 184 mg/kg/day for 5 days (3M 2007a) or up to 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a) did not result in significant alterations in hematological parameters. Oral doses of 30 mg/kg/day, but not 6 mg/kg/day, for 90 days resulted in significant reductions in red blood cell counts, hemoglobin, and hematocrit, and an increase in red cell distribution width in male rats (Butenhoff et al. 2012a; van Otterdijk 2007b). This dose level also caused a reduction in MCH and reduced MCH concentration in male rats. The lower hemoglobin and hematocrit observed in males were still detected at the end of a 3-week recovery period. These hematological effects were considered minor and not evidence of an adverse effect on red blood cell turnover by the investigator based on lack of alterations in bone marrow or the spleen.

PFDoDA

Laboratory Animal Studies. Gavage administration of 2.5 mg/kg/day for 42 days resulted in decreases in mean corpuscular volume and reticulocytes and increases in mean corpuscular hemoglobin concentration in male rats (Kato et al. 2015). In animals allowed to recover for 14 days, decreases in red blood cells, hemoglobin, hematocrit, and leukocyte levels and increases in reticulocytes were observed. In females administered 2.5 mg/kg/day for 42 days and allowed to recover for 14 days, decreases in hemoglobin, hematocrit, and mean corpuscular hemoglobin and increases in neutrophil levels were observed (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Several studies in rats have identified the hematological system as a target of PFHxA toxicity. Decreases in red blood cell counts, hemoglobin levels, and/or hematocrit levels and increases in reticulocyte levels have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005), 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b), 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009), or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015). A decrease in hemoglobin levels was also observed in rats administered 150 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Hematological alterations were not observed at doses ≤100 mg/kg/day. Hematological alterations were only observed in female rats in the Klaunig et al. (2015) study and only in males in the Kirkpatrick (2005) study; sex-specific differences were not observed in the Chengelis et al. (2009b) or Loveless et al. (2009) intermediate-duration studies.

2.8 MUSCULOSKELETAL

Overview. Several epidemiological studies have evaluated possible associations between perfluoroalkyls and bone mineral density, risk of bone fractures, and risk of osteoarthritis; the results of these studies are summarized in Table 2-9, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 6. Several cross-sectional community and general population studies have found associations between serum PFOA and the risk of osteoarthritis, particularly in participants under the age of 55 years. However, associations were not found in a study of mostly male workers. Mixed results were found in studies of PFOS, with studies finding a decreased risk of osteoarthritis, increased risk in women under 50 years of age, or no association. One general population study found increased risks of osteoarthritis associated with serum PFHxS and PFNA. The data provide some suggestive evidence of a relationship between serum perfluoroalkyls and osteoarthritis. Assessing whether there is an association between perfluoroalkyl exposure and osteoarthritis is complicated by the lack of mechanistic data to support this association and it is noted that there are a number of factors that contribute to the osteoarthritis risk, and that some of these factors may be affected by perfluoroalkyls, including elevations in uric acid levels. Epidemiological information on bone mineral density is limited to a study of women and a study of children both examining PFOA, PFOS, PFHxS, and PFNA; the database was not considered adequate for assessing possible associations. No epidemiological studies evaluating musculoskeletal outcomes were identified for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA. No morphological alterations were noted in bone or skeletal muscle in

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Steenland et al. 2015 Occupational (n=3,713 workers)	Estimated cumulative exposure	Osteoarthritis risk	NS (p=0.92 for trend), no lag NS (p=0.13 for trend), 10-year lag
Innes et al. 2011 Community (C8) (n=49,432 adults)	13.6–28.0 ng/mL (2nd PFOA quartile)	Osteoarthritis risk (physician diagnosed)	**OR 1.16 (1.03–1.31)*, 2nd quartile** **OR 1.22 (1.02–1.45)*, 2nd quartile participants <55 years of age**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	3.7 ng/mL (mean PFOA)	Total femur neck mineral density	**β -0.017 (-0.033 to -0.001)*, women** β 0.001 (-0.025–0.022), men
		Osteoporosis risk (women)	**OR 1.84 (1.17–2.90; p=0.008)*, per ln-PFOA increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.99 ng/mL (mean serum PFOA)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	4.70 and 3.31 ng/mL (geometric mean PFOA in males and females)	Total lumbar spine bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.98 (0.75–1.28), premenopausal women OR 1.53 (0.63–3.74), postmenopausal women OR 0.84 (0.67–1.07), men
		Hip fracture	OR 1.59 (0.57–4.46), premenopausal women OR 0.48 (0.06–4.16), postmenopausal women OR 0.64 (0.39–1.06), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Wrist fracture	OR 1.07 (0.65–1.77), premenopausal women OR 1.21 (0.46–3.13), postmenopausal women OR 1.12 (0.75–1.70), men
		Spine fracture	OR 1.83 (0.59–5.61), premenopausal women OR 0.84 (0.46–1.53), postmenopausal women OR 1.54 (0.85–2.79), men
Uhl et al. 2013 General population (NHANES) (n=1,888 male and 1,921 female adults)	>5.89 ng/mL (4th PFOA quartile)	Osteoarthritis risk (self-reported)	**OR 1.98 (1.24–3.19)*, 4th quartiles females** OR 0.82 (0.40–1.70), 4th quartile males **OR 4.95 (1.27–19.4)*, 4th quartile women 20–49 years of age** OR 1.33 (0.82–1.16), 4th quartile women 50–84 years of age
PFOS			
Innes et al. 2011 Community (C8) (n=49,432 adults)	≥29.4 ng/mL (4th PFOS quartile)	Osteoarthritis risk (physician diagnosed)	**OR 0.76 (0.68–0.85)*, 4th quartile**
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	12.7 ng/mL (mean PFOS)	Total femur neck mineral density	**β -0.016 (-0.029 to -0.002)*, women** **β -0.013 (-0.024 to -0.002)*, men**
		Osteoporosis risk (women)	OR 1.14 (0.68–1.94; p=0.619), per ln-PFOS increase
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	2.79 ng/mL (mean serum PFOS)	Bone mineral density	NS (p>0.05)
Lin et al. 2014 General population (NHANES) (n=2,339 participants)	19.23 and 12.09 ng/mL (geometric mean PFOS in males and females)	Total lumbar spine bone mineral density	**β -0.022 (-0.038 to -0.007)*, premenopausal women** NS (p>0.01), postmenopausal women NS (p>0.01), men

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Total hip bone mineral density	NS (p>0.01), premenopausal women, postmenopausal women, men
		All fracture types	OR 0.97 (0.75–1.24), premenopausal women OR 1.59 (0.88–2.86), postmenopausal women OR 0.92 (0.73–1.16), men
		Hip fracture	OR 1.12 (0.62–2.03), premenopausal women OR 0.83 (0.23–3.00), postmenopausal women OR 1.07 (0.76–1.52), men
		Wrist fracture	OR 1.04 (0.63–1.72), premenopausal women OR 1.22 (0.61–2.45), postmenopausal women OR 1.09 (0.72–1.66), men
		Spine fracture	OR 0.52 (0.15–1.86), premenopausal women OR 1.12 (0.26–4.78), postmenopausal women OR 1.27 (0.67–2.42), men
PFHxS			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	2.5 ng/mL (mean PFHxS)	Total femur bone mineral density	**β -0.014 (-0.074 to -0.014)*, women** β -0.026 (-0.065–0.013), men
		Osteoporosis risk (women)	**OR 1.64 (1.14–2.38; p=0.008)*, per ln-PFHxS increase**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	1.09 ng/mL (mean serum PFHxS)	Bone mineral density	NS (p>0.05)

Table 2-9. Summary of Skeletal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Khalil et al. 2016 General population (NHANES) (n=1,914 participants)	1.9 ng/mL (mean PFNA)	Total femur bone mineral density	**β -0.040 (-0.077 to -0.003)*, women** β 0.007 (-0.031–0.045), men
		Osteoporosis risk (women)	**OR 1.45 (1.02–2.05; p=0.001)*, per ln-PFNA increase.**
Khalil et al. 2018 General population (n=48 obese 8–12-year-old children)	0.24 ng/mL (mean serum PFNA)	Bone mineral density	**Inverse association (p<0.05)*** NS (p>0.05) after adjustment for multiple testing

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 6 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluoro-nonanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

laboratory animals following exposure to PFOA, PFOS, PFHxS, PFBS, PFBA, or PFHxA; these data are summarized in Tables 2-1, 2-3, 2-4, and 2-5 and Figures 2-6, 2-8, 2-9, and 2-10. No laboratory animal data were available for PFNA, PFDA, PFUnA, PFHpA, PFDoDA, or FOSA.

PFOA

Epidemiological Studies. Several studies have examined the possible association between serum PFOA levels and the risk of osteoarthritis; the possible mechanisms associated with these findings have not been elucidated. In an occupational study (80% male), no association between estimated cumulative serum PFOA levels and the risk of osteoarthritis was found (Steenland et al. 2015). Innes et al. (2011) examined adult participants in the C8 Health Project and found that the odds of reporting osteoarthritis were higher in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles compared to participants in the 1st quartile. When segregated by age and BMI, the strongest associations between serum PFOA levels and osteoarthritis were found in subjects under 55 years of age and in nonobese (BMI <30) subjects. Increases in the risk of osteoarthritis associated with serum PFOA levels were observed in female NHANES participants (Uhl et al. 2013); there were no associations in men. When stratified by age, the associations were found in women 20–49 years of age, but not in older women (50–84 years old) (Uhl et al. 2013). An association between increases in risk of osteoporosis and serum PFOA levels was found in another study of female NHANES participants (Khalil et al. 2016). Two studies of adult NHANES participants found no associations between serum PFOA and bone mineral density of the total femur (Khalil et al. 2016), hip (Lin et al. 2014), or lumbar spine (Khalil et al. 2016; Lin et al. 2014); however, an inverse association was found in the neck portion of the femur in the Khalil et al. (2016) study. A study in obese children did not find an association between serum PFOA levels and measures of bone mineral density (Khalil et al. 2018). Additionally, Lin et al. (2014) did not find associations between serum PFOA levels and the risk of bone fractures (total fractures, hip fractures, wrist fractures, or spine fractures) in premenopausal women, postmenopausal women, or men.

Laboratory Animal Studies. In male rats exposed head-only to up to 84 mg/m^3 APFO dusts for up to 2 weeks, examinations of the sternebrae were unremarkable (Kennedy et al. 1986). Similarly, no gross or microscopic alterations were reported in the sternum from rats following dietary exposure to 100–110 mg/kg/day APFO for 90 days (Griffith and Long 1980) or in the femur, sternum, or thigh skeletal muscle from Cynomolgus monkeys dosed with up to 20 mg/kg/day APFO administered via a capsule for 26 weeks (Butenhoff et al. 2002). *In utero* exposure to 0.3 mg/kg/day PFOA resulted in morphometrical alterations in the femur (increases in the periosteal area) and decreases in bone mineral density in the tibia

of 13- or 17-month-old mice (Koskela et al. 2016). No alterations in biomechanical properties were found.

PFOS

Epidemiological Studies. Several epidemiological studies have evaluated the potential of PFOS to induce skeletal damage. In the participants of the C8 Health Study, a decreased risk of osteoarthritis was found in participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles (Innes et al. 2011). In contrast, Uhl et al. (2013) found an increased risk of osteoarthritis in NHANES participants with serum levels of >20.97 ng/mL. When categorized by sex and age, the osteoarthritis risk was approximately 5 times higher in women aged 20–49 years with serum PFOS levels in the 4th quartile. Another study of NHANES participants (Khalil et al. 2016) did not find an increased risk of osteoporosis in women. However, the study did find an inverse association between serum PFOS and femur neck bone mineral density, but no associations with total femur or lumbar spine bone mineral density. No associations between serum PFOS levels and measures of bone mineral density were observed in a study of obese children (Khalil et al. 2018).

Laboratory Animal Studies. Treatment of monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks had no significant effect on the gross or microscopic appearance of the femur, sternum, or thigh skeletal muscle (Seacat et al. 2002). Similar observations were made in rats treated with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. A study of NHANES participants found an increase in the risk of osteoporosis among women that was associated with serum PFHxS levels (Khalil et al. 2016). An inverse association between serum PFHxS (fourth quartile) and total femur bone mineral density was also found in women. There were no associations between serum PFHxS and femur neck or lumbar spine bone mineral density (Khalil et al. 2016). In contrast, no association between serum PFHxS levels and bone mineral density were observed in obese children (Khalil et al. 2018).

Laboratory Animal Studies. No histological alterations were observed in bone or muscle of mice administered up to 3 mg/kg/day prior to mating and during mating, gestation, and lactation periods (Chang et al. 2018).

PFNA

Epidemiological Studies. Khalil et al. (2016) found an increase in the risk of osteoporosis in women NHANES participants that was associated with serum PFNA levels. Increasing serum PFNA levels did not result in alterations in bone mineral density of the lumbar spine or femur neck, but was inversely associated with total femur bone mineral density in women with serum PFNA levels in the fourth quartile. A study of 48 obese children found an inverse association between serum PFNA levels and bone mineral density; however, the association was no longer significant after adjusting for multiple testing (Khalil et al. 2018).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days (3M 2001) or 90 days (Lieder et al. 2009a) did not induce morphological alterations in skeletal muscle.

PFBA

Laboratory Animal Studies. PFBA administered to rats by gavage in doses of up to 184 mg/kg/day for 5 days did not induce morphological alterations in skeletal muscle (3M 2007a). Administration of 150 mg/kg/day PFBA for 28 days or 30 mg/kg/day for 90 days did not induce gross or microscopic alterations in bone (femur and sternum) or skeletal muscle (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

Laboratory Animal Studies. An intermediate-duration gavage study did not find histological alterations in the bone or muscle of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b).

2.9 HEPATIC

Overview. Epidemiological studies on perfluoroalkyls have examined three potential hepatic outcomes: liver disease, alterations in serum enzyme and bilirubin levels, and alterations in serum lipid levels. Summaries of the epidemiological studies examining these outcomes are presented in Tables 2-10, 2-11, and 2-12, with more detailed descriptions presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 7. There are limited epidemiological data on potential associations between serum perfluoroalkyls and risk of liver disease. Occupational exposure and community studies did not find increased risk of liver disease associated with PFOA or PFOS. As assessed by serum enzyme and bilirubin levels, the epidemiological studies provide suggestive evidence of liver damage. Increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT) levels and decreases in serum bilirubin levels have been reported in occupational, community, and/or general population studies. These increases in serum enzyme levels, particularly ALT, are associated with increasing levels of PFOA, PFOS, and PFHxS; it is noted that there is considerable variability across studies and not all of the studies adjusted for potential confounders. No consistent results were found for PFNA. The results of available epidemiological studies suggest associations between increases in serum lipids, particularly total cholesterol and LDL cholesterol, and serum PFOA, PFOS, PFNA, and PFDA. For PFHxS, PFUnA, PFHpA, PFBS, PFBA, and PFDoDA, there are too few studies or the results are too inconsistent to determine if they also would affect serum lipid levels at environmental exposure levels. No epidemiological studies examining hepatic endpoints were identified for PFHxA or FOSA.

Numerous animal studies have evaluated the hepatotoxicity of perfluoroalkyls following inhalation, oral, and dermal exposure; summaries of these studies are presented in Tables 2-1, 2-2, 2-3, 2-4, 2-5, and 2-6 and the NOAEL and LOAEL values are graphically presented in Figures 2-6, 2-7, 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFHpA.

The results of these studies provide strong evidence that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBS, PFBA, PFDoDA, and PFHxA toxicity. Observed effects in rodents include increases in liver weight; hepatocellular hypertrophy, hyperplasia, and necrosis; and decreases in serum cholesterol and triglyceride levels. As discussed in greater detail in Section 2.20, these effects are believed to be initiated by PPARα; however, studies in PPARα-null mice suggest that other mechanisms are also involved. Increases in liver weight have also been observed in monkey studies for PFOA and PFOS; these studies have also found alterations in serum lipid levels and hepatocellular hypertrophy (PFOS only).

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Liver problems (self-reported)	SPR 1.01 (0.64–1.59)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median 2005/2006)	Liver disease	HR 0.97 (0.92–1.03), no lag per ln increase in PFOA HR 0.98 (0.93–1.04), 10-year lag
		Enlarged liver, fatty liver, or cirrhosis	HR 0.97 (0.91–1.04), no lag per ln increase in PFOA HR 1.00 (0.94–1.07), 10-year lag
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Non-hepatitis liver disease risk	NS (p=0.86), no lag NS (p=0.40), 10-year lag
PFOS			
Alexander et al. 2003 Occupational (n=2,083)	NR	Liver cirrhosis deaths	SMR 0.81 (0.10–2.94)
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Liver disease	OR 1.21 (0.56–2.60)
		Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)

Table 2-10. Summary of Liver Disease in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2004a Occupational (n=652 exposed, n=659 for non-exposed)	NR	Cholelithiasis or acute cholecystitis	**RRE_pC 8.6 (1.1–>100)*** **RRE_pC 25 (2.1–>100)*, workers with ≥10 years high exposure potential**
		Liver disease	RRE_pC 1.2 (0.2–8.6)
		Biliary duct disorders	RRE_pC 1.6 (0.8–2.9) **RRE_pC 2.6 (1.2–5.5)*, workers with ≥10 years high exposure potential**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NR = not reported; NS = not significant; OR = odds ratio; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RRE_pC = risk ratio episode of care; SMR = standardized mortality ratio; SPR = standard prevalence ratio

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA current workers) 6,810 ng/mL (mean former workers)	AST	NS (p>0.05) (34 current workers)
		ALT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		GGT	NS (p>0.05) (34 current workers) **Association (p<0.01)* (56 current, former, non-exposed workers)**
		Total bilirubin	**Inverse association (p<0.01)* (56 current, former, non-exposed workers)**
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	ALT	NS (p=0.32)
		AST	NS (p=0.80)
		GGT	NS (p=0.81)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	ALT	NS (p=0.82, 0.30, 0.73) differences between exposure groups for each measurement period
		AST	NS (p=0.33, 0.45, 0.83) differences between exposure groups for each measurement period
		GGT	NS (p=0.24, 0.41, 0.78) differences between exposure groups for each measurement period
		Total bilirubin	NS (p=0.48, 0.11, 0.58) differences between exposure groups for each measurement period
		Direct bilirubin	NS (p=0.82, 0.05, 0.74) differences between exposure groups for each measurement period

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2170 ng/mL (mean 8th PFOA decile) 12,150 ng/mL (mean 10th PFOA decile)	GGT Elevated GGT	**Association (p=0.05)*** OR 1.0 (0.3–2.9), 10th decile
		Total bilirubin	**Inverse association (p=0.001)***
		ALT Elevated ALT	NS (p=0.06) OR 1.2 (0.5–3.4), 10th decile
		AST	NS (p=0.55)
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total bilirubin	**Association (p=0.006)***
		AST	**Association (p=0.009)***
		ALT	NS (p>0.05)
		GGT	NS (p>0.05)
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	GGT	**Association (p=0.016)***
		AST	NS (p=0.317)
		ALT	NS (p=0.124)
		Bilirubin	NS (p=0.590)
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	AST	**Association (p=0.02)***
		ALT	NS (p=0.38)
Darrow et al. 2016 Community (C8) (n=28,831)	Estimated cumulative 16.5 ng/mL (median PFOA in 2005/2006)	ALT	**Association (p<0.0001 for trend)*, estimated cumulative levels** **Association (p<0.0001 for trend)*, 2005/2006 levels**
		GGT	NS (p=0.1021), cumulative levels NS (p=0.1552), 2005/2006 levels
		Bilirubin	**Inverse association (p=0.0029 for trend)*, estimated cumulative levels** **Inverse association (p=0.0036 for trend)*, 2005/2006 levels**

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	ALT Abnormal ALT	NS (p>0.05) NS (p>0.05)
		AST Abnormal AST	NS (p>0.05) **Inverse association (p=0.03)***
		GGT Abnormal GGT	NS (p>0.05) NS (p>0.05)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.03–1.37)*, 3rd decile**
		GGT Abnormal GGT	**Correlation (p<0.001)*** NS (p=0.213 for trend)
		Direct bilirubin Abnormal bilirubin	NS (p>0.05) NS (p=0.496 for trend)
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	ALT	NS (p=0.05)
		AST	NS (p=0.22)
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	ALT	**Association (p<0.001)***
		Elevated ALT	**Association (p=0.007 for trend)***
		AST	**Association (p<0.01)***
		Elevated AST	NS (p=0.058 for trend).
		GGT	**Association (p<0.01)***
		Elevated GGT	**Association (p=0.042 for trend)***
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p<0.001 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	5.05 and 4.06 ng/mL (geometric mean PFOA in males and females)	ALT	**Association (p=0.005)***
		GGT	**Association (p=0.019)***
		Total bilirubin	NS (p=0.645)
Yamaguchi et al. 2013 General population (n=608)	2.1 ng/mL (mean PFOA)	ALT	**Association (p=0.02)***
		AST	**Association (p=0.001)***
		GGT	**Association (p=0.03)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Grice et al. 2007 Occupational (n=1,400)	1,300–1,970 ng/mL (high potential workers)	Cholelithiasis	OR 0.91 (0.57–1.46)
		Cholecystitis	OR 1.15 (0.65–2.06)
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean PFOS in 1997 in Decatur and Antwerp)	AST	NS (p=0.14 for trend), 1995 NS (p=0.67 for trend), 1997
		ALT	NS (p=0.38 for trend), 1995 NS (p=0.46 for trend), 1997
		GGT	NS (p=0.71 for trend), 1995 NS (p=0.34 for trend), 1997
Olsen et al. 2003a Occupational (n=518)	2460 ng/mL (median 4th PFOS quartile)	AST	NS (p>0.05), no adjustments
		ALT Risk of abnormal ALT	**Higher levels (p<0.05)*, males only with no adjustments** OR 2.1 (0.6–7.3)
		GGT Risk of abnormal GGT	**Difference (p<0.05)*, females only with no adjustments** OR 2.0 (0.7–5.8)
Gallo et al. 2012 Community (C8) (n=46,452)	NR	ALT Abnormal ALT	**Correlation (p<0.001)*** **OR 1.19 (1.04–1.37)*, 5th decile**
		GGT Abnormal GGT	NS (p>0.05) **Association (p=0.047 for trend)***
		Direct bilirubin Abnormal bilirubin	**Correlation (p<0.001)*** **Association (p=0.015 for trend)***

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	11.3 ng/mL (median PFOS)	ALT	NS (p>0.01)
		Elevated ALT	NS (p=0.370 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.438 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.654 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	**Association (p=0.028 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	27.39 and 22.20 ng/mL (geometric mean PFOS in males and females)	ALT	NS (p=0.066)
		GGT	NS (p=0.808)
		Total bilirubin	NS (p=0.223)
Yamaguchi et al. 2013 General population (n=608)	5.8 ng/mL (mean PFOS)	ALT	**Association (p=0.03)***
		AST	**Association (p=0.01)***
		GGT	**Association (p=0.03)***
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	ALT	**Association (p<0.01)***
		Elevated ALT	NS (p=0.484 for trend)
		AST	**Association (p<0.001)***
		Elevated AST	NS (p=0.230 for trend)
		GGT	NS (p>0.01)
		Elevated GGT	NS (p=0.415 for trend)
		Total bilirubin	**Association (p<0.01)***
		Elevated bilirubin	**Association (p=0.041 for trend)***
Lin et al. 2010 General population (NHANES) (n=2,216)	2.29 and 1.72 ng/mL (geometric mean PFHxS in males and females)	ALT	NS (p=0.691)
		GGT	NS (p=0.898)
		Total bilirubin	NS (p=0.063)

Table 2-11. Summary of Alterations in Serum Hepatic Enzymes and Bilirubin Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	ALT	NS, longitudinal analysis
		AST	NS, longitudinal analysis
		GGT	NS, longitudinal analysis
		Bilirubin	NS, longitudinal analysis
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	ALT	**Association (p<0.001)***
		Elevated ALT	NS (p=0.042 for trend)
		AST	NS (p>0.01)
		Elevated AST	NS (p=0.516 for trend)
		GGT	**Association (p<0.01)***
		Elevated GGT	NS (p=0.126 for trend)
		Total bilirubin	NS (p>0.01)
		Elevated bilirubin	NS (p=0.614 for trend)
Lin et al. 2010 General population (NHANES) (n=2,216)	0.89 and 0.72 ng/mL (geometric mean PFNA in males and females)	ALT	NS (p=0.131)
		GGT	NS (p=0.857)
		Total bilirubin	NS (p=0.053)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transferase; OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa 2004 Occupational (n=35)	NR	Total cholesterol	**Association (p=0.03)***
		Non-HDL cholesterol	**Association (p=0.03)***
		HDL cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		Total triglycerides	NS (p>0.05)
Costa et al. 2009 Occupational (n=37 current workers; n=16 former workers; n=107 non-exposed workers)	12,930 ng/mL (mean PFOA in current workers) 6,810 ng/mL (mean PFOA in former workers)	Total cholesterol	**Association (p=0.005)* (current workers)** **Association (p<0.05)* (56 current, former, non-exposed workers)**
		HDL cholesterol	NS (p>0.05) (34 current workers)
		Triglycerides	NS (p>0.05)
Gilliland 1992; Gilliland and Mandel 1996 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Total cholesterol	NS (p=0.62)
		Total LDL	NS (p=0.87)
		Total HDL	NS (p=0.66)
Olsen et al. 2000 Occupational (n=111, 80, and 74 in 1993, 1995, and 1997)	5,000, 6,400, and 6,400 ng/mL (mean PFOA in 1993, 1995, and 1997) Workers divided into three groups: 0–<1,000, 1,000–<10,000, and ≥10,000 ng/mL	Total cholesterol	NS (p=0.45, 0.48, 0.08) differences between exposure groups for each measurement period
		LDL cholesterol	NS (p=0.84, 0.96, 0.11) differences between exposure groups for each measurement period
		HDL cholesterol	NS (p=0.32, 0.70, 0.40) differences between exposure groups for each measurement period
		Triglycerides	NS (p=0.77, 0.07, 0.13) differences between exposure groups for each measurement period

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen and Zobel 2007 Occupational (n=552)	2,170 ng/mL (mean of 8th PFOA decile) 12,150 ng/mL (mean of 10th PFOA decile)	Total cholesterol	NS (p=0.20)
		Elevated total cholesterol	OR 1.1 (0.5–2.6), 10th decile
		LDL cholesterol	NS (p=0.81)
		Elevated LDL cholesterol	OR 1.2 (0.5–2.8), 10th decile
		HDL cholesterol	**Association (p=0.01)***
		Decreased HDL cholesterol	OR 1.8 (0.7–4.8), 10th decile
		Triglycerides	**Association (p=0.0001)***
		Elevated triglycerides	OR 1.8 (0.8–4.4), 10th decile
Sakr et al. 2007a Occupational (n=454)	1,130 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.011)***
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
		Total bilirubin	**Association (p=0.006)***
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.008)***
		VLDL cholesterol	**Association (p=0.031)***
		HDL cholesterol	NS (p=0.680)
		Triglycerides	NS (p=0.384)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Elevated cholesterol	NS (p=0.56), no lag NS (p=0.62), 10-year lag
Wang et al. 2012 Occupational (n=55)	2,157.74 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.36)
		LDL cholesterol	NS (p=0.43)
		HDL cholesterol	**Inverse association (p=0.01)***
		Triglycerides	NS (p=0.37)
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Total cholesterol Abnormal cholesterol	NS (p>0.05) NS (p>0.05)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fitz-Simon et al. 2013 Community (C8) (n=560 adults)	140.1 and 68.2 ng/mL (mean PFOA at first and second examinations)	Total cholesterol	**-1.65% (-0.32 to -2.97)*, 50% decrease in PFOA**
		LDL cholesterol	**-3.58% (-1.47 to -5.66)*, 50% decrease in PFOA**
		HDL cholesterol	-1.33% (0.21 to -2.85), 50% decrease in PFOA
		Triglycerides	0.78% (5.34 to -3.58), 50% decrease in PFOA
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	77.7 ng/mL (mean PFOA in children) 61.8 ng/mL (mean PFOA in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	OR 1.1 (1.0–1.3), 2nd quintile
		LDL cholesterol	**Association (p=0.001)*, children 5th quintile** **Association (p=0.004)*, adolescents 5th quintile**
		Abnormal LDL levels	OR 1.2 (1.0–1.5), 2nd quintile
		HDL cholesterol	NS (p=0.88), children 5th quintile NS (p=0.20), adolescents 5th quintile
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	80.3 ng/mL (mean PFOA) 13.2–26.5 ng/mL (2nd PFOA quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.21 (1.12–1.31)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2012 Community (n=132)	378.30 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.85)
		LDL cholesterol	NS (p=0.97)
		Triglycerides	NS (p=0.73)
		HDL cholesterol	NS (p=0.39)
Winquist and Steenland 2014a Community (C8) (n=28,541)	142–<234 ng/mL (estimated 2nd quintile for cumulative PFOA)	Hypercholesterolemia	**HR 1.24 (1.15–1.33)*, 2nd quintile for estimated cumulative exposure**
Eriksen et al. 2013 General population (n=753)	7.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01)***
Fisher et al. 2013 General population (n=2,368)	2.46 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.22)
		High cholesterol levels	OR 1.5 (0.86–2.62), 4th quartile
		Non HDL cholesterol	NS (p=0.13)
		LDL cholesterol	NS (p=0.63)
		HDL cholesterol	NS (p=0.96)
Fu et al. 2014a General population (n=133)	1.43 ng/mL (median PFOA)	Total cholesterol	**Association (p=0.015)***
		Elevated cholesterol	OR 0.55 (0.09–3.31)
		LDL cholesterol	**Association (p=0.022)***
		Elevated LDL	OR 0.71 (0.14–3.49)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.67 (0.13–3.51)
		Triglycerides	NS (p=0.298)
		Elevated triglyceride	OR 1.97 (0.59–6.55)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	4.2 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.0170 for trend)***
		Elevated cholesterol	**OR 1.44 (1.11–1.88, p=0.0253 for trend)*, log transformed PFOA**
		LDL cholesterol	**Association (p=0.0027 for trend)***
		Elevated LDL	NS (p=0.0539 for trend)
		HDL cholesterol	NS (p=0.1769 for trend)
		Decreased HDL	NS (p=0.1493 for trend)
		Triglycerides	NS (p=0.9943 for trend)
		Elevated triglycerides	NS (p=0.5975 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Total cholesterol	β -2.256 (-11.490–6.978, p=0.630)
		LDL cholesterol	β 3.899 (-4.810–12.608, p=0.377)
		Triglycerides	β 0.020 (-0.134–0.175, p=0.796)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	Total cholesterol	**β 0.09 mg/dL (0.04–0.14, p<0.001)***
		LDL cholesterol	**β 0.11 mg/dL (0.03–0.19, p=0.006)***
		HDL cholesterol	β 0.04 mg/dL (-0.04–0.12, p=0.34)
		Triglycerides	**β 0.14 mg/dL (0.02 to 0.27, p=0.03)***
Liu et al. 2018b General population (NHANES, n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Total cholesterol	Association (p<0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	Association (p<0.01)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015a General population (n=111 for 7-year-old and n=88 for 15-year-old girls	1.1–3.1, 3.2–4.4, and 4.5–16.4 ng/mL (maternal PFOA for 1st, 2nd, and 3rd tertiles)	Total cholesterol in 7-year-olds	**Association (β 13.75, 0.05–27.45)*, 1st tertile** NS, 2nd and 3rd tertiles
		Total cholesterol in 15-year-olds	**Association (β 17.19, 0.405–33.93)*, 1st tertile** NS, 2nd and 3rd tertiles
		LDL cholesterol in 7-year-olds	**Association (β 14.01, 3.26–24.76)*, 1st tertile** NS, 2nd and 3rd tertiles

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		LDL cholesterol in 15-year-olds	**Association (β 14.261, 0.25–28.26)*, 1st tertile** NS, 2nd and 3rd tertiles
		HDL cholesterol	NS (β -0.40, -1.82–1.01), 3rd tertile 7-year-olds NS (β -0.520, -2.10–1.06), 3rd tertile 15-year-olds
		Triglycerides	NS (β -0.020, -0.068–0.029), 3rd tertile, 7-year-olds NS (β -0.013, -0.051–0.025), 3rd tertile, 15-year-olds
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	2.32 ng/mL (maternal geometric mean PFOA)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.03 (-0.12–0.21)
		HDL cholesterol	β -0.04 (-0.15–0.08)
		Triglycerides	β -0.01 (-0.17–0.16)
Nelson et al. 2010 General population (NHANES) (n=860)	4.6 ng/mL (mean PFOA)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.84)
		Non-HDL cholesterol	**Association (p=0.05)* β 1.38 (0.12–2.65), per ng/mL increase in PFOA**
		HDL cholesterol	NS (p=0.34)
Skuladottir et al. 2015 General population (n=854 pregnant women)	4.1 ng/mL (mean PFOA)	Total cholesterol	**Association (p=0.01 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	2.25 ng/mL (50th PFOA percentile)	Total cholesterol	NS (β 2.58; -4.32–9.47), per ln-unit increase in PFOA
		LDL cholesterol	NS (β 0.35 -3.97–8.48), per ln-unit increase in PFOA
		HDL cholesterol	**Association (β 3.42 0.56–6.28)*, 4th quartile**
		Triglycerides	NS (β 0.00 (-0.07–0.06), per ln-unit increase in PFOA
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	9.3 ng/mL (median PFOA)	Triglycerides	NS (p=0.91), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	HDL cholesterol	β 0.15 (-0.17–0.46)
		Triglycerides	**β 2.3 (0.77–8.38)***
Zeng et al. 2015 General population (n=225 adolescents, 12–15 years old)	1.1 and 0.92 ng/mL (mean PFOA in boys and girls)	Total cholesterol	**Association (p=0.001)***
		LDL cholesterol	**Association (p=0.002)***
		HDL cholesterol	NS (p=0.06)
		Triglycerides	**Association (p<0.001)***
PFOS			
Olsen et al. 1999 Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Total cholesterol	NS (p=0.96 for trend), 1995 **Association (p=0.006 for trend)*, 1997**
		LDL cholesterol	NS (p=0.87 for trend), 1995 **Association (p=0.01 for trend)*, 1997**
		HDL cholesterol	**Inverse association (p=0.04 for trend)*, 1995** NS (p=0.34) 1997
		Triglycerides	NS (p=0.35 for trend), 1995 NS (p=0.67 for trend), 1997

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Olsen et al. 2003a Occupational (n=518)	2,460 ng/mL (median 4th PFOS quartile)	Total cholesterol	NS (p>0.05), no adjustments **Association (p=0.04)*, with adjustments**
		HDL cholesterol	NS (p>0.05), no adjustments
		Triglycerides	**Higher levels (p<0.05)*, males only with no adjustments** **Association (p=0.01)*, with adjustments**
Frisbee et al. 2010 Community (C8) (n=12,476 children and adolescents)	23.6 ng/mL (mean PFOS in children) 21.9 ng/mL (mean PFOS in adolescents)	Total cholesterol	**Association (p<0.001)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal cholesterol	**OR 1.3 (1.1–1.4)*, 2nd quintile**
		LDL cholesterol	**Association (p=0.002)*, children 5th quintile** **Association (p<0.001)*, adolescents 5th quintile**
		Abnormal LDL levels	**OR 1.2 (1.0–1.5)*, 2nd quintile**
		HDL cholesterol	**Association (p=0.007)*, children 5th quintile** **Association (p=0.001)*, adolescents 5th quintile**
		Triglycerides	NS (p=0.1), children 5th quintile NS (p=0.1), adolescents 5th quintile
Steenland et al. 2009b Community (C8) (n=46,294)	22.4 ng/mL (mean PFOS) 13.3–19.5 ng/mL (2nd quartile)	Total cholesterol	**Association (p<0.001 for trend)***
		Abnormal cholesterol	**OR 1.14 (1.05–1.23)*, 2nd quartile**
		LDL cholesterol	**Association (p<0.05 for trend)***
		HDL cholesterol	NS (p>0.05)
		Triglycerides	**Association (p<0.05 for trend)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Château-Degat et al. 2010 General population (n=723)	25.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.086)
		LDL cholesterol	NS (p=0.242)
		HDL cholesterol	**Association (p<0.001)*, men** **Association (p=0.001)*, women**
		Triglycerides	**NS (p=0.162), men** **Inverse association (p=0.040)*, women**
Eriksen et al. 2013 General population (n=753)	36.1 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.02)***
Fisher et al. 2013 General population (n=2,368)	8.04 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.35)
		High cholesterol levels	OR 1.36 (0.87–2.12), 4th quartile
		Non HDL cholesterol	NS (p=0.14)
		LDL cholesterol	NS (p=0.42)
		HDL cholesterol	NS (p=0.33)
Fu et al. 2014a General population (n=133)	1.47 ng/mL (median PFOS)	Total cholesterol	NS (p=0.287)
		Elevated cholesterol	OR 2.27 (0.47–10.92)
		LDL cholesterol	NS (p=0.357)
		Elevated LDL	OR 2.27 (0.50–10.37)
		HDL cholesterol	NS (p=0.260)
		Elevated HDL	OR 0.29 (0.06–1.50)
		Triglycerides	NS (p=0.711)
		Elevated triglycerides	OR 1.26 (0.41–3.90)
Geiger et al. 2014b General population (NHANES) (n=815 12–18-year-old adolescents)	17.7 ng/mL (mean PFOS)	Total cholesterol	NS (p=0.0512 for trend)
		Elevated cholesterol	**OR 1.35 (1.11–1.64, p=0.0183 for trend)*, log transformed PFOS**
		LDL cholesterol	**Association (p=0.0081 for trend)***
		Elevated LDL	**OR 1.48 (1.15–1.90, p=0.0178 for trend)*, log transformed PFOS**

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		HDL cholesterol	NS (p=0.9703)
		Decreased HDL	NS (p=0.9873 for trend)
		Triglycerides	NS (p=0.1104 for trend)
		Elevated triglycerides	NS (p=0.2418 for trend)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Total cholesterol	β -0.450 (-10.667–9.768, p=0.931)
		LDL cholesterol	β 2.507 (-6.879–11.893, p=0.598)
		Triglycerides	β -0.020 (-0.186–0.146, p=0.809)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	Total cholesterol	**β 0.08 mg/dL (0.05–0.12, p<0.001)***
		LDL cholesterol	**β 0.10 mg/dL (0.05–0.16, p<0.001)***
		HDL cholesterol	**β 0.06 mg/dL (0.003–0.13, p=0.04)***
		Triglycerides	β 0.04 mg/dL (0.05–0.13, p=0.36)
Liu et al. 2018b General population (NHANES, n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Maisonet et al. 2015b General population (n=111 for 7-year-old and n=88 for 15-year-old girls)	23.5–94.5 ng/mL (3rd tertile maternal PFOS)	Total cholesterol	NS (β -0.10, -0.73–0.54), 7-year-olds **Association (β -0.77, -1.40 to -0.13)*, 15-year-olds**
		LDL cholesterol	NS (β 0.02, -0.48–0.53), 7-year-olds **Association (β -0.54, -1.08 to -0.003)*, 15-year-olds**
		HDL cholesterol	NS (β -0.04, -0.33–0.25), 7-year-olds NS (β -0.18, -0.47–0.12), 15-year-olds
		Triglycerides	NS (β -0.004, -0.015–0.006), 7-year-olds NS (β -0.004, -0.011–0.004), 15-year-olds

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	5.80 ng/mL (maternal geometric mean PFOS)	Total cholesterol	β -0.02 (-0.10–0.15)
		LDL cholesterol	β 0.02 (-0.12–0.15)
		HDL cholesterol	β -0.03 (-0.14–0.09),
		Triglycerides	β 0.05 (-0.06–0.17)
Nelson et al. 2010 General population (NHANES) (n=860)	25.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01)***
		LDL cholesterol	NS (p=0.27)
		Non-HDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.78)
Skuladottir et al. 2015 General population (n=854 pregnant women)	22.3 ng/mL (mean PFOS)	Total cholesterol	**Association (p=0.01 for trend)***
Starling et al. 2014a General population (n=854 pregnant women)	13.03 ng/mL (50th PFOS percentile)	Total cholesterol	**Association (p<0.05)***
		LDL cholesterol	NS (β 6.48, -0.07–13.03), per ln-unit increase in PFOS
		HDL cholesterol	**Association (β 4.39, 2.37–6.42)*, per ln-unit increase in PFOS**
		Triglycerides	NS (β -0.02, -0.09–0.04), per ln-unit increase in PFOS
Timmermann et al. 2014 General population (n=499 children, 8–10 years old)	41.5 ng/mL (median PFOS)	Triglycerides	NS (p=0.78), normal weight children **Association (p=0.002)*, obese children**
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	HDL cholesterol	β 0.02 (-0.17–0.2)
		Triglycerides	β 0.3 (-0.63–1.22)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	32.4 and 34.2 ng/mL (mean PFOS in boys and girls)	Total cholesterol	**Association (p<0.001)***
		LDL cholesterol	**Association (p<0.001)***
		HDL cholesterol	NS (p=0.72)
		Triglycerides	**Association (p=0.05)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Fisher et al. 2013 General population (n=2,368)	2.18 ng/mL (mean PFHxS)	Total cholesterol	**Association (p=0.005)***
		High cholesterol levels	**OR 1.27 (1.11–1.45)*, 4th quartile**
		Non HDL cholesterol	**Association (p=0.002)***
		LDL cholesterol	**Association (p=0.02)***
		HDL cholesterol	NS (p=0.67)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Total cholesterol	β 0.989 (-9.526–11.503, p=0.853)
		LDL cholesterol	β -4.222 (-13.979–5.534, p=0.393)
		Triglycerides	β 0.081 (-0.092–0.253, p=0.355)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.04–0.06, p=0.01)***
		LDL cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.02)***
		HDL cholesterol	β 0.03 mg/dL (-0.02–0.07, p=0.26)
		Triglycerides	β 0.04 mg/dL (-0.02–0.11, p=0.20)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.61 ng/mL (maternal geometric mean PFHxS)	Total cholesterol	β 0.02 (-0.09–0.12)
		LDL cholesterol	β -0.01 (-0.12–0.09)
		HDL cholesterol	β -0.01 (-0.11 to 0.10)
		Triglycerides	**β 0.11 (0.01–0.21)***
Nelson et al. 2010 General population (NHANES) (n=860)	2.6 ng/mL (mean PFHxS)	Total cholesterol	NS (p=0.07)
		LDL cholesterol	NS (p=0.10)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.11)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.60 ng/mL (50th PFHxS percentile)	Total cholesterol	NS (β 3.00, -1.75–7.76), per ln-unit increase in PFHxS
		LDL cholesterol	NS (β 1.92, -2.50–6.33), per ln-unit increase in PFHxS
		HDL cholesterol	**Association (β 1.46; 0.19–2.73)*, per ln-unit increase in PFHxS**
		Triglycerides	NS (β -0.01, -0.05–0.03), per ln-unit increase in PFHxS
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	HDL cholesterol	**β 0.22 (0 to 0.43, p<0.05)***
		Triglycerides	**β 1.18 (0.12–2.25, p<0.05)***
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	2.1 and 2.1 ng/mL (mean PFHxS in boys and girls)	Total cholesterol	NS (p=0.23)
		LDL cholesterol	NS (p=0.17)
		HDL cholesterol	NS (p=0.54)
		Triglycerides	NS (p=0.15)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Total cholesterol	NS, longitudinal analysis
		Triglycerides	NS, longitudinal analysis
Fu et al. 2014a General population (n=133)	0.37 ng/mL (median PFNA)	Total cholesterol	**Association (p=0.002)***
		Elevated cholesterol	OR 1.03 (0.24–4.46)
		LDL cholesterol	**Association (p=0.004)**
		Elevated LDL	OR 2.51 (0.59–10.74)
		HDL cholesterol	NS (p=0.191)
		Lowered HDL	OR 1.06 (0.20–5.57)
		Triglycerides	NS (p=0.460)
		Elevated triglycerides	OR 0.80 (0.26–2.49)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Total cholesterol	β -1.624 (-10.218–6.970, p=0.709)
		LDL cholesterol	β 2.304 (-6.558–11.167, p=0.607)
		Triglycerides	β 0.065 (-0.092–0.221, p=0.820)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	Total cholesterol	**β 0.05 mg/dL (0.01–0.09, p=0.01)***
		LDL cholesterol	**β 0.07 mg/dL (0.01–0.14, p=0.01)***
		HDL cholesterol	β 0.05 mg/dL (0.02–0.12, p=0.13)
		Triglycerides	β -0.07 mg/dL (0.11–0.01, p=0.89)
Manzano-Salgado et al. 2017b General population (n=1,230 children; evaluated at 4 years of age)	0.66 ng/mL (maternal geometric mean PFNA)	Total cholesterol	β-0.00 (-0.11–0.12)
		LDL cholesterol	β 0.01 (-0.10–0.12)
		HDL cholesterol	β -0.03 (-0.14–0.08),
		Triglycerides	β 0.03 (-0.07–0.14)
Nelson et al. 2010 General population (NHANES) (n=860)	1.3 ng/mL (mean PFNA)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.08)
		Non-HDL cholesterol	**Association (p=0.04)***
		HDL cholesterol	NS (p=0.31)
Starling et al. 2014a General population (n=854 pregnant women)	0.39 ng/mL (50th PFNA percentile)	Total cholesterol	NS (β 0.01, -5.98–6.00), per ln-unit increase in PFNA
		LDL cholesterol	NS (β -2.15, -7.31–3.02), per ln-unit increase in PFNA
		HDL cholesterol	**Association (β 2.84; 0.97–4.71)*, per ln-unit increase in PFNA**
		Triglycerides	NS (β -0.02, -0.07–0.03), per ln-unit increase in PFNA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	HDL cholesterol	**β 0.3 (0.05–0.56)***
		Triglycerides	**β 1.54 (0.27–2.8)***

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.8 and 0.9 ng/mL (mean PFNA in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	**Association (p=0.05)***
		HDL cholesterol	NS (p=0.37)
		Triglycerides	**Association (p=0.007)***
PFDA			
Fu et al. 2014a General population (n=133)	0.19 ng/mL (median PFDA)	Total cholesterol	**Association (p=0.048)***
		Elevated cholesterol	OR 3.84 (0.87–16.95)
		LDL cholesterol	NS (p=0.251)
		Elevated LDL	OR 2.17 (0.52–9.04)
		HDL cholesterol	**Association (p=0.007)***
		Elevated HDL	OR 2.21 (0.49–10.07)
		Triglycerides	NS (p=0.317)
		Elevated triglycerides	OR 0.51 (0.17–1.58)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Total cholesterol	β -3.330 (-7.484–0.824, p=0.115)
		LDL cholesterol	β -1.858 (-5.694–1.979, p=0.339)
		Triglycerides	β -0.036 (-0.103–0.032, p=0.302)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	Total cholesterol	**β 0.04 mg/dL (0.02–0.06, p<0.001)***
		LDL cholesterol	**β 0.04 mg/dL (0.02–0.06, p=0.03)***
		HDL cholesterol	**β 0.05 mg/dL (0.02–0.09, p=0.003)***
		Triglycerides	β -0.01 mg/dL (-0.047–0.057,p=0.85)
Starling et al. 2014a General population (n=854 pregnant women)	0.09 ng/mL (50th PFDA percentile)	Total cholesterol	NS (β 1.84, -2.12–5.79), per ln-unit increase in PFDA
		LDL cholesterol	NS (β 0.19, -3.30–3.69), per ln-unit increase in PFDA
		HDL cholesterol	**Association (β 2.54, 1.22–3.87)*, per ln-unit increase in PFDA**
		Triglycerides	NS (β -0.03, -0.07–0.01), per ln-unit increase in PFDA

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	HDL cholesterol	β 0.24 (0.04–0.52)
		Triglycerides	β 0.64 (-0.77–2.05)
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	1.0 and 1.0 ng/mL (mean PFDA in boys and girls)	Total cholesterol	NS (p=0.74)
		LDL cholesterol	NS (p=0.85)
		HDL cholesterol	NS (p=0.47)
		Triglycerides	NS (p=0.92)
PFUnA			
Fu et al. 2014a General population (n=133)	0.26 ng/mL (median PFUnA)	Total cholesterol	NS (p=0.184)
		Elevated cholesterol	OR 3.70 (0.76–18.03)
		LDL cholesterol	NS (p=0.270)
		Elevated LDL	OR 4.16 (0.96–18.00)
		HDL cholesterol	NS (p=0.279)
		Elevated HDL	OR 0.54 (0.11–2.57)
		Triglycerides	NS (p=0.755)
		Elevated triglycerides	OR 0.74 (0.25–2.21)
Kang et al. 2018 General population (n=150 children ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Total cholesterol	**β 7.906 (2.681–13.131, p=0.003)***
		LDL cholesterol	**β 7.101 (2.448–11.754, p=0.003)***
		Triglycerides	β 0.043 (-0.042–0.129, p=0.317)
Koshy et al. 2017 General population (WTCHR, n=180 children; n=222 children in comparison group)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	Total cholesterol	β 0.02 mg/dL (0–0.04, p=0.06)
		LDL cholesterol	β 0.01 mg/dL (-0.02–0.04, p=0.49)
		HDL cholesterol	**β 0.04 mg/dL (0.01–0.07, p=0.01)***
		Triglycerides	β -0.04 mg/dL (-0.09–0.003, p=0.07)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Starling et al. 2014a General population (n=854 pregnant women)	0.22 ng/mL (50th PFUnA percentile)	Total cholesterol	NS (β 0.89, -3.28–5.06), per ln-unit increase in PFUnA
		LDL cholesterol	NS (β -2.36, -5.97–1.25), per ln-unit increase in PFUnA
		HDL cholesterol	**Association (β 4.05, 2.75–5.35)*, per ln-unit increase in PFUnA**
		Triglycerides	NS (β -0.04, -0.08–0.00), per ln-unit increase in PFUnA
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	HDL cholesterol	β 0.11 (-0.11–0.34)
		Triglycerides	β 0.61 (-0.48–1.7)
PFHpA			
Fu et al. 2014a General population (n=133)	0.04 ng/mL (median PFHpA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
Yang et al. 2018 General population (n=148 men; 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	HDL cholesterol	β -0.33 (-0.77–0.11)
		Triglycerides	β -0.92 (-3.12–1.28)
PFBS			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	0.5 and 0.4 ng/mL (mean PFBS in boys and girls)	Total cholesterol	**Association (p=0.04)***
		LDL cholesterol	NS (p=0.14)
		HDL cholesterol	NS (p=0.15)
		Triglycerides	NS (p=0.81)

Table 2-12. Summary of Serum Lipid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFBA			
Fu et al. 2014a General population (n=133)	0.11 ng/mL (median PFBA)	Total cholesterol	NS (p>0.05)
		LDL cholesterol	NS (p>0.05)
		HDL cholesterol	NS (p>0.05)
		Triglycerides	NS (p>0.05)
PFDoDA			
Zeng et al. 2015 General population (n=225 children, 12–15 years old)	4.5 and 4.4 ng/mL (mean PFDoDA in boys and girls)	Total cholesterol	NS (p=0.37)
		LDL cholesterol	NS (p=0.44)
		HDL cholesterol	NS (p=0.68)
		Triglycerides	NS (p=0.40)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 7 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HDL = high density lipoprotein; LDL = low density lipoprotein; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; VLDL = very low-density lipoprotein; WTCHR = World Trade Center Health Registry

To address concern over the relevance of liver enlargement in rodents to human health risk, the European Society of Toxicologic Pathology (ESTP) convened an expert panel to define what constitutes an adverse hepatic effect and whether hepatic effects induced by nuclear hormone receptors such as PPARα, constitutive androstane receptor (CAR), or pregnane X receptor (PXR) are rodent-specific adaptive reactions; the findings of the panel are summarized by Hall et al. (2012). As discussed by Hall et al. (2012), criteria were established for determining whether increases in liver organ weight and liver cell hypertrophy observed in studies of rodents exposed to agents inducing enzyme induction can be considered adaptive responses and of little relevance to humans. According to the ESTP criteria, increases in liver weight without histological evidence, such as (1) degenerative or necrotic changes including hepatocyte necrosis, inflammation, and steatotic vascular degeneration; (2) biliary/oval cell proliferation, degeneration, fibrosis, and cholestasis; or (3) necrosis and degeneration of other resident cells within the liver, are not considered adverse or relevant for human risk assessment. In the absence of histological changes, increases in liver organ weight are not considered relevant for human risk assessment unless at least two of the following three parameters are present: (1) at least 2–3 times increase in ALT levels; (2) biologically significant change in other biomarkers of hepatobiliary damage (alkaline phosphatase, AST, GGT, etc.); or (3) biologically significant change in another clinical pathology marker indicating liver dysfunction (albumin, bilirubin, bile acids, coagulation factors, cholesterol, triglycerides, etc.). ATSDR has adopted the criteria from Hall et al. (2012) for determining the adversity of the liver effects reported in the rodent perfluoroalkyl studies. Doses associated with increases in liver weight and hepatocellular hypertrophy were not considered adverse effect levels unless hepatocellular degenerative or necrotic changes or evidence of biliary or other liver cell damage were also present. The lowest doses associated with the liver weight increases and hepatocellular hypertrophy are noted in the LSE tables even though the dose levels are considered NOAELs.

PFOA

Epidemiological Studies—Liver Disease. Three studies of highly exposed populations have examined possible associations between PFOA and increased risk of liver disease. In workers, no association between estimated cumulative serum PFOA levels and the risk of non-hepatitis liver disease was observed (Steenland et al. 2015). Similarly, two studies of residents living near the Washington Works PFOA facility reported no increases in liver disease. In a study by Anderson-Mahoney et al. (2008), no significant increases in self-reported liver problems were found in residents primarily served by the Lubeck Public Water Service District or Little Hocking Water District; the study did not measure serum PFOA levels. In a C8 Health Project study that included workers at the Washington Works facility,

estimated cumulative serum PFOA levels were not associated with any liver disease or enlarged liver, fatty liver, or cirrhosis (Darrow et al. 2016).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. The possible association between PFOA exposure and hepatic enzymes has been examined in seven occupational exposure studies that have found inconsistent results. A small study of Italian perfluoroalkyl workers did not find associations between serum PFOA and ALT, AST, or GGT activities when only current workers were examined (Costa et al. 2009). In analysis of all workers (current, former, and non-exposed workers), associations between serum PFOA levels and ALT and GGT activities were found; total bilirubin was also inversely associated with serum PFOA. Another small study of workers at a fluorochemical facility in China found an association between serum PFOA and AST activity, but not ALT activity (Wang et al. 2012). Gilliland and Mandel (1996; data also reported in Gilliland 1992) did not find associations between serum fluorine levels (used as a surrogate for serum PFOA) and ALT, AST, or GGT levels in workers. In a follow-up study of this facility, there were no differences between AST, ALT, GGT, or total bilirubin levels between workers in three exposure groups (Olsen et al. 2000); the mean serum PFOA levels in this study ranged from 5,000 to 6,400 ng/mL at three time points and the serum PFOA levels in the lowest exposure group ranged from 0 to <1,000 ng/mL. Increases in GGT and decreases in total bilirubin levels associated with increases in serum PFOA were observed in a study of workers exposed to high levels of PFOA and PFOS (Olsen and Zobel 2007); ALT activity was not affected. In a cross-sectional study of active workers at a PFOA facility, a modest but statistically significant positive association between serum PFOA and GGT activity was found (Sakr et al. 2007b). No associations were found for bilirubin levels or ALT and AST activities.

The possible associations between serum PFOA and serum enzyme and bilirubin levels were examined in two longitudinal occupational exposure studies. Sakr et al. (2007a) examined the relationship between serum PFOA and liver enzymes in a longitudinal study of 454 workers who had two or more measurements of serum PFOA from 1979 until the study was conducted. The average length of employment among workers with multiple PFOA measurements was 11 years, and, on average, 10.8 years elapsed between their first and last serum PFOA measurement. The means of the first and last PFOA measurement were 1,040 and 1,160 ng/mL, respectively. After adjustment for potential confounders, serum PFOA was associated with AST activity, but not ALT, GGT, or total bilirubin. The second study included 179 workers involved in the demolition of 3M perfluoroalkyl manufacturing facilities examined over a mean period of 164 days (Olsen et al. 2012). In workers with prior exposure to

PFOA who had a decrease in serum PFOA levels during the study period, there was a significant increase in ALT levels. An increase in serum PFOA levels did not significantly alter AST or total bilirubin levels.

Community and general population exposure studies have also examined possible associations between serum PFOA levels and alterations in serum hepatic enzyme and bilirubin levels. As with the occupational exposure studies, several studies of populations living near PFOA facilities have found inconsistent results. Darrow et al. (2016) found associations between ALT and bilirubin (inverse association) and estimated cumulative and 2005/2006 serum PFOA levels in participants of the C8 Health Project (6.5% of the participants also worked at the facility); there were no associations with GGT activity. Gallo et al. (2012) also reported a significant correlation between serum PFOA levels and ALT activity in C8 Health Project participants. Unlike the Darrow et al. (2016) study, a significant correlation between serum PFOA levels and GGT activity, but no correlation with direct bilirubin levels, was found. An earlier study of residents in the same area, as well as a study of residents near a facility in China, did not find associations between serum PFOA and ALT, AST, or GGT (Emmett et al. 2006b; Wang et al. 2012).

More consistent results were found in three general population studies. In studies utilizing data from NHANES, Gleason et al. (2015) and Lin et al. (2010) reported associations between serum PFOA levels and ALT, AST, and GGT activities; total bilirubin was also found to be associated with serum PFOA in the Gleason et al. (2015) study, but not in the Lin et al. (2010) study. A general population study conducted in Japan (Yamaguchi et al. 2013) also found associations between serum PFOA levels and AST, ALT, and GGT activities.

Although a number of epidemiological studies have found associations between serum PFOA and serum hepatic enzyme and bilirubin levels, many of the investigators noted that liver biomarker levels were typically within the normal range. Four studies examining the risk of having biomarker levels outside of the normal range provide useful information for evaluating the health impact of the enzyme level alterations. For ALT, Gallo et al. (2012) and Gleason et al. (2015) found increased risks of abnormal levels in C8 and NHANES participants, respectively. In contrast, Olsen and Zobel (2007) and Emmett et al. (2006b) did not find increased risks of abnormal ALT levels in workers and C8 participants, respectively. No alterations in the risk of abnormal AST levels associated with elevated serum PFOA levels were observed in NHANES participants (Gleason et al. 2015). Emmett et al. (2006b) found a decrease in the risk of abnormal AST levels with increasing serum PFOA levels in community members. Associations between the risk of elevated GGT and serum PFOA were found in the study conducted by

Gleason et al. (2015), but not in the Olsen and Zobel (2007), Gallo et al. (2012), or Emmett et al. (2006b) studies. Similarly, Gleason et al. (2015) reported an association between serum PFOA and the risk of elevated bilirubin levels, whereas Gallo et al. (2012) did not find this association in the higher exposed population.

One limitation to the interpretation of the serum hepatic enzyme data is confounding factors that should be considered in analyses; these include age, body mass index (BMI), serum lipid levels (triglycerides and total cholesterol), alcohol consumption, smoking, physical activity, and glucose levels (Deb et al. 2018; Kim et al. 2008). Although many of the studies accounted for age, BMI, smoking, and alcohol consumption, none of the studies adjusted for all of these potential confounders.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined the possible associations between serum PFOA levels and serum lipid levels; the results of these studies are presented in Table 2-12. Summaries of the changes in serum total cholesterol and LDL cholesterol levels, as well as the risk associated with elevated serum cholesterol and LDL cholesterol levels, are presented in Figures 2-11, 2-12, 2-13, and 2-14.

A study of workers at a manufacturing facility in Italy found higher total cholesterol and non-high-density lipoprotein (HDL)-cholesterol levels (non-HDL cholesterol was estimated by subtracting HDL cholesterol from total cholesterol) in the PFOA-exposed workers, as compared to levels in workers who were not exposed to PFOA (Costa 2004). A second study at this facility (Costa et al. 2009) also found an association between serum PFOA levels and total cholesterol levels, but no association with HDL cholesterol levels. No associations were found for HDL cholesterol or triglyceride levels. In another small study of workers at a fluorochemical facility in China (Wang et al. 2012), no associations between serum PFOA and total cholesterol, LDL cholesterol, or triglyceride levels were observed; the study did find an inverse association between serum PFOA and HDL cholesterol levels.

Several studies have examined workers at 3M facilities in Cottage Grove, Minnesota, Decatur, Alabama, and/or Antwerp, Belgium; workers at these facilities were also exposed to high levels of PFOS. Gilliland and Mandel (1996; data also reported in Gilliland 1992) examined workers at the Cottage Grove facility in 1990 and found no associations between serum fluorine levels (used as a surrogate for PFOA) and total cholesterol, LDL cholesterol, or HDL cholesterol. In a follow-up to this study, Olsen et al. (2000) examined workers in 1993, 1995, and 1997; only 17 workers were examined at all three time periods,

Figure 2-11. Serum Total Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in cholesterol levels)

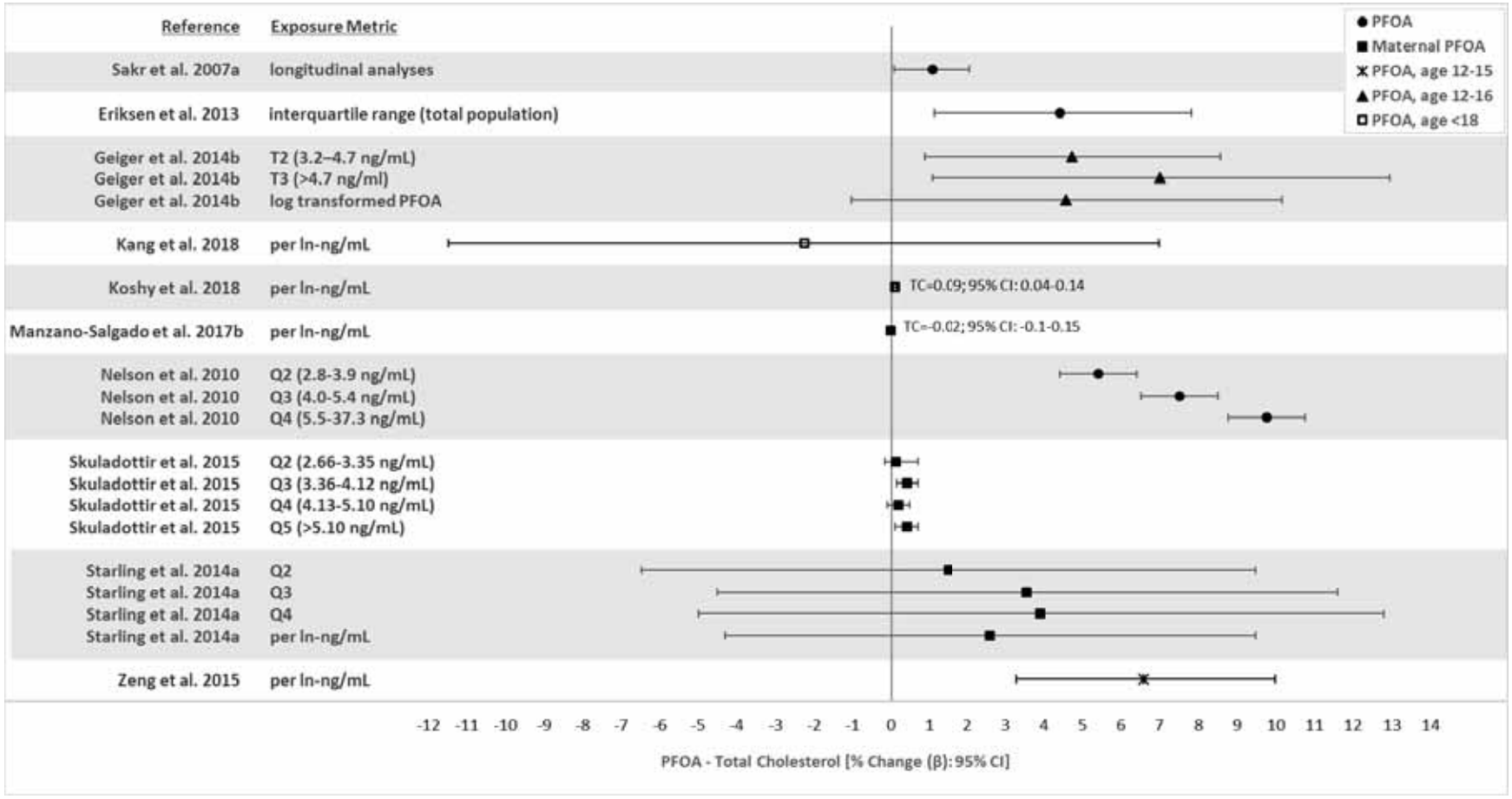


Figure 2-12. Risk of Abnormal Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)

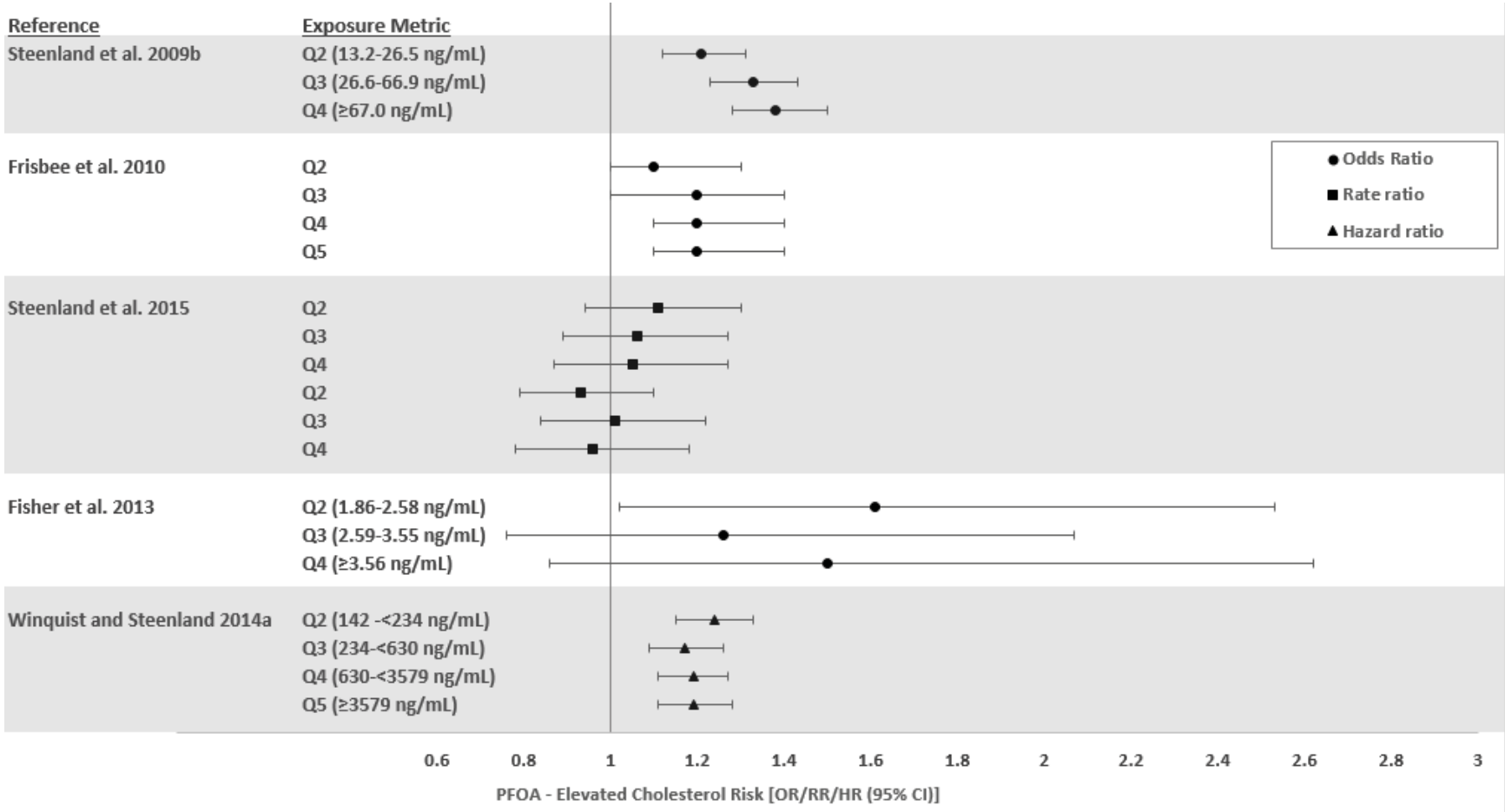


Figure 2-13. Serum LDL Cholesterol Levels Relative to Serum PFOA Levels
(Presented as percent change in LDL cholesterol levels)

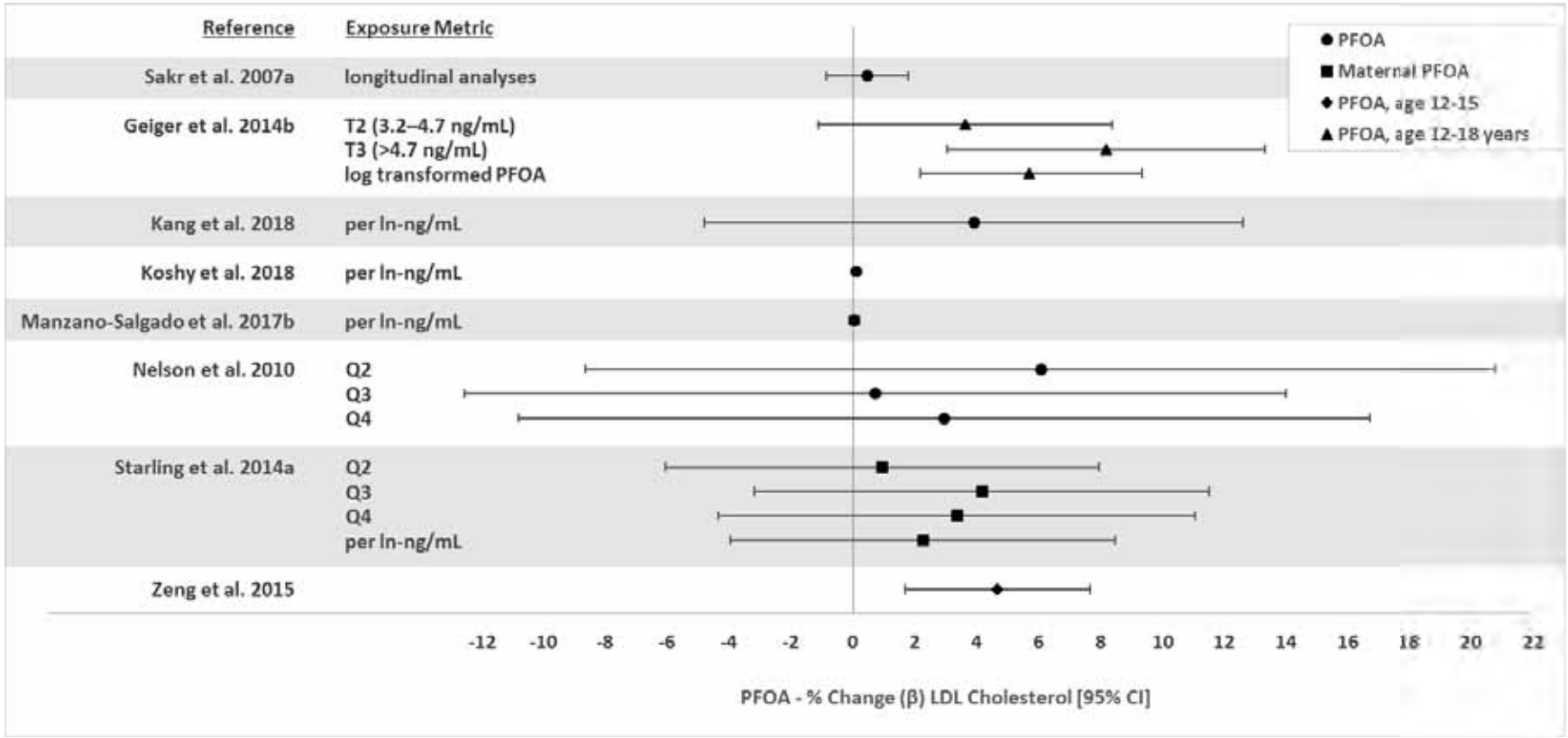
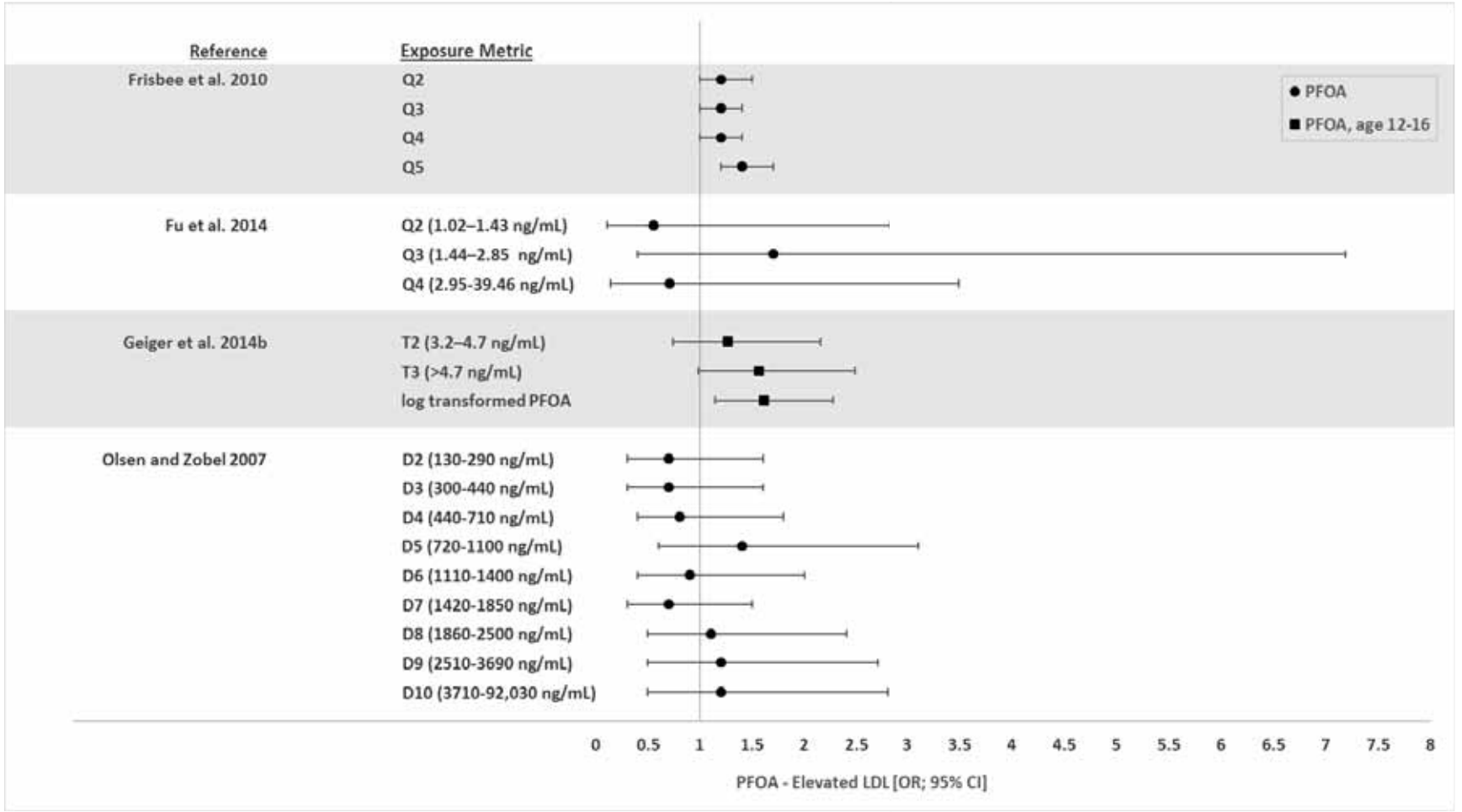


Figure 2-14. Risk of Abnormal LDL Cholesterol Levels Relative to PFOA Levels (Presented as Adjusted Ratios)



21 workers were examined in 1995 and 1997, and 68 workers were examined in 1993 and 1995. The study did not adjust for the use of cholesterol-lowering medication. When workers were categorized by blood PFOA levels (0–<1,000, 1,000–<10,000, and >10,000 ng/mL), no significant differences in serum cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found at any of the monitoring periods. A study in workers at the three 3M facilities, most of whom were not taking cholesterol-lowering medications, did not find associations between serum PFOA levels and total cholesterol or LDL cholesterol levels; however, serum PFOA levels were associated with elevated triglyceride levels and inversely associated with HDL cholesterol levels (Olsen and Zobel 2007). The study did not find increases in the risk of elevated total cholesterol (≥200 mg/dL), elevated LDL cholesterol (≥130 mg/dL), elevated triglyceride (≥150 mg/dL), or decreased HDL cholesterol (≤40 mg/dL) levels in workers with serum PFOA levels in the highest deciles. In addition to these cross-sectional studies, two longitudinal studies were conducted at these facilities. Using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995, Olsen et al. (2003a) found that serum PFOA was a significant predictor of cholesterol and triglyceride levels, which was primarily due to 21 workers at the Antwerp facility (mean serum level 8,400 ng/mL) whose serum PFOA levels increased over time. In a longitudinal study, Olsen et al. (2012) examined workers (none of the subjects reported using cholesterol-lowering medication) involved in the demolition of 3M perfluoroalkyl manufacturing facilities; serum PFOA and lipid levels were measured prior to the demolition and after demolition (mean time interval of 164 days). The mean baseline serum PFOA levels were 881 ng/mL in 14 3M workers with prior PFOA or PFOS exposure and 28.9 ng/mL in the remaining 165 workers. Among the 119 workers whose serum PFOA/PFOS levels (mean increase 50.9 ng/mL) increased during the observation period, there was a significant increase in HDL cholesterol levels, but no change in total cholesterol or non-HDL cholesterol levels. No significant alterations in serum lipid levels were observed in the 55 workers whose serum PFOA/PFOS levels decreased during the observation period. In workers whose baseline levels of PFOA and PFOS were <15 and <50 ng/mL, respectively, there were no significant differences between pre- and post-exposure serum lipid levels.

Investigators have also examined workers at the DuPont Washington Works facility in West Virginia. In a cross-sectional study, Sakr et al. (2007b) found associations between serum PFOA levels and total cholesterol, LDL cholesterol, and very-low-density lipoprotein (VLDL) cholesterol levels in all subjects and in a subset of subjects not taking cholesterol-lowering medication. The study did not find any association between serum PFOA and HDL cholesterol or triglyceride levels. In a second study, Steenland et al. (2015) did not find an association between estimated serum PFOA levels and the occurrence of elevated cholesterol levels that required medication. In a longitudinal study of workers

who had at least two serum PFOA measurements between 1979 and 2004, Sakr et al. (2007a) found a positive association between serum PFOA and total cholesterol levels; no associations with triglycerides, LDL cholesterol, or HDL cholesterol were found. Total cholesterol levels increased 1.06 mg/dL for each 1,000 ng/mL increase in serum PFOA.

Several studies have been conducted of residents living near the Washington Works facility. A study by Emmett et al. (2006b) of adults and children living in a community serviced by the Little Hocking Water Authority did not find an association between serum PFOA levels and total cholesterol levels; the study included an adjustment for the use of cholesterol-lowering medication. Four larger-scale studies of participants in the C8 Science Panel studies found associations between serum PFOA levels and serum lipid levels (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a). Positive associations between serum PFOA levels and total cholesterol and LDL cholesterol were found in a study of over 12,000 children and adolescents, with mean serum PFOA levels of 32.6 ng/mL in children (aged 1.0–11.9 years) and 26.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Serum PFOA was also positively associated with triglyceride levels. Additionally, there was an increased risk of elevated cholesterol (≥170 mg/dL) in subjects with serum PFOA levels in the 4th or 5th quintiles. Increased odds of high LDL cholesterol (≥110 mg/dL) were also observed for the 5th PFOA quintile (OR 1.4, 95% CI 1.2–1.7). The investigators noted that the dose-response relationship between serum PFOA and serum lipids was nonlinear, with greater increases in lipids observed at the lower serum PFOA levels. Similar findings were reported in a study of >46,000 adults with a median serum PFOA level of 26.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b). Associations were found between serum PFOA levels and total cholesterol, LDL cholesterol, and non-HDL cholesterol; a positive association between serum PFOA and triglycerides was also found. No associations between serum PFOA levels and HDL cholesterol levels were found. Increased risks of having high total cholesterol (≥240 mg/dL) were found in subjects with serum PFOA levels in the 2nd, 3rd, and 4th quartiles. The investigators noted that the odds of high total cholesterol from the 1st to the 5th quartile were approximately 40% for PFOA, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL. Steenland et al. (2009b) also found an association between serum PFOA levels and total cholesterol levels in a study of 10,746 adults taking cholesterol-lowering medication. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol-lowering medication was associated with lower serum PFOA levels, which may be indicative of reverse causality. Although serum PFOA levels were significantly lower in subjects taking cholesterol-lowering

medication, the difference between the groups was low (4%). Using estimated cumulative serum PFOA levels as the exposure metric, Winquist and Steenland (2014a) found increased risks of hypercholesterolemia at estimated cumulative exposure levels ≥142 ng/mL. In a longitudinal study by Fitz-Simon et al. (2013), adults participating in the C8 Health Project and not taking cholesterol-lowering medication were examined twice, with an average of 4.4 years between examinations. Mean serum PFOA levels were 74.8 ng/mL at the first examination and 30.8 ng/mL at the second examination. In subjects whose serum PFOA levels halved between examinations, there was a 3.6% decrease in LDL cholesterol levels and 1.7% decrease in total cholesterol levels. However, there were very small changes in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels decreased by >64% and there were slight increases in LDL cholesterol and total cholesterol levels in subjects whose serum PFOA levels fell by <50%. Changes in PFOA levels were not associated with changes in HDL cholesterol or triglyceride levels. Similarly, Wang et al. (2012) found no associations between serum PFOA levels and total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides in a study of adults living near a PFOA manufacturing facility in China; the mean serum PFOA level was 378.30 ng/mL and did not include an adjustment for the use of cholesterol-lowering medication.

General population studies were conducted in the United States, Canada, Denmark, Norway, Spain, Japan, Korea, China, and Taiwan; these studies have examined possible associations between serum PFOA levels and serum lipid levels in children, adolescents, pregnant women, and adults. In a study of 8–10-year-old children (median serum PFOA of 9.3 ng/mL), Timmermann et al. (2014) found an association between serum PFOA and triglyceride levels among obese children; this association was not found among normal weight children. In a study of adolescents (12–18 years of age) participating in NHANES (mean serum PFOA level of 4.2 ng/mL), Geiger et al. (2014b) found associations between serum PFOA and total cholesterol and LDL cholesterol levels; no associations were found for HDL cholesterol or triglycerides. The study also found increased risks of elevated total cholesterol levels (>170 mg/dL) associated with serum PFOA levels. No alterations in the risk of elevated LDL cholesterol or triglycerides or decreased HDL cholesterol were found. Associations between serum total cholesterol, LDL cholesterol, and triglycerides have also been observed in a study of Taiwanese adolescents (12–15 years of age, median PFOA level of 9.3 ng/mL) (Zeng et al. 2015); no association was found for HDL cholesterol. A fourth study found associations between maternal PFOA levels and total cholesterol and LDL cholesterol in 7- and 15-year-old girls, but no associations for girls whose maternal PFOA levels were in the 2nd or 3rd tertiles (Maisonet et al. 2015b). No associations were found for HDL cholesterol or triglyceride levels. A study of children enrolled in the World Trade Center Health Registry found associations between serum PFOA levels and elevated serum cholesterol, LDL cholesterol, and

triglyceride levels, but no association with HDL cholesterol (Koshy et al. 2017). Another study of children aged 3–18 years found no associations between serum PFOA levels and total cholesterol, LDL cholesterol, or triglycerides (Kang et al. 2018). Manzano-Salgado et al. (2017b) found no association between maternal serum PFOA levels and serum lipid levels in 4-year-old children.

Studies in adults have found mixed results for serum lipids. Using NHANES data for adults not taking cholesterol-lowering medication (mean serum PFOA level of 4.6 ng/mL), Nelson et al. (2010) found an association between serum PFOA levels and non-HDL cholesterol levels; no associations were found for total cholesterol, LDL cholesterol, or HDL cholesterol. Another study of NHANES participants that statistically adjusted for use of cholesterol-lowering medication found no associations between serum PFOA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels (Liu et al. 2018b). Associations between serum PFOA levels and total cholesterol levels were also found in a study of Danish adults not taking cholesterol-lowering medication (mean serum PFOA level of 7.1 ng/mL) (Eriksen et al. 2013). A study in Chinese adults (median PFOA level of 1.43 ng/mL) also found associations between serum PFOA and total cholesterol and LDL cholesterol, with no associations for HDL cholesterol or triglycerides (Fu et al. 2014a). This study did not find increased risks of elevated total cholesterol, LDL cholesterol, or triglycerides or decreased HDL cholesterol associated with serum PFOA. A second study of Chinese men found an association between serum PFOA and triglyceride levels, but no association with HDL cholesterol levels (Yang et al. 2018). A study of pregnant women in Denmark also found an association between serum PFOA (mean serum PFOA level of 4.1 ng/mL at gestation week 30) and total cholesterol levels (Skuladottir et al. 2015). No associations between serum PFOA levels and total cholesterol, LDL cholesterol, or non-HDL cholesterol levels were found in Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFOA level of 2.46 ng/mL (Fisher et al. 2013). In a second study of pregnant women (median PFOA level of 2.25 ng/mL at gestation week 18), no associations between plasma PFOA and total cholesterol, LDL cholesterol, or triglycerides were found (Starling et al. 2014a). The study did find an association between plasma PFOA and HDL cholesterol.

A number of epidemiological studies have reported associations between serum PFOA levels and serum lipid levels; the most consistently found alteration was for increased serum total cholesterol levels. Associations between serum PFOA and serum cholesterol levels have been observed in occupational (Costa 2004; Costa et al. 2009; Sakr et al. 2007a, 2007b), community (Fitz-Simon et al. 2013; Frisbee et al. 2010; Steenland et al. 2009b; Winquist and Steenland 2014a), and general population (Eriksen et al. 2013; Fu et al. 2014a; Geiger et al. 2014b; Skuladottir et al. 2015; Zeng et al. 2015) studies, whereas

other investigators have not found associations in worker populations (Gilliland and Mandel 1996; Olsen et al. 2000; Olsen and Zobel 2007; Steenland et al. 2015; Wang et al. 2012), community populations (Emmett et al. 2006b; Wang et al. 2012), or general populations (Fisher et al. 2013; Nelson et al. 2010; Starling et al. 2014a). Longitudinal studies conducted in workers and highly exposed residents strengthen the interpretation of this association between serum PFOA and serum lipid levels. Serum PFOA levels were found to be a significant predictor of serum cholesterol levels in workers examined at least twice in a ≥5-year period (Olsen et al. 2003a; Sakr et al. 2007a). Similarly, a study of highly-exposed residents examined twice with approximately 4 years between examinations found that there was a 1.7% decrease in serum total cholesterol levels in subjects whose serum PFOA levels decreased by 50% between examinations (Fitz-Simon et al. 2013). As noted in Steenland et al. (2010a), there is considerable variation in the strength of the association between PFOA and serum cholesterol, with the greatest changes in serum cholesterol occurring at lower PFOA levels. The change in cholesterol levels per ng/mL change in serum PFOA ranged from 0.0007, calculated from data from the Olsen et al. (2000) occupational exposure study, to 2.0 calculated from data from the Nelson et al. (2010) general population study; the mean serum PFOA levels in these studies were ~22,000 and 4 ng/mL respectively. In a clinical trial, administration of APFO to patients with advanced solid tumors at doses of 50–1,200 mg weekly for 6 weeks resulted in decreases in serum cholesterol levels; the marked decreases in serum cholesterol levels were observed at serum PFOA concentrations of 175,000–230,000 ng/mL (Convertino et al. 2018). These results are similar to those observed in laboratory animals, suggesting that the dose-response curve may be biphasic. Steenland et al. (2010a) and Frisbee et al. (2010) suggested that this may be due to a steep dose-response curve at low PFOA levels, which flattens out at higher PFOA levels and may be indicative of saturation. A similar pattern was also observed in the risks of elevated cholesterol per increases in serum PFOA levels (Figure 2-14). Several investigators have explored whether PFOA and cholesterol could be jointly affected or whether the associations were due to reverse causality (i.e., increased cholesterol resulted in increased serum PFOA levels). Butenhoff et al. (2012c) explored the issues of whether PFOA distributes into serum lipoprotein fractions, and whether increases in serum lipoproteins would result in increases in serum PFOA. They concluded that there was limited distribution to plasma lipoproteins, and did not consider it a non-causal factor. The Steenland et al. (2009b) study found slightly lower serum PFOA levels (4%) among individuals taking cholesterol medication, as compared to those not taking medication and noted that this was primarily a function of the large sample size. This finding does not support reverse causality.

Laboratory Animal Studies. Information from inhalation studies in animals is limited. Head-only exposure of male rats to 810 mg/m^3 APFO dusts for 4 hours caused liver enlargement, but

microscopically, the liver tissue appeared normal (Kennedy et al. 1986). Exposure head-only of male rats to 0, 1, 7.6, or 84 mg/m^3 APFO dusts 6 hours/day, 5 days/week for 2 weeks resulted in significant increases in absolute and relative liver weight at 7.6 and 84 mg/m^3 on exposure day 10; in rats from the 84 mg/m^3 group, absolute and relative liver weights were still significantly increased 28 days after exposure ceased (Kennedy et al. 1986). The activities of serum enzymes markers of liver function were unremarkable except for alkaline phosphatase, which was significantly increased in the 7.6 and 84 mg/m^3 groups immediately after exposure on day 10 and remained elevated in the 84 mg/m^3 group on day 14 of recovery. Histopathological changes were restricted to the 7.6 and 84 mg/m^3 groups and consisted of panlobular and centrilobular hepatocellular hypertrophy and necrosis. Panlobular hepatocellular hypertrophy was seen only after the 10th exposure, but was limited to the centrilobular hepatocytes 14 or 28 days after exposure terminated, and was absent 42 days following cessation of exposure. Inhalation exposure of pregnant rats to 25 mg/m^3 APFO dusts 6 hours/day during GDs 6–15 induced an 18% increase in absolute liver weight (Staples et al. 1984); no significant effect was reported in rats exposed to ≤10 mg/m^3.

Nose-only exposure of male CD rats to 67 mg/m^3 ammonium perfluorononanoate dusts for 4 hours induced significant increases (28–37%) in absolute and relative liver weight, assessed 5 and 12 days after exposure (Kinney et al. 1989). Histopathological examinations were not conducted in this study.

The liver is the main target organ for perfluoroalkyls in animals following short- or long-term oral exposures. The hepatic response to exposure to many perfluoroalkyls, particularly in rodents, is initiated by the activation of the nuclear hormone receptor, PPARα, which triggers a characteristic sequence of morphological and biochemical events characterized by liver hypertrophy and alteration of a wide range of enzymes, particularly those involved in lipid metabolism. It appears that PFOA can also damage the liver via a method independent of PPARα resulting in increases in liver weight, hepatocellular hypertrophy, microvesicular steatosis, and cholangiopathy (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b).

The most sensitive liver effect observed in rats and mice after acute oral exposure to PFOA is an increase in liver weight (Cook et al. 1992; Das et al. 2017; Eldasher et al. 2013; Haughom and Spydevold 1992; Ikeda et al. 1985; Iwai and Yamashita 2006; Kawashima et al. 1995; Kennedy 1987; Liu et al. 1996; Loveless et al. 2006; Pastoor et al. 1987; Permadi et al. 1992, 1993; Qazi et al. 2012; White et al. 2009; Wolf et al. 2007, 2008a; Xie et al. 2003; Yahia et al. 2010; Yang et al. 2001, 2002b). In rats orally administered 50 mg/kg/day PFOA for 1, 3, or 7 days, a 10% increase in liver weight was observed after

the first dose; however, the relative liver weight was not significantly different from controls (Pastoor et al. 1987). After 3 days of exposure, the relative liver weight was significantly higher (36%) than controls. Similarly, in mice, exposure to 390 mg/kg/day PFOA in the diet resulted in a significant increase in liver weight after 5 days of exposure, but not after 2 days of exposure (Permadi et al. 1992). The lowest LOAELs for increased relative liver weight in rats were 4.7 mg/kg/day in a 7-day study (Kawashima et al. 1995) and 2 mg/kg/day in a 14-day study (Liu et al. 1996); these studies also identified NOAELs of 2.4 and 0.2 mg/kg/day, respectively. In mice, the lowest LOAEL for increases in liver weight was 1 mg/kg/day PFOA administered in the diet for 10 days (Yang et al. 2001) or administered via gavage for 7 days (Eldasher et al. 2013; Wolf et al. 2008a). Pastoor et al. (1987) noted that oral administration of 50 mg/kg/day PFOA to rats for 7 days resulted in a 2-fold increase in absolute and relative liver weight, but no significant change in total deoxyribonucleic acid (DNA), indicating that the hepatomegaly represented hypertrophy rather than hyperplasia. Few acute-duration studies included histological examinations of the liver. Centrilobular and midzonal hypertrophy was observed in mice administered 1 or 3 mg/kg/day PFOA via gavage for 7 days; panlobular hypertrophy with cytoplasmic vacuolation was observed at 10 mg/kg/day (Wolf et al. 2008a). Qazi et al. (2010a) reported hepatocellular hypertrophy in mice exposed to 3.5 mg/kg/day PFOA in the diet for 10 days. Elcombe et al. (2010) reported hepatocellular hypertrophy in rats orally exposed to 18 mg/kg/day for 7 days, but not after 1 day of exposure. Increases in steatosis and triglyceride levels were observed in the livers of mice administered 10 mg/kg/day for 7 days (Das et al. 2017). A related liver effect was the finding of reduced serum cholesterol and triacylglycerol levels in rats administered 16 mg/kg/day PFOA in the diet for 7 days (Haughom and Spydevold 1992) and decreases in serum cholesterol and triglyceride levels in rats administered 18 mg/kg/day PFOA via gavage for 7 days (Elcombe et al. 2010).

Similar to the acute-duration studies, intermediate-duration oral exposure to PFOA resulted in increases in absolute and relative liver weights in rats (Biegel et al. 2001; Butenhoff et al. 2004b; Griffith and Long 1980; Perkins et al. 2004) and mice (Abbott et al. 2007; Ahmed and Abd Ellah 2012; Albrecht et al. 2013; Griffith and Long 1980; Kennedy 1987; Lau et al. 2006; Son et al. 2008; Wolf et al. 2007; Yang et al. 2009). The lowest dose resulting in increases in liver weight in rats was 0.96 mg/kg/day, observed following gavage administration of APFO for 28 days (Loveless et al. 2008); the lowest dose in mice was 0.5 mg/kg/day, observed in two 28-day studies using APFO (Kennedy 1987; Son et al. 2008). No significant alterations in liver weight were observed in rats administered 0.29 mg/kg/day for 28 days (Loveless et al. 2008) or in mice exposed to 0.2 mg/kg/day for 21 days (Kennedy 1987). Hepatocellular hypertrophy was the predominant histopathological alteration in rats (Cui et al. 2009; Griffith and Long 1980; Loveless et al. 2008; Perkins et al. 2004) and mice (Albrecht et al. 2013; Filgo et al. 2015a; Griffith

and Long 1980; Loveless et al. 2008; Tan et al. 2013); the severity of the hypertrophy was dose-related (Filgo et al. 2015a; Loveless et al. 2008). At higher doses, focal necrosis was observed (LOAEL of 29 mg/kg/day in rats and 0.96 mg/kg/day in mice exposed for 28 days) (Loveless et al. 2008). Fatty changes were observed in rats administered 20 mg/kg/day for 28 days (Cui et al. 2009) and mice administered 9.6 mg/kg/day (Loveless et al. 2008). No significant alterations in liver weight or histopathology were observed in rats allowed to recover for 8 weeks following a 13-week exposure to 0.6–6.5 mg/kg/day (Perkins et al. 2004). Intermediate-duration exposure to PFOA also resulted in decreases in serum HDL cholesterol levels in rats and mice administered ≥0.29 or 0.96 mg/kg/day, respectively, for 28 days (Loveless et al. 2008). Serum cholesterol levels were decreased in rats administered 0.29 or 0.96 mg/kg/day (no changes were observed at higher doses) and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). Similarly, serum triglyceride levels were decreased in rats administered 0.29–9.6 mg/kg/day and in mice administered 9.6 or 29 mg/kg/day (Loveless et al. 2008). In a study of mice fed a western-type diet, increases in plasma cholesterol levels were observed after 6 weeks of dietary exposure to 0.55 mg/kg/day in BALB/c or C57BL/6 mice (Rebholz et al. 2016). The results of this study suggest that diet (fat intake and/or cholesterol levels) may influence the response to PFOA and may account for some of the differences observed in humans and rats fed a standard diet, which is typically low in fat.

Chronic exposure of rats to PFOA resulted in hepatocellular hypertrophy, hepatocellular necrosis, and portal mononuclear cell infiltration after a 1-year exposure to a LOAEL of 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c). A 2-year exposure to 15 mg/kg/day resulted in hepatocellular hypertrophy, cystoid degeneration, and portal mononuclear cell infiltration (3M 1983; Butenhoff et al. 2012c). The study also found significant increases in ALT and AST levels in male rats exposed to 1.5 mg/kg/day. A second chronic exposure study found significant increases in relative liver weight in rats exposed to 13.6 mg/kg/day in the diet for 2 years; no non-neoplastic lesions were noted in the liver (Biegel et al. 2001).

Studies in monkeys suggest that longer-term exposure may also result in liver effects. Significant increases in absolute and relative liver weight were observed in Cynomolgus monkeys exposed to 20/30 mg/kg/day administered via capsules for 26 weeks (Butenhoff et al. 2002). A significant increase in absolute, but not relative, liver weight was also observed in monkeys administered 3 or 10 mg/kg/day. However, no histological alterations were observed in the livers at the doses tested. Similarly, no histological alterations were observed in the livers of Cynomolgus monkeys administered 2 or 20 mg/kg/day via capsules for 30 days (Thomford 2001) or Rhesus monkeys administered 3 or

10 mg/kg/day via gavage for 90 days (Griffith and Long 1980). Significant increases in serum triglyceride levels were observed in the 10 and 20/30 mg/kg/day groups; the increases were statistically significant at only some of the time points (Butenhoff et al. 2002). At 10 mg/kg/day, increases in serum triglyceride levels at 4, 10, and 14 weeks of exposure were significantly higher than pre-treatment levels. Increases in cholesterol levels were only observed in the 20/30 mg/kg/day group after 13 weeks of exposure, but not after 26 weeks. No alterations in serum cholesterol or triglyceride levels were observed in the Thomford (2001) study.

Several studies have examined PPARα-null mice to assess whether PFOA-induced liver effects can also occur via a mechanism independent of PPARα-receptor activation. Similar to wild-type mice, exposure to PFOA resulted in significant increases in liver weight (Abbott et al. 2007; Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a; Yang e al. 2002b). Abbott et al. (2007) found that the effect level was slightly higher in PPARα-null mice than wild-type mice (3 versus 1 mg/kg/day) following oral exposure on GDs 1–17 (liver weights measured at weaning). Wolf et al. (2008a) and Minata et al. (2010) reported the same effect level (1 or 5 mg/kg/day, respectively) in PPARα-null mice and wild-type mice administered PFOA via gavage for 7 days or 4 weeks. Wolf et al. (2008a) found dose-related increases in hepatocellular cytoplasmic vacuoles at ≥1 mg/kg/day and suggested that the increase in liver weight was due to the accumulation of PFOA in the hepatocytes rather than a toxic response. Hepatocyte proliferation was also observed at 10 mg/kg/day. Unlike the Wolf et al. (2008a) study, the Minata et al. (2010) 4-week study reported hepatocellular hypertrophy and microvesicular steatosis in the PPARα-null mice (no incidence data were provided and it is unclear at what dose levels these effects were found); cytoplasmic vacuolation was also reported in the hepatocytes. Filgo et al. (2015a) also reported hepatocellular hypertrophy in PPARα-null mice; the LOAEL was 3 mg/kg/day, which was higher than the LOAEL of 0.3 mg/kg/day found in wild-type mice. Minata et al. (2010) also reported cholangiopathy in both the wild-type and PPARα-null mice, but noted that the effect was more intensive in the PPARα-null mice. No significant alterations in steatosis or triglyceride accumulation were observed in PPARα-null mice administered 10 mg/kg/day for 7 days, but were observed in wild-type mice (Das et al. 2017). Additionally, significant decreases in serum total cholesterol levels at 5.2 and 10.2 mg/kg/day and increases at 20.7 mg/kg/day were observed in the PPARα-null mice; significant decreases in total cholesterol were observed in the wild-type mice at 10.2 and 20.7 mg/kg/day doses. Serum triglyceride levels were increased in both strains at 5.2 and 10.2 mg/kg/day doses and in the PPARα-null mice at 20.7 mg/kg/day (Minata et al. 2010).

Intermittent application of 20, 200, or 2,000 mg/kg APFO to the skin of rats for 2 weeks resulted in the presence of one or more foci of coagulative necrosis in the livers from all treated groups (Kennedy 1985). The Kupffer cells within the foci of hepatocellular necrosis contained large vesicular nuclei and were markedly increased in number. At 2,000 mg/kg/day, these changes were seen in three out of five rats killed on the 10th day of exposure, in three out of five rats killed on recovery day 14, and in one out of five rats killed on recovery day 42. This lesion occurred in two out of five rats from the 20 mg/kg/day dose group killed on day 10 of exposure. Serum ALT activity appeared elevated at termination of exposure in a dose-related manner, but without achieving statistical significance. A similar trend was seen for AST activity, but achieving statistical significance in the high-dose group. The blood concentrations of organofluorine on the 10th day of exposure were 10.2, 52.4, 79.2, and 117.8 µg/mL in the control, low-, mid-, and high-dose groups, respectively. A study in mice reported that application of 6.25 mg/kg/day PFOA on the dorsal surface of each ear for 4 days resulted in a 52% increase in absolute liver weight (Fairley et al. 2007); no significant effect occurred after application of 2.5 mg/kg/day.

Summary. Epidemiological studies examining the hepatotoxicity of PFOA have examined three outcomes—risk of liver disease, evidence of hepatocellular damage (as measured by alterations in serum hepatic enzymes and bilirubin levels), and alterations in serum lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides)—among workers, residents living near a PFOA manufacturing facility with high levels of drinking water contamination, and the general population. Exposure to PFOA does not appear to be associated with increased risks of liver disease in workers or highly exposed community members. The epidemiological studies have found associations between serum PFOA levels and increases in serum ALT, AST, and GGT enzyme levels and decreases in serum bilirubin levels. However, the results have not been consistently found, and serum enzyme levels were typically within the normal range. Four studies examined the risk of serum enzyme levels outside of the normal range; the results were mixed for the risk of elevated ALT, with two studies finding an increased risk and two studies finding no association. A number of occupational, community, and general population studies have found associations between serum PFOA levels and serum total cholesterol levels; several studies have also found no associations. Studies examining the change in cholesterol levels per change in serum PFOA levels have found greater increases in serum cholesterol levels associated with serum PFOA levels at the lower range of PFOA levels and the dose-response curve suggests a biphasic relationship. Positive associations have also been observed for LDL cholesterol, although associations have not been consistently found. In general, no consistent associations were found between serum PFOA and HDL cholesterol or triglyceride levels.

Studies in laboratory animals have found strong associations between PFOA exposure and hepatotoxicity. Liver effects have been observed in rats exposed to airborne APFO dusts; in rats, mice, and monkeys following oral exposure for acute-, intermediate-, or chronic-durations; and in rats following dermal exposure. The observed effects typically include increases in liver weight, hepatocellular hypertrophy, and decreases in serum cholesterol and triglyceride levels. Other effects that have been observed include hyperplasia, necrosis, and fatty degeneration. Available evidence suggests that the increased liver weight, hypertrophy, and serum lipid alterations are likely due to PPARα initiation and therefore, may not be relevant to humans. However, other mechanisms of liver toxicity are also involved, as evidenced by liver effects observed in PPARα-null mice (Das et al. 2017; Minata et al. 2010; Wolf et al. 2008a). In contrast to the results observed in epidemiological studies, a clinical trial study in humans with advanced solid tumors exposed to very large doses of PFOA (Convertino et al. 2018) and human exposure to other PPARα agonists, such as fibrates (Staels et al. 1998), suggest that hypolipidemic effects, similar to those observed in rodents, may occur in humans exposed to PFOA, although humans may not be as sensitive as rodents.

PFOS

Epidemiological Studies—Liver Disease. Several studies have examined the possible association between PFOS exposure and liver diseases. No increases in deaths from cirrhosis of the liver were found in workers at the 3M facility in Decatur, Alabama (Alexander et al. 2003). Another study of workers at this facility found no significant alterations in the episodes of care for all liver disorders or all biliary duct disorders (Olsen et al. 2004a). However, among workers with at least 10 years of high potential exposure to PFOS, there were significant increases in episodes of care for cholelithiasis or acute cholecystitis and for all biliary tract disorders. A third study of workers at a PFOS facility in Decatur, Alabama did not find increases in cholelithiasis, cholecystitis, or liver disease (including cirrhosis and hepatitis) (Grice et al. 2007).

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A series of studies conducted by Olsen and associates evaluated liver function (as assessed by serum liver enzymes) in workers at several 3M facilities involved in PFOS production. Using health data collected in 1995 and 1997, Olsen et al. (1999) did not find associations between serum PFOS and serum ALT, AST, or GGT enzymes at PFOS levels <6,000 ng/mL; a positive association with total bilirubin levels was found. No conclusions were drawn from the few workers with serum PFOS ≥6,000 ng/mL due to their small number (seven in 1995 and five in 1997 data). Similarly, no association of ALT, AST, or GGT and serum PFOA levels

were observed in groups of workers at these facilities examined in 1993 (111 subjects), 1995 (80 subjects), and/or 1997 (74 subjects) (Olsen et al. 2000). A subsequent evaluation of workers from the same plants, but that included women and a longitudinal analysis of the workers, reported that, after adjusting for potential confounding factors, there were no substantial changes in hepatic parameters (Olsen et al. 2003a). GGT levels in females and ALT levels in males with PFOS levels in the 4th quartile were significantly elevated in comparisons between individuals with serum PFOS levels in the 4th quartile to those with levels in the 1st quartile; however, there were no statistical adjustments for potential confounders. In contrast to these findings in workers, Gallo et al. (2012) reported significant increases in the risks of elevated ALT, GGT, and bilirubin levels in a study of C8 participants. Conflicting results have been found in general populations studies. Studies using the NHANES data set (Gleason et al. 2015; Lin et al. 2010) did not find associations between serum PFOS and ALT, AST, GGT, or total bilirubin levels. No increases in the risk of elevated levels of ALT, AST, or GGT were found (Gleason et al. 2015), although there was an increased risk of elevated total bilirubin levels. In a study of adults in Japan (Yamaguchi et al. 2013), significant correlations between serum PFOS and ALT, AST, and GGT levels were found.

Epidemiological Studies—Serum Lipids. Occupational, community, and general population studies have examined possible associations between serum PFOS levels and serum lipids; these data are summarized in Table 2-12. A graphical presentation of differences in total cholesterol and LDL cholesterol levels relative to serum PFOS levels and the risks of elevated total cholesterol and LDL cholesterol are presented in Figures 2-15, 2-16, 2-17, and 2-18.

In the Olsen occupational studies, significantly higher serum total cholesterol levels were found in workers with serum PFOS levels between 3,000 and 6,000 ng/mL (Olsen et al. 1999, 2003a). However, the studies found mixed results for associations between serum PFOS and other serum lipids, with one study finding an association with LDL cholesterol (Olsen et al. 1999) and the other finding an association with triglycerides (Olsen et al. 2003a). Longitudinal analysis was conducted using data for 174 workers with medical surveillance data in 2000 and 1997 and/or 1995 (Olsen et al. 2003a). No significant differences in serum PFOS levels were observed across the three time periods, and serum PFOS levels were not a significant predictor of cholesterol or triglyceride levels.

Two large-scale studies of participants in the C8 Science Panel studies found associations between serum PFOS levels and serum lipid levels (Frisbee et al. 2010; Steenland et al. 2009b). Associations between serum PFOS levels and total cholesterol, LDL cholesterol, and HDL cholesterol were found in a study of

Figure 2-15. Serum Total Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in cholesterol levels)

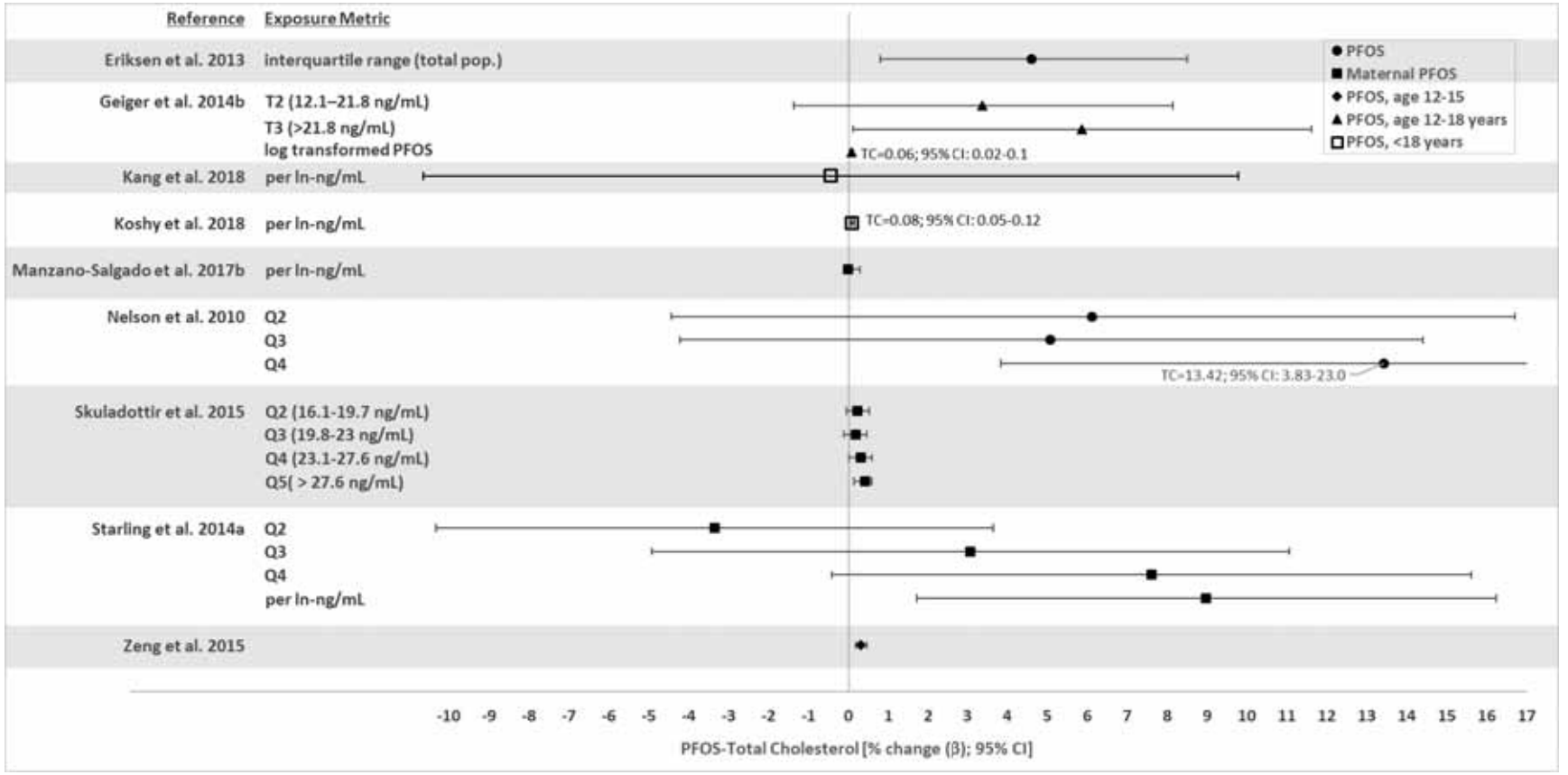


Figure 2-16. Risk of Abnormal Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)

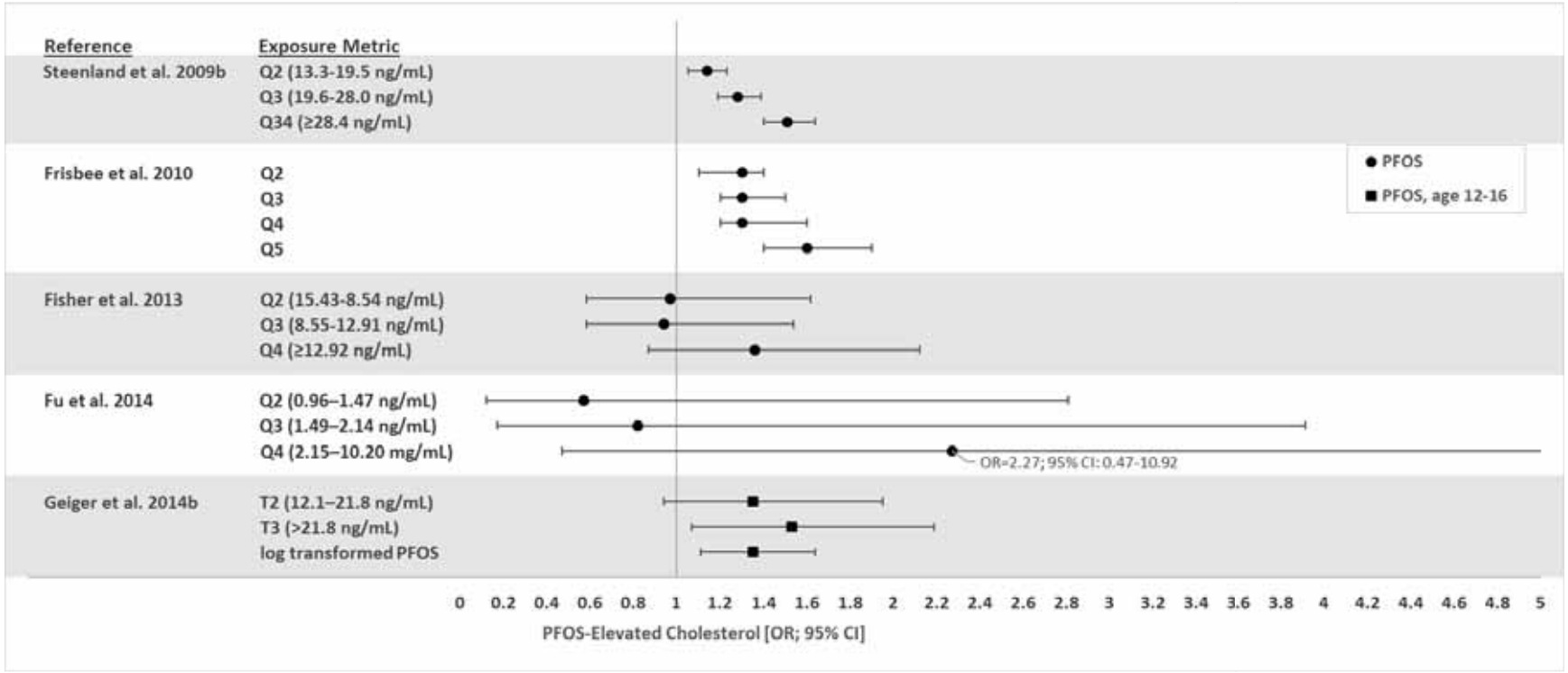


Figure 2-17. Serum LDL Cholesterol Levels Relative to Serum PFOS Levels
(Presented as percent change in LDL cholesterol levels)

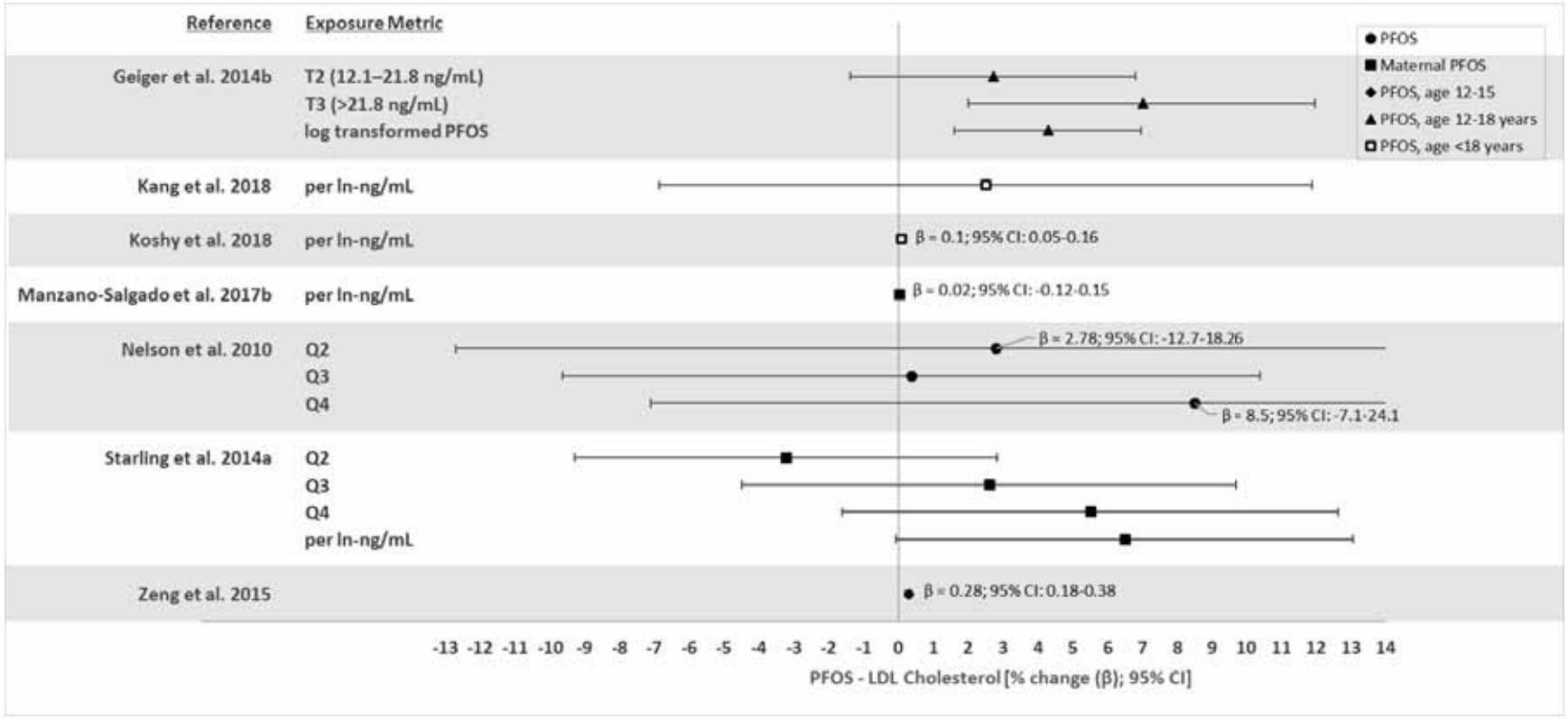
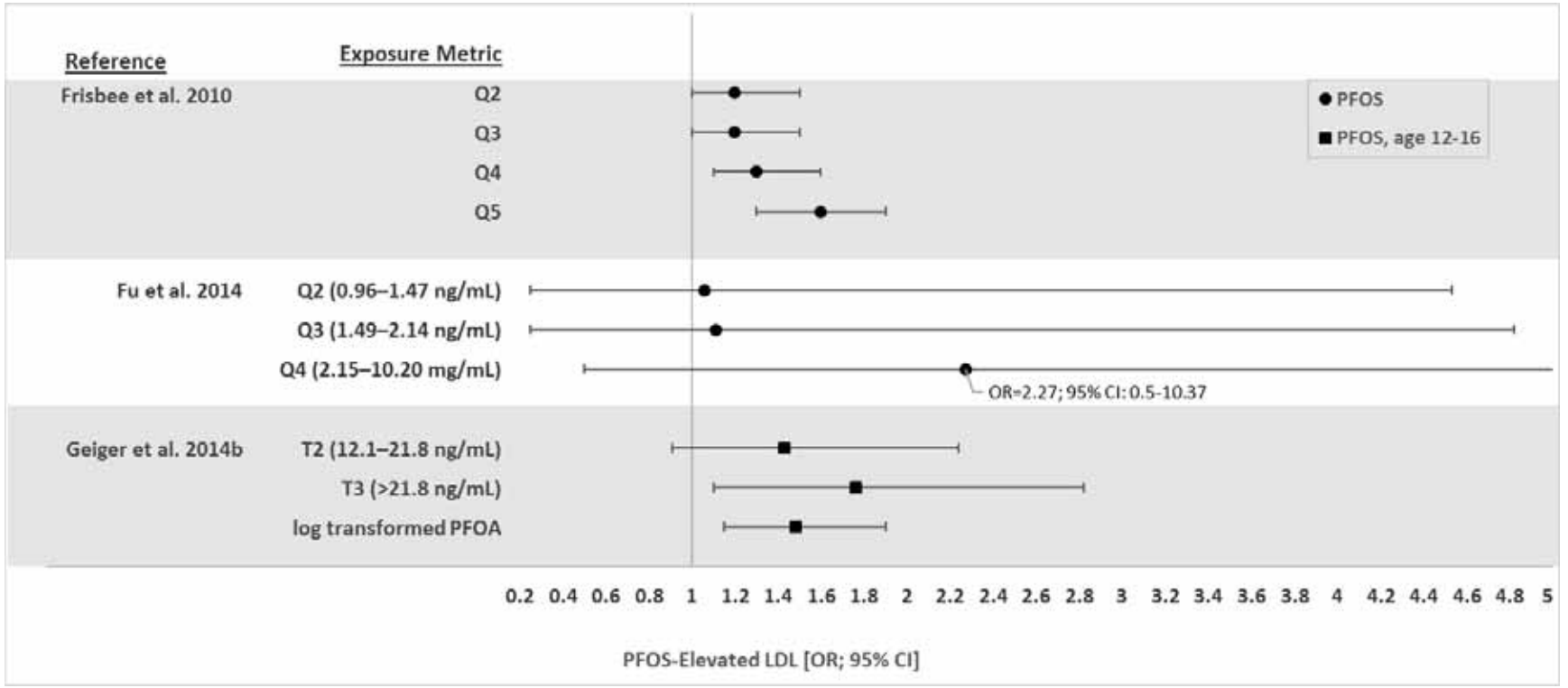


Figure 2-18. Risk of Abnormal LDL Cholesterol Levels Relative to PFOS Levels (Presented as Adjusted Ratios)



over 12,000 children and adolescents; the mean serum PFOS levels were 20.7 ng/mL in children (aged 1.0–11.9 years) and 19.3 ng/mL in adolescents (aged 12.0–17.9 years) (Frisbee et al. 2010). Similar findings were reported in a study of adults with a median serum PFOS level of 19.6 ng/mL; the study excluded subjects who reported taking cholesterol-lowering medication (Steenland et al. 2009b).

Associations were found between serum PFOS and total cholesterol, LDL cholesterol, and triglyceride levels, but not with HDL cholesterol. Participants with serum PFOS levels in the 2nd, 3rd, and 4th quartiles also had elevated risks of high cholesterol levels. Steenland et al. (2009b) noted that the odds of high cholesterol from the 1st to the 5th quintile was approximately 50% for PFOS, which may be important given that the Framingham study found that the risk of coronary heart disease was about 1.8 times higher in subjects with total cholesterol levels >240 mg/dL as compared to subjects with levels <200 mg/dL.

Steenland et al. (2009b) also examined over 10,000 participants who were taking cholesterol-lowering medication; an association between serum PFOS and total cholesterol levels was found in this group. Using both groups of subjects (taking or not taking cholesterol-lowering medication), the investigators analyzed whether taking cholesterol medication was associated with lower serum PFOA or PFOS levels, which may be indicative of reverse causality; no differences in serum PFOS levels were found between the two groups.

General population studies were conducted in the United States, Canada, and several European and Asian countries; these studies have found mixed results for associations between serum PFOS levels and serum lipids. Some studies have found associations between serum PFOS levels and serum total cholesterol (Nelson et al. 2010; Skuladottir et al. 2015; Starling et al. 2014a) and HDL cholesterol (Châteu-Degat et al. 2010); inverse associations between serum PFOS and HDL cholesterol (Starling et al. 2014a) and triglycerides (Châteu-Degat et al. 2010) were also found. However, other studies in adults have not found associations between serum PFOS and total cholesterol (Châteu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b), non-HDL cholesterol (Fisher et al. 2013), LDL cholesterol (Châteu-Degat et al. 2010; Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Starling et al. 2014a), HDL cholesterol (Fisher et al. 2013; Fu et al. 2014a; Liu et al. 2018b; Yang et al. 2018), or triglycerides (Fu et al. 2014a; Starling et al. 2014a; Liu et al. 2018b; Yang et al. 2018). Additionally, two studies did not find increased risks of elevated cholesterol levels (Fisher et al. 2013; Fu et al. 2014a). Several of these studies controlled for use of cholesterol-lowering medication (Châteu-Degat et al. 2010; Eriksen et al. 2013; Fisher et al. 2013; Nelson et al. 2010; Liu et al. 2018b). Overall, studies of children and adolescents have found associations for serum lipid levels. Geiger et al. (2014b) found increases in

the risk of elevated cholesterol and LDL cholesterol in children and adolescents aged 12–18 years; an association between serum PFOS and LDL cholesterol levels was also found. Zeng et al. (2015) found associations between serum PFOS and serum total cholesterol, LDL cholesterol, and triglyceride levels in children aged 12–15 years. Koshy et al. (2017) found an association between serum PFOS levels and serum total cholesterol, LDL cholesterol, and HDL cholesterol in children enrolled in the World Trade Center Health Registry. Timmermann et al. (2014) also found an association between serum PFOS and triglycerides only in obese Danish children (8–10 years of age), but not in normal weight children. In contrast, Maisonet et al. (2015b) found an inverse association between maternal serum PFOS and total cholesterol and LDL cholesterol in 15-year-old girls; no association was found when the girls were 7 years of age. Kang et al. (2018) did not find an association between serum PFOS and cholesterol, LDL cholesterol, or triglyceride levels in children aged 3–18 years, and Manzano-Salgado et al. (2017b) did not find associations between maternal serum PFOS and cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels in 4-year-old children.

Laboratory Animal Studies. Unpublished data summarized by OECD (2002) indicate that inhalation exposure of rats to lethal concentrations (1,890–45,970 mg/m^3) of PFOS dusts for 1 hour resulted in varying discoloration of the liver.

Consistent with the results for PFOA, acute-duration oral exposure of rats to PFOS resulted in increases in liver weight (Elcombe et al. 2012b; Era et al. 2009; Haughom and Spydevold 1992), hepatocellular hypertrophy (Elcombe et al. 2012b), and decreases in serum cholesterol and/or triglyceride levels (Elcombe et al. 2012a, 2012b; Haughom and Spydevold 1992). The lowest adverse effect level for increased liver weight, hypertrophy, and decreased serum cholesterol was 1.79 mg/kg/day in rats exposed to PFOS in the diet for 7 days (Elcombe et al. 2012b); however, a similar study by this group did not find significant alterations in liver weight or ALT, AST, or serum cholesterol levels after 7 days of exposure to 1.72 mg/kg/day (Elcombe et al. 2012a). Likewise, in mice, increases in liver weight (Fuentes et al. 2006; Qazi et al. 2009b, 2010a; Wan et al. 2011), hepatocellular hypertrophy (Qazi et al. 2010a), and decreases in serum cholesterol levels (Qazi et al. 2010a) were observed following acute exposure to PFOS. The lowest LOAEL for liver weight was 3 mg/kg/day in mice administered PFOS via gavage on GDs 6–18 (Fuentes et al. 2006); no effects were observed at 1.5 mg/kg/day. The only acute-duration mouse study that included histopathological examination of the liver and measurement of serum cholesterol levels identified a LOAEL of 8.5 mg/kg/day in mice exposed to PFOS in the diet for 10 days (Qazi et al. 2010a).

Intermediate-duration exposure to PFOS resulted in increased liver weight in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003; Thibodeaux et al. 2003) and mice (Bijland et al. 2011; Thibodeaux et al. 2003; Wan et al. 2011, 2014b; Xing et al. 2016; Yahia et al. 2008), hepatocellular hypertrophy in rats (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Seacat et al. 2003), decreased serum cholesterol levels in rats (Curran et al. 2008; Elcombe et al. 2012a; Luebker et al. 2005b; Seacat et al. 2003), decreased total cholesterol, triglyceride, non-HDL cholesterol, and HDL cholesterol levels in mice (Bijland et al. 2011), and increased serum AST and GGT levels in mice (Xing et al. 2016). A mouse study (Bijland et al. 2011) also showed dramatic decreases in the hepatic production of VLDL and HDL (Bijland et al. 2011). Another mouse study (Lee et al. 2015b) did not find increases in hepatic lipid levels in dams, although there were alterations in fetal livers. Only one of the intermediate-duration mouse studies included histopathological examination of the liver. Xing et al. (2016) reported cytoplasmic vacuolization, focal necrosis, and hepatocellular hypertrophy in mice exposed to PFOS via gavage for 30 days; however, the study did not report incidence; the lowest dose tested was 2.5 mg/kg/day. The lowest adverse effect level for liver effects in rats was 0.14 mg/kg/day for a significant increase in relative liver weight in female rats, but not male rats, exposed to PFOS in the diet for 28 days (Curran et al. 2008). This study also found significant decreases in serum cholesterol levels and increases in absolute and relative liver weights in males and females at 2.98 mg/kg/day and hepatocellular hypertrophy at 5.89 mg/kg/day. Seacat et al. (2003) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol levels in rats following a 14-week dietary exposure to 1.33 mg/kg/day; however, no significant alterations in liver weight or liver histopathology were observed in rats exposed to 1.77 mg/kg/day PFOS in the diet for 4 weeks (Seacat et al. 2003). In contrast, Elcombe et al. (2012a) reported increases in liver weight, hepatocellular hypertrophy, and decreased serum cholesterol in rats exposed to 1.54 mg/kg/day PFOS in the diet for 28 days.

Data on the chronic toxicity of PFOS to the liver in rodents are limited to a study in rats (Butenhoff et al. 2012b; Thomford 2002b). Hepatotoxicity characterized by centrilobular hypertrophy, centrilobular eosinophilic hepatocytic granules, and centrilobular hepatocytic vacuolation was noted in rats exposed to PFOS in the diet for 2 years. Among rats sacrificed at the end of the study, significant increases in the incidence of centrilobular hepatocellular hypertrophy were observed in male and female rats exposed to ≥0.25 mg/kg/day (Thomford 2002b). When animals sacrificed at interim periods (14 or 52 weeks) and unscheduled deaths were included with animals sacrificed at exposure termination, the incidence of centrilobular hepatocellular hypertrophy was also increased in males exposed to 0.1 mg/kg/day. At ≥0.1 mg/kg/day, significant increases in the incidences of eosinophilic clear cell altered foci and cystic hepatocellular degeneration were observed in male rats. An increase in cystic degeneration was observed

in male rats exposed to ≥0.025 mg/kg/day. However, this was mainly due to a high incidence in unscheduled deaths; among animals sacrificed at exposure termination, the incidence was only increased in males exposed to 1.04 mg/kg/day. An increased incidence of single cell necrosis was observed in males and females at 1.04 mg/kg/day (all groups combined). Observations made in a group of rats exposed to 1.17 mg/kg/day PFOS for 52 weeks and allowed to continue on the control diet for an additional year showed that hepatotoxicity was not a persistent response, as hepatotoxicity was generally absent at the end of the recovery period. At termination, electron microscopy showed mild to moderate smooth endoplasmic reticulum hyperplasia and minimal to mild hepatocellular hypertrophy primarily in rats dosed with 1.5 mg/kg/day PFOS, the highest dose tested.

In a study of Cynomolgus monkeys administered via gavage three doses of PFOS over 315 days, decreases in HDL cholesterol levels were found; the investigators noted that the levels were still within the normal variation (Chang et al. 2017). No alterations in other serum clinical chemistry parameters were found. Treatment of Cynomolgus monkeys with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks did not induce gross or microscopic morphological alterations in the liver and did not increase cell proliferation (Thomford 2002a). In a 26-week study in Cynomolgus monkeys, exposure to 0.75 mg/kg/day PFOS, administered via a capsule resulted in increased absolute liver weight after 183 days of treatment (Seacat et al. 2002). Significant decreases in serum total cholesterol were also observed at 0.75 mg/kg/day after 91, 153, and 182 days of exposure. On day 182, total cholesterol decreased to 35 and 53% of predosing values in males and females, respectively. The HDL cholesterol levels were significantly lower in males at 0.03 and 0.75 mg/kg/day on days 153 and 182 and in females at 0.15 and 0.75 mg/kg/day on days 153 and 182; the lack of pre-treatment HDL cholesterol measurements precludes within-group comparisons. Serum bilirubin was significantly lower in males at 0.75 mg/kg/day on days 91, 153, and 182. Light microscopy of liver sections showed centrilobular vacuolation, hypertrophy, and mild bile stasis in some monkeys exposed to 0.75 mg/kg/day. Electron microscopy showed lipid-droplet accumulation in some males and females exposed to 0.75 mg/kg/day. Increased glycogen content was also noted at this dose level. No histological alterations were observed in the livers of monkeys exposed to 0.75 mg/kg/day for 26 weeks and allowed to recover for 7 months or 1 year. Similarly, serum cholesterol returned to pretreatment levels 36 days post exposure and HDL cholesterol levels returned to pretreatment levels after 61 days of recovery.

Summary. Epidemiological studies have examined the possible associations between PFOS exposure and liver disease in workers and hepatocellular damage and alterations in serum lipid levels in workers and the general population. The available occupational exposure studies or general population studies do not

consistently suggest an association between PFOS exposure and increases in the risk of liver disease or biliary tract disorders. A small number of occupational exposure studies have not found associations between serum PFOS levels and increases in ALT, AST, or GGT levels. Overall, the epidemiological studies suggest an association between serum PFOS levels and increases in serum total cholesterol levels and possibly serum LDL cholesterol levels. Studies of workers at a PFOS manufacturing facility found elevated serum total cholesterol levels in workers with high serum PFOS levels; however, a longitudinal analysis at the same facility did not find that serum PFOS was a significant predictor of cholesterol levels. Studies of residents living in an area with very high PFOA water levels found increases in serum total cholesterol levels associated with elevated serum PFOS levels in children, adolescents, and adults. Mixed results have been found for associations between serum PFOS and increases in serum total cholesterol levels in general population studies. Associations have been found between serum PFOS levels and serum LDL-cholesterol levels among non-occupational populations.

In laboratory animals, oral exposure to PFOS results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipid levels. A small number of studies also reported focal necrosis and centrilobular hepatocytic vacuolization. The proposed mechanism of action for the increased liver weight, hepatocellular hypertrophy, and decreased serum lipid levels involves PPARα receptor activation. Due to species differences for this mechanism, these effects observed in rodents are not considered relevant to humans. The applicability of the hepatic hypertrophy and serum lipid alterations observed in rodent studies to humans has been questioned due to species differences in the presumed mechanism of action for these effects in rodents.

PFHxS

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. Lin et al. (2010) did not find associations between serum ALT and GGT levels with serum PFHxS levels in a general population study using the NHANES data set.

Epidemiological Studies—Serum Lipids. Eight studies have evaluated the potential association between serum PFHxS levels and serum lipids in the general population. A study utilizing the NHANES data set for adults not taking cholesterol-lowering medication reported an association between serum PFHxS and non-HDL cholesterol, but no associations with total cholesterol, LDL cholesterol, or HDL cholesterol (Nelson et al. 2010). In a study of Canadian adults not taking cholesterol-lowering medication with a geometric mean serum PFHxS level of 2.16 ng/mL, associations were found for total cholesterol, LDL

cholesterol, and non-HDL cholesterol (Fisher et al. 2013). The study also found increased odds of having a high cholesterol level with increasing PFHxS levels. Associations between serum PFHxS levels and HDL cholesterol and triglyceride levels were found in a study of Chinese men (Yang et al. 2018). In pregnant women in Norway with median serum PFHxS levels of 0.60 ng/mL, serum PFHxS levels were associated with serum HDL cholesterol, but not with total cholesterol, LDL cholesterol, or triglycerides (Starling et al. 2014a). No associations between serum PFHxS and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels were found in a study of Taiwanese children aged 12–15 years (mean serum PFHxS of 2.1 ng/mL) (Zeng et al. 2015) or Korean children aged 3–18 years (mean serum PFHxS of 0.793 ng/mL) (Kang et al. 2018). A study of Spanish children aged 4 years found an association between maternal serum PFHxS and triglyceride levels, but not with cholesterol, LDL cholesterol, or HDL cholesterol (Manzano-Salgado et al. 2017b). A fourth study in children reported associations between serum PFHxS levels and serum cholesterol and LDL cholesterol, but not HDL cholesterol or triglycerides, in World Trade Center Health Registry enrollees (Koshy et al. 2017).

Laboratory Animal Studies. Acute-duration gavage administration of PFHxS resulted in increases in liver weight, steatosis, and increases in hepatic triglyceride levels in mice; increases in liver weight and steatosis were also observed in similarly exposed PPARα-null mice (Das et al. 2017). An intermediate-duration study with PFHxS in rats reported that gavage doses of ≥3 mg/kg/day induced a significant increase in absolute and relative liver weight in males (Butenhoff et al. 2009a). Light microscopy revealed minimal to moderate enlargement of centrilobular hepatocytes. Clinical chemistry tests showed a significant decrease in serum cholesterol at ≥0.3 mg/kg/day and decreased serum triglycerides at 10 mg/kg/day. None of these alterations were observed in female rats. Centrilobular hepatocellular hypertrophy was observed in mice administered ≥0.3 mg/kg/day PFHxS for 42–60 days (Chang et al. 2018); at 3 mg/kg/day single cell necrosis and microvascular fatty changes were also observed. In male mice, dietary exposure to PFHxS in a western-type diet resulted in >50% decreases in plasma triglyceride, total cholesterol, non-HDL cholesterol, and HDL cholesterol levels and approximately 75% decreases in the hepatic production of VLDL (Bijland et al. 2011). Increases in liver weight and hepatic triglyceride levels were also observed.

PFNA

Epidemiological Studies—Hepatic Serum Enzymes and Bilirubin Levels. A health evaluation of workers at a U.S. polymer production facility using PFNA did not find alterations in ALT, AST, GGT, or bilirubin levels related to increases in exposure intensity score in a longitudinal analysis (Mundt et al.

2007). Associations between serum PFNA and ALT and GGT levels were observed in a NHANES data study (Gleason et al. 2015); however, another study (Lin et al. 2010) utilizing the NHANES data did not find associations between serum PFNA and these enzymes. Neither study found associations for AST or total bilirubin.

Epidemiological Studies—Serum Lipids. Longitudinal analysis of serum lipid levels in the occupational exposure study (Mundt et al. 2007) did not find significant differences in serum total cholesterol or triglycerides over time. In general population studies, associations have been observed between serum PFNA levels and total cholesterol levels in adults (Fu et al. 2014a; Nelson et al. 2010) and children (Koshy et al. 2017; Zeng et al. 2015). No associations with cholesterol were found in a study in pregnant women (Starling et al. 2014a) or studies in children (Kang et al. 2018; Manzano-Salgado et al. 2017b). Several studies have also found associations with LDL cholesterol (Fu et al. 2014a; Koshy et al. 2017; Zeng et al. 2015) or non-HDL cholesterol (Nelson et al. 2010), but others did not find associations for LDL cholesterol (Nelson et al. 2010; Kang et al. 2018; Starling et al. 2014a). Most studies did not find an association between serum PFNA and HDL cholesterol (Fu et al. 2014a; Nelson et al. 2010; Koshy et al. 2017; Manzano-Salgado et al. 2017b; Zeng et al. 2015) or triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Manzano-Salgado et al. 2017b;Starling et al. 2014a). Exceptions were the Starling et al. (2014a) study of pregnant women, which found a positive association for HDL cholesterol, Yang et al. (2018) study of men, which found associations for HDL cholesterol and triglycerides, and Zeng et al. (2015), which found an association with triglycerides in children. Fu et al. (2014a) did not find increased risks of elevated cholesterol, LDL cholesterol, or triglyceride levels or lowered HDL cholesterol levels in adults.

Laboratory Animal Studies. Ten studies have evaluated the hepatic toxicity of PFNA. The observed effects are consistent with effects observed for other perfluoroalkyls. Alterations in serum lipid levels consisted of decreases in serum HDL cholesterol levels in rats administered via gavage ≥1 mg/kg/day PFNA for 14 days (Fang et al. 2012a), decreases in serum triglyceride and cholesterol levels in mice receiving gavage doses of ≥1 mg/kg/day PFNA (Wang et al. 2015a), and decreases in serum cholesterol levels in mice administered 0.5 mg/kg/day PFNA (Singh and Singh 2018). Increases in liver weight were observed in rats nose-only exposed to ≥67 mg/m^3 PFNA (Kinney et al. 1989), in mice administered via gavage 10 mg/kg/day PFNA for 7 days (Das et al. 2017), in mice exposed to 0.5 mg/kg/day PFNA in the diet for 14 days (Kennedy 1987), in mice administered ≥0.2 mg/kg/day PFNA via gavage for 14 days (Wang et al. 2015a), and in the offspring of mice administered via gavage ≥0.83 mg/kg/day PFNA on GDs 1–17 or 1–18 (Das et al. 2015; Wolf et al. 2010). The increases in liver weight, hepatocellular

hypertrophy, and deceases in serum lipid levels are considered adaptive and not relevant to humans (Hall et al. 2012). Hepatocellular vacuolation was observed in mice administered via gavage 5 mg/kg/day PFNA for 14 days (Fang et al. 2012b). In PPARα-null mice, increases in liver weight were observed in non-pregnant mice administered via gavage ≥1.5 mg/kg/day PFNA for 18 days, but were not found in pregnant animals (Wolf et al. 2010). Das et al. (2017) found increases in liver weight, steatosis, and increases in liver triglyceride levels in PPARα-null mice administered 10 mg/kg/day PFNA for 10 days.

PFDA

Epidemiological Studies—Serum Lipids. Six general population studies have evaluated the potential relationships between serum PFDA and serum lipids and reported inconsistent results. Fu et al. (2014a) found an association between serum PFDA and total cholesterol in adults and Koshy et al. (2017) found associations between serum PFDA and total cholesterol in children. A study of men did not find associations between serum PFDA and HDL cholesterol or triglycerides (Yang et al. 2018). Studies in pregnant women (Starling et al. 2014a) and other studies in children (Kang et al. 2018; Zeng et al. 2015) did not find associations. Fu et al. (2014a), Starling et al. (2014a), and Koshy et al. (2017) found positive associations with HDL cholesterol; this was not found in the Zeng et al. (2015) study. Koshy et al. (2017) also found an association with LDL cholesterol. The other studies did not find associations between serum PFDA and LDL cholesterol (Fu et al. 2014a; Starling et al. 2014a; Zeng et al. 2015), and none found association with triglycerides (Fu et al. 2014a; Kang et al. 2018; Koshy et al. 2017; Starling et al. 2014a; Zeng et al. 2015). Only the Fu et al. (2014a) study looked for alterations in the risk of elevated cholesterol, LDL cholesterol, or triglyceride levels or decreased HDL cholesterol levels, but the study did not find significant increases in the risk.

Laboratory Animal Studies. Hepatic effects observed in laboratory animals exposed to PFDA include alterations in liver weight and morphology. Increases in liver weight have been observed in mice following a single gavage dose of PFDA; the alterations were observed 2 days after exposure to 40 mg/kg/day (Brewster and Birnbaum 1989) or 30 days after exposure to ≥20 mg/kg/day (Harris et al. 1989). Repeated dietary exposure to 2.4 mg/kg/day PFDA for 1 week (Kawashima et al. 1995) or 78 mg/kg/day for 10 days (Permadi et al. 1992, 1993) also resulted in increases in liver weight. Oral doses ≥9.5 mg/kg/day also resulted in increases in hepatic cholesterol levels in rats (Kawashima et al. 1995) and hepatic lipids in mice (Brewster and Birnbaum 1989). These acute doses were also associated with hepatocellular hypertrophy and evidence of peroxisome proliferation. Thirty days after a single gavage dose of ≥20 mg/kg/day PFDA, effects included periportal to panlobular hepatocellular

hypertrophy characterized by swollen hepatocytes with abundant granular eosinophilic cytoplasm and enlarged and hyperchromatic nuclei (Harris et al. 1989).

In intermediate-duration exposure studies, an increased incidence of minimal single cell hepatocellular necrosis were observed in rats administered 0.5 mg/kg/day PFDA for 28 days (Frawley et al. 2018).

PFUnA

Epidemiological Studies—Serum Lipids. Of the five studies evaluating potential associations between serum PFUnA and serum lipids, only a study by Kang et al. (2018) in children found an association between serum PFUnA and total cholesterol and LDL cholesterol. The other studies did not find associations between serum PFUnA and total cholesterol or LDL cholesterol (Fu et al. 2014a; Koshy et al. 2107; Starling et al. 2014a) or with HDL cholesterol or triglycerides (Yang et al. 2018). None of the studies found associations with triglyceride levels. Starling et al. (2014a) and Koshy et al. (2017) found associations of serum PFUnA levels with HDL cholesterol levels; Fu et al. (2014a) did not find an association for this parameter. No alterations in the risk of abnormal serum lipid levels were found in the adults examined by Fu et al. (2014a).

Laboratory Animal Studies. Only one animal study was identified that examined the liver following oral exposure to PFUnA. In an intermediate-duration study of rats administered PFUnA via gavage, increases in relative liver weight were observed in males at 0.3 mg/kg/day and in females at 1.0 mg/kg/day, and mild to moderate centrilobular hepatocellular hypertrophy was observed in males and females at 1.0 mg/kg/day (Takahashi et al. 2014).

PFHpA

Epidemiological Studies—Serum Lipids. Epidemiological data on PFHpA are limited to a study in adults conducted by Fu et al. (2014a), which found no associations between serum PFHpA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels and a study in men conducted by Yang et al. (2018), which found no associations between serum PFHpA and HDL cholesterol or triglycerides.

PFBS

Epidemiological Studies—Serum Lipids. In the only epidemiological study examining serum lipids and possible associations with serum PFBS, Zeng et al. (2015) found an association with total cholesterol levels in children. No associations were found between serum PFBS and LDL cholesterol, HDL cholesterol, or triglycerides.

Laboratory Animal Studies. Treatment of male rats with 900 mg/kg/day PFBS by gavage for 28 days induced a significant increase in absolute and relative liver weight (25–30%) relative to controls, which was no longer detected following a 14-day recovery period (3M 2001). Clinical chemistry tests of liver function were unremarkable and there were no chemical-related microscopic alterations. No alterations in liver weight, serum chemistry parameters (ALT, AST, cholesterol), or liver morphology were observed in rats administered gavage doses as high as 600 mg/kg/day PFBS for 90 days (Lieder et al. 2009a). Significant increases in liver weight were observed at 300 and 1,000 mg/kg/day in a 2-generation study (Lieder et al. 2009b); the alterations were only observed in male rats. An increase in hepatocellular hypertrophy was also observed in the male P0 and F1 rats administered via gavage 1,000 mg/kg/day. Dietary exposure to mice resulted in decreases in plasma triglyceride levels and hepatic cholesterol levels, but no alterations in liver weight or plasma cholesterol, HDL cholesterol, or non-HDL cholesterol (Bijland et al. 2011).

PFBA

Epidemiological Studies—Serum Lipids. Only one epidemiological study examined hepatic outcomes; this study (Fu et al. 2014a) did not find any associations between serum PFBA levels and total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides in adults.

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect liver weight, nor did it cause gross or microscopic morphological alterations in the liver (3M 2007a). In addition, clinical chemistry tests did not indicate altered liver function. Similarly, administration of approximately 20 mg/kg/day PFBA in the diet to male rats for 2 weeks did not significantly affect relative liver weight, but the same dose of PFOA induced a 45% increase in liver weight (Ikeda et al. 1985). Dietary administration of doses of approximately 78 mg/kg/day PFBA to male mice for 10 days induced a 63% increase in absolute liver weight (Permadi et al. 1992, 1993).

PFBA intermediate-duration studies have consistently found increases in liver weight and histological alterations. Dosing rats with PFBA by gavage for 28 days resulted in significant increases in absolute and relative liver weight and decreases in serum cholesterol at ≥30 mg/kg/day and hepatocellular hypertrophy at 150 mg/kg/day (Butenhoff et al. 2012a; van Otterdijk 2007a). Administration of 150 mg/kg/day PFBA induced hepatocyte hypertrophy. These liver effects were no longer detected after a 21-day recovery period. In a similar 90-day study, administration of 30 mg/kg/day PFBA resulted in increased absolute liver weight and panlobular hepatocyte hypertrophy (Butenhoff et al. 2012a; van Otterdijk 2007b); no liver effects were observed at 6 mg/kg/day. None of the liver alterations were observed after a 21-day recovery period.

PFDoDA

Epidemiological Studies—Serum Lipids. A general population study of adolescents (Zeng et al. 2015) did not find any associations between serum PFDoDA and total cholesterol, LDL cholesterol, HDL cholesterol, or triglyceride levels.

Laboratory Animal Studies. Dosing of male Sprague-Dawley rats with 10 mg/kg/day PFDoDA by gavage for 14 days induced a 35% increase in total serum cholesterol; doses of 1 or 5 mg/kg/day had no significant effect (Shi et al. 2007). In a subsequent study, the same group of investigators reported that in rats dosed via gavage with 1 or 5 mg/kg/day PFDoDA, there was a trend for decreased serum triglycerides, but the differences with controls were not statistically significant (Zhang et al. 2008); at 10 mg/kg/day, serum triglyceride levels were significantly increased. Liver triglyceride and liver cholesterol levels were increased at ≥5 mg/kg/day. Absolute liver weight was significantly reduced in the 5 mg/kg/day group (19%) relative to controls, but this may have been due to a marked reduction in body weight (shown in Shi et al. [2007], but not in Zhang et al. [2008]).

In a 42-day PFDoDA gavage administration study, increases in relative liver weight were observed in males at ≥0.5 mg/kg/day and hepatocellular hypertrophy was observed at 2.5 mg/kg/day (Kato et al. 2015). The study also found decreases in serum cholesterol at 0.1 and 0.5 mg/kg/day, but not at 2.5 mg/kg/day. In pregnant females (most dying before the end of the study), single cell hepatocyte necrosis was observed at 2.5 mg/kg/day (Kato et al. 2015). Prebiliary infiltration of inflammatory cells (males), disposition of bilirubin (females), and hepatocellular hypertrophy (females) were observed in males and nonpregnant females administered 2.5 mg/kg/day PFDoDA for 42 days followed by a 42-day recovery period (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Increases in liver weight, decreases in serum cholesterol levels, and centrilobular hepatocellular hypertrophy have been observed in rats administered 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005) or ≥100 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009). In a chronic-duration study, gavage administration of 200 mg/kg/day for 2 years resulted in increases in the incidence of hepatocellular necrosis in female rats (Klaunig et al. 2015). At 100 mg/kg/day, decreases in triglyceride levels were observed in male rats.

FOSA

Laboratory Animal Studies. In the only study examining hepatic effects, Seacat and Luebker (2000) reported no alterations in liver weight in rats receiving a single gavage dose of 5 mg/kg FOSA.

2.10 RENAL

Overview. Epidemiological and laboratory animal studies have evaluated the potential of perfluoroalkyls to be renal toxicants. Human studies have evaluated the risk of kidney disease, alterations in renal function, damage to the kidney, and alterations in uric acid levels. The results of epidemiological studies evaluating kidney disease and renal function are summarized in Table 2-13; Table 2-14 contains the studies evaluating alterations in uric acid levels. More detailed descriptions of these studies can be found in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 8. Although there are a couple of studies finding associations between PFOA exposure and kidney disease, the results are not consistent across study populations. However, there is some indication that perfluoroalkyls may affect renal function. Decreases in estimated glomerular filtration rate and increases in uric acid levels associated with serum PFOA or PFOS have been reported in a number of epidemiological studies. However, these alterations may be due to reverse causality (i.e., increases in serum perfluoroalkyl levels could be due to a decrease in glomerular filtration and shared renal transporters for perfluoroalkyls and uric acid). Based on the small number of epidemiological studies or the inconsistency of the results, possible associations between other perfluoroalkyls (PFHxS, PFNA, PFDA, PFBS, PFDoDA, or PFHxA) and renal functions cannot be assessed. No studies were available for PFUnA, PFHpA, PFBA, or FOSA.

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n =53)	12,930 ng/mL (mean PFOA in current workers)	Serum urea	NS (p>0.05)
		Serum creatinine	NS (p>0.05)
		Total proteins	NS (p>0.05)
		α1 globulins, α2 globulins, β globulins, or γ globulins	NS (p>0.05)
		α2 globulins	**Association (p<0.01), current, former and non-exposed workers.**
Lundin et al. 2009 Occupational (n=3,992)	NR	Nephritis and nephrosis deaths	SMR 5.2 (0.6–18.9)
Raleigh et al. 2014 Occupational (n=9,027)	NR	Chronic kidney disease deaths	HR 0.73 (0.21–2.48), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Chronic kidney disease risk	NS (p=0.92), no lag NS (p=0.99), 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084)	7,800 ng/mL-year (mean PFOA)	Chronic kidney disease deaths	**SMR 3.79 (1.03–9.71)*, 2nd quartile**
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Kidney disease (self-reported)	**SPR 2.26 (1.45–3.51)***
Dhingra et al. 2016b Community (C8) (n=28,541)	Estimated cumulative PFOA	Chronic kidney disease	NS (p=0.80 for trend), no lag NS (p=0.81 for trend), 5-year lag NS (p=0.88 for trend), 10-year lag NS (p=0.30 for trend), 20-year lag
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	Serum creatinine	NS (p>0.05)
		BUN	NS (p>0.05)
		Total serum proteins	NS (p>0.05)

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Watkins et al. 2013 Community (C8) (9,660 children)	28.3 ng/mL (median PFOA)	GFR	**Inverse association (p=0.02)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.01)*, 4th quartile**
		Serum uric acid	**Association (p<0.01)***
Shankar et al. 2011a General population (NHANES) (n=4,587)	>5.9 ng/mL (4th PFOA quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.73 (1.04–2.88)*, 4th quartile**
PFOS			
Olsen et al. 1998a Occupational (n=178 in 1995; n=149 in 1997)	2,440 and 1,930 ng/mL (mean PFOS in 1995 in Decatur and Antwerp) 1,960 and 1,480 ng/mL (mean in 1997 in Decatur and Antwerp)	Serum creatinine	**Association (p<0.06)*, 1997 only**
		BUN	NS (p>0.1)
Watkins et al. 2013 Community (C8) (9,660 children)	20.0 ng/mL (median PFOS)	GFR	**Inverse association (p=0.0001)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	7.9–12.8 ng/mL (2nd PFOS quartile) ≥19.4 ng/mL (4th PFOS quartile)	GFR	**Inverse association (p<0.05)*, 2nd quartile**
Shankar et al. 2011a General population (NHANES) (n=4,587)	>29.5 ng/mL (4th PFOS quartile) 11.2–17.8 ng/mL (2nd PFOS quartile)	GFR	**Inverse association (p<0.001 for trend)***
		Chronic kidney disease	**OR 1.82 (1.02–3.27)*, 4th quartile**
PFHxS			
Watkins et al. 2013 Community (C8) (9,660 children)	5.2 ng/mL (median PFHxS)	GFR	**Inverse association (p=0.003)***

Table 2-13. Summary of Renal Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	GFR	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	BUN Creatinine	Small, but not clinically significant
Watkins et al. 2013 Community (C8) (9,660 children)	1.5 ng/mL (median PFNA)	GFR	**Inverse association (p=0.002)***
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	GFR	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BUN = blood urea nitrogen; GFR = glomerular filtration rate; HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SMR = standardized mortality ratio

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Costa et al. 2009 Occupational (n=53)	12,930, ng/mL (mean PFOA in current workers)	Serum uric acid	**Association (p=0.039)***
Sakr et al. 2007b Occupational (n=1,025)	490 ng/mL (median PFOA)	Serum uric acid	**Association (reported by investigator)***
Steenland et al. 2010b Community (n=54,591)	11.5–20.6 ng/mL (2nd quintile PFOA)	Hyperuricemia risk	**OR 1.33 (1.24–1.43)* (2nd quintile)**
Gleason et al. 2015 General population (NHANES) (n=4,333)	3.7 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	**Association (p<0.001)***
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	4.3 ng/mL (mean PFOA), >5.4 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p=0.0001)***
		Hyperuricemia risk	**OR 1.62 (1.10–2.37)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4.7 ng/mL (4th PFOA quartile)	Serum uric acid	**Association (p<0.01)***
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFOA)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	**OR 2.16 (1.29–3.61)* (full cohort)** **OR 2.76 (1.37–5.56)* (boys only)**
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	3.5–5.1 ng/mL (3rd PFOA quartile)	Serum uric acid	**Association (p<0.0001)***
		Hyperuricemia risk	**OR 1.90 (1.35–2.69)*, 3rd quartile**
PFOS			
Steenland et al. 2010b Community (n=54,591)	17.5–23,26 ng/mL (3rd PFOS quintile)	Hyperuricemia risk	**OR 1.11 (1.04–1.20)* (3rd quintile)**

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES, n=4,333)	11.3 ng/mL (median PFOS)	Serum uric acid	**Association (p<0.01)***
		Hyperuricemia risk	NS (p=0.502)
Geiger et al. 2013 General population (NHANES) (n=1,772 adolescents and adults)	18.4 ng/mL (mean PFOS), >25.5 ng/mL (4th PFOS quartile)	Serum uric acid	NS (p=0.0575)
		Hyperuricemia risk	**OR 1.65 (1.10–2.49)* (4th quartile)**
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥19.4 ng/mL (4th PFOS quartile)	Serum uric acid	**Association (p<0.05)***
Qin et al. 2016 General population (n=225 adolescents)	28.9 ng/mL (median PFOS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.35 (0.95–1.93) (full cohort)
Shankar et al. 2011b General population (NHANES) (n=3,883 adults)	11.2–17.8 ng/mL (2nd PFOS quartile)	Serum uric acid	**Association (p=0.0018)***
		Hyperuricemia risk	**OR 1.46 (1.11–1.91)*, 2nd quartile**
PFHxS			
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.8 ng/mL (median PFHxS)	Serum uric acid	NS (p>0.01)
		Hyperuricemia risk	NS (p=0.110 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥4 ng/mL (4th PFHxS quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	1.3 ng/mL (median PFHxS)	Serum uric acid	**Association (p<0.05)***
		Hyperuricemia risk	OR 1.39 (0.93–2.07)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	Serum uric acid	Small, but not clinically significant

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gleason et al. 2015 General population (NHANES) (n=4,333)	1.4 ng/mL (median PFNA)	Serum uric acid	**Association (p<0.001)***
		Hyperuricemia risk	NS (p=0.42 for trend)
Kataria et al. 2015 General population (NHANES) (n=1,960 adolescents)	≥1.5 ng/mL (4th PFNA quartile)	Serum uric acid	NS (p>0.05)
Qin et al. 2016 General population (n=225 adolescents)	0.8 ng/mL (median PFNA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.28 (0.83–1.96)
PFDA			
Qin et al. 2016 General population (n=225 adolescents)	0.9 ng/mL (median PFDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.26 (0.82–1.92)
PFBS			
Qin et al. 2016 General population (n=225 adolescents)	0.5 ng/mL (median PFBS)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.23 (0.86–1.75)
PFDoDA			
Qin et al. 2016 General population (n=225 adolescents)	2.7 ng/mL (median PFDoDA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 0.93 (0.65–1.34)

Table 2-14. Summary of Uric Acid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxA			
Qin et al. 2016 General population (n=225 adolescents)	0.2 ng/mL (median PFHxA)	Serum uric acid	NS (p>0.05)
		Hyperuricemia risk	OR 1.08 (0.77–1.61)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 8 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Laboratory animal studies have primarily evaluated kidney morphology; these studies are summarized in Tables 2-1, 2-3, 2-4, 2-5, and 2-6. The NOAEL and LOAEL values for these studies are illustrated in Figures 2-6, 2-8, 2-9, and 2-10. In general, the laboratory animal studies have not found evidence of impaired renal function or morphological damage following exposure to PFOA, PFOS, PFHxS, PFDA, PFUnA, PFBS, PFBA, PFDoDA, or PFHxA. No laboratory animal studies examining renal endpoints were available for PFNA, PFHpA, or FOSA.

PFOA

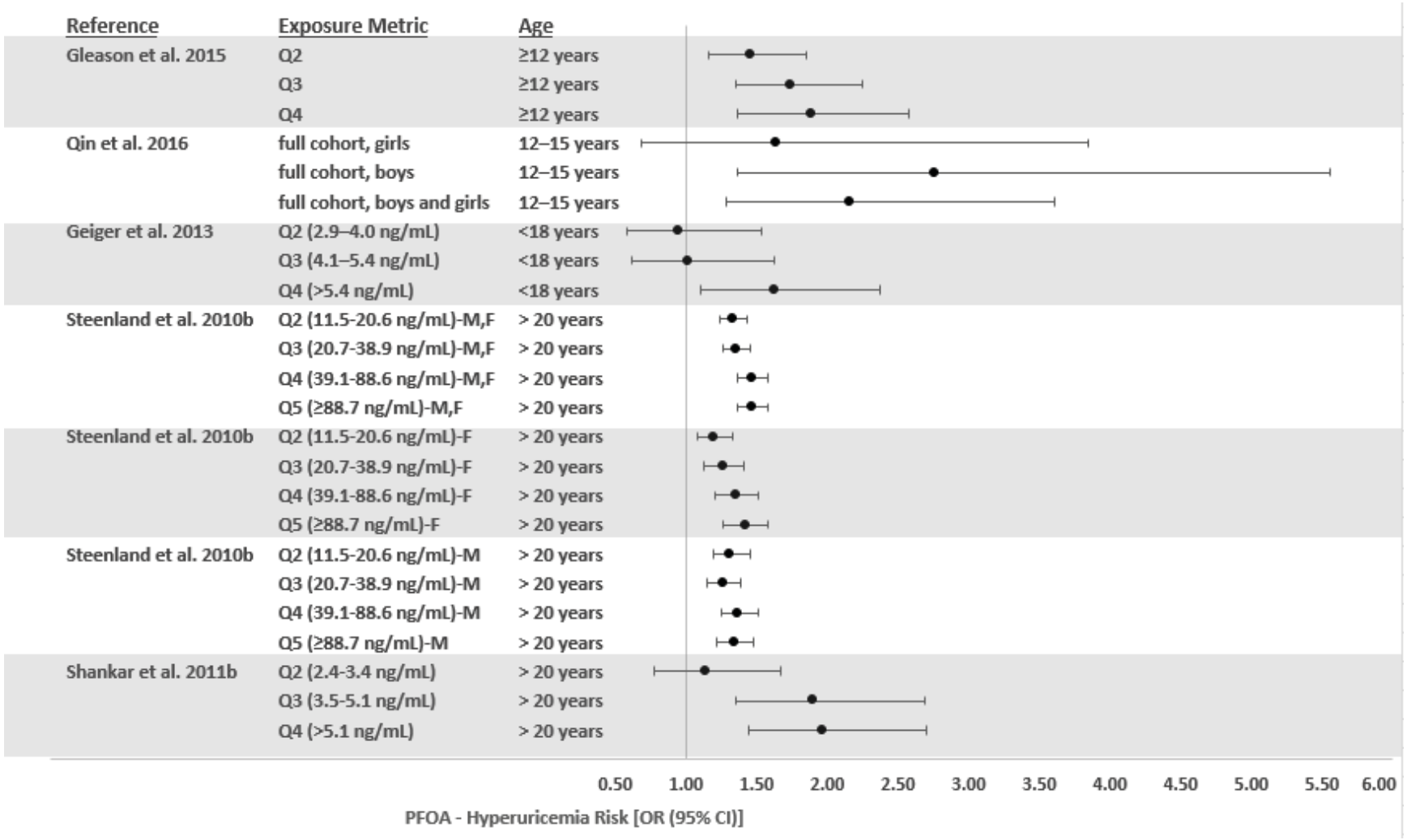
Epidemiological Studies—Kidney Disease. Several epidemiological studies have examined the possible association between PFOA exposure and increased risk of kidney disease. In a cohort mortality study of workers at the DuPont PFOA facility in West Virginia, Steenland and Woskie (2012) found an increase in deaths from chronic renal disease when compared to DuPont workers at other regional facilities. When estimated cumulative PFOA exposure was estimated based on the worker's job history and data from a biomonitoring survey conducted from 1979 to 2004, there was a significant positive trend for nonmalignant kidney disease when the workers were divided in estimated cumulative exposure quartiles. Two studies of workers at the 3M APFO facility in Cottage Grove, Minnesota did not find increases in deaths from chronic kidney disease (Raleigh et al. 2014) or nephritis and nephrosis (Lundin et al. 2009) as compared to mortality rates for the state of Minnesota. Similar results were found when chronic kidney disease deaths were compared to those in a cohort of workers in St. Paul Minnesota who worked at a non-APFO facility (Raleigh et al. 2014). An occupational exposure study (Steenland et al. 2015) and C8 community study (Dhingra et al. 2016b) found no associations between estimated cumulative PFOA exposure and the risk of chronic kidney disease. Another study of the community living near the Washington Works facility found a higher prevalence of self-reported kidney disease as compared to rates reported in NHANES (Anderson-Mahoney et al. 2008).

Epidemiological Studies—Biomarkers of Renal Function. Several biomarkers of renal function have been evaluated in epidemiological studies; these include BUN, serum creatinine, glomerular filtration rate, and uric acid levels (discussed in the following section). Kidney function, assessed by levels of BUN and serum creatinine, was not associated with exposure to PFOA in the occupational exposure studies by Olsen et al. (2003a) or Costa et al. (2009) or a community exposure study by Emmett et al. (2006b).

Three studies have found inverse associations between serum PFOA and glomerular filtration rate. Using the NHANES data for the 1999–2008 cycles, Shankar et al. (2011a) found an inverse association between serum PFOA levels and estimated glomerular filtration rate in adults. The likelihood of chronic kidney disease, defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2, was significantly higher in adults with the highest serum PFOA (>5.9 ng/mL, OR 1.73, 95% CI 1.04–2.88) levels than in adults with serum PFOA levels in the lowest quartile. The study also investigated whether the association between serum PFOA levels and chronic kidney disease was due to reverse causality (i.e., decreased glomerular filtration leads to a decrease in perfluoroalkyl filtration) and found a stronger negative correlation between estimated glomerular filtration rate and serum PFOA levels in subjects without chronic kidney disease, suggesting that it was not due to reverse causality. In another study utilizing NHANES data, an inverse association was found in adolescents with serum PFOA levels in the 4th quartile (Kataria et al. 2015). Similarly, an inverse association between serum PFOA and glomerular filtration rate was found in children participating in the C8 Health Project (Watkins et al. 2013). Unlike Shankar et al. (2011a), Watkins et al. (2013) suggested that the association between serum perfluoroalkyl levels and estimated glomerular filtration rates may be a consequence of reverse causation because no associations were found between estimated serum PFOA levels 3 or 10 years prior to enrollment in the study or at the time of study enrollment and estimated glomerular filtration rates; predicted serum PFOA levels were based on environmental PFOA levels, self-reported residential history, and PBPK modeling.

Epidemiological Studies—Alterations in Uric Acid Levels. Associations between serum PFOA levels and serum uric acid levels have been found in several occupational, community, and general population studies. Costa et al. (2009) and Sakr et al. (2007b) reported associations between serum PFOA levels and serum uric acid levels in workers with high serum PFOA levels. In adult participants of the C8 Health Project, positive linear trends between serum uric acid levels and serum PFOA levels were found (Steenland et al. 2010b). When the subjects were categorized by PFOA levels, significantly increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (≥11.5 ng/mL). Four studies utilizing NHANES data have found associations between serum PFOA and serum uric acid levels in adults (Gleason et al. 2015; Shankar et al. 2011b) and adolescents (Geiger et al. 2013; Kataria et al. 2015). A study in Taiwanese adolescents also found this association between PFOA and uric acid (Qin et al. 2016). Several studies have also found increases in the risk of hyperuricemia in a highly exposed population (Steenland et al. 2010b) and the general population (Gleason et al. 2015; Geiger et al. 2013; Qin et al. 2016; Shankar et al. 2011b). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-19.

Figure 2-19. Risk of Hyperuricemia Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Laboratory Animal Studies. No gross or microscopic alterations were observed in the kidneys from male rats following head-only inhalation exposure to up to 84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986). Significantly elevated absolute and relative kidney weight was reported in male rats dosed with ≥3 mg/kg/day PFOA by gavage in water for 70 days (Butenhoff et al. 2004b), but histological evaluation of the kidney was not conducted in this study. Rats that received much higher doses (100–110 mg/kg/day) of APFO for 90 days in the diet showed no significant morphological alterations in the kidneys, and BUN and the urinalysis were unremarkable (Griffith and Long 1980). Also, male mice dosed with up to 47 mg/kg/day APFO in the drinking water for 21 days showed no morphological alterations in the kidneys, and BUN and serum creatinine levels were not significantly affected (Son et al. 2008). Treatment of Cynomolgus monkeys with daily doses of up to 20 mg/kg/day APFO, administered via a capsule, for 26 weeks (Butenhoff et al. 2002) or Rhesus monkeys dosed with up to 10 mg/kg/day by gavage for 90 days (Griffith and Long 1980) did not cause morphological alterations in the kidneys, and blood chemistries and urinalyses provided no evidence of alterations in kidney function. In a 2-year dietary study in rats, relative kidney weight from males dosed with 15 mg/kg/day APFO was significantly elevated (14%) at the 1-year interim evaluation relative to controls, but gross and microscopic appearance (at 1 year and at termination), BUN, and urinalyses (several times during the study) were not significantly affected (3M 1983; Butenhoff et al. 2012c). No gross or microscopic alterations were seen in the kidneys from rats that received dermal applications of up to 2,000 mg/kg/day APFO to the shaven skin for 2 weeks (Kennedy 1985).

Summary. Epidemiological studies have examined possible associations between exposure to PFOA and increases in the risk of kidney disease and alterations in renal function. Mixed results for associations between serum PFOA and risks of kidney disease have been reported in occupational exposure studies and studies of highly exposed residents with more studies not finding associations. Several general population and community studies have found inverse associations between serum PFOA and glomerular filtration rate; however, there is suggestive evidence that this association may be due to reverse causation rather than a direct effect. Associations between serum PFOA levels and serum uric acid levels have been consistently observed in occupational, community, and general populations. Laboratory animal studies have not found evidence of alterations in renal function or histological alterations.

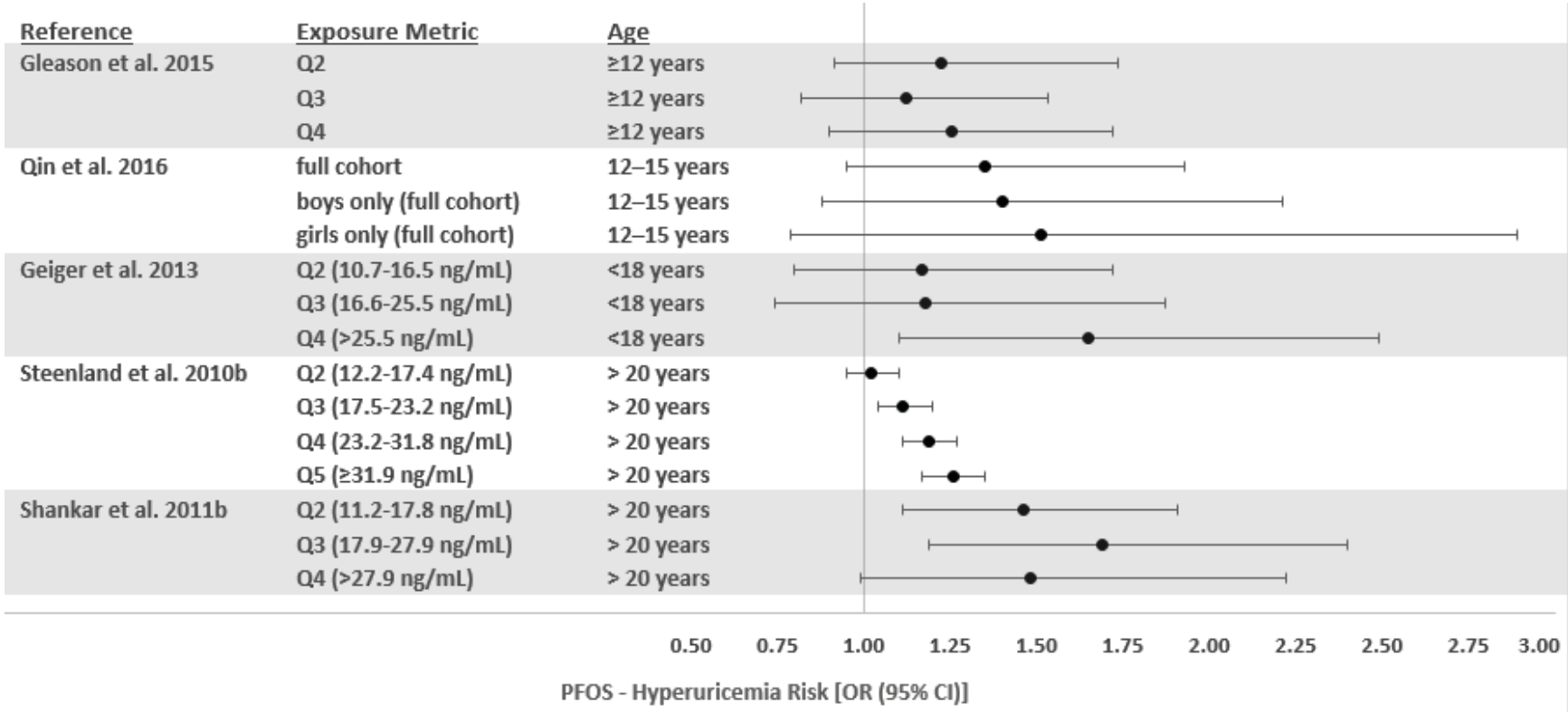
PFOS

Epidemiological Studies—Biomarkers of Renal Function. Three studies have found inverse associations between serum PFOS levels and glomerular filtration rate in adults (Shankar et al. 2011a), adolescents (Kataria et al. 2015), and children (Watkins et al. 2013). In the Watkins et al. (2013) study of C8 Health Project participants, a concentration-related linear trend between decreasing estimated glomerular filtration rates and increases in serum PFOS levels was observed in children and adolescents 1–<18 years old. In adolescents 12–19 years of age participating in NHANES, the estimated glomerular filtration rate was lower in participants with serum PFOA levels in the 2nd, 3rd, and 4th quartiles than those with levels in the 1st quartile (Kataria et al. 2015). In addition to the inverse association between serum PFOS and estimated glomerular filtration rate observed in adult NHANES participants, Shankar et al. (2011a) also found increased risks of chronic kidney disease (defined as a glomerular filtration rate of <60 mL/minute/1.73 m^2) in participants with serum PFOS levels in the 4th quartile.

Epidemiological Studies—Alterations in Uric Acid Levels. In a study of C8 Health Project participants, a linear trend between serum uric acid levels and serum PFOS levels was found (Steenland et al. 2010b). When the subjects were categorized by serum PFOS levels, increased risks of hyperuricemia (>6.0 mg/dL for women, >6.8 mg/dL for men) were observed for subjects with serum PFOS levels in the 3rd, 4th, and 5th quintiles. Similar findings were found in NHANES adult participants (Shankar et al. 2011b). A study of adolescent NHANES participants found associations between serum PFOS and serum uric acid levels (Kataria et al. 2015); a second study did not find an association (Geiger et al. 2013). The Geiger et al. (2013) study did find an increased risk of hyperuricemia for adolescents with serum PFOS levels in the 4th quartile. A study of Taiwanese adolescents did not find associations between serum PFOS and uric acid or an increased risk of hyperuricemia (Qin et al. 2016). The ORs for the risk of hyperuricemia in these studies are summarized in Figure 2-20.

Laboratory Animal Studies. No significant morphological alterations or clinical evidence of impaired kidney function was reported in male and female rats dosed with up to 1.77 mg/kg/day PFOS (potassium salt) (Seacat et al. 2003) or 5.89 mg/kg/day (Curran et al. 2008) for 4 weeks. Extending the treatment to 14 weeks resulted in an increase in BUN in male (23% increase) and female rats (41% increase), but histopathology of the kidneys and urinalyses were unremarkable (Seacat et al. 2003). The NOAEL values were 0.34 and 0.4 mg/kg/day in males and females, respectively. Gavage administration of three doses of PFOS to Cynomolgus monkeys over 315 days did not result in alterations in BUN or serum creatinine or

Figure 2-20. Risk of Hyperuricemia Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



total protein levels (Chang et al. 2017). Treatment of Cynomolgus monkeys with up to 0.75 mg/kg/day PFOS (potassium salt) administered via a capsule for 26 weeks did not cause morphological alterations in the kidneys, nor did it affect BUN, serum creatinine, or urinary parameters (Seacat et al. 2002). Similar results were reported in a 4-week study in monkeys dosed with up to 2 mg/kg/day PFOS (Thomford 2002a). A mild increase in BUN was reported in rats treated with approximately 0.25 or 1.04 mg/kg/day PFOS in the diet for 53 weeks in a 2-year study (Butenhoff et al. 2012b; Thomford 2002b). However, there were no significant gross or microscopic alterations in the kidneys at week 53 or at termination.

PFHxS

Epidemiological Studies—Biomarkers of Renal Function. A small number of epidemiological studies have evaluated biomarkers of renal function. In a study of C8 Health Project child participants (aged 1–<18 years), an inverse association between serum PFHxS and estimated glomerular filtration rate was observed (Watkins et al. 2013). A study of adolescent participants in NHANES did not find this association (Kataria et al. 2015). It is noted that the reported median PFHxS level in the Watkins et al. (2013) study (5.2 ng/mL) exceeded the lower end of the 4th quartile serum PFHxS level in the Kataria et al. (2015) study (≥4 ng/mL).

Epidemiological Studies—Alterations in Uric Acid Levels. In NHANES participants ≥12 years of age (Gleason et al. 2015) and adolescent NHANES participants (Kataria et al. 2015), no associations between serum PFHxS levels and serum uric acid levels or risk of hyperuricemia (Gleason et al. 2015) were found. A study of Taiwanese adolescents found an association between serum PFHxS levels and serum uric acid levels, but did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Male rats treated by gavage with 10 mg/kg/day PFHxS for at least 42 days showed a significant increase in BUN levels, but there were no significant gross or microscopic alterations in the kidneys (Butenhoff et al. 2009a); the NOAEL was 3 mg/kg/day. No significant effect on BUN was reported in female rats. No histological alterations were observed in the kidneys of mice following intermediate-duration administration of ≤3 mg/kg/day PFHxS (Chang et al. 2018).

PFNA

Epidemiological Studies—Biomarkers of Renal Function. Two epidemiological studies have evaluated the possible associations between serum PFNA and alterations in renal function biomarkers. In a study of

children participating in the C8 Health Project, an inverse association between serum PFNA and estimated glomerular filtration rate was observed, but not in adolescents participating in NHANES (Watkins et al. 2013). Mundt et al. (2007) noted that there were small, but not clinically significant, alterations in BUN, creatinine, and serum uric acid levels in workers exposed to PFNA.

Epidemiological Studies—Alterations in Uric Acid Levels. Gleason et al. (2015) found an association between serum PFNA and serum uric acid levels in NHANES participants; this association was not found in studies of adolescents (Kataria et al. 2015; Qin et al. 2016). Studies by Gleason et al. (2015) and Qin et al. (2016) did not find increases in the risk of hyperuricemia associated with serum PFNA levels.

PFDA

Epidemiological Studies—Alterations in Uric Acid Levels. Epidemiological studies examining renal outcomes are limited to a study of Taiwanese adolescents that found no association between serum PFDA levels and serum uric acid levels and did not find increased risks of hyperuricemia (Qin et al. 2016).

Laboratory Animal Studies. Administration of a single dose of up to 80 mg/kg PFDA to female C57BL/6N mice by gavage did not induce gross or microscopic changes in the kidneys (Harris et al. 1989). However, 2 out of 10 mice that died following administration of a dose of 320 mg/kg showed mild acute necrosis of the proximal convoluted tubules. No histological alterations were observed in the kidneys of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Laboratory Animal Studies. Treatment of male and female rats with 1.0 mg/kg/day PFUnA via gavage for 41–46 days resulted in significant increases in BUN levels (35–61% in males, 19–45% in females) and alkaline phosphatase activity (86–140% in males, 83% in females) and significant decreases in total protein (11% in males, 10–13% in females) and albumin (7% in males) levels (Takahashi et al. 2014); the NOAEL was 0.3 mg/kg/day.

PFBS

Epidemiological Studies—Alterations in Uric Acid Levels. Serum PFBS levels were not associated with serum uric acid levels or increases in the risk of hyperuricemia in a study of adolescents in Taiwan (Qin et al. 2016).

Laboratory Animal Studies. Treatment of female rats with 900 mg/kg/day PFBS by gavage for 28 days caused a significant increase (9–11%) in absolute and relative kidney weight, but caused no significant alterations in the microscopic appearance of the kidneys (3M 2001). The weight of the kidneys returned to control levels following a recovery period of approximately 14 days; the NOAEL for kidney weight effects was 900 mg/kg/day PFBS. In a 90-day rat study, PFBS did not result in alterations in kidney weights, but did result in hyperplasia of the medullary and papillary tubular and ductal epithelial cells in the inner medullary region at 600 mg/kg/day, but not at 200 mg/kg/day (Lieder et al. 2009a). Minimal to moderate papillary epithelial tubular/acinar hyperplasia was also observed in a 2-generation rat study at 300 mg/kg/day; the study identified a NOAEL of 100 mg/kg/day (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No alterations in renal morphology or clinical indications of impaired renal function were reported in rats treated with PFBA in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day by gavage for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFDoDA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. No histopathological alterations were observed in rats administered up to 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015).

PFHxA

Epidemiological Studies—Alterations in Uric Acid Levels. In adolescents, no associations between serum PFHxA levels and serum uric acid levels or the risk of hyperuricemia were observed (Qin et al. 2016).

Laboratory Animal Studies. Renal papillary necrosis was determined to be one of the causes of death in rats administered 450 mg/kg/day PFHxA for 4 days (Kirkpatrick 2005). No increases in renal lesions were observed in surviving rats administered a TWA dose of 315 mg/kg/day for 32–44 days (Kirkpatrick 2005). No histological alterations were observed in the kidneys of rats administered up to 200 mg/kg/day NaPFHx for 90 days (Chengelis et al. 2009b). In a 2-year gavage study, treatment of female rats with 200 mg/kg/day PFHxA resulted in mild renal tubular degeneration and mild to severe papillary necrosis (Klaunig et al. 2015); the NOAEL was 100 mg/kg/day. In addition, urinalysis revealed an increased mean urine volume and reduced specific gravity. There were no histological alternations in the kidneys of males.

2.11 DERMAL

Overview. No studies were located regarding dermal effects in humans. Studies in laboratory animals have not found dermal effects following head-only inhalation exposure to PFOA (see Table 2-1) or oral exposure to PFOA, PFOS, or PFBA (see Tables 2-3, 2-4, and 2-5). Dermal exposure to PFOA has resulted in skin damage (see Table 2-6).

PFOA

In an inhalation head-only exposure study, no histopathological alterations were observed in the abdominal skin of male rats exposed to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986).

No microscopic alterations were observed in the skin following oral exposure of rats to ≤100–110 mg/kg/day APFO via the diet for 90 days (Griffith and Long 1980) or monkeys exposed to up to 20 mg/kg/day PFOA or 0.75 mg/kg/day PFOS for 26 weeks (Butenhoff et al. 2002; Seacat et al. 2002).

Application of a single dose of 5,000 mg/kg of an aqueous paste of APFO to a clipped area of the skin of rats, and left in place covered for 24 hours produced mild skin irritation (Kennedy 1985); no irritation was

apparent with a dose of 3,000 mg/kg. In a 2-week dermal exposure study, skin irritation was observed in rats exposed to 200 mg/kg/day (Kennedy 1985). Acute necrotizing dermatitis was observed in two out of five rats exposed to 2,000 mg/kg/day; this lesion was observed after the 10th treatment. Application of 500 mg/kg APFO to the intact or abraded skin of young rabbits and left covered for 24 hours was non-irritating, as scored according to the Draize procedure immediately after removal of the cover and 48 hours later (Griffith and Long 1980).

PFOS

Administration of up to approximately 1.04 mg/kg/day PFOS to rats in the diet for 2 years did not induce morphological alterations in the skin (Butenhoff et al. 2012b; Thomford 2002b).

PFBA

There were no significant gross or microscopic alterations in the skin of rats receiving gavage doses of ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day PFBA for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

2.12 OCULAR

Overview. No information was located regarding ocular effects in humans. Ocular irritation has been observed in laboratory animals following exposure to airborne APFO dust or instillation of PFOA into the eye (see Tables 2-1 and 2-6). However, ocular effects have not been found following oral exposure to PFOA, PFOS, PFBS, PFBA, or PFHxA (see Tables 2-3, 2-4, and 2-5).

PFOA

Rats exposed to 18,600 mg/m^3 APFO dusts for 1 hour exhibited a red material around the eyes and lacrimation during exposure (Griffith and Long 1980). Male rats exposed to ≥810 mg/m^3 APFO dusts for 4 hours showed corneal opacity and corrosion, which was confirmed by fluorescein staining (Kennedy et al. 1986). Examination of the eyes of male rats exposed intermittently to up to 84 mg/m^3 APFO for 2 weeks using a bright light and a slit-lamp biomicroscope on days 5 and 9 of exposure did not reveal any significant exposure-related alterations (Kennedy et al. 1986). Microscopic examination of the eyes from these rats at termination and following a recovery period of up to 42 days was unremarkable.

In oral exposure studies, examination of the eyes from rats exposed to approximately 100–110 mg/kg/day APFO in the diet for 90 days did not reveal any significant gross or microscopic alterations (Griffith and Long 1980). Similar results were reported in rats that received dietary doses up to 15 mg/kg/day APFO for 2 years (3M 1983; Butenhoff et al. 2012c) and in monkeys dosed with up to 20 mg/kg/day APFO for 26 weeks (Butenhoff et al. 2002).

No significant gross alterations were observed in the eyes of rats following repeated dermal exposure to APFO (Kennedy 1985). Microscopic examination of the eyes also did not reveal treatment-related changes. In a study in rabbits, 0.1 g APFO was instilled once in the conjunctival sac of the right eye and examinations were conducted after 1, 24, 48, and 72 hours and 5 and 7 days after the application (Griffith and Long 1980). APFO produced moderate irritation of the eye characterized by iridal and conjunctival effects. The effects were most pronounced 1 hour after instillation. The irritation was persistent, but by day 7, it had subsided. In a different experiment in which 0.1 g APFO was instilled for 5 or 30 seconds before washing with 200 mL of water, there was limited conjunctival irritation, but the effects were immediate and persistent.

PFOS

No gross or microscopic alterations were observed in the eyes from rats exposed to ≤1.77 mg/kg/day PFOS in the diet for 4 weeks or ≤1.56 mg/kg/day for 14 weeks (Seacat et al. 2003). Similar findings were reported in monkeys dosed daily with up to 2 mg/kg/day PFOS administered via a capsule for 4 weeks (Thomford 2002a) or up to 0.75 mg/kg/day PFOS administered via a capsule for 26 weeks (Seacat et al. 2002), and in rats dosed with up to 1.04 mg/kg/day in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFBS

No gross or microscopic alterations were observed in the eyes of rats administered ≤900 mg/kg/day PFBS via gavage for 28 days (3M 2001).

PFBA

Examination of the eyes of rats orally exposed to ≤150 mg/kg/day PFBA for 28 days or ≤30 mg/kg/day for 90 days did not reveal any significant alterations in the eyes (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFHxA

No ophthalmological alterations were observed in rats administered up to 500 mg/kg/day NaPFHx for 90–93 days (Chengelis et al. 2009b; Loveless et al. 2009).

2.13 ENDOCRINE

Overview. Epidemiological studies have examined a number of endocrine targets including thyroid gland and hormones, reproductive hormones, and insulin levels. A discussion of the thyroid effects is included in this section; the reproductive hormone effects are discussed in Section 2.16, Reproductive, and the insulin effects (as well as other effects associated with glucose metabolism and utilization) are discussed in Section 2.18, Other Noncancer. Summaries of results of epidemiological studies evaluating thyroid outcomes are presented in Table 2-15; more in-depth summaries of the studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9. Although some associations between serum PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnA and thyroid stimulating hormone (TSH), triiodothyronine (T3), or thyroxine (T4) levels or thyroid disease have been found, the results are not consistent across studies and a larger number of studies have not found associations. A small number of studies have evaluated PFDoDA and most studies have not found consistent associations between serum perfluoroalkyl levels and thyroid hormone levels. No epidemiological studies examining endocrine health outcomes were identified for PFHpA, PFBS, PFBA, PFHxA, or FOSA.

Laboratory animal studies have primarily evaluated potential morphological alterations in endocrine tissues following oral exposure; these studies are summarized in Tables 2-3, 2-4, and 2-5. Some alterations in thyroid hormone levels have been observed in laboratory animals exposed to PFOA, PFOS, PFHxS, or PFDA. Histopathological alterations have been observed in the thyroid of some laboratory animal studies for PFHxS, PFBA, and PFHxA; the investigators noted that these effects were likely secondary to the hepatocellular hypertrophy, although the mechanism has not been established for these compounds. In general, the pituitary, parathyroid, thyroid, and adrenal glands do not appear to be

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	TSH	**Association (p=0.004)***
Olsen et al. 1998b Occupational (n=111 in 1993 and n=80 in 1995)	10,000–<30,000 ng/mL (PFOA range)	TSH	NS (p=0.09 for trend), 1993 group Association (p=0.002), 1995 group
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	TSH	The investigators noted that the levels were within the reference range
		T4	
		T3	
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative PFOA	Thyroid disease risk	NS (p=0.98 for trend) no lag, males NS (p=0.55 for trend) 10-year lag, males NS (p=0.97 for trend) no lag, females NS (p=0.27 for trend) 10-year lag, females
Olsen and Zobel 2007 Occupational (n=552)	2,210 ng/mL (mean PFOA)	Free T4	**Association (p=0.01)***
		T4	NS (p=0.29)
		T3	**Association (p=0.05)***
		TSH	NS (p=0.08)
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported thyroid problems	**SPR 1.56 (1.22–1.98)***
Emmett et al. 2006b Community (n=371)	354 ng/mL (median PFOA)	TSH	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	52.6, 91.0, 98.6, and 124.3 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p≤0.0001)*, women ≤50 years** **Association (p<0.001), men and women >50 years**
		T3 uptake	**Inverse association (p=0.0001)* women ≤50 years** **Inverse association (p=0.005)*, women >50 years** **Inverse association (p=0.037)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	29.3 and 67.7–2,071 ng/mL (median and 4th quartile PFOA)	Thyroid disease	**OR 1.44 (1.02–2.03)*, per interquartile shift**
		Hypothyroidism	**OR 1.54 (1.00–2.37)*, per interquartile shift**
		Subclinical hypothyroidism	OR 0.98 (0.86–1.15), per interquartile shift
		Subclinical hyperthyroidism	OR 0.81 (0.58–1.15), per interquartile shift
		TSH	β -1.1 (-5.3–3.4), 4th quartile
		Total T4	β -0.1 (-1.7–1.4), 4th quartile
Winquist and Steenland 2014b Community (C8 and occupational) (n=28,541)	114.7–<202.2 ng/mL-year (2nd quintile estimated cumulative PFOA)	Functional thyroid disease	**HR 1.24 (1.02–1.51;p=0.031)* (women), retrospective analysis** HR 1.01 (0.94–1.07 per log linear increase in PFOA, p=0.853) (men), retrospective analysis NS (p=0.549) (women), prospective analysis NS (p=0.087) (men), prospective analysis

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Hyperthyroidism	NS (p=0.074) (women), retrospective analysis NS (p=0.858) (men), retrospective analysis NS (p=0.268) (women), prospective analysis NS (p=0.760) (men), prospective analysis
		Hypothyroidism	NS (p=0.076) (women), retrospective analysis NS (p=0.684) (men), retrospective analysis NS (p=0.247) (women), prospective analysis **HR 1.24 (1.03–1.49)* (men), prospective analysis**
Berg et al. 2017 General population (n=370 pregnant women)	1.53 ng/mL (median maternal serum PFOA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	1.33 ng/mL (geometric mean PFOA)	TSH	NS (p=0.871)
		Free T4	NS (p=0.896)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFOA in cases and controls)	Hypothyroxinemia risk	OR 0.94 (0.74–1.18)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Total T4	NS (p=0.07)
		Free T4	NS (p=0.11)
		T3	**Association (β 6.05, p=0.03)***
		TSH	NS (p=0.37)
Dufour et al. 2018 General population (n=214 pregnant women)	0.80 ng/mL (cord blood mean PFOA); 0.44–0.68 ng/mL (2nd quartile cord blood PFOA)	Hypothyroidism	**OR 4.42 (1.23–21.14)*, 4th quartile**
		TSH in infants	NS (p=0.196)
Jain 2013 General population (NHANES) (n=1,525)	NR	Total T3	**Association (p=0.013)***
		TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	2.74 ng/mL (median PFOA)	TSH	NS (p=0.4055)
		T4	NS (p=0.2221)
Kang et al. 2018 General population (150 children, 3–18 years)	1.88 ng/mL (median serum PFOA)	Free T4	NS (p=0.075)
		TSH	NS (p=0.565)
Lewis et al. 2015 General population (NHANES) (n=1,682)	1.42–2.55 ng/mL (range of median PFOA for different age groups)	TSH	**Association (p<0.05)*, 12–20-year-old females**
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)*, 60–80-year-old females**
		Total T3	**Association (p<0.05)*, 60–80-year-old females**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Melzer et al. 2010 General population (NHANES) (n=3,966)	9.47 and 10.39 ng/mL (4th PFOA quartile mean in women and men)	Thyroid disease risk	**OR 1.64 (1.09–2.46)*, females** OR 1.58 (0.74–3.39), males
Preston et al. 2018 General population (n=732 mothers and 480 infants)	5.6 ng/mL (maternal median serum PFOA)	Total T4	β 0.09 (-0.08–0.27)
		Free T4	**β -1.87 (-3.40 to -0.31)***
		TSH	β 0.28 (-9.26–10.8)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Raymer et al. 2012 General population (n=256)	10.4 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.91 ng/mL (cord blood median PFOA)	Cord blood T4	NS (p=0.99)
		Cord blood T3	NS (p=0.99)
		Cord blood TSH	NS (p=0.24)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	9.17 ng/mL (geometric mean PFOA)	TSH	NS (p=0.176)
		Free T4	NS (p=0.536)
		T4	NS (p=0.097)
		T3	NS (p=0.208)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	3.14 ng/mL (mean cord blood PFOA)	Cord blood T4	β -0.031 (-0.414–0.342)
		Cord blood T3	β 0.025 (-0.054–0.103)
		Cord blood TSH	β 0.059 (-0.136–0.254)
Wang et al. 2013a General population (n=903 pregnant women)	2.13 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	2.39 ng/mL (median PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Webster et al. 2016 General population (NHANES) (n=1,525)	4.2 ng/mL (geometric mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	**Association (p<0.05)***
		Total T3	NS (p<0.05)
Wen et al. 2013 General population (NHANES) (n=1,181)	4.15 ng/mL (geometric mean PFOA)	TSH	NS (p=0.916), men NS (p=0.732), women
		Total T4	NS (p=1.0), men NS (p=0.705), women
		Total T3	NS (p=0.673), men **Association (p=0.035)*, women**
		Thyroglobulin	NS (p=0.226), men NS (p=0.341), women
		Subclinical hypothyroidism risk	OR1.29 (0.40–4.10), men **OR 7.42 (1.14–48.12, p<0.05), women**
		Subclinical hyperthyroidism risk	**OR 0.38 (0.16–0.95, p<0.05)*, men** OR 0.99 (0.13–7.59), women
Yang et al. 2016a General population (n=157 pregnant women)	1.95 ng/mL (mean PFOA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of mean PFOS)	TSH	NS (p=0.95)
		Cortisol	NS (p=0.45)
Olsen et al. 2003a Occupational (n=518)	1,320 and 800 ng/mL (mean PFOS at the Decatur and Antwerp facilities, respectively)	T3	**Association (p=0.04)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Knox et al. 2011a Community (n=50,113 adults ≥20 years of age)	17.3, 24.8, 25.7, and 29.1 ng/mL (mean PFOA in women ≤50 years, men ≤50 years, women >50 years, men >50 years, respectively)	T4	**Association (p<0.0001)*, women ≤50 or >50 years** **Association (p=0.0001)*, men ≤50 or >50 years**
		T3 uptake	**Inverse association (p<0.0001)* women ≤50 years** **Inverse association (p=0.0001)*, women >50 years** **Inverse association (p=0.009)*, men ≤50 years** **Inverse association (p=0.0001)*, men >50 years**
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	20.0 ng/mL (median PFOS)	Thyroid disease	OR 0.8 (0.62–1.08), per interquartile shift
		Hypothyroidism	OR 0.91 (0.63–1.31), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.86–1.13), per interquartile shift
		Subclinical hyperthyroidism	OR 0.80 (0.62–1.02), per interquartile shift
		TSH	β -1.0 (-0.3–2.3), per interquartile shift
		Total T4	β 1.1 (0.6–1.5), per interquartile shift
Berg et al. 2015 General population (n=391)	8.1–11.0 ng/mL (3rd PFOS quartile)	TSH	**Association (p=0.03)*, 3rd quartile**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Berg et al. 2017 General population (n=370 pregnant women)	8.03 ng/mL (median maternal serum PFOS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	**Association (p<0.05)*** NS (p<0.05) after adjustment for other perfluoroalkyls or persistent organic pollutants
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	19.57 ng/mL (geometric mean PFOS)	TSH	NS (p=0.896)
		Free T4	NS (p=0.623)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	7.59 and 7.08 ng/mL (geometric mean PFOS in cases and controls)	Hypothyroxinemia risk	OR 0.88 (0.63–1.24)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Total T4	NS (p=0.28)
		Free T4	NS (p=0.42)
		T3	NS (p=0.19)
		TSH	NS (p=0.98)
Dallaire et al. 2009 General population (n=623)	18.28 ng/mL (geometric mean PFOS)	TSH	**Inverse association (p≤0.05)***
		T3	**Inverse association (p≤0.05)***
		T4-binding globulin	**Inverse association (p≤0.01)***
		Free T4	**Association (p≤0.05)***
Dufour et al. 2018 General population (n=214 pregnant women)	0.88 ng/mL (cord blood mean PFOS); 0.73–1.01 ng/mL (3rd quartile cord blood PFOS)	Hypothyroidism	**OR 3.22 (1.08–10.92)*, 3rd quartile** OR 2.95 (0.98–10.07), 4th quartile
		TSH in infants	NS (p=0.679)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	7.96 ng/mL (median PFOS)	TSH	NS (p=0.3537)
		T4	NS (p=0.1134)
Kang et al. 2018 General population (150 children, 3–18 years)	5.68 ng/mL (median serum PFOS)	Free T4	NS (p=0.987)
		TSH	NS (p=0.628)
Lewis et al. 2015 General population (NHANES) (n=1,682)	3.76–11.1 ng/mL (range of median PFOS for different age groups)	TSH	NS (p>0.05)
		Free T4	**Association (p<0.05)*, 20–<40-year-old females**
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 and 50.96 ng/mL (4th PFOS quartile mean in women and men)	Thyroid disease risk	OR 1.15 (0.7–1.91, p=0.568), females OR 1.58 (0.72–3.47, p=0.251), males **OR 2.68 (1.03–6.98, p=0.043)*, males 4th quartile versus combined 1st and 2nd quartiles**
Preston et al. 2018 General population (n=732 mothers and 480 infants)	24.0 ng/mL (maternal median serum PFOS)	Total T4	β 0.01 (-0.14–0.16)
		Free T4	β -1.04 (-2.36–0.29)
		TSH	β 0.90 (-7.27–9.80) **β-16.4 (-29.8 to -0.38)*, TPOAb positive mothers**
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile.**

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raymer et al. 2012 General population (n=256)	37.4 ng/mL (mean PFOS)	TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.66 ng/mL (cord blood median PFOS)	Cord blood T4	NS (p=0.10)
		Cord blood T3	NS (p=0.37)
		Cord blood TSH	NS (p=0.73)
Shrestha et al. 2015 General population (n=87 with thyroid disease)	31.6 ng/mL (geometric mean PFOS)	TSH	NS (p=0.094)
		Free T4	**Association (p=0.044)***
		T4	**Association (p=0.001)***
		T3	NS (p=0.287)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.24 ng/mL (mean cord blood PFOS)	Cord blood T4	**β -0.458 (-0.916 to -0.001, p<0.05)***
		Cord blood T3	β 0.027 (-0.072–0.125)
		Cord blood TSH	**β 0.346 (0.101–0.591, p<0.05)***
Wang et al. 2013a General population (903 pregnant women)	12.77 ng/mL (geometric mean PFOS)	TSH	**Association (p=0.03)***
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	12.73 ng/mL (median PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	13.9 ng/mL (geometric mean PFOS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p<0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	14.2 ng/mL (geometric mean PFOS)	TSH	NS (p=0.931), men NS (p=0.358), women
		Total T4	NS (p=0.840), men NS (p=0.433), women
		Total T3	NS (p=0.404), men NS (p=0.384), women
		Thyroglobulin	NS (p=0.342), men NS (p=0.061), women
		Subclinical hypothyroidism risk	**OR 1.98 (1.19–3.28, p<0.05)*, men** **OR 3.03 (1.14–8.07, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.92 (0.19–4.46), men OR 1.90 (0.33–6.80), women
Yang et al. 2016a General population (n=157 pregnant women)	5.08 ng/mL (mean PFOS)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFHxS			
Berg et al. 2017 General population (n=370 pregnant women)	0.44 ng/mL (median maternal serum PFHxS)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.75 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.956)
		Free T4	NS (p=0.567)
Chan et al. 2011 General population (n=94 women with hypothyroxinemia and 175 matched controls)	1.28 and 1.37 ng/mL (geometric mean PFHxS in cases and controls)	Hypothyroxinemia risk	OR 1.12 (0.89–1.41)
Crawford et al. 2017 General population (n= 99 30–-44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Total T4	NS (p=0.50)
		Free T4	NS (p=0.84)
		T3	NS (p=0.22)
		TSH	NS (p=0.71)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFHxS)	Hypothyroidism	OR 1.92 (95% CI 0.87–4.25),detected versus non-detected
		TSH in infants	NS (p=0.894)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	1.51 ng/mL (median PFHxS)	TSH	NS (p=0.8144)
		T4	NS (p=0.5147)
Kang et al. 2018 General population (150 children, 3–18 years)	0.793 ng/mL (median serum PFHxS)	Free T4	NS (p=0.308)
		TSH	NS (p=0.901)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lewis et al. 2015 General population (NHANES) (n=1,682)	0.69–1.81 ng/mL (range of median PFHxS for different age groups)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	2.4 ng/mL (maternal median serum PFHxS)	Total T4	β -0.05 (-0.14–0.04)
		Free T4	β -0.60 (-1.39–0.19)
		TSH	β 2.89 (-2.12–8.17)
		Neonatal T4	**β -1.1 (-2.1 to -0.1)*, 4th quartile**
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.38 ng/mL (cord blood median PFHxS)	Cord blood T4	NS (p=0.83)
		Cord blood T3	NS (p=0.15)
		Cord blood TSH	NS (p=0.15)
Wang et al. 2013a General population (n=903 pregnant women)	0.62 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	0.81 ng/mL (median PFHxS)	TSH	**Association (p<0.05)**
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.9 ng/mL (geometric mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	2.0 ng/mL (geometric mean PFHxS)	TSH	NS (p=0.608), men NS (p=0.720), women
		Total T4	NS (p=0.641), men **Association (p=0.022)*, women**
		Total T3	NS (p=0.917), men **Association (p<0.001)*, women**
		Thyroglobulin	NS (p=0.455), men NS (p=0.725), women
		Subclinical hypothyroidism risk	OR 1.57 (0.76–3.25), men **OR 3.10 (1.22–7.86, p<0.05)*, women**
		Subclinical hyperthyroidism risk	OR 0.56 (0.24–1.20.92), men **OR 12.27 (1.07–4.80.90)*,women**
Yang et al. 2016a General population (n=157 pregnant women)	0.63 ng/mL (mean PFHxS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFNA			
Mundt et al. 2007 Occupational (n=592)	NR	TSH	Investigators noted differences between groups was small and not clinically relevant
		T4	
		T3	
Lopez-Espinosa et al. 2012 Community (n=10,725 children aged 1–17 years)	1.5 ng/mL (median PFNA)	Thyroid disease	OR 1.05 (0.78–1.41), per interquartile shift
		Hypothyroidism	OR 1.11 (0.77–1.60), per interquartile shift
		Subclinical hypothyroidism	OR 0.99 (0.88–1.12), per interquartile shift
		Subclinical hyperthyroidism	OR 0.78 (0.61–1.01), per interquartile shift

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		TSH	β -1.1 (CI 0.7–1.5), per interquartile shift
		Total T4	B 0.8 (-0.4–2.0), per interquartile shift
Berg et al. 2017 General population (n=370 pregnant women)	0.56 ng/mL (median maternal serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.79 ng/mL (geometric mean PFNA)	TSH	NS (p=0.789)
		Free T4	NS (p=0.424)
Crawford et al. 2017 General population (n= 99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Total T4	NS (p=0.34)
		Free T4	**Association (β 0.08, p<0.01)***
		T3	**Association (β 5.65, p=0.02)***
		TSH	NS (p=0.91)
Dufour et al. 2018 General population (n=214 pregnant women)	0.18 ng/mL (cord blood mean PFNA), 0.23–0.68 ng/mL (4th quartile cord blood PFNA)	Hypothyroidism	OR 1.17 (0.37–3.92), 4th quartile
		TSH in infants	NS (p=0.064)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ji et al. 2012 General population (n=633)	2.09 ng/mL (median PFNA)	TSH	NS (p=0.1354)
		T4	NS (p=0.7436)
Kang et al. 2018 General population (150 children, 3–18 years)	0.938 ng/mL (median serum PFNA)	Free T4	**β 0.052 (0.007–0.097, p=0.025)***
		TSH	NS (p=0.840)
Preston et al. 2018 General population (n=732 mothers and 480 infants)	0.6 ng/mL (maternal median serum PFNA)	Total T4	β -0.05 (-0.16–0.05)
		Free T4	β -0.57 (-1.52–0.40)
		TSH	β -0.27 (-6.19–6.03)
		Neonatal T4	β 0.05 (-0.29–0.39)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.2 ng/mL (cord blood median PFNA)	Cord blood T4	NS (p=0.70)
		Cord blood T3	NS (p=0.93)
		Cord blood TSH	NS (p=0.14)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	7.55 ng/mL (mean cord blood PFNA)	Cord blood T4	β -0.067 (-0.252–0.009)
		Cord blood T3	β -0.03 (0.069–0.103)
		Cord blood TSH	β 0.045 (-0.051–0.142)
Wang et al. 2013a General population (n=903 pregnant women)	0.37 ng/mL (geometric mean PFNA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (n=285 pregnant women)	1.51 ng/mL (median PFNA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Webster et al. 2016 General population (NHANES) (n=1,525)	1.5 ng/mL (geometric mean PFNS)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wen et al. 2013 General population (NHANES) (n=1,181)	1.54 ng/mL (geometric mean PFNA)	TSH	NS (p=0.973), men NS (p=0.407), women
		Total T4	NS (p=0.097), men NS (p=0.632), women
		Total T3	NS (p=0.063), men NS (p=0.258), women
		Thyroglobulin	NS (p=0.537), men NS (p=0.395), women
		Subclinical hypothyroidism risk	OR 1.30 (0.65–2.60), men OR 2.54 (0.40–16.05), women
		Subclinical hyperthyroidism risk	OR 2.41 (0.48–12.04), men OR 1.91 (0.83–4.38), women
Yang et al. 2016a General population (n=157 pregnant women)	0.52 ng/mL (mean)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDA			
Berg et al. 2015 General population (n=391 pregnant women)	0.31–2.34 ng/mL (4th PFDA quartile)	T3	**Association (p=0.03) (4th quartile)**
Berg et al. 2017 General population (n=370 pregnant women)	0.23 ng/mL (median maternal serum PFNA) and 0.32–2.34 ng/mL (4th quartile serum PFNA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	**β -0.02 (-0.044 to -0.005, p<0.05)***
		Free T3	NS (p>0.05)
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bloom et al. 2010 General population (n=31)	0.21 ng/mL (geometric mean PFDA)	TSH	NS (p=0.365)
		Free T4	NS (p=0.107)
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)
Ji et al. 2012 General population (n=633)	0.91 ng/mL (median PFDA)	TSH	NS (p=0.2721)
		T4	NS (p=0.2176)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0592 ng/mL (median serum PFDA)	Free T4	NS (p=0.153)
		TSH	NS (p=0.420)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.1 ng/mL (cord blood median PFDA)	Cord blood T4	NS (p=0.40)
		Cord blood T3	NS (p=0.07)
		Cord blood TSH	NS (p=0.22)
Wang et al. 2013a General population (903 pregnant women)	0.09 ng/mL (geometric mean PFDA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)
Wang et al. 2014 General population (285 pregnant women)	0.46 ng/mL (median PFDA)	TSH	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Total T3	**Association (p<0.01)***
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFDA)	TSH	**Inverse association (p<0.01)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Berg et al. 2015 General population (n=391)	0.4–0.96 ng/mL (4th PFUnA quartile)	Free T3	**Association (p=0.00)*, 4th quartile**
Berg et al. 2017 General population (n=370 pregnant women)	0.26 ng/mL (median maternal serum PFUnA) and 0.39–1.46 (4th quartile serum PFUnA)	Total T4	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T3	**β -0.02 (-0.033 to -0.003, p<0.05)**
		TSH	NS (p>0.05)
		Thyroxine binding capacity	NS (p>0.05)
Bloom et al. 2010 General population (n=31)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p=0.527)
		Free T4	NS (p=0.204)
Ji et al. 2012 General population (n=633)	1.75 ng/mL (median PFUnA)	TSH	NS (p=0.5368)
		T4	NS (p=0.0642)
Kang et al. 2018 General population (150 children, 3–18 years)	0.0652 ng/mL (median serum PFUnA)	Free T4	NS (p=0.581)
		TSH	NS (p=0.510)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.26 ng/mL (cord blood median PFUnA)	Cord blood T4	NS (p=0.86)
		Cord blood T3	NS (p=0.35)
		Cord blood TSH	NS (p=0.37)
Tsai et al. 2017 General population (n=118 mother-infant pairs)	15.94 ng/mL (mean cord blood PFNA)	Cord blood T4	β 0.045 (-0.223–0.313)
		Cord blood T3	β 0.048 (-0.008–0.104)
		Cord blood TSH	β 0.077 (0.063–0.216)
Wang et al. 2013a General population (n=903 pregnant women)	0.20 ng/mL (geometric mean PFUnA)	TSH	NS (p>0.05)
		Elevated TSH risk	NS (p>0.05)

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2014 General population (n=285 pregnant women)	3.26 ng/mL (median PFUnA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.001)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.45 ng/mL (mean PFUnA)	TSH	**Inverse association (p<0.05)***
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
PFDoDA			
Ji et al. 2012 General population (n=633)	0.92 ng/mL (median PFDoDA)	TSH	NS (p=0.6925)
		T4	NS (p=0.7153)
Shah-Kulkarni et al. 2016 General population (n=279 pregnant women)	0.08 ng/mL (cord blood median PFDoDA)	Cord blood T4	NS (p=0.69)
		Cord blood T3	NS (p=0.30)
		Cord blood TSH	NS (p=0.20)
Wang et al. 2014 General population (285 pregnant women)	0.36 ng/mL (median PFDoDA)	TSH	NS (p>0.05)
		Free T4	**Inverse association (p<0.001)***
		Total T4	**Inverse association (p<0.01)***
		Total T3	NS (p>0.05)
Yang et al. 2016a General population (n=157 pregnant women)	0.046 ng/mL (mean PFDoDA)	TSH	**Inverse association (p<0.01)***
		Free T4	**Inverse association (p<0.05)***
		Total T4	**Inverse association (p<0.05)***
		Free T3	**Inverse association (p<0.01)***
		Total T3	**Inverse association (p<0.01)***

Table 2-15. Summary of Thyroid Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Me-FOSA-AcOH			
Jain 2013 General population (NHANES) (n=1,525)	NR	TSH	NS (p>0.05)
		Free T3	NS (p>0.05)
		Total T3	NS (p>0.05)
		Free T4	NS (p>0.05)
		Total T4	NS (p>0.05)
		Thyroglobulin	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 9 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

sensitive targets following exposure to PFOA, PFOS, PFDA, PFBS, or PFBA. Endocrine effects have not been examined in laboratory animal studies on PFNA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. A number of epidemiological studies have examined the potential of PFOA to damage the thyroid. Steenland et al. (2015) did not find an association between serum PFOA and the risk of thyroid disease in male or female workers at the Washington Works facility. The occupational exposure studies do not suggest an association between serum PFOA and alterations in thyroid hormone levels. One study (Olsen and Zobel 2007) reported associations between serum PFOA levels and free T4 and T3 levels in workers at 3M facilities; it is noted that the investigators did not consider the results clinically relevant since the levels were within the normal range. A study reported an association between serum PFOA and TSH, but this was only observed at one time point (Olsen et al. 1998b); another study of the 3M Cottage Grove facility, reported an association between serum fluorine levels and TSH levels (Gilliland 1992). A fifth occupational study reported that TSH, T4, and T3 levels were within the reference range (Sakr et al. 2007b).

Three studies of the community affected by the Washington Works facility reported increases in self-reported thyroid disease (Anderson-Mahoney et al. 2008), any type of functional thyroid disease (Lopez-Espinosa et al. 2012; Winquist and Steenland 2014b), or hypothyroidism (Lopez-Espinosa et al. 2012). No associations between estimated cumulative serum PFOA and hyperthyroidism or hypothyroidism were found in retrospective analysis (Winquist and Steenland 2014b). However, in prospective analysis, an association between estimated cumulative serum PFOA and hypothyroidism was found in men (Winquist and Steenland 2014b). Consistent with the occupational exposure data, no association between serum PFOA and TSH levels was found (Emmett et al. 2006b; Knox et al. 2011a; Lopez-Espinosa et al. 2012). Increases in serum PFOA were also associated with increases in T4 levels and decreases in T3 uptake in adults (Knox et al. 2011a).

A number of studies have examined the thyroid outcomes associated with serum PFOA levels in the general population. An association between serum PFOA and thyroid disease risk was found in female NHANES participants, but not in males (Melzer et al. 2010). Another study utilizing NHANES data (Wen et al. 2013) found an increased risk of subclinical hypothyroidism among women, but not men, and a decreased risk of subclinical hyperthyroidism among men, but not women. An increased risk of hypothyroidism was also observed in a study of pregnant women (DuFour et al. 2018). A case-control

study of women did not find that serum PFOA levels were associated with the risk of hypothyroxinemia (Chan et al. 2011). Although five studies found associations between serum PFOA and T3 levels (Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Webster et al. 2016; Wen et al. 2013), five other studies did not find these associations (Berg et al. 2017; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Yang et al. 2016a). No associations between serum PFOA and TSH or T4 levels were found in the general population studies (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2013a, 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), with the exception of two studies which found an association for TSH and T4 levels (Lewis et al. 2015) or free T4 (Preston et al. 2018).

Studies examining possible relationships between cord blood PFOA and cord blood thyroid hormone levels have not found associations for T4, T3, or TSH (Dufour et al. 2018; Shah-Kulkarni et al. 2016; Tsai et al. 2017). Preston et al. (2018) found an inverse association between maternal serum PFOA and neonatal T4 levels.

In a clinical trial of patients with advanced solid tumors administered 50–1,200 mg APFO (approximately 0.10–2.4 mg/kg/day) for 6 weeks, increases in free T4 levels were observed with no apparent alterations in TSH (Convertino et al. 2018).

Laboratory Animal Studies. Repeated intermittent head-only exposure of male rats to up to 84 mg/m^3 APFO dusts for 2 weeks did not result in significant gross or microscopic alterations in the thyroid or adrenal gland (Kennedy et al. 1986).

In a 2-generation study in rats, daily treatment of the parental generation with 0, 1, 3, 10, or 30 mg/kg/day APFO by gavage in water for 70–90 days produced an increased incidence of hypertrophy and/or vacuolation of the zona glomerulosa of the adrenal gland from high-dose males (Butenhoff et al. 2004b). The respective incidences were 0/10, 0/10, 0/10, 2/10, and 7/10. This effect was also observed in F1 generation males treated with the same dose level. No explanation was apparent for this finding. In rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years, there were no significant morphological alterations in the adrenals (3M 1983; Butenhoff et al. 2012c). A study in monkeys treated with APFO also reported effects on the adrenal glands. Griffith and Long (1980) reported diffuse lipid depletion in the adrenals from Rhesus monkeys dosed daily for 90 days with 30 mg/kg/day APFO by gavage. This dose level was lethal to some monkeys; no such effect was seen in monkeys dosed with 10 mg/kg/day.

For the most part, morphological evaluations of other endocrine glands in animals treated with PFOA have been negative. For example, male and female rats dosed via the diet with approximately 100–110 mg/kg/day APFO for 90 days showed no gross or microscopic alterations in the pituitary or thyroid glands (Griffith and Long 1980). Similar observations were reported in the pituitary, thyroid, and parathyroid glands from male and female rats dosed with up to 15 mg/kg/day APFO in the diet for 2 years (Butenhoff et al. 2012c; 3M 1983).

Administration of up to 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 weeks did not significantly alter free T4, total T4, free T3, total T3, or TSH (Thomford 2001). Serum T4 and total T4 were significantly reduced in Cynomolgus monkeys dosed with 10 mg/kg/day APFO administered via a capsule for up to 6 months, but were still within the normal range (Butenhoff et al. 2002). No significant changes were seen on serum free T3, total T3, or TSH, or thyroid histology.

The only relevant dermal information is that no morphological alterations were observed in the thyroid of rats following dermal application of up to 2,000 mg/kg/day APFO for 2 weeks in the Kennedy (1985) study.

PFOS

Epidemiological Studies. A number of epidemiological studies have examined the risk of thyroid disease and alterations in thyroid hormone levels to evaluate whether the thyroid gland is a target of PFOS toxicity. In studies of NHANES participants, no increases in the risk of thyroid disease were observed in men or women (Lewis et al. 2015; Melzer et al. 2010). Melzer et al. (2010) did find an increase in the risk of having thyroid disease and currently taking thyroid medication among men, and Wen et al. (2013) found increased risks of subclinical hypothyroidism among men and women. Dufour et al. (2018) also found an association between cord blood PFOS and risk of maternal hypothyroidism. Although some studies have found alterations in thyroid hormone levels, the results are not consistent across studies. Associations between serum PFOS and TSH levels were observed in three general population studies (Berg et al. 2015, 2017; Wang et al. 2014); however, one of the studies (Berg et al. 2017) found that the association was no longer significant after adjustments for exposure to other perfluoroalkyls and persistent organic compounds. In contrast, two other studies found inverse associations for TSH (Dallaire et al. 2009; Yang et al. 2016a). A third study also found an inverse association with TSH but only among pregnant women who were positive for thyroid peroxides antibodies (Preston et al. 2018). An occupational exposure study (Olsen et al. 1998a) and ten general population studies (Bloom et al. 2010;

Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Wen et al. 2013) did not find associations between serum PFOS and TSH levels. Conflicting results were also reported for T3 levels, with some studies reporting associations (Olsen et al. 2003a), inverse associations (Dallaire et al. 2009), or no association (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Lewis et al. 2015; Raymer et al. 2012; Shrestha et al. 2015; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). Most studies did not find an association with T4 levels (Berg et al. 2017; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Raymer et al. 2012; Preston et al. 2018; Wang et al. 2014; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a), but three studies did find associations between T4 levels and serum PFOS (Dallaire et al. 2009; Lewis et al. 2015; Shrestha et al. 2015). In NHANES participants with two indicators of thyroid stress (low iodine levels and high thyroid peroxidase antibody), serum PFOS levels were significantly ($p<0.05$) associated with increases in free and total T3, decreases in free T4, and increases in TSH levels (Webster et al. 2016).

Conflicting results were also found in studies using cord blood PFOS as the biomarker of exposure. Tsai et al. (2017) found an inverse association with cord blood T4 and a positive association with cord blood TSH; no association was found for T3. Shah-Kulkarni et al. (2016) found no associations for cord blood T4, T3, or TSH. It is noted that cord blood serum PFOS levels were much higher in the Tsai et al. (2017) study compared to the Shah-Kulkarni et al. (2016) study.

Laboratory Animal Studies. Chang et al. (2008b) conducted a study of thyroid function in rats exposed to PFOS (potassium salt). Administration of a single dose of 15 mg/kg by gavage in water (only dose level tested) reduced serum total T4 significantly at 2, 6, and 24 hours after dosing. This effect was attributed to a PFOS-induced transient increase in tissue availability of thyroid hormones and turnover of T4 with a resulting reduction in serum total T4. Chang et al. (2008b) concluded that PFOS did not induce a classical hypothyroid state or alter the hypothalamic-pituitary-thyroid axis. In another acute-duration study, dosing of pregnant mice with 6 mg/kg/day PFOS (potassium salt) on GDs 6–18 did not affect maternal serum levels of free or total T3 or T4 (Fuentes et al. 2006).

Changes in thyroid hormones have also been reported following intermediate-duration exposure to PFOS. For example, in a 2-generation gavage study in which dosing of rats started before mating and continued through gestation, doses ≥0.4 mg/kg/day (the lowest dose tested) caused a significant and dose-related reduction in total T4 in maternal serum on postpartum day 5 (Luebker et al. 2005b). Free T4 and TSH were not significantly affected. Exposure of pregnant rats to ≥1 mg/kg/day PFOS on GDs 2–20 induced

significant reductions in total T4 and free T4 and less marked reductions in T3 during pregnancy, particularly on GD 7 (Thibodeaux et al. 2003); however, serum TSH values were not significantly altered. A similar study in pregnant mice reported a decrease in total T4 on GD 6 in mice dosed with 20 mg/kg/day PFOS on GDs 1–17 (Thibodeaux et al. 2003). No alterations in total T4 were reported in mice dosed with 15 mg/kg/day. No information was provided regarding other thyroid hormones. Decreases in T4 levels were observed in male and female rats exposed to PFOS in the diet for 28 days (Curran et al. 2008); T3 levels were decreased in female rats exposed to 50 or 100 mg/kg/day and in male rats at 100 mg/kg/day. No histological alterations were observed in the thyroid. Another study with PFOS found no thyroid histological effects in rats exposed to 10.3 mg/kg/day for 1 day, 8.17 mg/kg/day for 7 days, or 7.34 mg/kg/day for 28 days (Elcombe et al. 2012a). Exposure of rats to ≥0.27 mg/kg/day PFOS in drinking water for 91 days resulted in decreases in total T4 levels (Yu et al. 2009a), but no changes in T3 or TSH levels (highest dose tested was 2.37 mg/kg/day). Curran et al. (2008) suggested that the apparent decreases in T4 levels, in the absence of TSH alterations and histological alterations in the thyroid, may be a result of measurement error when analog assays (chemiluminometric immunoassay and radioimmunoassay) are used due to binding interference. A decrease in serum total T4 levels was observed in Cynomolgus monkeys administered three doses of PFOS (average dose of 13.3 mg/kg in males and 14 mg/kg in females) over 315 days (Chang et al. 2017). The investigators did not consider this an adverse effect because the values were within the normal variation and there were not changes in free T4 levels or TSH levels. In another study in Cynomolgus monkeys, T3 was numerically lower than controls in one female and one male monkey dosed with 2 mg/kg/day PFOS by capsule for 4 weeks (Thomford 2002a). However, it is difficult to determine whether the effect was treatment-related based on only two animals. In a 26-week study in Cynomolgus monkeys, the highest dose of PFOS tested, 0.75 mg/kg/day, induced a significant increase in serum TSH (approximately twice control value, but still within the reference range) and a decrease in total T3 at termination, but not at earlier time points; variations in other thyroid hormones, including T4, were inconsistent regarding dose and over time (Seacat et al. 2002). The clinical relevance of the lowered total T3 values was not apparent since there was no indication of a clinical hypothyroid response, and thyroid histology was not altered by treatment with PFOS.

Examination of the adrenal glands from rats dosed with up to 1.77 mg/kg/day PFOS via the diet for 4 or 14 weeks did not show any significant gross or microscopic alterations (Seacat et al. 2003). No significant gross or microscopic lesions were reported in the adrenals, thyroid and parathyroid, or pituitary gland from rats dosed with up to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b).

PFHxS

Epidemiological Studies. Fifteen general population studies have evaluated possible associations between serum PFHxS levels and alterations in thyroid hormone levels. With the exception of a study of pregnant women, which found an association between serum PFHxS levels and TSH levels (Wang et al. 2014), and a study of NHANES participants, which found associations between serum PFHxS and total T4 and T3 in women (Wen et al. 2013), the epidemiological studies did not find associations for TSH, T3, or T4 (Berg et al. 2017; Bloom et al. 2010; Crawford et al. 2017; Jain 2013; Ji et al. 2012; Kang et al. 2018; Lewis et al. 2015; Preston et al. 2018; Wang et al. 2013a, 2014; Webster et al. 2016; Yang et al. 2016a). No associations were also found between cord blood PFHxS levels and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016). Chan et al. (2011) did not find an increase in the risk of hypothyroxinemia associated with serum PFHxS levels. Wen et al. (2013) found increases in the risk of subclinical hypothyroidism and subclinical hyperthyroidism among women, but not men and Dufour et al. (2018) did not find an association between cord blood PFHxS levels and risk of hypothyroidism in pregnant women.

Laboratory Animal Studies. Hypertrophy and hyperplasia of the follicular cells were observed in the thyroids of male rats treated with ≥3 mg/kg/day PFHxS for at least 42 days (Butenhoff et al. 2009a). The NOAEL was 1 mg/kg/day. The investigators noted that the observed changes in rats are consistent with the known effects of inducers of microsomal enzymes where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid due to an increase in plasma turnover of T4 and associated stimulation of TSH. Neither thyroid hormones nor TSH were measured in the study. In studies of pregnant rats, 20–30 and 60% decreases in serum thyroxine were observed in the dams administered 5 mg/kg/day or 25 mg/kg/day PFHxS on GD 7–22 (Ramhøj et al. 2018). In mice administered up to 3 mg/kg/day PFHxS prior to mating and during mating, gestation, and lactation, no alterations in TSH were observed in the parental males or females (Chang et al. 2018); this study also found no histological alterations in the thyroid gland.

PFNA

Epidemiological Studies. Inverse associations between serum PFNA levels and T4 levels (Wang et al. 2014) and TSH levels (Yang et al. 2016a) have been reported in general population studies. However, several other studies have not found alterations in TSH, T4, or T3 levels associated with serum PFNA

levels (Berg et al. 2017; Bloom et al. 2010; Jain 2013; Ji et al. 2012; Lopez-Espinosa et al. 2012; Preston et al. 2018; Wang et al. 2013a; Webster et al. 2016; Wen et al. 2013; Yang et al. 2016a). The investigators for an occupational exposure study reported that differences in TSH, T4, and T3 levels were small and clinically insignificant in groups of workers exposed to low levels, high levels, or no PFNA (Mundt et al. 2007). Preston et al. (2018) found an inverse association between serum PFNA levels and TSH levels, but only in pregnant women who were positive for maternal thyroid peroxides antibodies. Crawford et al. (2017) found associations between serum PFNA and free T4 and T3 levels in women, but no associations with total T4 or TSH. No associations between cord blood PFNA levels and cord blood T4, T3, or TSH were found in two studies (Shah-Kulkarni et al. 2016; Tsai et al. 2017). No associations were found for thyroid disease, hypothyroidism, or subclinical hypo- or hyperthyroidism among residents living near the Washington Works PFOA facility (Lopez-Espinosa et al. 2012), in NHANES participants (Wen et al. 2013), or in pregnant women (Dufour et al. 2018).

PFDA

Epidemiological Studies. Most general population studies did not find associations between serum PFDA levels and TSH, T3, or T4 levels (Berg et al. 2017; Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a). The exceptions were studies in pregnant women that found positive associations (Berg et al. 2015; Wang et al. 2014) or inverse associations with T3 (Berg et al. 2017), or an inverse association with TSH levels (Yang et al. 2016a). No associations between cord blood PFDA and cord blood T4, T3, or TSH were found in a study by Shah-Kulkarni et al. (2016).

Laboratory Animal Studies. Administration of a single dose of 80 mg/kg PFDA to female C57BL/6N mice by gavage resulted in 2- and 4-fold increases in serum T3 and T4, respectively, relative to controls 30 days after dosing (Harris et al. 1989). No alterations were observed in the adrenal glands of rats administered 0.5 mg/kg/day PFDA for 28 days or mice receiving weekly gavage doses of 5 mg/kg for 4 weeks (Frawley et al. 2018).

PFUnA

Epidemiological Studies. Inverse associations between serum PFUnA and serum TSH (Yang et al. 2016a) T4 (Wang et al. 2014), or T3 (Berg et al. 2015, 2017) have been reported in pregnant women. However, other general population studies have not found association between PFUnA and TSH, T4, or T3 levels (Bloom et al. 2010; Ji et al. 2012; Kang et al. 2018; Wang et al. 2013a, 2014; Yang et al. 2016a)

or between cord blood PFUnA and cord blood T4, T3, or TSH (Shah-Kulkarni et al. 2016; Tsai et al. 2017).

PFBS

Laboratory Animal Studies. Treatment of rats with up to 900 mg/kg/day PFBS by gavage for 28 days did not alter the gross or microscopic appearance of the adrenal, pituitary, or thyroid/parathyroid glands (3M 2001). Levels of thyroid hormones in serum were not available in this study. A study in pregnant mice administered ≥200 mg/kg/day PFBS on GDs 1–20 found decreases in maternal levels of total T4, free T4, and total T3 and increases in TSH levels (Feng et al. 2017).

PFBA

Laboratory Animal Studies. Treatment of rats with up to 184 mg/kg/day PFBA by gavage for 5 days did not affect the gross or microscopic morphology of the adrenal, thyroid, or pituitary glands (3M 2007a). Treatment with ≥30 mg/kg/day for 28 or 90 days significantly increased the incidence of hyperplasia/ hypertrophy of the follicular epithelium of the thyroid gland (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). These changes were not observed following a 3-week recovery period. Van Otterdijk (2007a, 2007b; Butenhoff et al. 2012a) suggested that the thyroid lesion likely reflected an increase in T4 producing follicular cells in response to feedback mechanisms from the increased turnover of T4 by the hypertrophic hepatocytes. None of these studies measured thyroid hormones or TSH in serum.

PFDoDA

Epidemiological Studies. Four general population studies have evaluated the effect of PFDoDA on thyroid hormone levels. Wang et al. (2014) reported inverse associations between serum PFDoDA and free T4 and total T4 in pregnant women; no associations were found for TSH or total T3. In another study of pregnant women (Yang et al. 2016a), inverse associations were found for TSH, free T4, total T4, free T3, and total T3. The third study (Ji et al. 2012) found no associations between serum PFDoDA and TSH or T4. Shah-Kulkarni et al. (2016) did not find associations between cord blood PFDoDA levels and cord blood T4, T3, or TSH levels.

Laboratory Animal Studies. Histological alterations were observed in the pancreas, adrenal gland, and/or thymus of rats administered 2.5 mg/kg/day PFDoDA for 42–47 days (Kato et al. 2015). Decreases in

zymogen granules were observed in the pancreas of male rats and edema of the pancreas interstitium was observed in females (most female rats died before the end of the study). Atrophy of the adrenal cortex was observed in males and in females exposed for 42 days and allowed to recover for 14 days. Atrophy of the thymic cortex was observed in females (most dying before the end of the study). A 28-day study found a 40% reduction in serum estradiol levels in pubertal female rats administered 3 mg/kg/day for 28 days (Shi et al. 2009b).

PFHxA

Laboratory Animal Studies. An increased incidence of thyroid follicular epithelial hypertrophy was observed in female rats administered 500 mg/kg/day NaPFHx for 93 days (Loveless et al. 2009). No alterations were observed in male rats in this study or in a second 90-day study in which male and female rats were administered doses as high as 200 mg/kg/day NaPFHx.

2.14 IMMUNOLOGICAL

Overview. Epidemiological studies have evaluated three categories of altered immune response related to exposure to perfluoroalkyls: immunosuppression (altered antibody response, infectious disease resistance), hypersensitivity (asthma, wheezing, eczema, atopic dermatitis, allergies), and autoimmunity. A summary of epidemiological studies evaluating immunological endpoints is presented in Table 2-16; more detailed descriptions of individual studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 10. Epidemiological data evaluating potential immunological effects are available for all perfluoroalkyls except PFBA. In general, the epidemiological studies identify the immune system as a target of perfluoroalkyl toxicity. The strongest evidence of the immunotoxicity of perfluoroalkyls in humans comes from epidemiological studies finding associations evaluating the antibody response to vaccines. Associations have been found for PFOA, PFOS, PFHxS, and PFDA. There is also some limited evidence for decreased antibody response for PFNA, PFUnA, and PFDoDA, although many of the studies did not find associations for these compounds. In general, decreases in disease resistance have not been found for PFOA, PFOS, PFHxS, or PFNA. There is marginal evidence for associations between PFOA, PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA and increased risk of asthma; the evidence was considered marginal due to the small number of studies evaluating the outcome and/or conflicting study results. There are limited data of effects on

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Anderson-Mahoney et al. 2008 Community (n=566 adults)	NR	Asthma	**SPR 1.82 (1.47–2.25)***
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	OR 1.1 (0.6–1.8)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	3.59 and 3.27 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.47 ng/mL (4th quartile)	Food allergies	**OR 9.09 (3.52–24.90)*, 4th quartile**
		Food sensitization	NS (p=0.74 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	2.04–10.12 ng/mL (maternal 3rd tertile PFOA)	Risk of number of days above the median	
		Fever	**OR 1.97 (1.07–3.62)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	OR 1.12 (0.82–1.54), 3rd tertile
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.5 and 1.0 ng/mL (mean serum PFOA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.67 (1.49–4.79)*, 3rd quartile**
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population. (n=1,400 pregnant women and young children)	5.6 ng/mL (maternal PFOA)	Risk of hospitalization for infectious disease in young children	IRR 0.96 (0.87–1.06) for trend **IRR 1.21 (1.04–1.42)* for trend, girls** **IRR 0.83 (0.73–0.95)* for trend, boys**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	2.713 ng/mL (mean maternal plasma PFOA)	Prevalence of allergic disease	OR 0.830 (0.591–1.16), 4th quartile
		Prevalence of wheezing	OR 1.09 (0.729–1.65), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	2.713 ng/mL (mean maternal serum PFOA)	Risk of total infectious diseases	OR 1.11 (0.806–1.54), 4th quartile, p=0.393 for trend.
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	4.1 and 4.4 ng/mL (median PFOA at age 5 and 7 years) 3.20 ng/mL (geometric mean maternal PFOA)	Tetanus antibody levels at age 5	NS, maternal PFOA NS, PFOA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFOA **β -35.8% (-51.9 to -14.2)*, per 2-fold increase in PFOA levels at age 5** NS, PFOA at age 7
		Diphtheria antibody levels at age 5	NS, maternal PFOA NS (p=0.69), PFOA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFOA **β -25.2% (-42.9 to -2.0)*, per 2-fold increase in PFOA levels at age 5** **β -25.4% (-40.9 to -5.8)*, per 2-fold increase in PFOA levels, PFOA at age 7**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	4.4 and 2.0 ng/mL (median PFOA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.637), PFOA at age 7 NS (p=0.856), PFOA at age 13
		Diphtheria antibody levels at age 13	Full cohort NS (p=0.742), PFOA at age 7 NS (p=0.129), PFOA at age 13 Cohort restricted to children without possible unscheduled booster vaccines NS (p=0.480), PFOA at age 7 **Association (p=0.029)*, PFOA at age 13**
Granum et al. 2013 General population (n=56 children age 3 years)	1.1 ng/mL (mean maternal PFOA)	Rubella antibody levels	**Inverse association (p=0.001)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p<0.001)***
		Number of episodes of gastroenteritis	**Association (p=0.048)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	4.3 and 4.0 ng/mL (median PFOA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	**OR 1.18 (1.01–1.39)*, per doubling PFOA**
		Current asthma	NS (p=0.26)
		Wheezing	NS (p=0.98)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Impinen et al. 2018 General population (n=641 infants followed through age 10)	**Exposure:** level 1.8 ng/mL (mean cord PFOA)	Number of common colds (0–2 years of age)	NS (p=0.089)
		Number of lower respiratory infections (0–10 years of age)	**β 0.28 (0.22–0.35; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General pop. (n=12 adults)	1.69 ng/mL (median PFOA)	Diphtheria antibody levels	NS (p=0.250), unadjusted
		Tetanus antibody levels	NS (p=0.970), unadjusted.
Looker et al. 2014 Community (C8) (n=411)	33.74 ng/mL (geometric mean) 13.8–31.5 ng/mL (2nd quartile)	Seroprotection from influenza A H3N2 virus	**OR 0.34 (0.14–0.83)*, 2nd quartile**
		Seroprotection from influenza A H1N1 virus	NS (p=0.02)
		Seroprotection from influenza type B virus	NS (p=0.68)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)
Okada et al. 2012 General population (n=343 pregnant women)	1.3 ng/mL (maternal median PFOA)	Cord IgE levels Males Females	 NS (p>0.05) **Inverse association (p<0.05)***
		Infant food allergy	OR 1.67 (0.52–5.37)
		Eczema	OR 0.96 (0.23–4.02)
		Wheezing	OR 1.27 (0.27–6.05)
		Otitis media	OR 1.51 (0.45–5.12)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	2.67 ng/mL (maternal mean PFOA)	Risk of allergic diseases Males Females	 OR 0.63 (0.63–1.37), 4th quartile **OR 0.64 (0.42–0.97)*, 4th quartile**
		Eczema Males Females	 OR 0.75 (0.48–1.18), 4th quartile OR 0.65 (0.39–1.09), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.02 and 0.50 ng/mL (median serum PFOA in cases and controls)	Asthma	**OR 2.76 (1.82–4.17)***
Smit et al. 2015 General population (n=1,024 children)	0.97 and 1.79 ng/mL (maternal mean PFOA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.80 (0.62–1.04), whole cohort
		Ever having eczema	OR 0.97 (0.81–1.17), whole cohort
		Current eczema	OR 1.01 (0.79–1.29), whole cohort
		Ever having wheezing	OR 0.91 (0.76–1.10), whole cohort
		Current wheezing	OR 0.97 (0.71–1.33), whole cohort
Steenland et al. 2013 Community (C8) (28,441)	Estimated cumulative	Ulcerative colitis	**OR 1.76 (1.04–2.99)*, 2nd quartile**
		Rheumatoid arthritis	NS (p>0.05)
		Crohn's disease	NS (p>0.05)
		Type I diabetes	NS (p>0.05)
		Lupus	NS (p>0.05)
		Multiple sclerosis	NS (p>0.05)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Asthma	NS (p=0.27), with no lag NS (p=0.53), with 10-year lag **Positive categorical trend (p=0.05)*, with no lag**
		Ulcerative colitis	**RR 6.57 (1.47–29.40)*, with 10-year lag**
		Rheumatoid arthritis	**Positive categorical trend (p=0.04)*, with no lag**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	4.13 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity), whole cohort **β -6.6% (-11.7 to -1.5)*, per 2-fold increase in PFOA levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -8.9% (-14.6 to -2.9)*, per 2-fold increase in PFOA levels, seropositive subcohort**
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	3.59 ng/mL (geometric mean)	Rhinitis	**OR 1.35 (1.10–1.66)***
		Current asthma	OR 1.28 (0.81–2.04)
		Wheeze	OR 0.94 (0.51–1.73)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization Plants Dust mites Pets Cockroach or shrimp Rodents Mold Food	 OR 0.88 (0.67–1.15) OR 0.93 (0.75–1.16) OR 1.17 0.81–1.68) OR 0.79 (0.55–1.13) OR 1.65 (0.59–4.60 OR 1.21 (0.85–1.72) OR 1.02 (0.60–1.73)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	2.28 ng/mL (geometric mean)	Seroconversion Hemagglutinin Immunohistochem.	 NS (p=0.07 for trend) NS (p=0.27 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2011 General population (n=244 children aged 2 years)	1.71 ng/mL (median cord PFOA)	Serum IgE levels	NS (p=0.870)
		Cord blood IgE levels	Association (p=0.047)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.51 and 1.00 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.24 (1.91–9.42)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines	
		IL-4	**Association (p=0.001 for trend)***
		IL-5	**Association (p=0.004 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFOS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.1 (0.6–1.9)
		IgE (cord blood)	OR 1.1 (0.6–1.9)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	14.98 and 8.74 ng/mL (geometric mean PFOS 2005–2006 and 2007–2010) 9.17–13.75 ng/mL (3rd quartile)	Food allergies	**OR 2.43 (1.05–5.59)*, 3rd quartile (trend not significant, p=0.27)**
		Food sensitization	NS (p=0.49 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	10.19–25.10 ng/mL (maternal 3rd tertile PFOS)	Risk of number of days above the median	
		Fever	**OR 2.35 (1.34–4.11)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Risk of number of days	
		Fever	**OR 1.65 (1.24–2.18)*, 3rd tertile**
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	45.5 and 33.4 ng/mL (mean serum PFOS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.63 (1.48–4.69)*, 4th quartile**
		Asthma severity	**Association (p=0.045 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Fei et al. 2010 General population (n=1,400 pregnant women and young children)	35.3 ng/mL (maternal PFOS)	Risk of hospitalization for infectious disease in young children	IRR 1.00 (0.91–1.09) for trend **IRR 1.18 (1.03–1.36)* for trend, girls** IRR 0.90 (0.80–1.12) for trend, boys
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	5.456 ng/mL (mean maternal plasma PFOS)	Prevalence of allergic disease	OR 0.815 (0.596–1.11), 4th quartile
		Prevalence of wheezing	OR 0.770 (0.526–1.12), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	5.456 ng/mL (mean maternal serum PFOS)	Risk of total infectious diseases	**OR 1.44 (1.06–1.96)*, 2nd quartile; p=0.008 for trend**
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	17.3 and 15.5 ng/mL (median PFOS at age 5 and 7 years) 27.3 ng/mL (geometric mean maternal PFOS)	Tetanus antibody levels at age 5	NS, maternal PFOS **β -28.5% (-45.5 to -6.1)*, per 2-fold increase in PFOS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFOS NS, PFOS at ages 5 and 7

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Diphtheria antibody levels at age 5	NS, maternal PFOS NS, PFOS at age 5
		Diphtheria antibody levels at age 7	**β -27.6% (-45.8 to -3.3)*, per 2-fold increase in PFOS levels at age 5** **β -30.3% (47.3 to -7.8)*, per 2-fold increase in PFOS levels at age 7**
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	15.3 and 6.7 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	Full cohort NS (p=0.240), PFOS at age 7 NS (p=0.237), PFOS at age 13 Cohort restricted to children without possible unscheduled booster vaccines **Association (p=0.043)*, PFOS at age 7** NS (p=0.144), PFOS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.07), age 7 NS (p=0.454), age 13
Granum et al. 2013 General population (n=56 children age 3 years)	5.6 ng/mL (mean maternal PFOS)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.501)
		Number of episodes of gastroenteritis	NS (p=0.367)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	17.0 and 16.8 ng/mL (median PFOS in asthmatics and non-asthmatics)	Asthma episode in last 12 months	NS (p=0.13), per doubling PFOS
		Current asthma	NS (p=0.24)
		Wheezing	NS (p=0.08)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	5.6 ng/mL (mean cord PFOS)	Number of common colds (0–2 years of age)	NS (p=0.173)
		Number of lower respiratory infections (0–10 years of age)	**β 0.50 (0.42–0.57; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	9.52 ng/mL (median PFOS)	Diphtheria antibody levels	**Inverse association (p=0.044)*, unadjusted**
		Tetanus antibody levels	NS (p=0.420), unadjusted
Looker et al. 2014 Community (C8) (n=411)	8.32 ng/mL (geometric mean PFOS)	Response to influenza A H3N2 virus vaccine	NS (p>0.05)
		Response to influenza A H1N1 virus vaccine	NS (p>0.05)
		Response to influenza type B virus vaccine	NS (p>0.05)
		Cold or flu infection	NS (p>0.05)
		Frequency of colds	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2012 General population (n=343 infants)	5.2 ng/mL (maternal median PFOS)	Cord IgE levels	NS (p>0.05)
		Infant food allergy	OR 3.72 (0.81–17.10)
		Eczema	OR 0.87 (0.15–5.08)
		Wheezing	OR 2.68 (0.39–18.30)
		Otitis media	OR 1.40 (0.33–6.00)
Okada et al. 2014 General population (n=2,603 infants)	5.56 ng/mL (maternal mean PFOS)	Risk of allergic diseases Males Females	 OR 0.95 (0.65–1.37), 4th quartile OR 0.79 (0.53–1.17), 4th quartile
		Eczema Males Females	 OR 0.98 (0.63–1.53), 4th quartile OR 0.84 (0.51–1.38), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	31.51 and 28.83 ng/mL (median serum PFOS in cases and controls)	Asthma	**OR 1.30 (1.00–1.69)***
Smit et al. 2015 General population (n=1,024 children)	4.88 and 20.6 ng/mL (maternal mean PFOS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.86 (0.67–1.10), whole cohort
		Ever having eczema	OR 0.98 (0.88–1.18), whole cohort
		Current eczema	OR 1.05 (0.82–1.33), whole cohort
		Ever having wheezing	OR 0.83 (0.69–1.00), whole cohort
		Current wheezing	**OR 0.60 (0.38–0.92)*, Ukraine cohort** OR 0.91 (0.62–1.36) Greenland OR 0.76 (0.56–1.01), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	20.8 ng/mL (geometric mean PFOS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	**β -7.4% (-12.8 to -1.7)*, per 2-fold increase in PFOS levels, whole cohort** **β -5.9% (-9.9 to -1.6)*, per 2-fold increase in PFOS levels, seropositive subcohort**
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -13.3% (-19.9–6.2)*, per 2-fold increase in PFOS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	15.0 ng/mL (geometric mean)	Rhinitis	OR 1.16 (0.90–1.50)
		Current asthma	OR 1.20 (0.88–1.63)
		Wheeze	OR 0.76 (0.45–1.19)
		Allergy	OR 1.05 (0.80–1.37)
		Allergic sensitization	
		Plants	**OR 0.17 (0.53–0.97)***
		Dust mites	OR 1.00 (0.73–1.38)
		Pets	OR 0.83 (0.56–1.22)
		Cockroach or shrimp	**OR 0.67 (0.48–0.93)***
		Rodents	OR 0.85 (0.29–2.45)
		Mold	**OR 1.33 (1.06–1.69)***
		Food	OR 0.74 (0.39–1.40)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	5.22 ng/mL (geometric mean)	Seroconversion	
		Hemagglutinin	NS (p=0.81 for trend)
		Immunohistochemistry	NS (p=0.12 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	5.50 ng/mL (median cord PFOS)	Serum IgE levels	NS (p=0.179)
		Cord blood IgE levels	Association (p=0.017)*
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	45.86 and 33.9 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 4.38 (2.02–9.50)*, males 4th quartile** NS (p=0.899 for trend), females
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Ashley-Martin et al. 2015 General population (1,258 women)	NR	IL-33/TSLP (cord blood)	1.0 (0.7–1.4)
		IgE (cord blood)	OR 1.0 (0.7–1.4)
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	2.09 and 2.19 ng/mL (geometric mean 2005–2006 and 2007–2010) >4.00 ng/mL (4th quartile)	Food allergies	**OR 3.06 (1.35–6.93)*, 4th quartile** (trend not significant, p=0.11)
		Food sensitization	NS (p=0.72 for trend)
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.32 ng/mL (maternal median PFHxS)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	NS (p>0.05)
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	3.9 and 2.1 ng/mL (mean serum PFHxS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.94 (1.65–5.25)*, 3rd quartile**
		Asthma severity	NS (p=0.722 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.322 ng/mL (mean maternal plasma PFHxS)	Prevalence of allergic disease	OR 0.841 (0.615–1.151), 4th quartile
		Prevalence of wheezing	OR 0.728 (0.497–1.06), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.322 ng/mL (mean maternal serum PFHxS)	Risk of total infectious diseases	OR 0.957 (0.7.3–1.41), 4th quartile, p=0.928 for trend Females only: **OR 1.81 (1.14–2.88)*, 3rd quartile; p=0.045 for trend** Males only: p=0223 for trend
Grandjean et al. 2012; Mogensen et al. 2015a General population (n=456 and n=464 children 5 and 7 years of age)	0.6 and 0.5 ng/mL (median PFHxS at age 5 and 7 years) 4.41 ng/mL (geometric mean maternal PFHxS)	Tetanus antibody levels at age 5	NS, maternal PFHxS **-19.0% (-29.8 to -6.6)*, per 2-fold increase in PFHxS levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFHxS **β -19.7% (-31.6 to -5.7)*, per 2-fold increase PFHxS levels at age 5** **β -22.3% (-36.3 to -5.2)*, per 2-fold increase PFHxS levels at age 7**
		Diphtheria antibody levels at age 5	NS, maternal PFHxS NS, PFHxS at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFHxS NS, PFHxS at age 5 or 7
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.5 and 0.4 ng/mL (median PFHxS at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.334), PFHxS at age 7 NS (p=0.568), PFHxS at age 13
		Diphtheria antibody levels at age 13	NS (p=0.264), PFHxS at age 7 NS (p=0.583), PFHxS at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFHxS levels)	Rubella antibody levels	**Inverse association (p=0.008)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	NS (p=0.078)
		Number of episodes of gastroenteritis	**Association (p=0.007)***
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	2.2 and 2.0 ng/mL (median PFHxS in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.66), per doubling PFHxS
		Current asthma	NS (p=0.99)
		Wheezing	NS (p=0.92)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.3 ng/mL (mean cord PFHxS)	Number of common colds (0–2 years of age)	NS (p=0.530)
		Number of lower respiratory infections (0–10 years of age)	NS (p=0.119)
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.37 ng/mL (median PFHxS)	Diphtheria antibody levels	NS (p=0.055), unadjusted
		Tetanus antibody levels	NS (p=0.390), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.324 ng/mL (maternal mean PFHxS)	Risk of allergic diseases Males Females	 OR 0.81 (0.56–1.16), 4th quartile OR 1.13 (0.75–1.69), 4th quartile
		Eczema Males Females	 OR 0.78 (0.51–1.19), 4th quartile OR 0.82 (0.49–1.36), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.38 and 1.07 ng/mL (median serum PFHxS in cases and controls)	Asthma	**OR 2.14 (1.48–3.11)***
Smit et al. 2015 General population (n=1,024 children)	1.53 and 2.14 ng/mL (maternal mean PFHxS in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.91 (0.69–1.18), whole cohort
		Ever having eczema	OR 1.03 (0.86–1.24), whole cohort
		Current eczema	OR 0.93 (0.73–1.20), whole cohort
		Ever having wheezing	OR 0.96 (0.79–1.17), whole cohort
		Current wheezing	OR 0.93 (0.68–1.27), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	2.47 ng/mL (geometric mean PFHxS)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity), whole cohort **β -6.0% (-9.6 to -2.2)*, per 2-fold increase PFHxS levels, seropositive subcohort**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	2.09 ng/mL (geometric mean PFHxS)	Rhinitis	OR 0.81 (0.57–1.16)
		Current asthma	OR 0.98 (0.51–1.87)
		Wheeze	OR 0.99 (0.68–1.44)
		Allergy	OR 0.83 (0.59–1.17)
		Allergic sensitization	
		Plants	OR 0.93 (0.62–1.39)
		Dust mites	OR 1.01 (0.84–1.22)
		Pets	OR 0.96 (0.71–1.30)
		Cockroach or shrimp	OR 0.72 (0.56–0.93)
		Rodents	OR 0.81 (0.54–1.21)
		Mold	OR 0.98 (0.65–1.47)
		Food	OR 1.03 (0.74–1.42)
Stein et al. 2016b General population (n=78 adults receiving influenza vaccine)	1.1 ng/mL (geometric mean PFHxS)	Seroconversion	
		Hemagglutinin	NS (p=0.22 for trend)
		Immunohistochem.	NS (p=0.34 for trend)
		Serum cytokine levels	
		IFN-γ	**Association (p=0.05 for trend)***
		IFN-α2	NS (p=0.09 for trend)
		TNF-α	**Association (p=0.04 for trend)***
		IP-10	NS (p=0.59 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	3.86 and 2.10 ng/mL (mean PFHxS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.97 (1.33–6.64)*, males 4th quartile** **OR 5.02 (2.05–12.30)*, females 4th quartile**
		T-helper cytokines	
		IL-4	NS (p>0.05 for trend)
		IL-5	NS (p>0.05 for trend)
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFNA			
Buser and Scinicariello 2016 General population (NHANES) (n=637 and 701 adolescents in 2005–2006 and 2007–2010)	0.93 and 1.13 ng/mL (geometric mean PFNA 2005–2006 and 2007–2010) >1.36 ng/mL (4th quartile)	Food allergies	NS (p=0.28 for trend)
		Food sensitization	**OR 0.51 (0.28–0.92)*, 4th quartile (trend not significant, p=0.15)**
Dalsager et al. 2016 General population (n=359 1–4-year-old children)	0.56–0.81 and 0.82–3.64 ng/mL (maternal 2nd and 3rd tertile PFNA)	Risk of number of days above the median	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	**OR 0.53 (0.31–0.92)*, 2nd tertile**
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)
		Risk of number of days	
		Fever	NS (p>0.05)
		Cough	NS (p>0.05)
		Nasal discharge	OR 1.12 (0.84–1.49), 3rd tertile
		Diarrhea	NS (p>0.05)
		Vomiting	NS (p>0.05)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.1 and 0.9 ng/mL (mean serum PFNA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 2.56 (1.41–4.65)*, 4th quartile**
		Asthma severity	NS (p=0.217 for trend)
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.402 ng/mL (mean maternal plasma PFNA)	Prevalence of allergic disease	OR 0.873 (0.562–1.35), 4th quartile
		Prevalence of wheezing	OR 1.11 (0.760–1.63), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.402 ng/mL (mean maternal serum PFNA)	Risk of total infectious diseases	OR 0.918 (0.672–1.25), 4th quartile, p=0.748 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	1.00 ng/mL (geometric mean PFNA at age 5 years) 0.60 ng/mL (geometric mean PFNA at age 7 years)	Tetanus antibody levels at age 5	NS, maternal PFNA NS, PFNA at age 5
		Tetanus antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
		Diphtheria antibody levels at age 5	NS, maternal PFNA **β -16.1% (-28.8 to -1.0)*, per 2-fold increase PFNA levels at age 5**
		Diphtheria antibody levels at age 7	NS, maternal PFNA NS, PFNA at age 5
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	1.1 and 0.7 ng/mL (median PFNA at age 7 and 13 years)	Tetanus antibody levels at age 13	NS (p=0.075), PFNA at age 7 NS (p=0.394), PFNA at age 13
		Diphtheria antibody levels at age 13	NS (p=0.243), PFNA at age 7 NS (p=0.693), PFNA at age 13

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Granum et al. 2013 General population (n=56 children age 3 years)	0.3 ng/mL (mean maternal PFNA levels)	Rubella antibody levels	**Inverse association (p=0.007)***
		Hemophilus influenza type B antibody levels	NS (p>0.05)
		Tetanus antibody levels	NS (p>0.05)
		Asthma diagnosis	NS (p>0.05)
		Atopic eczema	NS (p>0.05)
		Eczema and itchiness	NS (p>0.05)
		Number of episodes of otitis media	NS (p>0.05)
		Number of episodes of common cold	**Association (p=0.035)***
		Number of episodes of gastroenteritis	NS (p=0.883)
Humblet et al. 2014 General population (NHANES) (n=1,877 adolescents)	0.9 and 0.8 ng/mL (median PFNA in asthmatics and nonasthmatics)	Asthma episode in last 12 months	NS (p=0.92), per doubling PFNA
		Current asthma	NS (p=0.97)
		Wheezing	NS (p=0.94)
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.2 ng/mL (mean cord PFNA)	Number of common colds (0–2 years of age)	NS (p=0.983)
		Number of lower respiratory infections (0–10 years of age)	**β 0.0.09 (0.03–0.14;p=0.001)***
		Rhinitis	NS
		Rhinoconjuctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kielsen et al. 2016 General population (n=12 adults)	0.66 ng/mL (median PFNA)	Diphtheria antibody levels	**Inverse association (p=0.004)*, unadjusted**
		Tetanus antibody levels	NS (p=0.250), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	1.36 ng/mL (maternal mean PFNA)	Risk of allergic diseases Males Females	 OR 0.95 (0.66–1.38), 4th quartile **OR 0.55 (0.36–0.82)*, 4th quartile**
		Eczema Males Females	 OR 0.96 (0.61–1.52), 4th quartile OR 0.63 (0.38–1.02), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	2.00 and 0.80 ng/mL (median serum PFNA in cases and controls)	Asthma	**OR 1.61 (1.12–2.31)***
Smit et al. 2015 General population (n=1,024 children)	0.62 and 0.73 ng/mL (maternal mean PFNA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.90 (0.70–1.14), whole cohort
		Ever having eczema	OR 0.94 (0.78–1.14), whole cohort
		Current eczema	OR 1.03 (0.82–1.30), whole cohort
		Ever having wheezing	OR 0.91 (0.75–1.09), whole cohort
		Current wheezing	OR 0.90 (0.66–1.23), whole cohort
Stein et al. 2016a General population (NHANES) (n=1,191 adolescents)	0.765 ng/mL (geometric mean)	Measles antibody titers	NS (95% CI included unity)
		Mumps antibody titers	NS (95% CI included unity)
		Rubella antibody titers	NS (95% CI included unity)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2016a General population (NHANES) (n=640 adolescents)	0.929 ng/mL (geometric mean)	Rhinitis	OR 1.24 (0.97–1.60)
		Current asthma	OR 1.26 (0.79–2.01)
		Wheeze	OR 0.99 (0.58–1.68)
		Allergy	OR 1.12 (0.85–1.47)
		Allergic sensitization	
		Plants	OR 0.96 (0.74–1.23)
		Dust mites	OR 1.05 (0.78–1.41)
		Pets	OR 1.26 (0.85–1.87)
		Cockroach or shrimp	OR 0.86 (0.60–1.24)
		Rodents	OR 2.25 (0.83–6.10)
		Mold	OR 1.31 (0.83–2.06)
		Food	OR 0.91 (0.55–1.50)
Stein et al. 2016b General pop. (n=78 adults receiving influenza vaccine)	0.77 ng/mL (geometric mean PFNA)	Seroconversion	
		Hemagglutinin	NS (p=0.33 for trend)
		Immunohistochem.	NS (p=0.40 for trend)
		Serum cytokine levels	NS (p>0.05 for trend)
		Serum chemokine levels	NS (p>0.05 for trend)
		Nasal cytokine levels	NS (p>0.05 for trend)
		Nasal chemokine levels	NS (p>0.05 for trend)
		Serum IgA levels	NS (p>0.05 for trend)
Wang et al. 2011 General population (n=244 children aged 2 years)	2.30 ng/mL (median cord PFNA)	Serum IgE levels	NS (p=0.837)
		Cord blood IgE levels	NS (p=0.908)
		Atopic dermatitis	NS (p>0.05)
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.07 and 0.87 ng/mL (mean PFNA in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.33 (1.46–7.58)*, males 4th quartile** NS (p=0.142 for trend), females
		T-helper cytokines	
		IL-4	**Association (p=0.031 for trend)***
		IL-5	**Association (p=0.011 for trend)***
		IFN-γ	NS (p>0.05 for trend)
		IL-2	NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.008 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Dalsager et al. 2016 General population (n=359 children aged 1–4 years)	0.27 ng/mL (median maternal serum PFDA level)	Symptoms of infection	NS (p>0.05)
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	1.2 and 1.0 ng/mL (mean serum PFDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 3.22 (1.75–5.94)*, 4th quartile**
		Asthma severity	**Association (p=0.005 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** **Association (p<0.05)*, non-asthmatics**
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.575 ng/mL (mean maternal plasma PFDA)	Prevalence of allergic disease	OR 0.906 (0.663–1.23), 4th quartile
		Prevalence of wheezing	OR 0.879 (0.602–1.28), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.575 ng/mL (mean maternal serum PFDA)	Risk of total infectious diseases	OR 0.799 (0.588–1.08), 4th quartile, p=0.114 for trend
Grandjean et al. 2012 General population (n=456 and n=464 children 5 and 7 years of age)	0.28 ng/mL (geometric mean PFDA at age 5 years) 0.28 ng/mL (geometric mean maternal PFDA)	Tetanus antibody levels at age 5	NS, maternal PFDA **β -19.9% (-33.1 to -3.9), per 2-fold increase PFDA levels at age 5**
		Tetanus antibody levels at age 7	NS, maternal PFDA **β -22.3 (-35.8 to -5.8), per 2-fold increase PFDA levels at age 5**
		Diphtheria antibody levels at age 5	NS, maternal PFDA NS, PFDA at age 5
		Diphtheria antibody levels at age 7	NS, maternal PFDA NS, PFDA at age 5

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Grandjean et al. 2017 General population (n=516 children examined at age 7 and 13 years)	0.4 and 0.3 ng/mL (median at age 7 and 13 years)	Tetanus antibody levels at age 13	**Association (p=0.022)*, PFDA at age 7** NS (p=0.258), PFDA at age 13
		Diphtheria antibody levels at age 13	**Association (p=0.008)*, PFDA at age 7** NS (p=0.726), PFDA at age 13
Kielsen et al. 2016 General population (n=12 adults)	0.30 ng/mL (median PFDA)	Diphtheria antibody levels	**Inverse association (p=0.009)*, unadjusted**
		Tetanus antibody levels	NS (p=0.130), unadjusted
Okada et al. 2014 General population (n=2,603 infants)	0.563 ng/mL (maternal mean PFDA)	Risk of allergic diseases Males Females	 OR 1.13 (0.78–1.64), 4th quartile OR 0.70 (0.47–1.04), 4th quartile
		Eczema Males Females	 OR 0.93 (0.60–1.44), 4th quartile OR 0.78 (0.49–1.25), 4th quartile
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	1.13 and 0.93 ng/mL (median serum PFDA in cases and controls)	Asthma	OR 1.24 (0.97–1.58)
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.42 ng/mL (maternal mean PFDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.92 (0.73–1.16), whole cohort
		Ever having eczema	OR 0.88 (0.73–1.06), whole cohort
		Current eczema	OR 0.95 (0.75–1.20), whole cohort
		Ever having wheezing	OR 0.85 (0.70–1.01), whole cohort
		Current wheezing	OR 0.76 (0.56–1.04), whole cohort
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	1.24 and 1.02 ng/mL (mean in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 3.45 (1.51–7.88)*, males 4th quartile** **OR 3.68 (1.43–9.48)*, females 4th quartile**
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	**Association (p=0.002 for trend)***

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	1.534 ng/mL (mean maternal plasma PFUnA)	Prevalence of allergic disease	OR 0.736 (0.538–1.00), 4th quartile
		Prevalence of wheezing	OR 1.04 (0.714–1.51), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	1.534 ng/mL (mean maternal serum PFUnA)	Risk of total infectious diseases	OR 1.03 (0.764–1.40), 4th quartile, p=0.786 for trend
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.1 ng/mL (mean cord PFUnNA)	Number of common colds (0–2 years of age)	**β 0.11 (0.08–0.14; p=<0.0001)***
		Number of lower respiratory infections (0–10 years of age)	**β 0.18 (0.13–0.23; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS
Kielsen et al. 2016 General population (n=12 adults)	0.21 ng/mL (median PFUnA)	Diphtheria antibody levels	**Inverse association (p=0.036)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.039)*, unadjusted**
Okada et al. 2014 General population (n=2,603 infants)	1.50 ng/mL (maternal mean PFUnA)	Risk of allergic diseases Males Females	 OR 1.13 (0.79–1.63), 4th quartile **OR 0.58 (0.39–0.86)*, 4th quartile**
		Eczema Males Females	 OR 1.16 (0.75–10.81), 4th quartile **OR 0.50 (0.30–0.81)*, 4th quartile**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Smit et al. 2015 General population (n=1,024 children)	0.16 and 0.68 ng/mL (maternal mean PFUnA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.96 (0.77–1.21), whole cohort
		Ever having eczema	OR 0.95 (0.79–1.15), whole cohort
		Current eczema	OR 1.07 (0.85–1.34), whole cohort
		Ever having wheezing	OR 0.84 (0.70–1.00), whole cohort
		Current wheezing	OR 0.87 (0.65–1.17), whole cohort
PFHpA			
Kielsen et al. 2016 General population (n=12 adults)	0.12 ng/mL (median PFHpA)	Diphtheria antibody levels	NS (p=0.750), unadjusted
		Tetanus antibody levels	NS (p=0.280), unadjusted
Smit et al. 2015 General population (n=1,024 children)	0.03 and 0.05 ng/mL (maternal mean PFHpA in Ukraine and Greenland cohorts)	Ever having asthma	OR 0.93 (0.71–1.22), whole cohort
		Ever having eczema	OR 0.93 (0.78–1.11), whole cohort
		Current eczema	OR 0.90 (0.70–1.15), whole cohort
		Ever having wheezing	OR 1.03 (0.84–1.25), whole cohort
		Current wheezing	**OR 0.62 (0.40–0.97)*, Ukraine cohort** OR 1.24 (0.79–1.93), Greenland cohort OR 0.88 (0.64–1.20), whole cohort
PFBS			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Zhu et al. (2016)	0.5 and 0.5 ng/mL (mean serum PFBS levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.90 (1.08–3.37)*, 4th quartile**
		Asthma severity	NS (p=0.092 for trend)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.48 and 0.48 ng/mL (median serum PFBS in cases and controls)	Asthma	OR 1.06 (0.93–1.20)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhu et al. 2016 General population (n=231 asthmatic and 225 non-asthmatic children) This is the same group of children evaluated by Dong et al. (2013)	0.53 and 0.48 ng/mL (mean serum PFBS in asthmatics and non-asthmatics)	Asthma diagnosis	**OR 2.59 (1.14–5.87)*, males 4th quartile** NS (p=0.505 for trend), females
		T-helper cytokines IL-4 IL-5 IFN-γ IL-2	 NS (p>0.05 for trend) **Association (p=0.023 for trend)*** NS (p>0.05 for trend) NS (p>0.05 for trend)
		Serum IgE	NS (p>0.05 for trend)
PFDoDA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	5.8 and 4.5 ng/mL (mean serum PFDoDA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	**OR 1.81 (1.02–3.23)*, 4th quartile**
		Asthma severity	**Association (p=0.024 for trend)***
		IgE	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	**Association (p<0.05)*, asthmatics** NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	**Association (p<0.05)*, asthmatics** Association (p<0.05)*, non-asthmatics
Goudarzi et al. 2016a General population (n=1,558 4-year-old children)	0.191 ng/mL (mean maternal plasma PFDoDA)	Prevalence of allergic disease	**OR 0.621 (0.454–0.847)*, 4th quartile**
		Prevalence of wheezing	OR 0.999 (0.684–1.45), 4th quartile
Goudarzi et al. 2017 General population (n=1,558 mother-child pairs); children examined up to 4 years of age	0.191 ng/mL (mean maternal serum PFDoDA)	Risk of total infectious diseases	OR 1.07 (0.790–1.46), 4th quartile, p=0.502 for trend
Kielsen et al. 2016 General population (n=12 adults)	0.039 ng/mL (median PFDoDA)	Diphtheria antibody levels	**Inverse association (p=0.038)*, unadjusted**
		Tetanus antibody levels	**Inverse association (p=0.038)*, unadjusted**

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Okada et al. 2014 General population (n=2,603 infants)	0.188 ng/mL (maternal mean PFDoDA)	Risk of allergic diseases Males Females	 OR 0.93 (0.65–1.34), 4th quartile OR 0.58 (0.39–0.85)*, 4th quartile
		Eczema Males Females	 OR 1.00 (0.64–1.55), 4th quartile OR 0.73 (0.45–1.18), 4th quartile
Smit et al. 2015 General population (n=1,024 children)	0.04 and 0.13 ng/mL (maternal mean PFDoDA in Ukraine and Greenland cohorts)	Ever having asthma	OR 1.03 (0.81–1.30), whole cohort
		Ever having eczema	OR 0.90 (0.75–1.08), whole cohort
		Current eczema	OR 0.88 (0.70–1.14), whole cohort
		Ever having wheezing	OR 0.97 (0.80–1.16), whole cohort
		Current wheezing	OR 0.87 (0.64–1.18), whole cohort
PFHxA			
Dong et al. 2013 General population (n=231 asthmatic and 225 non-asthmatic children)	0.3 and 0.2 ng/mL (mean serum PFHxA levels in the asthmatic and non-asthmatic children, respectively; serum levels were not reported for full cohort)	Asthma diagnosis	OR 1.60 (0.90–2.86), 4th quartile
		Asthma severity	NS (p=0.854)
		IgE	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Absolute eosinophil counts	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
		Eosinophil cationic protein	NS (p>0.05), asthmatics NS (p>0.05), non-asthmatics
Qin et al. 2017 General population (n=132 children aged 10–15 years and 168 matched controls)	0.20 and 0.18 ng/mL (median serum PFHxA in cases and controls)	Asthma	OR 0.99 (0.80–1.21)

Table 2-16. Summary of Immunological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Impinen et al. 2018 General population (n=641 infants followed through age 10)	0.4 ng/mL (mean cord FOSA)	Number of common colds (0–2 years of age)	NS (p=0.477)
		Number of lower respiratory infections (0–10 years of age)	**β 0.10 (0.06–0.14; p=<0.0001)***
		Rhinitis	NS
		Rhinoconjunctivitis	NS
		Asthma diagnosis	NS
		Current asthma	NS
		Asthma ever	NS
		Allergic sensitization	NS

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 10 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk and bold indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; IFN-α-2 = interferon-α2; IFN-γ = interferon-γ; IgA = immunoglobulin A; IgE = immunoglobulin E; IP-10 = interferon-γ-inducible protein 10; IRR= incidence risk ratio; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= relative risk; SPR = standard prevalence ratio; TNF-α =tumor necrosis factor-α

autoimmunity; epidemiological studies provide suggestive evidence of an association between serum PFOA and the risk of ulcerative colitis. The small number of studies investigating immunotoxicity following exposure to PFHpA and PFHxA did not find associations.

Laboratory animal studies have also evaluated immunosuppression (disease resistance, antibody response, NK cell activity, delayed-type hypersensitivity response, monocyte phagocytosis), hypersensitivity (airway resistance, local lymph node assay), and autoimmunity. In addition, laboratory animal studies have examined secondary outcomes (lymphoid organ weights, lymphocyte counts or subpopulations, lymphocyte proliferation, cytokine levels, serum antibody levels, histological alterations in immune organs). Summaries of the laboratory animal studies are presented in the LSE tables for PFOA, PFOS, and other perfluoroalkyls (Tables 2-3, 2-4, 2-5, and 2-6); the NOAEL and LOAEL values are presented in Figures 2-8, 2-9, and 2-10. No laboratory animal studies were identified for PFUnA, PFHpA, PFDoDA, or FOSA. Studies in laboratory animals identify the immune system as a sensitive target of toxicity following exposure to PFOA and PFOS. The observed effects include impaired responses to T-dependent antigens, impaired response to infectious disease, and secondary outcomes (decreases in spleen and thymus weights and in the number of thymic and splenic lymphocytes). A small number of studies evaluated the immunotoxicity of other perfluoroalkyls and most did not evaluate immune function. No alterations in spleen or thymus organ weights or morphology were observed in studies on PFHxS, PFBA, and PFDA. A study on PFNA found decreases in spleen and thymus weights and alterations in splenic lymphocyte phenotypes.

The National Toxicology Program (NTP 2016b) concluded that exposure to PFOA or PFOS is presumed to be an immune hazard to humans based on a high level of evidence that PFOA and PFOS suppressed the antibody response from animals and a moderate level of evidence from studies in humans. It was noted that the strongest evidence is for suppression of the antibody response and increased hypersensitivity (PFOA only).

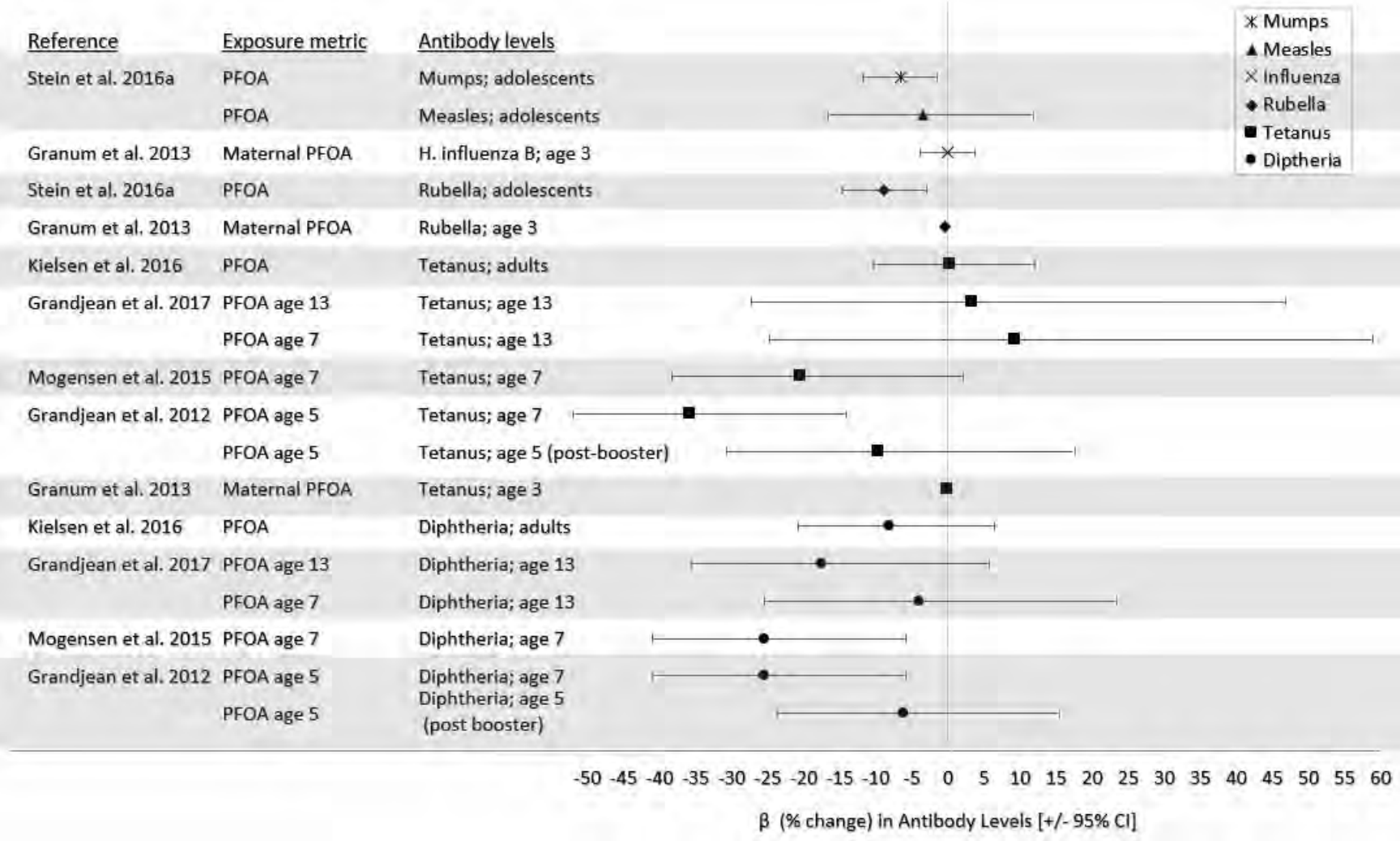
PFOA

Epidemiological Studies—Immunosuppression Outcomes. Studies evaluating the immunosuppressive effects of PFOA have examined disease resistance and antibody responses. One study found associations between maternal serum PFOA and the number of episodes of the common cold and other respiratory tract infections and the number of episodes of gastroenteritis with vomiting or diarrhea in 3-year-old children (Granum et al. 2013). Another study found an association between maternal PFOA and the risk

of having a greater number of days with a fever greater than the median (Dalsager et al. 2016), although there was no increase in the number of days with a fever. A third study found an increased risk of lower respiratory tract infections associated cord PFOA from birth to 10 years of age (Impinen et al. 2018). However, other studies have not found associations between PFOA levels and the frequency of the common cold or flu in adults (Looker et al. 2014), between maternal PFOA levels and otitis media in 1.5–3-year-old children (Granum et al. 2013; Okada et al. 2012), between maternal PFOA and the risk of hospitalization for infectious diseases in young children (Fei et al. 2010), between maternal PFOA and the risk of number of days with cough, nasal discharge, diarrhea, or vomiting (Dalsager et al. 2016), between cord PFOA and number of common colds (Impinen et al. 2018), or between maternal serum PFOA and total number of infectious diseases between birth and 2 years of age (Goudarzi et al. 2017).

Several studies have evaluated the antibody response to vaccination in adults and children; the changes in the response to antibody levels relative to serum PFOA levels are graphically presented in Figure 2-21; the figure does not include data from other studies that used different statistical methods. In adults, decreases in antibody response against influenza A H3N2 virus were associated with increasing serum PFOA levels; however, there were no associations with two other strains of influenza virus (influenza A H1N1 and influenza B) (Looker et al. 2014). Another study of adults also did not find an altered immune response to influenza A H1N1 virus (Stein et al. 2016b). A small-scale study of 12 adults did not find significant alterations in the response to diphtheria or tetanus booster vaccines associated with serum PFOA levels (Kielsen et al. 2016). Increasing current serum PFOA levels were associated with lower antibody levels for mumps and rubella, but not for measles, in a cross-sectional study of adolescents (Stein et al. 2016a). A series of prospective studies by Grandjean and associates (Grandjean et al. 2012, 2017; Mogensen et al. 2015a) evaluated tetanus and diphtheria antibody levels in children at 5, 7, and 13 years of age. Diphtheria antibody levels at age 7 and 13 were inversely associated with serum PFOA levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and with serum PFOA at age 13 (Grandjean et al. 2017), respectively. Decreases in tetanus antibody levels at age 7 were associated with increases in serum PFOA levels at age 5, but not at age 7 (Grandjean et al. 2012; Mogensen et al. 2015a) and tetanus antibody levels were not associated with serum PFOA at age 7 or 13 (Grandjean et al. 2017). In studies comparing maternal serum PFOA with antibody levels in children, no associations were found for tetanus antibodies at age 3 (Granum et al. 2013), age 5 (Grandjean et al. 2012), or age 7 (Grandjean et al. 2012) or for diphtheria at age 5 or 7 (Grandjean et al. 2012). It is noted that Grandjean and associates also found an inverse association between serum polychlorinated biphenyls (PCBs) and serum antibody concentrations against tetanus and diphtheria in children living in the Faroe

Figure 2-21. Antibody Responses Relative to Serum PFOA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOA)



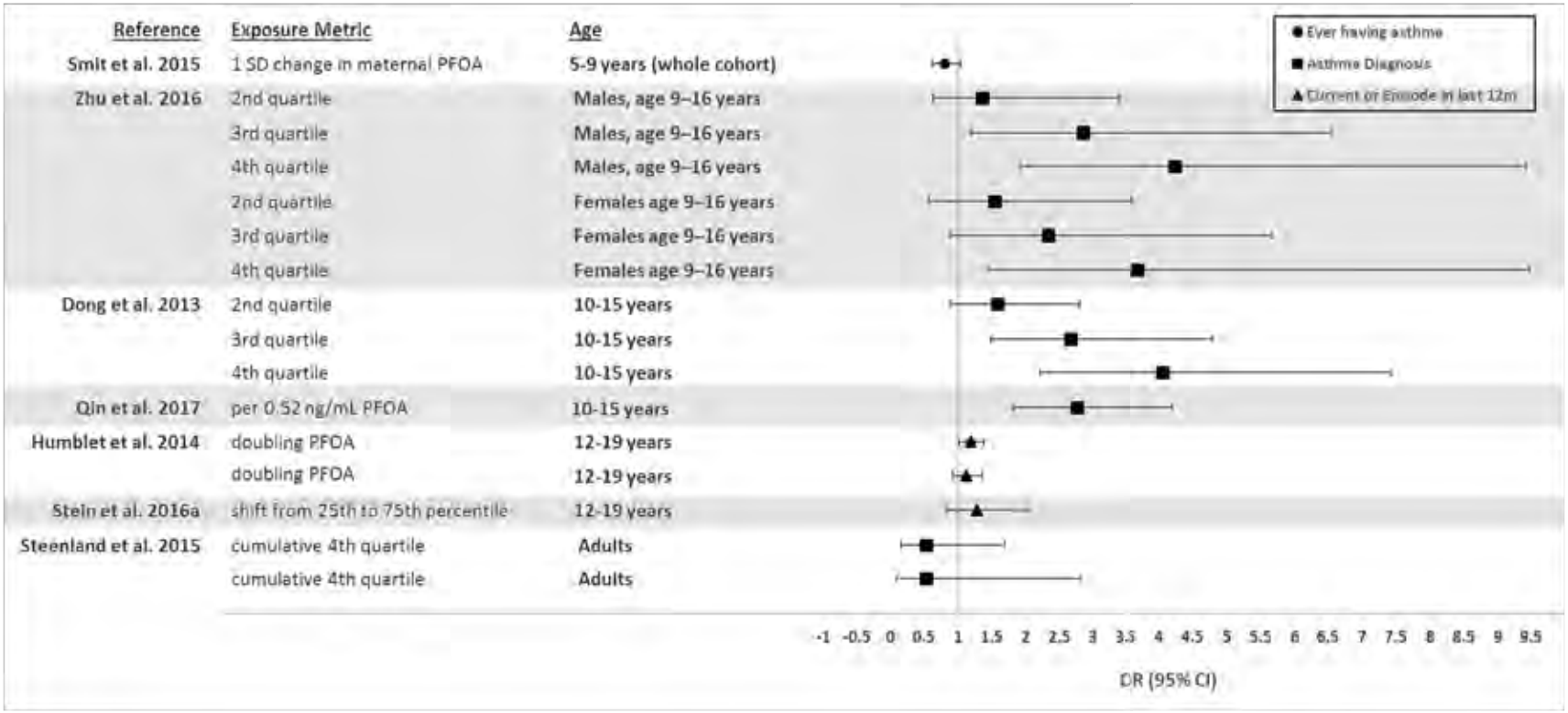
Islands (Heilmann et al. 2010). Statistically adjusting for PCB exposure (milk and serum PCB levels) did not alter the results (Grandjean et al. 2012). Lower levels of rubella antibodies at age 3 were associated with increasing maternal PFOA (Granum et al. 2013).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOA is associated with suppression of the antibody response based on the available human studies. NTP (2016b) also concluded that there is low confidence that exposure to PFOA is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Of the different types of hypersensitivity effects, the most widely studied endpoint is asthma; the possible association between exposure to PFOA and asthma has been studied in occupational, community, and general population studies. Several studies have found associations between current serum PFOA levels and diagnosis of asthma in children (Dong et al. 2013; Humblet et al. 2014; Qin et al. 2017) and adults (Anderson-Mahoney et al. 2008; Zhu et al. 2016). A case-control study found significantly higher serum PFOA levels in asthmatic adolescents as compared to adolescents without asthma (Zhou et al. 2017).

However, other studies have found no association between estimated cumulative serum PFOA levels and incidence of asthma being treated with medication in workers (Steenland et al. 2015) or asthma in the general population (Stein et al. 2016a). In children, no associations between maternal serum PFOA levels and asthma-related health outcomes were observed in 3-year-old children (Granum et al. 2013), 5–9-year-old children (Smit et al. 2015) or 1–10-year-old children (Impinen et al. 2018), or between current PFOA levels and current asthma in adolescents (Stein et al. 2016a). However, the Stein et al. (2016a) study did find an association with rhinitis in adolescents. No associations between maternal PFOA and wheezing were found in infants up to 18 months of age (Okada et al. 2012), infants 12 or 24 months of age (Okada et al. 2014), children 3 years of age (Granum et al. 2013), children 5–9 years of age (Smit et al. 2015), children 2–10 years old (Impinen et al. 2018), or between current serum PFOA levels and wheezing in adults (Stein et al. 2016a). The ORs for asthma diagnosis relative to serum PFOA levels are graphically presented in Figure 2-22; studies using different statistical methods are not included. No associations between maternal PFOA and prevalence of allergic diseases or wheezing were found in 4-year-old children (Goudarzi et al. 2016a). No associations between maternal PFOA and eczema were found in infants up to 18 months of age (Okada et al. 2012), children 3 years of age (Granum et al. 2013), or children 5–9 years of age (Smit et al. 2015). Similarly, no association was found between cord blood PFOA and atopic dermatitis in children 2 years of age (Wang et al. 2011).

Figure 2-22. Risk of Asthma Diagnosis Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



No associations were found between risks of allergy or allergic sensitization and current serum PFOA levels in adults (Stein et al. 2016a) or between cord PFOA in 2–10-year-old children (Impinen et al. 2018). Two studies examining the possible association between current serum PFOA levels in adults and food allergies have found mixed results, with one study finding an association (Buser and Scinicariello 2016) and one not finding an association (Stein et al. 2016a); a study in infants did not find an association between the risk of food allergy and maternal serum PFOA levels (Okada et al. 2012). It is noted that IgE levels, which were used to assess food allergies, is not a sensitive measure of clinical food allergy. No association was found for food sensitization (Buser and Scinicariello 2016).

Associations between serum PFOA and IgE, eosinophil counts, and eosinophil cationic protein levels were observed in asthmatic children (9–16 years of age), but not in non-asthmatic children (Dong et al. 2013; Zhu et al. 2016). Significantly higher IL-4 and IL-5 levels were observed in male children with asthma with the highest PFOA levels (Zhu et al. 2016). Two studies found associations between PFOA and IgE levels in infants. An inverse association was found between maternal PFOA and IgE levels in female infants but not in male infants (Okada et al. 2012), whereas Wang et al. (2011) found a correlation between cord blood PFOA and child IgE levels in males only or in males and females combined. A third study did not find an association between cord blood PFOA and IgE levels in infants (Ashley-Martin et al. 2015). NTP (2016b) concluded that there is low confidence that exposure to PFOA during childhood is associated with increased hypersensitivity responses.

Epidemiological Studies—Autoimmune Outcomes. There are limited data that can be used to evaluate the possible association between PFOA exposure and the risk of autoimmune diseases. Significant increases in the risk of ulcerative colitis were observed in an occupational exposure study (Steenland et al. 2015) and a C8 Science Panel study (Steenland et al. 2013). Although both studies found consistent results, it should be noted that the community study also included participants with occupational exposure to PFOA. The occupational study also found an association between PFOA exposure and rheumatoid arthritis; this was not observed in the community study. The community study (Steenland et al. 2013) also found no associations for other autoimmune diseases (Crohn's disease, Type I diabetes, lupus, and multiple sclerosis). A third study examined neural- and non-neural-specific antibodies and found no associations with cord blood PFOA or current serum PFOA in 7-year-old children (Osuna et al. 2014).

NTP (2016b) concluded that there is low confidence that exposure to PFOA is associated with ulcerative colitis.

Laboratory Animal Studies. The results of several mouse studies support the epidemiological data suggesting that exposure to PFOA can result in immunosuppression. Significant alterations in IgM levels in response to T-dependent antigens, such as sheep red blood cells (sRBCs) or horse red blood cells were observed in acute and intermediate oral mouse studies (DeWitt et al. 2008, 2009, 2016; Loveless et al. 2008; Yang et al. 2002a); the lowest-adverse-effect level was 3.75 mg/kg/day in mice exposed to PFOA in the drinking water for 15 days (DeWitt et al. 2008). Rats appear to be less sensitive than mice; no alterations in IgM levels were observed in rats administered PFOA via gavage for 28 days (Loveless et al. 2008). In a mouse developmental toxicity study, exposure to PFOA on GDs 6–17 was not associated with alterations in IgM or IgG levels in the offspring (Hu et al. 2010). Limited data suggest that alterations in NK cells or delayed type hypersensitivity are not sensitive endpoints for PFOA in laboratory animals. Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the numbers of T cells, NK cells, or helper T cells (Iwai and Yamashita 2006), and tests for delayed-type hypersensitivity response in mice challenged with bovine serum albumin following exposure to 30 mg/kg/day PFOA via drinking water for 15 days were negative (DeWitt et al. 2008).

Two studies have evaluated hypersensitivity in mice. Application of ≥18.8 mg/kg/day PFOA to the dorsal surface of the ears of mice and subsequently injected with ovalbumin resulted in a significant increase in serum total IgE compared to mice exposed only to ovalbumin (Fairley et al. 2007). Ovalbumin-specific airway hyperreactivity also increased in mice co-exposed to ovalbumin and 25 mg/kg PFOA relative to mice exposed to ovalbumin alone. The investigators suggested that PFOA exposure may increase the IgE response to environmental allergens (Fairley et al. 2007). In contrast to the results of the dermal study, no increases in airway hyperresponsiveness were observed in ovalbumin-sensitized mice exposed *in utero* and post-weaning to PFOA in the diet (Ryu et al. 2014). In nonsensitized mice, PFOA did induce airway hyperresponsiveness in 12-week-old pups.

Numerous studies have evaluated secondary outcomes in monkeys, rats, and mice. In the spleen and thymus, exposure to PFOA resulted in decreases in organ weight, decreases in the number of cells, and/or atrophy (DeWitt et al. 2008; Loveless et al. 2008; Qazi et al. 2009a, 2012; Son et al. 2009; Yang et al. 2000, 2001, 2002b). Acute exposure resulted in decreases in absolute thymus weight at 11.5 mg/kg/day (Yang et al. 2001), decreases in spleen weight at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000), and severe thymic atrophy at 30 mg/kg/day (Qazi et al. 2012; Yang et al. 2000). Exposure of male rats to 50 mg/kg/day PFOA by gavage for 14 days did not significantly affect the absolute or relative spleen weight nor did it alter lymphocyte subsets (Iwai and Yamashita 2006).

Decreases in relative spleen weight were observed at ≥0.96 mg/kg/day PFOA, and absolute spleen weight and absolute and relative thymus weights were decreased at 9.6 and 29 mg/kg/day (Loveless et al. 2008). The lowest-adverse-effect levels for spleen and thymus weight changes identified in mouse intermediate studies were 3.75 mg/kg/day PFOA for decreases in absolute spleen weight (DeWitt et al. 2008) and 9.6 mg/kg/day for decreases in absolute and relative thymus weight (Loveless et al. 2008). In rats, no alterations in spleen weight were observed following chronic exposure to 15 mg/kg/day in the diet (3M 1983; Butenhoff et al. 2012c).

Decreases in the number of splenic and thymic lymphocytes were observed in mice administered via gavage ≥9.6 mg/kg/day PFOA for 28 days (Loveless et al. 2008). In contrast, administration of 29 mg/kg/day PFOA by gavage for 28 days did not result in alterations in the number of splenic or thymic lymphocytes in rats (Loveless et al. 2008). A 10-day exposure of mice to 3.0 mg/kg/day PFOA resulted in decreases in the number of bone marrow B-lymphoid cells (Qazi et al. 2012); a decrease in bone marrow myeloid cells was also observed at 30 mg/kg/day. Examination of the B-lymphoid cell subpopulations showed decreases in pro/pre B cells, immature B cells, and early mature B cells, with the greatest reductions observed for pro/pre B cells. When mice were allowed to recover for 10 days following a 10-day exposure to 30 mg/kg/day PFOA in the diet, only a partial recovery of B-lymphoid cells was observed. Significant increases in CD4-CD8- and CD4-CD8+ thymic lymphocytes were observed in mice exposed to 47.21 mg/kg/day for 21 days; increases in CD4+CD8+ lymphocytes were observed at 17.63 and 47.21 mg/kg/day (Son et al. 2009). Similarly, there were decreases in splenic CD4-CD8- lymphocytes at 47.21 mg/kg/day and CD4-CD8+ lymphocytes at ≥0.49 mg/kg/day and increases in splenic CD4+CD8- lymphocytes at 17.63 and 47.21 mg/kg/day.

Two studies examined the immune response to mitogens in mice exposed to PFOA. Marked decreases in total leukocytes, lymphocytes, and neutrophils levels and increases in tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were observed in the peritoneal cavity, bone marrow, and spleen cells in response to lipopolysaccharide (LPS) stimulation in mice exposed to approximately 40 mg/kg/day PFOA for 10 days (Qazi et al. 2009a). Exposure of splenic lymphocytes isolated from PFOA-exposed mice to concavalin A (ConA) or LPS resulted in decreases in lymphocyte proliferation (Yang et al. 2002a).

A number of studies have evaluated the potential of PFOA to induce histological alterations in immune organs. In monkeys, administration of approximately 20 mg/kg/day PFOA administered via a capsule to Cynomolgus monkeys for 4 or 26 weeks did not affect the gross or microscopic morphology of the spleen (Butenhoff et al. 2002; Thomford 2001). Administration via gavage of 30 mg/kg/day PFOA to Rhesus

monkeys for 90 days induced atrophy of lymphoid follicles in the spleen and lymph nodes and slight to moderate hypocellularity of the bone marrow (Griffith and Long 1980). No histological alterations were observed in the spleen or thymus of rats exposed intermittently to ≤84 mg/m^3 APFO dusts for 2 weeks (Kennedy et al. 1986), ≤29 mg/kg/day administered via gavage for 28 days (Loveless et al. 2008), or dermal doses of ≤2000 mg/kg/day for 2 weeks (Kennedy 1985) or in the spleen and mesenteric lymph nodes of rats exposed to ≤110 mg/kg/day PFOA in the diet for 90 days (Griffith and Long 1980) or ≤15 mg/kg/day PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c).

Studies in wild-type mice and PPARα-null mice demonstrate that PFOA-induced immunomodulation results from PPARα-dependent and -independent mechanisms (DeWitt et al. 2016; Yang et al. 2002b). Exposure to 30 or 33 mg/kg/day PFOA resulted in decreases in spleen weight, thymus weight, number of splenic lymphocytes, number of thymic lymphocytes, and CD4+ and CD8+ splenic and thymic lymphocytes in wild-type mice. Similar exposures of PPARα knockout mice did not result in alterations in spleen weight, number of splenic lymphocytes, or their phenotypes. Although decreases in thymus weight, number of thymic lymphocytes, and their phenotypes were observed in the knockout mice, the magnitudes of the changes were lower in the knockout mice than in the wild-type mice. However, similar responses were observed in T-cell-dependent antibody responses. Exposure to 30 mg/kg/day PFOA resulted in 16 and 14% decreases in the response to sRBCs in wild-type and knockout mice, respectively (DeWitt et al. 2016).

In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOA is associated with suppression of the antibody response, very low confidence that PFOA is associated with the ability to respond to infectious disease, and moderate confidence that PFOA is associated with increased hypersensitivity.

Summary. Epidemiological studies have evaluated several aspects of immunotoxicity including immunosuppression, hypersensitivity, and autoimmunity. A number of general population studies have found significant inverse associations between serum PFOA levels and antibody responses to vaccines. However, no consistent associations were found between serum PFOA and disease resistance, as measured by episodes of the common cold, cough, fever, or hospitalization for infectious disease. In tests of hypersensitivity, there is some evidence of an association between serum PFOA and asthma diagnosis in children and adults, although this finding was not consistent across studies; increased risk of allergy or allergic sensitization does not appear to be associated with serum PFOA. Based on the findings of an occupational exposure and community exposure study, there is some suggestive association between

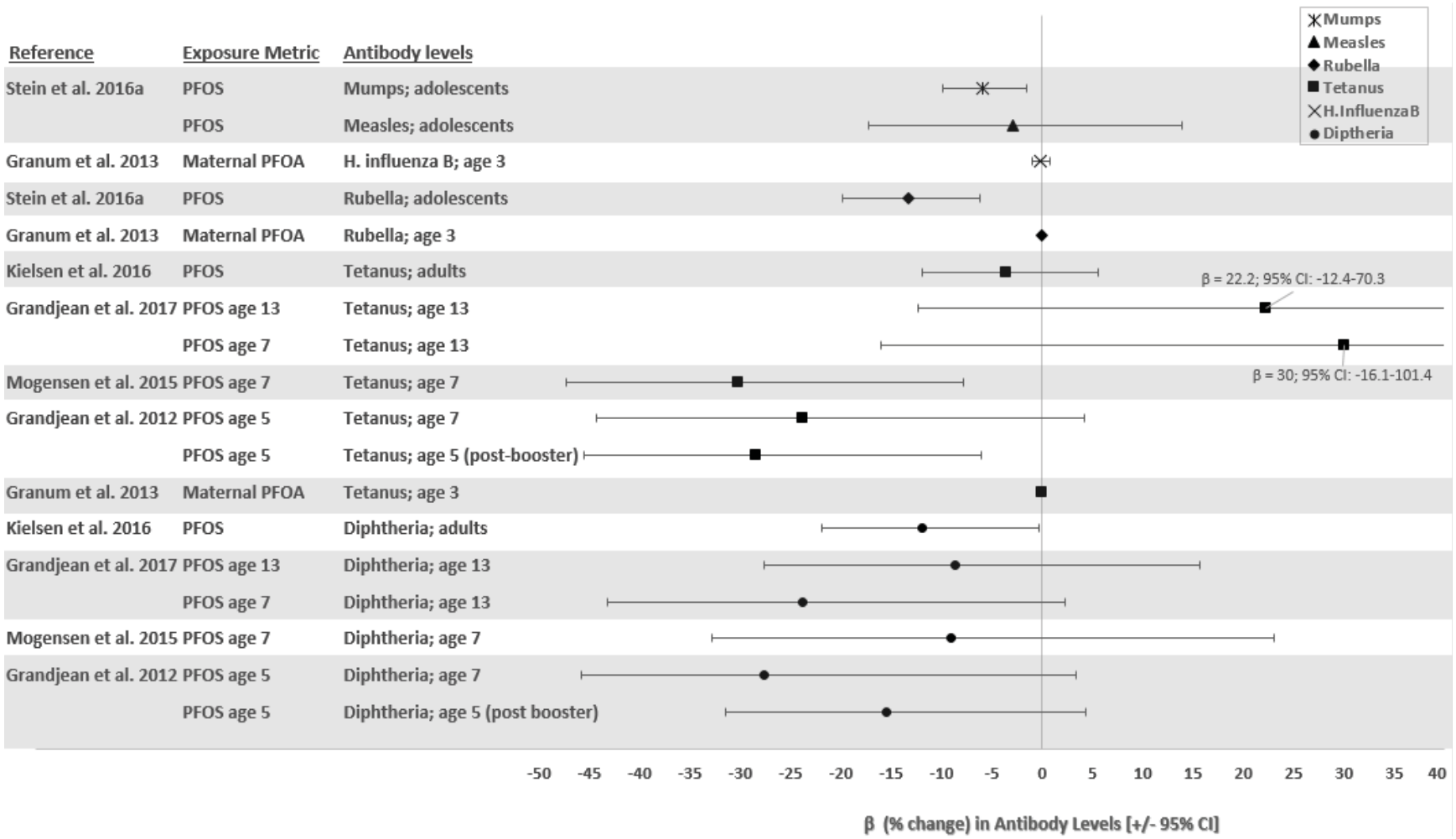
serum PFOA and an increased risk of ulcerative colitis, but not for other autoimmune diseases. Animal studies suggest that the immune system is a sensitive target of PFOA toxicity. A number of studies in mice have demonstrated evidence of immunosuppression and increased hypersensitivity. Laboratory animal studies have also found secondary immune outcomes in the spleen and thymus, which included decreases in organ weight and decreases in the number of lymphocytes.

PFOS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have evaluated the potential of PFOS to cause immunosuppression. In studies that evaluated infectious disease resistance, no alterations in the risk of otitis media were observed in infants monitored through 18 months or 3 years of age (Granum et al. 2013; Okada et al. 2012), common cold or other upper respiratory infections (Granum et al. 2013), gastroenteritis with vomiting or diarrhea (Granum et al. 2013), hospitalizations due to infectious diseases in children (Fei et al. 2010), or symptoms of infection such as nasal discharge, cough, diarrhea, or vomiting in children (Dalsager et al. 2016). In contrast, other studies have found associations between PFOS and infectious diseases. Associations between the number of days with symptoms of infection and maternal PFOS levels were observed in children (Dalsager et al. 2016) and between maternal serum PFOS and the risk of total infectious disease in early life (age 4 years) (Goudarzi et al. 2017). Associations were also found between cord PFOS levels and the number of common colds from 0 to 2 years of age and the number of lower respiratory tract infections between 0 and 10 years of age (Impinen et al. 2018).

Other studies evaluating immunosuppression found significant alterations in the response to vaccines; the changes in the response to antibody levels relative to serum PFOS levels are graphically presented in Figure 2-23; studies utilizing different statistical methods are not included in this figure. In children receiving a tetanus vaccination at age 5, there were associations between serum PFOS levels at age 5 and tetanus antibody levels at age 5 (Grandjean et al. 2012) and between serum PFOS levels at age 7 and tetanus antibody levels at age 7 when the analysis was restricted to children who were not likely to have had a booster vaccine after age 5 (Grandjean and Budtz-Jorgensen 2013). However, no associations were found between tetanus antibody levels at age 5 and maternal PFOS or child PFOS levels (Grandjean et al. 2012), between tetanus antibody levels at age 7 and maternal PFOS or child PFOS levels at age 5 or 7 years (Grandjean et al. 2012; Mogensen et al. 2015a), or between tetanus antibody levels at age 14 and child PFOS levels at age 13 (Grandjean et al. 2017). Similarly, diphtheria antibody levels at age 7 were

Figure 2-23. Antibody Responses Relative to Serum PFOS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFOS)

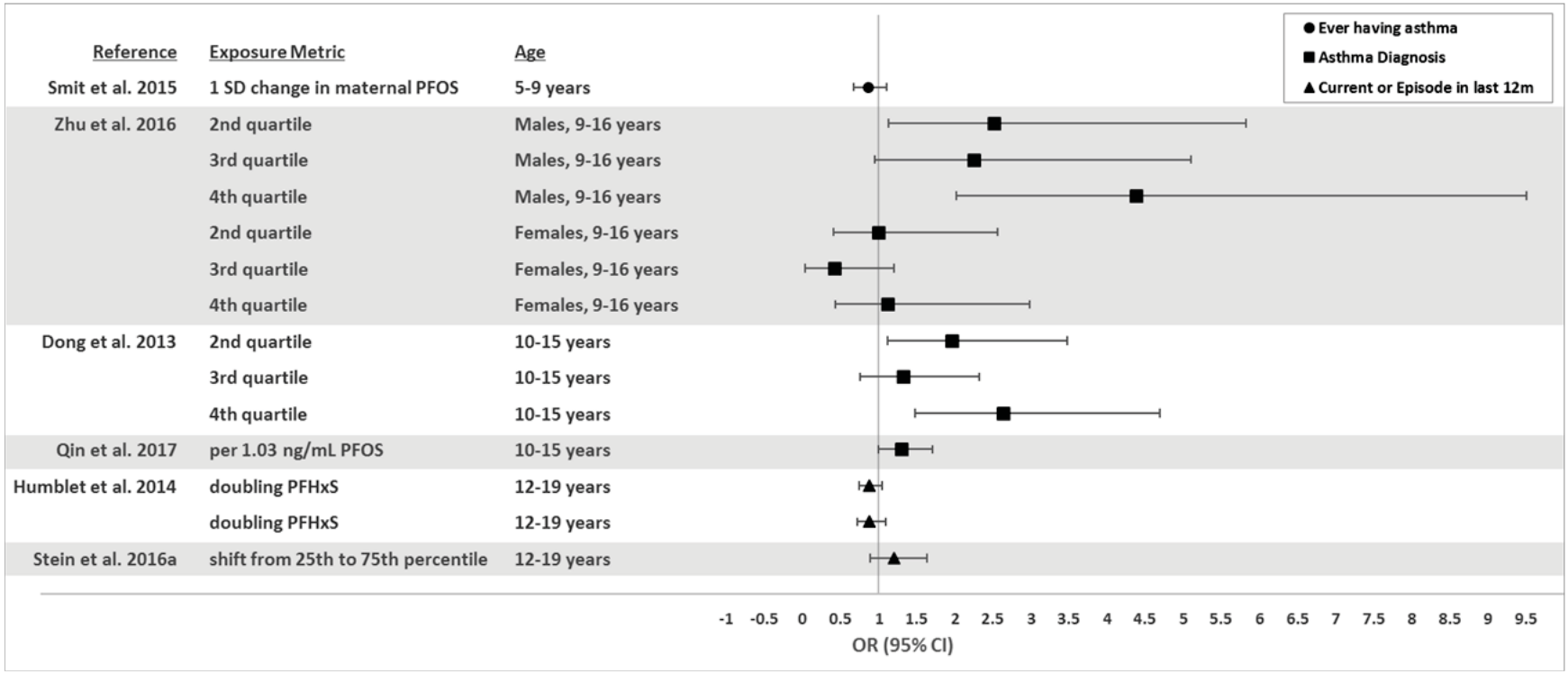


significantly associated with serum PFOS levels at age 5 and 7 (Grandjean et al. 2012; Mogensen et al. 2015a), but antibody levels at age 5 were not associated with maternal PFOS or child PFOS at age 5 years (Grandjean et al. 2012) and antibody levels at age 13 were not associated with child PFOS levels at age 7 or 13 years (Grandjean et al. 2017). In another study of children (Granum et al. 2013), decreased rubella antibody levels were associated with higher maternal PFOS levels, but no associations were found for tetanus or Haemophilus influenza type B antibodies. In adolescents, recent serum PFOS levels were inversely associated with mumps and rubella antibody levels, but not with measles antibody levels (Stein et al. 2016a). In studies in adults, recent PFOS levels were inversely associated with diphtheria antibody levels 30 days after booster administration (Kielsen et al. 2016), but not with tetanus antibody levels 30 days after booster administration (Kielsen et al. 2016) or influenza types A H3N2, A H1N1, or B antibody levels 21 days post-vaccination (Looker et al. 2014).

NTP (2016b) concluded that there is moderate confidence that exposure to PFOS is associated with suppression of the antibody response and that there is low confidence that exposure to PFOS is associated with increased incidence of infectious disease (or lower ability to resist or respond to infectious disease).

Epidemiological Studies—Hypersensitivity Outcomes. Several studies examined the risk of hypersensitivity associated with serum PFOS in children and adolescents; however, the results are inconsistent. In three case-control studies, increased risks of asthma were observed. Qin et al. (2017) reported increased risk of asthma in children associated with serum PFOS levels. Dong et al. (2013) reported an increased risk of asthma diagnosis and increased severity of asthma episodes in children with PFOS levels in the 4th quartile. Zhu et al. (2016) also reported an association between asthma diagnosis and serum PFOS levels in the 4th quartile; however, the association was only significant in males. A third case-control study found significantly elevated serum PFOS levels in asthmatic adolescents (Zhu et al. 2016). Prospective and cross-sectional studies in children (Granum et al. 2013) did not find an association between maternal PFOS levels and the risk of asthma diagnosis in 3-year-old children; between cord PFOS and asthma diagnosis, current asthma, or ever having asthma in 2–10-year-old children (Impinen et al. 2018); or between maternal PFOS and asthma diagnosis in adolescents (Humblet et al. 2014; Stein et al. 2016a). Data evaluating associations between serum PFOS and the risk of asthma diagnosis are presented in Figure 2-24.

Figure 2-24. Risk of Asthma Diagnosis Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



No associations between maternal PFOS or cord PFOS and eczema, atopic dermatitis, or wheezing or total allergic diseases have been found in children (Goudarzi et al. 2016a; Granum et al. 2013; Impinen et al. 2018; Okada et al. 2012, 2014; Smit et al. 2015; Wang et al. 2011). Similarly, no associations between recent serum PFOS levels in adolescents and food allergies or sensitizations (Buser and Scinicariello 2016; Stein et al. 2016a) or maternal PFOS levels and food allergies in infants (Okada et al. 2012) were observed. However, in a cross-sectional study of adolescents, recent PFOS levels were associated with mold allergies and inversely associated with the risk of plant or cockroach or shrimp allergies (Stein et al. 2016a). In related studies, cord blood PFOS levels were associated with an increase in cord IgE levels, but not in infant serum IgE levels (Wang et al. 2011). Two other studies did not find associations between maternal PFOS levels and cord IgE levels (Ashley-Martin et al. 2015; Okada et al. 2012).

NTP (2016b) concluded that there is very low confidence that exposure to PFOS is associated with changes in the hypersensitivity response in children.

Laboratory Animal Studies. A limited number of laboratory animal studies examined PFOS-induced immunosuppression. Guruge et al. (2009) reported an impaired response to an influenza A virus challenge in mice administered 0.025 mg/kg/day PFOS via gavage for 21 days (Guruge et al. 2009). Several studies have found an impaired response to sRBCs (Dong et al. 2009, 2011; Peden-Adams et al. 2008); however, decreases in NK cell activity were observed at higher doses (0.83–2.08 mg/kg/day) (Dong et al. 2009). Qazi et al. (2009a) reported several alterations in parameters associated with the innate immune system in mice exposed to approximately 40 mg/kg/day PFOS in the diet for 10 days. These alterations included marked decreases in total leukocyte and lymphocyte levels and increases in TNF-α and IL-6 levels in the peritoneal cavity and bone marrow in response to LPS stimulation; no alterations were observed in mice exposed to a 20-fold lower dose. As discussed in Section 2.17, a developmental toxicity study (Keil et al. 2008) found an altered response to sRBCs in mice exposed to PFOS *in utero*.

No alterations in spleen or thymus weights were observed in mice exposed to 0.025 mg/kg/day PFOS (Guruge et al. 2009); at a higher dose (0.42 mg/kg/day), significant decreases in relative spleen and thymus weights were observed (Dong et al. 2009; Zheng et al. 2009). Decreases in splenic and thymic cellularity were also observed at ≥0.42 mg/kg/day PFOS (Dong et al. 2009; Qazi et al. 2009b, 2012; Zheng et al. 2009). Bone marrow cells (B-lymphoid and myeloid cells) were also significantly decreased in mice exposed to 30 mg/kg/day PFOS for 10 days (Qazi et al. 2012). Within the B-lymphoid cell population, there were decreases in the number of pro/pre B cells and immature cells (Qazi et al. 2012).

Significant alterations in all splenic T cell CD4 and CD8 subpopulations were observed at ≥0.00331 mg/kg/day PFOS (Peden-Adams et al. 2008) and thymic lymphocyte phenotypes were altered at 0.42 mg/kg/day PFOS (Dong et al. 2009).

Rats treated with 1.77 mg/kg/day PFOS for 4 weeks, 6.34 mg/kg/day for 28 days, 1.56 mg/kg/day for 14 weeks, or 1.04 mg/kg/day for 2 years did not show significant morphological alterations in the spleen, thymus, or mesenteric lymph nodes (Butenhoff et al. 2012b; Lefebvre et al. 2008; Seacat et al. 2003; Thomford 2002b). Similar findings were reported in Cynomolgus monkeys dosed with up to 2 mg/kg/day for 4 weeks or up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002; Thomford 2002a).

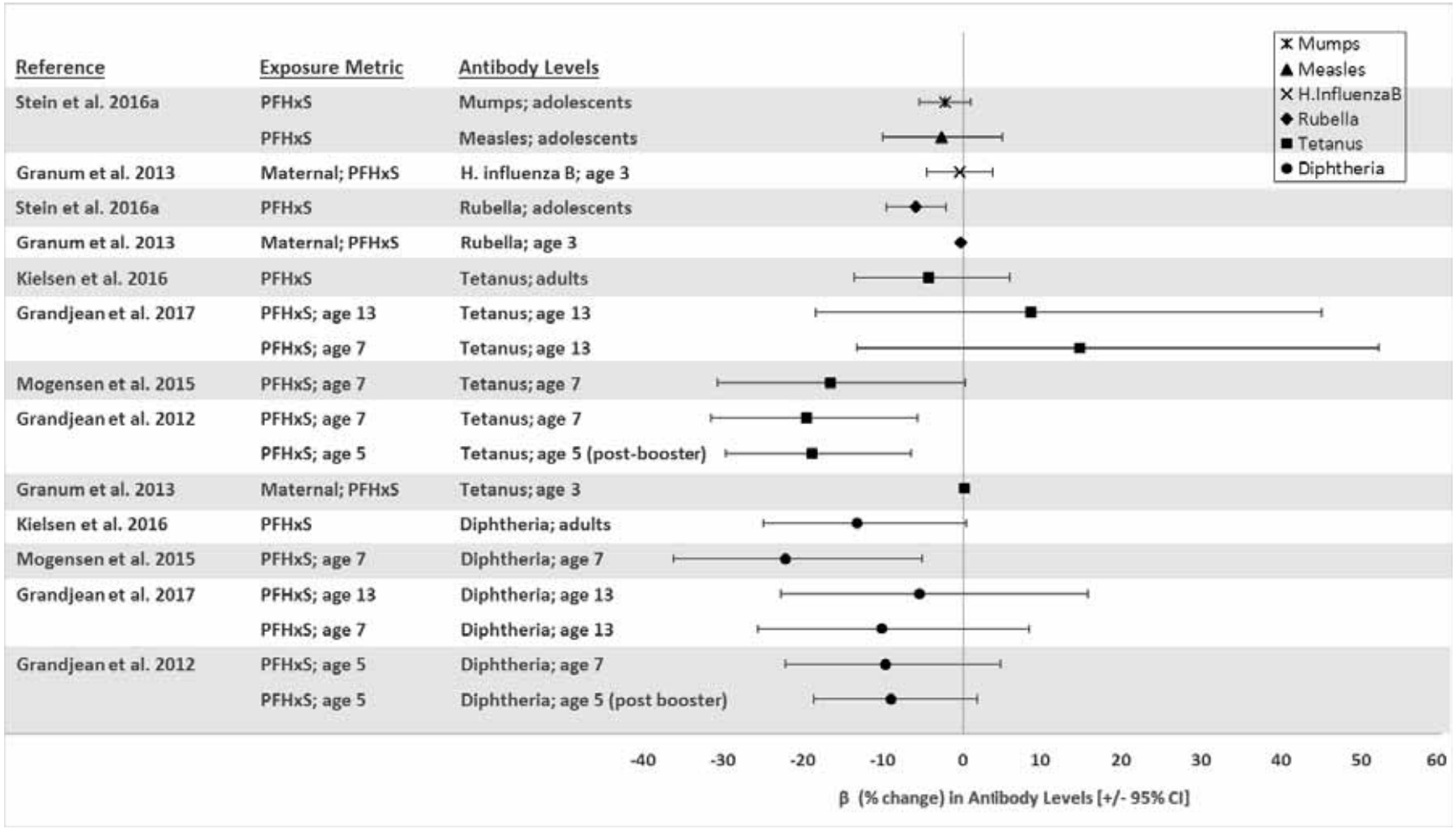
In a systematic review of the available laboratory animal data, NTP (2016b) concluded that there is high confidence that exposure to PFOS is associated with suppression of the antibody response, moderate confidence that PFOS is associated with the ability to respond to infectious disease, and low confidence that PFOS is associated with increased hypersensitivity.

Summary. A number of epidemiological studies have examined the potential immunotoxicity of PFOS. The database provides convincing evidence of an association between serum PFOS levels and immunosuppression, particularly impaired antibody responses to vaccines in adults and children. Mixed results have been observed in studies evaluating infectious disease resistance. Similarly, inconsistent results have been examined in studies evaluating associations between serum PFOS and hypersensitivity outcomes, such as asthma; no associations were found for eczema, dermatitis, food allergies/ sensitizations. Laboratory animal studies, particularly studies in mice, provide strong evidence of the immunotoxicity of PFOS. The strongest evidence comes from studies reporting impaired antibody responses resulting from oral exposure to relatively low doses of PFOS. Other immune effects include decreased response to infectious disease, decreases in spleen and thymus weights, and decreases in splenic and thymic cellularity and bone marrow cells.

PFHxS

Epidemiological Studies—Immunosuppression Outcomes. Several epidemiological studies have examined the potential of PFHxS to suppress the immune system. Altered antibody responses relative to serum PFHxS levels are graphically presented in Figure 2-25. Inverse associations were observed between tetanus antibody levels in 5- and 7-year-old children and serum PFHxS levels at age 5 or 7 years

Figure 2-25. Antibody Responses Relative to Serum PFHxS Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFHxS)

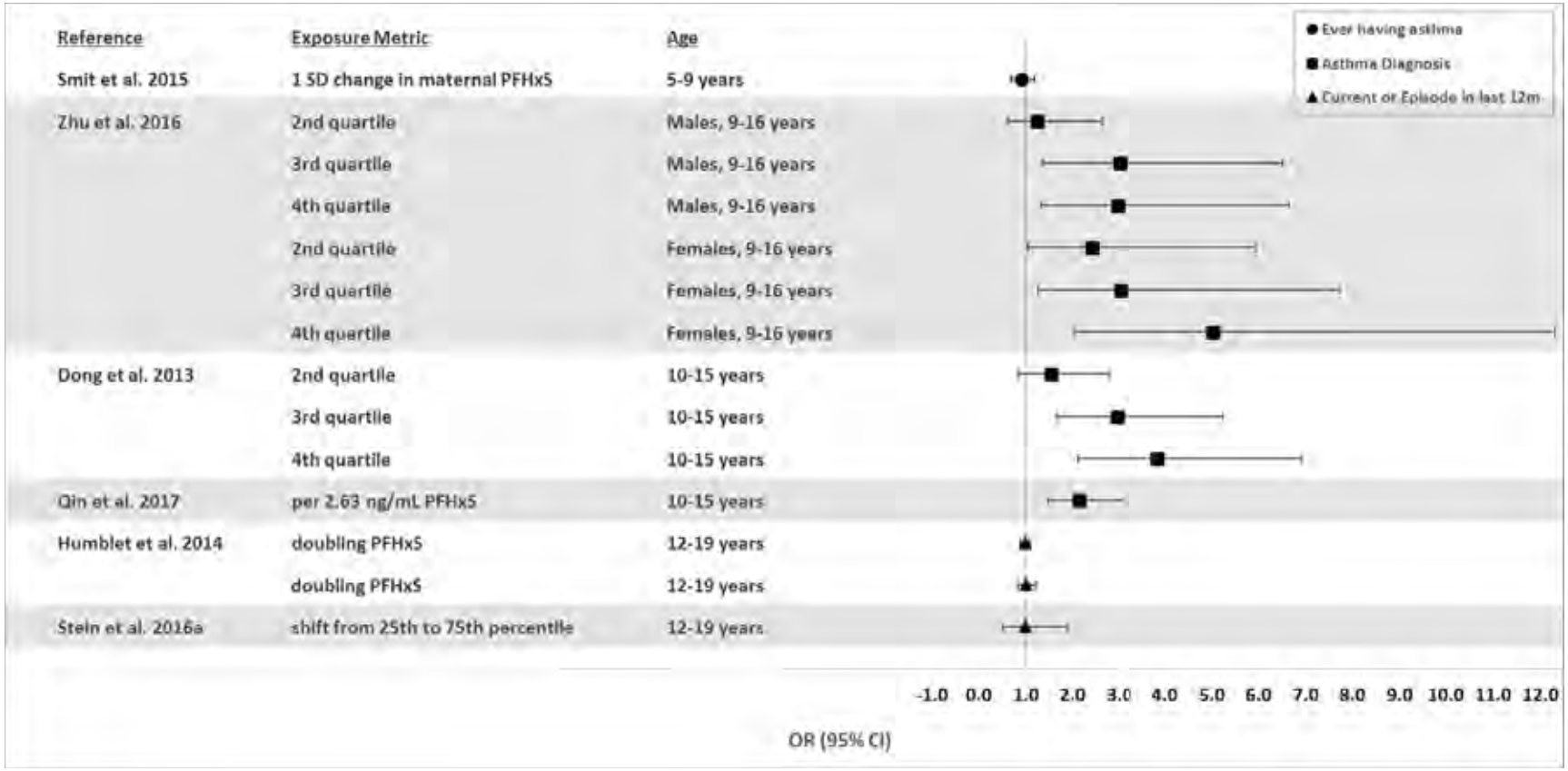


(Grandjean et al. 2012; Mogensen et al. 2015a); but there were no associations between serum PFHxS levels at age 7 or 13 and tetanus antibody levels at age 13 (Grandjean et al. 2017). No associations were found between maternal PFHxS levels and tetanus antibody levels in the children. These studies found no associations between diphtheria antibody levels at ages 5, 7, or 13 and serum PFHxS levels in the mother or in the children. A study in 3-year-old children found an inverse association between maternal PFHxS levels and rubella antibody levels, but no association with influenza type B or tetanus antibody levels (Granum et al. 2013). In adolescents, serum PFHxS levels were also inversely associated with rubella antibody titers in a seropositive subcohort (Stein et al. 2016a); no associations were found for measles or mumps antibody titers. Another study in adolescents did not find associations between recent serum PFHxS levels and tetanus or diphtheria antibody levels (Kielsen et al. 2016). A study in adults did not find associations between PFHxS levels and response to influenza vaccine; some alterations in serum cytokine levels were observed, but chemokine and IgA levels were not altered (Stein et al. 2016b).

In general, the available studies do not suggest an association between serum PFHxS and decreased infectious disease resistance. No alterations in the frequency of fever, cough, nasal discharge, otitis media, diarrhea, or vomiting were observed in children (Dalsager et al. 2016; Granum et al. 2013). Cord PFHxS levels were not associated with increased prevalence of common colds in children 0–2 years of age or lower respiratory tract infections in children 0–10 years of age (Impinen et al. 2018). No association between maternal PFHxS levels and total infectious disease prevalence was found in children up to the age of 4 years (Goudarzi et al. 2017); however, when boys and girls were analyzed separately, an association was found in girls. An association between maternal PFHxS levels and the number of episodes of gastroenteritis was found in children (Granum et al. 2013).

Epidemiological Studies—Hypersensitivity Outcomes. Data evaluating associations between serum PFHxS and the risk of asthma diagnosis are presented in Figure 2-26. No associations were observed between asthma diagnosis, wheezing, and/or eczema or total allergic diseases in children and maternal serum PFHxS levels (Goudarzi et al. 2016a; Granum et al. 2013; Smit et al. 2015) or with recent PFHxS levels in adolescents (Humblet et al. 2014; Okada et al. 2014). In contrast, case-control studies in asthmatic children did find associations between recent PFHxS serum levels and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013). Another case-control study found significantly elevated serum PFHxS levels in adolescents with asthma (Zhu et al. 2016). Dong et al. (2013) also reported associations between serum PFHxS levels and

Figure 2-26. Risk of Asthma Diagnosis Relative to PFHxS Levels (Presented as Adjusted Odds Ratios)



eosinophil counts and eosinophil cationic protein levels in asthmatic children, but not in non-asthmatics. No associations were found with IgE levels in either case-control study (Dong et al. 2013; Zhu et al. 2016) or in a study measuring cord blood IgE (Ashley-Martin et al. 2015).

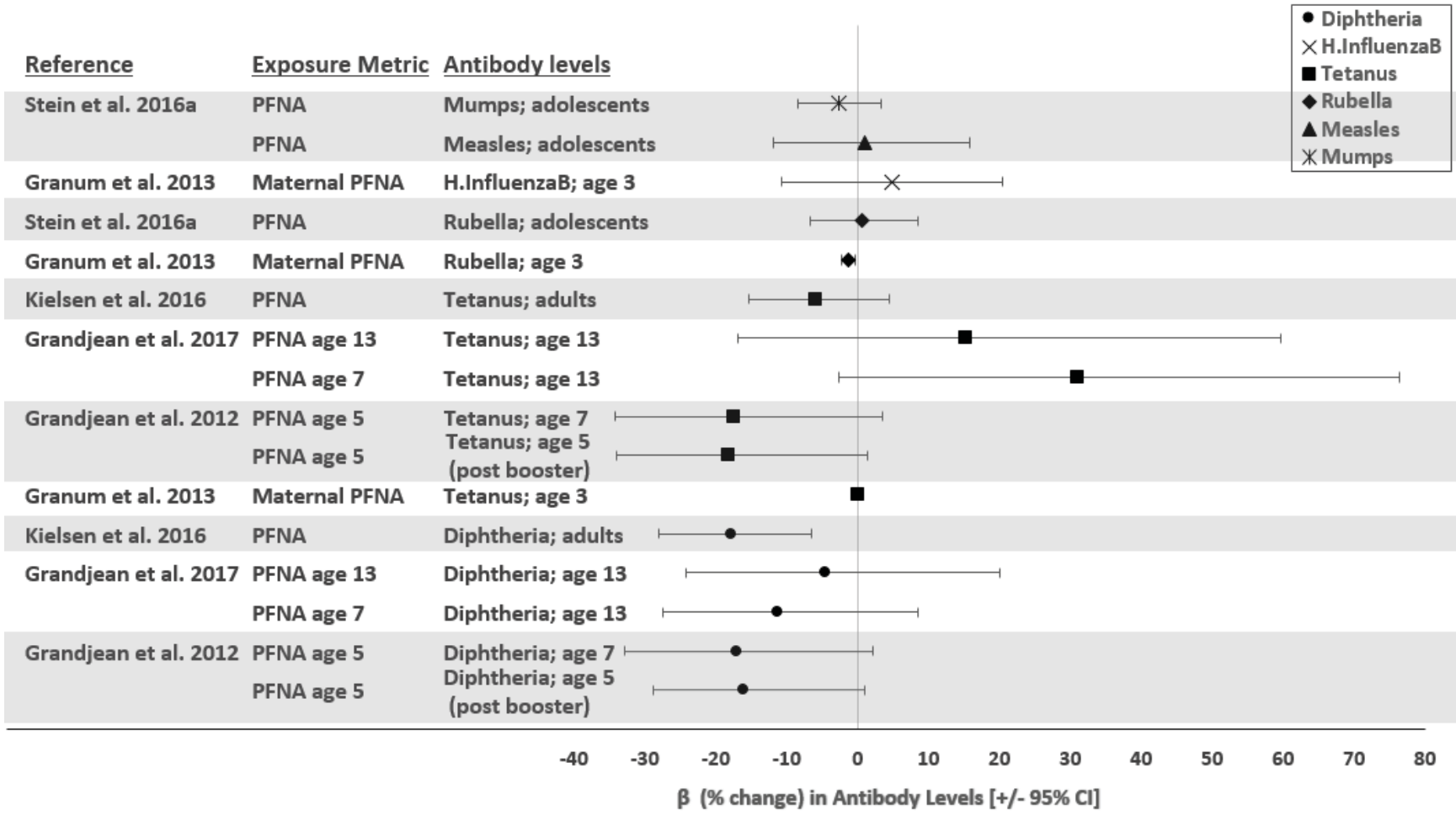
An increased risk of food allergies associated with serum PFHxS levels, but not increased sensitivity to foods, was found in adolescents (Buser and Scinicariello 2016). Another study found no associations between serum PFHxS levels and allergic sensitization to plants, dust mites, pets, cockroaches/shrimp, rodents, mold, or food in adolescents (Stein et al. 2016a).

Laboratory Animal Studies. In the only available study evaluating immunotoxicity for PFHxS, Butenhoff et al. (2009a) did not find histological alterations in the spleen, thymus, or lymph nodes of rats administered 10 mg/kg/day PFHxS via gavage for 42–56 days.

PFNA

Epidemiological Studies—Immunosuppression Outcomes. Most studies examining a possible association between serum PFNA levels and immunosuppression have not found associations. No associations were found between maternal or child PFNA levels and tetanus antibody levels at ages 3, 5, 7, or 13 (Grandjean et al. 2012, 2017; Granum et al. 2013) or in adults (Kielsen et al. 2016). Some studies have found associations between serum PFNA and diphtheria antibody levels, but the results were not consistent. Grandjean and associates found a significant inverse association between diphtheria antibodies levels at age 5 (Grandjean et al. 2012) and serum PFNA levels at age 5, but not for antibody levels at age 13 and PFNA levels at age 7 or 13 (Grandjean et al. 2017). Kielsen et al. (2016) also reported an inverse association (unadjusted for potential confounders) between serum PFNA and diphtheria antibody levels in a small study of adults. An inverse association between maternal serum PFNA and rubella antibody levels was observed in children (Granum et al. 2013), but there was no association for influenza type B antibody levels. Similarly, no associations were found between recent PFNA serum levels and measles, mumps, or rubella antibody titers in adolescents (Stein et al. 2016a). Data evaluating associations between serum PFNA and altered antibody response are presented in Figure 2-27.

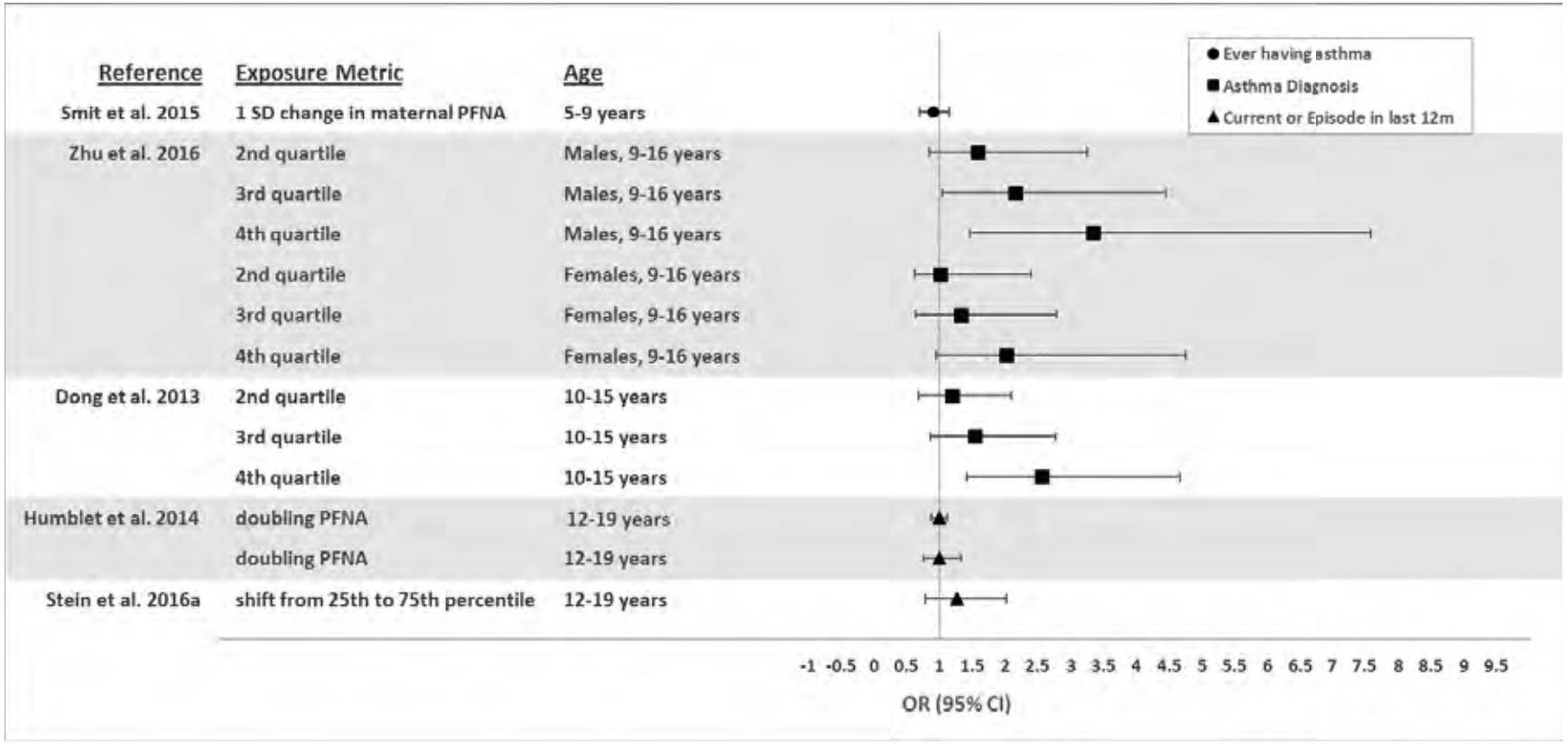
Figure 2-27. Antibody Responses Relative to Serum PFNA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFNA)



The epidemiological data provide mixed results on whether there are associations between decreased infectious disease resistance and PFNA levels. No alterations in the risk of increased number of days with fever, cough, nasal discharge, diarrhea, or vomiting were observed in children (Dalsager et al. 2016), although the study did find a significant increase in the number of days above the median for nasal discharge. In a prospective study of children to the age of 4 years, no associations between maternal PFNA levels and prevalence of total infectious diseases were found (Goudarzi et al. 2017). Another study found that the number of episodes of the common cold in children was associated with maternal serum PFNA; no associations were found for otitis media or gastroenteritis (Granum et al. 2013). No associations between cord PFNA levels and the prevalence of common colds were found in children up to 2 years of age (Impinen et al. 2018), but cord PFNA levels were positively associated with the prevalence of lower respiratory infections in children up to the age of 10 years (Impinen et al. 2018).

Epidemiological Studies—Hypersensitivity Outcomes. Case-control studies of asthmatic children have reported associations between serum PFNA and asthma diagnosis (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016), but no association with asthma severity (Dong et al. 2013); another study found significantly higher serum PFNA levels in adolescents with asthma (Zhu et al. 2016). However, cross-sectional or retrospective studies (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a) have not found associations. A prospective study of children to the age of 10, did not find associations between cord PFNA levels and current asthma, ever having asthma, asthma diagnosis, or wheezing (Impinen et al. 2018). Data evaluating associations between serum PFNA and the risk of asthma diagnosis are presented in Figure 2-28. Another study found no associations between maternal PFNA levels and prevalence of total allergic diseases or wheezing (Goudarzi et al. 2016a). No associations were found in adolescents between PFNA and food allergies (Buser and Scinicariello 2016), allergies (Stein et al. 2016a), or allergic sensitizations to plants, dust mites, pets, cockroach/shrimp, rodents, mold, or food (Stein et al. 2016a). However, inverse associations between serum PFNA and food sensitizations were observed in adolescents (Buser and Scinicariello 2016) and between maternal serum PFNA and allergic diseases in infants (Okada et al. 2014). No increases in the risk of other hypersensitivity effects (wheezing, eczema, or atopic dermatitis) were observed (Humblet et al. 2014; Okada et al. 2014; Smit et al. 2015; Stein et al. 2016a; Wang et al. 2011).

Figure 2-28. Risk of Asthma Diagnosis Relative to PFNA Levels (Presented as Adjusted Odds Ratios)



Laboratory Animal Studies. Administration of PFNA for 14 days resulted in decreases in thymus and/or spleen weights at ≥3 mg/kg/day in rats and mice (Fang et al. 2008, 2009, 2010); at 1 mg/kg/day, an increase in thymus weight was observed in rats (Fang et al. 2009). Fang et al. (2009) reported increases in the ratio of thymic cortex to medulla in rats presumably administered ≥3 mg/kg/day PFNA. In the spleen, there were decreases in the percentage of F4/80+ and CD49b+ cells at ≥1 mg/kg/day and in CD11c+ cells at ≥3 mg/kg/day (Fang et al. 2008). Increases in pro-inflammatory cytokines were observed in the serum at ≥3 mg/kg/day (Fang et al. 2009) and spleen at 5 mg/kg/day (Fang et al. 2010).

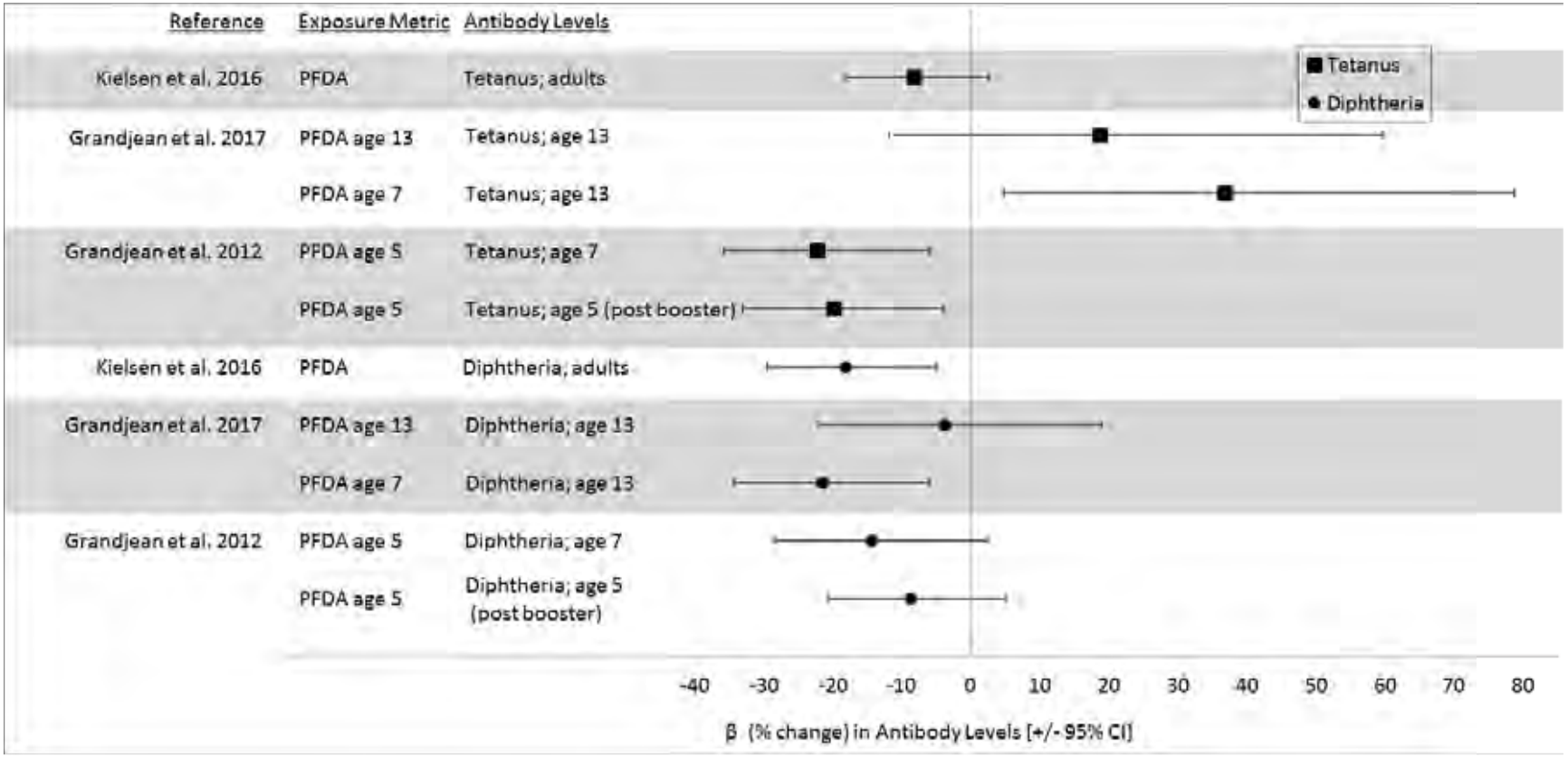
No alterations were observed in the response of splenic T lymphocytes to ConA at 5 mg/kg/day (Fang et al. 2008).

Two weeks after a single intraperitoneal administration of 46 mg/kg PFNA to male and female B57BL/6J mice, a number of immunological alterations included significant decreases in relative spleen weight and splenic leukocyte counts, alterations in splenic T-lymphocyte phenotypes (increased ratios of CD4+ and CD8+ cells), a decrease in viable thymic cells, a marked decrease in CD4+CD8+ thymic lymphocytes, and an increase in CD4+ and CD8+ thymic lymphocytes, and increased levels of tumor necrosis factor-α in response to exposure to the LPS (Rockwell et al. 2013). Similar effects were observed 4 weeks post-exposure (Rockwell et al. 2017). Comparison of the results 2 weeks post-exposure to 4 weeks post-exposure showed a partial recovery in spleen weight and specific thymic lymphocyte subpopulations, but no recovery of the ratio of specific splenic lymphocytes, thymocyte viability, or response to LPS (Rockwell et al. 2017). Some sex-related differences were noted, with females appearing to be more sensitive than males (Rockwell et al. 2017).

PFDA

Epidemiological Studies—Immunosuppression Outcomes. Studies examining possible associations between serum PFDA levels and response to vaccines have reported mixed results; see Figure 2-29 for a graphical presentation of the antibody response relative to PFDA levels. Inverse associations were observed between serum PFDA levels at age 5 and tetanus antibody levels at ages 5 and 7 (Grandjean et al. 2012) and serum PFDA levels at age 7 and antibody levels at age 13 (Grandjean et al. 2017).

Figure 2-29. Antibody Responses Relative to Serum PFDA Levels in Epidemiological Studies
(Presented as percent difference in antibody concentration per 2-fold increase in serum PFDA)

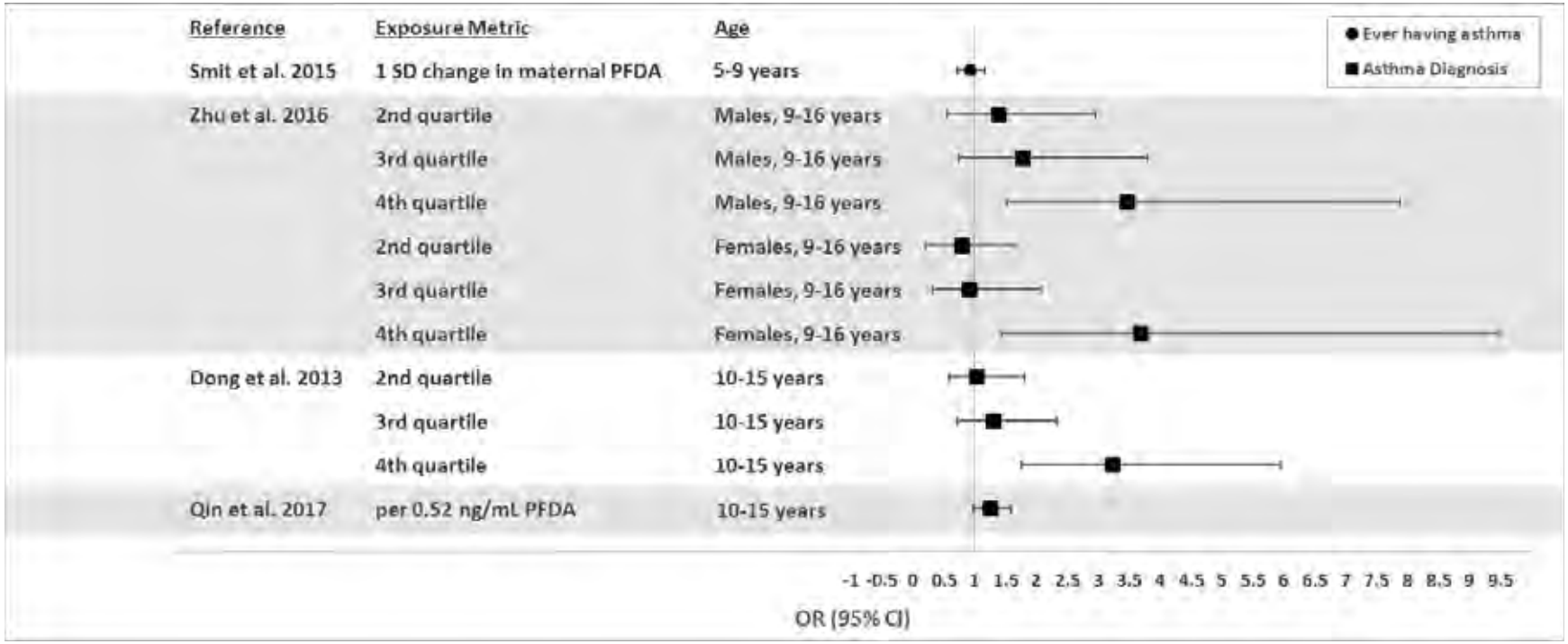


Similarly, diphtheria antibody levels at age 13 were inversely associated with serum PFDA levels at age 7 years (Grandjean et al. 2017), but no associations were observed at other time periods (Grandjean et al. 2012). In adults, diphtheria antibody levels were inversely associated with serum PFDA levels, but there was no association for tetanus antibody levels (Kielsen et al. 2016); this study did not adjust for potential confounders. Two studies examined the possible association between serum PFDA levels and infectious disease resistance, no association was found between maternal serum PFDA levels and symptoms of infection in children aged 1–4 years (Dalsager et al. 2016) and the prevalence of total infectious disease in children 0–4 years of age (Goudarzi et al. 2017).

Epidemiological Studies—Hypersensitivity Outcomes. In case-control studies, associations between asthma diagnosis and asthma severity were observed in children (Dong et al. 2013; Zhu et al. 2016); associations with serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were also observed. A case-control study in adolescents found significantly higher serum PFDA levels among the asthmatic cases (Zhu et al. 2016). A fourth case-control study did not find an association between serum PFDA and asthma risk in children (Qin et al. 2017). A cross-sectional study of children did not find associations between maternal PFDA levels and asthma, eczema, or wheezing in children (Smit et al. 2015). Another cross-sectional study found no association between allergic diseases or eczema in infant and maternal PFDA levels (Okada et al. 2014). In a prospective study, the prevalences of total allergic diseases or wheezing in 4-year-old children were not associated with maternal PFDA levels (Goudarzi et al. 2016a). Data evaluating associations between serum PFDA and the risk of asthma diagnosis are presented in Figure 2-30.

Laboratory Animal Studies. A single gavage dose of 80 mg/kg PFDA did not significantly alter relative thymus weight in female C57BL/6N mice, but it caused a 28% decrease in relative spleen weight 30 days after dosing (Harris et al. 1989). Lethal doses (160 and 320 mg/kg) induced atrophy and lymphoid depletion in both the thymus and spleen. No significant alterations in tests of humoral- or cell-mediated immunity, or alterations in the number of total splenic cells or splenic B-cells, T-cells, T-cell subsets, natural killer cells or macrophages were observed in rats administered up to 0.5 mg/kg/day for 28 days (Frawley et al. 2018). In tests of innate immunity, the study found decreases in the specific activity of fixed tissue macrophages in the liver in rats administered 0.25 or 0.5 mg/kg/day; the investigators suggested that interpretation of this finding may be confounded by the increased number of hepatocytes.

Figure 2-30. Risk of Asthma Diagnosis Relative to PFDA Levels (Presented as Adjusted Odds Ratios)



In mice receiving weekly doses of PFDA for 4 weeks, decreases in the number of splenic T cells, T-cell subsets, and macrophages were observed at ≥1.25 mg/kg (Frawley et al. 2018). No alterations in humoral-mediated or cell-mediated immune tests or host-resistance to the influenza virus were found.

PFUnA

Epidemiological Studies. Six epidemiological studies have evaluated the potential immunotoxicity of PFUnA in humans. Kielsen et al. (2016) reported inverse associations between serum PFUnA (unadjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. Goudarzi et al. (2017) found no association between maternal PFUnA levels and the risk of total infectious diseases in children up to the age of 4 years. However, Impinen et al. (2018) found cord PFUnA levels were associated with increases in the prevalence of common colds in children up to 2 years of age and the prevalence of lower respiratory tract infections in children up to the age of 10 years.

No significant associations between maternal PFUnA levels and the risk of asthma diagnosis, eczema, or wheezing were observed in children (Smit et al. 2015). Similarly, no associations were found between cord PFUnA levels and risk of current asthma, ever having asthma, asthma diagnosis, or wheezing in children up to the age of 10 years (Impinen et al. 2018). Maternal PFUnA levels were not associated with the prevalences of total allergic diseases or wheezing in 4-year-old children (Goudarzi et al. 2016a). Okada et al. (2012) found inverse associations between maternal serum PFUnA and risk of allergies or eczema in female infants, but not in males, and Impinen et al. (2018) found no association between serum PFUnA and allergic sensitization.

PFHpA

Epidemiological Studies. In general, the two available human immunotoxicity studies did not find associations between serum PFHpA levels and diphtheria or tetanus antibody levels in adults (Kielsen et al. 2016) or risk of asthma diagnosis, eczema, or wheezing in children (Smit et al. 2015). The Smit et al. (2015) study did find an inverse association between maternal PFHpA levels and current wheezing in one subcohort; however, this was not observed in the other subcohort with higher mean maternal PFHpA levels.

PFBS

Epidemiological Studies. The epidemiological database for PFBS consists of three case-control studies in asthmatic children (Dong et al. 2013; Qin et al. 2017; Zhu et al. 2016). Two studies reported increases in asthma diagnosis, but no association with serum IgE levels (Dong et al. 2013; Zhu et al. 2016); the third study (Qin et al. 2017) did not find an association between serum PFBS and asthma risk.

Laboratory Animal Studies. No significant histological alterations were observed in spleen, thymus, or lymph nodes of rats administered via gavage 900 mg/kg/day PFBS for 28 days (3M 2001).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in the spleen, thymus, or mesenteric lymph nodes from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days, 150 mg/kg/day for 28 days, or 30 mg/kg/day for 90 days (3M 2007a; Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b).

PFDoDA

Epidemiological Studies. Six epidemiological studies examining potential immunotoxic endpoints were identified. Kielsen et al. (2016) found inverse associations between recent serum PFDoDA levels (not adjusted for potential confounders) and diphtheria and tetanus antibody levels in adults. No associations between maternal PFDoDA levels and the risk of total infectious diseases were found in children up to the age of 4 years (Goudarzi et al. 2017). Associations between serum PFDoDA levels and the risk of asthma diagnosis, severity of asthma, serum IgE levels, absolute eosinophil counts, and eosinophil cationic protein levels were observed in a case-control study of asthmatic children (Dong et al. 2013). A cross-sectional study of children did not find associations between maternal serum PFDoDA levels and risk of asthma diagnosis, eczema, or wheezing (Smit et al. 2015). Another study did not find associations between maternal serum PFDoDA levels and the risk of allergic disease or eczema in infants (Okada et al. 2014). In contrast, a prospective study of 4-year-old children found an inverse association between maternal PFDoDA levels and the prevalence of mother-reported total allergic diseases, but no association with the prevalence of wheezing (Goudarzi et al. 2016a).

PFHxA

Epidemiological Studies. Two epidemiological studies examined potential immunotoxic endpoints. Dong et al. (2013) found no associations between serum PFHxA levels in asthmatic and nonasthmatic children and asthma diagnosis, asthma severity, or IgE levels. Qin et al. (2017) did not find an association between serum PFHxA levels and asthma risk in children.

Laboratory Animal Studies. Thymic atrophy was observed in 3/9 female rats administered a TWA dose of 315 mg/kg/day PFHxA for 32–44 days (Kirkpatrick 2005). Thymic atrophy and necrosis was also observed in most male and female rats administered 450 mg/kg/day PFHxA for 4 days; all animals died early or were sacrificed *in extremis* (Kirkpatrick 2005).

FOSA

Epidemiological Studies. The only available epidemiological study found an association between cord FOSA levels and an increased prevalence of lower respiratory tract infections in children up to the age of 10 years (Impinen et al. 2018); no association was found for common colds in children up to the age of 2 years. This study also found no associations between cord FOSA and current asthma, ever having asthma, asthma diagnosis, wheezing, or allergic sensitization (Impinen et al. 2018).

2.15 NEUROLOGICAL

Overview. There are limited data on the neurotoxicity of perfluoroalkyls in humans or laboratory animals; epidemiological data come from three studies examining memory and animal studies primarily evaluated for morphological alterations; the results of these human studies are summarized in Table 2-17 with more detailed descriptions in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 11. The epidemiological studies found decreases in the risk of memory loss associated with serum PFOA, PFOS, PFHxS, and PFNA. The potential to induce neurodevelopmental effects (including the risk of attention deficit hyperactivity disorder [ADHD]) has been more widely studied; these data are discussed in Section 2.17, Developmental. No epidemiological studies examining potential neurological effects were found for PFDA, PFUnA, PFHpA, PFBS, PFBA, PFDoDA, PFHxA, or FOSA.

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	14.1–27.0 ng/mL (2nd PFOA quintile)	Memory loss (self-reported)	**OR 0.88 (0.79–0.97)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	4.08 ng/mL (median PFOA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.92 (0.78–1.09)
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	8.1 ng/mL (median serum PFOA)	Memory and learning scores	**Association (p=0.03)***
		Executive function scores	**Inverse association (p=0.04, p=0.03)***
		Visual and spatial function scores	NS (p>0.05)
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFOS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	20.5–27.1 ng/mL (3rd PFOS quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 3rd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	22.63 ng/mL (median PFOS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.90 (0.78–1.03)

Table 2-17. Summary of Neurological Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shrestha et al. 2017 General population (n=126 older adults, aged 55–74 years)	33.7 ng/mL (median serum PFOS)	Memory and learning scores	**Association (p=0.04)***
		Executive function scores	NS (p>0.05)
		Visual and spatial function scores	**Association (p=0.05)***
		Reaction time	NS (p>0.05)
		Motor function	NS (p>0.05)
PFHxS			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	5.7–232.6 ng/mL (5th PFHxS quintile)	Memory loss (self-reported)	**OR 0.89 (0.79–0.99)*, 5th quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	2.05 ng/mL (median PFHxS)	Difficulty remembering or periods of confusion (self-reported)	OR 0.93 (0.82–1.06)
PFNA			
Gallo et al. 2013 Community (C8) (n=21,024 older adults; >50 years of age)	1.0–1.2 ng/mL (2nd PFNA quintile)	Memory loss (self-reported)	**OR 0.86 (0.78–0.96)*, 2nd quintile**
Power et al. 2013 General population (NHANES) (n=1,766 older adults aged 60–<85 years)	1.01 ng/mL (median PFNA)	Difficulty remembering or periods of confusion (self-reported)	OR 0.91 (0.79–1.04)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 11 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NHANES = National Health and Nutrition Examination Survey; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

The results of the laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. No morphological alterations in the brain and nerves were observed in studies of PFOA, PFOS, PFBS, or PFBA. No alterations in neurological function tests were observed in studies of PFOA, PFHxS, PFHxA, PFBS, PFBA, or PFDoDA. Impaired learning and memory were observed in a study of PFOS and decreases in grip strength were observed in a study of PFUnA. Potential neurological effects were not examined in animals exposed to PFNA, PFHpA, or FOSA.

PFOA

Epidemiological Studies. Gallo et al. (2013) found a decreased risk of self-reported memory loss in older adult (>50 years of age) C8 participants with serum PFOA levels in the 2nd, 3rd, 4th, or 5th quintiles. When the participants were categorized by diabetic status, the risk of memory loss was higher among the diabetics than nondiabetics (p=0.014). In sensitivity analyses, the association between serum PFOA levels and memory impairment was compared within and across water districts. Within a water district, the association between serum PFOA and memory impairment was significant, but there was no association between the geometric mean concentration of PFOA in a district and memory impairment. A general population study conducted by Shrestha et al. (2017) of 55–74-year-old participants also found higher memory and learning scores (6% increase) and 16–18% decreases in perseverative errors and responses. In a third study, no association between serum PFOA and self-reported difficulty remembering or periods of confusion was found in NHANES participants aged 60–<85 years (Power et al. 2013).

Laboratory Animal Studies. Exposure of rats to 18,600 mg/m^3 APFO dusts for 1 hour induced excessive salivation. Intermittent, head-only exposure of male rats exposed to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal gross or microscopic alterations in the brain (Kennedy et al. 1986).

A small number of studies have examined the potential toxicity of perfluoroalkyls to the nervous system in animals, but comprehensive testing has not been conducted. No alterations in performance on a novel recognition test were observed in rats administered a single 50 mg/kg dose of PFOA (Kawabata et al. 2017). No overt signs of neurotoxicity or altered response to stimuli were observed in rats and mice administered up to 1,000 mg/kg PFOA via gavage and observed for 14 days (Sato et al. 2009). Exposure of rats to up to approximately 110 mg/kg/day PFOA via the diet for 90 days did not induce gross or microscopic alterations in the brain, spinal cord, or peripheral nerves (Griffith and Long 1980). Similar results were reported in rats fed a diet that provided approximately 15 mg/kg/day PFOA for 2 years (3M

1983; Butenhoff et al. 2012c). Rhesus monkeys exposed to doses of PFOA that caused lethality (≥30 mg/kg/day by gavage) showed signs of hypoactivity and prostration, but examination of the brain did not reveal treatment-related alterations (Griffith and Long 1980). Treatment of Cynomolgus monkeys with doses of up to 20 mg/kg/day PFOA administered via a capsule did not induce morphological alterations in the brain or sciatic nerve (Butenhoff et al. 2002).

Similarly, no gross or microscopic alterations were reported in the brain from rats dermally exposed to APFO in the Kennedy (1985) study.

PFOS

Epidemiological Studies. Three studies have examined the influence of serum PFOS levels on self-reported memory in older adults. Gallo et al. (2013) found an inverse association between serum PFOS levels and the risk of memory loss in C8 Health Study participants. No association for difficulty remembering or periods of confusion was found in the second study of NHANES participants (Power et al. 2013). A second general population study of older adults found associations between serum PFOS levels and 11% higher scores on tests of visual reproduction delayed recall and 8% higher scores on tests of visual and spatial function (Shrestha et al. 2017), but found no associations on tests of executive function, reaction time, affective state, or motor function.

Laboratory Animal Studies. No histological alterations were observed in the brain, spinal cord, and/or sciatic nerve of rats administered a single gavage dose of up to 500 mg/kg PFOS (Sato et al. 2009), rats treated with up to 1.6–1.8 mg/kg/day PFOS for 4 or 14 weeks (Seacat et al. 2003), rats exposed to 8.5 mg/kg/day PFOS in the diet for 13 weeks (Kawamoto et al. 2011), rats exposed to 1.04 mg/kg/day PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b), or Cynomolgus monkeys dosed with up to 0.75 mg/kg/day PFOS for 26 weeks (Seacat et al. 2002). However, ultrasonic stimulation resulted in bursts of locomotion immediately followed by tonic convulsions in mice administered 125 mg/kg PFOS and rats administered 250 mg/kg PFOS (Sato et al. 2009); the effect was observed 1–7 days postexposure and frequently resulted in death. Similarly, tonic convulsions following ultrasonic stimulation were observed in rats exposed to 8.5 mg/kg/day PFOS in the diet for 6 weeks (Kawamoto et al. 2011); this effect was not observed at ≤2.0 mg/kg/day. Impaired spatial learning and memory, assessed using the Morris water maze test, was observed in mice administered 2.15 or 10.75 mg/kg/day PFOS, but not 0.43 mg/kg/day, for 3 months (Long et al. 2013). Similarly, impaired performance on retention tasks, as assessed by the water maze test, was observed in mice administered 3 or 6 mg/kg/day

PFOS for 4 weeks (Fuentes et al. 2007c). Histopathological examination of the hypothalamus in male Sprague-Dawley rats administered PFOS via gavage for 28 days revealed degeneration of gonadotropic cells of the pituitary gland at ≥1.0 mg/kg/day and dense chromatin, condensed ribosomes, and loss of morphology in the hypothalamus at ≥3.0 mg/kg/day (López-Doval et al. 2014).

PFHxS

Epidemiological Studies. A decrease in the risk of self-reported memory loss was observed in older adult participants of the C8 Health Study who had serum PFHxS levels in the 5th quintile (Gallo et al. 2013). No association between serum PFHxS levels and self-reported difficulty remembering or periods of confusion was reported in a study of NHANES participants (Power et al. 2013).

Laboratory Animal Studies. In a reproductive study in rats dosed with PFHxS, a functional observational battery (FOB) and motor activity tests were conducted in males on exposure days 36 and 39 and in females on postpartum day 17 (Butenhoff et al. 2009a). The battery assessed autonomic functions, reactivity and sensitivity to stimuli, excitability, gait and sensorimotor coordination, limb grip strength, and abnormal clinical signs. No significant alterations were reported in males or females dosed with up to 10 mg/kg/day PFHxS.

PFNA

Epidemiological Studies. Self-reported memory loss was shown to be inversely associated with serum PFNA levels in a study of older C8 Health Study participants (Gallo et al. 2013). Another study of NHANES participants did not find an association with self-reported difficulty remembering or periods of confusion (Power et al. 2013).

PFUnA

Laboratory Animal Studies. In the only study located for PFUnA, a decrease in grip strength was observed in male and female rats administered 1.0 mg/kg/day PFUnA for 41–46 days and allowed to recover for 14 days (Takahashi et al. 2014). No other alterations in performance on FOB tests were found.

PFBS

Laboratory Animal Studies. A significant decrease in tail flick latency to a thermal stimulus was observed in all groups of male rats administered via gavage PFBS for 28 days. However, other tests of sensory reactivity to stimuli, grip strength, and motor activity were not affected (3M 2001), and the significance of this isolated finding is difficult to ascertain. Gross and microscopic examination of the brain, spinal cord, and sciatic nerve did not show any significant alterations. In a 90-day study, no significant alterations in motor activity or performance on functional observation tests were observed in rats at PFBS doses as high as 600 mg/kg/day (Lieder et al. 2009a).

PFBA

Laboratory Animal Studies. Administration of up to 184 mg/kg/day PFBA by gavage for 5 consecutive days to rats had no significant effect on the gross or microscopic morphology of the brain or spinal cord (3M 2007a). In a 28-day gavage study, male rats dosed with 150 mg/kg/day, but not 30 mg/kg/day, showed a delay in bilateral pupillary reflex at the end of the treatment period (Butenhoff et al. 2012a; van Otterdijk 2007a). Results from other tests, including hearing ability, static righting reflex, grip strength, and motor activity, were comparable between groups, and histological examinations of the brain (including the optic nerve), spinal cord, and sciatic nerve were unremarkable. In a 90-day study, pupillary reflex tests conducted in weeks 8 and 12 showed delayed dilation under dark conditions in rats dosed with 30 mg/kg/day (2/40 in controls versus 7/39 in high-dose rats; p=0.071 according to the Fisher Exact Test) (Butenhoff et al. 2012a; van Otterdijk 2007b). Since no abnormalities were recorded during a 3-week recovery period, and there were no histopathological alterations in the eyes, the effect was not considered biologically significant by the investigators. Tests for hearing ability, static righting reflex, grip strength, and motor activity showed no associations with treatment with PFBA. In addition, there were no significant gross or microscopic alterations in the brain, spinal cord, or sciatic nerve.

PFDoDA

Laboratory Animal Studies. Single-dose administration of 50 mg/kg resulted in impaired performance on a novel object recognition test, but did not result in alterations in other tests of memory, anxiety, or open field activity (Kawabata et al. 2017). A second study conducted functional observation tests in rats administered PFDoDA for 42 days (Kato et al. 2015). No alterations in sensorimotor reactivity, grip strength, or spontaneous motor activity were observed at 2.5 mg/kg/day. However, in rats allowed to

recover for 14 days, decreases in forelimb grip strength were observed in males and females at 2.5 mg/kg/day; a decrease in motor activity was also observed in females at 2.5 mg/kg/day but this was only observed during the first week of recovery (Kato et al. 2015).

PFHxA

Laboratory Animal Studies. Administration of up to 500 mg/kg/day NaPFHx for 92–93 days (Loveless et al. 2009) or 200 mg/kg/day PFHxA for 104 weeks (Klaunig et al. 2015) had no effect on locomotion or performance in the FOB test.

2.16 REPRODUCTIVE

Overview. A number of epidemiological studies have evaluated the reproductive toxicity of perfluoroalkyls; summaries of these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12. These studies have evaluated the following categories of reproductive outcomes: alterations in reproductive hormone levels; effects on sperm; effects on menopause onset, menstrual cycle length, endometriosis, and breastfeeding duration; and effects on fertility. Overviews of the studies examining these specific endpoints are presented in Tables 2-18, 2-19, 2-20, and 2-21, respectively. In addition to these reproductive outcomes, several epidemiological studies have evaluated the influence of perfluoroalkyls on sexual maturation; these data are discussed in Section 2.17, Developmental. Although some studies examining reproductive hormone levels have found associations with PFOA, PFOS, PFHxS, PFNA, PFUnA, PFDoDA, or PFHxA levels, the findings are not consistent across studies or there are too few studies to interpret the results. Alterations in reproductive hormone levels have not been found in studies of FOSA. Some associations between serum perfluoroalkyls (PFOA, PFOS, PFHxS, PFNA, PFDA) levels and sperm parameters have been found; often, only one sperm parameter was altered and it is difficult to assess the adversity of this alteration. There is some suggestive evidence of an association between serum PFOA, PFOS, PFHxS, or PFNA levels and an increased risk of early menopause; however, this may be due to reverse causation since an earlier onset of menopause would result in a decrease in the removal of perfluoroalkyls in menstrual blood. Epidemiological studies provide mixed evidence of impaired fertility (increased risks of longer time to pregnancy and infertility); there is also some evidence for PFOA, PFOS, PFHxS PFNA, PFHpA, and PFBS but the results are not consistent across studies or were only based on a single study. The small number of studies evaluating fertility for PFDA, PFUnA, PFDoDA, and FOSA did not find associations. Reproductive outcomes have not been evaluated in epidemiological studies on PFBA.

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992 Occupational (n=115)	NR (serum fluorine levels used as surrogate for serum PFOA)	Bound testosterone	**Inverse association (p=0.05)***
		Free testosterone	**Inverse association (p=0.03)***
		Estradiol	**Association (p=0.03)***
		LH	NS (p=0.93)
		FSH	NS (p=0.91)
		Prolactin	**Association (p=0.0002)***
Olsen et al. 1998b Occupational (n=111 males in 1993 and 80 males in 1995)	0–80,000 ng/mL (PFOA range)	Prolactin	**Association (p=0.01 for trend)*, 1993** NS (p=0.58 for trend), 1995
		Estradiol	NS (p=0.66 and 0.56 for trend), 1993 and 1995
		17α-Hydroxy-progesterone	NS (p=0.21 and 0.18 for trend), 1993 and 1995
		Bound testosterone	NS (p=0.07 and 0.85 for trend), 1993 and 1995
		Free testosterone.	NS (p=0.15 or 0.82 for trend), 1993 and 1995
Sakr et al. 2007b Occupational (n=1,025)	428 ng/mL (mean PFOA)	Estradiol	**Association (p=0.017)*, males**
		Testosterone	**Association (p=0.034)*, males**
Knox et al. 2011 Community (C8) (n=25,957 women)	11.3–19.8 ng/mL (2nd PFOA quintile)	Estradiol concentration	NS (p>0.05), menopausal and perimenopausal subgroups
Barrett et al. 2015 General population (n=178 women)	3.61 and 2.31 ng/mL (mean PFOA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	3.46 ng/mL (mean PFOA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Estradiol	NS (p=0.751)
		Prolactin	NS (p=0.349)
		FSH	NS (p=0.581)
		LH	**Correlation (p=0.011)***
		Free testosterone	**Correlation (p=0.015)***
		Total testosterone	NS (p=0.440)
Specht et al. 2012 General population (n=604 men)	1.3–4.8 (range of PFOA means of different sites)	SHBG	NS (p=0.39 for trend)
Tsai et al. 2015 General population (n=540 adolescents and young adults aged 12–30 years)	2.74 ng/mL (geometric mean PFOA)	SHBG	**Association (p<0.05)*, females 12–17 years old**
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	**Association (p=0.03)***
		FSH	**Association (p=0.01)***

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFOA in boys and girls)	Testosterone	β -0.0549 (-0.1186–0.0088), boys β -0.1627 (-0.1627–0.0233), girls
		Estradiol	**β 0.0921 (0.0186–0.1656)*, boys** β 0.1015 (-0.0023–0.0033), girls
PFOS			
Olsen et al. 1998a Occupational (n=327)	1,480–2,440 ng/mL (range of PFOS means at different time periods)	DHEAS	NS (p=0.60)
		FSH	NS (p=0.91)
		17-HP	NS (p=0.99)
		LH	NS (p=0.69)
		Prolactin	NS (p=0.25)
		SHBG	NS (p=0.77)
		Free testosterone	NS (p=0.90)
		Bound testosterone	NS (p=035)
		Estradiol	NS (p=0.14), after removal of 1 outlier
Knox et al. 2011 Community (C8) (n=25,957 women)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Estradiol concentration Perimenopausal subgroup Menopausal subgroup	 **Inverse association (p=0.0001)*** **Inverse association (p=0.007)***
Barrett et al. 2015 General population (n=178 women)	16.44 and 14.18 ng/mL (mean PFOS in nulliparous and parous women)	Follicular estradiol	**Inverse association (β -0.013, 95% CI -0.023 to -0.001)*, whole cohort** **Inverse association (β -0.025, 95% CI -0.043 to -0.007)*, nulliparous subcohort**
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	8.46 ng/mL (mean PFOS)	Total testosterone	**Inverse association (p<0.05)***
		Free testosterone	**Inverse association (p<0.05)***
		Free androgen index	**Inverse association (p<0.05)***
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Estradiol	NS (p>0.05)
		Prolactin	NS (p>0.05)
		FSH	NS (p>0.05)
		LH	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Total testosterone	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	7.78 ng/mL (geometric mean PFOS)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, males 12–17 years old**
		Testosterone	**Inverse association (p<0.05)*, females 12–17 years old**
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Testosterone	NS (p>0.05)
		Estradiol	NS (p>0.05)
		Inhibin B	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free antigen index	NS (p>0.05)
		LH	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	29.9 and 28.8 ng/mL (median serum PFOS in boys and girls)	Testosterone	**β -0.0029 (-0.0055 to -0.0003)*, boys** β 0.0005 (-0.0018–0.0028), girls
		Estradiol	β 0.0024 (-0.0007–0.0055), boys β 0.0005 (-0.0023–0.0033), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Barrett et al. 2015 General population (n=178 women)	1.22 and 1.65 ng/mL (mean PFHxS in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.81 ng/mL (mean PFHxS)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	1.4 and 1.2 ng/mL (median serum PFHxS in boys and girls)	Testosterone	β 0.0173 (-0.0211–0.0588), boys β -0.0182 (-0.0451–0.0087), girls
		Estradiol	**β 0.0462 (0.0020–0.0925)*, boys** β 0.0017 (-0.0154–0.0496), girls
PFNA			
Barrett et al. 2015 General population (n=178 women)	0.67 and 0.60 ng/mL (mean PFNA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	1.23 ng/mL (mean PFNA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	**Association (p<0.05)***
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
Tsai et al. 2015 General population (n=540 male and female adolescents and young adults aged 12–30 years)	1.10 ng/mL (geometric mean PFNA)	SHBG	NS (p>0.05)
		FSH	NS (p>0.05)
		Testosterone	NS (p>0.05)
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.8 and 0.9 ng/mL (median serum PFNA in boys and girls)	Testosterone	**β -0.4233 (-0.6998 to -0.1467)*, boys** β -0.1018 (-0.2684–0.0648), girls
		Estradiol	β 0.3204 (-0.0115–0.6522), boys β 0.1252 (-0.0758–0.3263), girls
PFDA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.24 ng/mL (mean PFDA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Joensen et al. 2013 General population (n=247 young men; mean age 19.6 years)	0.38 ng/mL (mean PFDA)	Total testosterone	NS (p>0.05)
		Free testosterone	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		LH	NS (p>0.05)
		Estradiol	NS (p>0.05)
		SHBG	NS (p>0.05)
		FSH	NS (p>0.05)

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.3 and 1.0 ng/mL (median serum PFDA in boys and girls)	Testosterone	**β -0.2565 (-0.4135 to -0.0994)*, boys** β -0.0626 (-0.1730–0.0477), girls
		Estradiol	β 0.0734 (-0.1189–0.2657), boys β 0.0131 (-0.1208–0.1469), girls
PFUnA			
Barrett et al. 2015 General population (n=178 women)	0.40 and 0.42 ng/mL (mean PFUnA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
Tsai et al. 2015 General population (n=540 males and females aged 12–30 years)	5.84 ng/mL (geometric mean PFUnA)	SHBG	NS (p>0.05)
		FSH	**Inverse association (p<0.05)*, females 12–17 years old**
		Testosterone	NS (p>0.05)
PFBS			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.5 and 0.5 ng/mL (median serum PFBS in boys and girls)	Testosterone	β -0.0387 (-0.3261–0.2487), boys β 0.1326 (-0.3576–0.6229), girls
		Estradiol	β 0.0149 (-0.3216–0.3513), boys β 0.3129 (-0.2771–0.9028), girls
PFDoDA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	2.4 and 3.1 ng/mL (median serum PFDoDA in boys and girls)	Testosterone	β 0.0056 (-0.0056–0.0168), boys **β -0.0119 (-0.0227 to -0.0010)*, girls**
		Estradiol	β -0.0007 (-0.0139–0.0124), boys β 0.0106 (-0.0026–0.0218), girls
PFHxA			
Zhou et al. 2016 General population (n=225 adolescents, 13–15 years of age)	0.2 and 0.2 ng/mL (median serum PFHxA in boys and girls)	Testosterone	**β -0.3095 (0.5942 to -0.0248)*, boys** β -0.1896 (-0.4387–0.0595), girls
		Estradiol	β 0.0600 (-0.2803–0.4003), boys β -0.1492 (-0.4515–0.1531), girls

Table 2-18. Summary of Alterations in Reproductive Hormone Levels in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Barrett et al. 2015 General population (n=178 women)	0.25 and 0.23 ng/mL (mean FOSA in nulliparous and parous women)	Follicular estradiol	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts
		Luteal progesterone	NS (95% CI included unity), whole cohort and parous and nulliparous subcohorts

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SHBG = sex hormone binding globulin

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	4.6 and 5.3 ng/mL (median PFOA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ curvilinear velocity Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology ↑ percentage of sperm head acrosome area ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	3.46 ng/mL (mean PFOA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	1.91–5.19 ng/mL (range of PFOA means)	Y-X chromosome ratio	NS (p>0.05)
Raymer et al. 2012 General population (n=256 men)	10.4 ng/mL (mean PFOA)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	3.8 ng/mL (median PFOA)	↑ percent motile sperm	**Association (p<0.05)***
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vested et al. 2013 General population (n=169 males aged 19–21 years)	3.8 ng/mL (median maternal PFOA)	Sperm concentration	**Inverse association (p=0.01)***
		Total sperm count	**Inverse association (p=0.001)***
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFOS			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	19.15 and 21.6 ng/mL (median PFOS in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility ↑ distance travelled Other parameters	 **Association (p<0.05)*** NS (p>0.05)
		Sperm morphology	NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	8.46 ng/mL (mean PFOS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
Kvist et al. 2012 General population (n=588 men)	8.20–51.65 ng/mL (range of mean PFOS) 51.65 ng/mL (mean for Greenland subcohort)	Y-X chromosome ratio	**Association (p<0.05)*, whole cohort Inverse association (p=0.044 for trend)*, Greenland subcohort**
Raymer et al. 2012 General population (n=256 men)	37.4 ng/mL (mean PFOS)	Semen volume	NS (p>0.05)
		Semen pH	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm concentration	NS (p>0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Toft et al. 2012 General population (n=588 males)	18.4 ng/mL (median PFOS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***
Vested et al. 2013 General population (n=169 males aged 19–21 years)	21.2 ng/mL (median maternal PFOS)	Sperm concentration	NS (p>0.05)
		Total sperm count	NS (p>0.05)
		Semen volume	NS (p>0.05)
		Percentage progressive spermatozoa	NS (p>0.05)
		Percentage morphologically normal spermatozoa	NS (p>0.05)
		Mean testicular volume	NS (p>0.05)
PFHxS			
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.81 ng/mL (mean PFHxS)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.1 ng/mL (median PFHxS)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	**Inverse association (p<0.05)***

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	1.0 and 1.65 ng/mL (median PFNA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology ↑ percentage of normal sperm ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	1.23 ng/mL (mean PFNA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)
Toft et al. 2012 General population (n=588 males)	1.2 ng/mL (median PFNA)	Percent motile sperm	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm volume	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percent normal sperm	NS (p>0.05)
PFDA			
Buck Louis et al. 2015 General population (n=96 in Michigan and 366 in Texas)	0.3 and 0.5 ng/mL (median PFDA in Michigan and Texas)	Sperm viability	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Sperm motility	NS (p>0.05)
		Sperm morphology ↑ sperm head length ↓ percentage sperm with coiled tails Other parameters	 **Association (p<0.05)*** **Association (p<0.05)*** NS (p<0.05)

Table 2-19. Summary of Male Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Joensen et al. 2013 General population (n=247 young men; mean age of 19.6 years)	0.38 ng/mL (mean PFDA)	Sperm volume	NS (p>0.05)
		Sperm concentration	NS (p>0.05)
		Sperm count	NS (p>0.05)
		Percentage progressive motile sperm	NS (p>0.05)
		Sperm morphology	NS (p>0.05)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Dhingra et al. 2016a Community (C8) (n=8,759; retrospective analysis)	>2,130 ng/mL·year (estimated cumulative PFOA exposure 5th quintile)	Menopause age	HR 1.11 (0.97–1.26, p=0.14), 5th quintile
		Early menopause risk	NS (p=0.45), 5-year lag NS (p=0.58), 10-year lag NS (p=0.57), 15-year lag NS (p=0.20), 20-year lag
Dhingra et al. 2016a Community (C8) (n=3,334, prospective analysis)	>4,670 ng/mL·year (estimated cumulative PFOA exposure 5th quintile) >80.8 ng/mL (measured 5th PFOA quintile)	Menopause age (estimated cumulative)	HR 1.10 (0.84–1.43, p=0.51), 5th quintile
		Menopause age (measured)	HR 1.12 (0.86–1.45, p=0.40), 5th quintile
Knox et al. 2011b Community (C8) (n=25,957)	11.3–19.8 ng/mL (2nd PFOA quintile)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.4 (1.1–1.8)*, 2nd quintile**
Buck Louis et al. 2012 General population (n=473)	2.65 and 2.15 ng/mL (geometric mean PFOA in women with or without endometriosis)	Endometriosis	**OR 1.89 (1.17–3.06)*, without parity adjustment** OR 1.62 (0.99–2.66), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 2.58 (1.18–5.64)*, without parity adjustment** OR 1.86 (0.81–4.24) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.70–3.99 and 4.00–20.60 ng/mL (3rd and 4th quartile serum PFOA)	Self-reported endometriosis	**OR 5.45 (1.19–25.04)*, 3rd quartile** OR 1.33 (0.82–2.17), 4th quartile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.98 (1.17–3.24)*, 2nd quartile**
		Breastfeeding duration ≤6 months	**OR 1.88 (1.31–2.72)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	3.1, 3.5, and 3.1 ng/mL (median serum PFOA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 0.98 (0.96–1.00)
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	1.5 ng/mL (median PFOA)	Irregular menstrual cycle	OR 1.4 (0.9–2.2)
		Long menstrual cycle	**OR 1.7 (1.1–2.6)***
		Short menstrual cycle	OR 0.7 (0.3–1.5)
Romano et al. 2016 General population (n=336 women)	5.5–7.6 ng/mL (maternal 3rd quartile PFOA)	Breastfeeding duration ≤3 months	**RR 1.63 (1.16–2.28)*, 3rd quartile**
		Breastfeeding duration ≤6 months	**RR 1.38 (1.06–1.79)*, 3rd quartile**
Taylor et al. 2014 General population (n=2,151 women)	>2.5–4.4 and >4.4 ng/mL (2nd and 3rd PFOA tertiles)	Menopause	**HR 1.36 (1.05–1.75)*, 3rd tertile**
		Hysterectomy	**HR 1.83 (1.31–2.56)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	2.40 ng/mL (median maternal PFOA)	Breastfeeding duration (in months)	**β -1.3 (-1.9 to -0.7)*, per doubling of serum PFOA levels**
		Exclusive breastfeeding (in months)	**β -0.5 (-0.7 to -0.3)*, per doubling of serum PFOA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	4.1 and 2.3 ng/mL (geometric mean PFOA for cases and controls)	Polycystic ovary syndrome risk	**OR 6.93 (1.79–29.92, p=0.003)*, 3rd tertile**

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Knox et al. 2011b Community (C8) (n=25,957)	11.9–17.0 and 17.1–22.4 ng/mL (2nd and 3rd PFOS quintiles)	Early menopause risk (menopausal subgroup)	**OR 1.5 (1.1–2.1)*, 2nd quintile**
		Early menopause risk (perimenopausal subgroup)	**OR 1.1 (1.1–1.8)*, 3rd quintile**
Buck Louis et al. 2012 General population (n=473)	7.20 and 6.11 ng/mL (geometric mean PFOS in women with or without endometriosis)	Endometriosis	OR 1.39 (0.98–1.98), without parity adjustment OR 1.25 (0.87–1.80), with parity adjustment
		Risk of moderate to severe endometriosis	**OR 1.86 (1.05–3.30)*, without parity adjustment** OR 1.50 (0.82–2.74) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	18.20–392.00 ng/mL (4th quartile PFOS)	Self-reported endometriosis	OR 3.48 (1.00–12.00), 4th quartile
Fei et al. 2010 General population (n=1,347 pregnant women)	3.91–5.20 ng/mL (2nd quartile for maternal PFOA)	Breastfeeding duration ≤3 months	**OR 1.89 (1.19–3.01)*, 4th quartile**
		Breastfeeding duration ≤6 months	**OR 1.56 (1.10–2.22)*, 2nd quartile**
Lum et al. 2017 General population (n=501 women)	12.3, 12.6, and 11.5 ng/mL (median serum PFOS in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.98–1.03), 3rd tertile
Lyngsø et al. 2014 General population (n=1,623 pregnant women)	8.0 ng/mL (median PFOS)	Irregular menstrual cycle	OR 1.0 0.6–1.6)
		Long menstrual cycle	OR 0.7 (0.4–1.2)
		Short menstrual cycle	OR 0.7 (0.3–1.5)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Romano et al. 2016 General population (n=336 women)	13.9 ng/mL (maternal median PFOS)	Breastfeeding duration ≤3 months	NS (p=0.065 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.111 for trend)
Taylor et al. 2014 General population (n=2,151 women)	>9–18.4 and >18.4 ng/mL (2nd and 3rd PFOS tertiles)	Menopause	HR 1.16 (0.91–1.48), 3rd tertile
		Hysterectomy	**HR 1.44 (1.12–1.85)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	19.47 ng/mL (median maternal PFOS)	Breastfeeding duration (in months)	**β -1.4 (-2.1 to -0.6)*, per doubling of serum PFOS levels**
		Exclusive breastfeeding (in months)	**β -0.3 (-0.6 to -0.1)*, per doubling of serum PFOS levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	8.2 and 4.9 ng/mL (geometric mean PFOS for cases and controls)	Polycystic ovary syndrome risk	**OR 5.79 (1.58–24.12, p=0.005)*, 3rd tertile**
PFHxS			
Buck Louis et al. 2012 General population (n=473)	0.48 and 0.43 ng/mL (geometric mean PFHxS in women with or without endometriosis)	Endometriosis	OR 1.14 (0.58–2.24), without parity adjustment OR 0.85 (0.42–1.73), with parity adjustment
		Risk of moderate to severe endometriosis	OR 2.12 (0.85–5.27), without parity adjustment OR 1.24 (0.47–3.31) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	2.20–19.40 ng/mL (4th quartile PFHxS)	Self-reported endometriosis	OR 1.47 (0.40–1.41), 4th quartile
Romano et al. 2016 General population (n=336 women)	1.5 ng/mL (maternal median PFHxS)	Breastfeeding duration ≤3 months	NS (p=0.124 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.087 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.9–1.8 and >1.8 ng/mL (2nd and 3rd PFHxS tertiles)	Menopause	**HR 1.42 (1.08–7.87)*, 2nd tertile**
		Hysterectomy	**HR 2.22 (1.66–2.98)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	1.45 ng/mL (median maternal PFHxS)	Breastfeeding duration (in months)	β -0.2, (-0.5–0.2), per doubling of serum PFHxS levels
		Exclusive breastfeeding (in months)	β -0.1 (-0.2–0.2), per doubling of serum PFHxS levels
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.1 and 0.7 ng/mL (geometric mean PFHxS for cases and controls)	Polycystic ovary syndrome risk	OR 1.20 (0.35–4.07), 3rd tertile
PFNA			
Buck Louis et al. 2012 General population (n=473)	0.69 and 0.58 ng/mL (geometric mean PFNA in women with or without endometriosis)	Endometriosis	**OR 2.20 (1.02–4.75)*, without parity adjustment** OR 1.99, 0.91–4.33), with parity adjustment
		Risk of moderate to severe endometriosis	OR 1.21 (0.35–4.19), without parity adjustment OR 0.99 (0.27–3.65) with parity adjustment
Campbell et al. 2016 General population (NHANES) (n=753 women aged 20–50 years)	1.20–15.40 ng/mL (4th quartile PFNA)	Self-reported endometriosis	OR 3.24 (0.81–12.91), 4th quartile
Lum et al. 2017 General population (n=501 women)	1.3, 1.2, and 1.1 ng/mL (median serum PFNA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile
Romano et al. 2016 General population (n=336 women)	0.9 ng/mL (maternal median PFNA)	Breastfeeding duration ≤3 months	NS (p=0.591 for trend)
		Breastfeeding duration ≤6 months	NS (p=0.349 for trend)

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Taylor et al. 2014 General population (n=2,151 women)	>0.80–1.5 and >1.5 ng/mL (2nd and 3rd PFNA tertiles)	Menopause	**HR 1.47 (1.14–1.90)*, 3rd tertile**
		Hysterectomy	**HR 1.39 (1.08–1.80)*, 2nd tertile**
Timmermann et al. 2017 General population (n=1,130 woman)	0.62 ng/mL (median maternal PFNA)	Breastfeeding duration (in months)	**β -1.3, (-2.0 to -0.7)*, per doubling of serum PFNA levels**
		Exclusive breastfeeding (in months)	**β -0.2 (-0.5 to -0.0)*, per doubling of serum PFNA levels**
Vagi et al. 2014 General population (n=52 cases and 50 controls)	1.2 and 0.9 ng/mL (geometric mean PFNA for cases and controls)	Polycystic ovary syndrome risk	OR 2.25 (0.67–8.00), 3rd tertile
PFDA			
Buck Louis et al. 2012 General population (n=473)	0.20 and 0.18 ng/mL (geometric mean PFDA in women with or without endometriosis)	Endometriosis	OR 2.95 (0.72–12.1), without parity adjustment OR 2.60 (0.62–10.9), with parity adjustment
		Risk of moderate to severe endometriosis	OR 0.72 (0.06–8.09), without parity adjustment OR 0.58 (0.04–7.42) with parity adjustment
Lum et al. 2017 General population (n=501 women)	0.4, 0.4, and 0.4 ng/mL (median serum PFDA in women with menstrual cycles of ≤24 days, 25–31 days, or ≥32 days	Menstrual cycle length	OR 1.01 (0.99–1.04), 3rd tertile

Table 2-20. Summary of Female Reproductive Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Timmermann et al. 2017 General population (n=1,130 woman)	0.28 ng/mL (median maternal PFDA)	Breastfeeding duration (in months)	**β -0.8 (-1.4 to -0.3) *, per doubling of serum PFDA levels**
		Exclusive breastfeeding (in months)	β -0.2 (-0.4–0.0), per doubling of serum PFDA levels

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

HR = hazard ratio; OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; RR = risk ratio

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Bach et al. 2015a General population (n=1,372 pregnant women)	2.0 ng/ml (median maternal PFOA)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 1.00 (0.98–1.01), per 0.1 ng/mL
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	5.6–7.7 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.86 (0.63–1.19), 4th quartile FR 0.74 (0.48–1.13), 4th quartile FR 0.99 (0.64–1.54), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.67 (0.70–4.00), 4th quartile OR 1.74 (0.46–6.55), 4th quartile OR 1.56 (0.55–4.42), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161 pregnant women)	4.1–5.4 ng/mL (2nd PFOA quartile) 7.2–41.5 ng/mL (4th PFOA quartile)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.78 (0.65–0.94)*, 2nd quartile** **FR 0.76 (0.59–0.96)*, 2nd quartile** **FR 0.74 (0.56–0.98)*, 4th quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.91 (1.16–3.13)*, 2nd quartile** **OR 2.30 (1.09–4.87)*, 2nd quartile** OR 1.48 (0.80–2.75), 4th quartile
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 3.112 and 5.016 ng/mL or withdrawing from study or not pregnant 3.101 and 4.749 ng/mL (geometric mean serum PFOA in females and males)	Fecundability Female serum PFOA Male serum PFOA	 OR 0.95 (0.82–1.11) OR 1.01 (0.88–1.17)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	2.79 ng/mL (geometric mean serum PFOA)	Fecundability	FR 1.15 (0.66–2.01)
Fei et al. 2009 General population (n=1,240 pregnant women)	3.91–5.20 ng/mL (2nd PFOA quartile, maternal)	Fecundability	**FOR 0.72 (0.57–0.90)*, 2nd quartile**
		Infertility	**OR 2.06 (1.22–3.51)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	3.91–5.20 and ≥6.97 ng/mL (2nd and 4th PFOA quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 **FOR 0.61 (0.46–0.80)*, 2nd quartile** FOR 0.63 (0.39–1.04), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	 **OR 3.39 (1.75–6.53)*, 2nd quartile** OR 1.30 (0.52–3.21; p=0.082 for trend), 4th quartile
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.65 ng/mL (median PFOA)	Fecundability Primiparous subgroup	FR1.04 (0.87–1.25) **FR 1.31 (1.03–1.68)***
		Infertility	OR 1.11 (0.74–1.66)
Lum et al. 2017 General population (501 couples)	≥4.20 ng/mL (3rd tertile serum PFOA)	Probability of pregnancy	OR 0.7 (0.5–1.1)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1.7 ng/mL (median maternal PFOA)	Fecundability	**FOR 0.89 (0.83–0.94, p<0.001)***
		Infertility	**OR 1.31 (1.11–1.53, p=0.001)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	5.58 and 5.61 ng/mL (median PFOA in women with no pregnancy and pregnant)	Fecundability	OR 1.18 (0.78–1.78)
		Not becoming pregnant within first six cycles	OR 1.21 (0.67–2.18)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>19.6–72.1 ng/mL (3rd tertile serum PFOA)	Endometriosis-related infertility	OR 1.05 (0.58–1.91)
Whitworth et al. 2012b General population (n=416 subfecund pregnant women and 474 controls)	1.66–2.24, 2.25–3.02, and ≥3.02 ng/mL (2nd, 3rd, and 4th PFOA quartiles)	Infertility Parous subgroup Primiparous subgroup	**OR 1.6 (1.1–2.3)*, 2nd quartile** **OR 2.4 (1.4–4.1)*, 3rd quartile** OR 0.5 (0.2–1.2), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	2.8 ng/mL (maternal median serum PFOA)	Fecundability	OR 1.0 (0.90–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Bach et al. 2015a General population (n=1,372 pregnant women)	8.3 ng/ml (median maternal PFOS) 10.85–36.10 ng/mL (4th PFOS quartile)	Fecundability	FR 1.09 (0.92–1.30), 4th quartile
		Infertility risk	OR 0.71 (0.47–1.07), 4th quartile
Bach et al. 2015c, 2015d General population (n=440 pregnant women)	36.3–103.8 ng/mL (4th PFOS quartile)	Fecundability Parous subgroup Nulliparous subgroup	FR 0.96 (0.75–1.24), 4th quartile FR 1.04 (0.70–1.55), 4th quartile FR 0.97 (0.62–1.51), 4th quartile
		Infertility Parous subgroup Nulliparous subgroup	OR 1.03 (0.54–2.00), 4th quartile OR 0.70 (0.16–3.11), 4th quartile OR 1.23 (0.452–3.39), 4th quartile
Bach et al. 2015c, 2015d (re-analysis of Fei et al. 2009, 2012 data) General population (n=1,161)	27.0–34.2, 34.3–43.8, and 43.9–106.7 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	Fecundability Parous subgroup Nulliparous subgroup	**FR 0.79 (0.66–0.95)*, 2nd quartile** FR 0.90 (0.70–1.14), 4th quartile **FR 0.68 (0.52–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	**OR 1.65 (1.01–2.68)*, 2nd quartile** OR 1.60 (0.78–3.28), 4th quartile **OR 2.71 (1.38–5.30)*, 3rd quartile**
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 11.764 and 20.867 ng/mL or withdrawing from study or not pregnant 11.088 and 19.765 ng/mL (geometric mean serum PFOS in females and males)	Fecundability Female serum PFOS Male serum PFOS	 OR 0.99 (0.85–1.17) OR 0.96 (0.80–1.15)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	9.29 ng/mL (geometric mean serum PFOS)	Fecundability	FR 0.89 (0.49–1.60)
Fei et al. 2009 General population (n=1,240 pregnant women)	26.1–33.3 ng/mL (2nd PFOS quartile, maternal)	Fecundability	**OR 0.70 (0.56–0.87)*, 2nd quartile**
		Infertility	**OR 1.70 (1.01–2.86)*, 2nd quartile**

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fei et al. 2012 (re-analysis of Fei et al. 2009 data) General population (n=1,240 pregnant women)	26.1–33.3 and 33.4–43.2 ng/mL (2nd and 3rd PFOS quartiles, maternal)	Fecundability Parous subgroup Nulliparous subgroup	 NS (p=0.32 for trend) **FOR 0.63 (0.43–0.91)*, 3rd quartile**
		Infertility Parous subgroup Nulliparous subgroup	 NS (p=0.26 for trend) **OR 2.50 (1.16–5.37, p=0.36 for trend)*, 3rd quartile**
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	10.60 ng/mL (median PFOS)	Fecundability	FR 0.90 (0.76–1.07)
		Infertility	OR 1.39 (0.93–2.07)
Lum et al. 2017 General population (501 couples)	≥15.20 ng/mL (3rd tertile serum PFOS)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vélez et al. 2015 General population (n=1,743 pregnant women)	4.7 ng/mL (median maternal PFOS)	Fecundability	FOR 0.96 (0.91–1.02, p=0.17)
		Infertility	OR 1.14 (0.98–1.34, p=0.09)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	35.75 and 36.29 ng/mL (median PFOS in women with no pregnancy and pregnant)	Fecundability	NS (p=0.29)
		Not becoming pregnant within first six cycles	OR 0.98 (0.54–1.77)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>9.36–138 ng/mL (3rd tertile serum PFOS)	Endometriosis-related infertility	OR 0.66 (0.36–1.21)
Whitworth et al. 2012b General population (n=416 subfecund women and 474 controls)	10.34–16.60 and ≥16.61 ng/mL (3rd and 4th PFOS quartile)	Infertility Parous subgroup Primiparous subgroup	**OR 1.4 (1.0–2.0)*, 3rd quartile** **OR 2.1 (1.2–3.8)*, 4th quartile** OR 0.7 (0.4–1.3), 4th quartile
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	14.6 ng/mL (maternal median serum PFOS)	Fecundability	OR 1.00 (0.88–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.5 ng/ml (median maternal PFHxS)	Fecundability	FR 1.00 (0.99–1.01), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.93–1.03), per 0.1 ng/mL
Crawford et al. 2017 General population (n=99 30–44-year-old women)	1.59 ng/mL (geometric mean serum PFHxS)	Fecundability	0.84 (0.46–1.54)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	1.94 ng/mL (median PFHxS)	Fecundability	FR 0.97 (0.85–1.11)
		Infertility	OR 0.99 (0.73–1.33)
Vélez et al. 2015 General population (n=1,743 pregnant women)	1 ng/mL (median maternal PFHxS)	Fecundability	**FOR 0.91 (0.86–0.97, p=0.002)***
		Infertility	**OR 1.27 (1.09–1.48, p=0.003)***
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	1.12 and 1.22 ng/mL (median PFHxS in women with no pregnancy and pregnant)	Fecundability	**OR 1.33 (1.01–1.75)***
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.20)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.39–1.69 ng/mL (3rd tertile serum PFHxS)	Endometriosis-related infertility	**OR 0.47 (0.26–0.87)***
Whitworth et al. 2016 General population (n=450 primiparous pregnant women)	7.0 ng/mL (maternal median serum PFHxS)	Fecundability	OR 0.97 (0.90–1.1)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFNA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.8 ng/mL (median maternal)	Fecundability	FR 1.00 (0.98–1.02), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.95–1.03), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 1.176 and 1.558 ng/mL or withdrawing from study or not pregnant 1.112 and 1.422 ng/mL (geometric mean serum PFNA in females and males)	Fecundability Female serum PFNA Male serum PFNA	 OR 1.00 (0.84–1.19) OR 1.09 (0.90–1.32)
Crawford et al. 2017 General population (n=99 30–44-year-old women)	0.84 ng/mL (geometric mean serum PFNA)	Fecundability	1.40 (0.79–2.49)
Jørgensen et al. 2014a, 2014b General population (n=938 pregnant women)	0.64 ng/mL (median PFNA)	Fecundability Primiparous subgroup	**FR 0.80 (0.69–0.94)*** FR 0.99 (0.88–1.22)
		Infertility	**OR 1.53 (1.08–2.15)***
Lum et al. 2017 General population (501 couples)	≥1.50 ng/mL (3rd tertile serum PFNA)	Probability of pregnancy	OR 0.8 (0.6–1.2)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.45 and 0.51 ng/mL (median PFNA in women with no pregnancy and pregnant)	Fecundability	OR 1.17 (0.88–1.54)
		Not becoming pregnant within first six cycles	OR 0.67 (0.37–1.25)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.50–7.10 ng/mL (3rd tertile serum PFNA)	Endometriosis-related infertility	**OR 0.52 (0.28–0.95)***

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=451 primiparous pregnant women)	0.43 ng/mL (maternal median serum PFNA)	Fecundability	OR 1.1 (0.92–1.3)
PFDA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFDA)	Fecundability	FR 1.00 (0.97–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.99 (0.92–1.07), per 0.1 ng/mL
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.385 and 0.448 ng/mL or withdrawing from study or not pregnant 0.349 and 0.416 ng/mL (geometric mean serum PFDA in females and males)	Fecundability Female serum PFDA Male serum PFDA	 OR 1.11 (0.95–1.29) OR 1.08 (0.93–1.26)
Lum et al. 2017 General population (501 couples)	≥0.05 ng/mL (3rd tertile serum PFDA)	Probability of pregnancy	OR 0.9 (0.6–1.3)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median PFDA in women with no pregnancy and pregnant)	Fecundability	OR 1.15 (0.89–1.49)
		Not becoming pregnant within first six cycles	OR 0.61 (0.33–1.12)
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.79–11.2 ng/mL ng/mL (3rd tertile serum PFDA)	Endometriosis-related infertility	OR 0.74 (0.40–1.35)
Whitworth et al. 2016 General population (n=429 primiparous pregnant women)	0.11 ng/mL (maternal median serum PFDA)	Fecundability	OR 1.00 (0.85–1.2)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Bach et al. 2015a General population (n=1,372 pregnant women)	0.3 ng/mL (median maternal PFUnA)	Fecundability	FR 1.01 (0.98–1.03), per 0.1 ng/mL
		Infertility risk	OR 0.98 (0.92–1.04), per 0.1 ng/mL
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>1.42–5.34 ng/mL (3rd tertile serum PFUnA)	Endometriosis-related infertility	OR 0.61 (0.33–1.13)
Whitworth et al. 2016 General population (n=447 primiparous pregnant women)	0.23 ng/mL (maternal median serum PFUnA)	Fecundability	OR 0.93 (0.78–1.1)
PFHpA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.11–0.66 ng/mL (3rd tertile serum PFHpA)	Endometriosis-related infertility	**OR 0.48 (0.26–0.86)***
PFBS			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.086–0.094 ng/mL (2nd tertile serum PFBS)	Endometriosis-related infertility	**OR 3.74 (2.04—6.84)***
PFDoDA			
Wang et al. 2017 General population (n=157 women with endometriosis-related infertility and 178 controls)	>0.27–1.02 ng/mL (2nd tertile serum PFDoDA)	Endometriosis-related infertility	OR 0.61 (0.34–1.11)

Table 2-21. Summary of Fertility Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Whitworth et al. 2016 General population (n=410 primiparous pregnant women)	0.04 ng/mL (maternal median serum PFDoDA)	Fecundability	OR 0.91 (0.77–1.1)
FOSA			
Buck Louis et al. 2013 General population (n=501 couples)	Couples achieving pregnancy: 0.110 and 0.112 ng/mL or withdrawing from study or not pregnant 0.126 and 0.129 ng/mL (geometric mean serum FOSA in females and males)	Fecundability Female serum FOSA Male serum FOSA	 **OR 0.81 (0.70–0.94)*** OR 0.89 (0.78–1.02)
Vestergaard et al. 2012 General population (n=222 nulliparous couples)	0.10 and 0.11 ng/mL (median FOSA in women with no pregnancy and pregnant)	Fecundability Not becoming pregnant within first six cycles	OR 1.01 (0.86–1.18) OR 0.81 (0.45–1.46)
Whitworth et al. 2016 General population (n=226 primiparous pregnant women)	0.03 ng/mL (maternal median serum FOSA)	Fecundability	OR 0.91 (0.71–1.2)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 12 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

FOR = fecundability odds ratio; FOSA = perfluorooctane sulfonamide; FR = fecundability ratio (probability of conceiving during a given menstrual cycle); OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Studies in laboratory animals have evaluated the potential histological alterations in reproductive tissues, alterations in reproductive hormones, and impaired reproductive functions. Summaries of these studies are presented in Tables 2-1, 2-3, 2-4, 2-5, and 2-6 and in Figures 2-6, 2-8, 2-9, and 2-10. Multigeneration studies on PFOA, PFOS, and PFBS have not found alterations in reproductive parameters in animals; similarly, no effect on fertility was observed for PFHxS or PFDoDA. One study found alterations in sperm parameters and decreases in fertility in mice exposed to PFNA. An increase in the incidence of Leydig cell hyperplasia (reclassified as gonadal stromal hyperplasia) has been observed in animals exposed to PFOA; one study for PFDoDA reported ultrastructural alterations in the testes. Studies on PFOS, PFHxS, PFBS, and PFBA have not found histological alterations. Delays in mammary gland development have been observed in mice exposed to PFOA; this effect has also been observed in perinatally exposed mice (see Section 2.17, Developmental). No laboratory animal studies examined reproductive endpoints for PFDA, PFUnA, PFHpA, or FOSA.

PFOA

Epidemiological Studies—Reproductive Hormone Levels. Three studies have evaluated potential effects of PFOA exposure on reproductive hormone levels in workers (Gilliland 1992; Olsen et al. 1998b; Sakr et al. 2007b). Sakr et al. (2007b) found associations between serum PFOA and estradiol and testosterone levels in male workers at the Washington Works facility. Similarly, Gilliland (1992) found associations between serum fluorine levels and estradiol and prolactin levels and inverse associations with bound and free testosterone levels in workers at the 3M Cottage Grove facility. In contrast, Olsen et al. (1998b) did not find associations between serum PFOA and estradiol or testosterone in male workers at the 3M Cottage Grove facility. The study did find an association with prolactin levels, but this was only found in workers examined in 1993, but not in those examined in 1995. In a general population study of men aged 30–66 years of age, correlations were found between serum PFOA levels and free testosterone levels and LH levels; no correlations were found for estradiol, prolactin, follicle stimulating hormone (FSH), or total testosterone levels (Raymer et al. 2012). Another study of similar aged men did not find an association between serum PFOA and sex hormone binding globulin levels (Specht et al. 2012). Studies of young men (median age 19 years) (Joensen et al. 2013) or adolescents and young men (12–30 years of age) (Tsai et al. 2015) did not find associations between serum PFOA and reproductive hormone levels. A third study (Vested et al. 2013) found an association between LH and FSH levels and maternal serum PFOA levels in young adult males; other hormones were not affected. A fourth study in adolescents (aged 13–15 years) found an association between serum PFOA and estradiol levels in boys, but not in girls, and did not find associations for testosterone levels (Zhou et al. 2016).

Two studies of women (Barrett et al. 2015; Knox et al. 2011b) did not find associations with estradiol levels or luteal progesterone levels. A third study of adolescent and young women (Tsai et al. 2015) found an association between serum PFOA and sex hormone binding globulin levels in adolescents (12–17 years), but not in young adults; no associations with FSH or testosterone were observed in either group.

Epidemiological Studies—Effects on Sperm. Six general population studies have evaluated the potential alterations in sperm parameters associated with PFOA exposure. Although some associations have been found, the results are not consistent across studies. Buck Louis et al. (2015) reported an increase in curvilinear velocity and some alterations in sperm morphology that were associated with serum PFOA levels. Toft et al. (2012) found a PFOA-related increase in the percentage of motile sperm in men with serum PFOA levels in the 3rd tertile. Vested et al. (2013) reported inverse associations between maternal serum PFOA levels and sperm concentration and total sperm count in young adults; no alterations in motility or morphology were observed. Other studies did not find alterations in sperm viability, count, concentration, motility, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012) or the Y-X chromosome ratio (Kvist et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Two studies examined possible associations between serum PFOA levels and alterations in menstrual cycle length. An increased risk of a long menstrual cycle (≥32 days) was observed in women with serum PFOA levels in the 3rd tertile and when serum PFOA was used as a continuous variable (Lyngsø et al. 2014). No alterations in the risk of having a short menstrual cycle (≤24 days) or irregular menstrual cycles (≥7 days difference between cycles) were observed. The second study did not find an association between serum PFOA and menstrual cycle length (Lum et al. 2017).

Four studies have evaluated the risk of early menopause. In a study of C8 Health Study participants, increases in the risk of early menopause was observed in perimenopausal (>42–≤51 years of age) and menopausal (>51–≤65 years of age) women with serum PFOA levels in the 2nd, 3rd, 4th, and 5th quintiles (Knox et al. 2011b). An increase in menopause risk was also observed in a cross-sectional study of NHANES participants with serum PFOA levels in the 3rd tertile (Taylor et al. 2014). Taylor et al. (2014) also found a higher risk of hysterectomy among women with serum PFOA levels in the 2nd and 3rd tertiles. Findings of higher levels of PFOA (and other perfluoroalkyls) among women with hysterectomies and that serum PFOA levels increased after menopause provide suggestive evidence that at least part of the

association may be due to reverse causation (Taylor et al. 2014). In contrast, no alterations in the risk of early menopause or age of menopause were associated with estimated cumulative serum PFOA levels in retrospective and prospective studies of C8 Health Study participants (Dhingra et al. 2016a); age of menopause was also not associated with measured serum PFOA levels in the prospective study (Dhingra et al. 2016a). Cross-sectional analysis also showed that early menopause was associated with measured serum PFOA levels, but not with modeled serum PFOA levels (Dhingra et al. 2017), providing support that reverse causation may contribute to the observed association.

Buck Louis et al. (2012) showed that the risk of endometriosis and the risk of moderate-to-severe endometriosis were associated with serum PFOA levels; however, adjustment for parity resulted in confidence intervals that included unity. A second study found an increased risk of self-reported endometriosis in women with serum PFOA levels in the 3rd quartile; for the 4th quartile women, the confidence intervals included unity (Campbell et al. 2016). A case-control study (Vagi et al. 2014) found an increased risk of polycystic ovary syndrome among women with serum PFOA levels in the 3rd tertile.

Two studies utilizing pharmacokinetic modeling have investigated whether the observed associations between PFOA exposure and early onset menopause or risk of endometriosis was due to reverse causation (Ngueta et al. 2017; Ruark et al. 2017). As discussed in Section 3.1.4, menstrual blood loss is a route of elimination of perfluoroalkyls. Therefore, variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOA levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

Three studies evaluated a possible association between maternal PFOA levels and breastfeeding duration. Two studies found increases in the risk of breastfeeding ≤3 or 6 months that were associated with maternal PFOA levels (Fei et al. 2010; Romano et al. 2016). Timmermann et al. (2017) found an inverse association between maternal PFOA levels and the duration of breastfeeding and the amount of time the women exclusively breastfed. Fei et al. (2010) reported that when the women were segregated by parity, the associations were only found in multiparous women. In contrast, Timmermann et al. (2017) found no differences in duration or breastfeeding exclusiveness between primiparous and multiparous women. It is noted that a number of factors can influence the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice. In general, these studies did not consider whether these factors may have influenced the observed associations.

Epidemiological Studies—Effects on Fertility. Several general population studies have examined the possible association between female serum PFOA levels and decreased fertility or infertility; the results are graphically presented in Figures 2-31 and 2-32, respectively. With the exception of the Buck Louis et al. (2013) and Vestergaard et al. (2012) prospective studies, all of the women were pregnant; thus, couples with unresolved infertility are underrepresented in these analyses. Maternal transfer of PFOA during pregnancy and lactation can result in lower serum PFOA levels in women (see Section 3.1.2 for additional information), as compared to nulliparous women; thus, parity should be considered when evaluating potential associations between serum PFOA and infertility. The Buck Louis et al. (2013) study is the only study that used maternal and paternal serum PFOA levels as the biomarkers of exposure. Most of the studies evaluated two aspects of fertility: fecundability, which is a measure of time to pregnancy, and risk of infertility, which is typically time to pregnancy of >12 months.

In a study of pregnant women participating in the Danish National Birth Cohort study, a decrease in fecundability and an increase in infertility were observed in women with serum PFOA (measured at gestation week 12) levels in the three highest quartiles (Fei et al. 2009). When the women were categorized by parity, decreased fecundability OR and increased infertility OR were only found in the parous group; the ORs for the nulliparous women included unity (Fei et al. 2009). A second re-analysis of these data (Bach et al. 2015a) using a different statistical approach confirmed the results of the whole group and the parous subgroup; this re-analysis also found a decrease in the fecundability risk among the nulliparous women. In another set of women participating in the Danish National Birth Cohort study (Bach et al. 2015c), no alterations in fecundability or infertility risk were observed in the whole cohort or when the women were categorized into parous and nulliparous subcohorts. It was noted that the median serum PFOA levels in this second study (4.0 ng/mL) were lower than the levels in the larger study (5.4 ng/mL). A decrease in fecundability and an increase in infertility risk were also observed in a Canadian study of pregnant women (Vélez et al. 2015). An increase in infertility risk was also found in a Norwegian study of subfecund pregnant women with serum PFOA levels in the three highest quartiles (Whitworth et al. 2012b); when the women were categorized based on parity, the infertility risk was only elevated in the parous women with serum PFOA levels in the 3rd and 4th quartiles. A multinational study also found an alteration in fecundability (Jørgensen et al. 2014a); however, this study found that higher serum PFOA levels resulted in a decrease in the time to pregnancy (fecundability ratio >1) among primiparous women.

Figure 2-31. Fecundability Relative to PFOA Levels (Presented as Adjusted Fecundability Ratios)

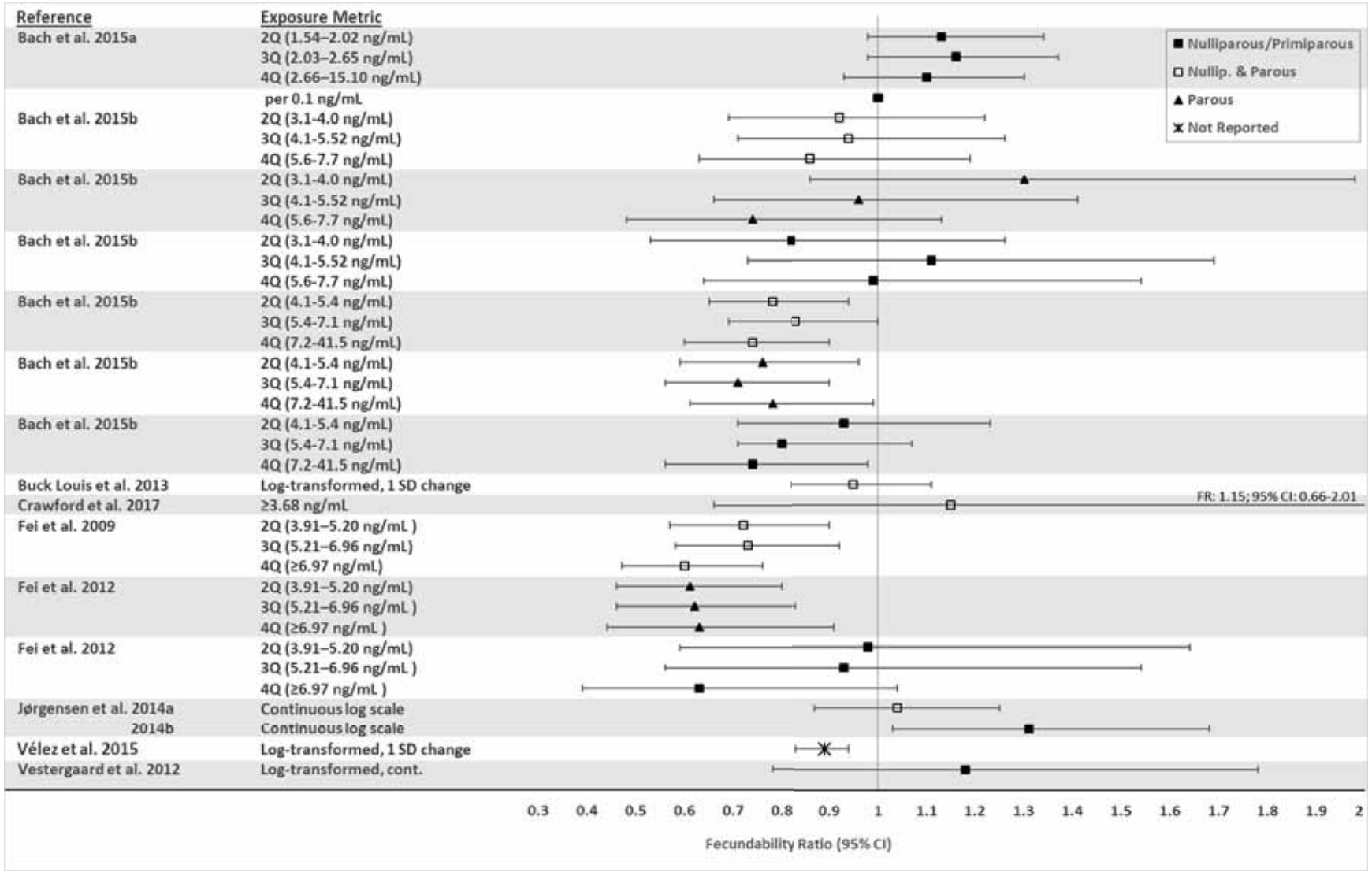
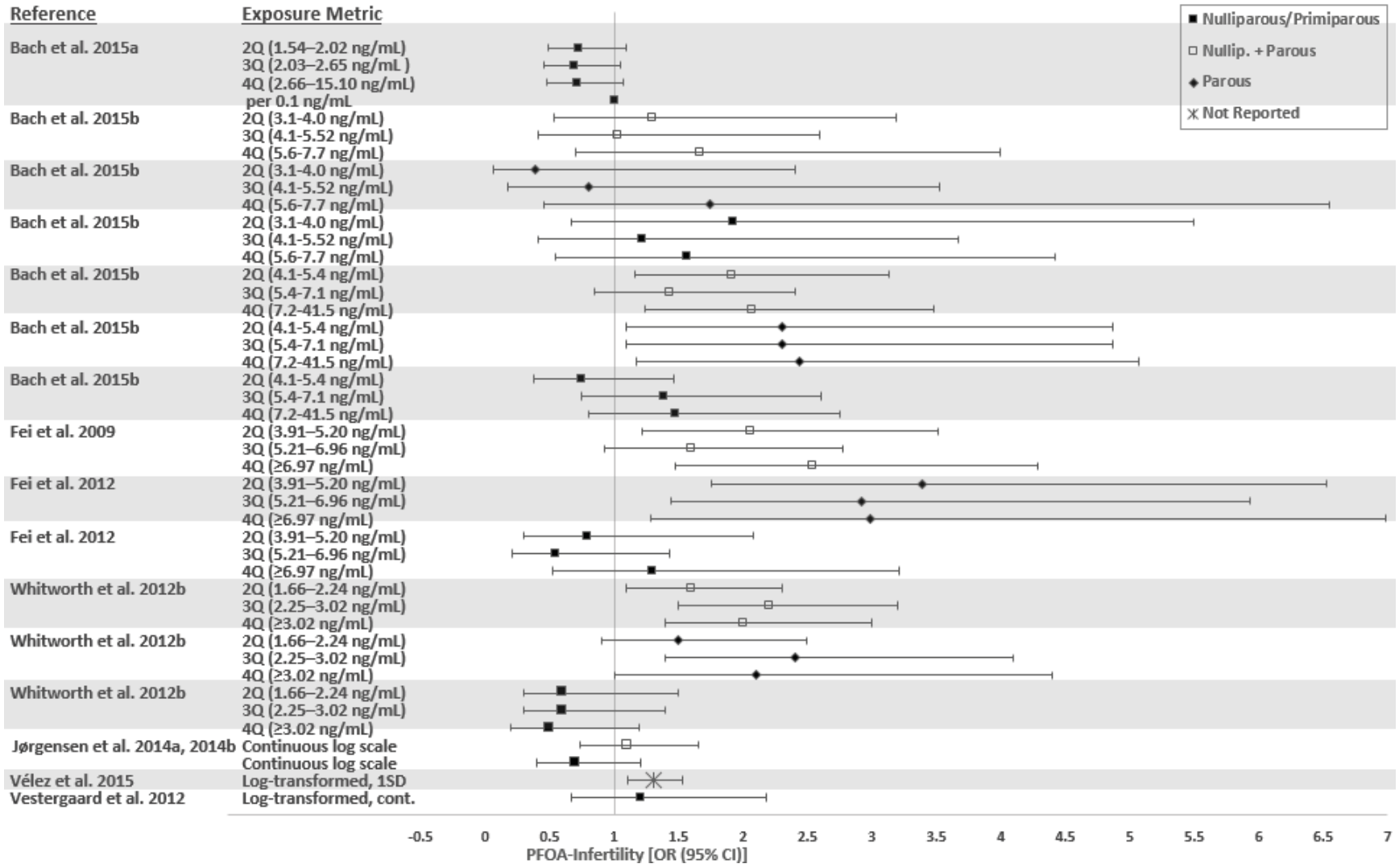


Figure 2-32. Infertility Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



Other studies of pregnant women have not found alterations in fecundability, fertility, and/or infertility (Bach et al. 2015a; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Wang et al. 2017; Whitworth et al. 2016). The two prospective studies which followed women intending to get pregnant for 6 months (Vestergaard et al. 2012) or 12 months (Buck Louis et al. 2013) also did not find associations between serum PFOA levels in women and fecundability; Buck Louis et al. (2013) also found no association when male serum PFOA was used as the biomarker of exposure.

Laboratory Animal Studies. Examination of the testes and epididymides of rats exposed intermittently head-only to up to 84 mg/m^3 APFO dusts for 2 weeks did not reveal any gross or microscopic treatment-related alterations (Kennedy et al. 1986).

Several studies have been conducted in rats to examine whether induction of Leydig cell tumors could be due to an endocrine-related mechanism. In a 14-day gavage study in which rats were dosed with up to 50 mg/kg/day PFOA, testes weight was not significantly affected and microscopic examination did not reveal any significant alterations (Cook et al. 1992). However, the weight of the accessory sex organ unit (ventral and dorsal lateral prostate, seminal vesicles, and coagulating glands) was significantly decreased in rats dosed with 25 mg/kg/day PFOA (17% decrease) and 50 mg/kg/day PFOA (18% decrease) relative to controls and to a pair-fed group. There was also a trend for reduced serum and interstitial fluid testosterone in PFOA-treated rats; serum LH was not altered and estradiol was significantly increased (63%) at ≥10 mg/kg/day. Challenge experiments conducted with human chorionic gonadotropin, gonadotropin-releasing hormone, or naloxone suggested that the decrease in serum testosterone was due to a lesion at the level of the testes. Serum levels of progesterone and 17α-hydroxyprogesterone were not altered by 50 mg/kg/day PFOA, but androstenedione levels were reduced 2-fold. The data suggested that the decrease in serum testosterone may be due to a decrease in the conversion of 17α-hydroxy-progesterone to androstenedione, and this could be attributed to the elevated serum levels of estradiol. The decrease in weight of the accessory sex organ unit could also be attributed to the elevated estradiol serum levels. In a subsequent study from the same group of investigators, rats dosed with 25 mg/kg/day PFOA for 14 days showed a significant increase in estradiol in serum and in testicular interstitial fluid relative to controls (Biegel et al. 1995). Treatment with PFOA for 14 days significantly increased aromatase activity in the liver (aromatase converts testosterone to estradiol), but not in testes, muscle, or adipose tissue, suggesting that PFOA increases serum estradiol by inducing aromatase activity in the liver. Treatment with PFOA also increased testicular interstitial fluid transforming growth factor α (TGFα). Collectively, the results were consistent with the hypothesis that increased estradiol levels ultimately produce Leydig cell hyperplasia and adenoma by acting as a mitogen or enhancing growth

factor secretion. A study of the dose-response relationship for PFOA and serum estradiol reported a significant increase in serum estradiol in rats dosed with ≥2 mg/kg/day, which was well correlated with total hepatic aromatase activity (Liu et al. 1996). Significant increases in serum estradiol were also reported during the first year of treatment of male rats with 13.6 mg/kg/day PFOA in a 2-year dietary study (Biegel et al. 2001).

Significant increases in the incidence of Leydig cell hyperplasia were observed in rats exposed to 13.6 mg/kg/day PFOA in the diet for 2 years (Biegel et al. 2001). Another 2-year study found an increased incidence of vascular mineralization in the testes of rats exposed to 15 mg/kg/day PFOA in the diet; no effects were observed at 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). In female rats, increases in the incidence of tubular hyperplasia of the ovaries were observed following a 2-year exposure to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c). A peer review of the histological slides from this study (3M 1983; Butenhoff et al. 2012c) concluded that the more current nomenclature for the tubular hyperplasia was gonadal stromal hyperplasia (Mann and Frame 2004). Additionally, the peer reviewers substantially disagreed with the incidence of lesions in the 1.5 mg/kg/day group and slightly disagreed with the incidence in the 15 mg/kg/day group. Based on the incidence reported by the peer reviewers, no statistically significant increases in the occurrence of gonadal stromal hyperplasia were observed in either group; a significant increase in grade 3 and above lesions were observed in the 15 mg/kg/day group.

In a 2-generation reproduction study in which male and female rats were dosed with up to 30 mg/kg/day PFOA by gavage in water for 70 days before mating and until sacrifice, there were no effects on estrous cycling, sperm number and quality, mating and fertility, or histopathology of the reproductive organs assessed in the parental and F1 generations (Butenhoff et al. 2004b). Intermediate-duration studies of rats and monkeys also did not find gross or microscopic alterations in the sex organs at termination; Cynomolgus monkeys were dosed with up to 20 mg/kg/day PFOA for 4 or 26 weeks (Butenhoff et al. 2002; Thomford 2001), Rhesus monkeys with up to 100 mg/kg/day PFOA for 13 weeks (Griffith and Long 1980), and rats with up to approximately 100–110 mg/kg/day PFOA for 13 weeks (Griffith and Long). Serum levels of estradiol and estriol were not significantly altered in the 4-week study conducted by Thomford (2001), but estrone was reduced in monkeys dosed with 2 and 20 mg/kg/day PFOA; no possible explanation was discussed. In the 26-week study (Butenhoff et al. 2002), no treatment-related alterations were reported in serum estrone, estriol, estradiol, or testosterone, indicating that the reduced serum estrone levels in the 4-week study was transitory. In 2-year dietary studies in rats, doses of 13.6 mg/kg/day PFOA significantly increased the incidence of Leydig cell hyperplasia (Biegel et al. 2001), whereas 15 mg/kg/day increased the incidence of vascular mineralization in the testes and

1.5 mg/kg/day increased the incidence of tubular hyperplasia in the ovaries (3M 1983; Butenhoff et al. 2012c).

A study in pregnant mice dosed with 5 mg/kg/day PFOA (only dose level tested) reported that the mammary gland showed changes suggesting substantial delay (possibly up to 10 days) in gland differentiation on PND 20 and alterations in milk protein gene expression on PND 20 (White et al. 2007). Subsequent studies by this group support the finding of delayed mammary gland differentiation. On PND 1, the mammary glands of mice administered 5 mg/kg/day on GDs 8–17 appeared immature; the morphology was similar to that seen in late pregnancy prior to parturition and the initiation of nursing (White et al. 2009). Another study found that the normal weaning-induced mammary gland involution was compromised on PND 22 in mice exposed to 1 mg/kg/day on GDs 1–17 or 0.001 mg/kg/day administered on GD 7–PND 22 (White et al. 2011); the investigators noted that the mammary gland structure was similar to mammary gland tissue at or near the peak of lactation (PND 10). Necrosis was observed in the placenta of mice administered via gavage 10 or 25 mg/kg/day PFOA on GDs 11–16 (Suh et al. 2011); no alterations were observed at 2 mg/kg/day.

A study of pregnant mice reported increases in serum estradiol levels, with no changes in progesterone levels, at 10 mg/kg/day when PFOA was administered on GDs 1–7 (Chen et al. 2017b); however, when PFOA was administered on GD 13, there were significant decreases in serum progesterone levels at 5 and 10 mg/kg/day with no changes in estradiol levels (Chen et al. 2017b). In peripubertal female mice, administration of 5 mg/kg PFOA 5 days/week for 4 weeks resulted in significant increases in serum progesterone levels during estrus and preestrus, but no changes in estradiol levels were observed (Zhao et al. 2010).

No gross or microscopic alterations were reported in the testes from rats dermally exposed to 2,000 mg/kg/day APFO (Kennedy 1985).

Summary. Epidemiological studies have examined a several types of reproductive endpoints. Due to inconsistent results, the available data are not suitable for determining whether there are associations between serum PFOA and reproductive hormones or effects on sperm. There is some suggestive evidence that increases in serum PFOA levels can result in earlier onset of menopause; however, this is based on the findings of two studies (a third study did not find an association) and may partially be due to reverse causation. Several general population studies found associations between serum PFOA and impaired fertility (increased time to pregnancy and/or infertility), while others have not found

associations. The available epidemiological data are considered inadequate for determining whether there is an association between serum PFOA and fertility. The database limitations include inconsistency across studies, small number of studies including measurements of male serum PFOA levels, findings in parous women but not nulliparous women, and the underrepresentation of couples not becoming pregnant. The results of a multi-generational study in rats do not suggest that the reproductive system is a sensitive target of PFOA toxicity. Additionally, histological alterations have not been observed in monkeys or rats following intermediate and/or chronic oral exposure.

PFOS

Epidemiological Studies—Reproductive Hormone Levels. In an occupational exposure study of workers at 3M Decatur and Antwerp facilities (Olsen et al. 1998a) and a general population study (Raymer et al. 2012), no associations between serum PFOS and reproductive hormones were found. Studies in adolescent and young adult males have found inverse associations between serum PFOS levels and total and free testosterone levels (Joensen et al. 2013), free androgen index (Joensen et al. 2013), and FSH levels (Tsai et al. 2015). Another study of young men did not find alterations in reproductive hormone levels (Vested et al. 2013).

In a study of females participating in the C8 Health Studies, serum PFOS levels were inversely associated with estradiol levels in both perimenopausal and menopausal women (Knox et al. 2011b). An inverse association with follicular estradiol levels was also observed in a general population study (Barrett et al. 2015); when segregated by parity, the inverse association was only found in nulliparous women. An inverse association between serum PFOS levels and testosterone levels was observed in adolescent females; no association was found in older females (Tsai et al. 2015). A general population study of adolescents (aged 13–15 years) found an inverse association between serum PFOS levels and testosterone levels in boys, but not in girls; the study also found no associations with estradiol levels in boys or girls (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. The available general population data do not provide evidence that PFOS damages sperm. One study (Buck Louis et al. 2015) found an association for one measure of sperm motility (distance travelled) but not for other measures. Another study (Toft et al. 2012) found an inverse association between serum PFOS levels and percentage of normal sperm. Other studies have not found alterations in sperm viability, count, motility, volume, or morphology (Buck Louis et al. 2015; Joensen et al. 2013; Raymer et al. 2012; Toft et al. 2012; Vested et al. 2013). A multinational

study (Kvist et al. 2012) found a nonlinear association between serum PFOS and Y-X chromosome ratio; however, when categorized by country, the only significant trend was a negative trend in the Greenland cohort. It is noted that these are studies of individuals exposed to background levels of PFOS, involved a single measurement of PFOS, and are not adequate for establishing causality.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No alterations in the risk of irregular, short, or long menstrual cycle lengths associated with serum PFOS levels were observed in a study of pregnant women (Lyngsø et al. 2014). Similarly, no association between serum PFOS levels and menstrual cycle length was observed in another study (Lum et al. 2017). A study of C8 Health Study participants found increases in the risk of early menopause in perimenopausal and menopausal women with serum PFOS levels in the $\geq 3^{rd}$ and $\geq 2^{nd}$ quintiles, respectively (Knox et al. 2011b). In contrast, a study of NHANES participants did not find an association between serum PFOS and the risk of early menopause (Taylor et al. 2014). The risk of endometriosis was not associated with serum PFOS levels (Buck Louis et al. 2012; Campbell et al. 2016). However, there was a greater risk of having moderate to severe endometriosis; adjusting for parity decreased the risk and the CIs included unity. General population studies found increases in the risk of having a hysterectomy in women having serum PFOS levels in the 2^{nd} and 3^{rd} tertiles (Taylor et al. 2014) and the risk of having polycystic ovary syndrome in women with serum PFOS levels in the 3^{rd} tertile (Vagi et al. 2014). Most of these endpoints were only examined in one study and the evidence is inconclusive to determine whether there is an association between PFOS exposure and these female reproductive outcomes.

Utilizing pharmacokinetic modeling, Ruark et al. (2017) and Ngueta et al. (2017) have investigated whether the observed associations between PFOS exposure and early onset menopause or risk of endometriosis was due to reverse causation. Menstrual blood loss is a route of elimination of perfluoroalkyls (see Section 3.1.4) and variability in menstruation such as menarche, menopause, and pharmacological management of menstruation (e.g., use of oral contraceptives) could affect serum perfluoroalkyl levels, and thereby contribute to observed statistical associations between serum PFOS levels and early onset menopause (Ruark et al. 2017) or endometriosis (Ngueta et al. 2017) outcomes.

Maternal serum PFOS levels have been associated with increases in the risk of breastfeeding for ≤3 or 6 months (Fei et al. 2010; Romano et al. 2016) and inversely associated with the length of breastfeeding and the length of exclusive breastfeeding (Timmermann et al. 2017). When the women were segregated by parity, the associations were only found in multiparous women (Fei et al. 2010). In contrast,

Timmermann et al. (2017) found no significant alterations in breastfeeding length or exclusiveness between primiparous women and multiparous women. In general, these studies did not consider whether other factors such as the duration of breastfeeding including diminished milk production, inadequate lactation support from health care providers after delivery, use of medication that is not compatible with breastfeeding, lack of spousal/family support, and individual choice may have influenced the observed associations. Additionally, the associations between maternal PFOS and breastfeeding duration may be due to reverse causality since longer breastfeeding would likely result in lower maternal PFOS levels.

Epidemiological Studies—Effects on Fertility. Several general population studies have evaluated whether there is a possible association between serum PFOS and time-to-pregnancy (as measured using a fecundability ratio) or infertility; graphical presentations of potential associations between fecundability and infertility relative to serum PFOA levels are presented in Figures 2-33 and 2-34, respectively. A couple of studies have found associations, but most have not found associations. Fei et al. (2009) found decreases in fecundability and increases in infertility risk among pregnant women with serum PFOS levels in the top three quartiles. When the women were categorized by parity (Fei et al. 2012), the decrease in fecundability and increase in infertility risk were only observed in nulliparous women with serum PFOS levels in the 3rd and 4th quartiles; no alterations were observed among parous women. A re-analysis of these data (Bach et al. 2015c) resulted in similar associations between PFOS and fecundability and infertility. Whitworth et al. (2012b) also found an increased risk of infertility among subfecund women with serum PFOS levels in the 3rd quartile; categorizing by parity resulted in increases in only parous women with serum PFOS levels in the 4th quartile. In contrast, other studies have not found alterations in fecundability or fertility associated with maternal serum PFOS levels (Bach et al. 2015a, 2015c; Buck Louis et al. 2013; Crawford et al. 2017; Jørgensen et al. 2014a; Lum et al. 2017; Vélez et al. 2015; Vestergaard et al. 2012; Wang et al. 2016; Whitworth et al. 2016).

Laboratory Animal Studies. Significant decreases in serum testosterone levels and epididymal sperm count were observed in mice administered 10 mg/kg/day PFOS for 21 days (Wan et al. 2011), in rats administered 5 mg/kg/day for 21 days (Li et al. 2018), and in mice administered 10 mg/kg/day for 5 weeks (Qu et al. 2016). No alterations were observed in mice administered 5 mg/kg/day PFOS or in mice administered 5 or 10 mg/kg/day PFOS for 14 days (Wan et al. 2011). No alterations in reproductive performance (number of litters, gestation length, number of implantation sites, or potential resorptions) were observed in rats administered 1 mg/kg/day PFOS throughout gestation and lactation (Buttenoff et al. 2009b). Lee et al. (2015a) did find a decrease in placental weight and placental capacity (ratio of fetal weight to placental weight) in mice administered ≥0.5 mg/kg/day PFOS via gavage on GDs 11–16.

Figure 2-33. Fecundability Relative to PFOS Levels (Presented as Adjusted Fecundability Ratios)

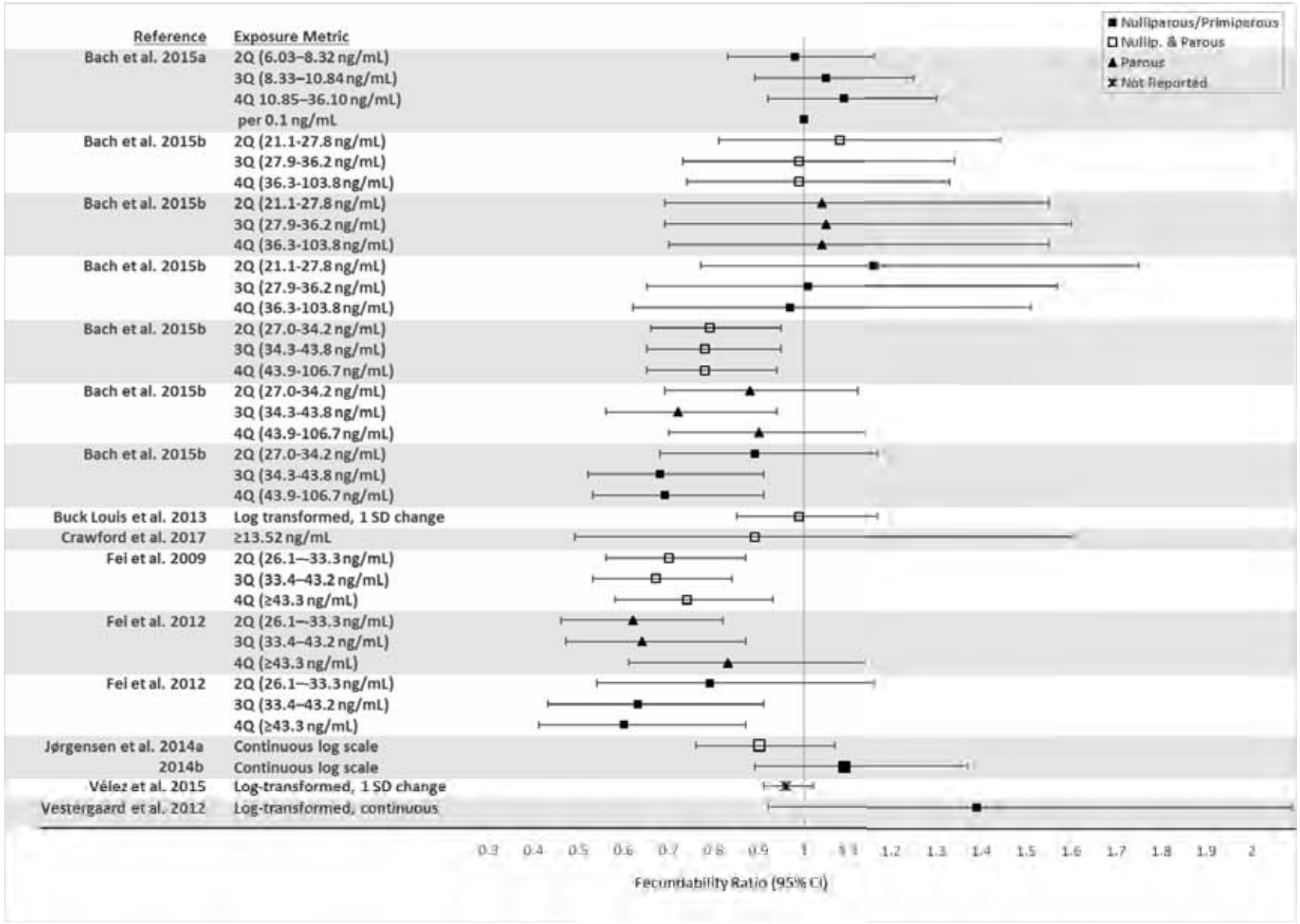
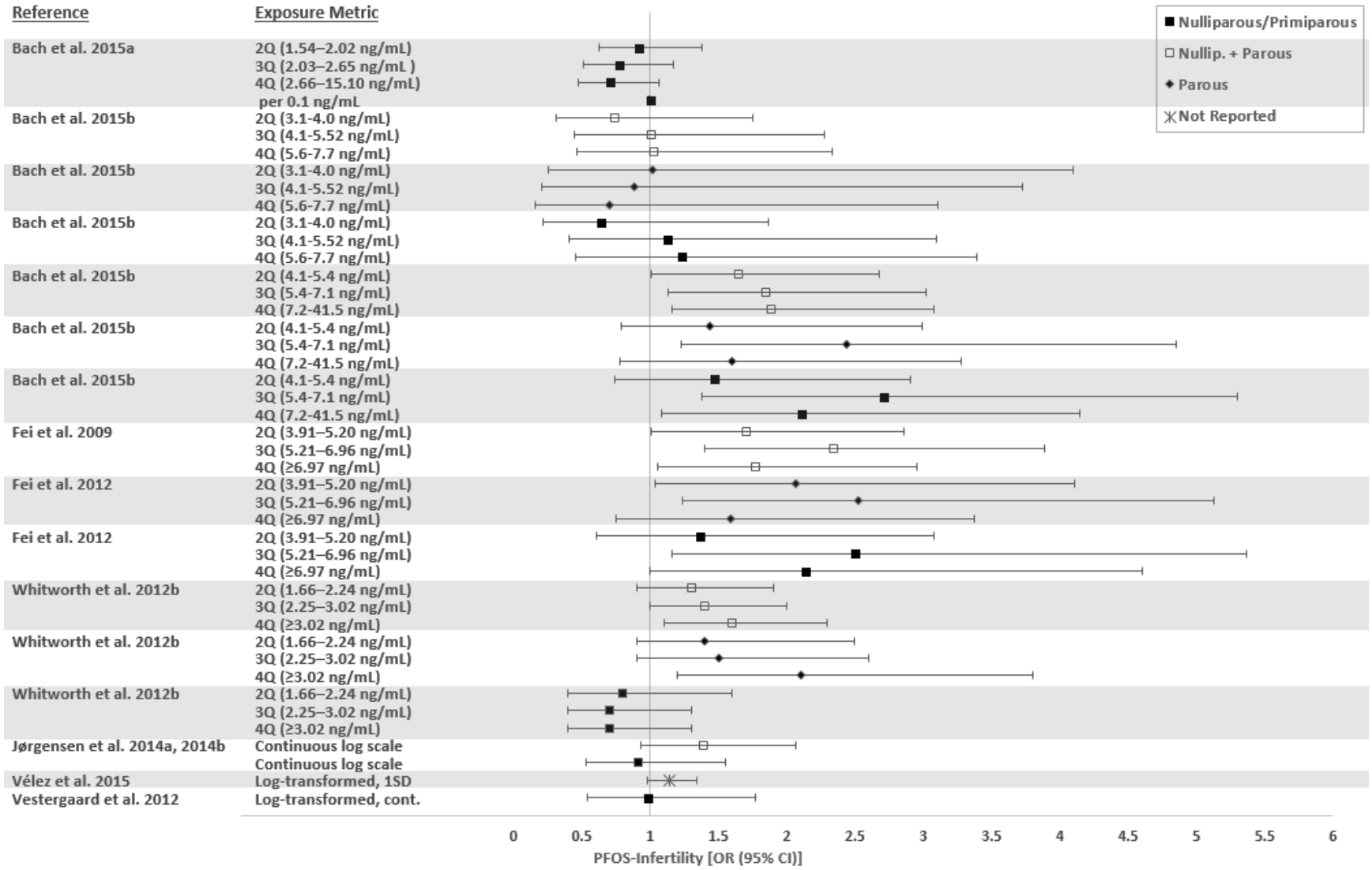


Figure 2-34. Infertility Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



Multigeneration studies with PFOS in rats did not provide indications of reproductive toxicity. Exposure of male and female rats to up to 3.2 mg/kg/day PFOS by gavage before mating and continuing during gestation did not affect mating or fertility parameters of the parental or F1 generation (Luebker et al. 2005a, 2005b). Dietary exposure of rats to 1.3–1.8 mg/kg/day PFOS for 4 or 14 weeks did not induce gross or microscopic alterations in the sex organs of males or females (Seacat et al. 2003). A similar study in Cynomolgus monkeys administered up to 0.75 mg/kg/day PFOS administered via a capsule also reported no significant morphological alterations in the sex organs, but serum estradiol was significantly decreased in males on days 62, 91, and 182 of the study (Seacat et al. 2002). In addition, treatment with PFOS had no significant effect on cell proliferation in the testes. Serum estradiol also was lower than in controls in one male and one female monkey dosed with 2 mg/kg/day PFOS for 4 weeks, but little can be concluded from results from just two animals (Thomford 2002a). In a 2-year dietary study in rats, administration of up to 1.04 mg/kg/day PFOS did not induce gross or microscopic alterations in the reproductive organs (Butenhoff et al. 2012b; Thomford 2002b). Overall, the reproductive system does not seem to be a sensitive target of PFOS toxicity, although some changes in testosterone and estradiol levels and decreases in sperm count have been observed.

PFHxS

Epidemiological Studies—Reproductive Hormone Levels. Three general population studies evaluated possible effects of PFHxS on reproductive hormone levels. In young men, no associations between serum PFHxS levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels were found (Joensen et al. 2013). Similarly, no alterations in follicular estrogen or luteal progesterone were observed in women (Barrett et al. 2015). An association between serum PFHxS levels and estradiol levels were observed in adolescent boys, but not in girls; no associations were observed for testosterone levels (Zhou et al. 2016).

Epidemiological Studies—Effects on Sperm. With the exception of the finding of an inverse association between serum PFHxS levels and percent normal sperm (Toft et al. 2012), general population studies have not found associations between PFHxS and sperm parameters (Joensen et al. 2013; Toft et al. 2012); it is noted that the Joensen et al. (2013) study of young men did not find alterations in sperm morphology.

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. Five general population studies have evaluated possible associations between serum PFHxS levels and female reproductive outcomes. Taylor et al. (2014) reported increases in the risk

of earlier menopause in women with serum PFHxS levels in the 3rd tertile and the risk of hysterectomy in women with serum PFHxS levels in the 2nd and 3rd tertiles. These findings may be due to reverse causation in that early menopause may result in higher serum PFHxS levels. Other studies did not find associations with the risk and severity of endometriosis (Buck Louis et al. 2012; Campbell et al. 2016) or polycystic ovary syndrome (Vagi et al. 2014).

Romano et al. (2016) did not find associations between maternal PFHxS levels and the risk of breastfeeding ≤3 or 6 months. Similarly, Timmermann et al. (2017) did not find associations between maternal PFHxS levels and the length of breastfeeding or length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Seven studies have evaluated possible effects on fertility associated with female serum PFHxS levels. Vélez et al. (2015) found increases in time to pregnancy (measured as a decreased fecundability OR) and risk of infertility, which were associated with serum PFHxS levels in pregnant women. Vestergaard et al. (2012) reported an increase in the fecundability OR, indicating a shorter time to pregnancy, when risk was calculated using continuous serum PFHxS; however, when the subjects were divided into two groups based on serum PFHxS levels above and below the median level, the fecundability ratio included unity in the above-median group (fecundability ratio 1.29, 95% CI 0.90–1.83), as compared to the below-median group. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study. Studies by Bach et al. (2015a), Crawford et al. (2017), Jørgensen et al. (2014a), and Whitworth et al. (2016) did not find alterations in time to pregnancy, fertility, or the risk of infertility.

Laboratory Animal Studies. Exposure to 10 mg/kg/day PFHxS did not result in alterations in reproductive organ weights or histopathology in male rats exposed for a minimum of 42 days beginning 14 days prior to cohabitation and female rats sacrificed on lactation day 21 or GD 25 (rats that did not deliver a litter) (exposure began 14 days prior to cohabitation) (Butenhoff et al. 2009a). Fertility was not affected by treatment with PFHxS and there were no significant effects on sperm parameters. Also, estrous cycling was not affected by dosing with PFHxS. A similarly designed study in mice also reported no alterations in reproductive toxicity parameters (Chang et al. 2018).

PFNA

Epidemiological Studies—Reproductive Hormone Levels. Reproductive hormone alterations associated with serum PFNA levels are limited to a finding for estradiol in young men (Joensen et al. 2013); no

associations with other reproductive hormones were found in this study. In another study of adolescent and young adults, no associations between serum PFNA and sex hormone binding globulin, FSH, or testosterone were found in males or females (subjects were segregated by sex and age range) (Tsai et al. 2015). Zhou et al. (2016) found an inverse association between serum PFNA and testosterone levels in boys, but not in girls, and did not find associations for estradiol levels. Another study did not find alterations in follicular estradiol or luteal progesterone levels in women (Barrett et al. 2015).

Epidemiological Studies—Effects on Sperm. Buck Louis et al. (2015) found associations between serum PFNA and increases in the percentage of normal sperm and a decrease in the percentage of sperm with coiled tails. No associations were found for other sperm parameters (Buck Louis et al. 2015; Joensen et al. 2013; Toft et al. 2012).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding Duration. No association between serum PFNA levels and menstrual cycle length was observed in a general population study (Lum et al. 2017). Increases in the risk of earlier menopause and hysterectomy were found in women with serum PFNA levels in the 3rd and ≥2nd serum PFNA tertiles (Taylor et al. 2014). The investigators examined the possibility that these effects may be due to reverse causation and found that serum PFNA levels increased post-menopause (Taylor et al. 2014). An increase in the risk of endometriosis was associated with serum PFNA levels in a general population study (Buck Louis et al. 2012); however, adjustment for parity resulted in OR CIs that included unity. A second study did not find an association between serum PFNA and self-reported endometriosis (Campbell et al. 2016). Vagi et al. (2014) did not find an increased risk of polycystic ovary syndrome that was associated with serum PFNA levels.

No associations between maternal PFNA levels and the risk of breastfeeding ≤3 or 6 months were found in a general population study (Romano et al. 2016). In contrast, Timmermann et al. (2017) found inverse associations between maternal PFNA levels and breastfeeding length and the length of exclusive breastfeeding. The study also found no differences in breastfeeding length or exclusiveness between primiparous and multiparous women.

Epidemiological Studies—Effects on Fertility. Jørgensen et al. (2014a) found increases in time to pregnancy (measured as a decrease in fecundability ratio) and an increase in infertility risk in a study of pregnant women. In sensitivity analysis, the fecundability ratio for primiparous women was 0.99 and the 95% CI range included unity (0.88–1.22). Wang et al. (2016) found an inverse association between

serum PFNA levels in women and the risk of endometriosis-induced infertility. Studies by Bach et al. (2015a), Buck Louis et al. (2013), Crawford et al. (2017), Lum et al. (2017), Vestergaard et al. (2012), and Whitworth et al. (2016) did not find associations between serum PFNA levels and fecundability ratio, fertility or risk of infertility.

Laboratory Animal Studies. Two acute-duration studies have evaluated the reproductive toxicity of PFNA in male rats (Feng et al. 2009, 2010). Gavage administration of 5 mg/kg/day for 14 days resulted in decreases in serum testosterone and increases in serum estradiol levels and atrophy of the seminiferous tubules (Feng et al. 2009). Electron microscopic examination of the testes revealed large vacuoles between the Sertoli cells and spermatogonia at 5 mg/kg/day; these changes as well as increases in serum Mullerian inhibiting substance and decreases in serum inhibin B cells were suggestive of damage to the secretory function of the Sertoli cells (Feng et al. 2010). In mice administered 0.5 mg/kg/day PFNA for 90 days, decreases in sperm motility, viability, and count and degenerative changes in the seminiferous tubules were observed (Singh and Singh 2018). When the mice were mated with unexposed females, significant decreases in litter size were observed at 0.5 mg/kg/day.

PFDA

Epidemiological Studies—Reproductive Hormone Levels. No associations were found between serum PFDA levels and testosterone, free androgen index, LH, estradiol, sex hormone binding globulin, or FSH levels in young men (Joensen et al. 2013). Similarly, no alterations in follicular estradiol or luteal progesterone levels were observed in women (Barrett et al. 2015). In adolescent boys, an inverse association between serum PFDA and testosterone was found; no association was found in girls (Zhou et al. 2016). This study also found no associations for estradiol levels in boys or girls.

Epidemiological Studies—Effects on Sperm. Two general population studies evaluated potential effects of PFDA exposure on sperm parameters. Buck Louis et al. (2015) found associations between serum PFDA levels and increases in sperm head length and decreases in the percentage of sperm with coiled tails. No alterations were found for sperm viability, count, volume, motility, or other morphological alterations (Buck Louis et al. 2015; Joensen et al. 2013).

Epidemiological Studies—Effects on Menstrual Cycle Length, Menopause Onset, Endometriosis, and Breastfeeding. Three studies examined alterations in female reproductive outcomes associated with serum PFDA levels. In two studies, no associations between serum PFDA levels and the risk or severity

of endometriosis were found (Buck Louis et al. 2012; Lum et al. 2017). In the third study, an inverse association between maternal PFDA levels and duration of breastfeeding was found (Timmermann et al. 2017). No association was found for the length of exclusive breastfeeding.

Epidemiological Studies—Effects on Fertility. Six studies examined the potential for PFDA to alter fertility. No alterations in time to pregnancy (measured as fecundability ratio) or risk of infertility were observed in pregnant women (Bach et al. 2015a). Additionally, no associations with the probability of pregnancy (Lum et al. 2017), endometriosis-related infertility (Wang et al. 2017), or fecundability (Whitworth et al. 2016) were observed in other general population studies. Two prospective studies also found no association between female serum PFDA levels (Buck Louis et al. 2013; Vestergaard et al. 2012) or male serum PFDA levels (Buck Louis et al. 2013) and time to pregnancy.

PFUnA

Epidemiological Studies—Reproductive Hormone Levels. An inverse association between serum PFUnA levels and FSH levels was observed in adolescent girls (Tsai et al. 2015). The study did not find alterations in sex hormone binding globulins or testosterone levels in adolescent and young adult males or females. Another study of women did not find alterations in follicular estradiol or luteal progesterone levels (Barrett et al. 2015).

Epidemiological Studies—Effects on Fertility. Three studies evaluated possible associations between maternal serum PFUnA levels and fertility. No alterations in time to pregnancy (measured as a fecundability ratio) or infertility risk (Bach et al. 2015a), endometriosis-related infertility risk, or fecundability (Whitworth et al. (2016) were observed.

PFHpA

Epidemiological Studies. Only one study examined potential fertility associations. Wang et al. (2017) found a decreased risk of endometriosis-related infertility in a case-control study.

PFBS

***Epidemiological Studies*—**Two studies have evaluated potential association for reproductive outcomes. Zhou et al. (2016) did not find associations between serum PFBS and testosterone or estradiol levels in adolescent boys or girls. Wang et al. (2017) found an association between serum PFBS levels and endometriosis-related infertility in a case-control study.

Laboratory Animal Studies. Administration of up to 900 mg/kg/day PFBS to rats by gavage for 28 days did not cause any significant gross or microscopic alterations in primary or secondary sex organs from males or females (3M 2001). A 2-generation study in which rats were exposed to gavage doses of potassium PFBS as high as 1,000 mg/kg/day did not result in alterations in fertility, sperm parameters, estrus cycling, or histological alterations in reproductive tissues (Lieder et al. 2009b).

PFBA

Laboratory Animal Studies. No significant gross or microscopic alterations were reported in primary and secondary reproductive organs from rats dosed with PFBA by gavage in doses of up to 184 mg/kg/day for 5 days (3M 2007a), 150 mg/kg/day for 28 days (Butenhoff et al. 2012a; van Otterdijk 2007a), or 30 mg/kg/day for 90 days (Butenhoff et al. 2012a; van Otterdijk 2007b).

PFDoDA

***Epidemiological Studies*—**A study in adolescent boys and girls found an inverse association between serum PFDoDA levels and testosterone levels in girls only; no associations were found for estradiol levels (Zhou et al. 2016). In the two studies evaluating fertility, no associations were found for endometriosis-related infertility (Wang et al. 2017) or fecundability (Whitworth et al. 2016).

Laboratory Animal Studies. Treatment of male rats with 1, 5, or 10 mg/kg/day PFDoDA by gavage for 14 days induced a dose-related decrease in testes weight, which achieved statistical significance at 10 mg/kg/day (Shi et al. 2007). Measurement of serum hormone levels showed a significant decrease in LH at 10 mg/kg/day and in testosterone at 5 and 10 mg/kg/day, no significant effect on FSH levels, and a significant decrease in serum estradiol only at 5 mg/kg/day. Alterations in the ultrastructure of the testes were seen in the 5 and 10 mg/kg/day groups and consisted of the presence of large clustered lipid droplets and enlarged mitochondria in Sertoli cells, large vacuoles, and expanded mitochondria in Leydig and

spermatogenic cells. Morphological features of apoptosis were seen in cells in the 10 mg/kg/day group. Assessment of messenger ribonucleic acid (mRNA) expression of genes involved in cholesterol transport and steroidogenesis provided evidence of altered cholesterol transport and steroid hormone synthesis, but no effects were noted for LH receptor and aromatase mRNA expression. Considering that serum total cholesterol was unaffected at 5 mg/kg/day and increased at 10 mg/kg/day and that aromatase expression was unaffected, the decrease in testosterone synthesis probably resulted from decreased steroidogenesis gene expression. In a longer-duration study (110 days) conducted by these investigators, decreased serum testosterone levels were observed at 0.2 and 0.5 mg/kg/day (Shi et al. 2009a). A third study (Kato et al. 2015) evaluated reproductive performance and found no alterations in estrous cycling during the first 14 days of exposure and no alterations in fertility, number of corpora lutea, or number of implantation sites in male and female rats administered 2.5 mg/kg/day PFDoDA for 14 days prior to mating and during gestation. In pregnant females administered 2.5 mg/kg/day, hemorrhages were observed at the implantation sites; only one female delivered live pups and 58% of the animals died or were sacrificed early. In females exposed for 42 days and not mated, continuous diestrus was observed at 2.5 mg/kg/day (Kato et al. 2015).

PFHxA

Epidemiological Studies—The only epidemiological study evaluating reproductive outcomes associated with PFHxA found an inverse association for testosterone levels in adolescent boys (Zhou et al. 2016) but did not find this association in girls and found no association with estradiol levels.

Laboratory Animal Studies. No alterations in mating, fertility, or gestation length were observed in rats administered TWA doses of 315 mg/kg/day PFHxA for 14 days prior to mating and during mating and gestation (Kirkpatrick 2005). Similarly, no alterations in mating, fertility, gestation length, number of implantation sites, estrous cycling, or sperm parameters were observed in rats administered up to 500 mg/kg/day NaPFHx for 70 days prior to mating, during the mating period, and throughout gestation and lactation (Loveless et al. 2009). A 90-day study did not find histological alterations in reproductive tissues of male or female rats administered up to 200 mg/kg/day NaPFHx (Chengelis et al. 2009b).

FOSA

Epidemiological Studies. One study examined reproductive hormone levels and did not find an association between serum FOSA and follicular estradiol or luteal progesterone levels in women (Barrett

et al. 2015). Two prospective epidemiological studies evaluated the possible association between FOSA and fertility. Vestergaard et al. (2012) did not find an increase in time to pregnancy, as measured as a fecundability ratio, or decrease in the likelihood of becoming pregnant within the first six menstrual cycles. In contrast, Buck Louis et al. (2013) found an increased time to pregnancy associated with serum FOSA levels in women, but not in men; the investigators noted that the results should be interpreted cautiously because only 10% of the blood samples had FOSA levels above the limit of detection. Another study found no association between maternal FOSA and fecundability (Whitworth et al. 2016).

2.17 DEVELOPMENTAL

Overview. A large number of epidemiological studies have examined the potential of developmental toxicity of perfluoroalkyls in the general population and in populations living in an area with high PFOA drinking water contamination. Epidemiological studies are available for 10 of the 12 perfluoroalkyls discussed in the profile; no developmental data were identified for PFHxA or PFBS. The discussion of these developmental outcomes is divided into four categories: pregnancy outcome, birth outcome, neurodevelopment, and sexual maturation. The epidemiological studies examining pregnancy outcome are summarized in Table 2-22; the pregnancy outcomes include miscarriage, stillbirth, preterm birth, and gestation age. Table 2-23 summarizes the epidemiological studies examining birth outcomes, which include birth weight, birth size, low birth weight, small for gestational age, birth defects, and sex ratio. Epidemiological studies examining neurodevelopmental endpoints, particularly risks for ADHD, are summarized in Table 2-24. Studies evaluating possible associations between serum perfluoroalkyl levels and development of the reproductive system are summarized in Table 2-25. Further details on these studies are presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 13. Studies examining childhood growth and examining the possible relationship between maternal serum perfluoroalkyl levels and body weight and BMI in children and adults are discussed in Section 2.3, Body Weight.

In general, the epidemiological studies did not find associations between perfluoroalkyl exposure and adverse pregnancy outcomes (miscarriage, preterm birth, or gestational age) for PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA. Mixed results have been found for birth outcomes, particularly birth weight. Some epidemiological studies have found associations between maternal PFOA or PFOS exposure and decreases in birth weight, and meta-analyses of these data have found that increases in maternal PFOA or PFOS were associated with 11–19 g or 1–5 g decreases in birth weight, respectively; accounting for maternal glomerular filtration rates attenuated these results by about 50%. No consistent associations for

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Preterm birth	OR 1.01 (0.55–1.86)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>39.4 ng/mL (5th PFOA quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.00 (0.63–1.58), 5th quintile OR 1.06 (0.57–1.97), 5th quintile OR 0.81 (0.38–1.71), 5th quintile
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Miscarriage	OR 0.9 (0.7–1.0)
		Stillbirth	OR 1.0 (0.5–1.8)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight or small for gestational age)	21.0–717.6 ng/mL (5th maternal PFOA quintile)	Stillbirth	OR 0.8 (0.5–1.5)
		Preterm birth (<37 weeks)	OR 1.0 (0.9–1.2)
		Preterm birth (<32 weeks)	OR 1.0 (0.7–1.3)
Savitz et al. 2012b Community (4,547 infants)	83.3–921.3 ng/mL (5th maternal PFOA quintile)	Preterm birth (<37 weeks)	OR 1.2 (0.9–1.6)
		Preterm birth (<32 weeks)	OR 1.4 (0.5–3.6)
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	48.8 ng/mL (maternal mean PFOA)	Miscarriage	OR 0.9 (0.5–1.6), >90th percentile
		Preterm birth	OR 0.9 (0.6–1.5), >90th percentile
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Gestational age	NS (p>0.05)
Buck Louis et al. 2016 General population (n=332 couples)	3.3 ng/mL (median serum PFOA in women)	Pregnancy loss	HR 0.93 (0.75–1.16)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Gestational age	NS (p>0.05)
		Preterm birth	OR 0.64 (0.40–1.02)
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd PFOA tertile)	Preterm birth	RR 1.31 (0.38–4.45), 3rd tertile

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2015 General population (n=56 cases and 336 controls)	1.58 ng/mL (maternal median PFOA)	Miscarriage before gestation week 12	OR 0.64 (0.36–1.18)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.318)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Gestational age	NS (p=0.431)
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Gestational age	β 0.16 (-0.02–0.33)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Preterm	OR 0.90 (0.60–1.35)
		Gestational age	β-0.05 (-0.12–0.08)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Preterm	OR 1.0 (0.9–1.3)
		Gestation length	β -0.05 (-0.16–0.06)
Whitworth et al. 2012a General population (n=901 infants)	≥3.04 ng/mL (maternal 4th PFOA quartile)	Preterm birth	**OR 0.1 (0.03–0.6)*, 4th quartile**
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Gestational age	**β -15.99 (-27.72 to -4.25, p<0.01)***

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Preterm birth	OR 1.07 (0.58–1.95)
Darrow et al. 2014 Community (C8) (n=1,129 women)	>23.3 ng/mL (5th PFOS quintile)	Miscarriage risk Parous subgroup Nulliparous subgroup	OR 1.41 (0.88–2.26), 5th quintile OR 1.12 (0.58–2.17), 5th quintile OR 2.02 (0.83–4.93), 5th quintile
Stein et al. 2009 Community (C8) (n=5,262 infants)	23.2–83.4 ng/mL (>90th PFOS percentile)	Miscarriage	OR 0.9 (0.7–1.3), >90th percentile
		Preterm birth	**OR 1.4 (1.1–1.7)*, >90th percentile**
Buck Louis et al. 2016 General population (n=332 couples)	12.2 ng/mL (median serum PFOS in women)	Pregnancy loss	HR 0.81 (0.65–1.00)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Preterm birth	**OR 2.45 (1.47–4.08)***
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Gestation length	NS (p>0.01)
		Preterm birth	OR 1.43 (0.50–4.11), 4th quartile
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Preterm birth	RR 1.11 (0.36–3.38), 3rd tertile
Jensen et al. 2015 General population (n=56 cases and 336 controls)	8.10 ng/mL (maternal median PFOS)	Miscarriage before gestation week 12	OR 1.16 (0.59–1.29)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.201)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Gestational age	NS (p=0.952)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Gestational age	β 0.11 (-0.06–0.29) **β 0.29 (0.05–0.53)*, boys only** β -0.07 (-0.35–0.20), girls only
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Preterm	OR 1.10 (0.70–1.74)
		Gestational age	β-0.06 (-0.19–0.06)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS); 18.9–25.6 ng/mL (2nd quartile maternal PFOS)	Preterm	**OR 2.0 (1.1–3.7)*, 2nd quartile**
		Gestation length	β -0.08 (-0.17–0.02)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Preterm birth	**OR 0.3 (0.1–1.0, p=0.03)*, 4th quartile**
PFHxS			
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Preterm birth	**RR 0.31 (0.11–0.90)*, 3rd tertile**
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.298 ng/mL (maternal median PFHxS)	Miscarriage before gestation week 12	OR 1.53 (0.99–2.38)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Gestational age	β 0.12 (-0.03–0.27)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Gestational length	NS (p>0.05)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Preterm	OR 0.85 (0.63–1.13)
		Gestational age	β -0.01 (-0.10–0.09)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Preterm	OR 1.0 (0.9–1.1)
		Gestation length	β 0.02 (-0.04–0.07)
PFNA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFNA in women)	Pregnancy loss	HR 0.86 (0.70–1.06)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Preterm birth	OR 0.88 (0.71–1.11)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.72 ng/mL (maternal median PFNA)	Miscarriage before gestation week 12	**OR 16.46 (7.39–36.62)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Gestational age	β -0.02 (-0.19–0.10)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Gestational length	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Preterm	OR 0.87 (0.62–1.22)
		Gestational age	β -0.00 (-0.11–0.11)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA)	Preterm	OR 1.2 (1.0–1.4)
		Gestation length	β-0.07 (-0.17–0.02)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Buck Louis et al. 2016 General population (n=332 couples)	1.2 ng/mL (median serum PFDA in women)	Pregnancy loss	HR 0.83 (0.66–1.04)
Jensen et al. 2015 General population (n=56 cases and 336 controls)	0.27 ng/mL (maternal median PFDA)	Miscarriage before gestation week 12	**OR 2.30 (1.18–4.47)***
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Gestational age	β 0.10 (-0.09–0.29)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Gestational length	NS (p>0.05)
PFUnA			
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Preterm birth	OR 0.87 (0.64–1.16)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Gestational age	β 0.09 (-0.07–0.25)
PFHpA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Gestational age	β 0.14 (-0.17–0.45)
PFBA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Gestational age	β 0.01 (-0.18–0.20)

Table 2-22. Summary of Pregnancy Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDoDA			
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Gestational age	β 0.07 (-0.24 to 0.39)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

OR = odds ratio; NS = not significant; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR= risk ratio

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Darrow et al. 2013 Community (C8) (n=1,330 women)	≥37.2 ng/mL (5th PFOA quintile)	Birth weight	NS (p=0.70 for trend)
		Low birth weight	OR 0.92 (0.44–1.95)
Nolan et al. 2009 Community (n=1,555 singleton infants)	NR	Birth weight	NS (p>0.05)
		Low birth weight	**OR 0.37 (0.16–0.86)***
Nolan et al. 2010 Community (n=1,548 singleton infants)	NR	Congenital anomalies	OR 1.1 (0.34–3.3)
Savitz et al. 2012a Community (C8) (11,737 singleton infants)	63.1–934.3 ng/mL (4th maternal PFOA quartile)	Low birth weight	**OR 0.37 (0.16–0.86)***
		Birth defect	OR 1.0 (0.8–1.3)
Savitz et al. 2012b Community (13,243 cases stillbirth, preterm birth, low birth weight, or small for gestational age)	7.7 ng/mL (estimated maternal median PFOA)	Birth weight	β -14.80 (-42.28–13.68), per 100 ng/mL increase in PFOA
		Low birth weight	OR 1.0 (0.86–1.15), per 100 ng/mL increase in PFOA
		Small for gestational age	OR 0.86 (0.67–1.11), per 100 ng/mL increase in PFOA
Savitz et al. 2012b Community (4,547 infants)	13.4 ng/mL (estimated maternal median PFOA)	Low birth weight	OR 1.07 (0.96–1.18), per 100 ng/mL increase in PFOA
		Small for gestational age	**OR 1.08 (1.01–1.16)*, per 100 ng/mL increase in PFOA** OR 0.8 (0.6–1.2), for serum PFOA levels ≥80th percentile
		Birth weight	OR -12.76 (-26.08–0.57), per 100 ng/mL increase in PFOA
Stein et al. 2009 Community (C8) (n=1,845 pregnancies)	50.0–<120.6 and 120.6–894.4 ng/mL (3rd and 4th maternal PFOA quartile)	Low birth weight	OR 0.8 (0.3–1.9), 4th quartile
		Birth defects	OR 1.7 (0.8–3.6), 4th quartile

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Stein et al. 2014c Community (C8) (n=10,262 infants)	61.3 ng/mL (estimated *in utero* mean PFOA)	Brain defects	OR 2.6 (1.3–5.1), interquartile range
		Gastrointestinal defects	OR 0.7 (0.3–1.4), interquartile range
		Kidney defects	OR 0.7 (0.3–1.8), interquartile range
		Craniofacial defects	OR 0.6 (0.3–1.3), interquartile range
		Eye defects	OR 1.1 (0.6–2.1), interquartile range
		Limb defects	OR 1.2 (0.7–2.0), interquartile range
		Genitourinary defects	OR 1.0 (0.6–1.7), interquartile range
		Heart defects	OR 1.2 (0.8–1.7), interquartile range
Alkhalawi et al. 2016 General population (n=156 mother-infant pairs)	2.43 ng/mL (geometric mean maternal PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.412 (-0.788 to -0.037)***
Apelberg et al. 2007b General population (n=341 singleton births)	1.6 ng/mL (cord serum median PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.70 and 0.39 ng/mL (maternal and cord median PFOA)	Gestational weight gain	NS (p>0.1), serum PFOA **OR 1.04 (1.02–1.06)*, cord PFOA**
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.7 ng/mL (median maternal plasma PFOA)	Birth weight	β -0.10 (-0.34–0.13)
		Infant leptin levels	β 0.01 (-0.15–0.13)
		Infant adiponectin levels	β 0.04 (-0.05–0.12)
Bach et al. 2016 General population (n=1,507 nulliparous women)	2.0 ng/mL (median PFOA)	Birth weight	NS, investigators noted no consistent alterations across PFOA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFOA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (n=233 couples)	5.01 and 4.05 ng/mL and 5.00 and 2.54 ng/mL (geometric mean PFOA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.93 (0.68–1.26), maternal PFOA OR 0.94 (0.72–1.23), paternal PFOA
Callan et al. 2016 General population (n=98 pregnant women)	0.86 ng/mL (median maternal serum PFOA)	Birth weight	β -48 g (-203–108)
		Birth length	β 0.06 (-0.70–0.81)
		Head circumference	β -0.40 (-0.96–0.16)
		Ponderal index	β-0.06 (-0.16–0.05)
Cao et al. 2018 General population (n=337 newborns)	1.59 ng/mL (mean cord serum PFOA); >1.59 ng/mL (3rd tertile cord PFOA)	Birth weight	NS (p=0.58)
		Birth length	**β -0.45 (-0.79 to -0.10)*, 3rd tertile**
		Ponderal index	NS (p=0.21)
Chen et al. 2012a General population (n=429 infants)	1.84 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 1.24 (0.75–2.05)
		Low birth weight	OR 0.53 (0.18–1.55)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	5.6 ng/mL (maternal median PFOA)	Birth weight	**β -10.63 (-20.79 to -0.47)***
		Birth length	**β -0.069 (-0.113 to -0.024)***
		Abdominal circumference	**β -0.059 (-0.106 to -0.012)***
		Head circumference	β-0.030 (-0.064–0.004)
		Low birth weight	OR 2.44 (0.27–22.25), 4th quartile
		Small for gestational age	OR 0.97 (0.55–1.70), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	1.52 ng/mL (cord blood geometric mean PFOA)	Birth weight	NS (p=0.473)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hamm et al. 2010 General population (n=252 pregnant women)	>2.1–18 ng/mL (maternal 3rd tertile PFOA)	Birth weight	Change in weight 14.80 (-107.29–136.89), 3rd tertile
		Small for gestational age	RR 0.99 (0.25–3.92), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	1.46 ng/mL (maternal median PFOA)	Birth weight	NS (p>0.05)
		Cord TSH	**Association (p<0.05)***
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	5.398 and 2.12 ng/mL (mean PFOA in cases and controls)	Thyroid stimulating immunoglobulin levels	**Inverse association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	1.6 ng/mL (mean maternal serum PFOA)	Birth weight	β -49.4 (-130.4–31.6)
		Birth length	β 0.01 (-0.37–0.40)
		Ponderal index	β -0.44 (-0.99–0.12)
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	2.33 ng/mL (median maternal serum PFOA)	Birth weight	β -359 (-596 to -122; p=0.003)*
		Birth length	**β -1.3 (-2.3 to -0.3, p=0.010)***
		Head circumference	NS (p=0.115)
		Small for gestational age	**OR 5.25 (1.68–16.4)*** **OR 6.55 (1.14–37.45)*, boys only** OR 4.73 (0.79–28.3), girls only
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	1.62 ng/mL (median maternal serum PFOA)	Birth weight	NS (p=0.590)
		Birth length	NS (p=0.656)
		Head circumference	NS (p=0.354)
		Small for gestational age	OR 0.66 (0.33–1.33)
Lee et al. 2013 General population (n=59 pregnant women)	2.73 ng/mL (maternal mean PFOA)	Birth weight	OR 0.54 (0.17–3.03)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.56 (0.16–2.01)
		Head circumference	OR 0.82 (0.24–13.65)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	1.11 ng/mL (cord blood mean PFOA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort	1.84, 0.96, and 2.51 ng/mL (maternal median PFOA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	**β -63.77 (-122.83 to -4.71, p=0.035)*, 2 SD increase in ln-transformed PFOA**
Li et al. 2017 General population (n=321 mother-infant pairs)	1.2 ng/mL (median cord serum PFOA)	Birth weight	**β -112.7 (-171.9 to -53.5)***
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	3.7 ng/mL (maternal median PFOA)	Birth weight	**Inverse association (p=0.0120 for trend)***
		Birth length	NS (p=0.0978)
		Ponderal index	NS (p=0.5920)
		Body weight at 20 months	NS (p=0.4147)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	2.35 ng/mL (mean maternal serum PFOA)	Birth weight	β -9.33 (-38.81–20.16)
		Birth length	β -0.01 (-0.15–0.14)
		Head circumference	β 0.07 (-0.17–0.03)
		Small for gestational age	OR 0.92 (0.72–1.19)
		Low birth weight	OR 0.90 (0.63–1.29)
		Low birth weight at term	OR 0.85 (0.53–1.34)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	1.4 ng/mL (median maternal serum PFOA)	Birth weight	**β -197 (-391 to -3, p=0.047)***
		Ponderal index	β -1.32 (-2.66–0.02, p=0.054)
		Cord total adiponectin	NS (p=0.377)
		Cord leptin	NS (p=0.830)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Monroy et al. 2008 General population (n=101 pregnant women)	1.81 and 1.58 ng/mL (maternal and cord median PFOA)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	3.16 and 5.00 ng/mL (maternal and paternal geometric mean PFOA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	5.8 ng/mL (median maternal plasma PFOA)	Birth weight for gestational age	β -0.02 (-0.08–0.03)
Shi et al. 2017 General population (n= 170 infants)	1.097 ng/mL (median cord serum PFOA)	Birth weight	β 163.28 (-127.66–454.23)
		Birth length	β 0.38 (-0.41–1.17)
		Ponderal index	β 0.06 (-0.10–0.22)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1 ng/mL (median maternal serum PFOA); 1.4–17.0 ng/mL (3rd tertile maternal PFOA)	Birth weight	**β -92.4 g (-166.2 to -18.5)*, 3rd tertile**
		Adiposity at birth	β -0.97% fat mass (-0.33–0.49), 3rd tertile
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	2.37 and 2.34 ng/mL (median maternal PFOA for boys and girls)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
Washino et al. 2009 General population (n=428 infants)	1.3 ng/mL (maternal median PFOA)	Birth weight	NS (p=0.207)
		Birth length	NS (p=0.631)
		Chest circumference	NS (p=0.460)
		Head circumference	NS (p=0.823)
Whitworth et al. 2012a General population (n=901 infants)	2.2 and ≥3.04 ng/mL (maternal median and 4th quartile PFOA)	Birth weight	NS (p=0.12)
		Small for gestational age	NS (p=0.92)
		Large for gestational age	NS (p=0.33)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	Birth weight	**β -267.30 (-573.27 to -37.18, p<0.05)***
		Birth length	**β -1.91 (-3.31 to -0.52, p<0.01)***
		Ponderal index	β -0.095 (-0.200–0.389)
PFOS			
Grice et al. 2007 Occupational (n=263 females)	1,300–1,970 ng/mL (range of PFOS)	Birth weight	NS (p=0.15)
Darrow et al. 2013 Community (C8) (n=1,330 women)	15.6 ng/mL (mean PFOS)	Birth weight	NS (p=0.045 for trend), whole cohort **Association (p=0.006), women (n=783) who conceived after blood sample collection**
		Low birth weight	OR 1.33 (0.60–2.96)
Stein et al. 2009 Community (C8) (n=5,262 infants)	17.7–<23.2 and 23.2–83.4 ng/mL (75th–90th and >90th PFOS percentile)	Low birth weight	**OR 1.6 (1.1–2.3)*, 75th-90th percentile**
		Birth defects	OR 1.3 (0.8–2.1)
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	9.04 ng/mL (geometric mean maternal serum PFOS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	**β -0.355 (-0.702 to -0.008)***
Apelberg et al. 2007b General population (n=341 singleton births)	5 ng/mL (PFOS cord serum median)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p>0.05)***
		Ponderal index	**Inverse association (p>0.05)***
Ashley-Martin et al. 2016 General population (1,723 pregnant women)	4.60 and 0.15 ng/mL (maternal and cord PFOS median)	Gestational weight gain	**Association (p<0.1), serum PFOS in underweight/normal weight subjects** **OR 1.03 (1.00–1.05)*, cord PFOS**
Ashley-Martin et al. 2017	4.6 ng/mL (median maternal plasma PFOS)	Birth weight	β 0.05 (-0.18–0.29)
		Infant leptin levels	β -0.09 (-0.23–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
General population (n=1,705 mother-infant pairs)		Infant adiponectin levels	β 0.02 (-0.11–0.07)
Bach et al. 2016 General population (n=1,507 nulliparous women)	8.3 ng/mL (PFOS median)	Birth weight	Inverse association reported by investigators
		Birth length	NS, investigators noted no consistent alterations across PFOS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFOS quartiles
Bae et al. 2015 General population (233 couples)	21.7 and 14.5 ng/mL and 21.5 and 10.8 ng/mL (geometric mean PFOS in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.16 (0.88–1.53), maternal PFOS OR 1.01 (0.78–1.33), paternal PFOS
Callan et al. 2016 General population (n=98 pregnant women)	1.99 ng/mL (median maternal serum PFOS)	Birth weight	β -69 g (-231–94)
		Birth length	β -0.22 (-1.0–0.57)
		Head circumference	β -0.39 (-0.98–0.20)
		Ponderal index	β -0.03 (-0.14–0.08)
Cao et al. 2018 General population (n=337 newborns)	1.43 ng/mL (mean cord serum PFOS)	Birth weight	NS (p=0.84)
		Birth length	NS (p=0.65)
		Ponderal index	NS (p=0.47)
Chen et al. 2012a General population (n=429 infants)	5.94 ng/mL (cord blood geometric mean PFOS)	Gestational age	**Inverse association (p<0.001)***
		Birth weight	**β -110.2 g (-176.0 to -44.5, p<0.001)*, per ln PFOS**
		Birth length	NS (p>0.05)
		Head circumference	**β -0.25 cm (-0.46–0.05 cm, p<0.05)*, per ln PFOS**
		Ponderal index	NS (p>0.05)
		Small for gestational age	**OR 2.27 (1.25–4.15)***
		Low birth weight	OR 2.61 (0.185–8.03)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
de Cock et al. 2014 General population (n=89 infants)	1.611 ng/mL (cord blood mean PFOS)	Weight	NS (p=0.802)
		Height	NS (p=0.975)
		BMI	NS (p=0.586)
		Head circumference	NS (p=0.649)
Fei et al. 2007, 2008a General population (n=1,400 pregnant women)	35.3 ng/mL (maternal median PFOS)	Birth weight	β -0.46 (-2.34–1.41)
		Birth length	β-0.002 (-0.011–0.006)
		Abdominal circumference	β -0.003 (-0.012–0.005)
		Head circumference	β 0.000 (-0.006–0.007)
		Gestation length	NS (p>0.01)
		Low birth weight	OR 4.82 (0.56–41.16), 4th quartile
		Small for gestation age	OR 0.98 (0.58–1.65), 4th quartile
Govarts et al. 2016 General population (n=202 infants)	2.63 ng/mL (cord blood geometric mean PFOS)	Birth weight	NS (p=0.798)
Hamm et al. 2010 General population (n=252 pregnant women)	>10–35 ng/mL (maternal 3rd tertile PFOS)	Birth weight	Change in weight 71.25 (54.97–197.48), 3rd tertile
		Small for gestational age	**RR 0.26 (0.10–0.70)*, 3rd tertile**
Kim et al. 2011 General population (n=44 pregnant women)	2.93 ng/mL (maternal median PFOS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	**Inverse association (p<0.05)***
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; N=13 controls)	5.326 and 4.05 ng/mL (mean PFOS in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Kobayashi et al. 2017 General population (n=177 mother-infant pairs)	5.7 ng/mL (mean maternal serum PFOS)	Birth weight	β -56.0 (-162.8–50.8)
		Birth length	β 0.32 (-0.19–0.82)
		Ponderal index	**β -1.07 (-1.79 to -0.36)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lauritzen et al. 2017 General population (n=159 mother-infant pairs)	16.4 ng/mL (median maternal serum PFOS)	Birth weight	**β -292 (-500 to -84; p=0.006)***
		Birth length	**β -1.2 (-2.1 to -0.3, p=0.007)***
		Head circumference	NS (p=0.073)
		Small for gestational age	OR 2.51 (0.93–6.77)
Lauritzen et al. 2017 General population (n=265 mother-infant pairs)	9.74 ng/mL (median maternal serum PFOS)	Birth weight	NS (p=0.167)
		Birth length	NS (p=0.987)
		Head circumference	NS (p=0.189)
		Small for gestational age	OR 0.71 (0.42–1.20)
Lee et al. 2013 General population (n=59 pregnant women)	10.77 ng/mL (maternal mean PFOS)	Birth weight	OR 0.98 (0.32–3.03)
		Birth length	OR 0.97 (0.29–3.27)
		Ponderal index	**OR 0.22 (0.05–0.90)***
		Head circumference	OR 1.34 (0.20–8.90)
Lee et al. 2016 General population (n=85 infants)	0.87 ng/mL (cord blood mean PFOS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	20.09, 5.04, and 7.81 ng/mL (maternal median PFOS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.109)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.0 ng/mL (median cord serum PFOS)	Birth weight	**β -95.0 (-154.0 to -36.0)*** **β -150.6 (-225.4 to -75.7)*, boys only** β -26.6 (-125.1–71.8), girls only
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	28.90 and 27.50 ng/mL (maternal median PFOS in boy and girl cases) 27.60 and 26.20 ng/mL (maternal median PFOS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 **RR 1.7 (1.0–2.8)*** RR 0.7 (0.4–1.4)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	19.6 ng/mL (maternal median PFOS)	Birth weight	**β -140.01 g (-238.14 to -41.89 g, p=0.0053 for trend)*, 3rd tertile**
		Birth length	**β -0.63 cm (-1.11 to -0.15 cm, p=0.103 for trend)* 3rd tertile**
		Ponderal index	NS (p=0.1120)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	6.05 ng/mL (mean maternal serum PFOS)	Birth weight	β 0.44 (-32.48–33.36)
		Birth length	β 0.03 (-0.12–0.17)
		Head circumference	β -0.00 (-0.10–0.10)
		Small for gestational age	OR 0.92 (0.70–1.22)
		Low birth weight	OR 1.06 (0.71–1.58)
		Low birth weight at term	OR 0.91 (0.55–1.50)
Minatoya et al. 2017 General population (n=168 mother-infant pairs)	5.1 ng/mL (median maternal serum PFOS)	Birth weight	β -29 (-289–232, p=0.828)
		Ponderal index	**β -2.25 (-4.01 to -0.50, p=0.012)***
		Cord total adiponectin	**β 0.12 (0.01–0.22, p=0.028)***
		Cord leptin	NS (p=0.691)
Monroy et al. 2008 General population (n=101 pregnant women)	14.54 and 6.08 ng/mL (maternal and cord median PFOS)	Birth weight	NS (p>0.05), maternal serum and cord blood PFOA
Robledo et al. 2015a, 2015b General population (n=234 couples)	12.44 and 21.6 ng/mL (maternal and paternal geometric mean PFOS)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Sagiv et al. 2018 General population (n=1,645 pregnant women)	25.7 ng/mL (median maternal plasma PFOS)	Birth weight for gestational age	β -0.04 (-0.08–0.01)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shi et al. 2017 General population (n= 170 infants)	0.974 ng/mL (median cord serum PFOS)	Birth weight	β 160.45 (-11.85–332.75)
		Birth length	β 0.33 (-0.14–0.79)
		Ponderal index	β 0.07 (-0.03–0.16)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (median maternal serum PFOS)	Birth weight	β -13.8 g (-53.8–26.3)
		Adiposity at birth	β 0.8 (-0.33–0.49)
Washino et al. 2009 General population (n=428 infants)	5.2 ng/mL (maternal median PFOS)	Birth weight Males Females	**β -148.8 g (-297.0 to -0.5 g, p=0.049)*, per log PFOS unit** NS (p=0.917) **β -269.4 g (-465.7 to -73.0 g, p=0.007)*, per log PFOS unit**
		Birth length	NS (p=0.167)
		Chest circumference	NS (p=0.718)
		Head circumference	NS (p=0.488)
Whitworth et al. 2012a General population (n=901 infants)	13.0 and ≥16.59 ng/mL (maternal median and 4th quartile PFOS)	Birth weight	NS (p=0.10)
		Small for gestational age	NS (p=0.51)
		Large for gestational age	NS (p=0.33)
PFHxS			
Alkhalawi et al. 2016 General population (n=156 mother-child pairs)	0.62 ng/mL (geometric mean maternal serum PFHxS)	Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Ponderal index	NS (p>0.05)
Ashley-Martin et al. 2016 General population (n=1,723 pregnant women)	1.00 and 0.10 ng/mL (maternal and cord PFHxS median)	Gestational weight gain	NS (p>0.1), serum PFHxS OR 1.01 (10.99–1.03), cord PFHxS
Ashley-Martin et al. 2017 General population (n=1,705 mother-infant pairs)	1.0 ng/mL (median maternal plasma PFHxS)	Birth weight	β 0.04 (-0.12–0.20
		Infant leptin levels	β 0.01 (-0.08–0.10)
		Infant adiponectin levels	β 0.02 (-0.08–0.04)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.5 ng/mL (maternal PFHxS median)	Birth weight	**Inverse association reported by investigators**
		Birth length	NS, investigators noted no consistent alterations across PFHxS quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFHxS quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.33 ng/mL (median maternal serum PFHxS)	Birth weight	β -103 g (-221–15)
		Birth length	β -0.20 (-0.78–0.38)
		Head circumference	β -0.31 (-0.74–0.12)
		Ponderal index	β -0.05 (-0.13–0.03)
Cao et al. 2018 General population (n=337 newborns)	0.16 ng/mL (mean cord serum PFHxS); 0.06–0.139 ng/mL (2nd tertile cord PFHxS)	Birth weight	NS (p=0.69)
		Birth length	NS (p=0.67)
		Head circumference	**β 1.33 (0.42–2.26)*, 2nd tertile**
		Ponderal index	NS (p=0.85)
Hamm et al. 2010 General population (n=252 pregnant women)	>1.4–43 ng/mL (maternal 3rd tertile PFHxS)	Birth weight	Change in weight (25.99, 95% CI -95.25–147.23), 3rd tertile
		Small for gestational age	RR 2.35 (0.63–8.72), 3rd tertile
Kim et al. 2011 General population (n=44 pregnant women)	0.55 ng/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
		Cord TSH	NS (p>0.05)
		Cord T3	NS (p>0.05)
		Cord T4	NS (p>0.05)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.228 and 1.17 ng/mL (mean PFHxS in cases and controls)	Thyroid stimulating immunoglobulin levels	**Association (p<0.05)***
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2013 General population (n=59 pregnant women)	1.35 ng/mL (maternal mean PFHxS)	Birth weight	OR 0.57 (0.19–1.75)
		Birth length	OR 0.44 (0.12–1.58)
		Ponderal index	OR 0.64 (0.19–2.23)
		Head circumference	OR 0.90 (0.13–6.13)
Lee et al. 2016 General population (n=85 infants)	0.60 ng/mL (cord blood mean PFHxS)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	2.05, 1.56, and 2.28 ng/mL (maternal median PFHxS for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.801)
Li et al. 2017 General population (n=321 mother-infant pairs)	3.9 ng/mL (median cord serum PFHxS)	Birth weight	β -30.0 (-83.4–23.5)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.96 and 0.90 ng/mL (maternal median PFHxS in boy and girl cases) 0.92 and 0.92 ng/mL (maternal median PFHxS in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.9–1.7) RR 1.1 (0.6–1.9)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Birth weight	NS (p>0.05)
Maisonet et al. 2012 General population (n=447 girls)	1.6 ng/mL (maternal median PFHxS)	Birth weight	**Inverse association (p=0.0314 for trend)***
		Birth length	**Inverse association (p=0.0008 for trend)***
		Ponderal index	NS (p=0.6802 for trend)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.58 ng/mL (mean maternal serum PFHxS)	Birth weight	β -8.60 (-32.00–14.80)
		Birth length	β -0.06 (-0.17–0.06)
		Head circumference	β -0.01 (-0.09–0.07)
		Small for gestational age	OR 0.98 (0.80–1.19)
		Low birth weight	OR 0.94 (0.71–1.23)
		Low birth weight at term	OR 0.97 (0.68–1.41)
Monroy et al. 2008 General population (n=101 pregnant women)	1.62 mg/mL (maternal median PFHxS)	Birth weight	NS (p>0.05)
Sagiv et al. 2018 General population (n=1,645 pregnant women)	2.4 ng/mL (median maternal plasma PFHxS)	Birth weight for gestational age	β 0.00 (-0.03–0.02)
Shi et al. 2017 General population (n= 170 infants)	0.157 ng/mL (median cord serum PFHxS)	Birth weight	β 108.80 (-53.84–271.45)
		Birth length	β 0.38 (-0.06–0.82)
		Ponderal index	β 0.03 (-0.06–0.12)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.8 ng/mL (median maternal serum PFHxS); 1.1–10.9 ng/mL (3rd tertile maternal PFHxS)	Birth weight	β -31.84 g (-105.8–42.2), 3rd tertile
		Adiposity at birth	**β -0.99% fat mass (-1.75 to -0.23)*, 3rd tertile**
PFNA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.8 ng/mL (PFNA median)	Birth weight	NS, investigators noted no consistent alterations across PFNA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFNA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFNA quartiles

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Bae et al. 2015 General population (233 couples)	1.60 and 1.37 ng/mL and 1.55 and 1.09 ng/mL (geometric mean PFNA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 0.94 (0.70–1.26), maternal PFNA OR 0.94 (0.71–1.24), paternal PFNA
Callan et al. 2016 General population (n=98 pregnant women)	0.30 ng/mL (median maternal serum PFNA)	Birth weight	β 14 g (-169–196)
		Birth length	β 0.20 (-0.68–1.09)
		Head circumference	β -0.14 (-0.80–0.52)
		Ponderal index	β -0.03 (-0.16–0.09)
Cao et al. 2018 General population (n=337 newborns)	0.13 ng/mL (mean cord serum PFNA)	Birth weight	NS (p=0.19)
		Birth length	NS (p=0.06)
		Ponderal index	NS (p=0.91)
Chen et al. 2012a General population (n=429 infants)	2.36 ng/mL (cord blood geometric mean PFNA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	**Association (p<0.01)***
		Head circumference	NS (p>0.05)
		Ponderal index	**Inverse association (p<0.05)**
		Small for gestational age	OR 0.97 (0.74–1.26)
		Low birth weight	OR 0.76 (0.47–1.23)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	1.931 and 0.633 ng/mL (mean PFNA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.36 ng/mL (cord blood mean PFNA)	Birth weight	NS (p>0.05)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.69, 0.61, and 0.56 ng/mL (maternal median PFNA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.065)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.2 ng/mL (median cord serum PFNA)	Birth weight	β -45.6 (-106.9–15.8)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.46 and 0.39 ng/mL (maternal median PFNA in boy and girl cases) 0.44 and 0.41 ng/mL (maternal median PFNA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.2 (0.6–2.5) RR 0.6 (0.3–1.2)
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Birth weight	NS (p>0.05)
Manzano-Salgado et al. 2017a General population (n=1,202 mother-infant pairs)	0.66 ng/mL (mean maternal serum PFNA)	Birth weight	β -10.27 (-38.14–17.61)
		Birth length	β -0.00 (-0.13–0.13)
		Head circumference	β -0.04 (-0.13–0.05)
		Small for gestational age	OR 0.85 (0.68–1.07)
		Low birth weight	OR 0.86 (0.63–1.17)
		Low birth weight at term	OR 0.91 (0.60–1.38)
Monroy et al. 2008 General population (n=101 pregnant women)	0.69 mg/mL (maternal median PFNA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	1.211 and 1.566 ng/mL (maternal and paternal geometric mean PFNA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Sagiv et al. 2018 General population (n=1,645 pregnant women)	0.7 ng/mL (median maternal plasma PFNA); 0.7–0.9 ng/mL (3rd quartile maternal PFNA)	Birth weight for gestational age	**β -0.20 (-0.33 to -0.06)*, 3rd quartile**
Shi et al. 2017 General population (n= 170 infants)	0.191 ng/mL (median cord serum PFNA)	Birth weight	β 52.68 (-206.01–311.36)
		Birth length	β 0.13 (-0.57–0.83)
		Ponderal index	β 0.01 (-0.13–0.15)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.4 ng/mL (median maternal serum PFNA); 0.5–6.0 ng/mL (2nd half maternal PFNA)	Birth weight	**β -92.1 g (-150.6 to -33.6)*, 2nd half**
		Adiposity at birth	**β -0.85% fat mass (-1.46 to -0.24)*, 2nd half**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	1.55 and 1.58 ng/mL (median maternal PFNA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	NS (p>0.05)
		Growth during childhood	NS (p>0.05)
PFDA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFDA median)	Birth weight	NS, investigators noted no consistent alterations across PFDA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFDA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFDA quartiles
Bae et al. 2015 General population (233 couples)	0.46 and 0.38 ng/mL and 0.49 and 0.46 ng/mL (geometric mean PFDA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.42), maternal PFDA OR 1.02 (0.78–1.34), paternal PFDA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Callan et al. 2016 General population (n=98 pregnant women)	0.12 ng/mL (median maternal serum PFDA)	Birth weight	β 4 g (-161–170)
		Birth length	β 0.36 (-0.44–1.15)
		Head circumference	β -0.07 (-0.67–0.53)
		Ponderal index	β -0.06 (-0.18–0.05)
Cao et al. 2018 General population (n=337 newborns)	0.12 ng/mL (mean cord serum PFDA)	Birth weight	NS (p=0.26)
		Birth length	NS (p=0.24)
		Ponderal index	NS (p=0.55)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.523 and 0.298 ng/mL (mean PFDA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.40, 0.16, and 0.22 ng/mL (maternal median PFDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.158)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDA)	Birth weight	β -47.3 (-112.9–18.2)
Liew et al. 2014 General population (n=156 children diagnosed with congenital cerebral palsy (cases) and 550 controls)	0.18 and 0.16 ng/mL (maternal median PFDA in boy and girl cases) 0.17 and 0.16 ng/mL (maternal median PFDA in boy and girl controls)	Congenital cerebral palsy Boys Girls	 RR 1.1 (0.7–1.7) RR 0.6 (0.3–1.1)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Birth weight	NS (p>0.05)
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.402 and 0.458 ng/mL (maternal and paternal geometric mean PFDA)	Birth weight	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal
Shi et al. 2017 General population (n= 170 infants)	0.075 ng/mL (median cord serum PFDA)	Birth weight	β -3.04 (-129.67–123.59)
		Birth length	β -0.002 (-0.354–0.34)
		Ponderal index	β -0.01 (-0.08–0.06)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.1 ng/mL (median maternal serum PFDA)	Birth weight	β 11.5 g (-37.3–60.4)
		Adiposity at birth	β 0.06 (-0.45–0.56)
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.46 and 0.43 ng/mL (median maternal PFDA for boys and girls)	Birth weight	**Inverse association (p>0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 3.14 (1.07–9.19)*, girls only**
PFUnA			
Bach et al. 2016 General population (n=1,507 nulliparous women)	0.3 ng/mL (PFUnA median)	Birth weight	NS, investigators noted no consistent alterations across PFUnA quartiles
		Birth length	NS, investigators noted no consistent alterations across PFUnA quartiles
		Head circumference	NS, investigators noted no consistent alterations across PFUnA quartiles
Callan et al. 2016 General population (n=98 pregnant women)	0.08 ng/mL (median maternal serum PFUnA)	Birth weight	β 102 g (-41–245)
		Birth length	β 0.32 (-0.37–1.02)
		Head circumference	β -0.29 (-0.81–0.24)
		Ponderal index	β 0.01 (-0.09–0.11)
		Optimal body weight	**β 5.3 (1.2–9.3)***

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Cao et al. 2018 General population (n=337 newborns)	0.10 ng/mL (mean cord serum PFUnA); >0.11 ng/mL (3rd tertile cord PFUnA	Birth weight	NS (p=0.08)
		Birth length	**β 0.41 (0.06–0.77)*, 3rd tertile**
		Ponderal index	NS (p=0.56)
Chen et al. 2012a General population (n=429 infants)	10.26 ng/mL (cord blood geometric mean PFUnA)	Gestational age	NS (p>0.05)
		Birth weight	NS (p>0.05)
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Ponderal index	NS (p>0.05)
		Small for gestational age	OR 0.93 (0.65–1.33)
		Low birth weight	OR 1.01 (0.53–1.91)
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.982 and 0.438 ng/mL (mean PFUnA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Lee et al. 2016 General population (n=85 infants)	0.22 ng/mL (cord blood mean PFUnA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.70, 0.16, and 0.13 ng/mL (maternal median PFUnA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.275)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFUnA)	Birth weight	β -29.7 (-85.7–26.3)
Shi et al. 2017 General population (n= 170 infants)	0.063 ng/mL (median cord serum PFUnA)	Birth weight	β -28.87 (-128.16–70.42)
		Birth length	β -0.20 (-0.47–0.07)
		Ponderal index	β 0.01 (-0.04–0.06).

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2016 General population (n=117 boys and 106 girls examined at age 2, 5, 8, and 11 years of age)	3.52 and 3.31 ng/mL (median maternal PFUnA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	NS (p>0.05)
		Small for gestational age	**OR 1.83 (1.01–3.32)*, girls only**
PFHpA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.284 and 0.324 ng/mL (mean PFHpA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFHpA)	Birth weight	β -103.7 (-211.3–3.8) **β -266.6 (-426.8 to -106.3)*, boys only** β 15.5 (-134.1–165.1), girls only
PFBA			
Kim et al. 2016a General population (n=27 infants with congenital hypothyroidism; n=13 controls)	0.464 and 0.220 ng/mL (mean PFBA in cases and controls)	Thyroid stimulating immunoglobulin levels	NS (p>0.05)
		TSH	NS (p>0.05)
		T3	NS (p>0.05)
		T4	NS (p>0.05)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFBA)	Birth weight	β -46.2 (-111.3–19.0)
PFDoDA			
Cao et al. 2018 General population (n=337 newborns)	0.04 ng/mL (mean cord serum PFDoDA)	Birth weight	NS (p=0.94)
		Birth length	NS (0.51)
		Ponderal index	NS (p=0.60)

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lee et al. 2016 General population (n=85 infants)	0.14 ng/mL (cord blood mean PFDoDA)	Birth weight	NS (p>0.05)
Lenters et al. 2016a, 2016b General population (n=513 infants in Greenland subcohort, n=557 infants in Ukraine subcohort, and n=180 infants in Poland subcohort)	0.13, 0.04, and 0.05 ng/mL (maternal median PFDoDA for Greenland, Ukraine, and Poland subcohorts)	Birth weight	NS (p=0.440)
Li et al. 2017 General population (n=321 mother-infant pairs)	0.1 ng/mL (median cord serum PFDoDA)	Birth weight	β -46.86 (-122.0–28.4) β 18.4 (-86.8–123.5), boys only **β -130.4 (-239.1 to -21.7)*, girls only**
Wang et al. 2016 General population (n=117 boys and 106 girls examined at 2, 5, 8, and 11 years of age)	0.37 and 0.37 ng/mL (median maternal PFDoDA for boys and girls)	Birth weight	**Inverse association (p<0.05)*, girls only**
		Birth length	NS (p>0.05)
		Head circumference	**Inverse association (p<0.05)*, girls only**
		Small for gestational age	NS (p>0.05)
FOSA			
Bae et al. 2015 General population (233 couples)	0.11 and 0.10 ng/mL and 0.10 and 0.12 ng/mL (geometric mean FOSA in male and female nulliparous parents and male and female parous parents, respectively)	Male birth	OR 1.07 (0.81–1.41), maternal FOSA OR 1.14 (0.86–1.51), paternal FOSA

Table 2-23. Summary of Birth Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Robledo et al. 2015a, 2015b General population (n=234 couples)	0.112 and 0.114 ng/mL (maternal and paternal geometric mean FOSA)	Birth weight	
		Boys	**Inverse association (p<0.05)*, maternal only**
		Girls	NS (p>0.05), maternal or paternal
		Birth length	NS (p>0.05), maternal or paternal
		Head circumference	NS (p>0.05), maternal or paternal
		Ponderal index	NS (p>0.05), maternal or paternal

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

BMI = body mass index; FSH = follicle stimulating hormone; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; LH = luteinizing hormone; NS = not significant; NR = not reported; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Stein et al. 2013 Community (C8) (n=320 children 6–12 years old)	115.9 ng/mL (estimated *in utero* mean PFOA)	Full scale IQ	**β 4.61 (0.68–8.54)*, 4th quartile**
		Reading and math skills	NS
		Scores on tests of ADHD (improvement)	**β -8.49 (-16.14 to -0.84)*, 4th quartile**
Stein et al. 2014a, 2014b Community (C8) (n=321 children 6–12 years old)	94.1–838.6 ng/mL (4th PFOA quartile measured 3–4 years prior to behavioral assessment)	Executive function scores (mother completed survey)	**β -6.39 (-11.43 to -1.35)*, 4th quartile boys** β -6.39 (-0.03–8.87), 4th quartile girls
		Executive function scores (teacher completed survey)	β -6.42 (-13.29–0.45), 4th quartile boys β -1.92 (-10.39–6.55), 4th quartile girls
		ADHD-like behaviors (mother completed survey)	β -3.82 (-8.96–1.31), 4th quartile boys **β 6.99 (2.47–11.51)*, 4th quartile girls** β 2.30 (-1.18–5.77), 4th quartile boys and girls
		ADHD-like behaviors (teacher completed survey)	β -9.25 (-18.78–0.27), 4th quartile boys β -3.65 (-10.85–3.51) 4th quartile girls **β -6.03 (-11.40 to -0.66)*, 4th quartile boys and girls**
		Behavioral problems and emotional disturbances (mother completed survey)	β -1.55 (-5.91–2.82), 4th quartile boys **β 4.63 (0.72–8.53)*, 4th quartile girls**
		Behavioral problems and emotional disturbances (teacher completed survey)	β -2.47 (-8.24–3.30), 4th quartile boys β -0.91 (-6.19–4.37), 4th quartile girls
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 year)	65.3–2,070.6 ng/mL (4th PFOA quartile)	ADHD	**OR 0.76 (0.64–0.90)*, 4th quartile**
		Learning problems 12–15 years old 5–18 years old	 OR 0.96 (0.73–1.26), 4th quartile OR 0.90 (0.76–1.06), 4th quartile

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	5.5 ng/mL (maternal median PFOA)	Social responsiveness scale score (measure of autistic behaviors)	β -2.0 (-4.4–0.4)
Chen et al. 2013 General population (239 children 2 years of age)	2.5 ng/mL (mean cord PFOA)	Poor performance on tests	NS OR 0.6 (0.08–4.8), whole test OR1.3 (0.3–6.2), cognitive tests OR 0.5 (0.06–4.0), language tests OR 0.8 (0.1–4.7), gross motor tests OR 2.8 (0.6–13.5), fine motor tests OR 0.3 (0.02–2.7), social tests OR 3.2 (0.7–14.3), self-help tests
Donauer et al. 2015 General population (n=349 infants at 5 weeks of age)	5.49 ng/mL (maternal geometric mean PFOA)	Social/easy going	NS (p>0.05)
		Hypotonic	**OR 3.79 (1.1–12.8)* per 10-fold increase in PFOA**
		High arousal/difficult	NS (p=0.3533)
Fei et al. 2008b General population (n=1,400 infants)	5.6 ng/mL (maternal median PFOA)	Apgar scores <10	OR 1.14 (0.57–2.25)
		Motor and mental development at 6 months	NS (p>0.05)
		Neurobehavioral milestones at 18 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 7-year-old children)	5.4 ng/mL (maternal median PFOA)	Behavioral problems	NS (p>0.15 for trend)
		Motor coordination	NS (p=0.89 for trend)
Forns et al. 2015 General population (n=843 infants)	40 ng/L (median PFOA breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 1.05 (0.77–1.44) at 6 months of age OR 1.0 (0.78–1.28) at 24 months of age
Goudarzi et al. 2016b General population (n=173 infants at 6 months and 133 at 18 months)	1.2 ng/mL (maternal median PFOA)	MDI/PDI at 6 months of age	NS (p>0.05), boys and girls **Inverse association (p<0.05)*, females**
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	3.23 ng/mL (mean PFOA)	Performance on task requiring behavioral inhibition	NS (p>0.05)
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	4.4 ng/mL (median PFOA)	ADHD (parent reported)	**OR 1.12 (1.01–1.23)*, per 1 ng/mL PFOA**
Høyer et al. 2015a General population (n=1,106 children aged 5–9 years)	1.4 and 1.9–9.8 ng/mL (maternal median and 3rd tertile PFOA)	Motor skills	β -0.2 (-1.2–0.9)
		Abnormal behavior	**OR 2.7 (1.2–6.3)*, 3rd tertile**
		Hyperactivity	**OR 3.1 (1.3–7.2)*, 3rd tertile**
Lien et al. 2016 General population (n=282 children aged 7 years)	1.55 ng/mL (cord blood weighted average PFOA)	Inattention	NS (p=0.7758)
		Hyperactivity/impulsivity	NS (p=0.2997)
		Emotional symptoms	NS (p=0.691)
		Conduct problems	NS (p=0.2664)
		Hyperactivity/inattention	NS (p=0.774)
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	3.7 ng/mL (maternal median serum PFOA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.04 (-0.08 to -0.01)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	4.06, 3.88, and 4.00 ng/mL (maternal median PFOA for ADHD, autism, and controls)	ADHD	RR 0.98 (0.82–1.16)
		Autism	RR 0.98 (0.73–1.31)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls; children were 5–17 years old at time of diagnosis)	1.80 and 1.83 ng/mL (cord blood median PFOA in cases and controls)	ADHD	OR 0.98 (0.91–1.02), per 1 ng/mL increase in PFOA
Oulhote et al. 2016 General population (n=567 7-year-old children)	3.19 ng/mL (maternal geometric mean PFOA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.09 ng/mL (geometric mean PFOA in 5-year-old children)	Total behavioral scores and higher internalizing problems, peer relationship, and autism screening scores	Associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.51 ng/mL (geometric mean PFOA in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	0.9056 ng/mL (cord mean PFOA)	Score on test evaluating ADHD	NS (p=0.72), 3rd tertile
		Males	NS (p=0.22), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	**Association (p=0.05 and 0.09)*, 2nd and 3rd tertiles**
		Females	NS (p=0.74), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	3.7 ng/mL (median maternal PFOA)	ADHD	NS (p=0.45 for trend of 3rd tertile)
		Depression	NS (p=0.28 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.21 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children aged 5 or 8 years)	5.4 ng/mL (maternal median PFOA)	Behavioral regulation	β 1.11(-1.22–3.44)
		Metacognition	β 0.58 (-1.77–2.93)
		Global executive functioning	β 1.06 (-1.33–3.45)
Vuong et al. 2018 General population (n=208 8-year-old children)	2.4 ng/mL (mean serum PFOA)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	**OR 3.18 (1.17–8.60)***
		At risk behavior regulation score	OR 1.56 (0.49–4.92)
		At risk global executive score	OR 2.69 (0.92–7.90)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	2.50 and 2.50 ng/mL (maternal median PFOA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS NS
Wu et al. 2012 General population (n=167 pregnant women at 2 hospitals)	18.32 and 9.76 ng/mL (mean maternal serum PFOA at each hospital)	5-minute Apgar score	**β -1.37 (-2.42 to -0.32, p<0.05)***
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.4 ng/mL (median maternal PFOA)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	5.5 ng/mL (median PFOA in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	2.4 ng/mL (median PFOA in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	14.8–<20.2, 20.22–<27.9, and 27.9–202.1 ng/mL (2nd, 3rd, and 4th PFOS quartiles)	ADHD	OR 0.99 (0.76–1.30), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.83 (0.70–0.98)*, 2nd quartile** **OR 0.68 (0.52–0.89)*, 3rd quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	13 ng/mL (maternal PFOS median)	Social responsiveness scale score (test of autism)	No association
Donauer et al. 2015 General population (n=349 infants)	13.25 ng/mL (maternal geometric mean PFOS)	Neurobehavioral outcomes	NS (p>0.05)
		Hypotonic	NS (p=0.3996)
		High arousal/difficult	NS (p=0.4678)
Fei et al. 2008b General population (n=1,400 infants)	35.3 ng/mL (maternal median PFOS)	Apgar scores <10	OR 1.20 (0.67–2.14)
		Neurobehavioral milestones Delay in age of sitting Earlier use of word-like sounds Delays in using 2-word sentences Other milestones	 **Association (p=0.041 for trend)*** **Association (p=0.039 for trend)*** **Association (p=0.050 for trend)*** NS (p>0.05)
		Motor and mental development at 6 months	NS (p>0.05)
Fei and Olsen 2011 General population (n=526–787 children)	34.4 ng/mL (maternal median PFOS)	Behavioral health	NS (p>0.39 for trend)
		Motor coordination	NS (p=0.41 for trend)
Forns et al. 2015 General population (n=843 infants)	110 ng/L (median PFOS breast milk level)	Risk of an abnormal score on neurobehavioral assessment questionnaire	OR 0.96 (0.76–1.20) at 6 months of age OR 0.93 (0.74–1.17) at 24 months of age
Goudarzi et al. 2016b General population (n=173 at 6 months and 133 at 18 months)	5.7 ng/mL (maternal median PFOS)	MDI/PDI at 6 months of age	NS (p>0.05)
		MDI/PDI at 18 months of age	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Gump et al. 2011 General population (n=83 children aged 9–11 years)	9.90 ng/mL (mean PFOS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	22.6 ng/mL (median PFOS)	ADHD (parent reported)	**OR 1.03 (1.01–1.05)*, per 1 ng/mL PFOS**
Høyer et al. 2015a General population (n=1,106 children)	10.0 and 16.6–87.3 ng/mL (maternal median and 3rd tertile PFOS)	Motor skills	β -0.1 (-1.2–1.1)
		Abnormal behavior	OR 1.5 (0.5–4.8), 3rd tertile
		Hyperactivity	OR 1.4 (0.4–4.9), 3rd tertile
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	19.8 ng/mL (maternal median serum PFOS)	Verbal comprehension (15-month-olds)	**β 0.03 (0.01–0.05)***
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	**β -0.01 (-0.01–0.00)***
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.79 ng/mL (cord blood weighted average PFOS)	Inattention	NS (p=0.8508)
		Hyperactivity/impulsivity	NS (p=0.6857)
		Emotional symptoms	NS (p=0.9431)
		Conduct problems	NS (p=0.4938)
		Hyperactivity/inattention	NS (p=0.5226)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	26.80, 25.40, and 27.40 ng/mL (maternal median PFOS for ADHD, autism, and controls)	ADHD	RR 0.87 (0.74–1.02) **RR 0.79 (0.64–0.98)*, 4th quartile**
		Autism	RR 0.92 (0.69–1.22)
Ode et al. 2014 General population (n=206 children with ADHD and 206 controls)	6.92 and 6.77 ng/mL (cord blood median PFOS in cases and controls)	ADHD	OR 0.98 (0.92–1.04), per 1 ng/mL increase in PFOS
Oulhote et al. 2016 General population (n=567 7-year-old children)	27.42 ng/mL (maternal geometric mean PFOS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	16.75 ng/mL (geometric mean PFOS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	15.27 ng/mL (geometric mean PFOS in 7-year-old children)	Behavioral development scores	No associations
Quaak et al. 2016 General population (n=76 infants 18 months of age)	1.5836 ng/mL (cord mean PFOS)	Score on test evaluating ADHD	NS (p=0.19), 3rd tertile
		Males	NS (p=0.35), 3rd tertile
		Females	NS (p=0.43), 3rd tertile
		Scores on test evaluating externalizing problem	NS (p=0.31), 3rd tertile
		Males	NS (p=0.74), 3rd tertile
		Females	NS (p=0.31), 3rd tertile
Strøm et al. 2014 General population (n=876 adults age 20 years)	21.4 ng/mL (median maternal PFOS)	ADHD	NS (p=0.38 for trend of 3rd tertile)
		Depression	NS (p=0.14 for trend of 3rd tertile)
		Scholastic achievement	NS (p=0.59 for trend of 3rd tertile)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	13.2 ng/mL (maternal median PFOS)	Behavioral regulation	**β 3.14 (0.68–5.61)***
		Metacognition	**β 3.10 (0.62–5.58)***
		Global executive functioning	**β 3.38 (0.86–5.90)***
		Global executive functioning composite score >60	**OR 2.19 (1.03–4.66)***
Vuong et al. 2018 General population (n=208 8-year-old children)	3.9 ng/mL (mean serum PFOS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.53 (0.67–3.52)
		At risk behavior regulation score	OR 0.40 (0.14–1.14)
		At risk global executive score	OR 1.04 (0.41–2.68)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	13.25 and 12.28 ng/mL (maternal median PFOS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	13.0 ng/mL (median maternal PFOS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	6.6 ng/mL (median PFOS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	3.6 ng/mL (median PFOS in 5-year-old children)	Reading scores At 8 years of age	 **Association (p<0.05)***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	2.9–<5.2 and 10.1–276.4 ng/mL (2nd and 4th PFHxS quartiles)	ADHD 5–18 years 12–15 years	 **OR 1.27 (1.06–1.52)*, 2nd quartile** **OR 1.46 (1.10–1.93)*, 2nd quartile**
		Learning problems 5–18 years old 12–15 years old	 OR 1.19 (1.00–1.41), 4th quartile OR 1.05 (0.79–1.40), 4th quartile
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	1.6 ng/mL (maternal PFHxS median)	Social responsiveness scale score (test for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	6.06 ng/mL (mean PFHxS)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.01)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	2.2 ng/mL (median PFHxS)	ADHD (parent reported)	**OR 1.06 (1.02–1.11)*, per 1 ng/mL PFOS**
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	1.6 ng/mL (maternal median serum PFHxS)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Communicative scores (38-month-olds)	NS (p>0.05)
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.84. 0.92, and 0.92 ng/mL (maternal median PFHxS for ADHD, autism, and controls)	ADHD	**RR 0.67 (0.54–0.83)*, 4th quartile**
		Autism	RR 1.10 (0.92–1.33)
Oulhote et al. 2016 General population (n=567 7-year-old children)	4.43 ng/mL (maternal geometric mean PFHxS)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.54 ng/mL (geometric mean PFHxS in 5-year-old children)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.53 ng/mL (geometric mean PFHxS in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	1.5 ng/mL (maternal median PFHxS)	Behavioral regulation	β 1.19 (-0.54–5.40)
		Metacognition	β 1.31 (-0.43–3.04)
		Global executive functioning	β 1.36 (-0.41–3.12)
		Global executive functioning composite score >60	**OR 1.71 (1.05–2.77)***
Vuong et al. 2018 General population (n=208 8-year-old children)	1.4 ng/mL (mean serum PFHxS)	Metacognition index score	NS (p>0.05)
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	NS (p>0.05)
		At risk metacognition score	OR 1.10 (0.58–2.09)
		At risk behavior regulation score	OR 0.54 (0.22–1.32)
		At risk global executive score	OR 0.65 (0.32–1.32)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.69 and 0.69 ng/mL (maternal median PFHxS for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.5 ng/mL (median maternal PFHxS)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.9 ng/mL (median PFHxS in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFHxS in 5-year-old children)	Reading scores At 8 years of age	 NS (p>0.05)
PFNA			
Stein and Savitz 2011 Community (C8) (n=10,546 children aged 5–18 years)	1.2–<1.5, 1.5–<2.0, and 2.0–24.1 ng/mL (2nd, 3rd, and 4th PFNA quartiles)	ADHD 5–18 years 12–15 years	 OR 0.99 (0.84–1.18), 4th quartile OR 1.00 (0.75–1.32), 4th quartile
		Learning problems 5–18 years old 12–15 years old	 **OR 0.81 (0.69–0.95)*, 3rd quartile** **OR 0.73 (0.55–0.98)*, 4th quartile**
Braun et al. 2014 General population (n=175 children 4 and 5 years old)	0.9 ng/mL (maternal PFNA median)	Social responsiveness scale score (tests for autism)	No association
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.82 ng/mL (mean PFNA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Hoffman et al. 2010 General population (NHANES) (n=571 children 12–15 years)	0.6 ng/mL (median PFNA)	ADHD (parent reported)	OR 1.32 (0.86–2.02), per 1 ng/mL PFNA

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jeddy et al. 2017 General population (n=432 mother-daughter pairs)	0.5 ng/mL (maternal median serum PFNA)	Verbal comprehension (15-month-olds)	NS (p>0.05)
		Vocabulary comprehension and production (15-month-olds)	NS (p>0.05)
		Nonverbal communication (15-month-olds)	NS (p>0.05)
		Social development (15-month-olds)	NS (p>0.05)
		Intelligibility scores (38-month-olds)	NS (p>0.05)
		Language scores (38-month-olds)	NS (p>0.05)
		Communicative scores (38-month-olds)	NS (p>0.05)
Lien et al. 2016 General population (n=282 children aged 7 years)	4.49 ng/mL (cord blood weighted average PFNA)	Inattention	**Inverse association (p=0.0129)***
		Hyperactivity/impulsivity	NS (p=0.0588)
		Emotional symptoms	NS (p=0.1902)
		Conduct problems	NS (p=0.6931)
		Hyperactivity/inattention	**Inverse association (p=0.0484)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.42, 0.41, and 0.43 ng/mL (maternal median PFNA for ADHD, autism, and controls)	ADHD	RR 0.80 (0.62–1.03)
		Autism	RR 0.80 (0.58–1.11)
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.61 ng/mL (maternal geometric mean PFNA)	Behavioral development scores	No association
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.01 ng/mL (geometric mean PFNA in 5-year-old children)	Total behavioral development score and higher externalizing problems score	**Association***

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Oulhote et al. 2016 General population (n=567 7-year-old children)	1.2 ng/mL (geometric mean PFNA in 7-year-old children)	Behavioral development scores	No association
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.9 ng/mL (maternal median PFNA)	Behavioral regulation	β 2.57 (-0.26–5.40)
		Metacognition	β 1.37 (-1.49–4.23)
		Global executive functioning	β 2.01 (-0.89–4.92)
Vuong et al. 2018 General population (n=208 8-year-old children)	0.8 ng/mL (mean serum PFNA)	Metacognition index score	**β 3.4 (0.4–6.3, p<0.05)***
		Behavior regulation index score	NS (p>0.05)
		Global executive functioning score	**Association (p<0.05)***
		At risk metacognition score	**OR 2.94 (1.52–5.69)***
		At risk behavior regulation score	**OR 2.75 (1.30–5.79)***
		At risk global executive score	**OR 3.07 (1.60–5.90)***
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	1.59 and 1.44 ng/mL (maternal median PFNA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years Full scale IQ Visual IQ Performance IQ	 NS (p>0.05) **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.9 ng/mL (median maternal PFNA)	Reading scores At 5 years of age At 8 years of age	 NS (p>0.05) NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	1.2 ng/mL (median PFNA in 3-year-old children)	Reading scores At 5 years of age At 8 years of age	 **Association (p<0.05)*** NS (p>0.05)
Zhang et al. 2018 General population (n=167 mother-child pairs)	0.7 ng/mL (median PFNA in 5-year-old children)	Reading scores At 8 years of age	 NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFDA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.26 ng/mL (mean PFDA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***
Liew et al. 2015 General population (n=215, ADHD cases, 213 autism cases, 545 controls)	0.15, 0.15, and 0.17 ng/mL (maternal median PFDA for ADHD, autism, and controls)	ADHD	**RR 0.76 (0.64–0.91)*** **RR 0.53 (0.43–0.66)*, 4th quartile**
		Autism	RR 0.79 (0.63–1.01) **RR 0.52 (0.35–0.77)*, 4th quartile**
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (maternal geometric mean PFDA)	Behavioral development scores	No associations
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.28 ng/mL (geometric mean PFDA in 5-year-old children)	Total behavioral development score and higher externalizing problems and hyperactivity/ inattention scores	**Associations***
Oulhote et al. 2016 General population (n=567 7-year-old children)	0.36 ng/mL (geometric mean PFDA in 7-year-old children)	Behavioral development scores	No associations
Vuong et al. 2016 General population (n=256 children 5 or 8 years of age)	0.2 ng/mL (maternal median PFDA)	Behavioral regulation	β 0.70 (-3.31–1.92)
		Metacognition	β 1.24 (-3.87–1.39)
		Global executive functioning	β -1.13 (-3.79–1.54)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.44 and 0.44 ng/mL (maternal median PFDA for 5- and 8-year-old children)	IQ score Age 5 years Age 8 years	 NS (p>0.05) NS (p>0.05)

Table 2-24. Summary of Neurodevelopmental Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFUnA			
Lien et al. 2016 General population (n=282 children aged 7 years)	7.96 ng/mL (cord blood weighted average PFUnA)	Inattention	NS (p=0.6177)
		Hyperactivity/impulsivity	NS (p=0.3642)
		Emotional symptoms	NS (p=0.0517)
		Conduct problems	NS (p=0.1207)
		Hyperactivity/inattention	NS (p=0.9991)
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	3.42 and 3.13 ng/mL (maternal median PFUnA for 5- and 8-year-old children)	IQ score	NS (p>0.05)
		IQ scores, age 8 years	
		Full scale IQ	NS (p>0.05)
		Visual IQ	NS (p>0.05)
		Performance IQ	**Inverse association (p<0.05)***
PFDoDA			
Wang et al. 2015b General population (n=120 children age 5 years and 120 children aged 8 years)	0.38 and 0.37 ng/mL (maternal median PFDoDA for 5- and 8-year-old children)	IQ score	
		Age 5 years	NS (p>0.05)
		Age 8 years	NS (p>0.05)
FOSA			
Gump et al. 2011 General population (n=83 children aged 9–11 years)	0.75 ng/mL (mean FOSA)	Performance on task requiring behavioral inhibition	**Inverse association (p<0.05)***

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

ADHD = attention deficit hyperactivity disorder; FOSA = perfluorooctane sulfonamide; MDI/PDI = mental and psychomotor development indices; NHANES = National Health and Nutrition Examination Survey; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18 years)	26 and 20 ng/mL (median PFOA in boys and girls)	Age of puberty Boys Girls	 OR 0.95 (0.84–0.07) **OR 0.54 (0.35–0.84)*, 2nd quartile**
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	34.8 and 30.1 ng/mL (median PFOA in boys and girls)	Estradiol	NS (interquartile difference of 4.3, 95% CI -0.4–9.1), boys NS (4.2, 95% CI -0.7–9.4), girls
		Total testosterone	**Inverse association (-4.9, 95% CI -8.7 to -0.8)*, boys** NS (-2.5, 95% CI -6.7–1.8), girls
		Insulin-like growth factor-1	NS (-0.4, 95% CI -3.4–2.7), boys **Inverse association (-3.6, 95% CI -6.6 to -0.5)*, girls**
Christensen et al. 2011 General population (n=448 girls)	3.7 ng/mL (maternal median PFOA)	Earlier age of menarche	OR 1.01 (0.61–1.68)
Itoh et al. 2016 General population (n=189 infants)	1.4 ng/mL (maternal median PFOA)	Cord estradiol	NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio	NS (p>0.05)
		Cord progesterone	NS (p>0.05)
		Cord prolactin	NS (p>0.05)
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p=0.040)*** NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Kristensen et al. 2013 General population (n=343 females approximately 20 years of age)	3.6 and 4.4–19.8 ng/mL (maternal median PFOA and 3rd PFOA tertile)	Age of menarche	**Association (p=0.01)***
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	1.7 ng/mL (median maternal serum PFOA)	Anogenital distance	NS (p=0.71), boys NS (p=0.71), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>4.1 ng/mL (maternal 3rd tertile PFOA)	Testosterone	**β 0.24 (0.05–0.43)*, 3rd tertile**
		SHBG	β 5.02 (-13.07–11.00), 3rd tertile
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	2.6 and 2.1 ng/mL (median cord blood PFOA Denmark and Finland cohorts)	Cryptorchidism	OR 0.51 (0.21–1.20), whole cohort **OR 0.35 (0.12–0.99, p=0.04 for trend)*, Finland cohort 3rd tertile**
PFOS			
Lopez-Espinosa et al. 2011 Community (C8) (n=3,076 boys and 2,931 girls aged 8–18)	20 and 18 ng/mL (PFOS median in boys and girls)	Age of puberty Boys Girls	 **OR 0.58 (0.37–0.90)*, 3rd quartile** **OR 0.55 (0.35–0.86)*, 3rd quartile**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	22.4 and 20.9 ng/mL (PFOS median in boys and girls)	Estradiol	**Inverse association (interquartile difference of -4.0, 95% CI -7.7 to -0.1)*, boys** NS (-0.3, 95% CI -4.6–4.2), girls
		Total testosterone	**Inverse association (-5.8, 95% CI -9.4 to -2.0)*, boys** **Inverse association (-6.6, 95% CI -10.1 to -2.8)*, girls**
		Insulin-like growth factor-1	**Inverse association (-5.9, 95% CI -8.3 to -3.3)*, boys** **Inverse association (-5.6, 95% CI -8.2 to -2.9)*, girls**
Christensen et al. 2011 General population (n=448 girls)	19.8 ng/mL (maternal median PFOS)	Earlier age of menarche	OR 0.68 (0.40–1.13)
Itoh et al. 2016 General population (n=189 infants)	5.2 ng/mL (maternal median PFOS)	Cord estradiol Males Females	 **Association (p=0.021)*** NS (p>0.05)
		Cord testosterone	NS (p>0.05)
		Cord testosterone: estradiol ratio Males Females	 **Inverse association (p=0.008)*** NS (p>0.05)
		Cord progesterone Males Females	 **Association (p=0.043)*** **Association (p=0.002)***
		Cord prolactin Males Females	 NS (p>0.05) **Association (p=0.001)***
		Cord LH	NS (p>0.05)
		Cord FSH	NS (p>0.05)
		Cord SHBG	NS (p>0.05)

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Cord insulin-like factor 3	NS (p>0.05)
		Cord inhibin Males Females	 **Association (p<0.001)*** NS (p>0.05)
Kristensen et al. 2013 General population (n=343 young women approximately 20 years of age)	21.1 ng/mL (maternal median PFOS)	Age of menarche	NS (p=0.28)
		Menstrual cycle length	NS (p>0.05)
		Total testosterone	NS (p>0.05)
		SHBG	NS (p>0.05)
		Free androgen index	NS (p>0.05)
		Dehydroepiandrosterone sulphate	NS (p>0.05)
		Anti-Müllerian hormone	NS (p>0.05)
		Number of follicles/ovary	NS (p>0.05)
Lind et al. 2017a General population (n=649 pregnant women)	8.1 ng/mL (median maternal serum PFOS)	Anogenital distance	β 0.5 (-1.2–2.2, p=0.55), boys **β -0.4(-3.8 to -0.7, p<0.01)*, girls**
Toft et al. 2016 General population (270 cases cryptorchidism, 75 cases hypospadias, and 300 controls)	>1.4 ng/mL (amniotic fluid 3rd tertile PFOS)	Cryptorchidism	OR 1.01 (0.66–1.53), 3rd tertile
		Hypospadias	OR 0.69 (0.35–1.38), 3rd tertile
		Testosterone	**Association (p=0.002)***
		Androstenedione	**Association (p=0.001)***
		Progesterone	**Association (p=0.001)***
		Cortisol	**Association (p<0.001)***
		DHEAS	NS (p=0.93)
		Insulin-like factor 3	**Inverse association (p<0.001)***
Vesterholm Jensen et al. 2014 General population (n=107 cases cryptorchidism [29 from Denmark and 78 from Finland] and 108 matched controls from Denmark and Finland)	9.1 and 5.2 ng/mL (median cord blood PFOS Denmark and Finland cohorts)	Cryptorchidism	OR 0.83 (0.44–1.58), whole cohort

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	8.1 and 7.0 ng/mL (PFHxS median in boys and girls)	Estradiol	NS (interquartile difference of -1.3, 95% CI -5.5–3.1), boys NS (2.1, 95% CI -2.2–6.5), girls
		Total testosterone	NS (-2.7, 95% CI -6.4–1.2), boys NS (0.2, 95% CI -3.5–4.0), girls
		Insulin-like growth factor-1	NS (-2.5, 95% CI -5.2–0.3), boys NS (-2.1, 95% CI -4.8–0.7), girls
Christensen et al. 2011 General population (n=448 girls)	1.6 ng/mL (maternal median PFHxS)	Earlier age of menarche	OR 0.89 (0.65–1.22)
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFHxS)	Anogenital distance	NS (p=0.56), boys NS (p=0.10), girls
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>1.9 ng/mL (maternal 3rd tertile PFHxS)	Testosterone	β 0.18 (0.00–0.35), 3rd tertile
		SHBG	β 5.31 (-21.61–11.00), 3rd tertile
PFNA			
Lopez-Espinosa et al. 2016 Community (C8) (n=1,169 boys and 1,123 girls aged 6–9 years)	1.7 and 1.7 ng/mL (PFNA median in boys and girls)	Estradiol	NS (interquartile difference of -2.5, 95% CI -6.2–1.4), boys NS (-2.4, 95% CI -6.3–1.7), girls
		Total testosterone	NS (-2.1, 95% CI -5.5–1.3), boys NS (-1.9, 95% CI -5.5–1.9), girls
		Insulin-like growth factor-1	**Inverse association (-3.5, 95% CI -6.0 to -1.0)*, boys** **Inverse association (-3.8, 95% CI -6.4 to -1.2)*, girls**
Lind et al. 2017a General population (n=649 pregnant women)	0.7 ng/mL (median maternal serum PFNA)	Anogenital distance	β -0.5 (-2.1–1.1, p=0.63), boys **β -1.8 (-3.5 to -0.1, p=0.05)*, girls**

Table 2-25. Summary of Effects on the Development of the Reproductive System in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Maisonet et al. 2015 General population (n=72 girls aged 15 years)	>0.6 ng/mL (maternal 3rd tertile PFNA)	Testosterone	β 0.05 (-0.14–0.24), 3rd tertile
		SHBG	β 7.91 (-8.69–24.52), 3rd tertile
PFDA			
Lind et al. 2017a General population (n=649 pregnant women)	0.3 ng/mL (median maternal serum PFDA)	Anogenital distance	β -0.6 (-2.0–0.9, p=0.97), boys **β -1.3 (-2.8–0.2, p=0.04)*, girls**
FOSA			
Christensen et al. 2011 General population (n=448 girls)	0.2 ng/mL (maternal median FOSA)	Earlier age of menarche	OR 0.91 (0.67–1.24)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 13 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

CI = confidence interval; DHEAS = dihydroepiandrosterone sulfate; FOSA = perfluorooctane sulfonamide; FSH = follicle stimulating hormone; LH = luteinizing hormone; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SHBG = sex hormone binding globulin

alterations in birth weight were found for other perfluoroalkyls (PFHxS, PFNA, PFDA, PFUnA, PFDoDA). Overall, no associations were found between serum PFOA, PFOS, PFHxS, PFNA, or PFUnA and increases in the risk of low birth weight or small for gestational age infants. The small number of studies (2 or less) examining potential developmental effects of PFHpA, PFBA, and FOSA do not allow for assessing possible associations with pregnancy outcomes or birth outcomes.

No consistent results for risks of birth defects have been found; these potential endpoints were only examined for a few perfluoroalkyls. The available epidemiological data do not suggest associations between perfluoroalkyls and IQ or scholastic achievement for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, or PFDoDA. Similarly, no associations were found between PFOA, PFOS, PFHxS, PFNA, or PFDA and increased risk of ADHD; several studies found decreased risk of ADHD. Inconsistent results have been found between PFOA and PFOS and delays in puberty or age of puberty, especially in girls.

Summaries of laboratory animal studies are presented in Tables 2-1, 2-3, 2-4, and 2-5 and the NOAEL and LOAEL values are presented in Figures 2-6, 2-8, 2-9, and 2-10; no data were available for PFHpA or FOSA. Laboratory animal studies provide strong evidence of the developmental toxicity of a number of perfluoroalkyls. Prenatal losses and decreases in pup survival were observed following exposure to PFOA, PFOS, PFNA, PFDA, PFDoDA and PFHxA; no deaths were observed in a single study of PFBS. Decreases in fetal weights, birth weight, and pup weight were observed in studies of PFOA, PFOS, PFNA, PFDA, PFUnA, PFBS, and PFHxA; no effects on weight were observed in studies on PFHxS or PFDoDA. In PFOA studies, delays in mammary gland development were observed at fairly low doses. Several studies have demonstrated biphasic alterations in motor activity in rodents exposed to PFOA, PFOS, and PFHxS; no effects on locomotor activity were observed in a study of PFDA. Studies in laboratory animals have examined a number of developmental endpoints, including pup survival, malformations, birth weight, mammary gland development, and neurodevelopment.

PFOA

Epidemiological Studies—Pregnancy Outcomes. The results of available epidemiological studies of women living near a PFOA facility and the general population do not suggest an association between serum PFOA levels and adverse pregnancy outcomes. No increases in risk of miscarriage (Darrow et al. 2014; Jensen et al. 2015; Savitz et al. 2012b; Stein et al. 2009), stillbirths (Savitz et al. 2012b), pregnancy loss (Buck Louis et al. 2016), or pre-term birth (Chen et al. 2012a; Darrow et al. 2013; Hamm et al. 2010; Manzano-Salgado et al. 2017a; Sagiv et al. 2018; Stein et al. 2009; Whitworth et al. 2012a) were found.

The Whitworth et al. (2012a) general population study reported a decrease in the risk of preterm births among women with serum PFOA levels in the 4th quartile. Most studies did not find an association between maternal PFOA levels and gestational age (Apelberg et al. 2007b; Chen et al. 2012a; Lauritzen et al. 2017; Li et al. 2017; Manzano-Salgado et al. 2017a) or gestational length (Lind et al. 2017; Sagiv et al. 2018). The exception is a study by Wu et al. (2012) of pregnant women with higher serum PFOA levels which found an inverse association between maternal serum PFOA levels and gestational age.

Epidemiological Studies—Birth Outcomes. Community and general population exposure studies have evaluated a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. In highly exposed populations, no association between maternal serum PFOA levels and birth weight were found (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b). Several general population studies have found associations between maternal serum PFOA and birth weight. Fei et al. (2007, 2008a), Lauritzen et al. (2017), Lenters et al. (2016a), Maisonet et al. (2012), Minatoya et al. (2017), Starling et al. (2017), and Wu et al. (2012) found inverse associations between maternal serum PFOA and birth weight. However, 23 other general population studies did not find associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Li et al. 2017; Lind et al. 2017; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Wang et al. 2016; Washino et al. 2009; Whitworth et al. 2012a). As illustrated in Figure 2-35, most studies found no association between maternal serum PFOA levels and the risk of low birth weight infants (typically defined as <2,500 g) (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Stein et al. 2009) or found a decreased risk of low birth weight infants (Nolan et al. 2009; Savitz et al. 2012a). Similarly, most studies found no increases in the risk for small for gestational age (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Savitz et al. 2012b; Wang et al. 2016; Whitworth et al. 2012a); these data are presented in Figure 2-36. One study (Savitz et al. 2012b) of C8 participants did find an increase in the risk of small for gestational age; however, when the maternal serum PFOA levels were categorized into percentiles, the risk was not increased in infants whose maternal serum PFOA levels were ≥80th percentile (21.0–717.6 ng/mL). A general population study (Lauritzen et al. 2017) also found an increased risk of small for gestational age (Lauritzen et al. 2017). Using data compiled from four European birth cohort studies in which cord serum PFOA was measured or estimated from breast milk levels, Govarts et al. (2018) did not find an association between cord PFOA and the risk of small for gestational age.

Figure 2-35. Risk of Low Birth Weight Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)

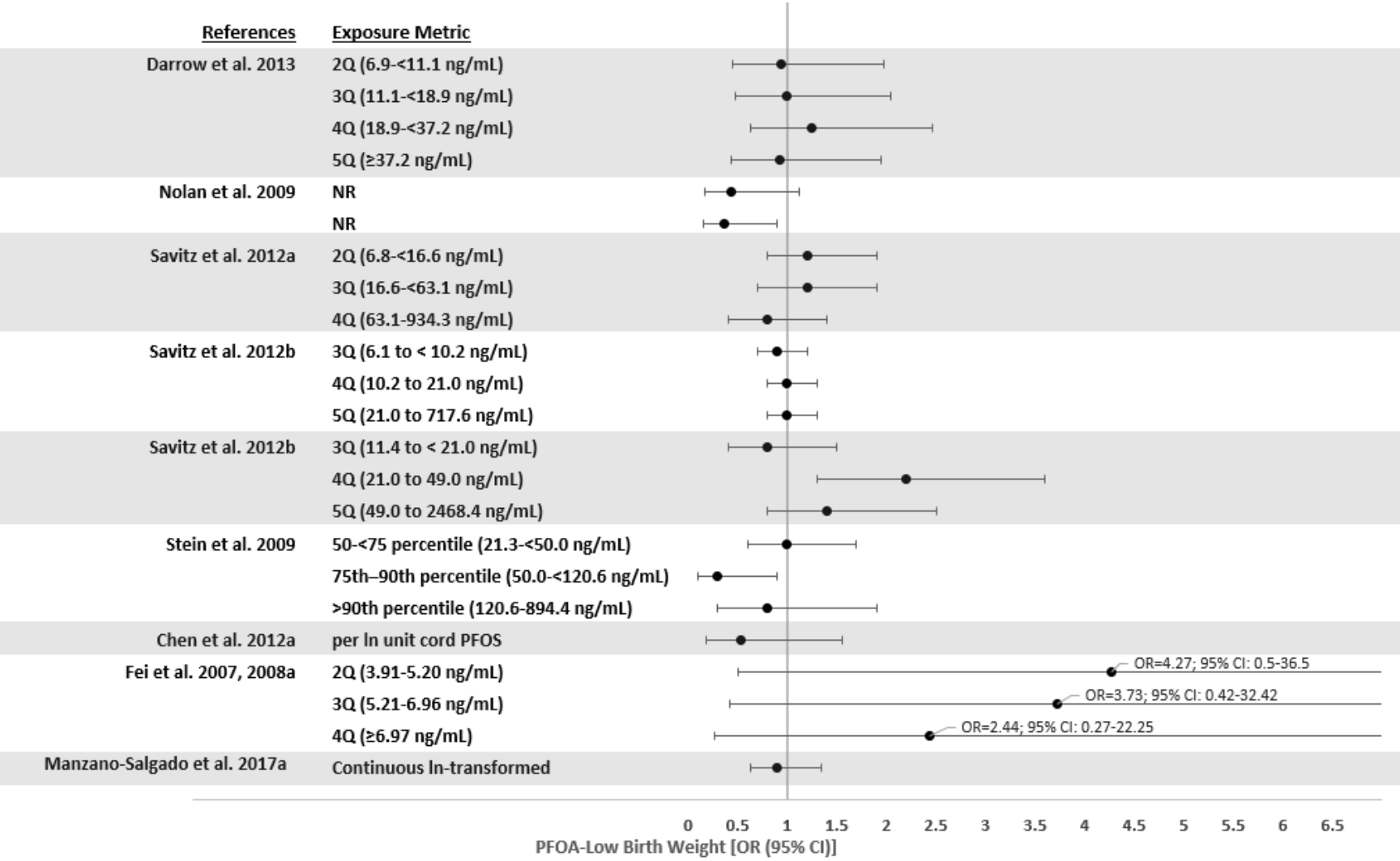
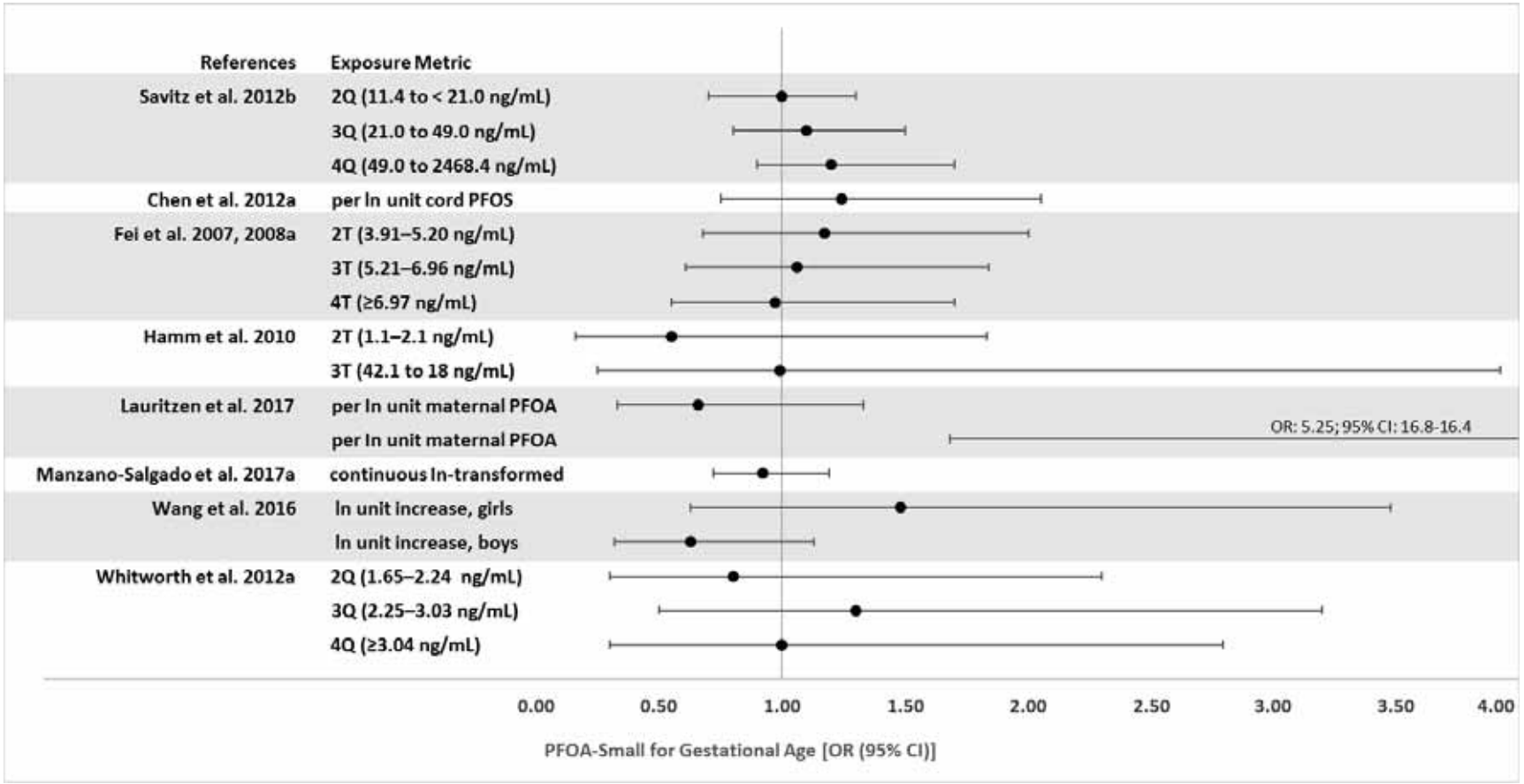


Figure 2-36. Risk of Small for Gestational Age Infant Relative to PFOA Levels (Presented as Adjusted Odds Ratios)



However, among women who smoked during pregnancy, cord serum PFOA was associated with an increased risk of small for gestational age infants (OR 2.177, 95% CI 1.022–4.643); no association was found among nonsmoking women (OR 0.511, 95% CI 0.869–2.632). Six general population studies found inverse associations between maternal serum PFOA levels and birth length, abdominal circumference, and/or ponderal index (ratio of birth weight to birth length) (Alkhalawi et al. 2016; Apelberg et al. 2007b; Cao et al. 2018; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Wu et al. 2012). However, most studies did not find associations between maternal serum PFOA levels and birth length; head, chest, or abdominal circumference; or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Studies examining newborn leptin and adiponectin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017) and adiposity (Starling et al. 2017) have not found associations with maternal PFOA levels.

In a systematic review of 19 epidemiological studies discussed above, Johnson et al. (2014) evaluated the possible association between PFOA exposure and fetal growth and concluded that there was sufficient evidence that PFOA reduces fetal growth based on a moderate rating of the human evidence. A meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007, 2008a), Fromme et al. (2010), Hamm et al. (2009), Kim et al. (2011), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012) studies showed an association between PFOA and birth weight; a 1 ng/mL increase in serum or plasma PFOA was associated with a -18.9 g (95% CI -29.8 to -7.9) change in birth weight. The results of this meta-analysis are also reported in Lam et al. (2014). Johnson et al. (2014) and Lam et al. (2014) discuss whether glomerular filtration rate was a possible confounder in evaluating the association between serum PFOA and birth weight. They concluded that there was insufficient evidence of an association between glomerular filtration rate and birth weight.

A second meta-analysis (Verner et al. 2015) of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies found a similar result, a 1 ng/mL increase in PFOA levels was associated with a 14.72 g (95% CI -21.66 to -7.78) decrease in birth weight. Verner et al. (2015) also utilized a PBPK model to simulate maternal PFOA levels at delivery and evaluate the influence of glomerular filtration rate on the association between maternal PFOA and birth weight. In contrast to the conclusions of Johnson et al. (2014) and Lam et al. (2014), Verner et al. (2015) found that a 1 ng/mL increase in PFOA was associated

with a 7.13 g (95% CI -8.46 to -5.80) decrease in birth weight; suggesting that glomerular filtration rate may be a confounding factor.

A third meta-analysis conducted by Negri et al. (2017) of the Apelberg et al. (2007b), Bach et al. (2016), Chen et al. (2012a), Darrow et al. (2013), Fei et al. (2007), Fromme et al. (2010), Hamm et al. 2009), Kim et al. (2011), Maisonet et al. (2012), Monroy et al. (2008), Washino et al. (2009), and Whitworth et al. (2012a) studies reported a -12.80 g (95% CI -23.21 to -2.38) change in birth weight associated with a 1 ng/mL increase in serum PFOA.

A fourth meta-analysis conducted by Steenland et al. (2018) included 24 studies; 11 of the 12 studies included by Negri et al. (2017) (the Monroy et al. 2008 study was excluded) plus studies by Wu et al. (2012), Robledo et al. (2015a), Callan et al. (2016), Lee et al. (2016), Wang et al. (2016), Lenters et al. (2016a); Minatoya et al. (2017), Shi et al. (2017), Li et al. (2017), Manzano-Selgado et al. (2017); Starling et al. (2017), and Sagiv et al. (2018). The study found a that a 1 ng/mL increase in serum PFOA was associated with a -10.5 g (95% CI -16.7 to -4.4) change in birth weight. In sensitivity analysis, inclusion of the Savitz et al. (2012b) study, which used predicted maternal serum concentrations based on estimated environmental exposure, resulted in a birth weight change of -1.0 g (95% CI -2.4–0.4) per 1 ng/mL increase in serum PFOA. Categorizing studies based on when maternal serum PFOA levels were sampled resulted in differences in birth weight change; -3.3 g (95% CI -9.6–3.0) when sampled early in pregnancy or shortly after conception and -17.8 g (-25.0 to -10.6) when sampled late in pregnancy. The investigators suggested that this may be indicative of reverse causality or confounding.

A small number of studies have examined the potential associations between PFOA exposure and risks of birth defects. In a study of C8 Health Study participants, no increases in the risk of brain, gastrointestinal, kidney, craniofacial, eye, limb, genitourinary, or heart defects were found (Stein et al. 2014c).

Epidemiological Studies—Neurodevelopmental Outcomes. A number of epidemiological studies have evaluated neurodevelopment at various ages using maternal serum PFOA or cord blood PFOA as a biometric of exposure. Fei et al. (2008b) did not find an increased risk of Apgar scores of <10 in newborns. Another study found an inverse association between maternal serum PFOA and the 5-minute Apgar score (Wu et al. 2012). Utilizing the Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) in 5-week-old infants, Donauer et al. (2015) found an increased risk of reduced muscle tone (hypotonia), which was associated with maternal serum PFOA levels, but found no associations on tests of social/easy going or high arousal/difficult. Goudarzi et al. (2016b) reported lower scores on tests of

mental and psychomotor development in female 6-month-old infants; no association was found when male and female infants were grouped together. When the infants were tested at 18 months of age, no association between maternal PFOA levels and mental and psychomotor indices were found. Fei et al. (2008b) did not find associations between maternal PFOA levels and the risk of delays in motor, cognitive, or language development in 6- and 18-month-old infants. It is noted that in the Fei et al. (2008b) study, the mothers were asked to recall at what age the infants reached a developmental milestone, whereas standardized tests of development were used in the other two studies. Although the Donauer et al. (2015) and Goudarzi et al. (2016b) studies suggest some delays in neurodevelopment in young infants, more research is needed before establishing a possible relationship with PFOA.

Studies in children have examined possible associations between PFOA and IQ, motor skills, behavior, and ADHD. An association between estimated *in utero* PFOA levels and IQ was found in 6–12-year-old children participating in the C8 Health Studies (Stein et al. 2013); higher IQ scores were found in children with the highest estimated PFOA exposure levels. The study did not find an association with reading or math skills. A general population study (Wang et al. 2015b) did not find an association between maternal serum PFOA levels and IQ scores in children 5 or 8 years of age. Jeddy et al. (2017) did not find an association between maternal PFOA levels and early communication development in 15-month-olds; among 38-month-olds, an inverse association was found for intelligibility scores, but there were no associations with other scores of communication development. In a prospective study, maternal PFOA levels were not associated with reading scores in 5- or 8-year-old children (Zhang et al. 2018). Reading scores at age 5 years were associated with serum PFOA levels when the children were 3 years of age and serum PFOA levels in 5-year-olds were not associated with reading scores at 8 years of age (Zhang et al. 2018). In a study of adults (20 years of age), Strøm et al. (2014) did not find an association between maternal PFOA levels and scholastic achievement. A community study of children and adolescents did not find an association between serum PFOA levels and learning problems in 12–15- or 5–18-year-olds (Stein and Savitz 2011). Two studies (Fei and Olsen 2011; Høyer et al. 2015a) did not find associations between maternal PFOA levels and motor coordination in 7-year-old children or motor skills in 5–9-year-old children.

Several studies have examined possible associations between maternal or child PFOA levels and scores on tests/surveys that assess behavioral problems. No associations were found between maternal PFOA levels and behavioral problems in 7-year-old children (Fei and Olsen 2011) or behavioral regulation problems in 5- or 8-year-old children (Vuong et al. 2016) or 7-year-old children (Oulhote et al. 2016). Similarly, no associations between serum PFOA levels and scores on behavioral tests were observed in 7-

year-old children (Oulhote et al. 2016) or 9–11-year-old children (Gump et al. 2011). No associations between breast milk PFOA levels and behavioral development in 6- and 24-month-old infants were observed (Forns et al. 2015). In contrast, Høyer et al. (2015a) found an association between maternal PFOA levels and behavioral problems in 5–9-year-old children; the risk was increased in children with maternal PFOA levels in the 3rd tertile. Stein et al. (2014a) found an association between the children's serum PFOA levels and survey results on behavioral problems and emotional disturbances in girls aged 6–12 years of age; this association was not found in boys or in boys and girls combined. Additionally, the association was only found when the survey was completed by mothers, but not when completed by the child's teacher. Oulhote et al. (2016) found associations between serum PFOA in 5-year-old children and behavioral survey scores, particularly for internalizing problems, peer relationships, and autism screening scores. In a study of 8-year-old children, Vuong et al. (2018) found an association between PFOA and at risk metacognition scores, but no associations with at risk behavior regulation or global executive scores.

Ten studies have looked for a possible association between PFOA and ADHD in children. Two studies of participants of the C8 Health Study found lower scores on tests for ADHD (Stein et al. 2013) or lower risks of ADHD (Stein and Savitz 2011) associated with estimated *in utero* PFOA or child PFOA levels, respectively. In a third community study in which parents and teachers completed surveys regarding ADHD-like behaviors (Stein et al. 2014a), no association between the child's serum PFOA (measured 3–4 years before the surveys were completed) and ADHD-like behaviors were found when the mothers completed the survey and an inverse association was found when the teachers completed the survey. Segregating the children by sex resulted in an association in girls (mother-completed survey only) and no associations in boys. Two general population studies have found associations between the risk of ADHD or increases in ADHD behavior in children. An increase in the risk of parent-reported ADHD diagnosis was observed in a study of 12–15-year-old NHANES participants (Hoffman et al. 2010). The second study (Høyer et al. 2015a) found increases in hyperactivity among 5–9-year-old children with maternal serum PFOA levels in the 3rd tertile. When this multinational cohort was segregated by country, the association was only found in the group of children from Greenland, but not in the Ukrainian cohort. Median serum PFOA levels were slightly higher in the Greenland cohort; it is also noted that the median maternal PFOS levels were 4 times higher in the Greenland cohort than in the Ukraine cohort. Other general population studies have not found associations. Two case-control studies of children did not find increased risks of being diagnosed with ADHD associated with maternal PFOA levels (Liew et al. 2015) or cord blood PFOA levels (Ode et al. 2014). Two studies did not find associations between cord blood PFOA levels and performance on tests evaluating for ADHD symptoms in 7-year-old children (Lien et al. 2016) or 18-month-old infants (Quaak et al. 2016). A third study found no association between maternal

PFOA levels and ADHD in 20-year-olds (Strøm et al. 2014). In addition to looking at possible relationships between PFOA and ADHD, two studies did not find associations between maternal PFOA levels and autism behaviors (Braun et al. 2014) or the risk of autism diagnosis (Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. Studies exploring possible associations between PFOA and alterations in the development of the reproductive system have examined several outcomes including hormone levels in cord blood, hormone levels in children and adolescents, anogenital distance, congenital malformations of reproductive organs, and age of puberty in boys and girls.

A multinational case-control general population study (Vesterholm Jensen et al. 2014) found a decrease in the risk of cryptorchidism in the Finnish cohort, but not in the Danish cohort or in the combined cohort. With the exception of inhibin levels, no associations between maternal serum PFOA levels and cord blood levels of reproductive hormones were found (Itoh et al. 2016). Cord inhibin was associated with maternal serum PFOA levels in male infants, but not in female infants (Itoh et al. 2016). Some alterations in reproductive hormone levels were found in 6–9-year-old boys and girls participating in the C8 Health Study (Lopez-Espinosa et al. 2016). In boys, an inverse association between serum PFOA levels and total testosterone levels were observed; no associations were found for estradiol levels or insulin-like growth factor 1. In girls, an inverse association was found for insulin-like growth factor 1 levels and no associations were found for estradiol or testosterone levels. In adolescent girls, an association between maternal PFOA levels and testosterone levels was found (Maisonet et al. 2015a). This association was not found in young adult females (Kristensen et al. 2013). Other reproductive hormones were not shown to be associated with maternal PFOA levels (Kristensen et al. 2013; Maisonet et al. 2015a). Lind et al. (2017a) found no association between maternal PFOA levels and anogenital distance in boys or girls.

In a community exposure study (Lopez-Espinosa et al. 2011), increasing levels of serum PFOA were associated with delays in menarche in girls aged 8–18 years. Serum PFOA levels in the 2nd, 3rd, and 4th quartiles were associated with 142-, 163-, and 130-day delays in the onset of menarche, respectively. Using PBPK modeling, Wu et al. (2015) examined whether the association between serum PFOA and delays in the onset of menarche observed in the Lopez-Espinosa et al. (2011) study were due to reverse causality using a Monte Carlo PBPK model. They found that rapid growth around the time of menstruation onset may contribute to the apparent association between PFOA and delay of menarche. In the PBPK simulated study, the delay in the onset of menarche was 48 days for the 4th quartile (OR 0.82, 95% CI 0.76–0.88). A delay in menarche was also observed in a general population study; a 162-day

delay was estimated in the daughters of women with maternal serum PFOA levels in the 3rd tertile (Kristensen et al. 2013). A second general population study did not find an association between maternal serum PFOA levels and an earlier age of menarche (Christensen et al. 2011).

The only study available on age of puberty in males (Lopez-Espinosa et al. 2011) did not find an association with serum PFOA levels.

Laboratory Animal Exposure Studies. Exposure of pregnant Sprague-Dawley rats to 25 mg/m^3 APFO on GDs 6–15 resulted in a statistically significant reduction (10.3%) in neonatal body weight on PND 1, but the difference over controls was no longer significant on PND 4 (Staples et al. 1984). Exposure concentrations ≤10 mg/m^3 did not affect neonatal body weight. The incidence of malformations and variations among the exposed groups and controls was comparable.

In utero exposure to PFOA resulted in prenatal losses and decreases in pup survival. An increase in resorbed embryos were observed in mice administered 10 mg/kg on GD 13 (Chen et al. 2017b). An increase in resorptions was observed in mice administered ≥5 mg/kg/day throughout gestation (Lau et al. 2006) or 2 mg/kg/day on GDs 11–16 (Suh et al. 2011). Prenatal losses were also observed in PFOA mouse studies administering ≥6 mg/kg/day (Abbott et al. 2007), 5 mg/kg/day (White et al. 2011), or 20 mg/kg/day (Lau et al. 2006) throughout gestation; an increase in the percentage of dams with total litter loss was also observed at 5 mg/kg/day (Wolf et al. 2007). Administration of 20 mg/kg/day PFOA on GDs 7–17 or 10–17 did not result in litter loss (Wolf et al. 2007); no effect on litter size was observed as a result of administration of 5 mg/kg/day on GDs 8–17 (White et al. 2009). Gestational exposure (GDs 1–17) to PFOA also resulted in perinatal losses in mice administered 3 mg/kg/day PFOA (Ngo et al. 2014) and decreases in pup survival in mice exposed to ≥0.6 mg/kg/day (Abbott et al. 2007), 3 mg/kg/day (Albrecht et al. 2013), or 5 mg/kg/day (Lau et al. 2006; Yahia et al. 2010; White et al. 2011, Wolf et al. 2007); 100% pup mortality was observed in the offspring of mice exposed to 10 mg/kg/day throughout gestation (Yahia et al. 2010). Decreased pup survival was also observed in mice exposed to 5 mg/kg/day PFOA on GDs 15–17 (Wolf et al. 2007). No alterations in fetuses/litter or survival were observed at 1 mg/kg/day PFOA (Lau et al. 2006; White et al. 2011). Butenhoff et al. (2004b) also reported increases in pup mortality on PNDs 6–8 in the offspring of rats administered 30 mg/kg/day PFOA throughout gestation and during lactation.

Decreases in birth weight have not been consistently found in mouse studies with PFOA. No significant alterations in birth weight were observed in mice exposed to 3 mg/kg/day (Albrecht et al. 2013), 5 or

10 mg/kg/day (Lau et al. 2006), or 20 mg/kg/day (Abbott et al. 2007); decreases in birth or fetal weight were observed at 5 mg/kg/day (Hines et al. 2009; Yahia et al. 2010), 10 mg/kg/day (Suh et al. 2011), and 20 mg/kg/day (Lau et al. 2006). A decrease in mean litter weight on PNDs 2–14 was observed in mice administered ≥0.5 mg/kg/day PFOA on GDs 6–17 (Hu et al. 2010) and a decrease in pup body weight on PND 20 was observed in mice exposed to 5 mg/kg/day on GDs 8–17 or 12–17 (White et al. 2007). *In utero* exposure of mice to PFOA throughout gestation resulted in decreases in pup body weight in mice exposed to 1 mg/kg/day (Abbott et al. 2007; Hines et al. 2009), ≥3 mg/kg/day (Lau et al. 2006; Wolf et al. 2007), and 5 mg/kg/day (Yahia et al. 2010; White et al. 2007, 2011). In a cross-fostering study, lactation-only exposure (maternal dose of 5 mg/kg/day PFOA) resulted in decreased body weight in female pups on some PNDs (2, 3, 4, and 22, but not on PNDs 7, 10, 15, or 17) (Wolf et al. 2007). Hines et al. (2009) monitored body weights from birth to 18 months of age in female mice exposed *in utero* to PFOA on GDs 1–17. At weaning, decreases in body weight were observed at 1 and 5 mg/kg/day; by 10 weeks of age, there were no differences in body weight between the controls and mice exposed to ≥1 mg/kg/day. Significant increases in body weight were observed in mice exposed to 0.1 and 0.3 mg/kg/day, and by 20–29 weeks of age, the increases in body weight were observed in mice exposed to 0.01, 0.1, or 0.3 mg/kg/day. The largest increase in body weight gain (9.6%) was observed at 0.1 mg/kg/day; because the weight increase was less than 10%, the 0.1 mg/kg/day was considered a NOAEL. At 40 weeks of age, the increased body weight was observed in the 0.1 and 0.3 mg/kg/day groups. At termination (18 months of age), there were no differences in body weight between the controls and mice exposed to 0.01–3 mg/kg/day; a decrease in body weight was observed at 5 mg/kg/day. During the period of increased body weight in the lower-dose animals, there were no changes in serum glucose levels or the response to a glucose challenge, but there were significant increases in insulin and leptin levels at 0.01 and 0.1 mg/kg/day. Although there were no changes in the percentage of body fat to body weight measurements in mice at 42 weeks of age, at 18 months of age, significant decreases in abdominal body fat and increases in intrascapular brown fat was observed at ≥1 mg/kg/day PFOA (Hines et al. 2009). Based on systematic review of pup body weight data from the Abbott et al. (2007), Hines et al. (2009), Lau et al. (2006), White et al. (2007, 2009, 2011), and Wolf et al. (2007) mouse studies, Koustas et al. (2014) concluded that there was sufficient evidence that exposure to PFOA adversely affected fetal growth in animals. A meta-analysis estimate was a decrease of 0.023 g pup body weight per 1 mg/kg/day increase in PFOA dose.

A few studies have examined the potential of PFOA to induce malformations/variations. Lau et al. (2006) reported reductions in ossification of the proximal phalanges at ≥1 mg/kg/day and supraoccipital at 10 or 20 mg/kg/day. This study also reported enlarged fontanels in pups exposed to ≥1 mg/kg/day and tail and

limb defects at ≥5 mg/kg/day; however, there was no clear dose-response for these effects. Koskela et al. (2016) found altered femur and tibial bone morphology and decreased tibial mineral density in the offspring of mice exposed to 0.3 mg/kg/day in the diet on GDs 1–21. An increased percentage of litters with microcardia was also observed in the offspring of mice exposed to 10 or 20 mg/kg/day (Lau et al. 2006). No increases in the occurrence of malformations/variations were observed in the offspring of rats administered 100 mg/kg/day on GDs 6–15 (Staples et al. 1984) or in a 2-generation study at doses as high as 30 mg/kg/day (Butenhoff et al. 2004b).

Delayed eye opening was observed in the offspring of mice administered ≥1 mg/kg/day PFOA on GDs 1–17 (Abbott et al. 2007) and in mice administered 5 mg/kg/day throughout gestation (Lau et al. 2006; Wolf et al. 2007). Neither Albrecht et al. (2013) nor Lau et al. (2006) found alterations in eye opening in mice exposed to 3 mg/kg/day PFOA on GDs 1–17. Lau et al. (2006) also reported advanced (earlier than controls) preputial separation at ≥1 mg/kg/day and delayed vaginal opening at 20 mg/kg/day. The effect in the male offspring is in contrast to the Butenhoff et al. (2004b) study, which found delays in preputial separation in rats exposed to 30 mg/kg/day PFOA; a delay in vaginal patency was also observed at this dose.

A series of studies conducted by White and associates found significant delays in mammary gland development in the offspring of mice administered 1 mg/kg/day PFOA via gavage on GDs 8–17 (White et al. 2011) or 5 mg/kg/day PFOA on GDs 1–17, 8–17, 12–17, 10–17, 13–17, or 15–17 (White et al. 2007, 2009, 2011). The delay was characterized as reduced ductal elongation and branching and delays in timing and density of terminal end buds and was observed at all observational periods (PNDs 10, 20, 22, and 42, and 63 and 18 months of age). Decreases in mammary epithelial growth, as assessed by developmental scoring, were observed in the offspring of mice exposed to 0.01 mg/kg/day on GDs 1–17 (Tucker et al. 2015), 0.3 mg/kg/day on GDs 1–17 (Macon et al. 2011), or 0.01 mg/kg/day on GDs 10–17 (Macon et al. 2011). Tucker et al. (2015) noted that the delays in mammary gland development began at puberty and continued during young adulthood. Albrecht et al. (2013) did not find any alterations in mammary gland development on PND 20 in mouse offspring following *in utero* exposure to PFOA on GDs 1–17. Delayed mammary gland development was also observed in offspring only exposed via lactation (maternal dose of 3 mg/kg/day PFOA on GDs 1–17); the effects were observed on PNDs 42 and 63, but not on PND 22 (White et al. 2009). In a multigeneration study conducted by White et al. (2011), delays in mammary gland development were not consistently observed in the F2 offspring of F1 females that were exposed *in utero* to 1 or 5 mg/kg/day PFOA. However, delays in mammary gland development were observed in the F1 and F2 offspring exposed to 0.001 mg/kg/day *in utero* (GDs 7–17) and

postnatally. The investigators (White et al. 2011) noted that the delay in mammary gland development did not appear to affect lactational support based on normal survival and growth of the F2 pups. Tucker et al. (2015) noted dose-related strain differences on the effect of PFOA on mammary gland differences; effects were observed in CD-1 mice at ≥0.01 mg/kg/day and in C57BL/6 mice at ≥0.3 mg/kg/day (the highest NOAEL for this strain was 0.1 mg/kg/day); it is noted that the serum PFOA concentrations at a given dose were lower in the C57Bl/6 mice than in the CD-1 mice. Yang et al. (2009) reported strain differences in mammary gland effects in peripubertal mice administered PFOA for 4 weeks beginning on PND 21. In BALB/c mice, reductions in ductal length and decreased numbers of terminal end buds and stimulated terminal ducts were observed at 5 and 10 mg/kg. In contrast, 5 mg/kg resulted in mammary gland growth stimulation in C57BL/6 mice, as evidenced by increased number of terminal end buds with no alterations in ductal length. Mammary gland inhibition was observed in the C57BL/6 mice administered 10 mg/kg. Stimulation of mammary gland growth was also observed in PPARα knockout mice similarly administered 5 mg/kg (Zhao et al. 2010). In a series of experiments to evaluate the mechanism of PFOA-induced alterations in mammary gland development, Zhao et al. (2010) found that PFOA did not result in alterations in ovariectomized C57BL/6 mice administered 5 mg/kg 5 days/week for 4 weeks. In ovary-intact mice, PFOA enhanced mammary gland responses to exogenous estradiol and progesterone. Increased levels of epidermal growth factor receptor, hepatocyte growth factor, cyclin D1, and proliferating cell nuclear antigen levels were also found in PFOA-exposed C57BL/6 and PPARα-knockout mice (Zhao et al. 2010).

A consistent finding in the five mouse studies evaluating the neurodevelopmental toxicity of PFOA is an increase in motor activity. Increases in horizontal and ambulatory locomotor activity (tested on PND 60) were observed in the offspring of mice exposed to 0.1 mg/kg/day in the diet on GD 7 through PND 21 (Sobolewski et al. 2014); a decrease in resting time was also observed in the males. Increased ambulatory activity was observed on PND 18 in the offspring of mice administered 1 mg/kg/day on GDs 1–17 (Goulding et al. 2017). Significant increases in open field activity were observed at PND 36 in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Johansson et al. (2008) and Onishchenko et al. (2011) demonstrated a biphasic alteration in motor activity: an initial period of decreased activity followed by increased activity. Johansson et al. (2008) administered a single dose of 8.7 mg/kg/day PFOA to mice on PND 10 and monitored spontaneous activity for a 1-hour period when the mice were 2 or 4 months of age. In the first 20-minute period, there was a decrease in spontaneous activity, followed by a 20-minute period with an activity level similar to controls, and a 20-minute period with significantly increased spontaneous activity. Similarly, Onishchenko et al. (2011) reported an increase in activity in a 48-hour period in the adult offspring of

mice exposed to 0.3 mg/kg/day PFOA throughout gestation; however, there was a decrease in activity during the initial 3 hours of testing. Johansson et al. (2008) also found an increased susceptibility of the cholinergic system in mice exposed to 0.58 or 8.7 mg/kg/day PFOA on PND 10. In control mice, an injection of nicotine resulted in increases in activity; mice exposed to 0.58 mg/kg/day also responded with an increase in activity, although the increase was less than that observed in the controls. In contrast, nicotine resulted in a decrease in activity in mice exposed to 8.7 mg/kg/day. Exposure to PFOA did not alter learning or memory, as evidenced by the lack of effect on maze tests (Cheng et al. 2013; Johansson et al. 2008). Tests of neurobehavioral development found altered motor coordination and impaired negative geotaxis reflex, but no effect on righting reflex or cliff avoidance, in the offspring of mice exposed to 1.6 mg/kg/day throughout gestation and lactation (Cheng et al. 2013). Decreases in initial novel object exploratory behavior were also observed at 0.1 mg/kg/day, but there were no alterations in recognition time for novel objects (Sobolewski et al. 2014).

Support for the heart effects observed in the mouse study conducted by Lau et al. (2006) comes from a series of studies in chicken embryos and hatchlings that demonstrate the developmental cardiotoxicity of PFOA (Jiang et al. 2012, 2013, 2016). The avian model was selected due to the similarity between avian and mammalian cardiovascular development and the lack of direct maternal influence (Jiang et al. 2012). The effects following *in ovo* exposure include thinning of the right ventricular wall in chick embryos and alterations in left ventricular posterior wall dimension, volume, heart rate, stroke volume, and ejection fraction in the hatchlings (Jiang et al. 2012). Tests with WY 14,643, a PPARα agonist, and PFOA provide evidence that the cardiotoxicity involves both PPARα and bone morphorgenic protein 2 (BMP2) pathways (Jiang et al. 2013). Comparisons of results following *in ovo* exposure and *in vitro* exposure suggest that the cardiotoxicity was not likely due to cytotoxicity, but rather an alteration in early cardio morphology and function processes (Jiang et al. 2016).

Summary. Epidemiological studies have examined a number of potential developmental outcomes in communities living near a PFOA facility and in general populations. Although not consistently reported, the available general population studies suggest an inverse association between maternal serum PFOA levels and birth weight; a number of studies have not found this association. Several systematic reviews of these data have concluded that there was sufficient evidence that maternal PFOA levels are associated with reductions in fetal growth. After correcting for glomerular filtration rate, a small decrease in birth weight was associated with increases in maternal serum PFOA. Two of the three studies evaluating possible effects of sexual maturation found small delays in the start of menarche associated with maternal serum PFOA levels. Overall, the data do not suggest associations between serum PFOA levels and

adverse pregnancy outcomes such as miscarriages or stillbirths, most birth outcomes (e.g., risk of low birth weight, risk of small for gestational age, birth length, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ or scholastic achievement, motor skills, and risk of ADHD). Animal studies provide strong evidence that developmental toxicity is a sensitive target of PFOA toxicity. Observed effects include prenatal losses and decreases in pup survival, decreases in birth weight, developmental delays such as delayed eye opening, delays in mammary gland development, and increased motor activity.

PFOS

Epidemiological Studies—Pregnancy Outcomes. No associations between maternal PFOS levels and the risk of miscarriages were observed in several studies (Darrow et al. 2014; Jensen et al. 2015; Stein et al. 2009). Three studies reported increases in the risk of preterm birth associated with maternal serum PFOS levels in the >90th percentile (>23.2 ng/mL) (Stein et al. 2009), maternal serum levels in the 2nd, 3rd, or 4th quartiles (≥18.9 ng/mL) (Sagiv et al. 2018), or cord blood PFOS levels in the 3rd and 4th quartiles (≥5.68 ng/mL) (Chen et al. 2012a), and one study reported a decrease risk in preterm birth (Whitworth et al. 2012a). Three other studies did not find associations for preterm birth (Fei et al. 2007, 2008a; Hamm et al. 2010; Manzano-Salgado et al. 2017a), one study found no association between serum PFOS and pregnancy loss (Buck Louis et al. 2016), and five studies found no associations between maternal PFOS levels and gestational age or length (Lauritzen et al. 2017; Li et al. 2017; Lind et al. 2017a; Manzano-Salgado et al. 2017a).

Epidemiological Studies—Birth Outcomes. Occupational, community, and general population exposure studies have examined the possible associations between maternal PFOS levels and a number of birth outcomes including birth weight; risk of low birth weight; risk of small for gestational age; birth length; head, chest, and abdominal circumferences; ponderal index; sex ratio; and birth defects. Most studies did not find associations between maternal serum PFOS levels and birth weight (Alkhalawi et al. 2016; Apelberg et al. 2007b; Ashley-Martin et al. 2016, 2017; Callan et al. 2016; Cao et al. 2018; Darrow et al. 2013; Bach et al. 2016; Fei et al. 2007, 2008a; Govarts et al. 2016; Hamm et al. 2010; Kim et al. 2011; Kobayashi et al. 2017; Lauritzen et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Maisonet et al. 2012; Manzano-Salgado et al. 2017a; Minatoya et al. 2017; Monroy et al. 2008; Robledo et al. 2015a; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017; Whitworth et al. 2012a), including an occupational exposure study (Grice et al. 2007) in which female workers were exposed to very high levels of PFOS (serum levels ranged from 1,300 to 1,970 ng/mL). Five studies did find inverse associations

between birth weight and maternal serum PFOS levels. In the Washino et al. (2009) study, an inverse association was found between maternal serum PFOS levels and birth weight; segregating by sex resulted in an inverse association in girls, but not in boys. The magnitude of the change was small, 148.8 g decrease in birth weight per log unit increase in maternal PFOS for combined. Maisonet et al. (2012) also reported small decreases in birth weight (140.1 g) in infants whose mother's serum PFOS levels were in the 3rd tertile. Lauritzen et al. (2017) also reported an inverse association between birth weight and maternal serum PFOS levels (292 g per ln unit increase in PFOS). Similarly, Chen et al. (2012a) reported an inverse association between cord blood PFOS and birth weight, but the magnitude was small (110.2 g decrease per ln unit increase in cord PFOS levels). Li et al. (2017) also reported a small decrease in birth weight associated with cord PFOS levels (95 g decrease per ln increase in cord PFOS levels); when infants were categorized by sex, the association was only found among boys. Although these studies found decreases in birth weight associated with PFOS levels, no studies found increases in the risk of low birth weight infants (Chen et al. 2012a; Darrow et al. 2013; Fei et al. 2007, 2008a; Manzano-Salgado et al. 2017a; Stein et al. 2009) or small for gestational age infants (Chen et al. 2012a; Fei et al. 2007, 2008a; Hamm et al. 2010; Lauritzen et al. 2017; Manzano-Salgado et al. 2017a; Whitworth et al. 2012a). The ORs for low birth weight and small for gestational age risks are presented in Figures 2-37 and 2-38. Analysis of data compiled from four European birth cohort studies found an inverse association between cord PFOS levels (measured levels and levels estimated from breast milk PFOS levels) and small for gestational age (OR 0.823, 95% CI 0.741–0.913) (Govarts et al. 2018). When subjects were segregated based on whether they smoked during pregnancy, a positive association was found among smokers (OR 1.627, 95% CI 1.024–2.588) and an inverse association was found among nonsmokers (OR 0.661, 95% CI 0.644–0.717). Three studies have evaluated leptin and adiponectin hormone levels or adiposity in newborns. Maternal PFOS levels were not associated with alterations in leptin levels (Ashley-Martin et al. 2017; Minatoya et al. 2017). Mixed results were found for adiponectin levels with one study finding no alterations (Ashley-Martin et al. 2017) and another finding an association (Minatoya et al. 2017). No association was found between maternal PFOS levels and adiposity at birth (Starling et al. 2017).

Verner et al. (2015) conducted a meta-analysis of the Apelberg et al. (2007b), Chen et al. (2012a), Fei et al. (2007), Hamm et al. (2010), Maisonet et al. (2012), Washino et al. (2009), and Whitworth et al. (2012a) studies and found that a 1 ng/mL increase in maternal PFOS levels was associated with a 5.00 g (95% CI -8.92 to -1.09) decrease in birth weight. When the data were re-analyzed utilizing a PBPK model to account for glomerular filtration rate, the magnitude of the effect of PFOS on birth weight decreased (Verner et al. 2015). A 1 ng/mL increase in PFOS was associated with a 2.72 g (95% CI -3.40 to -2.04) decrease in birth weight. A second meta-analysis conducted by Negir et al. (2017)

Figure 2-37. Risk of Low Birth Weight Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)

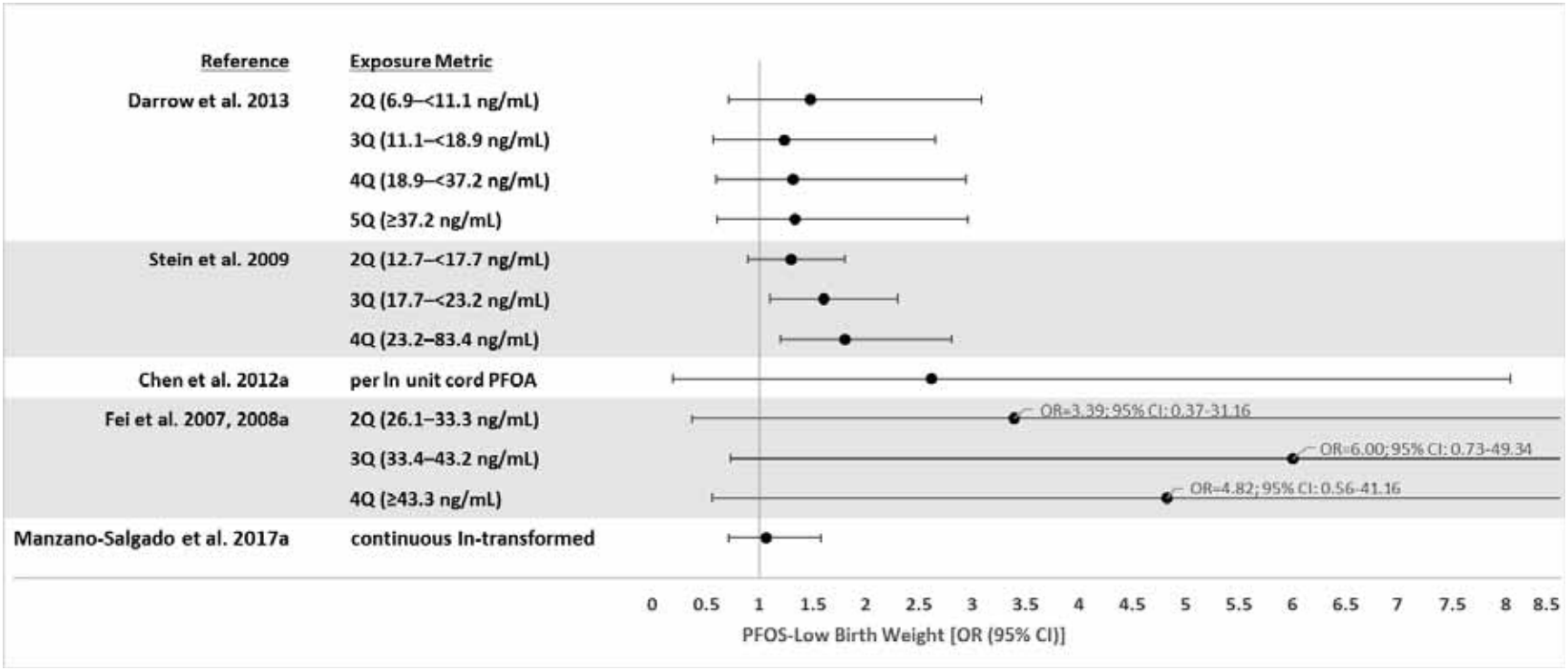
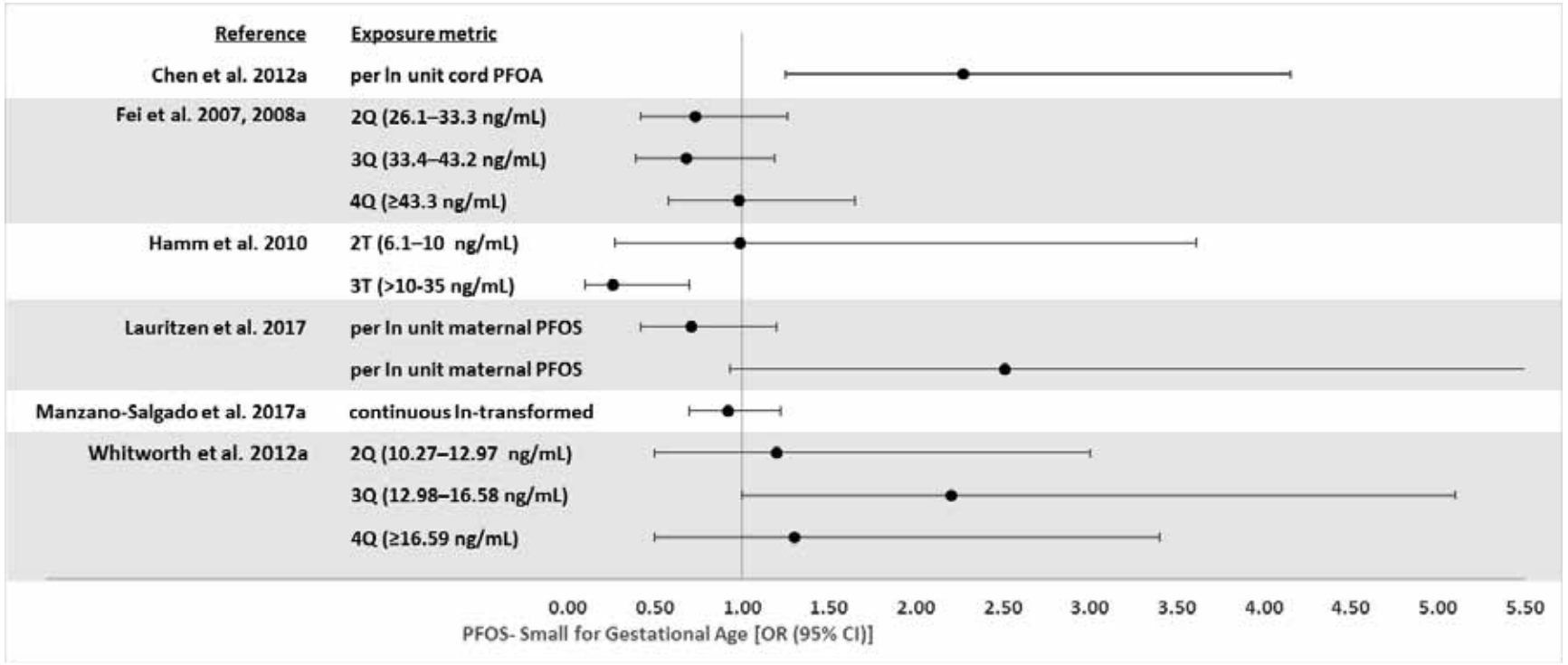


Figure 2-38. Risk of Small for Gestational Age Infant Relative to PFOS Levels (Presented as Adjusted Odds Ratios)



utilized data from the Fei et al. (2007), Monroy et al. (2008), Washino et al. (2009), Hamm et al. (2010), Chen et al. (2012a), Maisonet et al. (2012), Whitworth et al. (2012a), and Bach et al. (2016) studies. The investigators found a -0.92 g (95% CI -3.43–1.60) change in birth weight per 1 ng/mL increase in serum PFOS.

Maternal PFOS was not associated with birth length (Alkhalawi et al. 2016; Apelberg et al. 2007b; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Kobayashi et al. 2017; Laurizten et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Washino et al. 2009) with the exception of the finding of small decreases in birth length (≤1.2 cm) that was associated with serum PFOS levels (Fei et al. 2007, 2008a; Lauritzen et al. 2017). Four studies reported inverse associations between ponderal index and cord blood PFOS levels (Apelberg et al. 2007b) or maternal serum PFOS levels (Alkhalawi et al. 2016; Lee et al. 2013; Minatoya et al. 2017); other studies did not find this effect (Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Maisonet et al. 2012; Robledo et al. 2015a; Shi et al. 2017). Two studies reported small decreases in head circumference, which were associated with maternal serum PFOS levels (Apelberg et al. 2007b) and cord blood PFOS (Chen et al. 2012a); other studies have not found associations (Bach et al. 2016; Callan et al. 2016; de Cock et al. 2014; Fei et al. 2007, 2008a; Lauritzen et al. 2017; Lee et al. 2013; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Washino et al. 2009).

One study reported no increases in the risk of birth defects associated with maternal serum PFOS levels (Stein et al. 2009); a second study found an increased risk of congenital cerebral palsy in girls, but not in boys (Liew et al. 2014). Bae et al. (2015) did not find associations between the odds of having a boy and paternal or maternal serum PFOS levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies examined several aspects of neurodevelopment, including age of reaching neurobehavioral milestones, IQ, motor development, behavior, ADHD, and autism. Fei et al. (2008b) did not find associations between maternal PFOS levels and the risk of having an Apgar score of <10 or in motor and mental development at 6 months. However, the study did find that some neurobehavioral milestones (delay in sitting, early use of word-like sounds, and delays in using two-word sentences) were associated with maternal PFOS levels. Goudarzi et al. (2016b) did not find alterations on mental and psychomotor development in 6- and 18-month-old infants that were associated with maternal serum PFOS levels. A third study of infants did not find alterations in neurobehavioral or muscle coordination tests (Donauer et al. 2015).

In the only study evaluating IQ, Wang et al. (2015b) did not find associations between maternal PFOS levels and IQ score in children 5 or 8 years of age. Zhang et al. (2018) did not find associations between maternal PFOS levels and reading scores in 5- or 8-year-old children. However, associations were found between the child's serum PFOS levels at age 3 years and reading scores at 5 years of age and serum PFOS levels at 5 years of age and reading scores at 8 years of age. Strøm et al. (2014) found no associations between scholastic achievement in 20-year-olds and maternal PFOS levels. In a study of children living in a community with high PFOA contamination, Stein and Savitz (2011) found decreases in the risk of learning problems in children 5–18 or 12–15 years of age. In contrast, Vuong et al. (2016) found increased risks of global executive functioning and metacognition problems that were associated with maternal PFOS levels. Another study found an association between maternal PFOS levels and verbal comprehension in 15-month-olds, but an inverse association with intelligibility scores in 38-month-olds (Jeddy et al. 2017). A subsequent study by Vuong et al. (2018) did not find associations between serum PFOS levels in 8-year-old children and metacognition or global executive functioning scores. Four studies have not found associations between maternal PFOS levels and behavioral health and motor coordination/skills in children (Fei and Olsen 2011; Høyer et al. 2015a; Oulhote et al. 2016), between breast milk PFOS levels and behavioral development in 6- and 24-month-old infants (Forns et al. 2015), or between serum PFOS levels age 5 or 7 years and behavioral development in 7-year-old children (Oulhote et al. 2016). A fifth study (Vuong et al. 2016) found an increased risk for problems with behavioral regulation. The available data do not suggest an association between maternal PFOS levels or cord blood PFOS levels and the risk of ADHD or ADHD behaviors (Hoffman et al. 2010; Liew et al. 2015; Ode et al. 2014; Quaak et al. 2016; Stein and Savitz 2011; Strøm et al. 2014), although Liew et al. (2015) found a decreased risk of ADHD diagnosis in children whose mothers had serum PFOS levels in the 4th quartile. Similarly, Høyer et al. (2015a) did not find increases in the risk of hyperactivity in children and Gump et al. (2011) found a decrease in impulsivity. Braun et al. (2014) and Liew et al. (2015) did not find associations between maternal PFOS and autism risk.

Epidemiological Studies—Development of Reproductive System. Several epidemiological studies have examined the possible associations between PFOS and the development of the reproductive system, including the risk of congenital defects to reproductive organs, alterations in reproductive hormone levels, and age of puberty; the results of these studies are summarized in Table 2-25. No alterations in the risk of cryptorchidism (Toft et al. 2016; Vesterholm Jensen et al. 2014) or hypospadias (Toft et al. 2016) were found in two studies. No association between maternal PFOS levels and anogenital distance was found in boys and an inverse association was found in girls (Lind et al. 2017a). Itoh et al. (2016) reported associations between maternal PFOS levels and alterations in cord blood hormone levels, in particular

estradiol in males, testosterone:estradiol ratio in males (inverse association), progesterone levels in males and females, prolactin levels in females, and inhibin levels in males. Similarly, Toft et al. (2016) found associations between amniotic fluid PFOS levels and levels of testosterone, androstenedione, progesterone, and insulin-like factor 3 (inverse association) in amniotic fluid. Lopez-Espinosa et al. (2016) also found a number of alterations in reproductive hormone levels in 6–9-year-old boys and girls. In the boys, inverse associations between serum PFOS levels and estradiol, total testosterone, and insulin-like growth factor 1 were observed. Inverse associations between total testosterone and insulin-like growth factor 1 and serum PFOS levels were also observed in the girls. A study of young adult women found no associations between reproductive hormone levels and maternal PFOS levels (Kristensen et al. 2013).

A study of 8–18-year-old children found delays in the age of puberty in boys and girls (Lopez-Espinosa et al. 2011) that were associated with serum PFOS levels. In the children with serum PFOS levels in the 3rd and 4th quartiles, the respectively delays were 131 and 190 days in boys and 141 and 138 days in girls. In contrast, two other studies have not found alterations in either the age of menarche or an earlier age of menarche that were associated with maternal PFOS levels (Christensen et al. 2011; Kristensen et al. 2013). The differences in the biomarker of exposure and the potential exposure to high levels of PFOA in the Lopez-Espinosa et al. (2011) community study make it difficult to compare the results of these three studies. As discussed in the PFOA section, Wu et al. (2009) reanalyzed the Lopez-Espinosa et al. (2011) data using a Monte Carlo PBPK model, which accounted for rapid growth occurring around puberty, and found much shorter delays in the age of menarche than found in the Lopez-Espinosa et al. (2011) study. In the girls with simulated serum PPFOS levels in the 4th quartile, the delay was 72 days (OR 0.75, 95% CI 0.70–0.81).

Laboratory Animal Exposure Studies. Increases in fetal mortality and decreases in pup survival have also been observed in rats and mice exposed to PFOS *in utero* (Abbott et al. 2009; Chen et al. 2012b; Fuentes et al. 2006; Grasty et al. 2003, 2005; Lau et al. 2003; Lee et al. 2015a; Luebker et al. 2005a, 2005b; Ngo et al. 2014; Thibodeaux et al. 2003; Xia et al. 2011; Yahia et al. 2008). Increases in the number of resorptions and dead fetuses were observed in mice administered ≥0.5 mg/kg/day (Lee et al. 2015a); increases in abortions between GD 22 and 28 were observed in rabbits treated with 3.75 mg/kg/day PFOS by gavage on GDs 6–20 (Case et al. 2001). Decreases in the number of live fetuses were observed in mice exposed to ≥2.0 mg/kg/day on GDs 11–16 and 20 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003) or GDs 0–17 (Yahia et al. 2008). Increases in perinatal losses were observed in the litters of mice administered ≥0.1 mg/kg/day PFOS on GDs 1–17 (Ngo et al. 2014). Pup survival is

affected at lower maternal doses. Significant decreases in pup survival were observed in rats at 1.6 mg/kg/day (dams were exposed for 6 weeks prior to mating and during gestation through lactation days 4 or 21) (Luebker et al. 2005a, 2005b) and in mice exposed to 4.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009); no alterations in pup survival were observed in rats or mice exposed to 1 mg/kg/day (Luebker et al. 2005b; Yahia et al. 2008). A series of studies by Grasty et al. (2003) in rats that were exposed for 4 days during different gestational periods showed that the pup was more susceptible if exposure occurred later in gestation. On PND 4, pup survival was 70, 50, 60, 20, or 5% for exposures on GDs 2–5, 6–9, 10–13, 14–17, or 17–20, respectively. Grasty et al. (2003) and others (Abbott et al. 2009; Chen et al. 2012b; Lau et al. 2003) also noted that most deaths occurred within the first 4 PNDs, with the highest rates occurring on PND 1. Lau et al. (2003) and Luebker et al. (2005a) found that cross fostering did not significantly improve pup survival; deaths were observed in the *in utero* only exposure group. However, Luebker et al. (2005a) showed that rats exposed *in utero* and during lactation had the highest pup mortality, as compared to other cross-fostered groups. The mechanism involved in the early pup mortality has not been identified, but there is some indication that pulmonary deficits may be a contributing factor. At high doses (50 mg/kg/day administered on GDs 19–20), pups demonstrated difficulty breathing within minutes of birth (Grasty et al. 2003). Histological examination of the lungs of pups exposed to 25 or 50 mg/kg/day on GDs 19–20 showed evidence of delayed lung maturation (Grasty et al. 2003, 2005), specifically, an increase in the proportion of solid lung tissue and a decrease in the proportion of small airway tissue. A comparison of the lungs of PFOS-exposed neonates to control fetuses (GD 21) showed that 17 and 50% of the lung tissue in the neonates exposed to 25 or 50 mg/kg/day, respectively, on GDs 19–20 was not histologically different from the control fetuses (Grasty et al. 2005). Administration of therapeutic agents known to enhance terminal lung maturation and accelerate surfactant production did not improve pup survival (Grasty et al. 2005). Histological damage has also been reported in pups exposed to lower PFOS levels. Lung atelectasis was observed in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No lung effects were observed in pups exposed to 1 mg/kg/day or in fetuses exposed to 20 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Alveolar hemorrhage, thickened epithelial walls of the pulmonary alveolus, focal lung consolidation, and focal infiltration of inflammation cells were observed in pups exposed to 2 mg/kg/day on GDs 0–21; no lung effects were observed at 0.1 mg/kg/day (Chen et al. 2012b).

Decreases in fetal body weight, birth weight, and pup body weight have been observed in rats, mice, and rabbits exposed to PFOS (Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007b; Grasty et al. 2003; Lau et al. 2003; Lee et al. 2015a; Li et al. 2016; Luebker et al. 2005a, 2005b; Rogers et al. 2014; Xia et al. 2011; Yahia et al. 2008). In rats, the lowest-adverse-effect level for decrease in fetal

body weight was 10 mg/kg/day following administration on GDs 2–20 (Thibodeaux et al. 2003) and the highest no-effect level was 5 mg/kg/day, also identified in the Thibodeaux et al. (2003) study. Decreases in rat pup birth weight and body weight on PND 4 were observed in the offspring of rats exposed to 0.4 mg/kg/day for 42 days prior to mating and gestation through lactation day 4 (Luebker et al. 2005b). Mice appear to be less sensitive to the effect of PFOS on pup body weight than rats (Lau et al. 2003). Exposure of rats to 2 mg/kg/day PFOS on GDs 2–21 resulted in significant decreases in birth weight and pup body weight on PNDs 1–3; exposure to 5 mg/kg/day resulted in decreases in pup body weight through PND 19. In contrast, no alterations in birth weight or pup body weight were observed in mice exposed to doses as high as 5 mg/kg/day on GDs 1–18. Fuentes et al. (2007b) reported the lowest LOAEL of 6 mg/kg/day for decreases in pup weight in mice exposed on GDs 12–18. Decreases in fetal body weight were observed in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008). Fuentes et al. (2006) did not find decreases in fetal body weight following exposure to 6 mg/kg/day on GDs 6–18. In rabbits, a decrease in fetal body weight was observed following exposure to 2.5 mg/kg/day on GDs 6–20, but not at 1 mg/kg/day (Case et al. 2001).

Several studies also reported delays in developmental milestones. Delays in eye opening were observed in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003) or 0.4 mg/kg/day for 42 days prior to mating and throughout the gestation and lactation periods (Luebker et al. 2005a) and in mice exposed to 8.5 mg/kg/day on GDs 15–18 (Abbott et al. 2009). Fuentes et al. (2007b) did not find a delay in eye opening in mouse pups exposed to 6 mg/kg/day on GDs 12–18, but did find a delay in pinna detachment at this dose level. A decrease in neuromuscular development, as evidenced by a delay in tail pull reflex, climbing ability, and forelimb grip strength, was observed in mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b).

Prenatal exposure to PFOS has resulted in malformations/anomalies/variations in rats, mice, and rabbits (Case et al. 2001; Era et al. 2009; Thibodeaux et al. 2003; Yahia et al. 2008). An increased incidence of cleft palate was observed in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and in mice exposed to 10 mg/kg/day on GDs 0–17 (Yahia et al. 2008), 15 mg/kg/day on GDs 1–17 (Thibodeaux et al. 2003), 20 mg/kg/day on GDs 1–17 (Era et al. 2009), and 50 mg/kg/day on GDs 11–15 (Era et al. 2009). Other skeletal and external alterations included sternal defects in rats exposed to 10 mg/kg/day on GDs 2–20 (Thibodeaux et al. 2003) and mice exposed to 1 mg/kg/day on GDs 0–17 (Yahia et al. 2008), delayed skeletal ossification in rabbits exposed to 2.5 mg/kg/day on GDs 6–20 (Case et al. 2001), wavy ribs and spina bifida occulta in mice exposed to 10 mg/kg/day on GDs 1–17 (Yahia et al. 2008), and tail abnormalities and delayed ossification of phalanges at 20 mg/kg/day (Yahia et al.

2008). Visceral abnormalities, consisting of enlarged right atrium at 10 mg/kg/day, and ventricular septal defects at 20 mg/kg/day were observed in mice exposed on GDs 1–17 (Thibodeaux et al. 2003). No malformations/anomalies/variations were found by Thibodeaux et al. (2003) in mice exposed to 1 mg/kg/day on GDs 1–17 or by Fuentes et al. (2006) in mice exposed to 6 mg/kg/day on GDs 6–18. In addition to the previously discussed histological alterations observed in the pups exposed to lethal doses, mild to severe intracranial dilatation of blood vessels was observed in fetuses exposed to 20 mg/kg/day on GDs 0–17 and in pups exposed to 10 mg/kg/day on GDs 0–18 (Yahia et al. 2008). No histological alterations were observed in the heart of rat pups exposed to 2 mg/kg/day on GDs 2–21 (Xia et al. 2011); the study also found no alterations in heart rate or blood pressure. Lee et al. (2015b) found increases in cholesterol levels in fetal livers of mice exposed to PFOA on GDs 1–17 and Wan et al. (2014b) found increases in relative liver weights in pups on PND 21.

A study with wild-type mice (129S1/Svlm) and PPARα-null mice evaluated the influence of PPARα on developmental toxicity of PFOS (Abbott et al. 2009). Decreases in pup survival and delays in eye opening were observed in both strains, although lower LOAELs were identified in the wild-type mice. The investigators concluded that neonatal lethality and delayed eye opening was not dependent on PPARα activation.

Neurodevelopmental studies have shown that prenatal and/or postnatal exposure to PFOS can affect motor activity, but does not appear to affect learning or memory. A significant decrease in locomotion was observed in male mice aged 5–8 weeks exposed to 0.3 mg/kg/day on GDs 1–17 when they were placed in a novel environment (Onishchenko et al. 2011). Hallgren et al. (2015) reported biphasic alterations in spontaneous activity in 2-month-old mice administered a single dose of 11.3 mg/kg on PND 10; locomotor activity was reduced during the first 20-minute period, was unchanged in the second period, and increased during the third period. Decreases in circadian activity were noted in males and increases in the number of inactive periods were noted in males and females when they were observed over a 48-hour period. The study also found increased inactivity in an elevated plus maze test. In an open field test of 70-day-old mice exposed to 6 mg/kg/day on GDs 12–18, an increase in the amount of time spent in the center of the field was found; no changes in vertical movement were found (Ribes et al. 2010). In 3-month-old mice exposed to 6 mg/kg/day on GDs 12–18, a decrease in the distance traveled was observed after 20–25 minutes in an open field apparatus; activity was not affected during the first 5 minutes of the test (Fuentes et al. 2007a). In a 15-minute open field test, prenatal exposure to 6 mg/kg/day PFOS on GDs 12–18 did not alter motor activity in 3-month-old mice (Fuentes et al. 2007b). In contrast, Butenhoff et al. (2009b) found a significant increase in locomotion in male rats exposed to

0.3 or 1.0 mg/kg/day PFOS throughout gestation and lactation. However, this effect was only observed in male rats on PND 17; no significant alterations were observed on PNDs 13, 21, or 61. An increase in locomotion was observed in female rats on PND 21 exposed to 1.0 mg/kg/day, but not at other time points. To evaluate the biological relevance of the increased activity, activity was analyzed by 1-minute sequential time periods. The investigators concluded that the increased activity observed in the 0.3 mg/kg/day males at PND 17 and 1.0 mg/kg/day females at PND 21 was not treatment-related due to the lack of significant changes in total or ambulatory activity and the similarity in habituation pattern between the treated groups and controls. In the 1.0 mg/kg/day PND 17 males, the pattern of habituation differed from controls and there was an increase in ambulatory activity; this increase in locomotor activity was considered to be related to PFOS exposure. The increased activity was observed in the last three time periods. Postnatal exposure (PND 10) to 11.3 mg/kg/day resulted in an initial decrease in motor activity followed by an increase in activity in 2- and 4-month-old mice (Johansson et al. 2008). In 2-month-old mice exposed to 0.75 mg/kg/day, there was a decrease in total activity during the first 20 minutes of testing, but not during the remaining 40 minutes of the test; no changes in activity were observed in the 4-month-old mice exposed to 0.75 mg/kg/day. Johansson et al. (2009) also found an altered response to nicotine exposure. Exposure to 11.3 mg/kg/day PFOS resulted in a decrease in motor activity in response to nicotine exposure, as compared to the increased activity observed in controls; no significant alteration was observed at 0.75 mg/kg/day. Two studies testing muscle coordination did not find alterations in the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation (Luebker et al. 2005a) or mice exposed to 6 mg/kg/day on GDs 12–18 (Fuentes et al. 2007b). A decrease in muscle coordination was observed in mice exposed to 0.3 mg/kg/day on GDs 1–17 (Onishchenko et al. 2011). Perinatal exposure to PFOS did not significantly alter learning or memory in rats exposed to 2 mg/kg/day on GDs 2–21 and tested on PND 21 (Lau et al. 2003), the offspring of rats exposed to 3.2 mg/kg/day for 6 weeks prior to mating and throughout gestation and lactation and tested on PNDs 21 and 70 (Luebker et al. 2005a), or mice exposed to 6 mg/kg/day on GDs 12–18 and tested at 3 months of age (Fuentes et al. 2007a). In contrast, decreases in spatial learning ability were observed in the offspring of mice exposed to 0.8 mg/kg/day on GD 1 through PND 1 or on PNDs 1–7 (Wang et al. 2015b).

The effect of pre- and/or postnatal exposure to PFOS on serum lipid levels, thyroid function, and immune function has also been evaluated by a small number of studies. In the offspring of rats exposed to 1.6 mg/kg/day for 6 weeks prior to mating through GD 20, a significant decrease in fetal serum cholesterol levels and an increase in LDL cholesterol levels were observed (Luebker et al. 2005b). In rats exposed through PND 4, there was a decrease in serum triglyceride levels in the pups exposed to

1 mg/kg/day (Luebker et al. 2005b). No alterations in thyroid histology or follicular morphology were observed in rats exposed to 1 mg/kg/day on GD 0–PND 20 (Chang et al. 2009), and no alterations in TSH levels were observed in the Chang et al. (2009) study or in rats exposed to 2 mg/kg/day on GDs 2–21 (Lau et al. 2003). Decreases in total and free T4 levels were observed in rats exposed to 1 mg/kg/day on GDs 2–21 (Lau et al. 2003); free T4 levels remained low through PND 35. Similarly, a cross-fostering study found decreases in T4 levels in rats exposed to 3.2 mg/kg/day *in utero*, during lactation only, and throughout gestation and lactation (Yu et al. 2009b). Altered immune function was observed in mice exposed to PFOS on GDs 1–17 (Keil et al. 2008). At 5 mg/kg/day, an altered IgM antibody response to sRBCs was observed in 8-week-old males; decreases in CD3+ and CD4+ lymphocytes were also observed. At 1 mg/kg/kg/day, there was decreased in NK cell activity in males; no effects were observed at 0.1 mg/kg/day.

Summary. A number of epidemiological studies have evaluated developmental outcomes in occupational, community (living near a PFOA facility), and general exposure populations. Overall, these studies have not found associations between serum PFOS and adverse pregnancy outcomes (miscarriage, preterm birth), most birth outcomes (risks of low birth weight or small for gestational age, birth length, head, chest or abdominal circumferences, ponderal index, sex ratio, or birth defects), or neurodevelopmental outcomes (IQ, motor development, behavior, ADHD, or autism). It is noted that some studies have found associations for these effects and for some effects, only a couple of studies examined the endpoint. Although most studies did not find associations between maternal PFOS and birth weight, a meta-analysis did find a small decrease in birth weight was associated with increasing maternal PFOS levels, after adjustment for glomerular filtration rate. There is also some suggestive evidence that PFOS levels may be associated with small delays in the age of puberty in boys and girls. Studies in laboratory animals clearly indicate that developmental toxicity is a sensitive outcome of PFOS exposure. Oral exposure studies have reported increases in fetal mortality and decreases in pup survival; decreases in fetal body weight, birth weight, and pup body weight; delays in developmental milestones such as eye opening; increases in skeletal malformations/anomalies/variations such as cleft palate and delayed skeletal ossification; and decreases in offspring motor activity.

PFHxS

Epidemiological Studies—Pregnancy Outcomes. Six studies, summarized in Table 2-22 have evaluated possible associations between pregnancy outcomes and maternal PFHxS levels. Jensen et al. (2015) did not find an association between maternal PFHxS levels and the risk of miscarriage. Hamm et al. (2010)

found a decreased risk of preterm births among women with serum PFHxS levels in the 3rd tertile and Manzano-Salgado et al. (2017a) and Sagiv et al. (2018) found no associations with risk of preterm birth. Other studies have found no associations with gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018).

Epidemiological Studies—Birth Outcomes. General population studies have evaluated possible associations between maternal PFHxS levels and birth outcomes including birth weight, length, small for gestation age, and birth defects; studies are summarized in Table 2-23. Bach et al. (2016) and Maisonet et al. (2012) reported inverse associations between maternal PFHxS levels and birth weight; however, other studies have not found associations (Alkhalawi et al. 2016; Ashley-Martin et al. 2017; Callan et al. 2016; Cao et al. 2018; Hamm et al. 2010; Kim et al. 2011; Li et al. 2017; Lee et al. 2013, 2016; Lenters et al. 2016a; Lind et al. 2017a; Manzano-Salgado et al. 2017a; Monroy et al. 2008; Sagiv et al. 2018; Shi et al. 2017; Starling et al. 2017). Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS levels and the risk of low birth weight infants. Hamm et al. (2010) and Manzano-Salgado et al. (2017a) did not find an association between maternal PFHxS level and the relative risk of small for gestational age. Ashley-Martin et al. (2017) did not find an association between maternal PFHxS levels and infant leptin or adiponectin levels, but Starling et al. (2017) found an inverse association between maternal PFHxS levels and adiposity at birth. Several studies did not find associations between maternal PFHxS levels and birth length, head circumference, or ponderal index (Alkhalawi et al. 2016; Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2013; Manzano-Salgado et al. 2017a; Shi et al. 2017). Maisonet et al. (2012) found an inverse association for birth length, but no association for ponderal index. Cao et al. (2018) found an association between head circumference and cord PFHxS levels. Only one study examined possible birth defects; Liew et al. (2014) did not find an association between maternal PFHxS levels and the risk of congenital cerebral palsy in a case-control study.

Epidemiological Studies—Neurodevelopmental Outcomes. Epidemiological studies, summarized in Table 2-24, have examined PFHxS-related alterations in risks of ADHD, autism, intelligence, and behavior. Wang et al. (2015b) did not find associations between maternal PFHxS levels and IQ in 5- or 8-year-old children and Jeddy et al. (2017) did not find associations between maternal PFHxS levels and verbal comprehension or vocabulary comprehension production in 15-month-old infants or intelligibility, language, or communication scores in 38-month-old children. Zhang et al. (2018) did not find associations between reading scores at 5 or 8 years of age and maternal PFHxS levels or PFHxS levels at age 3 or 5 years. Vuong et al. (2016) found a higher risk of performing poorly on tests of global executive function with increasing maternal PFHxS levels. However, no association was found between

serum PFHxS levels and metacognition or global executive function in 8-year-old children (Vuong et al. 2018). No association between serum PFHxS levels and the risk of learning problems was found in children living in a community with high PFOA levels (Stein and Savitz 2011). Gump et al. (2011) found an inverse association between serum PFHxS levels and performance on tasks requiring behavioral inhibition; Vuong et al. (2016, 2018) did not find alterations in behavioral regulation associated with maternal PFHxS levels or 8-year-old's PFHxS levels and Oulhote et al. (2016) did not find associations between behavioral development scores in 7-year-old children and maternal PFHxS levels or PFHxS levels at 5 or 7 years of age. Two studies evaluated the risk of ADHD and reported conflicting findings. Stein and Savitz (2011) reported increases in risk of ADHD in 5–18- and 12–15-year-olds with serum PFHxS levels in the 2nd, 3rd, or 4th quartile, whereas Liew et al. (2015) reported an inverse association between maternal PFHxS levels and risk of ADHD. This study also did not find an increase in the risk of autism; Braun et al. (2014) also found no association between maternal PFHxS levels and performance on tests assessing autism.

Epidemiological Studies—Development of the Reproductive System. No associations between reproductive hormone levels and serum PFHxS levels (Lopez-Espinosa et al. 2016) or maternal serum PFHxS levels (Maisonet et al. 2015a) were found in boys and girls 6–9 years of age or in girls 15 years of age. Lind et al. (2017a) did not find an association between maternal PFHxS levels and anogenital distance in boys or girls. Christensen et al. (2011) did not find an association between maternal PFHxS levels and risk of an earlier menarche. Summaries of these epidemiological studies are presented in Table 2-25.

Laboratory Animal Studies. Administration of 9.2 mg/kg/day PFHxS on PND 10 resulted in a decrease in spontaneous motor activity during the first 20 minutes of the test and an increase in activity in the last 20 minutes of the test (Viberg et al. 2013). The study also assessed the influence of PFHxS on nicotine-induced behavior. In the 9.2 mg/kg/day PFHxS group, exposure to nicotine did not significantly affect spontaneous motor activity, which was in contrast to the nicotine-induced increases in spontaneous motor activity observed in the controls and lower PFHxS groups. Studies evaluating the developmental toxicity of PFHxS did not find alterations in litter size, pup survival, or pup body weight in rats exposed to 10 mg/kg/day PFHxS or mice exposed to 3 mg/kg/day for 14 days prior to mating and throughout gestation and lactation (Butenhoff et al. 2009a; Chang et al. 2018). Although the rat study did not find alterations in litter size (Butenhoff et al. 2009a), the mouse study found a decrease in the number of pups per litter, without a change in the pup to implantation site ratio at ≥1 mg/kg/day (Chang et al. 2018). Similarly, no alterations in litter size, perinatal loss, or sex ratio were observed in the offspring of rats

administered up to 25 mg/kg/day PFHxS on GDs 7–22 (Ramhøj et al. 2018). The study did find decreases in male birth weights (3.5%) at ≥5 mg/kg/day and 30 and 45% decreases in pup serum thyroxine levels at 5 and 25 mg/kg/day (Ramhøj et al. 2018).

PFNA

Epidemiological Studies—Pregnancy Outcomes. Seven studies (summarized in Table 2-22) have examined pregnancy outcomes. Jensen et al. (2015) found an increase in the risk of having a miscarriage before gestation week 12, which was associated with maternal serum PFNA levels. Another study found no alteration in the risk of pregnancy loss (Buck Louis et al. 2016). No alterations in the risk of preterm birth was found in studies conducted by Chen et al. (2012a), Manzano-Salgado et al. (2017a), and Sagiv et al. (2018). Other studies found no association between PFNA and gestational age (Li et al. 2017; Manzano-Salgado et al. 2017a) or length (Lind et al. 2017a; Sagiv et al. 2018)

Epidemiological Studies—Birth Outcomes. Several studies have examined the possible associations between birth outcomes and maternal PFNA levels, these studies are summarized in Table 2-23. Most studies did not find an association between birth weight and maternal PFNA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Manzano-Saldago et al. 2017a; Monroy et al. 2008; Robledo et al. 2015a; Shi et al. 2017). No alterations in the risk of low birth weight or small for gestational age were found in studies conducted by Chen et al. (2012a) and Manzano-Salgado et al. (2017a). Wang et al. (2016) did find an inverse association between maternal PFNA levels and birth weight in girls only and Starling et al. (2017) and Sagiv et al. (2018) found inverse associations in boys and girls combined; Starling et al. (2017) also found an inverse association between maternal PFNA levels and adiposity. Chen et al. (2012a) found an association between maternal PFNA levels and birth length, but other studies have not found alterations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Most studies did not find alterations in ponderal index or head circumference (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Manzano-Salgado et al. 2017a; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016); Chen et al. (2012a) reported an inverse association between cord PFNA levels on ponderal index. No associations between maternal PFNA or paternal PFNA levels and the odds of a male birth were observed in a general population study (Bae et al. 2015). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy that were associated with maternal PFNA levels.

Epidemiological Studies—Neurodevelopmental Outcomes. Several potential neurodevelopmental outcomes have been examined in epidemiological studies; these studies are summarized in Table 2-24. No association between maternal PFNA levels and full-scale IQ scores were observed in 8-year-old children (Wang et al. 2015b); however, an association was found for visual IQ. Maternal PFNA levels were not associated with IQ scores in 5-year-old children (Wang et al. 2015b). Stein and Savitz (2011) found a decrease in the risk of learning problems in 5–18- or 12–15-year-olds with serum PFNA levels in the two highest quartiles or in the 4th quartile, respectively. No associations were found between maternal PFNA levels and verbal and vocabulary comprehension in 15-month-olds or language skills and intelligence scores in 38-month-olds (Jeddy et al. 2017). Vuong et al. (2016) did not find an association between maternal PFNA levels and metacognition or global executive functioning in 5- or 8-year-old children. In a subsequent study (Vuong et al. 2018), associations were found between serum PFNA levels at age 8 years and metacognition and global executive function scores, which were indicative of poorer performance; When the children were categorized by sex, the associations were only found in boys. The study also found associations between PFNA levels and at risk metacognition and global executive functioning scores. Reading scores in 5-year-old children were associated with serum PFNA levels when the children were 3 years of age but were not associated with maternal PFNA levels (Zhang et al. 2018), and reading levels at 8 years of age were not associated with maternal, 3-year-old, or 5-year-old serum PFNA levels (Zhang et al. 2018).

Mixed results have been found in studies on behavior. Gump et al. (2011) found a decrease in behavioral response inhibition that was associated with serum PFNA levels in children aged 9–11 years, and Lien et al. (2016) reported inverse associations between cord blood PFNA levels in inattention and hyperactivity/inattention in 7-year-old children, but no effect on hyperactivity/impulsivity. Vuong et al. (2016) did not find an association between maternal PFNA levels and behavior regulation, but serum PFNA levels in 8-year-old children were associated with higher at risk behavioral regulation scores (Vuong et al. 2018). Three studies have not found associations between PFNA levels and ADHD risk (Hoffman et al. 2010; Liew et al. 2015; Stein and Savitz 2011). Similarly, maternal PFNA levels do not appear to be associated with autism (Braun et al. 2014; Liew et al. 2015).

Epidemiological Studies—Development of the Reproductive System. An inverse association between PFNA levels and insulin-like growth factor 1 was found in boys and girls aged 6–9 years (Lopez-Espinosa et al. 2016). No associations were found between PFNA and estradiol or total testosterone in 6–9 years olds (Lopez-Espinosa et al. 2016) or between maternal PFNA and testosterone or sex hormone binding globulin levels in 15-year-old girls (Maisonet et al. 2015a). Additionally, no association between

maternal serum PFHxS levels and risk of earlier age of menarche were observed in girls (Christensen et al. 2011). Summaries of these three studies are presented in Table 2-25.

Laboratory Animal Studies. Three studies were identified that examined the developmental toxicity of PFNA in laboratory animals. Full litter resorptions were observed in mice administered 10 mg/kg/day on GDs 1–17; maternal weight loss was also observed at this dose level (Das et al. 2015). At ≤1.5 mg/kg/day, decreases in postnatal survival were observed (Das et al. 2015; Wolf et al. 2010). Decreases in birth weight were observed in female offspring of rats administered 5 mg/kg/day PFNA on GDs 1–20 (Rogers et al. 2014). Postnatal growth was decreased on PNDs 1–24 in the offspring of mice administered ≥3 mg/kg/day PFNA on GDs 1–17 (Das et al. 2015); the decreases in body weight persisted in the males through PND 287 and in the females through PND 50. No skeletal or visceral abnormalities were observed in mouse pups (Das et al. 2015). Reductions in nephron endowment (number of functioning nephrons at birth) were observed in male rat pups on PND 22 (Rogers et al. 2014). This study also found increases in systolic blood pressure in pups at 10 weeks of age; no alterations were observed at 26 or 52 weeks of age. Delays in eye opening and decreased in pup body weight gain were observed in offspring of mice administered 2.0 mg/kg/day on GDs 1–18 (Wolf et al. 2010). Studies in PPARα knockout mice did not find alterations in pup survival, birth weight, pup body weight gain, or day of eye opening at maternal doses as high as 2.0 mg/kg/day (Wolf et al. 2010). Comparison between the results in tests using wild-type mice and knockout mice suggests that PPARα plays a role in PFNA developmental toxicity (Wolf et al. 2010).

PFDA

Epidemiological Studies—Pregnancy Outcomes. Four epidemiological studies examined pregnancy outcomes. Jensen et al. (2015) found an increased risk of miscarriage that was associated with maternal PFDA levels. The remaining studies found no associations between maternal PFDA levels and pregnancy loss (Buck Louis et al. 2016), gestational age (Li et al. 2017), or gestational length (Lind et al. 2017a).

Epidemiological Studies—Birth Outcomes. A small number of epidemiological studies examined risks of adverse birth outcomes associated with maternal PFDA exposure; these studies are summarized in Table 2-23. Wang et al. (2016) found an inverse association between maternal PFDA levels and birth weight in female infants only. This study also found an increased risk for small for gestational age among female infants. Other studies have not found associations (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Lind et al. 2017a; Robledo et al. 2015a; Shi et

al. 2017; Starling et al. 2017). Starling et al. (2017) also found no association with adiposity at birth. Epidemiological studies have not found associations between birth length, ponderal index, and/or head circumference and maternal PFDA levels (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Robledo et al. 2015a; Shi et al. 2017; Wang et al. 2016). Liew et al. (2014) did not find alterations in the risk of congenital cerebral palsy in boys or girls and Bae et al. (2015) did not find alterations in odds of a male birth associated with maternal or paternal PFDA levels. Additionally, Kim et al. (2016b) did not find associations between serum PFDA levels and thyroid parameters.

Epidemiological Studies—Neurodevelopmental Outcomes. Several studies have evaluated the potential of PFDA to adversely affect neurodevelopment; see Table 2-24 for a summary of the studies. Wang et al. (2015b) did not find associations between maternal PFDA levels and IQ in 5- and 8-year-old children. Similarly, Vuong et al. (2016) did not find alterations in scores on tests of global executive functioning and metacognition in 5- or 8-year-old children. This study also found no alteration in behavioral regulation. In contrast, Gump et al. (2011) found increases in impulsivity. Oulhote et al. (2016) found an association between serum PFDA levels in 5-year-old children and total behavioral development score and higher externalizing and hyperactivity/inattention scores in 7-year-old children; the study did not find associations between behavioral development at age 7 years and maternal PFDA levels or 7-year-old PFDA levels. Liew et al. (2015) found decreases in the risk of ADHD and autism in children.

Epidemiological Studies—Developmental of the Reproductive System. In the only study examining reproductive outcomes, Lind et al. (2017a) found an inverse association between maternal PFDA levels and anogenital distance in girls, but not in boys.

Laboratory Animal Studies. An increase in fetal mortality was observed in mice exposed to 12.8 mg/kg/day PFDA on GDs 6–15 (Harris and Birnbaum 1989); this dose level was also associated with a marked decrease in fetal weight/litter (50% lower than controls), 100% incidence of variations in ossification of the braincase, decreases in maternal body weight, and maternal mortality. Decreases in fetal body weight/litter were observed at ≥1 mg/kg/day. The study did not find alterations in the occurrence of cleft palate, soft tissue malformations, or skeletal malformations. In mice exposed to 10.8 mg/kg/day PFDA on PND 10, there was no effect on spontaneous activity, habituation, performance on an elevated maze test, or response to a nicotine injection (Johansson et al. 2008). These results differ from the Johansson et al. (2008) findings when mice were exposed to PFOA or PFOS and the findings of Viberg et al. (2013) in mice exposed to PFHxS.

PFUnA

Epidemiological Studies—Pregnancy Outcomes. A limited number of epidemiological studies evaluated pregnancy outcomes. Jensen et al. (2015) did not find an alteration in the risk of miscarriage before gestation week 12. No association between gestational age and maternal PFUnA levels were found in a study conducted by Li et al. (2017).

Epidemiological Studies—Birth Outcomes. The results from a study conducted by Wang et al. (2016) found an inverse association between maternal PFUnA levels and birth weight and an increased risk of small for gestation age among female infants. Callan et al. (2016) reported an association between maternal PFUnA levels and optimal body weight but did not find an association with birth weight. The remaining epidemiological studies have not found alterations in infant size (birth weight, birth length, ponderal index, head circumference) (Bach et al. 2016; Callan et al. 2016; Cao et al. 2018; Chen et al. 2012a; Lee et al. 2016; Lenters et al. 2016a; Li et al. 2017; Shi et al. 2017) or the risks of low birth weight (Chen et al. 2012a) or small for gestational age (Chen et al. 2012a). No association between serum PFUnA levels and thyroid parameters were observed in infants (Kim et al. 2016a). The results of the epidemiological studies examining associations between birth outcome and PFUnA are presented in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. The results of two studies examining possible associations between neurodevelopmental outcome and PFUnA are summarized in Table 2-24. Wang et al. (2015b) found no association between maternal PFUnA levels and IQ score in 5- and 8-year-old children; the study did find an inverse association with scores on tests assessing performance IQ. Lien et al. (2016) found no associations between cord blood PFUnA levels and performance on behavioral tests.

Laboratory Animal Studies. One study was identified that examined the potential developmental toxicity of PFUnA (Takahashi et al. 2014); the study found decreases in pup body weight at birth and on PND 4 in the offspring of rats administered via gavage 1.0 mg/kg/day PFUnA.

PFHpA

Epidemiological Studies. In the only epidemiological study evaluating developmental outcomes, Li et al. (2017) found no association between cord PFHpA levels and gestational age. The study did find an inverse association for birth weight in boys only, but not in girls and in boys and girls combined.

PFBS

Laboratory Animal Studies. No alterations in pup survival, body weight, or development were observed at doses as high as 1,000 mg/kg/day in a 2-generation rat study of potassium PFBS (Lieder et al. 2009b). In contrast to these findings, Feng et al. (2017) reported decreases in pup body weight, delays in eye opening, vaginal opening, and first estrous in the offspring of mice administered PFBS on GDs 1–20. York (2002) reported decreases in fetal body weight at 1,000 mg/kg/day in a rat study; however, a subsequent study (York 2003a) found decreases in body weights in the fetuses of rats administered 2,000 mg/kg/day, but not 1,000 mg/kg/day.

Reproductive and endocrine effects were also observed in the offspring at 200 and 500 mg/kg/day; these effects consisted of decreases in number of ovarian follicles and corpora lutea at diestrus, decreases in uterine weight and endometrial and myometrial thickness; increases in the average number of days in estrous stage; decreases in estrogen and progesterone levels; increases in luteinizing hormone levels; decreases in total T4, free T4, and total T3; and increases in TSH levels (Feng et al. 2017).

PFBA

Epidemiological Studies. Li et al. (2017) did not find an association between cord PFBA levels and gestational age or birth weight. In a study conducted by Kim et al. (2016a), no associations were found between serum PFBA levels and thyroid parameters in infants.

Laboratory Animal Studies. A delay (approximately 1 day) in eye opening was observed in the offspring of mice administered via gavage 35 mg/kg/day PFBA on GDs 1–17 (Das et al. 2008).

PFDoDA

Epidemiological Studies—Pregnancy Outcomes. In the only study examining pregnancy outcome (Table 2-22), Li et al. (2017) found no association between cord serum PFDoDA levels and gestational age.

Epidemiological Studies—Birth Outcomes. General population studies conducted by Cao et al. (2018), Lee et al. (2016), and Lenters et al. (2016a) did not find associations between cord blood PFDoDA or maternal PFDoDA levels and birth weight, birth length, and/or ponderal index. Wang et al. (2016) found an inverse association between maternal PFDoDA levels and birth weight and head circumference in female infants; no alteration in the risk of small for gestation age was found. Li et al. (2017) also found an association between cord PFDoDA levels and birth weight in girls only; no association was found in boys or in boys and girls combined. The results of these three studies are summarized in Table 2-23.

Epidemiological Studies—Neurodevelopmental Outcomes. As summarized in Table 2-24, only one study examined neurodevelopmental outcomes. In this study, maternal PFDoDA levels were not associated with IQ scores in 5- or 8-year-old children (Wang et al. 2015b).

Laboratory Animal Studies. One study evaluated the developmental toxicity of PFDoDA; no alterations in the number of live pups born, birth weight, growth, or the prevalence of external, visceral, or skeletal anomalies were observed at 0.1 or 0.5 mg/kg/day (Kato et al. 2015). At the next highest dose (2.5 mg/kg/day), only 1 of the 12 dams delivered live pups; 2 of these pups died on PND 0 and decreases in body weight gain were observed in the remaining pups.

PFHxA

Laboratory Animal Studies. Administration of 500 mg/kg/day NaPFHx on GDs 1–20 resulted in 10% decreases in fetal weight in rats (Loveless et al. 2009). Similarly, decreases in pup body weight (17–18% during the lactation period) were observed in the offspring of rats administered 500 mg/kg/day NaPFHx for 70 days prior to mating, during mating, and throughout gestation and lactation (Loveless et al. 2009). This study also found no alterations in pup clinical signs, survival, or developmental landmarks. No alterations in litter size, pup survival, or pup body weight, or occurrence of internal malformations were observed in the offspring of rats administered 315 mg/kg/day PFHxA (TWA dose) prior to mating through lactation day 4 (Kirkpatrick 2005).

FOSA

Epidemiological Studies. Robledo et al. (2015a) found an inverse association between maternal FOSA levels and birth weight in boys, but not in girls; paternal FOSA levels were not associated with birth weight. The study did not find alterations in birth length, head circumference, or ponderal index (see Table 2-23). Bae et al. (2015) did not find alterations in the odds of a male birth that was associated with maternal or paternal FOSA levels. As summarized in Table 2-24, only one study evaluated possible associations between FOSA and neurodevelopmental outcomes. Gump et al. (2011) reported an inverse association between serum FOSA levels and performance on tasks requiring behavioral inhibition. In the only study examining development of the reproductive system, Christensen et al. (2011) did not find an association between maternal serum FOSA levels and the risk of an earlier age of menarche in girls (see Table 2-25).

2.18 OTHER NONCANCER

Overview. A number of epidemiological studies have examined the possible associations between perfluoroalkyls and outcomes related to diabetes; the results of these studies are summarized in Table 2-26, with additional study details presented in the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 14. Overall, the epidemiological studies do not provide support for an association between serum perfluoroalkyl levels and increases in the risk of diabetes or related outcomes (e.g., increases in blood glucose, glucose tolerance) for PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFHpA, or FOSA. Additionally, results of studies on PFOA, PFOS, and PFHxS do not suggest an association between perfluoroalkyls and gestational diabetes. No epidemiological studies examining other noncancer endpoints were identified for PFBS, PFBA, PFDoDA, or PFHxA. Only four laboratory animal studies examined other noncancer endpoints reporting inflammation of the salivary glands in rats exposed to PFOA, pancreatic acinar cell hyperplasia in rats exposed to PFOA, and an increase in serum glucose levels in rats administered PFNA (Table 2-5). The fourth study did not find increases in serum glucose in rats exposed to PFOS (Table 2-4).

PFOA

Epidemiological Studies. A cohort mortality study conducted by Leonard et al. (2008; Leonard 2006) of workers at the Washington Works facility found a significant increase in deaths from diabetes, as

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Leonard 2006 Occupational (n=6,027)	>250–≤750 ng/mL PFOA	Diabetes deaths	**SMR 183 (112–283)* (males only)**
Leonard et al. 2008 Occupational (n=6,027)	NR	Diabetes deaths	**SMR 197 (123–298)***
Lundin et al. 2009 Occupational (n=3,992)	Probable exposure	Diabetes deaths	**SMR 2.0 (1.2–3.2)***
Raleigh et al. 2014 Occupational (n=9,027)	NR	Diabetes deaths	SMR 0.76 (0.50–1.11)
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative	Risk of diabetes	RR 1.10 (0.77–1.57) no lag RR1.12 (0.76–1.66) 10-year lag
Steenland and Woskie 2012 Occupational (n=1,088)	580 ng/mL (median PFOA)	Diabetes deaths	**SMR 1.90 (1.35–2.61)* no lag** SMR 1.90 (0.98–3.33) 10-year lag SMR 1.73 (0.83–3.18) 20-year lag
Anderson-Mahoney et al. 2008 Community (n=566)	NR	Self-reported diabetes	**SPR 1.54 (1.16–2.05)***
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 without diabetes)	68.4 ng/mL, 92.8 ng/mL, 86.5 ng/mL, 82.3 ng/mL (mean and serum PFOA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all) Adults (>20 years) Youth (≤20 years)	**OR 0.69 (0.65–0.74)*** **OR 0.74 (0.70–0.79)*** **OR 0.72 (0.54–0.97)***
		Type 2 diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.87 (0.89–0.91)*** **OR 0.91 (0.89–0.94)*** **OR 0.92 (0.88–0.96)***
		Uncategorized diabetes Adults (>20 years) Youth (≤20 years)	**OR 0.92 (0.88–0.97)*** OR 1.13 (0.82–1.56) OR 1.18 (0.90–1.55)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Karnes et al. 2014 Community (n=32,254 C8 participants)	Estimated cumulative	Self-reported diabetes	HR 1.00 (0.99–1.00, p=0.60), retrospective analysis HR 1.00 (1.00–1.01, p=0.31), prospective analysis
		Fasting blood glucose	NS (p>0.05)
MacNeil et al. 2009 Community (n=13,922 C8 participants)	122.7 ng/mL (mean PFOA)	Validated diabetes	OR 0.72 (0.52–1.00) (10^{th} decile)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 1.06 (0.89–1.28, p=0.50)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	9.7 and 9.0 ng/mL (median serum PFOA in males and females at age 9 years)	Glucose At age 15 At age 21	 β 1.87 (-1.19–4.93) β -1.01 (-14.62–30.07)
		Insulin At age 15 At age 21	 β -12.99 (-25.95–2.23) β -13.98 (-36.23–16.00)
		HOMA-IR At age 15 At age 21	 β -12.54 (-25.59–2.77) β -14.16 (-36.60–16.28)
		HOMA-β At age 15 At age 21	 **β -11.10 (-20.28 to -1.01)*** β -7.82 (-22.44–9.66)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	3.7 and 3.4 ng/mL (median serum PFOA in males and females at age 15 years)	Glucose at age 21	β 5.83 (-3.70–16.92)
		Insulin at age 21	β -0.59 (-44.76–79.43)
		HOMA-IR at age 21	β 0.93 (-44.45–83.55)
		HOMA-β at age 21	β -11.70 (-37.16–24.67)
Fisher et al. 2013 General population (n=2,700)	2.46 ng/mL (geometric mean PFOA)	Insulin	NS (p=0.12)
		Blood glucose	NS (p=0.17)
		HOMA-IR	NS (p=0.10)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	5.3 ng/mL (geometric mean maternal PFOA)	HOMA-IR	β -0.7 (-9.8–9.4)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	4.2 ng/mL (geometric mean PFOA in child)	HOMA-IR	**β -10.1 (-17.3 to -2.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	2.1–3.34, 3.34–5.1, and >5.1 ng/mL (serum PFOA for 2nd, 3rd, and 4th quartiles)	Diabetes	**OR 2.13 (1.30–3.46)*, 2nd quartile males** OR 1.47 (0.87–2.48), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	1.67 ng/mL (maternal median serum PFOA)	Fasting glucose	β-1.3 (3.0–0.5),
		Fasting insulin	β -4.0 (-12.2–5.0)
		2-hour glucose in oral glucose tolerance test	β-2.6 (-6.9–1.8)
		HOMA-IR	β -5.2 (-14.2–4.7)
		HOMA-β	β -0.4 (-8.0–8.0)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	1.88 ng/mL (median serum PFOA)	Fasting blood glucose	β 1.262 (-1.108–3.633, p=0.294)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	1.81 and 1.39 ng/mL (median serum PFOA in WTCHR group and comparison group)	HOMA-IR	β -0.05 (-0.21–0.12, p=0.58)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	1.51 ng/mL (mean log PFOA)	Insulin	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
General population (NHANES) (n=969 adults)	1.48 ng/mL (mean log PFOA)	Insulin	Association (p<0.05)
		β-cell function	Association (p<0.05)
		Fasting blood glucose	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	3.3 ng/mL (median PFOA)	Diabetes	OR 0.97 (0.61–1.53, p=0.88)
		HOMA-IR	NS (p=0.20)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	1.86 ng/mL (geometric mean serum PFOA)	Fasting glucose	NS (p>0.05)
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	**Association (p<0.05)***
		β cell function	**Association (p<0.05)***
Melzer et al. 2010 General population (NHANES) (n=3,966)	10.39 ng/mL (M) 9.47 ng/mL (F) (4th PFOA quartile)	Self-reported diabetes	OR 0.69 (0.41–1.16, p=0.158)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	4.6 ng/mL (mean PFOA)	HOMA (adolescent)	NS (p=0.16) (M), NS (p=0.11) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	1.68 ng/mL (geometric mean PFOA)	Gestational diabetes	NS (p=0.86 for trend)
		Impaired glucose tolerance	NS (p=0.36 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.4–17.0 ng/mL (3rd quartile maternal serum PFOA)	Maternal glucose levels	**β -0.025 (-0.046 to -0.004)*, 3rd quartile**
Su et al. 2016 General population (n=571)	5.8–8.0 ng/mL (2nd PFOA quartile)	Diabetes	**OR 0.39 (0.16–0.96)* (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	**Inverse association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	5.48–112 ng/mL (3rd tertile serum PFOA)	Type 2 diabetes	**OR 1.54 (1.04–2.28)*, 3rd tertile**
Wang et al. 2018 General population (n=385 pregnant women)	7.3 ng/mL (median maternal serum PFOA); ≥10.1 ng/mL (3rd tertile serum PFOA)	Fasting blood glucose	β -0.005 (-0.018–0.008, p=0.465)
		Fasting insulin	β 0.069 (-0.005–0.143, p=0.068)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.014 (-0.013–0.041, p=0.305)
		Gestational diabetes mellitus	HR 2.11 (0.76–5.86, p=0.151), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	1.90 ng/mL (median serum PFOA)	Fasting blood glucose	β 0.545 (-1.8–2.887)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	3.07 and 3.94 ng/mL (geometric mean PFOA in women with or without gestational diabetes)	Gestational diabetes	**OR 1.86 (1.14–3.02)***
PFOS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	21.8, 25.2, 25.1, 23.1 ng/mL (mean and serum PFOS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.61–0.70)***
		Type 2 diabetes	**OR 0.86 (0.82–0.90)***
		Uncategorized diabetes	OR 0.93 (0.86–1.03)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOS)	Type 2 diabetes	HR 0.87 (0.74–1.02, p=0.08)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		HOMA-β	**Association (p<0.05)***
		HbA1c	**Association (p<0.05)***
Domazet et al. 2016 General population (n=501 children assessed at ages 9, 15, and 21 years)	44.5 and 39.9 ng/mL (median serum PFOS in males and females at age 9 years)	Glucose	
		At age 15	**β 0.88 (0.07–1.60)***
		At age 21	β 0.64 (-0.55–1.84)
		Insulin	
		At age 15	β -0.29 (-4.26–3.67)
		At age 21	β -1.01 (-8.00–6.62)
		HOMA-IR	
		At age 15	β -12.54 (-25.59–2.77)
		At age 21	β -0.83 (-7.91–6.71)
		HOMA-β	
		At age 15	β -0.29 (-4.17–3.76)
		At age 21	β -1.66 (-5.70–2.67)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Domazet et al. 2016 General population (n=501children assessed at ages 9, 15, and 21 years)	22.3 and 20.8 ng/mL (median serum PFOS in males and females at age 15 years)	Glucose at age 21	β 0.81 (-1.27–2.72)
		Insulin at age 21	β 4.78 (-6.82–17.77)
		HOMA-IR at age 21	β 4.74 (-6.69–17.91)
		HOMA-β at age 21	β 1.81 (-4.77–9.09)
Fisher et al. 2013 General population (n=2,700)	8.04 ng/mL (geometric mean PFOS)	Insulin	NS (p=0.88)
		Blood glucose	NS (p=0.96)
		HOMA-IR	NS (p=0.25
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	24.4 ng/mL (geometric mean maternal PFOS)	HOMA-IR	β -0.6 (-8.2–7.6)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	6.2 ng/mL (geometric mean PFOS in child)	HOMA-IR	**β -10.1 (-16.4 to -3.3)***
He et al. 2018 General population (NHANES) (n=7,904 adults)	>25.5 ng/mL (4th quartile serum PFOS)	Diabetes	OR 1.75 (1.00–3.04), 4th quartile males OR1.41 (0.82–2.41), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	8.37 ng/mL (maternal median serum PFOS)	Fasting glucose	β -0.1 (-2.3–2.2)
		Fasting insulin	β 2.7 (-8.5–15.2)
		2-hour glucose in oral glucose tolerance test	β 2.9 (-2.8–8.9)
		HOMA-IR	β -2.9 (-7.1–14.1)
		HOMA-β	β -2.6 (-9.7–16.6)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	5.68 ng/mL (median serum PFOS)	Fasting blood glucose	β 0.707 (-1.921–3.336, p=0.595)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	3.72 and 2.78 ng/mL (median serum PFOS in WTCHR group and comparison group)	HOMA-IR	β -0.06 (-0.18–0.06, p=0.31)
Lin et al. 2009 General population (NHANES) (n=474 adolescents)	3.11 ng/mL (log mean PFOS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (969 adults)	3.19 ng/mL (log mean PFOS)	Insulin	**Association (p<0.05)***
		HOMA-IR	**Association (p<0.05)***
		β-cell function	**Association (p<0.05)***
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	13.2 ng/mL (median PFOS)	Diabetes	OR 1.43 (0.94–22.16, p=0.09)
		HOMA	NS (p=0.51)
Liu et al. 2018b General population (NHANES) (n=1,871 adults)	5.28 ng/mL (geometric mean serum PFOS)	Fasting glucose	**Inverse association (p<0.05)***
		Insulin	NS (p>0.05)
		2-hour glucose in glucose tolerance test	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HbA1C	NS (p>0.05)
		β cell function	NS (p>0.05)
Melzer et al. 2010 General population (NHANES) (n=3,966)	57.73 ng/mL (M) 50.96 ng/mL (F) (4th quartile mean PFOS)	Self-reported diabetes	OR 0.87 (0.57–1.31, p=0.491)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	25.3 ng/mL (mean PFOS)	HOMA (adolescent)	NS (p=0.18) (M), NS (p=0.22) (F)
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (1,274 pregnant women)	4.58 ng/mL (geometric mean PFOS)	Gestational diabetes	NS (p=0.70 for trend)
		Impaired glucose tolerance	NS (p=0.74 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	2.4 ng/mL (maternal median serum PFOS)	Maternal glucose levels	β -0.009 (-0.020–0.003)
Su et al. 2016 General population (n=571)	>4.8 ng/mL (4^{th} PFOS quartile)	Diabetes	**OR 3.37 (1.18–9.65)***
		Fasting blood glucose	**Association (p<0.01 for trend)***
		Glucose tolerance test	**Association (p≤0.01 for trend)***
		Glycated hemoglobin	**Association (p=0.04 for trend)***
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	26.3–41.4 ng/mL (2^{nd} tertile serum PFOS)	Type 2 diabetes	**OR 1.63 (1.25–2.12)*, 2^{nd} tertile**
Wang et al. 2018 General population (n=385 pregnant women)	5.4 ng/mL (median maternal serum PFOS); ≥7.3 ng/mL (3^{rd} tertile serum PFOS)	Fasting blood glucose	β -0.009 (-0.019–0.002, p=0.108)
		Fasting insulin	β 0.013 (-0.048–0.074, p=0.672)
		HOMA-IR	β 0.074 (-0.011–0.158, p=0.087)
		Blood glucose in oral glucose tolerance test	β 0.006 (-0.015–0.028, p=0.562)
		Gestational diabetes mellitus	HR 0.71 (0.29–0.75, p=0.453), 3^{rd} tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.00 ng/mL (median serum PFOS)	Fasting blood glucose	β -1.237 (-2.63–1.59)
Zhang et al. 2015a General population (n=258)	13.10 and 12.04 ng/mL (geometric mean PFOS in women with or without gestational diabetes)	Gestational diabetes	OR 1.13 (0.75–1.72)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHxS			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	3.4, 3.8, 4.2, and 5.2 ng/mL (mean and serum PFHxS in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.59 (0.54–0.64)***
		Type 2 diabetes	**OR 0.74 (0.71–0.77)***
		Uncategorized diabetes)	**OR 0.84 (0.78–0.90)***
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	4.82 ng/mL (geometric mean serum PFOA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fisher et al. 2013 General population (n=2,700)	2.18 ng/mL (geometric mean PFHxS)	Insulin	NS (p=0.89)
		Blood glucose	NS (p=0.98)
		HOMA-IR	NS (p=0.20)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.5 ng/mL (geometric mean maternal PFHxS)	HOMA-IR	β -2.07 (-5.9–2.0)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	2.2 ng/mL (geometric mean PFHxS in child)	HOMA-IR	β -1.7 (-3.8–0.5)
He et al. 2018 General population (NHANES) (n=7,904 adults)	0.9–1.64, 1.64–2.9, and >2.9 (2^{nd}, 3^{rd}, and 4^{th} quartile serum PFHxS)	Diabetes	**OR 1.99 (1.19–3.33)*, 2^{nd} quartile males** OR 1.22 (0.71–2.11), 4^{th} quartile, females

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.31 ng/mL (maternal median serum PFHxS)	Fasting glucose	**β 1.7 (0.2–3.2)***
		Fasting insulin	**β 7.7 (0.1–15.9)***
		2-hour glucose in oral glucose tolerance test	β 2.9 (-0.8–6.8)
		HOMA-IR	**β 9.5 (1.0–18.8)***
		HOMA-β	β 2.3 (-4.3–9.4)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.793 ng/mL (median serum PFHxS)	Fasting blood glucose	β 0.925 (-1.779–2.164, p=0.500)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.67 and 0.53 ng/mL (median serum PFHxS in WTCHR group and comparison group)	HOMA-IR	β -0.09 (-0.18–0.003, p=0.04)
Lin et al. 2009 General population (NHANES) (n=474 adolescents);	0.95 ng/mL (log mean)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
General population (NHANES) (n=969 adults)	0.60 ng/mL (log mean PFHxS)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		B-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)
Lind et al. 2014 General population (n=1,016)	2.1 ng/mL (median PFHxS)	Diabetes	OR 1.00 (0.74–1.35, p=0.98)
		HOMA	NS (p=0.29)
Nelson et al. 2010 General population (NHANES) (n=306 adolescent and 524 adults)	2.6 ng/mL (mean PFHxS)	HOMA (adolescent)	NS (p=0.20) (M), **Inverse association (p=0.001)* (F)**
		HOMA (adult)	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Shapiro et al. 2016 General population (n=1,274 pregnant women)	1.02 ng/mL (geometric mean PFHxS)	Gestational diabetes	NS (p=0.73 for trend)
		Impaired glucose tolerance	NS (p=0.44 for trend)
Starling et al. 2017 General population (n=604 mother-infant pairs)	1.1–10.9 ng/mL (3rd quartile maternal serum PFHxS)	Maternal glucose levels	**β -0.023 (-0.044 to -0.002)*, 3rd quartile**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	2.15 and 2.01 ng/mL (serum PFHxS in cases and controls, respectively)	Type 2 diabetes	OR 1.26 (0.86–1.86), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	3.80 ng/mL (median serum PFHxS)	Fasting blood glucose	β -0.29 (-1.9–1.32)
PFNA			
Conway et al. 2016 Community (n=820 with type 1 diabetes, 4,291 with type 2 diabetes, 1,349 with uncategorized, and 60,439 with no diabetes)	1.4, 1.5, 1.5, and 1.6 ng/mL (mean and serum PFNA in type 1 diabetics, type 2 diabetics, uncategorized diabetics, and no diabetes groups)	Type 1 diabetes (all)	**OR 0.65 (0.57–0.74)***
		Type 2 diabetes	**OR 0.94 (0.88–1.00)***
		Uncategorized diabetes)	OR 0.95 (0.85–1.06)
Cardenas et al. 2017 General population (n=957 adults at high risk of developing type 2 diabetes)	0.53 ng/mL (geometric mean serum PFNA)	Type 2 diabetes	HR 0.99 (0.87–1.12, p=0.82)
		Fasting blood glucose	**Association (p<0.05)***
		Fasting insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		HOMA-β	NS (p>0.05)
		HbA1c	NS (p>0.05)
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.6 ng/mL (geometric mean maternal PFNA)	HOMA-IR	β 1.4 (-8–11.7)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	1.7 ng/mL (geometric mean PFNA in child)	HOMA-IR	β -0.6 (-3.2–2.6)
He et al. 2018 General population (NHANES) (n=7,904 adults)	>1.64 (4th quartile serum PFNA)	Diabetes	OR 1.19 (0.73–1.95), 4th quartile males OR 1.01 (0.62–1.65), 4th quartile, females
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.65 ng/mL (maternal median serum PFNA)	Fasting glucose	β 0.03 (-2.1–2.2)
		Fasting insulin	**β 12.1 (0.7–24.8)***
		2-hour glucose in oral glucose tolerance test	β 1.3 (-6.5–4.2)
		HOMA-IR	β 12.2 (-0.5–26.4)
		HOMA-β	**β 12.4 (0.2–23.7)***
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.938 ng/mL (median serum PFNA)	Fasting blood glucose	β 0.428 (-1.785–2.641, p=0.703)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.61 and 0.49 ng/mL (median serum PFNA in WTCHR group and comparison group)	HOMA-IR	β 0.01 (-0.13–0.14, p=0.89)
Lin et al. 2009 General population (NHANES) (n=474 adolescents) General population (NHANES) (n=969 adults)	0.35 ng/mL (log mean PFNA)	Insulin	**Inverse association (p<0.05)***
		HOMA-IR	NS (p>0.05)
		β-cell function	**Inverse association (p<0.05)***
		Blood glucose	NS (p>0.05)
	0.21 ng/mL (log mean PFNA)	Insulin	NS (p>0.05)
		HOMA-IR	NS (p>0.05)
		β-cell function	NS (p>0.05)
		Blood glucose	NS (p>0.05)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Lind et al. 2014 General population (n=1,016)	0.7 ng/mL (median PFNA)	Diabetes	OR 1.30 (0.85–1.97, p=0.22)
		HOMA	NS (p=0.90)
Nelson et al. 2010 General population (NHANES) (n=306 adolescents and 524 adults)	1.3 ng/mL (mean PFNA)	HOMA (adolescent)	NS (p=0.83) (M), (p=0.20) (F)
		HOMA (adult)	NS (p>0.05)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.5–6.0 ng/mL (2nd half maternal serum PFNA)	Maternal glucose levels	**β -0.025 (-0.042 to -0.009)*, 2nd half**
Su et al. 2016 General population (n=571)	>5.1 ng/mL (4th PFNA quartile)	Diabetes	**OR 0.31 (0.11–0.88)* 4th quartile (inverse association)**
		Fasting blood glucose	NS (p=0.10 for trend)
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.11 for trend)
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.60 and 0.61 ng/mL (serum PFNA in cases and controls, respectively)	Type 2 diabetes	OR 0.99 (0.67–1.48), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.50 ng/mL (median serum PFNA)	Fasting blood glucose	β -0.627 (-2.54–1.29)
Zhang et al. 2015a General population (n=258)	1.23 and 1.20 ng/mL (geometric mean PFNA in women with or without gestational diabetes)	Gestational diabetes	OR 1.06 (0.70–1.60)
PFDA			
Fleisch et al. 2017 General population (n=665 children 7.7 (median) years of age)	0.3 ng/mL (geometric mean PFDA in child)	HOMA-IR	**β -14.7 (-22.1 to -6.5)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Jensen et al. 2018 General population (n=158 pregnant women with high risk of gestational diabetes mellitus)	0.26 ng/mL (maternal median serum PFDA)	Fasting glucose	β 1.3 (-3.6–1.0)
		Fasting insulin	β -0.2 (-11.2–12.1)
		2-hour glucose in oral glucose tolerance test	β -3.3 (-8.7–2.5)
		HOMA-IR	β -1.5 (-13.5–12.1)
		HOMA-β	β 3.9 (-6.4–15.2)
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.0592 ng/mL (median serum PFDA)	Fasting blood glucose	β -0.201 (-1.280–0.878, p=0.713)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.14 and 0.11 ng/mL (median serum PFDA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.11–0.03, p=0.26)
Starling et al. 2017 General population (n=604 mother-infant pairs)	0.2–3.5 ng/mL (2nd half maternal serum PFDA)	Maternal glucose levels	**β -0.024 g (-0.041 to -0.007)*, 2nd half**
Sun et al. 2018 General population (n=793 female cases and 793 female controls)	0.13 and 0.16 ng/mL (serum PFDA in cases and controls, respectively)	Type 2 diabetes	OR 0.71 (0.48–1.05), 3rd tertile
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.40 ng/mL (median serum PFDA)	Fasting blood glucose	β -2.543 (-4.65 to -0.44)

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Zhang et al. 2015a General population (n=258)	0.41 and 0.40 ng/mL ng/mL (geometric mean PFDA in women with or without gestational diabetes)	Gestational diabetes	OR 1.04 (0.70–1.53)
PFUnA			
Kang et al. 2018 General population (n=150 children, ages 3–18 years)	0.652 ng/mL (median serum PFUnA)	Fasting blood glucose	β 1.350 (-0.020–2.721, p=0.053)
Koshy et al. 2017 General population (n=180 children enrolled in the WTCHR)	0.12 and 0.04 ng/mL (median serum PFUnA in WTCHR group and comparison group)	HOMA-IR	β -0.04 (-0.10–0.02, p=0.21)
Lind et al. 2014 General population (n=1,016)	0.3 ng/mL (median PFUnA)	Diabetes	OR 0.95 (0.59–1.52; p=0.81)
		HOMA	NS (p=0.32)
Su et al. 2016 General population (n=571)	6.4–9.2 ng/mL (3rd PFUnA quartile)	Diabetes	**OR 0.24 (0.08–0.78)* 3rd quartile (inverse association)**
		Fasting blood glucose	**Inverse association (p<0.01 for trend)***
		Glucose tolerance test	**Inverse association (p<0.01 for trend)***
		Glycated hemoglobin	NS (p=0.17 for trend)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.30 ng/mL (median serum PFUnA)	Fasting blood glucose	**β -1.821 (-3.45 to -0.189)***

Table 2-26. Summary of Outcomes Related to Diabetes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFHpA			
Lind et al. 2014 General population (n=571)	0.05 ng/mL (median PFHpA)	Diabetes	OR 1.02 (0.77–1.34, p=0.90)
		HOMA	NS (p=0.56)
Yang et al. 2018 General population (n=148 men, 81 diagnosed with metabolic syndrome)	0.20 ng/mL (median serum PFHpA)	Fasting blood glucose	β -1.101 (-5.54–3.34)
FOSA			
Lind et al. 2014 General population (n=571)	0.11 ng/mL (median FOSA)	Diabetes	OR 1.07 (0.75–1.53, p=0.71)
		HOMA	NS (p=0.070)

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 14 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

(F) = females; FOSA = perfluorooctane sulfonamide; HOMA = homeostatic model assessment; HR = hazard ratio; IR = insulin resistance; (M) = males; NHANES = National Health and Nutrition Examination Survey; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; SMR = standardized mortality ratio; SPR = standardized prevalence ratio; WTCHR = World Trade Center Health Registry

compared to workers at other DuPont facilities in the region. In an update of the Leonard et al. (2008) study, Steenland and Woskie (2012) found an increased risk of diabetes deaths when compared to other regional DuPont employees, but not when compared to the U.S. population. However, when the workers were categorized by estimated cumulative exposure levels, the exposure-response trend was not statistically significant. Lundin et al. (2009) also found an increase in deaths from diabetes in workers exposed to APFO at the 3M Cottage Grove facility in Minnesota, as compared to Minnesota death rates. The increase was only found in workers with probable exposure to APFO, but not with definite exposure; no deaths from diabetes were observed in the workers with definite exposure to APFO. As noted by Steenland and Woskie (2012), diabetes mortality may not be a good surrogate for the underlying diabetes incidence data. Raleigh et al. (2014) did not find an increase in diabetes deaths at the Cottage Grove facility and Steenland et al. (2015) did not find an increased risk of diabetes associated with estimated cumulative PFOA exposure at the Washington Works facility.

In community exposure studies, Anderson-Mahoney et al. (2008) found an increased prevalence of self-reported diabetes in residents living near the Washington Works facility, as compared to expected rates taken from NHANES. Conway et al. (2016) found increases in the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes in C8 Health Study participants. When the participants were categorized by age, the increases in type 1 diabetes and type 2 diabetes prevalences were found in adults and children; uncategorized diabetes was not increased in either group. In contrast, Karnes et al. (2014) did not find an increased risk of self-reported diabetes associated with estimated cumulative PFOA levels and MacNeil et al. (2009) did not find an increased risk of validated diabetes in C8 Health Study participants.

General population studies found either an inverse association between serum PFOA and risk of diabetes (Su et al. 2016), an association (He et al. 2018; Sun et al. 2018), or no association (Cardenas et al. 2017; Lind et al. 2014; Melzer et al. 2010). Additionally, most general population studies have not found associations between serum PFOA levels and insulin (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b), blood glucose levels (Fisher et al. 2013; Lin et al. 2009; Liu et al. 2018b; Su et al. 2016; Yang et al. 2018), homeostatic model assessment for insulin resistance (HOMA-IR) (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010), or glucose tolerance (Liu et al. 2018b; Su et al. 2016). Cardenas et al. (2017) did find associations between serum PFOA and glycemic parameters in cross-sectional analyses; however, in longitudinal analyses, no associations were found between serum PFOA and fasting blood glucose, fasting insulin, HOMA-IR, HOMA-β, or HbA1c. Studies in children

have not found associations between serum PFOA and blood glucose, blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2017; Koshy et al. 2017).

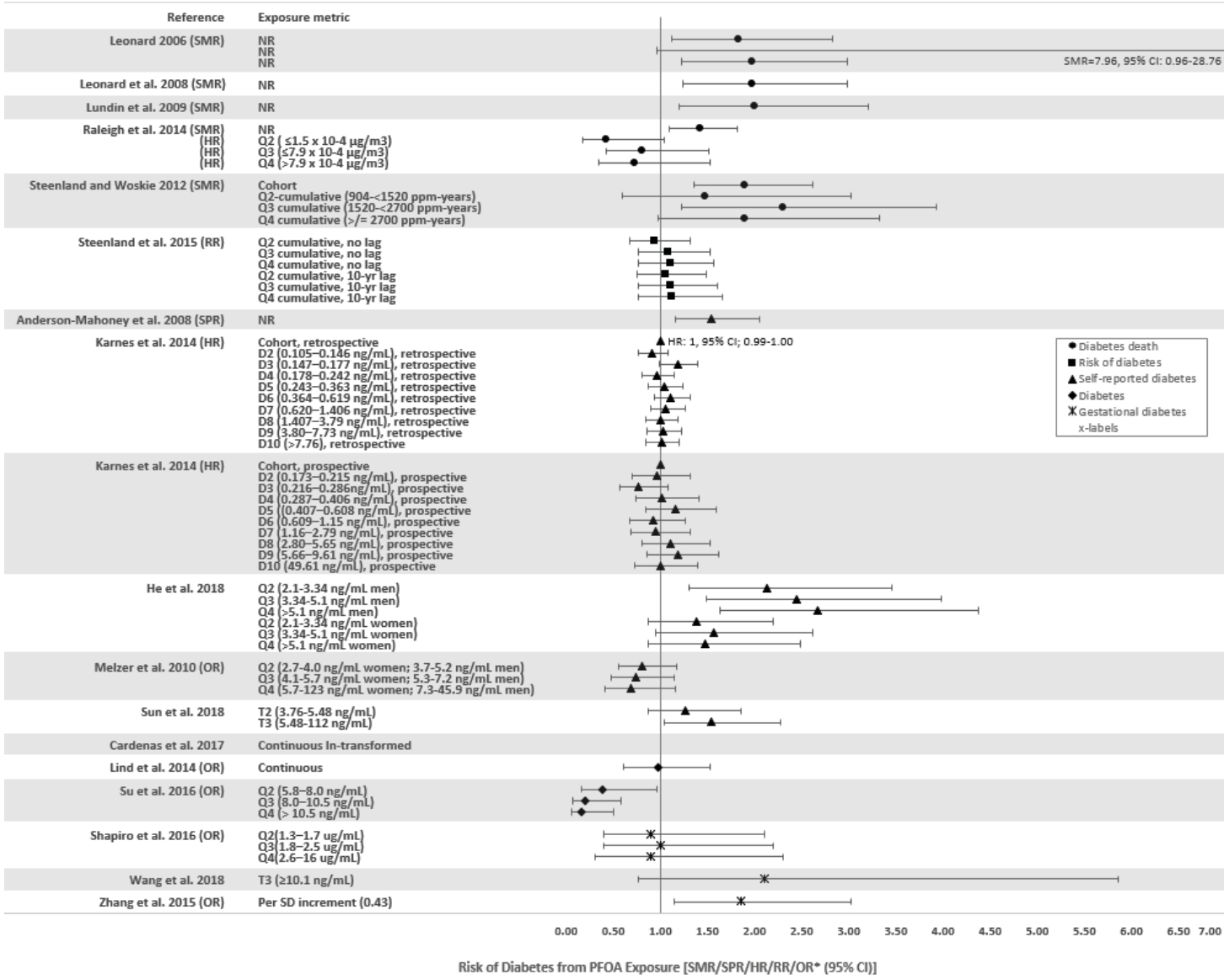
Three studies evaluated the risk of gestational diabetes and found mixed results. In a case-control study, Zhang et al. (2015a) found an increased risk of gestational diabetes associated with serum PFOA, whereas Shapiro et al. (2016) and Wang et al. (2018) did not find associations between serum PFOA and gestational diabetes or impaired glucose tolerance. Additionally, Starling et al. (2017) found an inverse association between blood glucose levels and serum PFOA in pregnant women. In contrast, Jensen et al. (2018) did not find associations between serum PFOA and fasting glucose, fasting insulin, HOMA-IR, HOMA-β, or blood glucose levels in a glucose tolerance test in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-39.

Laboratory Animal Studies. Two chronic-duration oral studies examined other noncancer endpoints. Inflammation of the salivary gland was observed in rats exposed to 1.5 mg/kg/day (3M 1983; Butenhoff et al. 2012c) and an increased incidence of acinar cell hyperplasia was observed in rats exposed to 13.6 mg/kg/day (Biegel et al. 2001).

PFOS

Epidemiological Studies. Inverse associations between serum PFOS and the prevalence of type 1 diabetes and type 2 diabetes were observed among participants of the C8 Health Study (Conway et al. 2016). In a general population study conducted by Su et al. (2016), an increased risk of diabetes was noted, as well as associations between serum PFOS levels and fasting blood glucose, response to glucose tolerance test, and glycated hemoglobin levels. Cardenas et al. (2017) also found associations between serum PFOS and fasting blood glucose, fasting insulin, HOMA-IR, and HOMA-β; however, these associations were not found in longitudinal analyses over a 3-year period. In a prospective case-control study, Sun et al. (2018) reported an association between serum PFOS and type 2 diabetes. Four other general population studies did not find increased risks of diabetes (Cardenas et al. 2017; He et al. 2019; Lind et al. 2014; Melzer et al. 2010). Several studies have not found associations between serum PFOS levels and insulin, blood glucose, or HOMA-IR levels (Fisher et al. 2013; Lin et al. 2009; Lind et al. 2014; Liu et al. 2018b; Nelson et al. 2010; Yang et al. 2018). In NHANES adult participants, Lin et al. (2009) found associations between serum PFOS and insulin and HOMA-IR and Liu et al. (2018b) found an inverse association with fasting glucose levels. No associations were found in adolescent participants

Figure 2-39. Diabetes Risk Relative to Serum PFOA Levels (Presented as Adjusted Ratios)



(Lin et al. 2009). Studies in children have not found associations between serum PFOS and fasting blood glucose, fasting blood insulin, HOMA-IR, and/or HOMA-β (Domazet et al. 2016; Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

No alterations in the risk of gestational diabetes were observed in three general population studies (Shapiro et al. 2016; Wang et al. 2018' Zhang et al. 2015a). Shapiro et al. (2016), Starling et al. (2017), and Wang et al. (2018) studies also found no association between serum PFOS and blood glucose levels, glucose tolerance or other glycemic measurements in pregnant women. The ORs for the risk of diabetes and gestational diabetes are graphically presented in Figure 2-40.

Laboratory Animal Studies. Perinatal exposure to 3 mg/kg/day PFOS did not result in alterations in serum insulin or glucose levels in the offspring on PND 63 (Wan et al. 2014b). However, when the offspring were fed a high fat diet, increases in fasting glucose levels were observed at 0.3 and 3 mg/kg/day and fasting serum insulin levels were increased at 3 mg/kg/day.

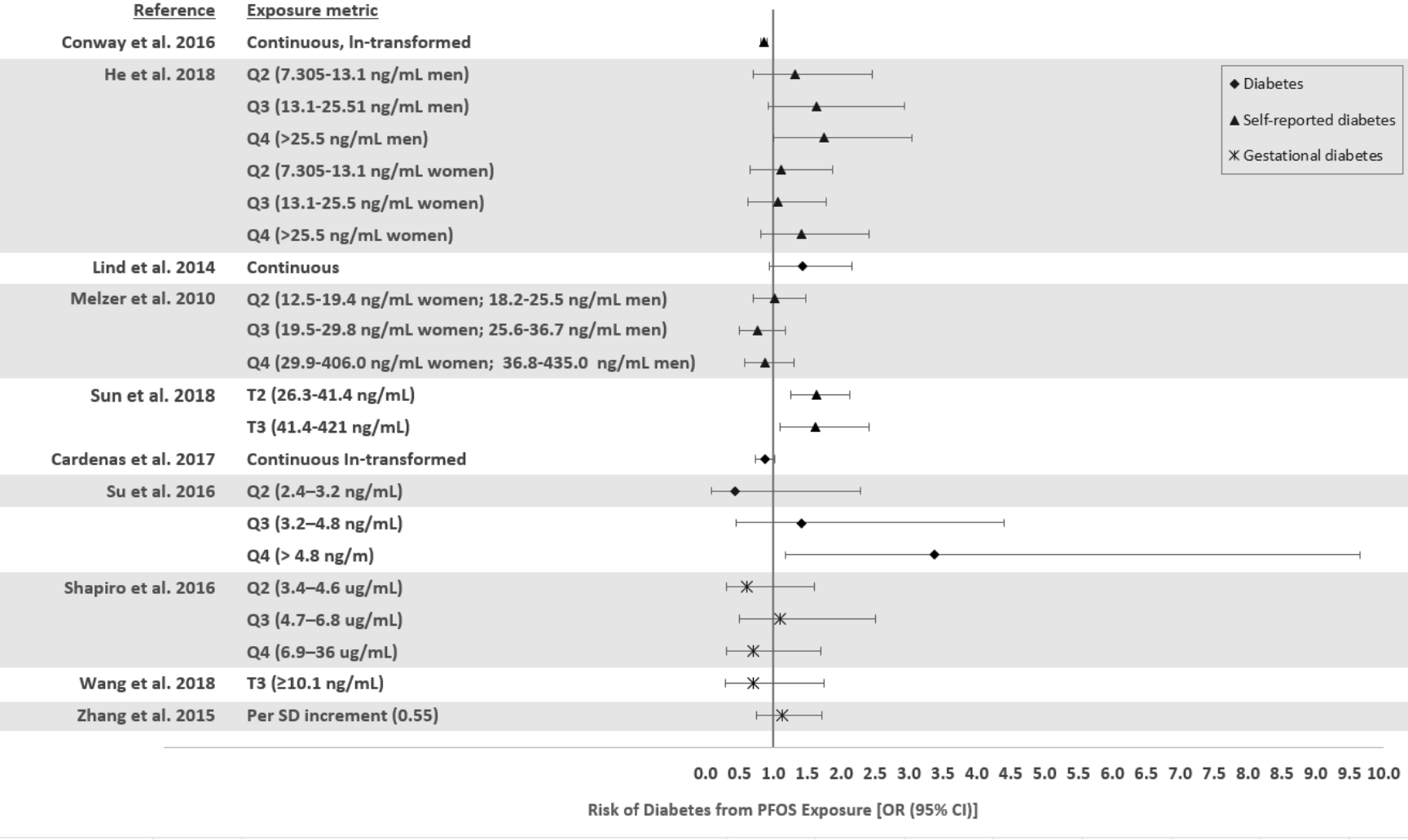
PFHxS

Epidemiological Studies. A study of C8 Health Project participants found inverse associations between serum PFHxS levels and the prevalence of type 1 diabetes, type 2 diabetes, and uncategorized diabetes (Conway et al. 2016). General population studies have examined diabetes-related outcomes and have not found associations between serum PFHxS levels and diabetes risk (Cardenas et al. 2017; He et al. 2018; Lind et al. 2014; Sun et al. 2018), gestational diabetes (Shapiro et al. 2016) or insulin, blood glucose, or HOMA-IR levels (Cardenas et al. 2017; Fisher et al. 2013; Jensen et al. 2018; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018). An inverse association between serum PFHxS levels and blood glucose levels was found in pregnant women (Starling et al. 2017). No associations between serum PFHxS and glycemic parameters were found in children (Fleisch et al. 2017; Kang et al. 2018; Koshy et al. 2017).

PFNA

Epidemiological Studies. An inverse association between serum PFNA levels and the risk of diabetes was observed in a general population study (Su et al. 2016) and for type 1 diabetes and type 2 diabetes in C8 Health Study participants (Conway et al. 2016). Four other studies did not find associations for diabetes (He et al. 2018; Lind et al. 2014; Sun et al. 2018) or gestational diabetes (Zhang et al. 2015a).

Figure 2-40. Diabetes Risk Relative to Serum PFOS Levels (Presented as Adjusted Odds Ratios)



The Su et al. (2016) study also reported an inverse association between PFNA levels and response on a glucose tolerance test. A study by Starling et al. (2017) also found an inverse association between PFNA levels and blood glucose levels in pregnant women. A study of adolescent NHANES participants found decreasing levels of insulin with increasing serum PFNA levels (Lin et al. 2009); this association was not found in adult NHANES participants (Lin et al. 2009). Several studies did not find associations between serum PFNA levels and fasting blood glucose, glucose tolerance, HOMA-IR, and/or HOMA-β (Cardenas et al. 2017; Fleisch et al. 2017; Jensen et al. 2018; Kang et al. 2018; Koshy et al. 2017; Lin et al. 2009; Lind et al. 2014; Nelson et al. 2010; Yang et al. 2018).

Laboratory Animal Studies. An increase in serum glucose levels was observed in rats administered via gavage 1 mg/kg/day PFNA for 14 days (Fang et al. 2012a).

PFDA

Epidemiological Studies. Two studies evaluated the potential association between PFDA and diabetes risk. Sun et al. (2018) did not find an association for type 2 diabetes risk and Zhang et al. (2015a) did not find an association between serum PFDA levels and the risk of gestational diabetes. Other studies have examined possible associations between serum PFDA and glycemic measurements. Fleisch et al. (2017) found an inverse association with HOMA-IR in children. Other studies in children (Kang et al. 2018; Koshy et al. 2017), adults (Yang et al. 2018), and pregnant women (Jensen et al. 2018) did not find associations for fasting blood glucose, fasting insulin, glucose tolerance, HOMA-IR, and/or HOMA-β.

PFUnA

Epidemiological Studies. Six epidemiological studies evaluating associations between PFUnA and diabetes-related outcomes have found conflicting results. Su et al. (2016) found inverse associations between serum PFUnA levels and diabetes risk, fasting blood glucose levels, and glucose tolerance test results, Yang et al. (2018) found an inverse association with fasting blood glucose levels, and Starling et al. (2017) found an inverse association with blood glucose levels in pregnant women. Whereas Lind et al. (2014) found no alterations in the risk of diabetes or HOMA, and Kang et al. (2018) and Koshy et al. (2017) found no associations between serum PFUnA levels and fasting blood glucose and HOMA-IR, respectively, in studies in children.

PFHpA

Epidemiological Studies. Lind et al. (2014) did not find associations between serum PFHpA levels and the risk of diabetes or HOMA alterations and Yang et al. (2018) did not find an association with fasting blood glucose levels.

FOSA

Epidemiological Studies. In the one epidemiological study identified, no associations between serum FOSA levels and the risk of diabetes or HOMA were found (Lind et al. 2014).

2.19 CANCER

Overview. A number of occupational exposure, community, and general population studies have examined possible associations between perfluoroalkyls and cancer risk; these studies are summarized in Table 2-27 and the *Supporting Document for Epidemiological Studies for Perfluoroalkyls*, Table 15. Occupational and community exposure studies have found increases in the risk of testicular and kidney cancer associated with PFOA. No consistent epidemiologic evidence for other cancer types were found for PFOA. For PFOS, one occupational exposure study reported an increase in bladder cancer, but this was not supported by subsequent occupational studies. General population studies have not consistently reported increases in malignant tumors for PFOS. A small number of epidemiology studies examined possible associations between other perfluoroalkyls and cancer risk. No consistent associations were observed for breast cancer risk for PFHxS, PFNA, PFHpA, or PFDoDA; increased breast cancer risks were observed for PFDA and FOSA, but this was based on a single study. No associations between PFOA, PFOS, PFHxS, PFNA, PFDA, or PFUnA and prostate cancer risk were found. However, among men with a first-degree relative with prostate cancer, associations were found for PFOA, PFOS, PFHxS, PFDA, and PFUnA, but not for PFNA. Epidemiological studies examining potential cancer effects were not identified for PFBS, PFBA, or PFHxA.

Laboratory animal studies have evaluated the carcinogenicity of PFOA and PFOS; the results of these studies are summarized in Tables 2-3 and 2-4. In laboratory animals, there is some evidence for increases in Leydig cell adenomas, pancreatic acinar cell adenomas, and hepatocellular adenomas in male rats exposed to PFOA in the diet. An increase in hepatocellular adenomas was observed in male rats exposed to dietary PFOS for 2 years; thyroid follicular cell adenomas were observed in rats exposed to PFOS for

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
PFOA			
Gilliland 1992; Gilliland and Mandel 1993 Occupational (n=389 deaths) Reference population: Minnesota general population	NR	All cancer deaths	SMR 0.86 (0.72–1.01), males SMR 0.75 (0.56–0.99), females
		Prostate cancer	SMR 2.03 (0.55–4.59) RR 1.13 (1.01–1.27) for a 1-year increase in employment length **RR 3.3 (1.02–10.6)* for a 10-year employment length**
Leonard 2006; Leonard et al. 2008 Occupational (n=6,027) Reference population: DuPont workers at other regional facilities	5–9,550 ng/mL (estimated range of PFOA)	All cancer deaths	SMR 100 (88–114), males SMR 149 (77–260), females
		Kidney cancer deaths	SMR 185 (95–323), males
		Biliary passages and liver cancer deaths	SMR 133 (53–274)
		Pancreatic cancer deaths	SMR 100 (50–180)
		Bladder or other urinary organ cancer deaths	SMR 131 (53–269)
		Prostate cancer deaths	SMR 65 (34–114)
		Bronchus, trachea, lung cancer deaths	SMR 81 (63–104)
Lundin et al. 2009 Occupational (n=3,993) Reference population: Minnesota general population; for HR analysis comparisons with workers with low exposure or <1 year of exposure	2,600–5,200 and 300–1,500 ng/mL (range of PFOA in subset of current workers with definite exposure jobs and probable exposure jobs)	All cancer deaths	SMR 0.9 (0.5–1.4), definite exposure SMR 0.9 (0.8–1.1), probable exposure
		Pancreas cancer deaths	SMR 0.9 (0.0–4.7), definite exposure SMR 1.0 (0.4–2.1), probable exposure
		Trachea, bronchus, and lung cancer deaths	SMR 1.2 (0.5–2.3), definite exposure SMR 1.0 (0.7–1.4), probable exposure
		Prostate cancer deaths	SMR 2.1 (0.4–6.1), definite exposure SMR 0.9 (0.4–1.8), probable exposure **HR 6.6 (1.1–37.7), high exposure ≥6 months** **HR 3.7 (1.3–10.4), definite exposure for ≥5 years**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Raleigh et al. 2014 Occupational (n=9,207) Reference population: Minnesota general population	>7.9x10^{-4} µg/m^3 (cumulative exposure, 4th PFOA quartile)	All cancer deaths	SMR 0.87 (0.78–0.97)
		Pancreatic cancer deaths	SMR 0.85 (0.50–1.34)
		Prostate cancer deaths	SMR 0.83 (0.53–1.23)
		Kidney cancer deaths	SMR 0.53 (0.20–1.16)
		Liver cancer deaths	SMR 0.81 (0.35–1.59)
		Breast cancer deaths	SMR 0.82 (0.41–1.47)
		Bladder cancer deaths	SMR 0.89 (0.38–1.76)
Raleigh et al. 2014 Occupational (n=9,207) Reference population: non-APFO exposed workers at a St. Paul facility	≤7.9x10^{-4} and >7.9x10^{-4} µg/m^3 (cumulative exposure,3rd and 4th PFOA quartile)	Pancreatic cancer deaths	HR 1.23 (0.50–3.00), 3rd and 4th quartiles combined
		Pancreatic cancer	HR 1.36 (0.59–3.11), 3rd and 4th quartiles combined
		Prostate cancer deaths	HR 1.32 (0.61–2.84), 4th quartile
		Prostate cancer	HR 1.11 (0.82–1.49), 4th quartile
		Kidney cancer deaths	HR 0.39 (0.11–1.32), 3rd and 4th quartiles combined
		Kidney cancer	HR 0.73 (0.21–2.48), 4th quartile
		Liver cancer deaths	HR 0.67 (0.14–3.27), 3rd and 4th quartiles combined
		Breast cancer deaths	HR 0.54 (0.15–1.94), 3rd and 4th quartiles combined
		Breast cancer	HR 1.27 (0.70–2.31), 4th quartile
		Bladder cancer deaths	HR 1.96 (0.63–6.15), 3rd and 4th quartiles combined
		Bladder cancer	HR 1.66 (0.86–3.18), 4th quartile
Steenland et al. 2015 Occupational (n=3,713)	Estimated cumulative exposure	Bladder cancer	**Inverse association (p=0.04 or p=0.06 for trend) with no lag or 10-year lag** **RR 0.23 (0.05–0.93), 4th quartile with no lag**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
		Colorectal cancer	NS (p=0.91 and 0.86 for trend), with no lag or 10-year lag
		Prostate cancer	NS (p=0.83 and 0.91 for trend), no lag or 10-year lag
		Melanoma	NS (p=0.16 and 0.55 for trend), no lag or 10-year lag
Steenland and Woskie 2012 Occupational (n=1,084 deceased workers) Reference population: DuPont workers at other regional facilities	≥2,700,000 ng/mL-years (estimated cumulative 4th PFOA quartile)	All cancer deaths	SMR 0.94 (0.76–1.16), 4th quartile
		Pancreatic cancer deaths	SMR 0.92 (0.30–2.16), 4th quartile
		Lung cancer deaths	SMR 0.75 (0.48–1.11), 4th quartile
		Prostate cancer deaths	SMR 0.57 (0.16–1.46), 4th quartile
		Bladder cancer deaths	SMR 0.36 (0.10–2.01), 4th quartile
		Kidney cancer deaths	**SMR 2.66 (1.15–5.24)*, 4th quartile** **SMR 2.82 (1.13–5.81)*, 10-year lag** **SMR 3.67 (1.48–7.57)*, 20-year lag**
Barry et al. 2013 Community and occupational (n=32,254)	Estimated cumulative exposure 24.2 and 112.7 ng/mL (median PFOA)	Testicular cancer	**HR 1.34 (1.00–1.79, p=0.05)* no lag** HR 1.28 (0.95–1.73, p=0.10) 10-year lag **HR 3.17 (0.75–13.45, p=0.04 for trend)*, 4th quartile**
		Kidney cancer	HR 1.10 (0.98–1.24, p=0.10), no lag (continuous) HR 1.58 (0.88–2.84, p=0.18 for trend), 4th quartile
		Breast cancer	**HR 0.94 (0.89–1.00, p=0.05)*, no lag** **HR 0.93 (0.88–0.99, p=0.03)*, 10-year lag**
		Colorectal cancer	HR 0.99 (0.92–1.07, p=0.84), no lag

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	86.6 ng/mL (mean PFOA)	Prostate specific antigen	NS ($p<0.05$)
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.5–27.8 ng/mL (2nd PFOA quartile)	Colorectal cancer	**OR 0.47 (0.31–0.74)*, 2nd quartile**
Vieira et al. 2013 Community (n=25,107)	30.8–109 and 110–655 ng/mL (estimated PFOA in high and very high exposure groups)	Kidney cancer	**AOR 2.0 (1.3–3.2)*, high exposure group** AOR 2.0 (1.0–3.9), very high exposure group
		Testes cancer	AOR 2.8 (0.8–9.2), very high exposure group
		Prostate cancer	AOR 1.5 (0.9–2.5), very high exposure group
		Breast cancer	AOR 1.4 0.9–2.3) very high exposure group, females only
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and115 matched controls)	2.5 and 1.6 ng/mL (median PFOA in cases and controls)	Breast cancer	AOR 1.20 (0.77–1.88, $p=0.43$)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 233 matched controls)	5.2 ng/mL (mean PFOA)	Breast cancer	RR 1.00 (0.90–1.11).
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	6.8 and 6.0 ng/mL (median PFOA in male and female cancer patients) 6.9 and 5.4 ng/mL (median PFOA in male and female controls)	Prostate cancer	IRR 1.18 (0.84–1.65)
		Bladder cancer	IRR 0.81 (0.53–1.24)
		Pancreas cancer	IRR 1.55 (0.85–2.80)
		Liver cancer	IRR 0.60 (0.26–1.37)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases and 186 controls)	2.3 and 1.9 ng/mL (mean PFOA in cases and controls)	Prostate cancer	OR 1.1 (0.7–1.7) **OR 2.6 (1.2–6.0)*, among subjects with a heredity risk and serum PFOA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.08 and 1.48 ng/mL (median serum PFOA in cases and controls, respectively)	Breast cancer	**OR 1.26 (1.01–1.58, p=0.039)*, continuous** **OR 2.64 (1.17–5.97, p=0.019)*, 3rd tertile**
PFOS			
Alexander et al. 2003 Occupational (n=2,083; 145 deaths) Reference population: Alabama general population	NR	All cancer deaths	SMR 0.84 (0.50–1.32), high potential exposure group
		Bladder and other urinary organs cancer	**SMR 12.77 (2.63–37.35)*, high potential exposure group** **SMR 16.12 (3.32–47.14)*, high exposure group ≥1 year exposure**
Alexander and Olsen 2007 Occupational (n=1,895; 1,488 deaths) Reference population: NIOSH SEER referent data	NR	Bladder cancer	SIR 1.74 (0.64–3.79), high potential exposure group SIR 1.43 (0.16–5.15) ≥10-year exposure group
Grice et al. 2007 Occupational (n=1,400 current, retired, or former workers)	1,300–1,970 ng/mL (PFOS levels in high potential exposure group)	Colon cancer	OR 1.69 (0.68–4.17)
		Melanoma	OR 1.01 (0.25–4.11)
		Prostate cancer	OR 1.08 (0.44–2.69)
Olsen et al. 2004a Occupational (current and retired workers)	NR	Malignant melanoma of the skin	RRE_pC 12 (1.0–>100) RRE_pC 10 (0.7–>100), >10 years employment
		Malignant neoplasm of the colon	RRE_pC 5.4 (0.5–>100) RRE_pC 12 (0.8–>100), >10 years employment
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	22.18 ng/mL (mean PFOS)	Prostate specific antigen	NS ($p<0.05$)

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Innes et al. 2014 Community (n=208 cases of colorectal cancer and 47,359 cancer-free adults)	13.6–20.1 ng/mL (2nd PFOS quartile)	Colorectal cancer	**OR 0.35 (0.24–0.53)***
Bonefeld-Jorgensen et al. 2011 General population (n=31 breast cancer cases and 115 matched controls)	45.6 and 21.9 ng/mL (median PFOS in cases and controls)	Breast cancer	**OR 1.03 (1.001–1.07, p=0.05)***
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	30.6 ng/mL (mean PFOS)	Breast cancer	RR 0.99 (0.98–1.01)
Eriksen et al. 2009 General population (n=713 for prostate cancer, n=332 for bladder cancer, n=128 for pancreatic cancer, n=67 for liver cancer, and n=772 controls)	35.1 and 32.1 ng/mL and 35.0 and 29.3 ng/mL (median PFOS in male and female cancer patients and males and females in the comparison group)	Prostate cancer	IRR 1.05 (0.97–1.14)
		Bladder cancer	IRR 0.93 (0.83–1.03)
		Pancreas cancer	IRR 0.99 (0.86–1.14)
		Liver cancer	IRR 0.59 (0.27–1.27)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	11 and 10 ng/mL (mean PFOS in cases and controls)	Prostate cancer	OR 1.0 (0.6–1.5) **OR 2.7 (1.04–6.8)*, among subjects with a heredity risk and serum PFOS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	35.50 and 18.2 ng/mL (median serum PFOS in cases and controls, respectively)	Breast cancer	**OR 1.02 (1.01–1.03, p=0.005)*, continuous** **OR 3.13 (1.20–8.15, p=0.020)*, 2nd tertile**
PFHxS			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	3.58 ng/mL (mean PFHxS)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	1.2 ng/mL (mean PFHxS)	Breast cancer	**RR 0.66 (0.47–0.94)***

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	1.1 and 0.940 ng/mL (mean PFHxS in cases and controls)	Prostate cancer	OR 1.3 (0.8–1.9) **OR 4.4 (1.7–12)*, among subjects with a heredity risk and serum PFHxS above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.52 and 1.14 ng/mL (median serum PFHxS in cases and controls, respectively)	Breast cancer	**OR 1.16 (1.02–1.32, p=0.029)*, continuous** **OR 2.69 (1.23–5.88, p=0.013)*, 3rd tertile**
PFNA			
Ducatman et al. 2015a, 2015b Community (C8) (n=25,412 men)	1.47 ng/mL (mean PFNA)	Prostate specific antigen	NS (p<0.05)
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	0.5 ng/mL (mean PFNA)	Breast cancer	RR 0.76 (0.30–1.94)
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.679 and 0.631 ng/mL (mean PFNA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.8) OR 2.1 (0.9–4.8), among subjects with a heredity risk and serum PFNA above the median
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	3.28 and 1.83 ng/mL (median serum PFNA in cases and controls, respectively)	Breast cancer	OR 1.07 (0.98–1.17, p=0.116), continuous **OR 2.43 (1.07–5.51, p=0.034)*, 2nd tertile** OR 2.07 (0.90–4.76, p=0.056), 3rd tertile
PFDA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.338 and 0.291 ng/mL (mean PFDA in cases and controls)	Prostate cancer	OR 1.4 (0.9–2.1) **OR 2.6 (1.1–6.1)*, among subjects with a heredity risk and serum PFDA above the median**

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	1.30 n and 1.01 ng/mL (median serum PFDA in cases and controls, respectively)	Breast cancer	OR 1.17 (0.97–1.40, p=0.094), continuous **OR 2.36 (1.04–5.36, p=0.041)*, 3rd tertile**
PFUnA			
Hardell et al. 2014 General population (n=201 cases of prostate cancer and 186 controls)	0.308 and 0.285 ng/mL (mean PFUnA in cases and controls)	Prostate cancer	OR 1.2 (0.8–1.9) **OR 2.6 (1.1–5.9)*, among subjects with a heredity risk and serum PFUnA above the median**
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	2.23 and 2.02 ng/mL (median serum PFUnA in cases and controls, respectively)	Breast cancer	OR 1.06 (0.97–1.15, p=0.207), continuous **OR 2.00 (0.88–4.53, p=0.019)*, 3rd tertile**
PFHpA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.11 and 0.08 ng/mL (median serum PFHpA in cases and controls, respectively)	Breast cancer	OR 6.98 (0.61–80.0, p=0.119), continuous OR 1.52 (0.54–4.24, p=0.425), 3rd tertile
PFDoDA			
Wielsøe et al. 2017 General population (n=77 cases and 84 controls)	0.40 and 0.21 ng/mL (median serum PFDoDA in cases and controls, respectively)	Breast cancer	OR 1.03 (1.01–1.06, p=0.447), continuous OR 0.93 (0.45–1.91, p=0.839), 3rd tertile

Table 2-27. Summary of Cancer Outcomes in Humans[a]

Reference and study population[b]	Serum perfluoroalkyl level	Outcome evaluated	Result[c]
FOSA			
Bonefeld-Jorgensen et al. 2014 General population (n=250 breast cancer cases and 115 matched controls)	3.5 ng/mL (mean FOSA)	Breast cancer	**RR 1.89 (1.01–3.54)*, among women with serum FOSA >5.75 ng/mL**

[a]See the *Supporting Document for Epidemiological Studies for Perfluoroalkyls,* Table 15 for more detailed descriptions of studies.
[b]Participants in occupational exposure may have also lived near the site and received residential exposure; community exposure studies involved subjects living near PFOA facilities with known exposure to high levels of PFOA.
[c]Asterisk indicates association with perfluoroalkyl; unless otherwise specified, values in parenthesis are 95% confidence intervals.

AOR = adjusted odds ratio; CI = confidence interval; FOSA = perfluorooctane sulfonamide; HR = hazard ratio; IRR = incidence rate ratio; NIOSH = National Institute for Occupational Safety and Health; NR = not reported; NS = not significant; OR = odds ratio; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid; RR = relative risk; RRE_pC = risk ratio episodes of care; SEER = Surveillance Epidemiology and End Results; SIR = standardized incidence ratio; SMR = standardized mortality ratio

1 year and allowed to recover for an additional year. A discussion of the relevance of the rodent carcinogenicity data to humans is included in Section 2.20.6.

EPA (2016e, 2016f) has concluded that there is suggestive evidence of the carcinogenic potential of PFOA and PFOS in humans. IARC (2017) concluded that PFOA is possibly carcinogenic to humans (Group 2B).

PFOA

Epidemiological Studies. Several studies have examined the possible association between occupational exposure to PFOA and increased cancer risk in workers at two U.S. facilities—3M facility in Cottage Grove, Minnesota (Gilliland and Mandel 1993; Lundin et al. 2009; Raleigh et al. 2014) and DuPont Washington Works facility in West Virginia (Leonard 2006; Leonard et al. 2008; Steenland and Woskie 2012; Steenland et al. 2015). In addition, the potential carcinogenicity of PFOA has been assessed in the community near the Washington Works facility (Barry et al. 2013; Innes et al. 2014; Vieira et al. 2013) and in the general population (Bonefeld-Jorgensen 2011, 2014; Eriksen et al. 2009; Hardell et al. 2014).

Occupational exposure studies have not found increases in the risk of all cancer deaths (Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012). The occupational exposure studies have consistently found no increases in the risk of pancreatic, liver, or respiratory tract cancers or deaths from these cancers (Leonard 2006; Leonard et al. 2008; Lundin et al. 2009; Raleigh et al. 2014; Steenland and Woskie 2012); a general population case-control study also found no associations between serum PFOA and pancreas or liver cancer (Eriksen et al. 2009). Additionally, two case-control studies did not find associations between serum PFOA levels and risk of breast cancer (Bonefeld-Jorgensen et al. 2011, 2014); a third case-control study found an association between serum PFOA and breast cancer (Wielsøe et al. 2017). Steenland et al. (2015) found an inverse association between estimated cumulative PFOA exposure and bladder cancer in workers; other studies have not found associations (Eriksen et al. 2009; Gilliland and Mandel 1993; Leonard 2006; Leonard et al. 2008; Raleigh et al. 2014; Steenland and Woskie 2012).

Some associations between PFOA and cancer effects have been observed, including prostate, kidney, and testicular cancers. Ten years of employment in the Chemical Division of the 3M Cottage Grove facility was associated with a 3.3-fold increase in the relative risk of prostate cancer mortality, as compared to no employment in PFOA production areas (Gilliland and Mandel 1993; data also reported in Gilliland 1992);

no increase in prostate cancer risk was observed when all workers in the Chemical Division were analyzed. The investigators noted that the prostate cancer findings are based on a small number of cases and could have resulted from chance or unrecognized confounding from exposure to other factors. An update of this study conducted by Lundin et al. (2009) did not find an increase in prostate cancer deaths in workers with definite PFOA exposure. When the cohort was divided into the three exposure categories and duration of definite exposure, increased risks for prostate cancer were found in the high-exposure category and in workers with definite exposure for at least 5 years, as compared with workers in the low-exposure category and with the shortest cumulative exposure duration, respectively. Interpretation of the Gilliland and Mandel (1993) and Lundin et al. (2009) studies is limited by the qualitative assessment of potential exposure and the fact that workers in the low exposure categories were likely research-and-development professionals rather than production workers (Raleigh et al. 2014). In the most recent evaluation of the Cottage Grove facility, which involved extensive exposure assessment, Raleigh et al. (2014) did not find increases in prostate cancer deaths when compared to the general population or to workers at another facility and did not find an increase in the incidence of prostate cancer when the workers were categorized by cumulative exposure levels. Studies of the Washington Works facility workers did not find increases in prostate cancer deaths (Leonard et al. 2008; Steenland and Woskie 2012) or incidence (Steenland et al. 2015). A case-control general population study by Hardell et al. (2014) did find an increase in prostate risk only among subjects with a heredity risk (first-degree relative with prostate cancer) and serum PFOA levels above the median. In a study of community members, Ducatman et al. (2015b) did not find an association between prostate-specific antigen (PSA) levels and serum PFOA levels in men 20–49 or 50–69 years of age.

In the earliest cancer assessment of workers at the Washington Works facility (Leonard 2006; Leonard et al. 2008), an increase in the number of deaths from kidney cancer relative to workers at other regional DuPont facilities was observed; however, the CI included unity. In a follow-up study that used serum PFOA levels collected in current workers to assess job title exposure (Steenland and Woskie 2012), an increase in kidney cancer deaths was observed in workers with the highest exposures when analyzed with no lag, a 10-year lag, or a 20-year lag. Steenland and Woskie (2012) also found an increase in deaths from mesothelioma; the investigators noted that this was likely due to asbestos exposure. Steenland and Woskie (2012) noted that tetrafluoroethylene, a rodent kidney carcinogen, is used in the manufacture of a variety of fluoropolymers and noted that the tetrafluoroethylene is well controlled due to its volatile and explosive properties. It is noted that in a multisite study of tetrafluoroethylene workers, which included workers at the Washington Works facility (Consonni et al. 2013), an increased risk of renal cancer (SMR 1.44, 95% CI 0.69–2.65) was found, although the CI included unity. Consonni et al. (2013) noted that

88% of the workers were also exposed to PFOA. When PFOA exposure was used as an exposure variable, the findings were the similar as when tetrafluoroethylene was used as the exposure variable, and thus, it was difficult for the investigators to evaluate separate associations for each compound. It is noted that increases in kidney deaths were not observed in the Cottage Grove facility (Raleigh et al. 2014), which did not use tetrafluoroethylene (Chang et al. 2014).

Three studies have examined the community living near the Washington Works facilities; some of these studies also included workers at the facility. Barry et al. (2013) reported an increased risk of testicular cancer that was associated with estimated cumulative PFOA exposure. Vieira et al. (2013) also reported an increase in testicular cancer, but the CIs of the adjusted odds ratio (AOR) included unity. When the participants were grouped by water district, an increased risk of testicular cancer (AOR 5.1, 95% CI 1.6–15.6) was observed in the Little Hocking water district, which had the highest PFOA levels in the water. The Vieira et al. (2013) study also found increased risks of kidney cancer among participants with high or very high exposure to PFOA; Barry et al. (2013) also concluded that there was an association between estimated cumulative PFOA exposure and kidney cancer, although the CIs for the hazard ratio included unity. The third study of the Washington Works community found an inverse association between serum PFOA and risk of colorectal cancer (Innes et al. 2014).

In their review of the available epidemiological data, IARC (2017) concluded that the evidence for testicular cancer was "considered credible and unlikely to be explained by bias and confounding, however, the estimate was based on small numbers." Similarly, IARC (2017) concluded that the evidence for kidney cancer was also credible but noted that chance, bias, and confounding could not be ruled out with reasonable confidence. They considered that there was limited evidence in humans for the carcinogenicity of PFOA.

Laboratory Animal Exposure Studies. Two studies have examined the carcinogenic potential of PFOA in rats. In the first study of male and female Sprague-Dawley rats exposed to PFOA in the diet for 2 years (3M 1983; Butenhoff et al. 2012c), significant increases in the incidence of fibroadenoma of the mammary gland in females and Leydig cell adenoma were found in males exposed to 15 mg/kg/day. A high incidence of pituitary adenoma occurred among all groups, including controls. The incidence of hepatocellular carcinoma was not significantly increased. The investigators noted that the incidence of fibroadenoma in the mammary gland in the 15 mg/kg/day group was similar to the incidence found in untreated aging rats and that the incidence of Leydig cell adenoma was similar to the spontaneous incidence of this tumor in aged rats. The mammary gland pathology slides from this study (3M 1983;

Butenhoff et al. 2012c) study were re-examined in 2005 by a Pathology Working Group (PWG) using current diagnostic criteria (Hardisty et al. 2010). The incidences of fibroadenoma found by the PWG were 36, 44, and 46% in the 0, 1, and 15 mg/kg/day groups, respectively; there were no statistically significant differences between the groups (Hardisty et al. 2010). Additionally, there were no significant differences in the incidence of adenocarcinoma, total benign neoplasms, or total malignant neoplasms between the groups. In the second study of male Sprague-Dawley rats exposed to PFOA in the diet for 2 years (Biegel et al. 2001), an increase in the incidence of hepatocellular adenomas was found, but there were no hepatocellular carcinomas in the treated group. PFOA also increased the incidence of Leydig cell adenomas. In addition, PFOA increased the incidence of pancreatic acinar cell adenomas; a pancreatic carcinoma was observed in one treated rat. Hepatic peroxisome proliferation was increased significantly at all interim evaluation time points (1, 3, 6, 9, 12, 15, 18, and 21 months), but there was no increase in cell proliferation. In Leydig cells, neither peroxisome proliferation nor cell proliferation were increased.

PFOA was a positive modulator of hepatocarcinogenesis in male Wistar rats in a biphasic (initiation with diethylnitrosamine followed by oral treatment with PFOA) or triphasic (initiation with diethylnitrosamine [DEN] followed by dosing with 2-acetylaminofluorene and then PFOA) promotion protocol (Abdellatif et al. 1991, 2004). PFOA induced a marked increase in acylCoA oxidase activity and only a slight increase in catalase activity (Abdellatif et al. 2004). Since PFOA did not significantly increase 8-hydroxy-deoxyguanosine (a marker of oxidative DNA damage *in vivo*) in isolated liver DNA, it appeared that PFOA did not require extensive DNA damage for its promoting activity (Abdellatif et al. 2004). PFOA was also found to act as a promoter in male Wistar rats in an initiation-selection-promotion protocol (Nilsson et al. 1991).

IARC (2017) concluded that there was limited evidence in experimental animals for the carcinogenicity of PFOA.

PFOS

Epidemiological Studies. Four studies have evaluated the carcinogenic potential in workers at a Decatur, Alabama perfluorooctanesulphonyl fluoride (PFOSF) based fluorochemical production facility. In the earliest study, no increase in all cancer deaths was found, as compared to the Alabama general population (Alexander et al. 2003). An increased risk of bladder cancer was observed in workers with high potential exposure and in workers with a high potential exposure for ≥1 year; the mortality ratio was based on three

cases in the high exposure group. In a reanalysis of workers at this facility conducted by Alexander and Olsen (2007), 11 cases of bladder cancer were identified from worker surveys (n=6) and death certificates (n=5). Only two of the six self-reported bladder cancer diagnosis were confirmed via medical records; the other four subjects declined to give consent for medical verification. When compared to incidence data from the National Institute for Occupational Safety and Health (NIOSH) Surveillance Epidemiology and End Results (SEER) referent data, the standardized incidence ratios for the high potential exposure group were elevated, but the CIs included unity. When compared with workers with <1 year of high exposure, workers with 5–<10 and ≥10 years of high exposure had relative risks of 1.92 (95% CI 0.30–12.06) or 1.52 (95% CI 0.21–10.99). Although the study did not adjust for smoking, the investigators noted that 83% of the living bladder cancer cases (five of the six subjects) reported cigarette use, as compared to 56% reported in the noncases. An additional limitation of the study is inclusion of four cases of bladder cancer that were not verified by medical records. The results of this study do not appear to confirm the findings of increased bladder cancer in the mortality study (Alexander et al. 2003). In a subsequent study of this facility, treatment for bladder cancer was not reported among current workers (Olsen et al. 2004a). The study did find increases in the number of episodes of care for malignant neoplasm of the prostate or malignant neoplasms of the colon, as compared to long-term workers in another division, but the CIs included unity. No increases in the risk ratio episodes of care were found for liver, rectum, or respiratory tract (Olsen et al. 2004a). A fourth study of this facility (Grice et al. 2007) examined possible associations between colon cancer, melanoma, and prostate cancer and PFOS exposure. The risks of these cancers were not associated with any of the PFOS-exposure categories for analyses that included all self-reported or only validated cancers.

General population case-control studies have evaluated several cancer types. Innes et al. (2014) reported an inverse association between PFOS and colorectal cancer. A small-scale study of 31 cases by Bonefeld-Jorgensen et al. (2011) found a slight increase in breast cancer risk, a finding not replicated in another larger study of a different population (Bonefeld-Jorgensen et al. 2014). A third case-control study found associations between serum PFOS and breast cancer in subjects with serum PFOS levels in the second tertile and higher (Wielsøe et al. 2017). Eriksen et al. (2009) and Hardell et al. (2014) did not find increases in the risk of prostate cancer associated with serum PFOS. However, an increased risk of prostate cancer was found among subjects with a first-degree relative with prostate cancer and PFOS levels above the median level (Hardell et al. 2014). Eriksen et al. (2009) also found no associations between serum PFOS and the risk of bladder cancer, pancreatic cancer, or liver cancer. Ducatman et al. (2015b) did not find an association between serum PFOS levels and PSA levels in men participating in the C8 studies.

Laboratory Animal Studies. In a 2-year PFOS dietary exposure study bioassay in male and female Sprague-Dawley rats (Butenhoff et al. 2012b; unpublished study by Thomford 2002b), a significant positive trend of hepatocellular adenoma was observed in males; the incidence was significantly higher than controls at 1.04 mg/kg/day. No hepatocellular adenomas were seen in a group of rats exposed to 1.17 mg/kg/day for 1 year and allowed to recover for the second year. High-dose males from the recovery group showed a significant increase in thyroid follicular cell adenoma relative to controls. No significant increase in this type of tumor was observed in rats exposed for 2 years. In females, there was a significant positive trend for incidences of hepatocellular adenoma, which was associated with a significant increase in the 1.04 mg/kg/day group. In females, there were also significant negative trends for mammary adenoma and fibroadenoma carcinoma combined.

PFHxS

Epidemiological Studies. Three case-control studies have examined the possible association between serum PFHxS and cancer. Bonefeld-Jorgensen et al. (2014) found in inverse association between PFHxS levels and breast cancer risk. In contrast, Wielsøe et al. (2017) found a positive association between serum PFHxS levels and breast cancer risk. No association between PFHxS and prostate cancer was observed (Hardell et al. 2014), with the exception of increased risk in men with a first-degree relative with prostate cancer and above-median serum PFHxS levels. No associations between serum PFHxS and PSA levels were observed in a cross-sectional study of men 20–49 or 50–69 years of age participating in the C8 Health Studies (Ducatman et al. 2015b).

PFNA

Epidemiological Studies. The carcinogenic potential of PFNA has been examined in three case-control studies. No consistent associations between serum PFNA levels and breast cancer (Bonefeld-Jorgensen et al. 2014; Wielsøe et al. 2017) or prostate cancer (Hardell et al. 2014) were found. Serum PSA levels were not associated with serum PFNA levels in men participating in the C8 Health Study (Ducatman et al. 2015b).

PFDA

Epidemiological Studies. Hardell et al. (2014) examined the possible association between the serum PFDA level and risk of prostate cancer and only found an association in men with a heredity risk factor and PFDA levels above the median. In a case-control study of breast cancer, Wielsøe et al. (2017) found an association among women with serum PFDA levels in the third quartile.

PFUnA

Epidemiological Studies. An increased risk of prostate cancer was found in men with first-degree relatives with prostate cancer and serum PFUnA levels above the median (Hardell et al. 2014). An increased breast cancer risk was found in women with serum PFUnA levels in the third quartile (Wielsøe et al. 2017).

PFHpA

Epidemiological Studies. One study evaluated possible associations between serum PFHpA and cancer risk and found no association for breast cancer (Wielsøe et al. 2017).

PFDoDA

Epidemiological Studies. In the only cancer study for PFDoDA, Wielsøe et al. (2017) did not find an increased risk of breast cancer in women associated with serum PFDoDA levels.

FOSA

Epidemiological Studies. Bonefeld-Jorgensen et al. (2014) reported an increased risk of breast cancer among women with serum FOSA levels >5.75 ng/mL.

2.20 MECHANISM OF TOXICITY

The primary effects observed in rodents exposed to perfluoroalkyls are liver toxicity, developmental toxicity, and immune toxicity. The cellular mechanisms by which hepatic effects are induced have been extensively studied, while more limited data are available on mechanisms for other effects. The available

data indicate that perfluoroalkyls produce a number of adverse effects through activation of the PPARα, a member of the nuclear receptor superfamily that mediates a broad range of biological responses (Issemann and Green 1990). However, some adverse effects of perfluoroalkyls occur through PPARα-independent mechanisms, which may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of gap junction intercellular communication (GJIC). In the sections below, cellular mechanisms of action that are mediated by PPARα and independent of PPARα are discussed, followed by discussions of mechanisms specific to the hepatic, developmental, immunotoxic, and hormone effects of perfluoroalkyls.

2.20.1 Cellular Mechanisms of Toxicity

PPARα-Dependent Mechanisms

Activation of the PPARα receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver. These events include marked hepatocellular hypertrophy due to an increase in number and size of peroxisomes, a large increase in peroxisomal fatty acid β oxidation, increased cytochrome 450-mediated ω hydroxylation of lauric acid, and alterations in lipid metabolism. Although there is uncertainty regarding the exact and possibly, multiple mechanisms for liver effects of perfluoroalkyls, peroxisome proliferation mediated by PPARα is a contributing mechanism. Proliferation of peroxisomes in laboratory animals exposed to perfluoroalkyls is discussed in Section 2.9 (Hepatic); as discussed in that section, hepatic peroxisome proliferation has been shown in rats exposed to PFOA and in mice exposed to PFDA.

Many, but not all, of the adverse effects induced by perfluoroalkyls in rodents are mediated through activation of the PPARα. Ligands, including perfluoroalkyls, bind to and activate PPARα, causing a conformational change in the receptor that leads to dissociation of co-repressors and enables heterodimerization with the retinoid X receptor (Corton et al. 2014). The activated receptor complex binds to a DNA direct repeat motif (the peroxisome proliferator response element or PPRE) located in the promoters of peroxisome proliferator responsive genes. The binding of the receptor complex leads to recruitment of co-activators, which acetylate histones and remodel chromatin, enabling RNA polymerase to transcribe the target gene(s). PPARα regulates lipid homeostasis by modulating the expression of genes involved in fatty acid uptake, activation, and oxidation. Activation of nuclear receptors including PPARα is a complex, dynamic process that depends on levels of expression of the receptors in different

tissues, competition among receptors for endogenous and exogenous ligands and for binding sites on chromatin, and availability and abundance of co-activators and/or co-repressors (Corton et al. 2014).

PPARα Receptor Activation. Many perfluoroalkyls, including PFOA, PFOS, PFUnA, PFHpA, and PFDoDA have been shown to activate PPARα in mammalian cells *in vitro* (Bjork and Wallace 2009; Bjork et al. 2011; Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). Cell systems used in these studies include COS-1 cells expressing mouse, rat, or human PPARα, and cultured rat, mouse, and human hepatocytes. In these studies, perfluoroalkyl sulfonate compounds were less potent than perfluoroalkyl carboxylate compounds in activating PPARα-induced gene expression, and the potency of stimulation within each class increased with carbon chain length (Bjork and Wallace 2009; Wolf et al. 2008b, 2012). In comparison with naturally occurring long-chain fatty acids such as linoleic and α linoleic acids, PFOA and PFOS are relatively weak ligands for PPARα (Vanden Heuvel et al. 2006)

PPARα-Dependent Gene Expression Changes. Perfluoroalkyls have been shown to induce changes in the expression of genes under the regulation of PPARα. The expression of PPARα target genes in the liver involved in fatty acid metabolism, cell cycle control, peroxisome biogenesis, and proteasome structure and organization were upregulated, while inflammatory response genes in the liver were downregulated in wild-type mice exposed orally to PFOA or the PPARα agonist WY-14,643 (Rosen et al. 2008a). Furthermore, PFOA and PFDA have been shown to downregulate, via PPARα activation, genes involved in bile transport in the livers of wild-type mice exposed by intraperitoneal administration (Cheng and Klaassen 2008a). Both compounds decreased expression of organic anion transporting polypeptides [*OATP1a1*, *1a4,* and *1b2*], and PFDA also downregulated sodium-taurocholate cotransporting polypeptide [*Nctp*], via activation of PPARα. Many of these expression changes may play roles in the hepatic effects of perfluoroalkyls.

Gene expression changes induced by perfluoroalkyls have been extensively studied in experiments aimed at determining the extent to which the adverse effects of these compounds are dependent on activation of PPARα or interaction with other nuclear receptors (Foreman et al. 2009; Rosen et al. 2008a, 2008b, 2010, 2017). These studies, comparing gene expression changes in wild-type and PPARα-null mice exposed to perfluoroalkyls, demonstrate the following:

- A majority of the gene expression changes induced in rodents by perfluoroalkyls tested to date, especially PFOA and PFNA, are dependent on activation of PPARα.

- Perfluoroalkyls also induce gene expression changes that are independent of PPARα.
- The extent to which gene expression changes induced by perfluoroalkyls are dependent on activation of PPARα varies by compound.

Species Differences in PPARα Activation. Species differences in response to PPARα activators have been reviewed by Corton et al. (2014). Studies of PPARα activation by structurally diverse ligands in various species have shown that rats and mice are the most sensitive species to PPARα agonists, whereas guinea pigs, hamsters, nonhuman primates, and humans are less responsive (Corton et al. 2014). However, the differences do not appear to result from differences in the PPARα gene itself: PPARα cDNA from humans is indistinguishable from the rodent PPARα. Species differences in response to exogenous PPARα activators may stem from any or all of the following: (1) differences in the expression of PPARα in a given tissue; (2) differences in the gene product structure; and (3) differences in the ligand-mediated transactivation of PPARα. Experiments quantifying mRNA and/or protein levels of PPARα show ~10-fold higher expression of PPARα in the livers of mice and rats compared with humans and guinea pigs, but available data are limited and require further study to validate these differences (Corton et al. 2014). In humans, variants of PPARα that may affect its transactivation potential have been identified. For example, humans produce higher levels of a truncated PPARα (that lacks a ligand binding domain) compared with mice and rats (Corton et al. 2014). The truncated form appears to inhibit the activity of the full-length receptor, possibly via sequestering critical co-activators. Other, non-truncated variants of PPARα have been identified in humans, but the sensitivity of these variants to PPARα activators does not differ markedly from that of the wild-type receptor.

Species and compound-related differences in PPARα transactivation by perfluoroalkyls have been demonstrated *in vitro* (Shipley et al. 2004; Takacs and Abbott 2007; Vanden Heuvel et al. 2006; Wolf et al. 2008b, 2012). In a comparison of human and mouse PPARα transactivation by different perfluoroalkyls in transfected COS-1 cells, Wolf et al. (2008b, 2012; see Table 2-28) found that some perfluoroalkyls exhibited marked species differences in transactivation potency (for example, PFUnA, PFDA, PFDoDA), while other compounds showed similar transactivation potency for both human and mouse PPARα (for example, PFNA, PFOA, perfluoropentanoic acid [PFPeA]).

Table 2-28. Transactivation of Human and Mouse PPARα in Transfected Cos-1 Cells Exposed to Perfluoroalkyls (In Order of Decreasing C_{20max} in the Mouse)

		Human		Mouse		C_{20max} (μM)[a]	
Perfluoroalkyl	Carbon number	NOEC (μM)	LOEC (μM)	NOEC (μM)	LOEC (μM)	Human	Mouse
PFNA	9	1	5	1	5	11	5
PFOA[b]	8	5, 0.5	10,1	0.5, 1	1, 3	7	6
PFUnA	11	50	75	5	10	86	8
PFHpA	7	<0.5	0.5	3	5	15	11
PFDA	10	100	>100[c]	<5	5	–	20
PFDoDA	12	75	90	3	5	NA	33
PFPeA	5	0.5	1	1	5	52	45
PFHxA	6	5	10	10	20	86	45
PFBA	4	30	40	30	40	75	51
PFHxS	6	5	10	10	20	81	76
PFOS	9	20	30	60	90	262	94
PFBS	4	20	30	120	150	206	317

[a]Perfluoroalkyl concentration yielding 20% of maximum response given by the most active compound (PFNA).
[b]Results from two separate experiments.
[c]Slope for human PPARα dose-response line was not significant.

– = not active; LOEC = lowest-observed-effect concentration; NA = not available; NOEC = no-observed-effect concentration; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFPeA = perfluoropentanoic acid; PFUnA = perfluoroundecanoic acid; PPAR = peroxisome proliferator activated receptor

Source: Wolf et al. 2008b, 2012

In addition to differences in transactivation of PPARα, Corton et al. (2014) noted that there are species differences in the transcripts controlled by PPARα. While PPARα activation leads to hypolipidemic changes in both humans and laboratory rodents, the gene sets responsible for these changes may differ. In a comparison between human and mouse hepatocytes exposed to the prototypical PPARα ligand WY-14,643, some genes (ACOX1, ECH1, PEX11A, and ACAA1) were induced in both species, while some (*Ehhadh*, *Pxmp4*, *Acot4*, and *Peci*) were induced only in mouse hepatocytes (Corton et al. 2014). Importantly, PPARα activators induce large increases in the expression of fatty acyl-CoA oxidase (ACO, which is believed to play a role in oxidative stress-induced liver cancer) in rodent hepatocytes, but relatively weak increases in human hepatocytes (Corton et al. 2014). Other hypothesized explanations for the species difference in response to exogenous PPARα ligands include variations in the structure of the

PPRE that alter the response of the human genes compared with rodents; differences between humans and rodents in the functions of genes under the regulation of PPARα; and differences in the ability of ligand-bound human and mouse PPARα receptor complex to recruit or interact with co-activators (Corton et al. 2014).

PPARα-Independent or Associative Mechanisms

Experiments using PPARα-null mice have demonstrated that perfluoroalkyls exert some adverse effects, including developmental and hepatic effects, through mechanisms other than activation of PPARα. These may include activation of other nuclear receptors, increased oxidative stress, dysregulation of mitochondrial function, and inhibition of GJIC. While some of these effects have been seen after exposure to PPARα activators (Corton et al. 2014), these mechanisms may also occur independent of PPARα activation.

Activation of Other Nuclear Receptors. Examination of gene expression changes, as well as studies using other knock-out mice, have shown that some of the PPARα-independent effects induced by perfluoroalkyls may be mediated by activation of other nuclear receptors, especially PPARγ, CAR, and ERα. In a series of experiments, Rosen et al. (2008b, 2010, 2017) compared the gene expression changes induced by perfluoroalkyls in wild-type and PPARα -null mice with gene expression changes induced by known agonists of PPARγ, CAR, and ERα. Using these data, the study authors estimated the percentage of gene expression changes that were independent of activation of PPARα, and identified other nuclear receptors potentially involved in the changes induced by the perfluoroalkyls. The results, summarized in Table 2-29, show that between 10 and 24% of gene expression changes induced by perfluoroalkyls are independent of PPARα. All four compounds tested (PFOA, PFOS, PFNA, and PFHxS) were shown to alter the expression of PPARγ- and CAR-regulated genes in PPARα-null mice, and PFNA and PFHxS also altered the expression of ERα-regulated genes in the knock-out mice. In contrast, none of the compounds altered the expression of genes commonly affected by an agonist of LXR in either wild-type or null mice.

Table 2-29. Gene Expression Changes Induced by Perfluoroalkyls

	Dose (mg/kg/day for 7 days)	% PPARα-independent gene changes	Gene expression changes similar to those induced by prototypical agonist							
			PPARγ		CAR		ERα		LXR	
			WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	~14	+	+	+	+	+	–	–	–
PFOS	10	~16	+	+	+	+	+	–	–	–
PFNA	1	~10	+	+/–	+	–	+	–	–	–
	3	~17	+	+	+	+	+	+	–	–
PFHxS	3	24	+	–	+	–	+	+	–	–
	10	22	+	+	+	+	+	+	–	–
WY-14,643[a]	0.1% in diet	2	+	–	–	–	+	–	–	–

[a]WY-14,643 is a PPARα agonist.

+ = significant ($p<0.0001$) similarity to gene expression changes induced by prototypical receptor agonist as assessed by running Fisher test; +/– = equivocal evidence; CAR = constitutive androstane receptor; ER = estrogen receptor; LXR = liver X receptor; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild-type

Sources: Rosen et al. 2017, 2008b, 2013; Wolf et al. 2008b

Gene expression changes typical of CAR and PXR activators (phenobarbital and pregnenolone 16 α-carbonitrile [PCN]) were also observed in rat liver after oral exposure to PFOA and PFOS (Ren et al. 2009). In addition, PFDA was shown to activate CAR-dependent genes in a study comparing wild-type and CAR-null mice exposed by intraperitoneal injection (Cheng and Klaassen 2008b).

These data suggest that perfluoroalkyls may induce gene expression changes through activation of other nuclear receptors including PPARγ, CAR, and ERα. Support for these findings are available from *in vitro* studies demonstrating binding and/or transactivation of PPARγ, CAR, and ERα by perfluoroalkyls. Both PFOA and PFOS activated PPARγ in cultured human, mouse, and rat hepatocytes, albeit with much lower potency than the known agonist rosiglitazone; neither LXRβ nor RXRα was activated in this system (Vanden Heuvel et al. 2006). Zhang et al. (2014) observed binding of PFOA and PFOS to human PPARγ in transfected *Escherichia coli*. However, in experiments conducted by Takacs and Abbott (2007), neither PFOA nor PFOS activated the mouse or human PPARγ.

Oxidative Stress. Perfluoroalkyls increase oxidative stress in the liver, kidney, and brain. Increases in oxidative stress may be mediated in part via PPARα activation, but may also result from activation of the Nrf2 receptor (Xu et al. 2016).

Oxidative stress may contribute to oxidative DNA damage, tumor promotion, perturbation of lipid homeostasis, and stimulation of inflammation, among other changes; thus, increases in oxidative stress can have diverse physiological effects. Evidence that perfluoroalkyls increase oxidative stress is available from *in vivo* and *in vitro* studies. For example, oxidative DNA damage (measured as 8-OH-dG levels) was significantly increased in the liver, but not the kidneys, of male rats exposed to PFOA via feed for 2 weeks (Takagi et al. 1991). In HepG2 cells cultured with PFOA or PFOS, significant increases in reactive oxygen species (ROS) (measured as 2'7'-dichlorofluorescein diacetate fluorescence) were observed, but there was no evidence of DNA damage measured with the comet assay (Eriksen et al. 2010). In this system, PFNA, PFBS, and PFHxA did not induce ROS production, but a significant increase in DNA damage was seen in cells exposed to PFNA (Eriksen et al. 2010).

In male, but not female, KM mouse pups administered a single subcutaneous injection of PFOS at 1, 2, 3, 4, or 5 weeks of age, brain total antioxidant capacity (T-AOC) was lower than controls at most time points, and significantly decreased after treatment on PND 21 (Liu et al. 2009). In the liver, T-AOC was decreased in male pups treated on PNDs 7 and 14, and in females treated on PND 21. Significant decreases in superoxide dismutase (SOD) activity were noted in the brain of males treated on PNDs 7 and 21, and in the liver of females treated on PND 14.

Increases in oxidative stress can lead to NFκB activation (Corton et al. 2014). NFκB activation plays a role in tumorigenesis, and NFκB transcription factors coordinate immune responses. Few studies have examined NFκB activation after exposure to perfluoroalkyls. An increase in NFκB mRNA level was seen in the hippocampus of neonatal rats exposed to PFOS *in utero* (Zeng et al. 2011). In addition, NFκB nuclear translocation was accelerated, and NFκB was activated, in breast cancer cells exposed to PFOA (Zhang et al. 2014). The activation of NFκB was associated with increased invasiveness of the breast cancer cells, as coexposure to an inhibitor of NFκB reduced the invasiveness induced by PFOA.

Gap Junction Intercellular Communication (GJIC) Inhibition. Perfluoroalkyls also have been shown to inhibit GJIC both *in vivo* and *in vitro* in rats (Corton et al. 2014). GJIC plays an important role in maintenance of tissue homeostasis, intercellular transmission of regulatory signals, and metabolic cooperation. Disruption of GJIC is thought to be involved in neurological, reproductive, and endocrine

abnormalities, as well as in carcinogenesis (Corton et al. 2014; EPA 2016h). There are limited data examining the effects of perfluoroalkyls on GJIC. The available studies showed that both PFOA and PFOS inhibited GJIC in the livers of rats exposed via diet for 1 week or 3 or 21 days, respectively (Hu et al. 2002; Upham et al. 1998, 2009). *In vitro* studies in WB-344 rat liver epithelial cells also showed inhibition of GJIC after exposure to PFOS (Hu et al. 2002) and to perfluorinated fatty acids with 7–10 carbons (Upham et al. 1998, 2009). In this system, PFOA activated extracellular receptor kinase, which may play a role in the inhibition of GJIC. In addition, inhibition of phosphatidylcholine-specific phospholipase C partially mitigated the GJIC inhibition, suggesting that PFOA-induced activation of this enzyme may also be involved in GJIC inhibition (Upham et al. 1998, 2009).

PFOS was also shown to inhibit GJIC in dolphin kidney epithelial cells and rat Sertoli cells *in vitro* (Hu et al. 2002; Wan et al. 2014a). In Sertoli cells, GJIC plays an important role in maintenance of the blood:testes barrier and in intercellular communication during spermatogenesis (EPA 2016i).

Impaired Mitochondrial Function. Mitochondrial function, including cellular respiration as well as mitochondrial membrane potential, has been shown to be perturbed by perfluoroalkyls. Available data suggest that PFOA and PFOS are relatively weak mitochondrial toxicants (EPA 2016h, 2016i). Mitochondrial proliferation was observed in rats exposed orally to PFOA for 28 days and in mice exposed to PFOA during gestation and lactation (Quist et al. 2015a, 2015b; Waters et al. 2009). In isolated rat liver mitochondria, higher concentrations of either PFOA or PFOS were noted to slightly increase resting respiration rate and decrease membrane potential, possibly due to these compounds' effects on membrane fluidity (Starkov and Wallace 2002). Testing of other perfluoroalkyls for effects on mitochondrial respiration rate and oxidative phosphorylation showed a wide range of inhibitory activities, with PFOS demonstrating the highest potency (3-fold higher than PFOA and 20–30-fold higher than PFBS and PFHxA) (Wallace et al. 2013).

2.20.2 Hepatic Toxicity Mechanisms

Hepatic effects of perfluoroalkyls in rodents likely result from a combination of PPARα-dependent and independent changes; see Table 2-30. For example, increased liver weight has been observed in both wild-type and PPARα-null mice orally exposed to PFOA or APFO (Nakagawa et al. 2012; Rosen et al. 2008a), PFOS (Qazi et al. 2009b; Rosen et al. 2010), PFNA (Das et al. 2017; Rosen et al. 2017), or PFHxS (Das et al. 2017; Rosen et al. 2017), but not in null mice exposed to PFBA by intraperitoneal injection (Foreman et al. 2009). Similarly, both wild-type and PPARα-null mice exposed to APFO

exhibited increased hepatocyte vacuolation and proliferation, while exposure to WY-14,643 did not induce such changes in the null mice (Wolf et al. 2008b). Das et al. (2017) showed that PFOA, PFNA, and PFHxS also increased hepatocyte cell size, percent lipid, and hepatic triglyceride levels, and decreased hepatic DNA content, in both wild-type and PPARα-null mice, while WY-14,643 did not, indicating that these effects were not dependent on PPARα activation. Similarly, Nakagawa et al. (2012) showed that at a lower APFO dose (1.0 mg/kg/day for 6 weeks), increases in hepatic triglyceride levels were observed in wild-type, PPARα-null, and humanized PPAR (hPPAR) mouse strains; however, at a higher dose (5 mg/kg/day), hepatic triglyceride levels were still increased in PPARα-null and hPPAR mice, but decreased in wild-type mice.

Table 2-30. Hepatic Effects of Perfluoroalkyls in Wild-Type and PPARα-Null Mice Exposed Orally

		↑ Relative liver weight		↑ % Lipid by cell area		↑ Hepatic triglycerides		↑ Hepatocyte cell size		↓ Hepatic DNA content	
	Dose (mg/kg/day)	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null	WT	PPARα-null
PFOA	3	+++	+++	+++	-	+++	-	+++	+++	+	+
PFOS	10	++	++	ND							
PFNA	1	++	+	ND							
	3	+++	++								
	10	+++	+++	+++	+++	+++	+++	+++	++	+	+
PFHxS	3	+	+	ND							
	10	+++	+++	+++	+++	+++	–	+++	+++	+	+
WY-14,643[a]	50	+++	–	+++	–	–	–	+++	–	+	–

[a]WY-14,643 is a PPARα agonist.

+ = statistically significant change from control (the number of plus signs indicates degree of change from controls); – = not statistically significantly different from control; DNA = deoxyribonucleic acid; ND = no data; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PPAR = peroxisome proliferator activated receptor; WT = wild type

Sources: Das et al. 2017; Rosen et al. 2008a, 2010, 2017

Lipid homeostasis is maintained through a balance between fatty acid synthesis or accumulation and fatty acid oxidation. Available data indicate that perfluoroalkyls affect both sides of this balance, but a growing body of evidence indicates that fatty acid accumulation induced by perfluoroalkyls tips the balance in favor of hepatic steatosis (Das et al. 2017). As discussed above, perfluoroalkyls alter lipid homeostasis via PPARα activation, which upregulates genes involved in fatty acid oxidation and reduces lipid levels. However, as noted above, Das et al. (2017) indicate that perfluoroalkyls also perturb lipid homeostasis via PPARα-independent mechanisms. In addition to the effects noted in Table 2-30, increased incidences of hepatic steatosis were seen in PPARα-null mice exposed to perfluoroalkyls (Das

et al. 2017; Minata et al. 2010; Nakagawa et al. 2012), but not in those exposed to the PPARα agonist WY-14,643 (Das et al. 2017). Additionally, microvesicular steatosis was observed in hPPAR mice (Nakagawa et al. 2012). The findings are consistent with earlier studies showing triglyceride accumulation in rodent livers after exposure to perfluoroalkyls (Kudo and Kawashima 1997, 2003; Kudo et al. 1999); hepatic steatosis and glucose intolerance in adult rats exposed to PFOS during the prenatal and postnatal periods (Lv et al. 2013); and inhibited hepatic secretion of VLDL, resulting in steatosis, in APOE3-Leiden mice (a rodent model with lipoprotein metabolism similar to humans) exposed to PFOS or PFHxS (Bijland et al. 2011).

Das et al. (2017) investigated whether the steatosis induced by PFOA, PFNA, and PFHxS was mediated by increased fatty acid or triglyceride synthesis or by inhibition of mitochondrial fatty acid transport or β-oxidation. Microarray analysis of mouse liver after exposure to these compounds showed upregulation of genes involved in fatty acid and triglyceride synthesis in both wild-type and PPARα-null mice. In contrast, *in vitro* experiments demonstrated that these perfluoroalkyls did not affect mitochondrial fatty acid oxidation in isolated rat liver mitochondria, and neither PFOA nor PFOS altered fatty acid oxidation in HepG2/C3A human liver cells. The authors suggested that perfluoroalkyls induce hepatic steatosis by perturbing lipid homeostasis in favor of the accumulation of fatty acids and triglycerides in the liver.

Data are also available to suggest that proinflammatory cytokines may also contribute to the hepatotoxicity of perfluoroalkyls. Studies in rodents have shown that *in vivo* exposure to PFOA (Qazi e al. 2013; Yang et al. 2014) or PFNA (Fang et al. 2012b, 2012c) have resulted in increases in IL-6, IL-1β, tumor necrosis factor-α (TNFα), C-reactive protein, and COX-2 at higher perfluoroalkyl doses (Fang et al. 2012b, 2012c; Yang et al. 2014) and decreases in TNFα, interferon-γ (IFN-γ), IL-4, and IL-6 levels at lower doses (Fang et al. 2012b; Qazi et al. 2013). Exposure to PFNA also resulted in increased expression of TNFα, IL-1β, and IL-6 mRNA (Fang et al. 2012b). Nakagawa et al. (2012) found increases in TNFα-mRNA in wild-type (2.9-fold), PPARα-null (1.9-fold), and humanized PPARα (1.9-fold) mouse strains exposed to 5 mg/kg/day doses of PFOA. Fang et al. (2012c) suggested that PFNA exposure stimulated liver Kupffer cells to release large amounts of TNFα and IL-1β and that the release of these cytokines activated the NFκB p65 pathway causing suppression of PPARα promoter activity and resulting in increases in liver triglyceride levels and steatosis.

2.20.3 Developmental Toxicity Mechanisms

Developmental effects observed in laboratory rodents exposed to perfluoroalkyls include prenatal loss, reduced neonate weight and viability, neurodevelopment toxicity, and delays in mammary gland differentiation, eye opening, vaginal opening, and first estrus (see Section 2.17 Developmental). During development, PPARα, PPARβ, and PPARγ mRNA and protein are expressed in the embryo of rodents and humans (Abbott 2009; Abbott et al. 2010). In humans, the fetal expression levels are equivalent to levels in adult tissues (Abbott et al. 2010). PPARα activation also appears to be involved in some, but not all, of the developmental effects of perfluoroalkyls in mice, and the role of PPARα in mediating developmental toxicity differs among the various compounds. For example, a gestational exposure study of PFOA resulted in decreases in postnatal survival in wild-type mice, but not in PPARα-null mice, while the occurrence of full-litter resorptions was similar between the two genotypes (Abbott 2009; Abbott et al. 2007). In contrast, gestational exposure to PFOS resulted in decreased pup survival in both wild-type and PPARα-null mice (Abbot et al. 2009). The developmental effects of PFNA, including reduced pup survival and body weight and delayed eye opening, were seen only in wild-type, and not in PPARα-null mice; however, maternal pregnancy rate was affected only in the null mice (Wolf et al. 2010). No alterations in postnatal survival or growth were observed in wild-type mice exposed to PFBA *in utero* (Das et al. (2008). The investigators suggested that the contrast of these findings to that of PFOA may be due to the shorter half-life of PFBA (daily administration did not result in reaching steady-state) and that PFBA is a less potent agonist of PPARα than PFOA.

Abbott et al. (2012) showed that PFOA altered expression of genes that are involved in homeostatic control of lipids and glucose, and postulated that decreased neonatal survival and body weights may be, in part, due to metabolic disruption. It has been suggested that PFOS interacts with pulmonary surfactants, and that this effect is responsible for neonatal mortality seen in rats. Grasty et al. (2003, 2005) showed that neonatal mortality in PFOS-exposed rats was highest when exposure occurred during the gestational period of lung maturation (GDs 17–20), and that the morphometry of the lungs in exposed neonates was consistent with immaturity. However, treatment of neonates with rescue agents that hasten lung maturation did not prevent neonatal mortality induced by PFOS, and examination of the pulmonary surfactant profile in exposed animals showed no difference from controls, leading Grasty et al. (2005) to conclude that neonatal mortality in neonatal rats exposed to PFOS was not due to immaturity. Other hypotheses pertaining to the mechanisms of developmental toxicity of perfluoroalkyls were not located. However, other molecular- and cellular-level effects of perfluoroalkyls, including increased oxidative

stress, dysregulation of mitochondrial function, and receptor-mediated events, may be involved in the observed developmental effects of these compounds.

2.20.4 Immunotoxicity Mechanisms

NTP (2016b) conducted a systematic review of the human, animal, and *in vitro* data examining immunotoxic effects of PFOA and PFOS. The conclusion of the systematic review was that both PFOA and PFOS are "presumed to be immune hazards to humans." Evidence was considered strong that both compounds were associated with suppression of the antibody response, while there was weaker evidence for PFOA-induced impairment of infectious disease resistance, increased hypersensitivity-related outcomes, and increased autoimmune disease incidence, and for PFOS-induced suppression of natural killer cell activity. A recent study comparing the T-cell dependent antibody response (TDAR) in female wild-type and PPARα knock-out mice after exposure to PFOA with or without antigen exposure showed that PFOA suppressed TDAR in both wild-type and knock-out mice, indicating that the mechanism for antibody response suppression is independent of PPARα activation (DeWitt et al. 2016). These investigators observed no treatment-related changes in splenic lymphocyte subpopulations in exposed mice of either genotype, suggesting that PFOA suppressed TDAR via impairment of B-cell/plasma cell function rather than by altering lymphocyte numbers. DeWitt et al. (2012) and Corsini et al. (2014) reviewed mechanistic data for perfluoroalkyl-induced suppression of antibody response, and postulated that perfluoroalkyls may modulate cell-signaling responses critical to antibody production, including c-Jun, NF-κB, and IL-6.

2.20.5 Endocrine Mechanisms

Perfluoroalkyls have been shown to induce alterations in thyroid hormone levels in rats, and associations between serum perfluoroalkyl concentrations and thyroid hormone levels have been reported in human epidemiological studies (see Section 2.13). Few data examining mechanisms of thyroid hormone disruption are available, but suggest that effects of perfluoroalkyls on thyroid function may be mediated by binding to the thyroid hormone receptor, and/or by altering expression of genes involved in thyroid function or thyroid hormone regulation. Several perfluoroalkyls were shown to bind to the human thyroid hormone receptor in cultured GH2 cancer cells and in molecular docking experiments (Ren et al. 2015). In the *in vitro* tests, all 16 of the tested compounds exhibited lower affinity for the receptor than T3 (Ren et al. 2015). Among the tested compounds, PFOS exhibited the strongest agonist activity (Ren et al. 2015). Alterations in the mRNA or protein levels of thyroid-regulating genes have been observed after oral exposure of male Sprague-Dawley rats to PFOS. PFOS exposure for 5 or 90 days resulted in

decreased hepatic levels of mRNA type 1 deiodinase (DIO1, which bioactivates T3 by deiodination of T4) (Martin et al. 2007; Yu et al. 2009a); after 5 days of exposure, hepatic mRNA for type 3 deiodinase (DIO3, which inactivates T3) was increased relative to controls (Martin et al. 2007). After 90 days, hepatic levels of uridine diphosphoglucuronosyl transferase 1A1 (UGT1A1, which plays a role in T4 turnover) mRNA and thyroid levels of DOI1 protein were increased, while there were no changes in thyroid levels of the sodium iodide symporter, thyrotropin (THS) receptor, or activity of thyroid peroxidase (Yu et al. 2009a).

Limited data from *in vitro* studies suggest the possibility that perfluoroalkyls may interact with the estrogen and androgen receptors. PFOA, PFOS, PFHxS, PFNA, and PFDA were all shown to be antagonists of the androgen receptor, while PFOA, PFOS, and PFHxS induced transactivation of the estrogen receptor (Kjeldsen and Bonefeld-Jorgensen 2013). Recently, analysis of gene expression data from the livers of wild-type and PPARα-null mice exposed to PFOA, PFOS, PFHxS, and PFNA by gavage for 7 days indicated similarities to gene expression changes induced by known ERα agonists (Rosen et al. 2017), providing indirect evidence for perfluoroalkyl changes in the liver mediated via ER activation. However, at oral doses up to 1 mg/kg, PFOA failed to induce treatment-related alterations in uterine weight, ER-dependent gene expression, or morphology of reproductive organs in uterotrophic assays using immature CD-1 mice (Dixon et al. 2012; Yao et al. 2014), suggesting that PFOA is either inactive *in vivo* or of very low estrogenic potency.

2.20.6 Cancer Mechanisms

PFOA induced hepatocellular adenomas, Leydig cell adenomas, and pancreatic acinar cell adenomas in rats (Biegel et al. 2001). Liver tumors induced by PFOA are believed to be mediated largely through PPARα activation, and considered to be of limited or no relevance to humans (EPA 2016h), based on species differences in response to PPARα (see details above under PPARα activation). An expert panel convened by EPA's Science Advisory Board in 2006 to review issues related to the toxicity of PFOA agreed that the weight of evidence supports the hypothesis that induction of liver tumors in rats by PFOA is mediated by a PPARα agonism mode of action (EPA 2006); this conclusion is also reflected in the EPA Health Effects Support Document for PFOA (EPA 2016h). A recent review by a panel of experts from academia, government, industry, and consulting groups updated the Klaunig et al. (2003) assessment of PPARα agonism as a liver cancer mode of action, and drew the same conclusion: while the PPARα mode of action for liver tumors is biologically plausible, species differences in response to PPARα activation indicate that liver tumors are unlikely to be induced by PPARα induction in humans (Corton et al. 2014).

Studies conducted in rainbow trout, an animal model that is similar to humans in terms of insensitivity to peroxisome proliferators, suggest that some perfluoroalkyls may induce liver cancer by alternate mechanisms (Benninghoff et al. 2011, 2012). The investigators (Benninghoff et al. 2011) found that PFOA, PFNA, PFDA, and PFUnA were potent inducers of vitellogenin, an estrogen-responsive biomarker protein at fairly high doses. Neither PFOA nor PFDA exposure resulted in vitellogenin expression at serum levels corresponding to general population serum levels of 2–7 ng/L. *In vitro*, PFOA, PFOS, PFHpA, PFNA, PFUnA, and PFDA also had weak to very weak affinities for estrogen receptors (ERα) for several species including humans, mice, and rats (Benninghoff et al. 2011). *In vivo* studies demonstrated that PFOA, PFOS, PFNA, and PFDA enhanced liver carcinogenesis in AFB_1 initiated fish via a mechanism that likely involves interactions with hepatic estrogen receptors (Benninghoff et al. 2012).

Although Leydig cell tumors are commonly induced by peroxisome proliferating agents such as perfluoroalkyls, the mode of action by which these tumors are induced, and thus their relevance to humans, is much less clear (Corton et al. 2014; EPA 2016h; Klaunig et al. 2003). One mode of action proposed for the induction of Leydig cell tumors involves PFOA-induced inhibition of testosterone biosynthesis, leading to increased production of gonadotropin releasing hormone and circulating LH, which promotes Leydig cell proliferation. Activation of PPARα may be involved in the decreased serum testosterone levels; PPARα-null mice did not exhibit the reduction in testosterone concentration seen in wild-type mice exposed to PFOA (Li et al. 2011). Evidence of decreased serum testosterone and increased serum estradiol was seen in studies of male rats exposed orally to PFOA for 14 days (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). Reduced testosterone levels may occur through the conversion of testosterone to estradiol via the enzyme aromatase. Hepatic aromatase activity was shown to be markedly increased in male rats exposed to APFO by gavage for 14 days, and aromatase activity was positively correlated with serum estradiol levels in these animals (Liu et al. 1996). The relevance of Leydig cell tumors induced by PFOA to human risk assessment is uncertain. For example, an intermediate-duration study in Cynomolgus monkeys exposed to PFOA did not find treatment-related alterations in serum estradiol, estrone, estriol, or testosterone (Butenhoff et al. 2002). Studies of humans occupationally exposed to PFOA have not consistently reported alterations in estradiol or testosterone levels (Klaunig et al. 2012). In addition, humans are less sensitive than rats to LH stimulation, and the average number of LH receptors per Leydig cell is 13-fold higher in rats than humans (Klaunig et al. 2012). In summary, the induction of Leydig cell tumors by PFOA may be mediated by effects on aromatase activity or testosterone biosynthesis, both of which may be related to PPARα activation (EPA

2016h). While the relevance of the PPARα mode of action to humans is uncertain, the data supporting this mode of action for Leydig cell tumors is not sufficient to rule out human relevance (EPA 2016h).

The mechanism of PFOA-induced pancreatic acinar cell tumors in rodents has not been elucidated, and relevant data are limited. A proposed mode of action involves stimulation of PPARα leading to reduced bile flow and/or changes in bile acid composition with subsequent increase in cholecystokinin (CCK), which stimulates pancreatic cell proliferation and tumor formation (EPA 2016h). Effects on bile acid composition induced by PFOA may be mediated by effects on bile acid transporters. PFOA exposure has been shown to decrease expression of OATPs and increase expression of MRP3 and MRP4 (Cheng and Klassen 2008a; Maher et al. 2008). In a study using wild-type and PPARα-null mice, increased biliary excretion of PFOA was seen in wild-type mice compared with null mice, and biliary excretion of bile acids was highest in the null mice (Minata et al. 2010). These observations suggest the possibility that increased excretion of PFOA could diminish the excretion of bile acids that require the same transporters. However, given the limitations in available data, information is insufficient to fully characterize the mode of action for PFOA-induced pancreatic tumors (EPA 2016e).

Mechanisms of carcinogenicity of PFOA are unknown. Liver and Leydig cell tumors produced by PFOS may be associated with PPARα activation or may involve other mechanisms. PFOS activates PPARβ/δ, γ, and CAR and PXR (Ren et al. 2009).

2.21 GENOTOXICITY

The genotoxicity of perfluoroalkyls has not been extensively studied, with the most information available for PFOA and PFOS. To supplement the information reported in the published literature, results of unpublished studies taken from publicly available reviews have been included in the following discussions. No studies of genotoxicity in humans exposed to perfluoroalkyls were located.

PFOA

The genotoxicity of PFOA has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. In general, results show that PFOA can produce DNA damage, but is not mutagenic at noncytotoxic concentrations.

Results of *in vitro* studies in bacteria show that PFOA induces DNA damage but is not mutagenic. DNA damage was observed in *Paramecium caudatum* following exposure to 100 μM for 12 and 24 hours (Kawamoto et al. 2010). Intracellular ROS was significantly increased but DNA damage was not reversed by the application of glutathione, a ROS inhibitor, indicating that intracellular ROS may not be the cause of PFOA-induced DNA damage. PFOA was not mutagenic in *Salmonella typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007) or in *S. typhimurium* TA98, TA100, TA102, and TA104 strains with or without metabolic activation using an Ames assay (Fernández Freire et al. 2008). Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA showing negative results in reverse mutation assays using *S. typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538), *Saccharomyces cerevisiae*, and *Escherichia coli* (WP2uvrA strain) with or without metabolic activation.

In vitro genotoxicity assays in mammalian cells show that PFOA induced DNA damage, although conflicting results have been reported for mutagenicity and increased micronuclei formation. Incubation of human hepatoma HepG2 cells with 50–400 μM PFOA caused DNA strand breaks and 100–400 μM increased the incidence of micronuclei, in a dose-related manner in both cases (Yao and Zhong 2005). These effects were accompanied by a significant increase in ROS, which the investigators suggested caused the DNA damage. Bjork and Wallace (2009) measured mRNA expression for DNA damage inducible *Ddit3* to assess DNA damage in primary rat and human hepatocyte cultures and in HepG2/C3a hepatoma cells. Significant increases in mRNA transcription for *Ddit3* were found in primary rat hepatocytes at 100 μM PFOA and in primary human hepatocytes and HepG2/C3a hepatoma cells at 200 μM PFOA. Although both studies provide evidence of DNA damage, the tested concentrations were very high as compared to what could be expected to occur in the environment. A significant increase in mutation frequencies was observed in hamster-human hybrid cells exposed to 200 μM PFOA for 1–16 days; a 79% decrease in cell viability was also observed at this concentration (Zhao et al. 2011). Concurrent treatment with a ROS inhibitor significantly decreased mutations, indicating that ROS may play an important role in mediating the genotoxic effects of PFOA. In contrast, Butenhoff et al. (2014) and Kennedy et al. (2004) summarized the results of various unpublished mutagenicity studies with PFOA. In mammalian cells, PFOA was negative for forward mutations using Chinese hamster ovary cells, for chromosomal aberrations in Chinese hamster ovary cells and human lymphocytes, and for cell transformation in C3H 10T1/2 cells.

Results of *in vivo* exposure of laboratory animals show that PFOA induced DNA damage, but not micronuclei formation. Administration of a single intraperitoneal injection of 100 mg/kg PFOA to male Fischer-344 rats resulted in a significant increase in the levels of 8-hydroxydeoxyguanosine (a marker of oxidative DNA damage) in liver DNA, but not in kidney DNA (Takagi et al. 1991). Oral administration of approximately 20 mg/kg/day PFOA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). Unpublished studies summarized by Butenhoff et al. (2014) and Kennedy et al. (2004) did not find increased micronuclei formation in mice orally exposed to PFOA.

PFOS

The genotoxicity of PFOS has been examined in bacterial and mammalian *in vitro* systems and in mammalian *in vivo* assays. However, compared to PFOA, less information is available. Results do not provide evidence for genotoxicity of PFOS, except for one *in vitro* study showing cell transformation and one report of increased micronuclei formation following *in vivo* exposure.

Results of *in vitro* studies in bacteria and mammalian cells show that PFOS did not induce DNA damage, mutagenicity or chromosome damage. In bacterial cell assays, as reviewed by OECD (2002), PFOS did not induce reverse mutations in *S. typhimurium* or *E. coli* with or without metabolic activation. A study published after this review also found that PFOS was not mutagenic in *S. typhimurium* TA1535/pSK1002 (*hisG46, rfa, uvrB*) with or without metabolic activation using the *umu* test (Oda et al. 2007).

In vitro genotoxicity assays of PFOS in mammalian cells were negative for DNA damage, mutagenicity, micronuclei formation, and chromosome damage, although one *in vitro* study reported cell transformation. PFOS did not result in DNA damage in Syrian hamster embryo cells at concentrations up to 50 µg/mL but did induce cell transformation at noncytotoxic concentrations (0.2 and 2 µg/mL) following 5 and 24 hours of exposure (Jacquet et al. 2012). Similarly, PFOS did not induce DNA damage or increased micronuclei formation in human hepatoma HepG2 cells following a 24-hour exposure to PFOS concentrations as high as 600 µM; cytotoxicity was observed at ≥300 µM (Florentin et al. 2011). Another study of with HepG2 cells did not find evidence of DNA damage at concentrations of 100 and 400 µM PFOS (Eriksen et al. 2010). As summarized by OECD (2002), PFOS did not induce chromosomal aberrations in human lymphocytes with or without metabolic activation and did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes.

Conflicting results have been reported on micronuclei formation following *in vivo* exposure to PFOS. Micronuclei frequency was increased and the ratio of polychromatic erythrocytes to normochromatic erythrocytes was decreased in bone marrow of rats following oral exposure to 0.6–2.5 mg/kg PFOS for 30 days (Celik et al. 2013; Eke and Celik 2016). As summarized by OECD (2002), PFOS did not induce micronuclei in the bone marrow of CD-1 mice in an *in vivo* assay.

Other Perfluoroalkyls

Little information is available on the genotoxicity of other perfluoroalkyl compounds, with available studies focused on DNA damage. No DNA damage was found in HepG2 cells incubated with 100 or 400 μM PFHxS or PFBS for 24 hours, although a "modest" increase in DNA damage was observed at 400 μM PFNA, a cytotoxic concentration (Eriksen et al. 2010). Oral administration of approximately 10 mg/kg/day PFDA in the diet for 2 weeks to male Fischer-344 rats induced hepatomegaly and also increased the levels of 8-hydroxydeoxyguanosine in liver DNA but not in kidney DNA (Takagi et al. 1991). In contrast, no DNA damage in liver or kidney was observed following administration of a single intraperitoneal injection of 100 mg/kg PFBA to male Fischer-344 rats (Takagi et al. 1991).

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Toxicokinetic data on perfluoroalkyls examined in this profile are available from studies in humans and animals. Most studies in animals administered perfluoroalkyls by the oral route. These data are briefly summarized below.

- Absorption
  - Perfluoroalkyls are absorbed following oral, inhalation, and dermal exposure.
  - Quantitative estimates of the fractional absorption of orally administered perfluoroalkyls in animals range from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA.
  - No quantitative estimates of the fractional absorption of perfluoroalkyls following inhalation or dermal exposure were identified.
- Distribution
  - Perfluoroalkyls are widely distributed in the body, with the highest concentrations in the liver, kidneys, and blood.
  - In the blood, perfluoroalkyls bind to albumin and other proteins.
  - Perfluoroalkyls can be transferred to the fetus during pregnancy and to nursing infants.
- Metabolism
  - Results of available oral and *in vitro* studies suggest that perfluoroalkyls are not metabolized and do not undergo chemical reactions in the body.
  - Although no studies examining metabolism of perfluoroalkyls following inhalation or dermal exposure were identified, metabolism by these exposure routes is not expected.
- Excretion
  - Studies of elimination rates (i.e., half-lives) of perfluoroalkyls show that elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect on rates of elimination of absorbed perfluoroalkyls.
  - Perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in feces and breast milk.

- Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion is not a major elimination pathway.
- Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species.
- In general, perfluoroalkyl sulfonates are eliminated more slowly than perfluoroalkyl carboxylates; elimination rate decreases with increasing chain length, and increases with increased branching.
- In humans, estimates for elimination $t_{1/2}$ range from hours (PFBA: 72–81 hours) to several years (PFOA: 2.1–8.5 years; PFOS: 3.1–7.4 years; PFHxS: 4.7–15.5 years).
- Evidence for sex differences in elimination of perfluoroalkyls in humans is not as strong as in rats. Menstruation may contribute to faster elimination of PFOS in younger women (≤50 years) when compared to men and older women.

3.1.1 Absorption

Inhalation Exposure. Studies of the absorption of perfluoroalkyls in humans following inhalation exposure were not located; elevated serum concentrations of perfluoroalkyls in workers in fluorochemical production industry have been reported (see Table 5-22), indicating that perfluoroalkyls are absorbed following inhalation exposure. Occupational exposures in these workers are likely to have included inhalation of aerosols of perfluoroalkyls complexed with airborne dusts. Higher serum levels in workers compared to the general population (see Table 5-20) probably reflect a predominant contribution from inhaled perfluoroalkyls.

Studies conducted in rodents provide direct evidence for absorption of inhaled perfluoroalkyls. PFOA was detected in plasma of rats within 30 minutes of initiating nose-only exposures to aerosols (mass median aerodynamic diameter [MMAD]=1.9–2.1 μm) of 1–25 mg ammonium PFOA/m^3. Plasma concentrations increased during the 6-hour exposure, with the highest concentrations observed at 9 hours (3 hours after cessation of exposure) in male rats and at 7 hours (1 hour after cessation of exposure) in females (Hinderliter et al. 2006a). Assuming an elimination $t_{1/2}$ of absorbed PFOA of approximately 160 hours in male rats, a peak plasma concentration at 9 hours would correspond to an absorption $t_{1/2}$ of approximately 1.3 hours (see discussion below, Equations 3-1 and 3-2). The earlier time of highest plasma concentration observed in female rats appears to be associated with faster elimination of absorbed PFOA in female rats, compared to male rats (see Section 3.1.4).

Nose-only exposure of male rats to dusts of ammonium perfluorononanoate induced significant increases in absolute and relative liver weight, assessed 5 and 12 days after exposure, providing indirect evidence of absorption of this compound through the respiratory airways (Kinney et al. 1989).

Oral Exposure. Studies of absorption of perfluoroalkyls through the gastrointestinal tract in humans are not available. A study of the general population of Europe and North America estimated that the greatest portion of the chronic exposure to PFOS and PFOA results from the intake of contaminated food, including drinking water (Trudel et al. 2008). Direct evidence of oral absorption of perfluoroalkyls was provided in studies that found associations between environmental levels (e.g., drinking water) and perfluoroalkyl concentrations in human serum (Emmett et al. 2006a; Hoffman et al. 2011; Hölzer et al. 2008; Seals et al. 2011; Wilhelm et al. 2008) and by reductions in serum levels after exposures from water were eliminated or reduced (Bartell et al. 2010; Emmett et al. 2009).

Animal data provide quantitative estimates of the fractional absorption of orally administered PFOA, PFOS, PFBA, PFHxA, PFHxS, PFHpA, PFNA, PFDA, PFUnA, and PFDoDA, with estimates ranging from >50% for PFHxS to >95% for PFOA, PFBA, PFNA, PFDA, PFUnA, and PFDoDA. Greater than 95% of an oral dose of ammonium [^{14}C]PFOA was absorbed in rats that received single gavage doses ranging from 0.1 to 25 mg/kg (Kemper 2003). In male and female mice, comparison of the 24-hour area under the curve (AUC) for oral and intravenous administration showed that 90–100% of the oral dose was absorbed for PFOA (females), PFNA (males and female), PFDA (males and females), PFUnA (males and females), and PFDoDA (males and female); however, absorption of PFOA in males was 80%, compared to 100% in females (Fujii et al. 2015a, 2015b). Gannon et al. (2011) estimated an absorption fraction of 99% based on 168-hour urinary excretion of ^{14}C in male and female rats and mice following single oral doses of 2 or 100 mg/kg ^{14}C-PFHxA. Based on comparison of the AUC for oral and intravenous administration, the estimated oral absorption fractions were 50% in female rats administered a single 10 mg/kg dose of potassium [$^{18}O_{3-}$]PFHxS (Sundström et al. 2012) and 79 and 55% in male and female rats administered a single dose of 4 mg/kg sodium [$^{18}O_{3-}$]PFHxS (Kim et al. 2016b). Sundström et al. (2012) stated that this estimate may not be reliable due to the short (24 hours) observation period. Based on 72-hour urinary excretion of ^{14}C, the estimated fractional absorption of a single dose (50 mg/kg) of ^{14}C-PFHxA was approximately 74% in male rats, 90% in female rats, and 80% in male and female mice (Iwai et al. 2011). A comparison of ^{14}C disposition in rats, mice, hamsters, and rabbits following an oral dose of 10 mg ammonium [^{14}C]PFOA/kg showed that similar fractions of the dose were absorbed (Hundley et al. 2006). The estimated absorbed fractions (i.e., ^{14}C in tissues, urine, and exhaled air measured 120–168 hours after the dose) in males were 89% in rats, 82% in mice, 92% in hamsters, and

88% in rabbits. Corresponding values for females were 76% in rats, 61%, in mice, 75% in hamsters, and 88% in rabbits. These estimates exclude ^{14}C excreted in feces, which may have been absorbed and secreted in bile before excretion (see Section 3.1.4). Fasting appears to increase absorption of PFOA. Plasma PFOA concentrations in rats, 24 hours following a gavage dose of 10 mg ammonium PFOA/kg, were 2–3 times higher when administered to fasted rats, compared to fed rats (Hinderliter et al. 2006b). The estimated absorption fractions of ingested ammonium [^{14}C]PFOA or potassium [^{14}C]PFOS (administered as a 4.2 mg/kg oral dose) were >93 and >95% in rats, respectively (Johnson and Ober 1979, 1999a). Based on combined urinary excretion and retention in the carcass (excluding the gastrointestinal tract and its contents), the estimated oral absorption fraction of [^{14}C]PFOS (administered as a single 4.2 mg/kg dose of potassium [^{14}C]PFOS) in male rats was >95% (Chang et al. 2012). The estimated absorption fraction of PFBA (administered as 30 mg/kg oral dose of PFBA) was >95% in rats (Chang et al. 2008a). Cumulative excretion of PFBA 24 hours after an oral dose (administered as 10, 30, or 100 mg/kg ammonium PFBA) was approximately 35% in urine and 4–11% in feces in male mice; and 65–69% in urine, and 5–7% in feces in female mice (Chang et al. 2008a).

Studies examining the rate of absorption of PFOA, PFHxA, PFBA, and PFBS show rapid absorption from the gastrointestinal tract, with values for absorption $t_{1/2}$ of <2 hours. For PFOA, the highest observed concentrations of ^{14}C in plasma occurred in male rats at approximately 10 hours (range 7.5–15 hours) following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals was approximately 170 hours (range 138–202 hours), corresponding to an elimination rate constant (k_e) of 0.0044 hour^{-1} (range 0.004–0.005 hour^{-1}). The corresponding absorption $t_{1/2}$ of approximately 1.5 hours (k_a=0.45 hour^{-1}) can be calculated from these observations (Equations 3-1 and 3-2):

$$t_{\max} = \ln\frac{k_a}{k_e} \cdot \frac{1}{(k_a - k_e)} \qquad \text{Eq. (3-1)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-2)}$$

Where t_{max} = time of maximum concentration of ^{14}C; k_e = elimination rate constant; and k_a = absorption constant. The absorption rate of PFOA appears to be greater in female rats compared to male rats. The time to peak concentrations of ^{14}C in plasma occurred at approximately 1.1 hour (range 0.6–1.5 hours) in female rats and 10 hours (range 7–15 hours) in male rats following single oral doses ranging from 0.1 to 25 mg ammonium PFOA/kg (Kemper 2003). The elimination $t_{1/2}$ of ^{14}C in plasma estimated in these same animals varied with dose and ranged from 3.2 hours at the lowest dose (k_e=0.23 hour^{-1}) to 16.2 hours at the highest dose (k_e=0.059 hour^{-1}). The estimated absorption $t_{1/2}$ from the observations

made at all doses (0.1, 1, 5, and 25 mg/kg), based on Equations 3-1 and 3-2, was approximately 0.25 hours (range 0.12–0.38 hours). The absorption $t_{1/2}$ of PFBA in male and female rats following administration of a single oral dose (30 mg/kg ammonium PFBA) was 0.23 hours (3.04 $hour^{-1}$) in males and 0.17 hours (4.15 $hour^{-1}$) in females (Chang et al. 2008a). In male and female mice administered 10–30 mg/kg ammonium PFBA, the absorption $t_{1/2}$ was <1 hour, although the absorption rate may be dose-dependent in males, with higher absorption $t_{1/2}$ at doses >30 mg/kg (Chang et al. 2008a). Estimated t_{max} values following administration of single doses (2 or 100 mg/kg) of ^{14}C-PFHxA to rats and mice ranged from 0.3 to 0.8 hours (Gannon et al. 2011). Similar results for were reported by Olsen et al. (2009) based on estimated compartmental pharmacokinetic parameters for PFBS in serum of male and female rats following a single intravenous or gavage dose of 30 mg potassium PFBS. Plasma concentration-time profiles were fit to a two-compartment elimination model. The absorption $t_{1/2}$ can be approximated from these data using Equation 3-1, with the elimination rate constant represented by the fast-phase elimination rate constant estimated for either the oral or intravenous dose. Using the oral or intravenous parameters yielded similar values for the absorption $t_{1/2}$ (0.12–0.16 hours). The estimated t_{max} values following the gavage dose were 0.42 hours in males and 0.33 hours in females. The fast-phase elimination rate constants following the gavage dose were 0.892 $hours^{-1}$ ($t_{1/2}$=0.79 hours) in males and 1.308 $hours^{-1}$ ($t_{1/2}$=0.53 hours) in females. The corresponding values for absorption $t_{1/2}$ were 0.14 hours (k_a=5.0 $hours^{-1}$) in males and 0.12 hours (k_a=5.8 $hours^{-1}$) in females. Use of the fast-phase elimination rate constants estimated following intravenous administration (male: 1.143 $hours^{-1}$; female: 1.956 $hours^{-1}$) yielded values for the absorption $t_{1/2}$ of 0.16 hours in males (k_a=4.30 $hours^{-1}$) and females (k_a=4.45 $hours^{-1}$).

Mechanisms of oral absorption of perfluoroalkyls have not been elucidated.

Dermal Exposure. Dermal exposures of rats to ammonium PFOA have been shown to produce systemic (e.g., liver, immunotoxicity) toxicity in animals (see Chapter 2). Estimates of the amount or rates of dermal absorption in humans or animals have not been reported. PFOA was detected in serum of mice following dermal application of PFOA dissolved in acetone (Franko et al. 2012). The investigators noted PFOA ingestion may have occurred during grooming and may have contributed to the body burden. Dermal absorption of PFOS was assessed following application of single doses of potassium PFOS (doses up to 0.30 mg/kg) and the diethanolamine salt of PFOS (doses up to 20 µg/kg) to clipped, intact skin of rabbits (Johnson 1995a, 1995b). Analysis of the liver 28 days after application showed no increase in content of total organic fluoride compared to controls, indicating that dermal absorption was not detectable at low dose levels using this methodology. Dermal penetration of PFOA has been studied in preparations of isolated rat, mouse, and human epidermis (Fasano et al. 2005; Franko et al. 2012). These

studies indicate that the rat and mouse skin may be more permeable to PFOA than human skin. Approximately 24% of a dermal dose of PFOA (0.5 mg in 1% acetone) was absorbed across isolated full thickness human skin in 24 hours and 45% of the dose was retained in skin (Franko et al. 2012); it is noted that the acetone, as well as the glycerol used to pretreat the skin may have enhanced PFOA absorption. Permeability was sensitive to pH and was higher when the skin was buffered at pH 2.5 ($5.5x10^{-2}$ cm/hour) compared to pH 5.5 ($4.4x10^{-5}$ cm/hour), well above the pKa for the terminal carboxylic acid of PFOA (Franko et al. 2012). This suggests that permeability of the unionized acid is greater than that of the dissociated anion. Lower permeability of ionized PFOA is also suggested by relatively low permeability of the ammonium salt of PFOA in isolated preparations of rat and human skin. Following application of the ammonium salt of PFOA to isolated human or rat epidermis (150 μL/cm^2 of a 20% aqueous solution of ammonium PFOA; approximately 30 mg ammonium PFOA/cm^2), approximately 0.048% of the dose was absorbed across human epidermis and 1.44% was absorbed across rat epidermis in 40 hours. The estimated dermal penetration coefficients were $9.49x10^{-7}$ cm/hour in the isolated human epidermis and $3.25x10^{-5}$ cm/hour in the isolated rat epidermis.

The available data suggest that absorption of PFOA and PFOS through the skin is limited and is of minimal concern as an exposure route. No dermal absorption data were located for other perfluoroalkyls.

3.1.2 Distribution

Available information on the distribution of perfluoroalkyls is obtained from oral exposure studies in laboratory animals and occupational exposure studies in which exposure is predominantly by inhalation. Studies specifically examining the distribution of perfluoroalkyls by inhalation or dermal exposure were not identified. As discussed in Section 3.1.3 (Metabolism), perfluoroalkyls do not undergo metabolism. Therefore, distribution is expected to be the same regardless of the route of administration.

Distribution in Blood. In a study of 60 healthy Chinese participants from the general population, whole blood:plasma ratios for PFOS, PFOA, PFHxA, and PFHxS were 0.65, 0.83, 3.0, and 0.57 (Jin et al. 2016). These results indicate that PFHxA, but not PFOA, PFOS, or PFHxS, enters cellular components of blood. In a study of perfluoroalkyl workers, serum:plasma ratios for PFHxS, PFOS, and PFOA were 1:1, and this ratio was independent of the concentrations measured (Ehresman et al. 2007). The ratio of whole blood:plasma (or serum) was approximately one-half, which corresponded to volume displacement by red blood cells, suggesting that these perfluoroalkyls do not enter cellular components of blood. In studies conducted in animals, most of the PFOA in blood is in the plasma fraction. In rats, 24 or 48 hours

following an oral dose of 11.4 mg ammonium [^{14}C]PFOA/kg, the red blood cell:plasma PFOA concentration ratio ranged from 0.2 to 0.3, suggesting that there was no selective retention of PFOA by red blood cells (Johnson and Ober 1999a). Blood:plasma (or serum) ratios of approximately 0.5 have also been observed in rats following intravenous injection of PFOA (Kudo et al. 2007).

Perfluoroalkyls in plasma bind to serum albumin. The dissociation constant for binding of PFOA to serum albumin is approximately 0.4 mM (0.38 mM, ±0.04 standard deviation [SD] for human serum albumin; 0.36 mM, ±0.08 SD for rat serum albumin) and involves 6–9 binding sites (Han et al. 2003). Given a dissociation constant (K_D) of 0.4 mM and an albumin concentration of approximately 0.6 mM, >90% of PFOA in serum would be expected to be bound to albumin when the serum concentration of PFOA is <1 mM (<440 mg/L). This is consistent with observations of the bound fraction of perfluoroalkyls in plasma of rats that received a gavage dose of 25 mg PFOA/kg (Han et al. 2003, 2005; Ylinen and Auriola 1990), and in human, rat, and monkey plasma incubated *in vitro* with perfluoroalkyls (e.g., PFHxA, PFOA, PFOS, PFNA, PFDA) (Kerstner-Wood et al. 2003; Ohmori et al. 2003). Comparison of dissociation constants for binding of PFOA and PFOS to human serum albumin indicates that PFOS (K_D: $8x10^{-8}$) has a higher binding affinity than PFOA (K_D: $1x10^{-4}$) for albumin, consistent with the longer $t_{1/2}$ of PFOS versus PFOA in humans (Beesoon and Martin 2015; see Section 3.1.4 for additional information). PFOS has also been shown to bind to human hemoglobin *in vitro* (Wang et al. 2016). PFBS was found to bind only to albumin, whereas PFOS, PFOA, and PFHxS were found to have the potential to bind to other human serum binding proteins, including plasma gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Kerstner-Wood et al. 2003).

Distribution to Extravascular Tissues. Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. An analysis of samples from human cadavers attempted to quantify PFOA, PFOS, FOSA, and PFHxA concentrations in serum and liver (Olsen et al. 2003c). The route of exposure was unknown. Mean serum PFOS concentration was 17.7 ng/mL (95% CI 13.0–22.5, range of <6.9 [limit of quantification] to 57 ng/mL, n=24) and was not different in males (18.2 ng/mL, n=13) and females (17.2 ng/mL, n=11). The mean liver concentration was 18.8 ng/g (95% CI 14.1–23.5; range <7.3–53.8 ng/g, n=30). The mean liver:serum concentration ratio was 1.3 (95% CI 0.9–1.7, n=23) and was not different in males (1.3, n=13) and females (1.3, n=10). Most liver and serum concentrations for PFOA, FOSA, and PFHxA were below the limit of quantification; these limits were <17.9–<35.9 ng/mL for PFOA, <7.5–<19.6 ng/g for FOSA, and <3.4–<18.5 ng/mL for PFHxA.

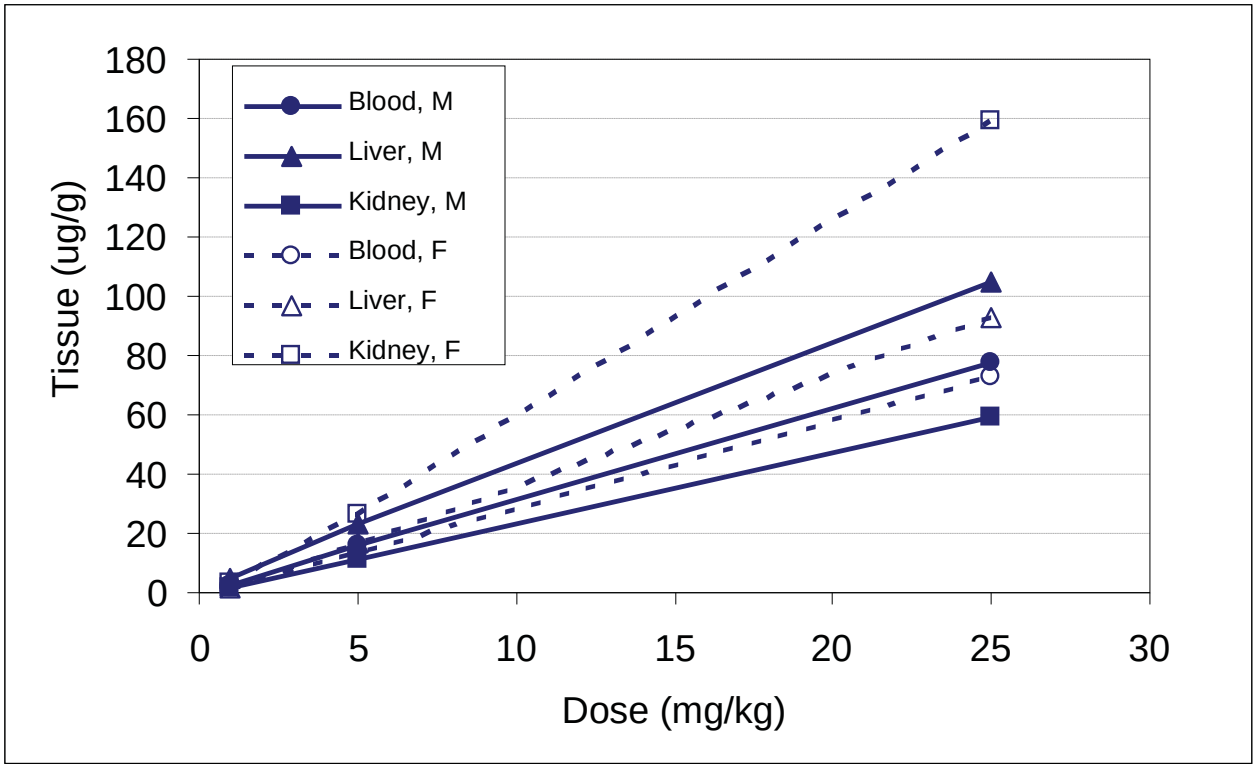
Studies conducted in nonhuman primates and rodents have provided additional information on the distribution of absorbed perfluoroalkyls to extravascular tissues. Distribution, as assessed from tissue perfluoroalkyl concentrations and tissue:serum ratios, exhibits profound species and sex differences as well as dose-dependencies (e.g., tissue levels that change disproportionately with dose). These differences have been attributed, in part, to species and sex differences in elimination kinetics of absorbed perfluoroalkyls and dose-dependence of elimination kinetics (see Section 3.1.4). In general, a consistent finding across species is that the liver receives a relatively high fraction of the absorbed dose and may also experience relatively high tissue concentrations compared with other tissues, with blood (i.e., plasma) and kidney also showing relatively high concentrations. The most extensive investigations of tissue distribution have been conducted in rodents.

Bogdanska et al. (2011) examined distribution of ^{35}S following dietary exposure to adult male C57/BL6 mice to low (environmentally relevant; 0.031 mg/kg/day) and high (experimentally relevant; 23 mg/kg/day) doses of [^{35}S]PFOS for 1–5 days. For both low and high doses after 1, 3, and 5 days of exposure, ^{35}S was distributed to the following tissues: blood, liver, lung, kidney, skin, whole bone, pancreas, spleen, thymus, heart, testes, epididymal fat, fat pads, brain, and muscle; ^{35}S was also detected in tissues throughout the gastrointestinal tract. Similar tissue:blood ratios were observed in both dose groups. In low-dose animals after 5 days of treatment, the highest tissue concentrations (excluding the gastrointestinal tract) were liver (tissue:blood=5.8), followed by lung (tissue:blood=1.4), whole bone, including marrow (tissue:blood=1.1), blood, and kidney (tissue:blood=0.94). In high-dose animals, the highest tissue concentrations were liver (tissue:blood=3.6), followed by lung (tissue:blood=1.6), blood, kidney (tissue:blood=0.81), and whole bone, including marrow (tissue:blood=0.72). A similar pattern of distribution was observed following intravenous administration of [^{14}C]potassium PFOS (4.2 mg/kg) to male rats (Johnson and Ober 1980). For both dose groups, the tissue:blood ratios for all other tissues were <1. In male and female CD-1 mice administered a single oral dose (4.2 mg/kg) of [^{14}C]PFOS, the highest concentrations of ^{14}C was observed in the liver, followed by serum, and then kidney, with similar tissue levels observed in males and females (Chang et al. 2012). In male and female rats fed diets containing 0, 2, 20, 50, or 100 mg/kg [^{13}C]sodium PFOS (equivalent to 0, 0.14, 1.33, 3.21, and 6.34 and 0, 0.15, 1.43, 3.73, and 7.58 mg/kg/day in males and females, respectively) for 28 days, PFOS levels were highest in liver, followed by spleen, heart, and serum. Liver:serum ratios for the 2, 20, 50, and 100 mg/kg/day diets were approximately 52, 42, 41, and 35, respectively, in males and 30, 47, 20, and 23, respectively, in females (Curran et al. 2008). Except for rats fed diets containing 20 mg/kg, the liver:serum ratio in males was higher than in females. No additional data were reported to determine if PFOS distribution differed between male and female rats.

Kemper (2003) determined the distribution of ^{14}C in male and female rats at the approximate time of maximum plasma concentration in both sexes, following single gavage doses of [^{14}C]PFOA (as ammonium PFOA, 0.1–25 mg/kg). This design allows a more direct comparison of patterns of tissue distribution in male and female rats at similar plasma concentrations, even though the elimination kinetics in the female rat are substantially faster than in male rats (see Section 3.1.4). The highest concentrations of ^{14}C were observed in blood, liver, and kidney (Figure 3-1). Liver, blood, and kidney accounted for approximately 22, 22, and 2% of the administered dose of 1 mg/kg in male rats; and 6, 7, and, 3% in female rats (the sex difference reflected more rapid excretory elimination in females). Although blood, liver, and kidney concentrations appeared to increase proportionately with increasing dose in male rats, in female rats, a disproportionately higher concentration in kidney was observed following the 25 mg/kg dose (Figure 3-1). Concentrations in other tissues ranged from 0.1 to 0.25 of that in liver or kidney; concentrations in bone and fat were <0.1 of that in liver or kidney. Profound sex differences and dose-dependencies in tissue concentrations of PFOA were also observed in rats that received oral doses of PFOA for 28 days at doses of 3, 10, or 30 mg PFOA/kg/day (Ylinen et al. 1990; Figure 3-2). Mean serum, kidney, or liver concentrations did not increase proportionally with dose in either sex. Kidney concentrations exhibited a disproportionate increase as the dose increased from 3 to 10 mg/kg/day, with little further increase at the 30 mg/kg/day dose. Sex differences in tissue distribution of PFOA in rats are not explained by sex differences in bioavailability since the differences persist in animals that received parenteral doses of PFOA (Johnson and Ober 1999b; Vanden Heuvel et al. 1991b, 1991c). The differences have been attributed to more rapid elimination of PFOA in female rats, compared to male rats (see Section 3.1.4).

A comparison of PFOA disposition in rats, mice, hamsters, and rabbits showed pronounced species and sex differences (Hundley et al. 2006; Table 3-1). In this study, rats, mice, hamsters, or rabbits received an oral dose of 10 mg ammonium [^{14}C]PFOA/kg and ^{14}C in tissues was measured at 120 or 168 hours (rabbits) hours post-dosing. In male rats, the highest concentrations of ^{14}C occurred in blood, liver and kidney, and all tissues combined accounted for approximately 60% of the dose. However, in female rats, concentrations of ^{14}C in all tissues were below limits of quantification. In mice, liver concentrations were similar in males and females, and liver showed the highest concentrations; ^{14}C levels in all tissues combined were lower in females compared to males. The opposite pattern was evident in hamsters and rabbits, with males having lower tissue levels than females, although, in common with rats and mice,

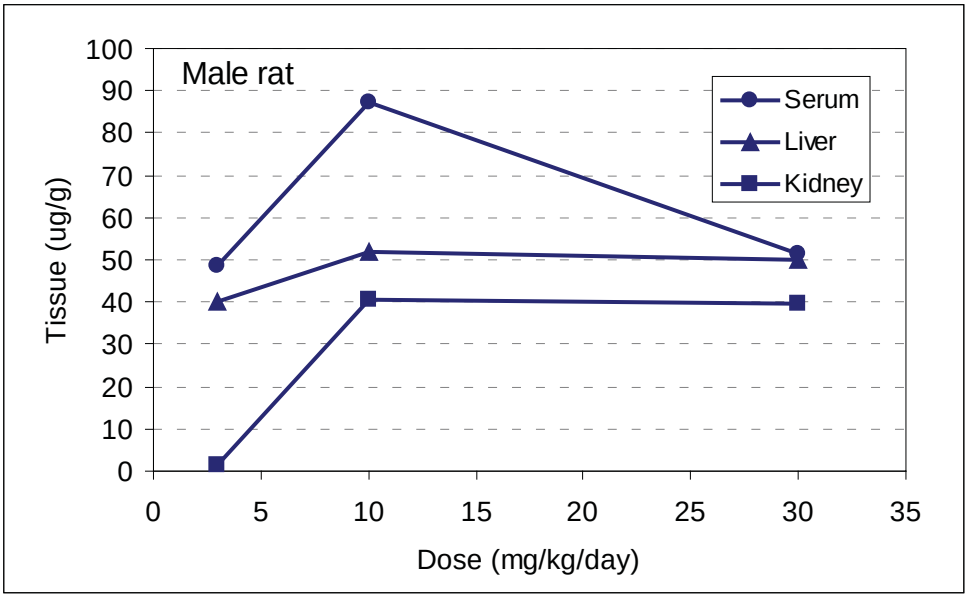
Figure 3-1. Tissue Concentrations of ^{14}C in Male and Female Rats Following a Single Gavage Dose of [^{14}C]PFOA at 1, 5, or 25 mg/kg*

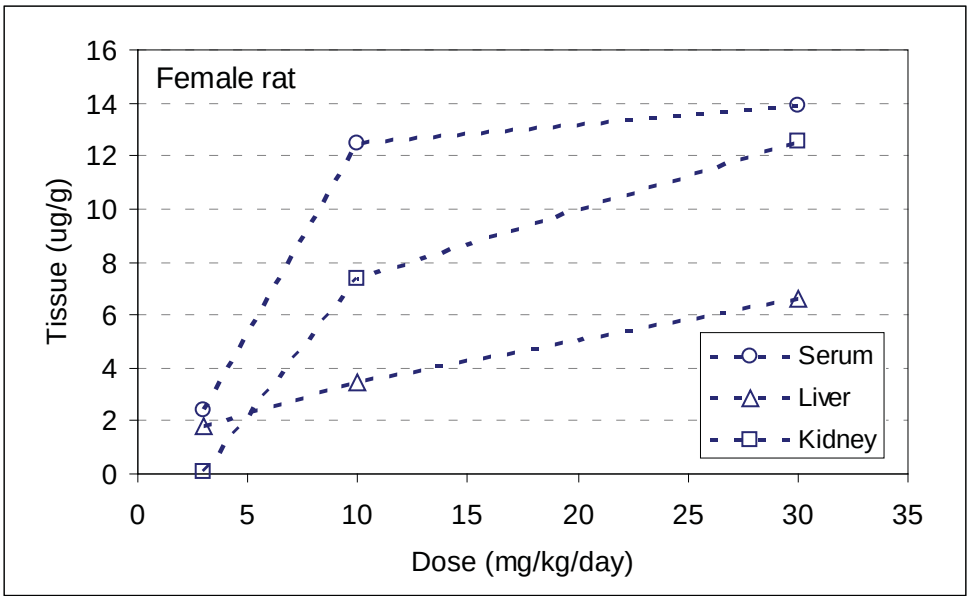


*Tissue levels are measured at time of maximum concentration in each tissue.

Source: Kemper 2003

Figure 3-2. Tissue Concentrations of ^{14}C in Male (Upper Panel) and Female (Lower Panel) Rats Following Oral Doses of PFOA for 28 Days at Doses of 3, 10, or 30 mg/kg/day





Source: Ylinen et al. 1990

Table 3-1. Tissue Distribution and Excretion of ^{14}C-Radioactivity from Both Sexes of Rats, Mice, Hamsters, and Rabbits Dosed with ^{14}C-Labeled APFO[a]

	µg Equivalent per g (mL) wet weight[b]							
	Rat		Mouse		Hamster		Rabbit	
Sample	Male	Female	Male	Female	Male	Female	Male	Female
Blood	23.5	<0.1	13.8	10.1	0.1	8.8	<0.1	0.1
Liver	40.0	<0.1	43.2	45.3	0.3	7.3	0.1	1.5
Kidneys	24.0	<0.1	2.9[c]	2.2[c]	0.2	7.1	0.1	0.4
Lungs	8.7	<0.1	1.4[c]	1.3[c]	<0.1	3.8	<0.1	0.1
Heart	6.4	<0.1	1.2[c]	0.6[c]	<0.1	2.9	<0.1	<0.1
Skin	4.8	<0.01	3.5	0.2	<0.1	3.4	<0.1	<0.1
Testes	3.2	–	0.9[c]	–	<0.1	–	<0.1	–
Muscle	1.9	<0.1	1.1	0.5	<0.1	0.9	<0.1	<0.1
Fat	1.7	<0.1	1.6	1.3	<0.1	1.5	<0.1	<0.1
Brain	0.6	<0.1	0.2[c]	0.8[c]	<0.1	0.3	<0.1	<0.1
	Percent of dose							
Tissues	59.6	0.6	73.6	50.0	0.7	26.5	<0.1	0.3
Urine	25.6	73.9	3.4	6.7	90.3	45.3	76.8	87.9
Feces	9.2	27.8	8.3	5.4	8.2	9.3	4.2	4.6
Expiration	3.6	1.5	5.2	4.4	1.3	2.9	No data	No data
Cage wash	0.6	0.8	4.9	4.9	0.6	2.1	0.5	4.8
Percent recovered	98.5	104.6	95.4	71.4	101.1	86.1	81.6	97.6

[a]The rabbits were sacrificed 168 hours after dosing; all other animals were sacrificed 120 hours after dosing.
[b]The µg equivalent calculations were based on the specific activity of ^{14}C-labeled APFO, which was 1.1×10^6 DPM/mg. The µg equivalent per g wet weight could not accurately be determined below 0.1 µg/g.
[c]Represents the µg equivalents for the entire organ.

APFO = ammonium perfluorooctanoate

Source: Hundley et al. 2006

blood, liver and kidney had the highest concentrations. Male rats that received a single oral dose of 5 mg FOSA/kg had liver FOSA concentrations that were 3–5 times higher than serum concentrations 1 day post-dosing (Seacat and Luebker 2000).

Sex differences in elimination that give rise to sex differences in tissue levels following oral exposure to perfluoroalkyls in rats are not evident in studies conducted with nonhuman primates. Rhesus monkeys that received 3 or 10 mg ammonium PFOA/kg/day for 90 days had liver concentrations of 48 µg/g (one male) or 50 µg/g (one female) at the low dose and 45 µg/g (one male) and 72 µg/g (one female) at the higher dose, with corresponding serum concentrations of 3 and 7 µg/mL, and 9 and 10 µg/mL, respectively (Griffith and Long 1980). Although limited to only one animal per sex, these results suggest

that liver levels did not increase proportionately with increasing dose. A similar observation was made in a study of male Cynomolgus monkeys (Butenhoff et al. 2004c). In male monkeys that received daily oral doses of 3 or 10 mg ammonium PFOA kg/day for 27 weeks, liver PFOA concentrations ranged from 11 to 18 μg/g at the low dose and from 6 to 22 μg/g at the higher dose. Mean serum concentrations measured after 6 weeks of exposure (which may have represented steady-state concentrations) were 77,000 ng/mL in the low-dose group and 86,000 ng/mL in the higher dose group. In this same study, an analysis of serum PFOA kinetics following an intravenous dose of PFOA revealed similar elimination kinetics in males and females (Butenhoff et al. 2004c; see Section 3.1.4). In Cynomolgus monkeys that received daily oral doses of PFOS (0, 0.03, 0.15, or 0.75 mg PFOS/kg/day) for 26 weeks, liver concentrations of PFOS and serum concentration were similar in males and females (liver:serum ratios ranged from 1 to 2) and increased in approximate proportion to the administered dose (Seacat et al. 2002).

Bogdanska et al. (2014) examined distribution of ^{35}S in 20 tissues following dietary exposure of adult male C57/BL6 mice to PFBS (16 mg/kg/day) for 1–5 days. ^{35}S was detected in all tissues and concentrations reached plateau levels after 3 days of exposure. After 5 days, tissue:blood ratios (excluding stomach and small intestine) were >1 for liver (tissue:blood=1.6), kidney (tissue:blood=1.3), whole bone (tissue:blood=1.1), and cartilage (tissue:blood=1.1). At all-time points, approximately 90% of the ingested ^{35}S was recovered in combined blood, liver, bone, skin, and muscle.

Iwabuchi et al. (2017) compared tissue distribution following single doses or 3-month dosing of PFOS (100 μg/kg), PFOA (100 μg/kg), PFHxA (100 μg/kg), and PFNA (50 μg/kg). Following administration of single doses, the tissue:serum (and/or whole blood) ratio was >1 for the liver for PFOS, PFOA, and PFNA, with tissue:serum ratios <1 for kidney, spleen, heart, and brain. For PFNA, the only tissue with a tissue:serum ratio >1 was kidney. After 3 months of exposure, tissue:serum ratios >1 were observed for the liver for PFOA and PFNA, and the liver and kidney for PFOS. For PFHxA, all tissue:serum ratios were <1. Similar to the single dose study, the lowest serum:tissue ratio for all compounds was observed for brain.

Subcellular Distribution. The subcellular distribution of perfluoroalkyls has been examined in rats (Han et al. 2004, 2005; Kudo et al. 2007; Vanden Heuvel et al. 1992b). Two hours following an oral dose of 25 mg ammonium [^{14}C]PFOA/kg, sex differences were noted in the subcellular distribution of ^{14}C in liver; females had approximately 50% of total ^{14}C in the cytosolic fraction compared to 26% in males (Han et al. 2005). The distributions to other cell fractions were: nuclear/cell debris fraction, 30% females, 40% males; lysosomes, 12% females, 14% males; mitochondria, 8% females, 16% males; and

ribosomes, <3% males and females. In kidney, 80 and 70% of the ^{14}C was associated with the cytosolic fraction in males and females, respectively, 16–22% in the nuclear/cell debris fraction, and the remainder in lysosome/mitochondria/ribosome fractions. In liver, approximately 55% of cytosolic ^{14}C was bound to proteins (>6,000 Da) in both males and females, whereas in kidney, 42% of the cytosolic fraction was bound to protein in males and 17% in females. The subcellular distribution of PFOA is dose-dependent. In rats, 2 hours following an intravenous dose of 0.041 mg [^{14}C]PFOA/kg, approximately 5% ^{14}C in the liver was associated with the cytosolic fraction, whereas approximately 45% was in the cytosolic fraction following a dose of 16.6 mg/kg (Kudo et al. 2007). A small component of tissue-associated PFOA and PFDA appeared to be bound covalently to protein. Following an intraperitoneal dose of 9.4 μmol/kg [^{14}C]PFDA or [^{14}C]PFOA (4.2 mg/kg), approximately 0.1–0.5% of liver ^{14}C was bound covalently (i.e., was not removed by repeated extraction with a methanol/ether and ethyl acetate; Vanden Heuvel et al. 1992b). Covalent binding was detected when cytosolic or microsomal fractions of rat liver were incubated *in vitro* with [^{14}C]PFDA (Vanden Heuvel et al. 1992b).

PFOA binds to rat kidney and urine α2u-globulin; dissociation constants were estimated to be approximately 1.5 and >2 mM (for a single binding site) for the proteins isolated from rat kidney and urine, respectively. These values suggest relatively low affinity for the protein, compared to other ligands that are known to induce hyaline droplet nephropathy (10^{-4}–10^{-7} M; Han et al. 2004).

Maternal-fetal Transfer. Perfluoroalkyls can be transferred to the fetus during pregnancy (Cariou et al. 2015; Chen et al. 2017a; Fei et al. 2007; Fisher et al. 2016; Fromme et al. 2010; Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2010, 2013; Inoue et al. 2004; Kato et al. 2014; Kim et al. 2011, Lee et al. 2013; Lien et al. 2013; Liu et al. 2011; Manzano-Salgado et al. 2015; Midasch et al. 2007; Monroy et al. 2008; Needham et al. 2011; Ode et al. 2013; Porpora et al. 2013; Yang et al. 2016a, 2016b). Studies that measured perfluoroalkyls in maternal and fetal cord blood of matched mother-infant pairs found relatively strong correlations (r>0.8) between maternal and fetal serum (or plasma); however, fetal/maternal serum ratios vary depending on the structure of the perfluoroalkyl (Table 3-2). With some exceptions, longer fluoroalkyl chain length and a terminal sulfonate group are associated with lower fetal/maternal ratios (Glynn et al. 2012; Gützkow et al. 2012; Hanssen et al. 2013; Kim et al. 2011; Liu et al. 2011; Needham et al. 2011). PFOS was detected in amniotic fluid obtained from amniocentesis (Jensen et al. 2012). The median concentration in amniotic fluid samples from 300 pregnancies (from the Danish amniotic fluid pregnancy-screening biobank) was 1.1 ng/mL.

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Glynn et al. 2012	PFOA	7	413	4	1	NR	0.89
	PFOS	8	413	29	5	NR	0.86
	PFNA	8	413	0.6	0.1	NR	0.53
Cariou et al. 2015	PFHxS	6	59	0.62	0.34	0.56	0.99
	PFOA	7	89	1.05	0.86	0.78	0.83
	PFOS	8	94	3.07	1.11	0.38	0.88
	PFNA	8	22	0.43	0.27	0.51	0.92
Chen et al. 2017a	PFHxS	6	32	0.53	0.33	0.62	ND
	PFOA	7	32	8.67	3.67	0.42	ND
	PFOS	8	32	1.56	1.24	0.79	ND
Fisher et al. 2016	PFHxS	6	315	NR	NR	0.23	NR
	PFOA	7	865	NR	NR	0.28	NR
	PFOS	8	648	NR	NR	0.14	NR
Fromme et al. 2010	PFHxS	6	53	0.60	0.30	0.50	0.89
	PFOA	7	53	2.60	1.70	0.65	0.94
	PFOS	8	53	3.50	1.10	0.31	0.89
	PFNA	8	53	0.60	<0.4	ND	ND
Gützkow et al. 2012	PFHxS	6	123	0.34	0.23	0.68	0.70
	PFOA	7	123	1.25	1.03	0.82	0.82
	PFOS	8	123	5.37	1.78	0.33	0.74
	PFNA	8	123	0.40	0.16	0.40	0.64
	PFDA	9	123	0.10	0.04	ND	ND
	PFUnA	10	123	0.19	0.06	0.32	0.67
Hanssen et al. 2013	PFHxS	6	7	0.26	0.17	0.65	ND
	PFOA	7	7	1.50	1.26	0.84	ND
	PFOS	8	7	10.70	3.93	0.37	ND
	PFNA	8	7	0.89	0.50	0.56	ND
	FOSA	8	7	0.41	0.45	1.10	ND
	PFUnA	10	7	0.33	0.16	0.48	ND
Han et al. 2018	PFBS	4	369	0.19	0.19	1.00	ND
	PFHxS	6	369	0.32	0.31	1.03	ND
	PFHpA	6	369	0.06	0.09	1.50	ND
	PFOA	7	369	42.83	34.67	0.81	ND
	PFOS	8	369	4.55	1.39	0.31	ND
	FOSA	8	369	0.13	0.13	1.00	ND
	PFNA	8	369	0.81	0.44	0.54	ND
	PFDA	9	369	0.55	0.21	0.38	ND
	PFUnA	10	369	0.47	0.17	0.36	ND
	PFDoDA	11	369	0.17	0.14	0.82	ND

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Inoue et al. 2004	PFOA	7	15	8.90	2.90	0.32	0.94
Kim et al. 2011	PFHxS	6	20	0.89	0.58	0.65	ND
	PFOA	7	20	1.60	1.10	0.69	ND
	PFOS	8	20	5.60	2.00	0.36	ND
	PFNA	8	20	0.79	0.37	0.47	ND
	PFDA	9	20	0.36	0.01	0.03	ND
	PFUnA	10	20	1.60	0.46	0.29	ND
Kato et al. 2014	PFHxS	6	78	1.20	0.60	0.50	0.89
	PFOA	7	78	3.30	3.10	0.89	0.88
	PFOS	8	78	8.50	3.50	0.31	0.82
	PFNA	8	78	0.66	0.41	0.62	0.79
	PFDA	9	78	0.20	ND	ND	ND
Lee et al. 2013	PFHS	6	70	1.35	0.67	0.57	ND
	PFOA	7	70	2.73	2.09	0.84	ND
	PFOS	8	70	10.77	3.44	0.35	ND
Liu et al. 2011	PFHxS	6	50	0.08	0.06	0.79	0.59
	PFOA	7	50	1.66	1.50	0.91	0.91
	PFOS	8	50	3.18	1.69	0.53	0.75
	PFNA	8	50	0.55	0.33	0.61	0.82
	PFDA	9	50	0.58	0.24	0.41	0.82
	PFUnA	10	50	0.56	0.30	0.53	0.70
	PFDoDA	11	50	0.08	ND	ND	ND
Manzano-Salgado et al. 2015	PFHxS	6	66	0.84	0.40	0.446	NR
	PFOA	7	66	2.97	1.90	0.746	NR
	PFOS	8	66	6.99	1.86	0.299	NR
	PFNA	8	66	0.85	0.32	0.4	NR
Midasch et al. 2007	PFOA	7	11	2.70	3.40	1.30	0.42
	PFOS	8	11	12.10	7.20	0.60	0.72
Monroy et al. 2008	PFHxS	6	101	4.05	5.05	1.25	ND
	PFOA	7	101	2.24	1.94	0.87	0.94
	PFOS	8	101	16.19	7.19	0.44	0.91
	PFNA	8	101	0.80	0.94	1.18	ND
Needham et al. 2011	PFHxS	6	12	12.30	9.10	0.74	0.05
	PFOA	7	12	4.20	3.10	0.72	0.91
	PFOS	8	12	19.70	6.60	0.34	0.82
	PFNA	8	12	0.76	0.37	0.50	0.84
	PFDA	9	12	0.34	0.10	0.29	0.91

Table 3-2. Serum (or Plasma) Concentrations in Matched Human Maternal-Infant Pairs

Study	Perfluoro-alkyl	Perfluoroalkyl chain length	N	Maternal (ng/mL)	Cord (ng/mL)	Ratio[a]	r
Ode et al. 2013	PFOA	7	263	2.30	2.80	1.30	0.74
	PFOS	8	263	17.00	7.40	0.45	0.76
	PFNA	8	263	0.31	0.26	0.93	0.51
Porpora et al. 2013	PFOA	7	38	2.90	1.60	0.55	0.70
	PFOS	8	38	3.20	1.40	0.44	0.72
Yang et al. 2016a	PFHxS	6	50	0.064	0.033	0.52	0.80
	PFOA	7	50	1.24	1.03	0.83	0.93
	PFOS	8	50	2.98	1.23	0.41	0.88
	PFNA	8	50	0.55	0.35	0.64	0.89
	PFDA	9	50	0.56	0.22	0.39	0.92
	PFUnA	10	50	0.55	0.23	0.42	0.88
	PFDoDA	11	50	0.085	0.058	0.68	0.76
Yang et al. 2016b	PFHxS	6	157	0.53	0.26	0.43	0.68
	PFOA	7	157	1.74	1.32	0.71	0.81
	PFOS	8	157	4.23	1.52	0.36	0.63
	PFNA	8	157	0.46	0.23	0.49	0.70
	PFDA	9	157	0.37	0.13	0.35	0.65
	PFUnA	10	157	0.38	0.14	0.36	0.63
	PFDoDA	11	157	0.040	0.026	0.61	0.52

[a]Ratio of cord:maternal perfluoroalkyl level.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFHpA = Perfluoroheptanoic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies in rats and mice provide further support for maternal-fetal transfer of perfluoroalkyls. Following gavage administration of 0.1–10 mg/kg/day PFOS to rats during gestation, PFOS was distributed to fetal serum, liver, and brain, with fetal concentrations increasing with maternal dose (Chang et al. 2009; Lau et al. 2003; Luebker et al. 2005a, 2005b; Thibodeaux et al. 2003). Levels in fetal serum and liver generally were similar and higher than in brain. Studies did not report on concentrations of PFOS in other fetal tissues. Paired fetal-maternal levels of PFOS were examined in rats following exposure (gavage) to potassium PFOS at doses of 0.1, 0.4, 1.6, or 3.2 mg/kg/day on GDs 0–20 (Luebker et al. 2005b). On GD 21, fetal:maternal serum ratios were 2.1, 1.7, 1.6, and 1.1 at doses of 0.1, 0.4, 1.6, and 3.2 mg/kg/day, respectively; these results suggest that fetal:maternal serum ratios varied inversely with dose. Fetal:maternal liver ratios (0.37–0.44) were similar across the dose range. In mice administered a single

gavage dose of 12.5 mg/kg [^{35}S]PFOS on GD 16, fetal organ:maternal blood ratios of ^{35}S on GD 18 were 2.8 for kidneys, 2.6 for liver, 2.3 for blood, 2.1 for lungs, and 1.2 for brain (Borg et al. 2010).

Maternal-fetal transfer of PFOA has also been studied in rats and mice (Das et al. 2008; Hinderliter et al. 2005). In rats, PFOA concentrations in amniotic fluid, placenta, and fetus (measured on days 10, 15, or 21 of gestation) increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Fetal plasma concentrations of PFOA measured on GD 21 were approximately 40% of maternal plasma concentration. Following gavage administration of 0.01, 1, or 5 mg/kg ammonium PFOA on GD 17 in mice, PFOA was detected in amniotic fluid and pup serum, with dose-dependent increases (Fenton et al. 2009). On PND 1, pup serum PFOA concentrations were approximately 1.7–2.0-fold greater than levels in maternal serum.

Following administration of ammonium PFBA (35, 175, or 350 mg/kg) to pregnant mice on GDs 0–17, fetal serum and liver levels of PFBA were determined on PND 1 (Das et al. 2008). The fetal:maternal serum ratio of PFBA was approximately 0.15 and did not vary with maternal dose. Fetal liver:serum ratios were 0.44, 0.75, and 0.78 at maternal doses of 35, 175, and 350 mg/kg, respectively. PFHxS was detected in fetal blood and in the liver of neonates following exposure of dams to potassium PFHxS (0.3, 1, 3, and 10 mg/kg) throughout gestation (Butenhoff et al. 2009a); concentrations in serum and liver increased with dose.

Maternal-infant Transfer. Perfluoroalkyls can be transferred to nursing infants (Barbarossa et al. 2013; Cariou et al. 2015; Fromme et al. 2010; Kärrman et al. 2007; Kim et al. 2011; Kuklenyik et al. 2004; Liu et al. 2011; Tao et al. 2008a, 2008b). Studies that measured perfluoroalkyls in maternal serum (or plasma) and breast milk in matched mother-infant pairs found highly variable correlations (Table 3-3). Relatively high correlations have been reported for PFOA (Kärrman et al. 2007; Liu et al. 2011). Transfer to breast milk appears to be a significant route of elimination of perfluoroalkyls during breastfeeding. Comparisons of serum concentrations of women who did or did not breastfeed their infants showed that breastfeeding significantly decreases maternal serum concentrations of PFOA, PFOS, PFHxS, and PFNA (Bjermo et al. 2013; Brantsaeter et al. 2013; Mondal et al. 2012, 2014; von Ehrenstein et al. 2009). The decrease was estimated to be 2–3% decrease per month of breastfeeding (Brantsaeter et al. 2013; Mondal et al. 2012, 2014). Concentrations of perfluoroalkyls in breast milk also decrease with breastfeeding duration (Tao et al. 2008b; Thomsen et al. 2010). Numerous perfluoroalkyls (including PFOS, PFOA, PFBS, PFHxS, PFNA, PFDA, PFDoDA, PFUnA, and FOSA) have been detected in breast milk samples in women in China, Korea, Japan, Malaysia, Cambodia, India, Korea, Vietnam, Indonesia,

Norway, Philippines, Sweden, and the United States (Forns et al. 2015; Fujii et al. 2012; Kang et al. 2016; Kärrman et al. 2007; Kim et al. 2011; Liu et al. 2010, 2011; Mondal et al. 2014; So et al. 2006b; Tao et al. 2008a). The mean concentrations for perfluoroalkyls in breast milk collected from 45 women in Massachusetts were 0.131 ng/mL (range of <0.032.0–617 ng/mL) for PFOS, 0.043.8 ng/mL (<0.0301–0.161 ng/mL) for PFOA, and 0.0145 ng/mL (<0.0120–0.0638 ng/mL) for PFHxS (Tao et al. 2008b). PFHpA, PFDA, PFUnA, PFDoDA, and PFBS were also detected in the breast milk; however, ≤4 samples had levels that exceeded the limit of quantitation. Serum concentrations in breastfed infants can be higher than maternal levels. Although cord:maternal serum ratios of PFOA, PFOS, and PFNA at birth are typically lower than 1 (see Table 3-2), infant serum levels increase several-fold during the first 6 months after birth (Fromme et al. 2010; Mondal et al. 2014; Post et al. 2012; Verner 2016a, 2016b). This increase is likely because breast milk concentrations of perfluoroalkyls and fluid intake per infant body weight are highest during this time period. Fromme et al. (2010) also showed increases in serum levels of PFNA in infants fed formula made with contaminated drinking water. Mogensen et al. (2015b) reported that following weaning, significant (<0.0001) decreases were observed in infant serum concentrations of PFOS, PFOA, and PFHxS.

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Cariou et al. 2015	PFHxS	6	9	2.28	0.026	0.011	0.36
	PFOA	7	10	1.22	0.041	0.034	0.72
	PFOS	8	19	3.62	0.040	0.011	0.85
Kärrman et al. 2007a	PFHxS	6	12	4.7	0.085	0.020	ND
	PFOA	7	12	3.8	0.49	0.120	0.88
	PFOS	8	12	20.7	0.20	0.010	0.83
	FOSA	8	12	0.24	0.013	0.070	ND
	PFNA	8	12	0.80	0.017	0.010	ND
Kim et al. 2011	PFHxS	6	20	0.89	0.007	0.008	NS
	PFOA	7	20	1.60	0.041	0.026	NS
	PFOS	8	20	5.60	0.061	0.011	0.60
	PFNA	8	20	0.79	<0.0088	ND	ND
	PFDA	9	20	0.36	<0.018	ND	ND
	PFUnA	10	20	1.60	<0.024	ND	ND

Table 3-3. Matched Serum (or Plasma) and Breast Milk Concentrations in Humans

Study	Perfluoroalkyl	Perfluoroalkyl chain length	N	Serum (ng/mL)	Milk (ng/mL)	Ratio[a]	r
Liu et al. 2011	PFHxS	6	50	0.08	ND	ND	ND
	PFOA	7	50	1.66	0.181	0.109	0.77
	PFOS	8	50	3.18	0.056	0.018	0.57
	PFNA	8	50	0.55	0.026	0.048	0.62
	PFDA	9	50	0.58	0.02	0.034	0.54
	PFUnA	10	50	0.56	0.026	0.046	0.44
	PFDoDA	11	50	0.08	ND	ND	ND
	PFTrDA	12	50	0.08	ND	ND	ND

[a]Milk to serum ratio.

FOSA = perfluorooctane sulfonamide; ND = no data (detected but below limit of quantification); NS = not significantly correlated; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFTrDA = perfluorotridecanoic acid; PFUnA = perfluoroundecanoic acid

Studies conducted in rats and mice provide further support for maternal-infant transfer of perfluoroalkyls through breast milk (Fenton et al. 2009; Hinderliter et al. 2005; Lau et al. 2003; Luebker et al. 2005a; Yu et al. 2009b). PFOA concentrations in breast milk of nursing rats increased with increasing maternal oral dose (3, 10, or 30 mg/kg/day, administered daily beginning on GD 4) (Hinderliter et al. 2005). Milk concentrations of PFOA measured on postpartum days 3, 7, 14, or 21 in rats were approximately 0.1 of maternal plasma concentration. In dams exposed to 0.1, 1, or 5 mg/kg PFOA by gavage on GD 17, a dose-dependent increase in PFOA concentrations in breast milk was observed on PND 2, with breast milk:serum ratios of approximately 0.15, 0.38, and 0.25 at 0.1, 1, and 5 mg/kg doses, respectively; milk/serum concentration ratios for PFOA ranged from 0.15 to 0.56 (Fenton et al. 2009). Following lactational exposure of control rat pups to PFOS in breast milk of dams treated with dietary PFOS (3.2 mg/kg diet; approximately equivalent to 0.33 mg/kg/day), pup serum and liver concentrations increased throughout the 35-day lactation period (Yu et al. 2009b). At PND 35, the pup liver:serum PFOS ratios were 2.55 and 2.43 in male and female pups, respectively. Results of a cross-foster study show that pups are exposed to PFOS through breast milk (Luebker et al. 2005a). Postnatal toxicity observed in cross-fostered pups that nursed from exposed dams provides additional evidence of maternal-infant transfer of PFOS in rats and mice (see Section 2.17).

Mechanisms of Distribution. Perfluoroalkyls in plasma bind to serum albumin and various other plasma proteins including gamma-globulin, alpha-globulin, alpha-2-macroglobulin, transferrin, and beta-lipoproteins (Bischel et al. 2011; Butenhoff et al. 2012d; Chen and Guo 2009; Han et al. 2003, 2005; Kerstner-Wood et al. 2003; Luo et al. 2012; Ohmori et al. 2003; Salvalaglio et al. 2010; Vanden Heuvel et al. 1992b; Wu et al. 2009; Ylinen and Auriola 1990; Zhang et al. 2009). The dissociation constant for albumin-bound PFOA in serum is approximately 0.4 mM (0.38 mM, ±0.04 SD for human serum albumin; 0.36 nM, ±0.08 SD for rat serum albumin) and involves 6–9 biding sites (Han et al. 2003). Noncovalent binding appears to be at the same sites as fatty acids (Chen and Guo 2009). Interactions between PFOS and human serum albumin include interaction of PFOS polar sulfonyl groups with albumin hydrophilic sites and interaction of perfluorinated groups with albumin hydrophobic sites (Luo et al. 2012).

Absorbed perfluoroalkyls distribute from plasma to soft tissues, with the highest extravascular concentrations achieved in liver. Mechanisms by which perfluoroalkyls enter the liver have not been elucidated and may involve interactions with organic anion transporters that function in the distribution of fatty acids or other organic anions (Andersen et al. 2008). PFOA appears to be a substrate for organic anion transporters in the luminal and basolateral membranes of renal tubular epithelial cells, which facilitates entry of PFOA into renal tubular cells (Kudo et al. 2002; Nakagawa et al. 2008; Vanden Heuvel et al. 1992b; Weaver et al. 2010). The subcellular distribution of PFOA is sex- and dose-dependent in rats (Han et al. 2005; Kudo et al. 2007) and the association with the membrane fraction of liver cells decreases with increasing dose (Kudo et al. 2007), consistent with limited capacity of membrane proteins that bind PFOA (e.g., membrane transport proteins). Intracellular PFOA binds to proteins; protein complexes formed have not been fully characterized. PFOA exhibits a low affinity for binding to rat kidney and urine alpha-2μ-globulin (dissociation constants 1.5 and >2 mM, respectively) (Han et al. 2004).

3.1.3 Metabolism

Results of available intraperitoneal and *in vitro* studies suggest that the perfluoroalkyls discussed in this profile are not metabolized and do not undergo chemical reactions in the body. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Studies conducted in male and female rats did not detect fluorine metabolites in the urine, plasma, or liver following a single injection of 4–150 mg/kg PFOA or 5–50 mg/kg PFDA (Goecke et al. 1992; Vanden Heuvel et al. 1991b, 1991c; Ylinen and Auriola 1990). Following a single intraperitoneal dose of approximately 4 mg/kg of ^{14}C-PFOA, only parent compound was excreted in the

urine and bile (Vanden Heuvel et al. 1991c). PFOA was not metabolized when incubated with microsomal fractions of human or rat intestine, kidney, or liver homogenates (Kemper and Nabb 2005). Although no studies examining metabolism of other perfluoroalkyls, including PFOS, following inhalation, oral, or dermal exposure were identified, metabolism by these exposure routes is not anticipated.

3.1.4 Excretion

As noted in Section 3.1.3 (Metabolism), there is presently no evidence that perfluoroalkyls undergo metabolism. The absence of significant metabolism is attributed to the high stability and low reactivity of carbon-fluorine bonds in perfluoroalkyls. Therefore, route-specific differences in excretion patterns are not expected. Selected studies in which elimination half-lives rates (i.e., $t_{1/2}$) of perfluoroalkyls have been determined (see summaries in Table 3-5) show that, in general, elimination $t_{1/2}$ values are similar following intravenous, intraperitoneal, and oral exposures. Findings suggest that the route of absorption has no substantial effect of rates of elimination of absorbed perfluoroalkyls (Butenhoff et al. 2004c; Chang et al. 2008a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Vanden Heuvel et al. 1991b; Ylinen et al. 1990). As discussed in this section, perfluoroalkyls are primarily eliminated in the urine, with smaller amounts eliminated in the feces, breast milk (see Section 3.1.2; Distribution, Maternal-fetal Transfer), and menstrual fluid. Perfluoroalkyls undergo biliary excretion, but substantial reabsorption occurs; therefore, biliary excretion does not represent a major elimination pathway. Perfluoroalkyls do not appear to be eliminated in sweat, as induction of perspiration by exercise or sauna does not alter clearance of PFOA, PFOA, PFHxA, or PFNA (Genuis et al. 2013). The elimination of perfluoroalkyls in menstrual fluid appears to contribute to sex differences in serum elimination rates (Wong et al. 2014, 2015; Zhang et al. 2013). Only free (unbound) perfluoroalkyls are available for redistribution, excretion, and renal reabsorption; the interaction of perfluoroalkyls with proteins plays a critical role in bioaccumulation, and the tissue environment highly favors protein bonding.

In humans, absorbed perfluoroalkyls are excreted in urine. Estimates of renal clearance of PFOA and PFOS from serum in humans ranged from 0.8 to 3.3 mL/day for PFOA (serum concentration range: 5–16 ng/mL) and 0.1–1.5 mL/day for PFOS (serum concentration range 9–49 ng/mL). These clearance values were <0.001% of glomerular filtration rate (Harada et al. 2005a). Assuming that 99% of the serum PFOA and PFOS was bound to albumin (see Section 3.1.2), <0.1% of filtered perfluoroalkyls were excreted in urine, suggesting extensive reabsorption of filtered PFOA and PFOS in the renal tubule. Renal clearance was not different in males and females. Mean renal clearances for PFOA were

2.12 mL/day (±0.80 SD, n=5) in males and 1.15 (±0.33 SD, n=5) in five females (mean age 22 and 23 years, respectively). Mean renal clearances for PFOS were 0.66 mL/day (±0.48 SD, n=5) in males and 0.91 (±0.56 SD, n=5) for females. Fujii et al. (2015a) reported renal clearances (mL/day/kg; mean±SD) for several perfluoroalkyls in humans (three males and five females), including PFOA (0.044±0.01), PFNA (0.038±0.01), PFDA (0.015±0.01), PFUnA (0.005±0.00), and PFDoDA (0.005±0.00). Zhang et al. (2013) reported renal clearances for several perfluoroalkyls (mL/day/kg) and found that clearance of PFOS was similar in younger females (≤50 years, 0.050 mL/day/kg, 95% CI 0.037–0.064) and a combined group of males and older females (grouped together since there were no significant differences in serum concentrations) (0.037 mL/day/kg, 95% CI 0.026–0.049). However, there appeared to be differences in renal clearance for PFOA; clearance rates were 0.30 mL/day/kg (95% CI 0.11–0.49) in young females and 0.77 mL/day/kg (95% CI 0.47–1.1) in the combined older women and all males group. Urinary excretion of perfluoroalkyls may show sex and age differences (Zhang et al. 2015b). Urinary excretion of PFOA as a fraction of estimated intake in male adults (n=29) was 31% (p=0.002) higher than in nonpregnant female adults (n=25). In addition, urinary excretion of PFOS was inversely correlated with age (r=0.334; p=0.015).

Absorbed PFOA and PFOS are also secreted into bile in humans, but the biliary pathway is not a major excretory pathway because PFOA and PFOS are reabsorbed after biliary secretion. Estimates of total body clearance, serum-to-urine clearance, and serum-to-bile clearance of PFOA and PFOS in humans are presented in Table 3-4 (Harada et al. 2007). Biliary clearances of PFOA and PFOS were 1.06 and 2.98 mL/kg body weight/day, respectively, and greatly exceeded total body clearance (0.150 and 0.106 mL/kg/day) and urinary clearance (0.030 and 0.015 mL/kg/day). Based on these estimates, approximately 89% of the PFOA secreted into bile and 97% of secreted PFOS was estimated to have been reabsorbed from the gastrointestinal tract. Fujii et al. (2015a) also reported that biliary clearances of several perfluoroalkyls (PFOA, PFNA, PFDA, PFUnA, PFDoDA) were much higher than total body clearance in humans, further supporting that perfluoroalkyls excreted in bile undergo extensive reabsorption.

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Serum $t_{1/2}$[a]	day	1,387	1,971
Total clearance[b]	mL/kg/day	0.150	0.106
Urinary clearance[c]	mL/kg/day	0.030	0.150

Table 3-4. Excretory Clearance of PFOA and PFOS in Humans

Parameter	Units	PFOA	PFOS
Biliary clearance[d]	mL/kg/day	1.06	2.98
Reabsorbed from bile[e]	%	89	97

[a]Estimates from Olsen et al. (2005).
[b]$\ln(t_{1/2})$xVd, where Vd is the volume of distribution (300 mL/kg).
[c]Estimates from Harada et al. (2005a).
[d]Estimates from Harada et al. (2007).
[e]1-(Total-Urinary)/Biliary.

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Harada et al. (2007)

Studies conducted in nonhuman primates and rodents provide further evidence that urine is the major route of excretion of perfluoroalkyls, accounting for >93% of absorbed PFOA and PFOS (Benskin et al. 2009; Butenhoff et al. 2004c; Chang et al. 2008a, 2012; Chengelis et al. 2009a; Hanhijarvi et al. 1982, 1987; Hundley et al. 2006; Johnson and Ober 1979, 1980, 1999a, 1999b; Kemper 2003; Kudo et al. 2001; Olsen et al. 2009; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c). Studies conducted in rats have shown that PFDA, PFNA, PFOA, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). PFOS, PFHxS, and PFBS are excreted in feces following intravenous dosing of rats, suggesting that these perfluoroalkyls may also be secreted into bile (Chang et al. 2012; Johnson et al. 1984; Olsen et al. 2009; Sundström et al. 2012). The percentage of the dose excreted in the feces appears to vary with compound, 8–13% for PFOS, <0.5% for PFHxS, and 0.13–0.36% for PFBS. Renal clearances of PFOA from plasma in rats were approximately 0.032 mL/minute/kg body weight in male rats and 0.73 mL/minute/kg in female rats; plasma concentrations of PFOA during these measurements ranged from approximately 0.8 to 80 µg/mL (Kudo et al. 2002). In the latter study, approximately >95% of plasma PFOA was bound to high molecular weight protein and the glomerular filtration rate was approximately 10 mL/minute/kg; therefore, urinary excretion of PFOA was approximately 6% of the rate of glomerular filtration of PFOA in males and 146% in females. These estimates indicate that net renal tubular reabsorption of filtered PFOA occurred in male rats, whereas net renal tubular secretion of PFOA occurred in female rats (i.e., clearance of free PFOA in plasma > glomerular filtration rate). The pronounced sex difference in renal clearance of PFOA has been attributed to modulation of renal excretory transport of PFOA by testosterone and estradiol (Kudo et al. 2002; Vanden Heuvel et al. 1992a; see Section 3.1.5).

Rates of elimination of perfluoroalkyls vary substantially across chemical species and animal species, and show sex differences and age-dependencies within certain species. Table 3-5 summarizes estimates of the elimination $t_{1/2}$ for perfluoroalkyls in humans and experimental animals. In compiling the estimates presented in Table 3-5, preference was given to the terminal $t_{1/2}$ when multiple $t_{1/2}$ values were reported. The significance of the terminal $t_{1/2}$ is that it determines the time required for complete elimination of the perfluoroalkyl as well as the exposure duration required to achieve a steady state. Most of the $t_{1/2}$ values in Table 3-5 were estimated from analyses of data on declining serum concentrations of perfluoroalkyls after a single dose or following cessation of a period of repeated dosing. Estimates of the terminal $t_{1/2}$ based on serum concentrations can vary with the length of the observation period following the last dose and with the modeling approach used to estimate the $t_{1/2}$. Longer observation times are required to estimate the slowest phases of elimination. As a result, estimates of $t_{1/2}$ based on observation periods of 1–2 days can be much shorter than estimates for the same perfluoroalkyl based on observation periods of several weeks. Direct comparisons of $t_{1/2}$ values should be made with consideration of whether or not the observation periods were comparable. Differences in estimation methodology can also contribute to differences in $t_{1/2}$ values. Values reported in Table 3-5 are based on fitting data to single or multi-compartment models, or noncompartmental modeling of the data. While the terminal $t_{1/2}$ provides a metric for comparing times required for complete elimination and steady state, it does not always provide a measure of how rapidly the perfluoroalkyl is cleared from the body. A more useful metric for this is the systemic clearance (Cl_S), typically estimated from the absorbed dose (AD) and the area under the serum concentration curve (AUC_S):

$$Cl_S = \frac{AD}{AUC_S} \qquad \text{Eq. (3-3)}$$

Equation 3-3 will provide an accurate estimate of systemic clearance following an oral dose if the oral dose is completely absorbed. Accurate estimation of AUC_S also depends on fitness of the underlying model used to predict serum concentrations. Estimates of systemic clearance based on pharmacokinetics analyses of serum data from animal studies are presented in Table 3-6.

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFOA—Human					
Human (n=26), adult, M (n=24) F (n=2)	NA	NA	NA	3.8 years (95% CI 3.1–4.4, GM 3.5)	Olsen et al. 2007a
Human (n=20) 15–50 years, M	NA	NA	NA	2.8 years (95% CI 2.4–3.4)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	2.4 years (95% CI 2.0–3.0)	Li et al. 2018
Human (n=66), >50 years, M, F	NA	NA	NA	2.6 years (SE 0.4, GM 1.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.1 years (SE 0.3, GM 1.5)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.9 years	Worley et al. 2017a
Human (n=5), 22±0.9, M	NA	NA	NA	2.3 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	2.6 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	3.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	2.9 years	Harada et al. 2005a
Human (n=200) 54±15, M, F	Oral	NA	NA	2.3 years (95% CI 2.1–2.4)	Bartell et al. 2010
Human (n=643), adult, M, F	Oral	NA	NA	2.9 years (<4 years) (95% CI 2.3–3.8) 10.1 years (>4 years)	Seals et al. 2011
Human (n=1,029), adult, M, F	Oral	NA	NA	8.5 years (<9 years) (95% CI 7.1–10.1)	Seals et al. 2011
Humans (n=17), adult, M, F	Oral	NA	NA	5.1 years (SD 1.7, GM 4.8)	Costa et al. 2009
Humans (n=6) adults, F	Inhalation	NA	NA	2.5 (range 1.8–3.1)	Gomis et a. 2016
PFOS—Human					
Human (n=26), adult, M (24) F (2)	NA	NA	NA	5.4 years (95% CI 3.9–6.9, GM 4.8)	Olsen et al. 2007a
Human (n≅1,000), >12–>80 years, M	NA	NA	NA	4.7 years	Wong et al. 2014

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Human (n≅1,000), >12–>80 years, F	NA	NA	NA	4.3 years (95% CI 4.1–4.5)	Wong et al. 2015
Human (n=66), >50 years, M, F	NA	NA	NA	27 years (SE 3.1, GM 18)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	6.2 years (SE 0.5, GM 5.8)	Zhang et al. 2013
Human (n=45), M, F	NA	NA	NA	3.3 years	Worley et al. 2017a
Human (n=20) 15–50 years M	NA	NA	NA	4.6 years (95% CI 3.7–6.1)	Li et al. 2018
Human (n=30) 15–50 years, F	NA	NA	NA	3.1 years (95% CI 2.7–3.7)	Li et al. 2018
Human (n=5), 22±0.9, M	NA	NA	NA	4.9 years	Harada et al. 2005a
Human (n=5), 68±5, M	NA	NA	NA	7.4 years	Harada et al. 2005a
Human (n=5), 23±3, F	NA	NA	NA	4.5 years	Harada et al. 2005a
Human (n=5), 69±5, F	NA	NA	NA	4.6 years	Harada et al. 2005a
PFHxS—Human					
Human (n=26), adult, M (24), F (2)	NA	NA	NA	8.5 years (95% CI 6.4–10.6, GM 7.3)	Olsen et al. 2007a
Human (n=20), ≤50 years, F	NA	NA	NA	7.7 years (SE 0.6, GM 7.1)	Zhang et al. 2013
Human (n=20) 15–50 years, M	NA	NA	NA	7.4 years (95% CI 6.0–9.7)	Li et al. 2018
Human (n=30) 15–50 years F	NA	NA	NA	4.7 years (95% CI 3.9–5.9)	Li et al. 2018
Human (n=45), M, F	NA	NA	NA	15.5 years	Worley et al. 2017a
Human (n=66), >50 years, M, F	NA	NA	NA	35 years (SE 3.9, GM 25)	Zhang et al. 2013
PFBA—Human					
Human (n=3), adult, M	NA	NA	NA	81 hours (SD 41)	Chang et al. 2008b
Human (n=9), adult, M (7), F (2)	NA	NA	NA	72 hours (SD 38)	Chang et al. 2008b
PFBS—Human					
Human (n=6), adult M (5), F(1)				665 hours (SD 266)	Olsen et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFNA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	4.3 years (SE 0.5, GM 3.2)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	2.5 years (SE 0.6, GM 1.7)	Zhang et al. 2013
PFDA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 1.5, GM 7.1)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.4, GM 4.0)	Zhang et al. 2013
PFUnA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	12 years (SE 2.0, GM 7.4)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	4.5 years (SE 0.5, GM 4.0)	Zhang et al. 2013
PFHpA—Human					
Human (n=66), >50 years, M, F	NA	NA	NA	1.2 years (SE 0.2, GM 0.82)	Zhang et al. 2013
Human (n=20), ≤50 years, F	NA	NA	NA	1.5 years (SE 0.3, GM 1.0)	Zhang et al. 2013
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	10 mg/kg/day	6 months	20.1 days	Butenhoff et al. 2004c
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	20.9 days (SD 12.5)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	32.6 days (SD 8.0)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.15 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, F	Oral	0.75 mg/kg/day	6 months	170 days	Seacat et al. 2002
Cynomolgus monkey, adult, M	IV	2 mg/kg	1 day	132 days (SE 7)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2 mg/kg	1 day	110 days (SE 15)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	5.3 days (SD 2.5)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	2.4 days (SD 1.7)	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	141 days (SE 30.)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	87 days (SE 27)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	40.3 hours (SD 2.4)	Chang et al. 2008b
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	41.0 hours (SD 4.7)	Chang et al. 2008b
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	15.0 hours (SD 9.8)	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	8.0 hours (SD 2.0)	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10 mg/kg	1 day	95.2 hours (SE 27.1)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10 mg/kg	1 day	83.2 hours (SE 41.9)	Olsen et al. 2009
PFOA—Rat					
Rat (CR), adult, M	Oral	11.4 mg/kg	1 day	115 hours	Johnson and Ober 1980
Rat (Sprague-Dawley), adult, M	Oral	0.1 mg/kg	1 day	202 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	138 hours (SD 32)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1 mg/kg	1 day	44 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	5 mg/kg	1 day	174 hours (SD 29)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25 mg/kg	1 day	157 hours (SD 38)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	185 hours (SD 19)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1 mg/kg	1 day	39 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	0.4 mg/kg	1 day	322 hours (SD 38)	Benskin et al. 2009

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	Oral	0.022 mg/kg/day	12 weeks	218 hours (95% CL 127–792)	De Silva et al. 2009
Rat (Wistar), adult, M	IV	21.5 mg/kg	1 day	136 hours (SD 24)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1 mg/kg	1 day	135 hours (SD 29)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, M	IP	3.9 mg/kg	1 day	216 hours (SE 30.9)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, M	IP	50 mg/kg	1 day	105 hours	Ylinen et al. 1990
Rat (Sprague-Dawley), adult, F	Oral	0.1 mg/kg	1 day	3.2 hours (SD 0.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.5 hours (SD 1.1)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1 mg/kg	1 day	3.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5 mg/kg	1 day	4.6 hours (SD 0.6)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25 mg/kg	1 day	16.2 hours (SD 9.9)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	2.8 hours (SD 0.5)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1 mg/kg	1 day	4.6 hours	Kim et al. 2016b
Rat (Wistar), adult, F	IV	21.5 mg/kg	1 day	1.9 hours (SD 0.7)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	20.1 mg/kg	1 day	1.9 hours (SD 0.7)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	3.9 mg/kg	1 day	2.9 hours (SE 0.2)	Vanden Heuvel et al. 1991c
Rat (Wistar), adult, F	IP	50 mg/kg	1 day	24 hours	Ylinen et al. 1990
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4.2 mg/kg	1 day	179 hours	Johnson and Ober 1979
Rat (Sprague-Dawley), adult, M	Oral	0.27 mg/kg	1 day	809 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.023 mg/kg/day	12 weeks	1,968 hours (95% CL 1.584–2.568)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1,495 hours (SE 50)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	635 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	15 mg/kg	1 day	1,707 hours (SE 270)	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, F	Oral	0.023 mg/kg/day	12 weeks	1,992 hours (95% CL 1,752–2,280)	De Silva et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	919 hours (SE 56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	564 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	15 mg/kg	1 day	989 hours (SE 48)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2 mg/kg	1 day	689 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	2 mg/kg	1 day	595 hours	Kim et al. 2016b
FOSA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	5.0 mg/kg	1 day	125 hours	Seacat and Luebker 2000
PFDA—Rat					
Rat (Sprague-Dawley), adult, M	IP	4.8 mg/kg	1 day	1,008 hours	Vanden Heuvel et al. 1991b
Rat (Wistar), adult, M	IV	25 mg/kg	1 day	958 hours (SD 207)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25 mg/kg	1 day	1,406 hours (SD 140)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	IP	4.8 mg/kg	1 day	552 hours	Vanden Heuvel et al. 1991b
PFNA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.2 mg/kg	1 day	974 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	0.029 mg/kg/day	12 weeks	1,128 hours (95% CL 935–1,416)	De Silva et al. 2009
Rat (Sprague-Dawley), adult M	Oral	1, 3, or 10 mg/kg	1 day	734.4 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, M	IV	22.6 mg/kg	1 day	710 hours (SD 55)	Ohmori et al. 2003
Rat (Sprague-Dawley), adult, F	Oral	1, 3, or 10 mg/kg	1 day	33.6 hours	Tatum-Gibbs et al. 2011
Rat (Wistar), adult, F	IV	22.6 mg/kg	1 day	58.6 hours (SD 9.8)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7 mg/kg	1 day	2.4 hours (SD 1.2)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7 mg/kg	1 day	1.2 hours (SD 0.2)	Ohmori et al. 2003

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	1.0 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg	1 day	2.4 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg	1 day	2.5 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.42 hour	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg	1 day	2.6 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg	1 day	2.2 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, M	Oral	2 mg/kg	1 day	1.7 hours (SD 0.6)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	10 mg/kg	1 day	0.5 hours (SD 0.1)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	2 mg/kg	1 day	1.5 hours (SD 0.2)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, F	Oral	10 mg/kg	1 day	0.7 hours (SD 0.3)	Gannon et al. 2011
Rat (Sprague-Dawley), adult, M	Oral	50 mg/kg/day	26 days	2.0 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	150 mg/kg/day	26 days	2.1 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, M	Oral	300 mg/kg/day	26 days	2.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	50 mg/kg/day	26 days	1.9 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	150 mg/kg/day	26 days	2.2 hours	Kirkpatrick 2005
Rat (Sprague-Dawley), adult, F	Oral	300 mg/kg/day	26 days	3.0 hours	Kirkpatrick 2005
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.030 mg/kg	1 day	382 hours	Benskin et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	4 mg/kg	1 day	645.6 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	4 mg/kg	1 day	41.28 hours	Kim et al. 2016b

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
Rat (Sprague-Dawley), adult, M	IV	4 mg/kg	1 day	496.8 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	688 hours (SE 14.4)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	IV	4 mg/kg	1 day	21.12 hours	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	39 hours (SE 1.9)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	9.22 hours (SE 0.75)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, M	IV	30 mg/kg	1 day	6.38 hours (SE 0.53)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	1.76 hours (SE 0.26)	Chang et al. 2008b
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	1.03 hours (SE 0.03)	Chang et al. 2008b
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10 mg/kg	1 day	2.1 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), Rat (SD), adult, M	IV	30 mg/kg	1 day	4.51 hours (SE 2,22)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, M	Oral	30 mg/kg	1 day	4.68 hours (SE 0.07)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	10 mg/kg	1 day	0.64 hours	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	30 mg/kg	1 day	3.96 hours (SE 0.21)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	Oral	30 mg/kg	1 day	7.42 hours (SE 0.79)	Olsen et al. 2009
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	1,027 hours	Chang et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	874 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	907 hours	Chang et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	731 hours	Chang et al. 2012

Table 3-5. Summary Elimination Half-Lives for Perfluoroalkyls Estimated in Humans and Experimental Animals

Species, age, and sex	Route	Dose	Exposure duration[a]	Elimination half-life[b]	Reference
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1 mg/kg	1 day	732 hours	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20 mg/kg	1 day	671 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1 mg/kg	1 day	597 hours	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20 mg/kg	1 day	643 hours	Sundström et al. 2012
PFNA—Mouse					
Mouse (CD-1), adult, M	Oral	1 or 10 mg/kg	1 day	823.2–1,653.6 hours	Tatum-Gibbs et al. 2011
Mouse (CD-1), adult, F	Oral	1 or 10 mg/kg	1 day	619.2–1,641.6 hours	Tatum-Gibbs et al. 2011
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10 mg/kg	1 day	13.34 hours (SE 4.55)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30 mg/kg	1 day	16.3 hours (SE 7.2)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100 mg/kg	1 day	5.22 hours (SE 2.27)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10 mg/kg	1 day	2.87 hours (SE 0.30)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30 mg/kg	1 day	3.08 hours (SE 0.26)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100 mg/kg	1 day	2.79 hours (SE 0.3)	Chang et al. 2008b
PFOS—Rabbit					
Rabbit (New Zealand), adult, F	Oral	0.085 mg/kg/day	102 days	87 days (SD 31)	Tarazona et al. 2016

[a]Exposure durations of 1 day indicate that a single dose was administered.
[b]Reported half-lives are arithmetic means for the terminal elimination phase if multiple elimination phases were observed.

CI = confidence interval; CL = confidence limit; F = female; GM = geometric mean; IP = intraperitoneal; IV = intravenous; M = male; NA = not applicable; PFBA = perfluorobutanoic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; FOSA = perfluorooctane sulfonamide; SD = standard deviation; SE = standard error

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFOA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	12.4 (SD 7.4)	Butenhoff et al. 2004c
Cynomolgus monkey, adult, F	IV	10	1 day	5.3 (SD 3.3)	Butenhoff et al. 2004c
PFOS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	2	1 day	1.10 (SE 0.06)	Chang et al. 2012
Cynomolgus monkey, adult, F	IV	2	1 day	1.65 (SE 0.04)	Chang et al. 2012
PFHxA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	569	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	535	Chengelis et al. 2009a
PFHxS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	1.3 (SE 0.1)	Sundström et al. 2012
Cynomolgus monkey, adult, F	IV	10	1 day	1.9 (SE 0.4)	Sundström et al. 2012
PFBA—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	2,371 (SE 293)	Chang et al. 2008a
Cynomolgus monkey, adult, F	IV	10	1 day	1,075 (SE 91)	Chang et al. 2008a
PFBS—Nonhuman primate					
Cynomolgus monkey, adult, M	IV	10	1 day	159	Chengelis et al. 2009a
Cynomolgus monkey, adult, F	IV	10	1 day	238	Chengelis et al. 2009a
Cynomolgus monkey, adult, M	IV	10	1 day	12,264 (SE 3384)	Olsen et al. 2009
Cynomolgus monkey, adult, F	IV	10	1 day	8,832 (SE 2880)	Olsen et al. 2009
PFOA—Rat					
Rat (Sprague-Dawley), adult, M	Oral	0.1	1 day	23.1 (SD 5.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	20.9 (SD 3.8)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	1	1 day	40.40 (SD 2.29)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	Oral	5	1 day	20.4 (SD 5.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	Oral	25	1 day	27.1 (SD 7.4)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	21.5 (SD 2.0)	Kemper 2003
Rat (Sprague-Dawley), adult, M	IV	1	1 day	47.39 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	0.1	1 day	778 (SD 144)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	655 (SD 173)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	1	1 day	645.12 (SD 43.44)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	5	1 day	1,164 (SD 118)	Kemper 2003
Rat (Sprague-Dawley), adult, F	Oral	25	1 day	842 (SD 166)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	816 (SD 221)	Kemper 2003
Rat (Sprague-Dawley), adult, F	IV	1	1 day	612.84 (SD 32.54)	Kim et al. 2016b
Rat (Wistar), adult, M	IV	21.5	1 day	50.4 (SD 14.4)	Kudo et al. 2002
Rat (Wistar), adult, F	IV	21.5	1 day	2,233 (SD 805)	Kudo et al. 2002
Rat (Wistar), adult, M	IV	20.1	1 day	135 (SD 29)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	20.1	1 day	2,233 (SD 805)	Ohmori et al. 2003
PFOS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	7.33 (SD 0.55)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	Oral	2	1 day	11.3 (SE 0.56)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	Oral	15	1 day	4.9 (SE 0.52)	Chang et al. 2012
Rat (Sprague-Dawley), adult, M	IV	2	1 day	9.24 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	8.52 (SD 0.37)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	Oral	2	1 day	22.2 (SE 0.28)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	15	1 day	5.4 (SE 20)	Chang et al. 2012
Rat (Sprague-Dawley), adult, F	IV	2	1 day	9.82 (SD 0.21)	Kim et al. 2016b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFDA—Rat					
Rat (Wistar), adult, M	IV	25	1 day	207 (SD 0.054)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	25	1 day	140 (SD 0.008)	Ohmori et al. 2003
PFNA—Rat					
Rat (Wistar), adult, M	IV	22.6	1 day	6.9 (SD 0.6)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	22.6	1 day	106 (SD 31)	Ohmori et al. 2003
PFHpA—Rat					
Rat (Wistar), adult, M	IV	17.7	1 day	1,604 (SD 558)	Ohmori et al. 2003
Rat (Wistar), adult, F	IV	17.7	1 day	3,071 (SD 781)	Ohmori et al. 2003
PFHxA—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	2,784	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	18,600	Chengelis et al. 2009a
PFHxS—Rat					
Rat (Sprague-Dawley), adult, M	Oral	4	1 day	7.15 (SD 0.06)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	4	1 day	9.01 (SD 0.05)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, M	IV	10	1 day	6.7 (SE 0.06)	Sundström et al. 2012
Rat (Sprague-Dawley), adult, F	Oral	4	1 day	124.83 (SD 3.40)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	4	1 day	227.93 (SD 6.73)	Kim et al. 2016b
Rat (Sprague-Dawley), adult, F	IV	10	1 day	53.4 (SE 4.38)	Sundström et al. 2012
PFBA—Rat					
Rat (Sprague-Dawley), adult, M	IV	30	1 day	851 (SE 61)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	IV	30	1 day	2,949 (SE 59)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, M	Oral	30	1 day	494 (SE 29)	Chang et al. 2008a
Rat (Sprague-Dawley), adult, F	Oral	30	1 day	1,527 (SE 145)	Chang et al. 2008a
PFBS—Rat					
Rat (Sprague-Dawley), adult, M	IV	10	1 day	946	Chengelis et al. 2009a
Rat (Sprague-Dawley), adult, F	IV	10	1 day	7,464	Chengelis et al. 2009a

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
Rat (Sprague-Dawley), adult, M	IV	30	1 day	2,856 (SE 816)	Olsen et al. 2009
Rat (Sprague-Dawley), adult, F	IV	30	1 day	11,265 (SE 960)	Olsen et al. 2009
PFOA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.13	1 day	14.2 (SD 8.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.13	1 day	11.8 (SD 6.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.3	1 day	13.1 (SD 7.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.3	1 day	9.0 (SD 1.9)	Fujii et al. 2015a, 2015b
PFOS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.7	Chang et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	5.0	Chang et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	6.0	Chang et al. 2012
PFHxS—Mouse					
Mouse (CD), adult, M	Oral	1	1 day	2.9	Sundström et al. 2012
Mouse (CD), adult, M	Oral	20	1 day	4.8	Sundström et al. 2012
Mouse (CD), adult, F	Oral	1	1 day	2.7	Sundström et al. 2012
Mouse (CD), adult, F	Oral	20	1 day	3.8	Sundström et al. 2012
PFNA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.14	1 day	3.9 (SD 1.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.14	1 day	5.1 (SD 2.3)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.4	1 day	4.0 (SD 1.7)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.4	1 day	2.4 (SD 1.0)	Fujii et al. 2015a, 2015b
PFDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.16	1 day	2.2 (SD 0.9)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.16	1 day	2.8 (SD 1.2)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.6	1 day	3.9 (SD 1.8)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.6	1 day	2.2 (SD 1.1)	Fujii et al. 2015a, 2015b

Table 3-6. Summary Systemic Clearance for Perfluoroalkyls Estimated in Experimental Animals

Species, age, and sex	Route	Dose (mg/kg)	Exposure duration	Systemic clearance (mL/day/kg)[a]	Reference
PFUnA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.17	1 day	2.8 (SD 1.0)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.17	1 day	3.4 (SD 1.5)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.7	1 day	5.7 (SD 2.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.7	1 day	3.1 (SD 1.7)	Fujii et al. 2015a, 2015b
PFDoDA—Mouse					
Mouse (FVB/NJcl), adult, M	IV	0.19	1 day	4.4 (SD 1.6)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	IV	0.19	1 day	4.8 (SD 2.4)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, M	Oral	1.9	1 day	9.4 (SD 4.1)	Fujii et al. 2015a, 2015b
Mouse (FVB/NJcl), adult, F	Oral	1.9	1 day	5.2 (SD 3.2)	Fujii et al. 2015a, 2015b
PFBA—Mouse					
Mouse (CD1), adult, M	Oral	10	1 day	280 (SE 72)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	30	1 day	296 (SE 640)	Chang et al. 2008b
Mouse (CD1), adult, M	Oral	100	1 day	784 (SE 112)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	10	1 day	564 (SE 24)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	30	1 day	696 (SE 32)	Chang et al. 2008b
Mouse (CD1), adult, F	Oral	100	1 day	1,336 (SE 64)	Chang et al. 2008b

[a]As reported in units of mL/day/kg or converted from mL/hour (x24), mL/hour (x24/body weight) or mL/minute (x60x24).

CI = confidence interval; F = female; IV = intravenous; M = male; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; SD = standard deviation; SE = standard error

Elimination of Perfluoroalkyls in Humans. Elimination $t_{1/2}$ values for PFOA, PFOS, PFHxS, PFBA, and PFBS have been estimated in humans (Bartell et al. 2010; Costa et al. 2009; Chang et al. 2008a; Glynn et al. 2012; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a, 2009; Seals et al. 2011; Spliethoff et al. 2008; Yeung et al. 2013; Wong et al. 2014, 2015; Worley et al. 2017a; Zhang et al. 2013). Estimates in humans are based on measurements of the decline in serum perfluoroalkyl concentrations following cessation or an abrupt decrease in exposure, or on measurements of renal plasma clearance

from serum in a general population sample from Japan (Harada et al. 2005a). The latter clearance estimates were converted to $t_{1/2}$ values, for display in Table 3-5 as follows (Equations 3-4 and 3-5):

$$k_e = \frac{Cl}{V} \qquad \text{Eq. (3-4)}$$

$$t_{1/2} = \frac{\ln(2)}{k_e} \qquad \text{Eq. (3-5)}$$

where k_e is the elimination rate constant (e.g., day^{-1}), Cl is the renal plasma clearance (e.g., mL plasma/day/kg), and V is the plasma volume (L/kg), which is assumed to be 4.3% of body weight (ICRP 1981). In general, these studies show that longer chain length is associated with slower elimination rates. For example, the elimination $t_{1/2}$ for PFBA was estimated to be 70–80 hours (Chang et al. 2008a), whereas the $t_{1/2}$ values for PFHxS, PFOS, and PFOA range from 2 to 35 years (Bartell et al. 2010; Harada et al. 2005a; Li et al. 2018; Olsen et al. 2007a; Seals et al. 2011; Worley et al. 2017a; Zhang et al. 2013). Longer $t_{1/2}$ values for PFOA have been reported with longer monitoring follow-up times, which allow the detection of slower elimination phases of multiphasic elimination kinetics (Seals et al. 2011). Perfluoroalkyl sulfonates are eliminated more slowly in humans than corresponding carboxylates of the same chain length (Zhang et al. 2013). Analytical methods typically used to measure serum perfluoroalkyls do not discriminate between linear and branched isomers and, as a result, these studies estimate elimination rates for the isomer mixture. A study that compared elimination rates of isomers of PFOA found that linear isomers tend to be eliminated more slowly than branched isomers (Zhang et al. 2013), consistent with results of studies conducted in rats (Benskin et al. 2009; De Silva et al. 2009).

An analysis of serum PFOS data from NHANES indicated that $t_{1/2}$ in females may be shorter (4.3 years) compared to males (4.7 years; Wong et al. 2014, 2015). The NHANES data are cross-sectional and, therefore, the estimates of $t_{1/2}$ required fitting the data to age patterns of PFOS intake. An improved fit to the data for females was achieved when estimated losses of PFOS in menstrual fluids were considered, suggesting that menstrual loss of PFOS may account for some, but not all, of the sex difference in the elimination rate (Verner and Longnecker 2015; Wong et al. 2015). Li et al. (2018) also found apparent sex differences in PFOS elimination in male and female residents in Sweden exposed to contaminated drinking water. The estimated $t_{1/2}$ for PFOS were 4.6 years in males and 3.1 years in females. Zhang et al. (2013) estimated serum $t_{1/2}$ for various age and sex strata in a population of 86 individuals. Serum $t_{1/2}$ for PFOS was lower for PFOS in younger females (≤50 years, $t_{1/2}$ = 6.2 years±0.3 SE, n=66) compared to males and older females ($t_{1/2}$ =27 years±3.1 SE, n=20). Zhang et al. (2013) attributed the difference in serum $t_{1/2}$ to clearance in menstrual fluids. However, the estimated serum $t_{1/2}$ of 27 years is much higher than values calculated from other studies; Zhang et al. (2013) noted that the serum $t_{1/2}$ should be

considered as an upper limit estimate. Estimated $t_{1/2}$ for PFOA was not different in younger females (2.1±0.3 SE, n=20) compared to males and older females (2.6±0.4 SE, n=66). Declines in serum PFOA concentrations were observed in populations following initiation of activated carbon filtration of public water supplies that had been contaminated with PFOA (Bartell et al. 2010). The estimated mean serum $t_{1/2}$ for a group of 200 adults followed for 1 year after filtration was initiated was 2.3 years (95% CI 2.1–2.4). Elimination rates were not different in males and females. Serum PFOA concentration ranged from 16 to 1,200 ng/mL. A larger follow-up study measured serum PFOA concentrations in two populations of former residents (n=1,672) of the same water districts (Seals et al. 2011). In one population (n=643), the serum $t_{1/2}$ increased with increasing elapsed time since leaving the water district. The $t_{1/2}$ values were 2.9 years (95% CI 2.3–3.8) for elapsed time of <4 years and 10.1 years for elapsed time of >4 years. In a second population with an elapsed time since residence of <9 years, the $t_{1/2}$ was 8.5 years (95% CI 7.1–10.1). Elimination rates (based on the annual percent decrease in serum concentrations) were faster in males (27%) compared to females (18%) for the first 4 years post-exposure; however, no difference was evident between sexes when elapsed time from exposure was >4 years.

Bartell (2012) and Russell et al. (2015) point out that most studies examining PFOA elimination half-lives fail to account for ongoing background exposure, which could result in an overestimation of elimination half-lives. Bartell (2012) estimated that the bias from background exposure could resulted in 1–26% overestimation of calculated PFOA half-lives and that greater overestimations can occur for half-lives based on longer follow-up times. Russell et al. (2015) estimated that the bias was greatest in populations with serum PFOA levels closest to background levels. In a re-analysis of the Olsen et al. (2007) occupational exposure data, Russell et al. (2015) estimated that overestimation was approximately 1.2% in workers with initial serum concentrations >500 ng/mL (100 times higher than NHANES general population data) and 13% for workers with lower initial serum PFOA levels. Restricting the elimination half-life calculation to workers with initial serum PFOA levels of >500 ng/mL would result in a half-life of 3.0 years (Russell et al. 2015), compared to 3.8 years calculated for the whole cohort (Olsen et al. 2007).

Analysis of kinetics of serum PFOS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) yielded a mean serum elimination $t_{1/2}$ estimate of 5.4 years (95% CI 3.9–6.9; geometric mean: 4.8 years, 95% CI 4.0–5.8) in subjects whose serum PFOS concentrations ranged from 37 to 3,490 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 4.9 and 6.8 years. Estimates based on renal clearance of PFOS from serum in subjects from the general population of Japan ranged from 2.9 to 7.4 years; these subjects had serum PFOS concentrations that

ranged from 4 to 49 ng/mL (Harada et al. 2005a). Estimates in males (7.4, 2.9 years) were similar to females (4.5, 4.6 years). This same study measured serum PFHxS concentrations in retired U.S. fluorochemical production workers (24 males, 2 females) and yielded a mean estimate of 8.5 years (95% CI 6.4–10.6; geometric mean: 7.3 years, 95% CI 5.8–9.2) for the serum elimination $t_{1/2}$ in subjects whose serum PFHxS concentrations ranged from 10 to 1,295 ng/mL (Olsen et al. 2007a). Estimates for the two females in the same study were 12.2 and 13.3 years.

The elimination rate of PFBA was estimated in fluorochemical workers who may have been exposed to various PFBA precursors (Chang et al. 2008a). In three male workers, the estimated mean $t_{1/2}$ based on serum PFBA kinetics was 81 hours (±41 SD). In a larger study of nine workers (seven males, two females), the mean $t_{1/2}$ was 72 hours (±38 SD). Estimates for the two female subjects were 56 and 118 hours. The combined mean value for the 12 estimates was 75 hours (±38 SD). Olsen et al. (2009) estimated serum $t_{1/2}$ of PFBS in six fluorochemical workers. The mean $t_{1/2}$ was 27.4 days (±11.1 SD). The group included a single female whose $t_{1/2}$ was 45.7 days. Based on these observations, PFBA (chain length 3) and PFBS (chain length 4) are eliminated substantially faster in humans than perfluoroalkyls having longer carbon chain lengths, such as PFHxS (chain length 6), PFOA (chain length 7), and PFOS (chain length 8).

Temporal trends in perfluoroalkyl serum concentrations have also been used to estimate population halving times (Glynn et al. 2012; Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Population halving times are influenced by temporal trends in intakes and may therefore not accurately reflect clearance. Population halving times for PFOS ranged from 4 to 5 years (Olsen et al. 2012; Spleithoff et al. 2008; Yeung et al. 2013). Glynn et al. (2012) monitored serum perfluoroalkyls in a population of pregnant women (n=413) in Sweden over the period 1996–2010. Halving times were 22 years (95% CI 16–38) for PFOA and 8.2 years (95% CI 6.3–12) for PFOS.

Elimination of Perfluoroalkyls in Nonhuman Primates. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, PFHxA, PFHxS, PFBA, and PFBS have been estimated in Cynomolgus monkeys (Buttenoff et al. 2004c; Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Seacat et al. 2002; Sundström et al. 2012). Estimated terminal $t_{1/2}$ values were 20–30 days for PFOA, 100–170 days for PFOS, 90–140 days for PFHxS, 40 hours for PFBA and 8–95 hours for PFBS. Elimination of perfluoroalkyls in monkeys is multiphasic and, as a result, estimates of the terminal $t_{1/2}$ can vary with the duration of the observation period and assumptions made in modeling elimination kinetics (Chang et al. 2012; Chengelis et al. 2009a; Olsen et al. 2009; Sundström et al. 2012). For example, the $t_{1/2}$ values for

PFBS were 8 and 15 hours in female and male monkeys, respectively, when monkeys were monitored for 48 hours following a single intravenous dose (Chengelis et al. 2009a), whereas the $t_{1/2}$ values were 95 and 83 hours in male and female monkeys, respectively, when the monitoring period was extended to 14 days and a three-compartment model was used to estimate the terminal $t_{1/2}$ (Olsen et al. 2009). Studies in monkeys confirm general trends observed in humans that perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and that elimination of longer-chain perfluoroalkyls occurs more slowly than short-chain perfluoroalkyls. Systemic clearances were lower for PFOS, PFHxS, and PFBS compared to the corresponding carboxylates, PFOA, PFHxA, and PFBA (Table 3-6). Systemic clearances were similar in male and female monkeys (Table 3-6).

Elimination of Perfluoroalkyls in Rats. Elimination $t_{1/2}$ values and systemic clearances for PFOA, PFOS, FOSA, PFDA, PFNA, PFHpA, PFHxA, PFHxS, PFBA, and PFBS have been estimated in rats (Benskin et al. 2009; Chang et al. 2008b, 2012; Chengelis et al. 2009a; De Silva et al. 2009; Johnson and Ober 1979; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Olsen et al. 2009; Seacat and Luebker 2000; Sundström et al. 2012; Vanden Heuvel et al. 1991b, 1991c; Ylinen et al. 1990). Consistent with observations made in humans and Cynomolgus monkeys, perfluoroalkyl sulfonates are more slowly eliminated than perfluoroalkyl carboxylates and short-chain perfluoroalkyls (e.g., PFBA, PFBS) are eliminated faster in rats than long-chain perfluoroalkyls (e.g., PFOA, PFOS, PFHxA, PFHxS); Tables 3-5 and 3-6. Linear PFOA isomers tend to be eliminated more slowly than branched isomers (Benskin et al. 2009; De Silva et al. 2009).

Elimination of perfluoroalkyls exhibits pronounced sex differences in rats, with faster elimination in females than in males (Benskin et al. 2009; Chang et al. 2008b; Chengelis et al. 2009a; Kemper 2003; Kim et al. 2016b; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012; Tatim-Gibbs et al. 2011; Vanden Heuvel et al. 1991c; Ylinen et al. 1990). Estimates of systemic clearance for PFOA in male rats ranged from 20 to 50 mL/day/kg, whereas estimates for female rats ranged from 600 to 2,200 mL/day/kg (Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003). Systemic clearances of PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS are also higher in female rats compared to male rats (Table 3-6). Pronounced sex difference in elimination rates in rats (faster elimination in females) was observed in rats following 30-minute nose-only exposures to aerosols (MMAD=1.9–2.1 μm) of 1–25 mg ammonium PFOA/m^3 (Hinderliter et al. 2006a). Plasma PFOA concentrations were not detectable 12 hours after exposure of female rats, and were approximately 90% of peak plasma concentrations 24 hours after the exposure in male rats. The slower elimination of PFOA in male rats resulted in steady-state plasma concentrations within 3 weeks of repeated exposures (6 hours/day, 5 days/week) in male rats, whereas in

female rats, daily periodic oscillations of plasma concentrations from peak to below detection occurred on each day of exposure. Steady-state plasma concentrations in male rats were approximately 10 times that of daily peak concentrations in female rats.

Pronounced dose dependence appears in the $t_{1/2}$ estimates for PFOA in female rats. With increasing dose, plasma elimination kinetics in female rats converts from monophasic to biphasic. Following an oral dose of PFOA of 0.1, 1, 5, or 25 mg/kg, the terminal $t_{1/2}$ values in female rats were 3.2, 3.5, 4.6, or 16.2 hours, respectively; no apparent dose dependence was observed in male rats over the same dose range (Kemper 2003). Dose-dependent elimination of PFOA has been attributed to a capacity-limited renal tubular secretion of PFOA in female rats (see discussion below on *Mechanisms of Excretion*). The divergence in elimination kinetics between male and female rats appears to be age-dependent, with faster elimination becoming evident in female rats after 30 days of age, consistent with the timing of sexual maturation and involvement of sex hormones in the modulation of the renal excretion of PFOA in rats (Hinderliter et al. 2006b).

Elimination of Perfluoroalkyls in Mice. Elimination $t_{1/2}$ values and systemic clearances for PFOS, PFHxS, and PFBA have been estimated in mice (Chang et al. 2008a, 2012; Sundström et al. 2012). Consistent with studies conducted in rats and monkeys, PFBA is eliminated more rapidly in mice than PFOS and PFHxS. Systemic clearances ranged from 5 to 6 mL/day/kg for PFOS (Chang et al. 2012), from 3 to 5 mL/day/kg for PFHxS (Sundström et al. 2012), and from 300 to 1,300 mL/day/kg for PFBA (Chang et al. 2008a). Sex differences in elimination in mice were observed for PFBA, but not PFOS or PFHxS. Systemic clearances of PFBA in female mice were approximately 2 times that of males (Chang et al. 2008a). Systemic clearance of PFBA in male and female mice appeared to be dependent on dose. Systemic clearance following a single oral dose of 100 mg PFBA/kg was approximately 2 times higher than the systemic clearance following a dose of 10 or 30 mg PFBA/kg. Possible explanations for the apparent dependence of clearance on dose are dose-dependent bioavailability or that the one-compartment model used to estimate elimination rates and serum AUC did not adequately fit the serum kinetics observed at the higher dose (Chang et al. 2008a). The latter could occur if renal tubular reabsorption of PFBA or plasma protein binding of PFBA is saturable in mice. Systemic clearance rates for PFOA were similar in male mice (13.1 mL/kg/day) and in female mice (9.0 mL/kg/day) (Fuji et al. 2015a, 2015b).

Elimination of Perfluoroalkyls in Other Species. Sex differences in elimination of PFOA have also been observed in hamsters; unlike the rat, male hamsters excreted absorbed PFOA more rapidly than female hamsters. Following a single gavage dose of 10 mg/kg as ammonium [^{14}C]PFOA, cumulative excretion

of ^{14}C in urine at 24 hours post-dosing was 96.4% of the dose in female rats and 8.7% in male rats; 24.6% and 84.5% in female and male hamsters, respectively; 4.1% in male and female mice; and 90.5 and 80.2% in female and male rabbits, respectively (Hundley et al. 2006).

Mechanisms of Excretion. Urinary excretion of perfluoroalkyls involves glomerular filtration and renal tubular secretion and reabsorption (for PFOA, see Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). Glomerular filtration of PFOA is limited by extensive binding of PFOA to albumin and other high molecular weight proteins in plasma (Han et al. 2003, 2005; Ohmori et al. 2003; Kerstner-Wood et al. 2003; Vanden Heuvel et al. 1992a, 1992b; Ylinen and Auriola 1990). Elimination of PFOA and other perfluoroalkyls shows pronounced sex differences in rats, with slower elimination in males for PFOA, PFOS, PFNA, PFHxA, PFHxS, PFBA, and PFBS (Chang et al. 2008a, 2012; Chengelis et al. 2009a; Kemper 2003; Kudo et al. 2002; Ohmori et al. 2003; Sundström et al. 2012). The sex difference in PFOA elimination in rats is dependent on testosterone (Hinderliter et al. 2006b; Kudo et al. 2002; Vanden Heuvel et al. 1992a). The significantly slower elimination of PFOA in adult male rats compared to female rats has been attributed to sex hormone modulation of organic anion transporters in kidney. At similar doses administered to male and female rats, PFOA undergoes net tubular reabsorption in male rats (i.e., urinary excretion rate < rate of glomerular filtration of PFOA) and net tubular secretion in female rats (i.e., urinary excretion rate > rate of glomerular filtration of PFOA) (Harada et al. 2005a; Kudo et al. 2002; Ohmori et al. 2003). In rats, several transporters have been shown to have affinity for C7–C9 perfluoroalkyl carboxylates. The transporters, OAT1 and OAT3, located on the basolateral membrane of the renal proximal tubule, appear to participate in secretion of C7–C9 perfluoroalkyl carboxylates into the tubular fluid (Nakagawa et al. 2008; Weaver et al. 2010). The transporters, OATP1a1 (rat), OAT4 (human), and URAT1 (human), located on the apical membrane, appear to mediate reabsorption of C8–C10 perfluoroalkyl carboxylates from the tubular fluid (Katakura et al. 2007; Nakagawa et al. 2009; Weaver et al. 2010; Yang et al. 2009, 2010). In rats and mice, expression of OAT1, OAT3, and OATP1a1 is controlled by male sex hormones and shows higher activities in males (Buist and Klaassen 2004; Gotoh et al. 2002; Kobayashi et al. 2002; Li et al. 2002; Lu et al. 1996; Lubojevic et al. 2004). The slower elimination of PFOA (and other long-chain perfluoroalkyl carboxylates) in male rats has been attributed to OATP1a1 (Weaver et al. 2010; Yang et al. 2009). Higher activity of OATP1a1 in male rats results in higher reabsorptive transport and lower rates of urinary excretion. However, saturation of this transporter could result in an increase in urinary elimination of perfluoroalkyls due to decreased tubular reabsorption. This is consistent with the apparent plateau in plasma concentration with increasing dose observed in cancer patients treated with PFOA (Convertino et al. 2018). Affinities of OATP1a1 (rat), OAT4 (human), and URAT1 (human) are highest for C7–C10 perfluoroalkyl carboxylates (Weaver et al.

2010; Yang et al. 2009, 2010). Affinity of rat OATP1a1 is strongly correlated with total clearance in rats (r^2=0.98; Yang et al. 2009).

Although sex differences for elimination of perfluoroalkyls have been detected in laboratory animals, human monitoring studies have not consistently detected sex differences in elimination $t_{1/2}$ of perfluoroalkyls; this may reflect limitations in the studies, including numbers and age of subjects (Bartell et al. 2010; Seals et al. 2011; Wong et al. 2014, 2015; Zhang et al. 2013). Menstruation may contribute to faster elimination of PFOS in women (Wong et al. 2014, 2015; Zhang et al. 2013). The effect of menstruation or other variables related to menstruation appear to contribute to faster elimination in younger (≤50 years) women compared to men and older women (Zhang et al. 2013). Two studies have found evidence for elimination of PFOS being affected by menstruation (Wong et al. 2014, 2015; Zhang et al. 2013). The estimated $t_{1/2}$ for PFOA was not different in younger females compared to males and older females. Mechanisms by which menstruation could affect PFOS clearance are not understood. Bulk elimination of blood would be expected to affect serum clearance of both PFOS and PFOA; therefore, other mechanisms must contribute that discriminate between perfluoroalkyl species. A better metric than serum $t_{1/2}$ for evaluating sex differences in elimination for this would be systemic or renal clearance of the perfluoroalkyl. Harada et al. (2005a) measured renal clearance in a small sample of young adults (five males and five females, age 22–23 years) and found that renal clearance was not different in males and females. Zhang et al. (2013) estimated renal clearance of PFOA and PFOS in a population of younger females (≤50 years, n=20), older females (>50 years), younger males (≤50 years), and older males (>50 years) and did not find significant sex or age differences. Studies that measured systemic clearance in monkeys also have not found significant sex differences in systemic clearance of PFOA (Buttenoff et al. 2004c) or PFOS (Chang et al. 2012).

Studies conducted in rats have shown that PFDA, PFNA, PFOA, PFOS, and PFHxA are secreted in bile and undergo extensive reabsorption from the gastrointestinal tract (Johnson et al. 1984; Kudo et al. 2001; Vanden Heuvel et al. 1991b, 1991c). Biliary secretion rates of PFOA are similar in male and female rats when renal excretion is blocked by ligation of the kidneys (Vanden Heuvel et al. 1991a, 1991b). This lack of sex influence on biliary secretion (compared to the sex influence on renal clearance) may reflect a relative sex insensitivity of OAT2 (or other organic anion transporter) expression in liver, compared to kidney; the latter is approximately 7–8 times higher in adult female rats compared to male rats (Kudo et al. 2002).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

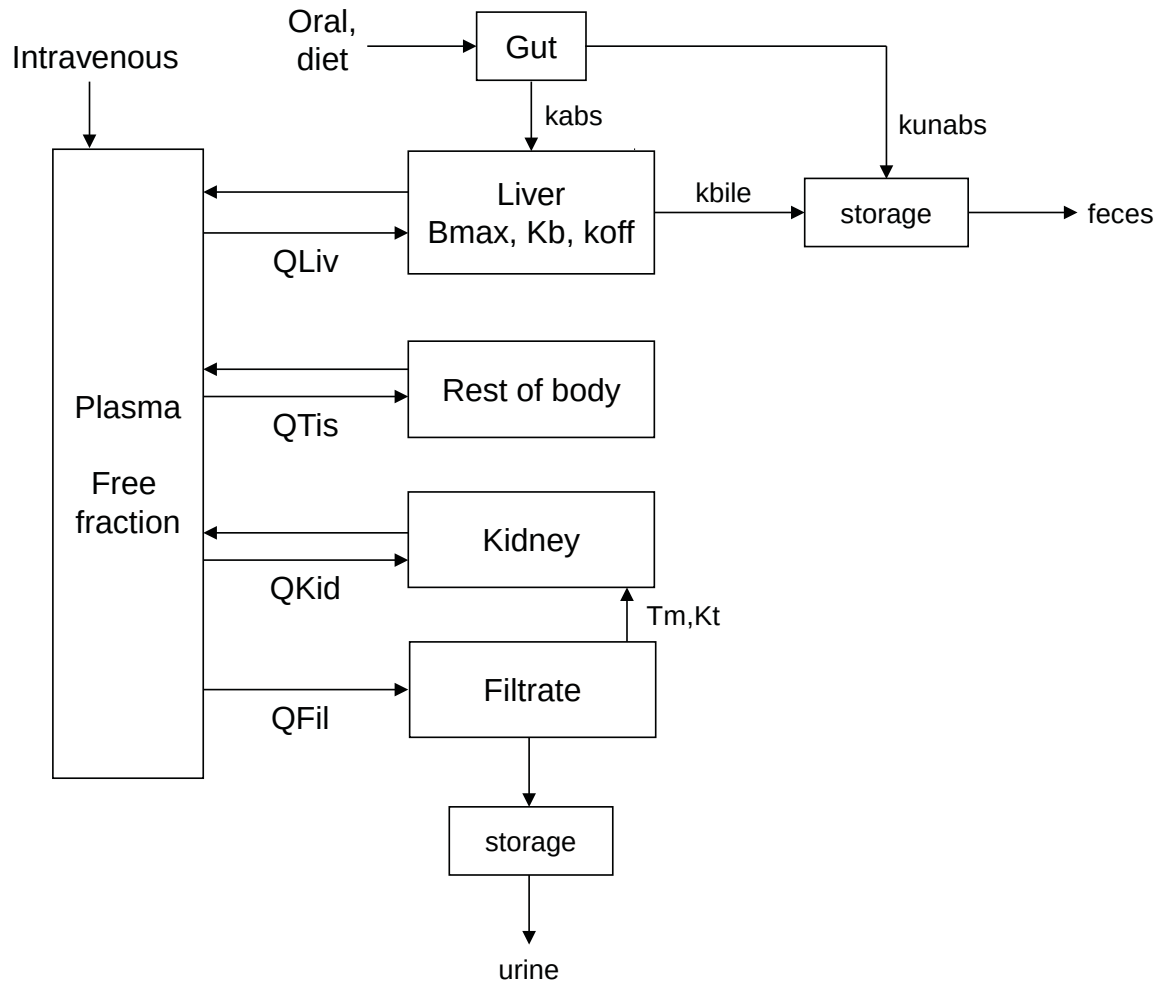
Several PBPK models of PFOA and PFOS have been reported. These include a human model for PFOA and PFOS (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b), models for PFOA and PFOS in monkeys (Loccisano et al. 2011), models for PFOA and PFOS in rats (Harris and Barton 2008; Loccisano et al. 2012a, 2012b; Tan et al. 2008; Worley and Fisher 2015a. 2015b), and a model for PFOA in mice (Rodriguez et al. 2009). Models of PFOA and PFOS kinetics during gestation and lactation in rats and mice also have been reported (Loccisano et al. 2012a, 2012b; Rodriguez et al. 2009). Various empirical and compartmental models have also been reported (Hoffman et al. 2011; Lorber and Egeghy 2011; Lou et al. 2009; Thompson et al. 2010; Verner et al. 2016; Wambaugh et al. 2013; Wu et al. 2009). Tardiff et al. (2009) utilized a human pharmacokinetic model to estimate an average daily oral dose corresponding to a Reference Dose for PFOA plasma concentration in humans. Cheng and Ng (2017) developed a permeability-limited PBPK model for PFOA in male rats that could be used for *in vitro* to *in vivo* extrapolation. Kim et al. (2018) developed a PBPK model for PFHxS in rats and humans. PBPK models were not identified for other perfluoroalkyls examined in this profile. Given the toxicokinetic differences between compounds, the PFOA, PFOS, and PFHxS PBPK models may not be appropriate for other compounds.

3.1.5.1 Loccisano et al. (2012a, 2012b) Rat Models

Loccisano et al. (2012a) developed a model for simulating the kinetics of PFOA and PFOS in male and female rats. The model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006). The female rat model (Loccisano et al. 2012a) was subsequently extended to include gestation and lactation (Loccisano et al. 2012b). The general structures of the models are depicted in Figures 3-3, 3-4, and 3-5. Complete lists of parameters and parameter values and the bases

for parameter values and evaluations of model predictions in comparison to observations are described in Loccisano et al. (2012a, 2012b).

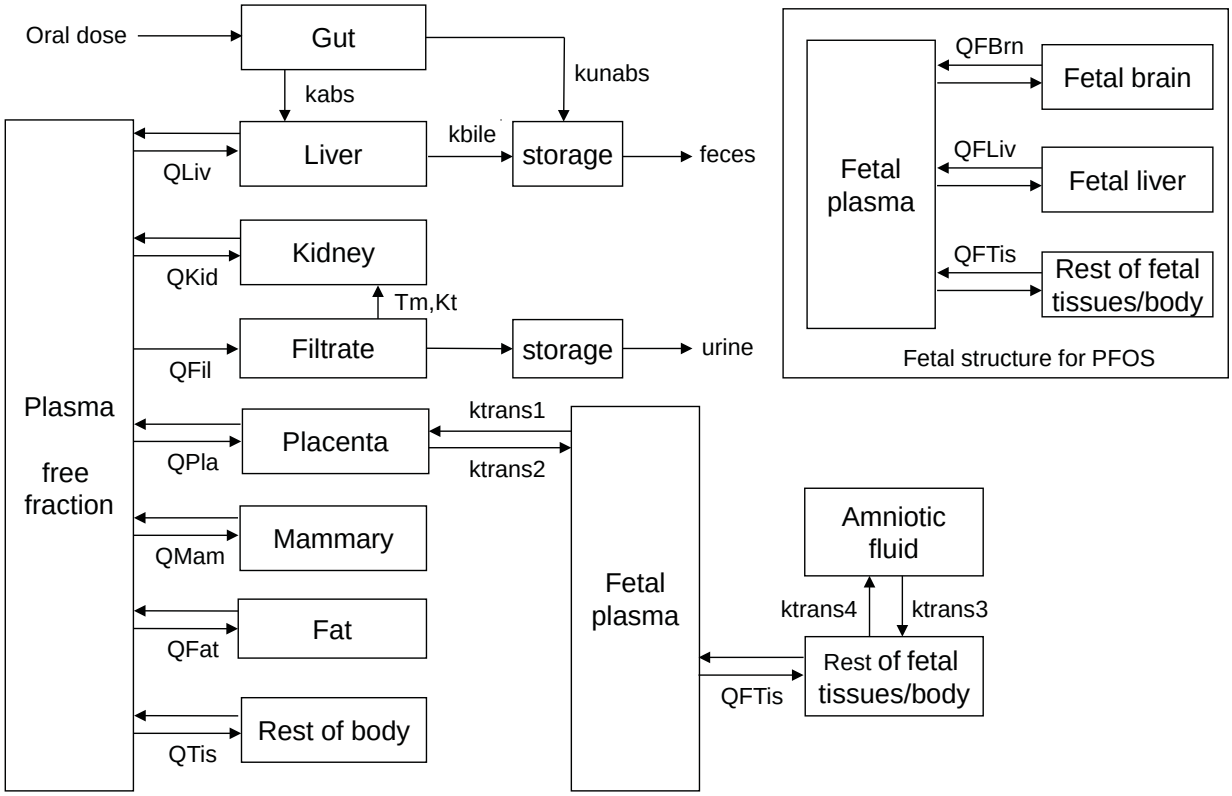
Figure 3-3. Structure of PBPK Model of PFOA and PFOS in the Rat



Bmax = liver binding capacity; kabs = first-order absorption rate constant; Kb = liver binding affinity constant; kbile = biliary excretion rate constant; Koff = liver binding dissociation constant; Kt = affinity constant; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QTis = blood flow in and out of tissues; Tm = transporter maximum

Source: Loccisano et al. 2012a (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

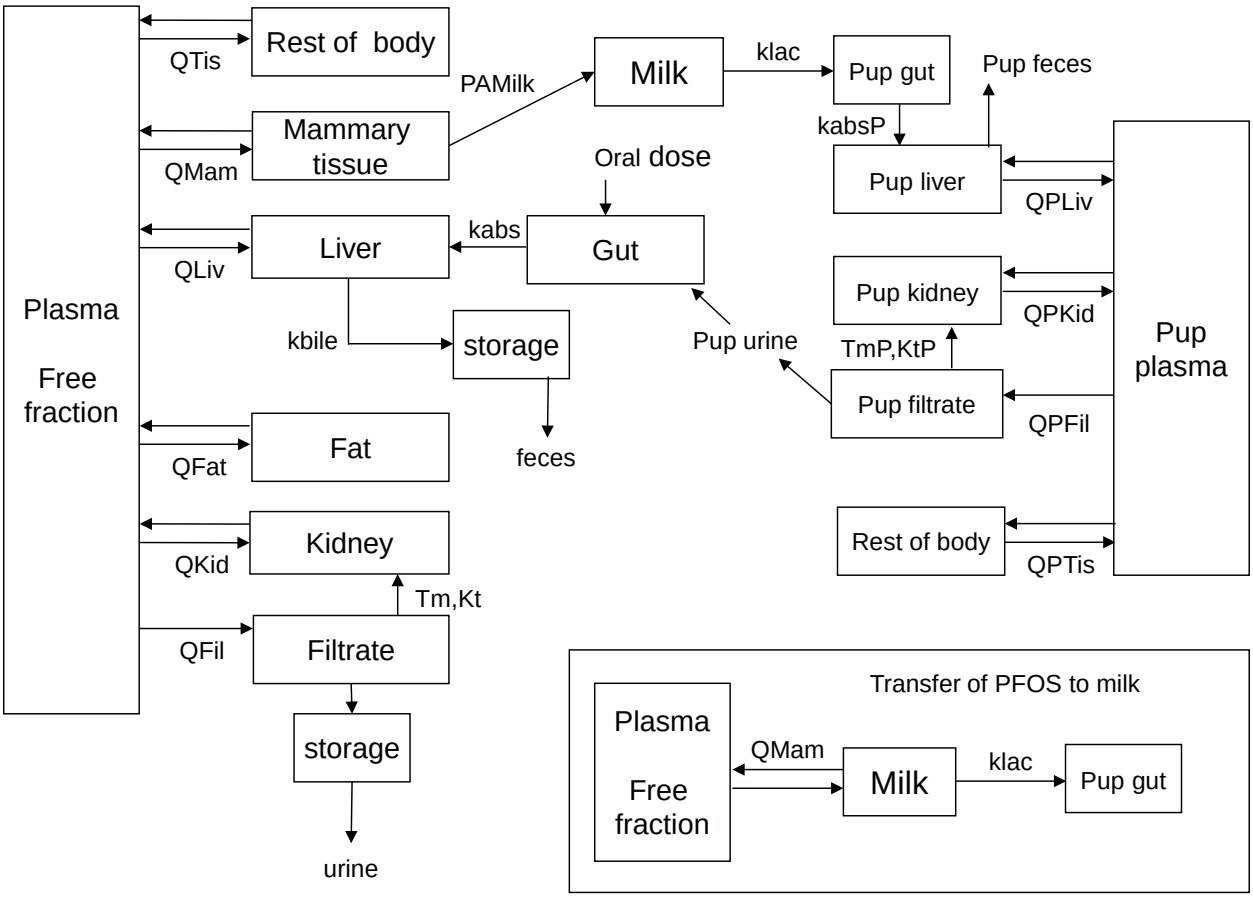
Figure 3-4. PBPK Model Structure for Simulating PFOA and PFOS Exposure During Gestation in the Rat (Dam, Left; Fetus, Right)



kabs = first-order absorption rate constant; kbile = biliary excretion rate constant; Kt = affinity constant; ktrans1/ktrans 2 = transfer between placenta and fetal plasma; ktrans3/ktrans4 = transfer between amniotic fluid and rest of the body; kunabs = rate of unabsorbed dose to appear in feces; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFBrn = blood flow in and out of fetal brain; QFil = clearance from plasma to glomerular filtrate; QFLiv = blood flow in and out of fetal liver; QFTis = blood flow in and out of fetal tissue; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPla = blood flow in and out of placenta; QTis = blood flow in and out of tissues; Tm = transporter maximum

Source: Loccisano et al. 2012b (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

Figure 3-5. PBPK Model Structure for Simulating PFOA/PFOS Exposure During Lactation in the Rat (Dam, Left; Pup, Right)



kabs = first-order absorption rate constant; kabsP = pup first-order absorption rate constant; kbile = biliary excretion rate constant; klac = transfer to pup through milk; Kt = affinity constant; KtP = pup affinity constant; PAMilk = transfer from mammary tissue to liver; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; QFil = clearance from plasma to glomerular filtrate; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QMam = blood flow in and out of mammary tissue; QPFil = clearance from pup plasma to glomerular filtrate; QPKid = blood flow in and out of pup kidney; QPLiv = blood flow in and out of pup liver; QPTis = blood flow in and out of pup tissue; QTis = blood flow in and out of tissues; Tm = transporter maximum; TmP = pup transporter maximum

Source: Loccisano et al. 2012b (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

The basic (i.e., adult nonpregnant rat) model includes compartments representing plasma (including a bound and free fraction), kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. Two storage compartments are included in the model: one receives perfluoroalkyl from the gastrointestinal tract (unabsorbed) and liver (bile) and the other receives perfluoroalkyl from the glomerular filtrate. The storage compartments were included in the model to simulate time delays between elimination from plasma and appearance of perfluoroalkyl in feces or urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed chemical. Absorbed PFOA and PFOS are assumed to be delivered to the liver where saturable binding of PFOS (but not PFOA) to liver proteins occurs. Saturable binding of PFOS in liver was included to simulate the relatively long retention times of PFOS in liver that have been observed in rats. Exchanges between PFOA or PFOS in liver (free fraction), kidney, and other tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:blood partition coefficient. PFOA and PFOS in plasma are simulated as instantaneous distributions into free and bound fractions. Extensive binding of PFOA and PFOS to plasma proteins has been demonstrated in various animal species including rats (see Section 3.1.2). For PFOA, the free fraction is assigned a constant of 4.5% in females and 0.6% in males. These values were optimized to fit observed kinetics of PFOA in plasma and urine of rats following intravenous and oral exposures (Loccisano et al. 2012a). Adequate fit to observed PFOS plasma kinetics following single doses of PFOS required introducing a time-dependence in binding of PFOS to protein (Loccisano et al. 2012a; Tan et al. 2008). The free fraction for PFOS in plasma decreases from an initial value (after dosing) of 2.2% to a minimum of 0.1% with a $t_{1/2}$ for the change of approximately 14 hours in a 0.25-kg rat ($k=0.035$ hours^{-1}/kg$^{-0.25}$). The relatively short $t_{1/2}$ for the change limits the effects of the time-dependent plasma kinetics over the first 1–2 days of dosing (including peak concentrations) and has no effect on longer-term kinetics or steady state. Although the time-dependence of the free fraction in plasma was needed to simulate short-term plasma PFOS kinetics in rats, the physiological mechanism for a dependence of plasma binding on the time following dosing (i.e., not on concentration of PFOS in plasma or some other dose surrogate) has not been established. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to feces (representing excretion following biliary transfer) is represented as a first-order process acting on the free fraction in liver. Excretion in urine is simulated as the balance between transfer from the free fraction to the glomerular filtrate and renal tubular reabsorption, which removes PFOA and PFOS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (µg/hour/kg body weight), representing the maximum rate of transport, and K_T (µg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate

is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFOA from plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFOA result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=4.1) in male rats compared to female rats (T_m/K_T=0.045), and correspondingly lower urinary clearance of PFOA from plasma in male rats. Reabsorption parameters for PFOS are the same in both sexes and result in reabsorptive clearances that are approximately twice that of PFOA in female rats (T_m/K_T=7.2).

The basic rat model was extended to simulate gestation with inclusion of additional compartments representing adipose and mammary tissue in the dam, placenta, and fetus (Figure 3-4); Loccisano et al. 2012b). Transfer of PFOA and PFOS to the fetus is simulated as a flow-limited transfer to the placenta, with first-order exchange between the placenta and the free fraction in fetal plasma. The free fraction in fetal plasma is simulated as a constant fraction for PFOA and PFOS (i.e., no dependence on time as in the adult). Within the fetus, PFOA in the free fraction of plasma exchanges with a single lumped compartment representing the fetal body, which exchanges with PFOA in amniotic fluid. The fetal PFOS model subdivides fetal tissue into brain, liver, and a lumped compartment for other tissues, all of which undergo flow-limited exchanges with the free fraction of PFOS in fetal plasma. Binding of PFOA and PFOS in fetal liver is assumed to be negligible. Differences in the structure of the fetal models for PFOA and PFOS reflect the differences in the availability of data for estimating parameter values for the various compartments (e.g., perfluoroalkyl concentrations in amniotic fluid, liver).

The lactation model extends the dam portion of the gestational model to include milk and pup (Figure 3-5; Loccisano et al. 2012b). Transfer of PFOA to milk occurs through the mammary gland with flow-limited exchange between plasma and mammary tissue and diffusion into milk from mammary tissue. The model also includes transfer from the pup to the dam, which occurs during maternal stimulation of the neonatal pup to induce elimination and during pup grooming. Data on PFOS in mammary tissue of rodents were not available to establish parameters for a mammary tissue compartment; therefore, the mammary tissue compartment was left out of the PFOS model, and transfer of PFOS to milk is simulated as diffusion directly from plasma. The pup model includes compartments representing the free fraction in plasma, liver, kidney, glomerular filtrate, and a lumped compartment representing all other pup tissues. This structure is essentially identical to the nonpregnant rat model (Loccisano et al. 2012a) with a few differences. Absorption from the gastrointestinal tract is assumed to be complete in pups, and binding in pup liver is assumed to be negligible in pups. There are no storage compartments for biliary or glomerular filtrate perfluoroalkyl in the pup model. Sex differences in renal

tubular reabsorption of PFOA are assumed to develop in response to sexual maturation and, therefore, are not present during lactation (i.e., parameter values are allometrically scaled to pup body weight from the male rat values). Reabsorptive transport parameters for PFOS are allometrically scaled from the lactating dam. The liver/plasma partition coefficient for PFOS in the pups was set lower than that in the dam, based on observations in rats. All other parameters for PFOA and PFOS in the pup were the same or allometrically scaled from values for the dam.

Optimization of parameter values and evaluations of the rat models are described in Loccisano et al. (2012a, 2012b). Data sets utilized in developing and evaluating the nonpregnant rat models included single-dose intravenous and gavage studies and short-term feeding studies (Johnson and Ober 1979; Kemper 2003; Kudo et al. 2007; Perkins et al. 2004). Data used in development and evaluation of the gestation and lactation models included data from gestational and/or lactational exposure studies in rats (Chang et al. 2009; Hinderliter et al. 2005; Kuklenyik et al. 2004; Luebker et al. 2002, 2005a, 2005b; Thibodeaux et al. 2003).

Applications for Dosimetry Extrapolation and Risk Assessment. The wealth of data on pharmacokinetics of PFOA and PFOS in rats allowed an extensive evaluation of the rat models for predicting plasma urinary and liver PFOA and PFOS following single intravenous or single and repeated oral dosing. Inclusion of renal tubular reabsorption parameters in the model provided accurate simulations of sex differences in elimination rates of PFOA from plasma and excretion in urine, and differences in rates of elimination of PFOA and PFOS. The gestation model successfully predicted fetal plasma and liver PFOA and PFOS at the end (or near the end) of pregnancy. Consistent with observations, the model predicts higher fetal plasma concentrations and lower fetal liver concentrations of PFOS compared to maternal, and lower internal exposure (plasma concentrations) to PFOA in the fetus compared to maternal (fetal liver data were not available for PFOA). The lactation model successfully predicted PFOA and PFOS in pup plasma following dosing of the dam. Predicted plasma concentrations of PFOA in nursing pups were approximately 10–50% lower than maternal concentrations, whereas maternal and pup concentrations of PFOS were similar. The model could be used to estimate liver doses and corresponding plasma profiles resulting from single or repeated dosing of adult male or female rats, and maternal-fetal and maternal-pup transfer of PFOA and PFOS. The rat model was evaluated with data from a 14-week oral dosing study and has not been tested for longer exposures. Harris and Barton (2008) developed a PBPK model for PFOS in the rat and found that time adjustments that increased renal clearance and decreased the liver-plasma partition coefficient as a function of time and dose improved predictions of plasma and liver PFOS in adult rats exposed for a period of 105 weeks. Although the

Harris and Barton (2008) model is very different from the Loccisano et al. (2012a) model, these results suggest the possibility that clearance of PFOS may be age- and/or dose-dependent in rats. This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

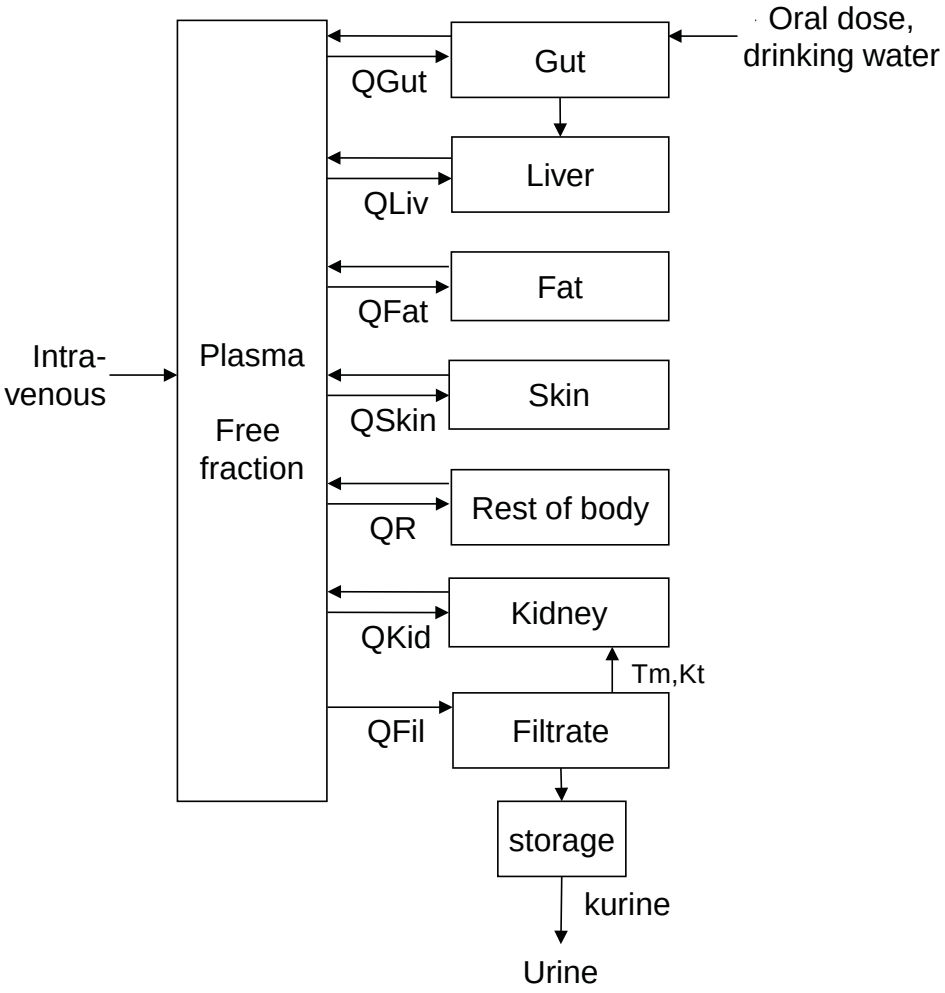
3.1.5.2 Loccisano et al. (2011, 2013) Monkey and Human Models

Loccisano et al. (2011) developed a model for simulating the kinetics of PFOA and PFOS in monkeys and humans. The human model described in Loccisano et al. (2011) was subsequently extended to include simulations of pregnancy and lactation (Loccisano et al. 2013). The monkey model was based, in part, on a multi-compartmental model developed by Tan et al. (2008; Andersen et al. 2006) for simulating the kinetics of plasma and urinary PFOA in monkeys. The structures of the monkey and human models are identical (Figure 3-6) and are very similar to the structure of the rat model (Loccisano et al. 2012a), with inclusion of compartments representing fat and skin, and absence of a storage compartment for biliary transfer. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Loccisano et al. (2011).

Parameters in the monkey and human models differ in several ways from the rat model. The free fraction in plasma is represented as a constant for both PFOA and PFOS; time-dependency for PFOS in the rat model is absent in the monkey and human models. The parameters for renal tubular reabsorption of PFOA and PFOS are the same for males and females. This is consistent with the absence of evidence for a sex difference in elimination kinetics in monkeys (Butenhoff et al. 2002, 2004a; Seacat et al. 2002).

Values for the affinity constant (K_T) and maximum (T_m) for tubular reabsorption were optimized to plasma concentration kinetics in monkeys. The value for K_T in monkeys was used in the human model. The value for T_m for PFOA in humans was set to yield a plasma elimination $t_{1/2}$ of 2.3 or 3.8 years. The latter two values were derived from estimates of the serum $t_{1/2}$ in populations exposed to PFOA in drinking water (2.3 years; Bartell et al. 2010) or in retired fluorochemical workers (3.8 years; Olsen et al. 2007a). The value for T_m for PFOS in humans was set to yield a plasma elimination $t_{1/2}$ of 5.4 years, based on observations in retired fluorochemical workers (Olsen et al. 2007a). Binding of PFOA and PFOS in the liver was assumed to be negligible in monkeys and humans. Tissue-plasma partition coefficients used in both models were derived from observations in rodents and were the same in the monkey and human models.

Figure 3-6. Structure of PBPK Model for PFOA and PFOS in Monkeys and Humans



Kt = half-saturation constant; kurine = urinary elimination rate; PBPK = physiologically based pharmacokinetic; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; QFat = blood flow in and out of fat; Qfil = clearance from plasma to glomerular filtrate; QGut = blood flow in and out of gut; QKid = blood flow in and out of kidney; QLiv = blood flow in and out of liver; QR = blood flow in and out of rest of body; QSkin = blood flow in and out of skin; Tm = transport maximum

Source: Loccisano et al. 2011 (reproduced with permission of Academic Press in the format reuse in a government report via Copyright Clearance Center; Regulatory Toxicology and Pharmacology: RTP by International Society of Regulatory Toxicology and Pharmacology)

Optimization of parameter values and evaluation of the monkey and human models are described in Loccisano et al. (2011). Data sets utilized in developing and evaluating the monkey model included single-dose intravenous and oral studies and repeated-dose oral studies conducted in Cynomolgus monkeys (Butenhoff et al. 2004c; Noker and Gorman 2003; Seacat et al. 2002). Data used in evaluating the human model consisted of serum measurements in people who experienced environmental exposures (Emmett et al. 2006a; Hölzer et al. 2008; Steenland et al. 2009b), adult Red Cross donors (Olsen et al. 2003b, 2008), and retired fluorochemical workers (Olsen et al. 2007a). In general, PFOA and PFOS intakes and exposure durations were not known with certainty in these populations and, as a result, these data do not yield confident evaluations of the ability of the human model to predict intake-plasma level relationships. Follow-up monitoring after a cessation or decrease in exposure can provide data that allow evaluation of the ability of the model to accurately simulate elimination kinetics. Predicted declines in serum PFOA concentrations encompassed observed group mean declines when the T_m for renal tubular reabsorption was set to yield an elimination $t_{1/2}$ of 2.3 or 3.8 years. Group mean declines in serum PFOS were predicted reasonably well for some populations, but not all populations, when the T_m for renal tubular reabsorption was optimized to yield an elimination $t_{1/2}$ of 5.4 years.

The human pregnancy model includes additional compartments representing the free fractions in plasma, amniotic fluid, and a lumped compartment for fetal tissue (Loccisano et al. 2013). The same conceptual approach was used in the rat pregnancy model (Loccisano et al. 2012b, Figure 3-4). Rate constants for placental transfer were initially those from the rat model, adjusted to yield predicated maternal/fetal plasma ratios that agreed with observed maternal/fetal ratios in cord blood (Apelberg et al. 2007b; Fei et al. 2007; Midasch et al. 2007; Washino et al. 2009). Transfers from amniotic fluid to fetus were the same as those used in the rat model, as there were no data on which to base estimates for humans. The lactation model included additional compartments for mammary milk and a lumped compartment representing the infant. Transfer of PFOA to milk is simulated as flow-limited exchange between plasma and milk, governed by mammary tissue blood flow and a milk/plasma partition coefficient. This structure obviated the need to simulate mammary tissue kinetics, for which there were no data in humans. The milk/plasma partition coefficient was calibrated to yield predictions of observed milk/plasma ratios (Fromme et al. 2010; Kärrman et al. 2007). Transfer from maternal milk to infants is the product of the milk concentration and milk production rate (assumed to be equal to sucking rate). The pregnancy model was evaluated by comparing predicted maternal/fetal plasma ratios for PFOA and PFOS with observations from various human monitoring studies (Fei et al. 2007; Fromme et al. 2010; Hanssen et al. 2010; Inoue et al. 2004; Kim et al. 2011; Midasch et al. 2007; Monroy et al. 2008; Tittlemier et al. 2004). The lactation model was evaluated by comparing predicted maternal plasma/milk ratios for PFOA and PFOS

with observations from various human monitoring studies (Fromme et al. 2009; Kärrman et al. 2007; Liu et al. 2011). In general, most model predictions were within plus or minus 2-fold of observations.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicts plasma concentrations and tissue levels of PFOA and PFOS following intravenous or oral dosing. A skin compartment is included in the model, which may serve for simulating absorption and distribution following deposition onto the skin surface; however, the dermal absorption model was not evaluated in Loccisano et al. (2011). The human model was calibrated to predict $t_{1/2}$ values estimated for human populations (e.g., 2.3 or 3.8 years for PFOA, 5.4 years for PFOS). As a result, comparisons made between observed and predicted serum concentrations evaluate whether or not the populations actually exhibit the $t_{1/2}$ to which the model was calibrated, and not the validity of the model to predict the internal distribution of PFOA or PFOS. It is not currently possible to assess with confidence whether the human model can accurately predict doses to liver or any other tissues. Fábrega et al. (2014) applied the human adult model to estimate plasma concentrations and tissue levels of PFOA and PFOS in human autopsy samples. Exposure inputs to the model were intakes of PFOA and PFOS estimated from public water supply concentrations in the local area where the subjects had resided (Catalonia, Spain) and concentrations in local market basket foods (Domingo et al. 2012a, 2012b). The human model predicted levels of PFOA in plasma and liver that were approximately 10- and 5-fold higher, respectively, than observed. Predicted plasma levels of PFOS were approximately 2-fold higher than observed, and predicted levels of PFOS in kidney were approximately 25% of observed. Fábrega et al. (2014) explored alternative values for tissue/plasma partition coefficients, determined from human autopsy issues (Maestri et al. 2006). The adjusted partition coefficients improved predictions of observed tissue PFOA and PFOS levels. Although the model could be applied to predicting plasma concentrations of PFOA and PFOS or intakes associated with specific plasma concentrations (e.g., oral MRLs), it is not clear what advantages the model offers over simpler empirical or compartmental models similarly calibrated to predict the serum $t_{1/2}$. The monkey model has been more thoroughly evaluated for predicting plasma and urinary kinetics of PFOA and PFOS. This was possible because of the availability of more extensive experimental data on plasma and urine PFOA and PFOS following intravenous and oral (single and repeated) dosing in male and female monkeys. Nevertheless, data on internal distribution were not available to allow evaluation of how well the monkey model predicts doses to the liver or other tissues. Predictions of plasma PFOA and PFOS concentrations from the monkey (and human) model were highly sensitive to values assigned to the maximum rate for tubular reabsorption (T_m) and other parameters that govern urinary elimination of PFOA and PFOS (e.g., free fraction in plasma and glomerular filtration rate; Loccisano et al. 2011). Optimization of the monkey models relied heavily on adjusting these same parameters and, for the human

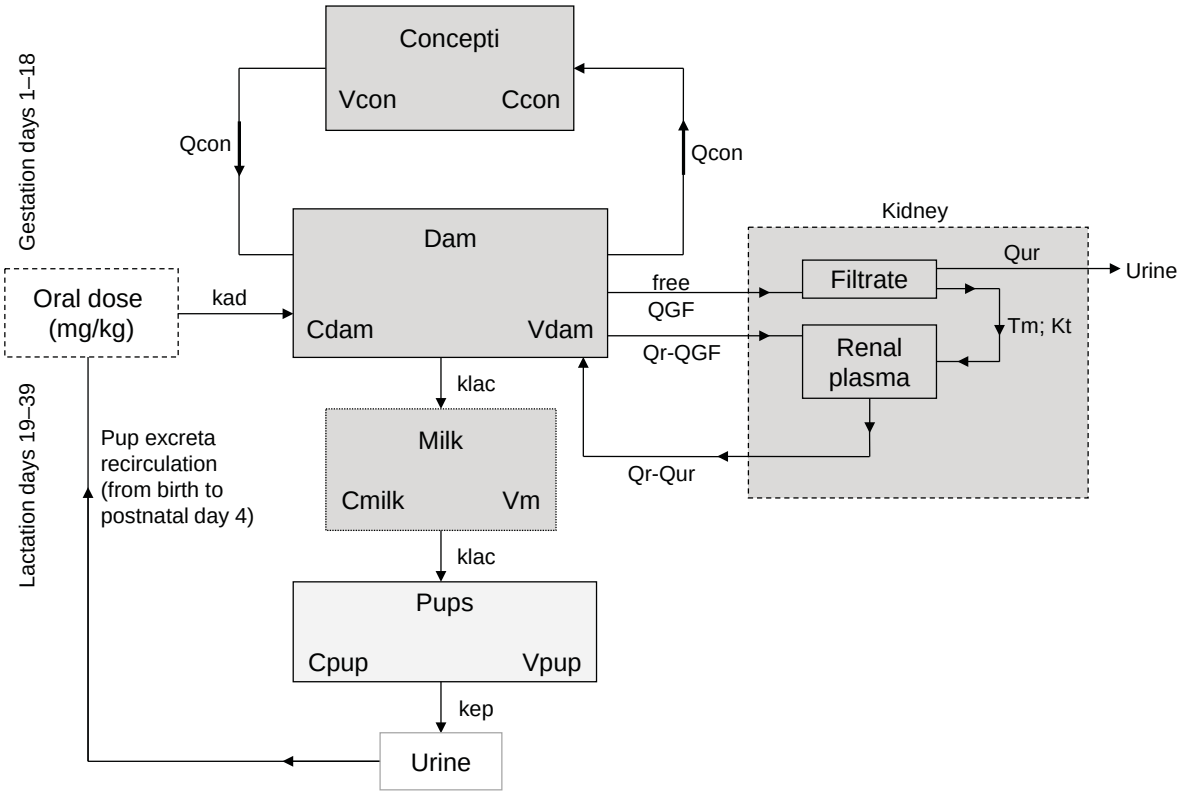
model, the target plasma elimination $t_{1/2}$ was achieved solely by adjusting T_m. Thus, despite the complexity of the models, their potential to accurately predict plasma elimination kinetics and, therefore, steady-state plasma concentrations and associated oral intakes, depends largely on how well they predict plasma clearance. If plasma clearance and the free-fraction in plasma can be reliably predicted empirically for the animal species of interest, then far simpler compartmental models can be used for dosimetry extrapolation of steady-state free plasma concentrations.

3.1.5.3 Rodriguez et al. (2009) Mouse Model

Rodriguez et al. (2009) developed a model for simulating the maternal-fetal and maternal-pup kinetics of PFOA in mice. The general structure of the model is depicted in Figure 3-7. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Rodriguez et al. (2009). The maternal, fetal, and pup systems are simulated as single well-mixed compartments. Absorption from the gastrointestinal tract is simulated as first-order with complete absorption of the ingested dose. Elimination of absorbed PFOA from the maternal system is simulated as the balance between glomerular filtration and renal tubular reabsorption. The latter is represented as a saturable process with parameters T_m and K_T. Transfer to the fetus is flow-limited and governed by a fetus/maternal partition coefficient and placental blood flow. Transfer from the maternal system to the pup by lactation is simulated as first-order governed by a lactation transfer rate constant. Elimination of PFOA from the pup is first-order to urine. Data sets utilized in developing and evaluating the mouse model included oral gestational dosing studies.

Applications for Dosimetry Extrapolation and Risk Assessment. The model predicted observed concentrations of PFOA in maternal, fetal, and pup serum following oral gestational exposures to mice (Abbott et al. 2007; Lau et al. 2006; White et al. 2007). Residuals for predictions are presented, which provide a quantitative measure of how well the model predicted observations (Rodriguez et al. 2009). Similar to the rat, the mouse model predicts higher internal exposure (serum PFOA concentrations) in the maternal system compared to the fetus. It also predicts accelerated loss of PFOA from the maternal system during lactation. The model simulates the maternal, fetal, and pup systems as single compartments. Although this serves for simulating plasma concentrations (the main objective of the modeling effort), it does not allow for simulation of tissue levels of PFOA in the maternal system, fetus, or pup.

Figure 3-7. Renal Resorption Pharmacokinetic Model of Gestation and Lactation used in the Analysis of CD-1 Mice



Ccon = concentration in concepti; Cdam = concentration in dam; Cmilk = concentration in milk; Cpup = concentration in pup; kad = first-order absorption rate; kep = urinary excretion rate; klac = transfer rate via milk; Kt = half-saturation constant; Qcon = blood flow to and from placenta; QGF = glomerular filtrate; Qr = renal plasma flow; Qur = urine flow; Tm = transport maximum; Vcon = volume in concepti; Vdam = volume in dam; Vmilk = volume in milk; Vpup = volume in pup

Source: Rodriguez et al. 2009 (reproduced with permission of Elsevier Inc. in the format reuse in a government report via Copyright Clearance Center; Reproductive Toxicology by Reproductive Toxicology Center; Washington, DC)

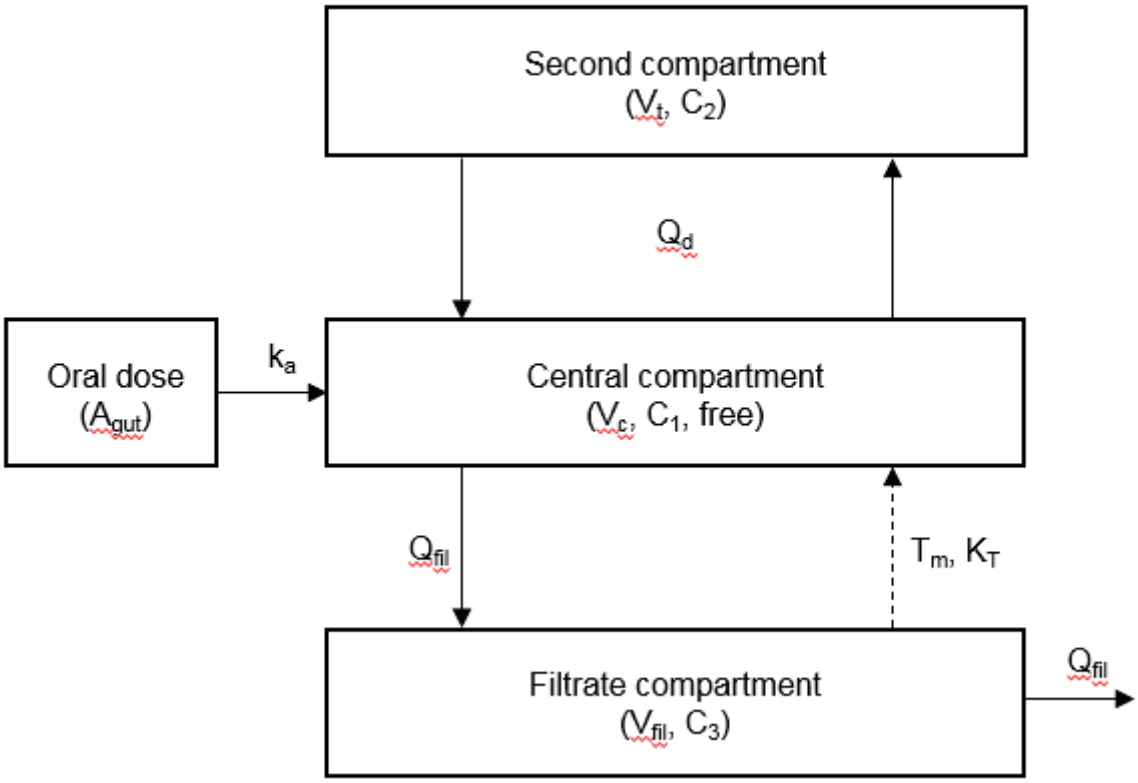
3.1.5.4. Wambaugh et al. 2013 (Andersen et al. 2006) Model

The Wambaugh et al. (2013) model is a three-compartment model based on the three-compartmental monkey model of Andersen et al. (2006). The structure of the two models are identical (Figure 3-8). Parameter values for the Wambaugh et al. (2013) model are presented in Table 3-7. The model includes a central compartment, a secondary distribution compartment, and a renal glomerular filtrate compartment. The central compartment (C1), which includes plasma, receives PFOA or PFOS from oral dosing (first-order k_a, $hour^{-1}$) and exchanges perfluoroalkyl with the secondary compartment (C2, which lumps all other tissues and distribution volumes into a single compartment) and with the glomerular filtrate (C3). A fraction of the perfluoroalkyl in C1 is free (Free) and available for exchange with C2 and C3. Exchanges between C1 and C2 are first order (k_{12}, k_{21}, $hour^{-1}$) with k_{21} assigned a value equal to the $R_{V2/V1}$, where $R_{V2/V1}$ is the ratio of the volumes of the two compartments (V_2/V_1). Transfer of perfluoroalkyl into the glomerular filtrate is first order and governed by the glomerular filtration rate (Q_{filc}, L/hour). Transfer for perfluoroalkyl from the glomerular filtrate to C1 (representing renal tubular reabsorption) is capacity limited (T_{maxc}, μmol/hr; K_T, μM). Perfluoroalkyl that is not reabsorbed is excreted.

Parameter values for the various species and strains were estimated from experimental pharmacokinetic data for each species and strain using Bayesian Markov Chain Monte Carlo (MCMC) analysis. Studies that provided data used to estimate parameter values are listed in Wambaugh et al. (2013). The parameter values shown in Table 3-7 are the mean values and posterior distributions (95% credible interval) from the MCMC analyses.

Applications for Dosimetry Extrapolation and Risk Assessment. Wambaugh et al. (2013) applied the model to predicting internal doses (mean and maximum serum concentrations and plasma AUC) for Benchmark Dose Software (BMDS) modeling and for comparing internal dosimetry from *in vivo* toxicity studies to estimates of potency (AC_{50}, maximum Efficacy) from *in vitro* studies. EPA applied the Wambaugh et al. (2013) model to deriving chronic oral reference doses (RfDs) for PFOA and PFOS (EPA 2016e, 2016f). The model was used to predict internal doses (time-integrated plasma PFOA or PFOS concentrations) achieved in toxicity studies conducted in various laboratory animal models (CD-1 mouse, C57Bl/6 mouse, Sprague-Dawley rat, Cynomolgus monkey). Plasma concentrations were then extrapolated to equivalent steady-state concentrations in humans using a model of first-order elimination of PFOA and PFOS from plasma. The same approach was used to derive MRLs for PFOA and PFOS (see Appendix A).

Figure 3-8. Andersen et al. (2006) Pharmacokinetic Model with Oral Absorption



A_{gut} is the amount of chemical in the gut; k_a is the first-order rate constant for absorption from the gut; Q_{fil} is the flow through the filtrate compartment; C_1, C_2, and C_3 are the chemical concentrations in the central, second, and filtrate compartments, respectively; V_c, V_t, and V_{fil} are the volumes of distribution of the central, second, and filtrate compartments; free is the free fraction of compound in the central compartment; Q_d is the flow between the central and second compartments; the saturable resorption process from the filtrate back into the central compartment is modeled with Michaelis-Menten kinetics, with a maximum rate $T_{maximum}$ and a half-maximum concentration K_T.

Reprinted from Wambaugh et al. (2013) by permission of Oxford University Press

Table 3-7. Estimated and Assumed Pharmacokinetic Parameters for the Modified Andersen et al. (2006) Model for PFOA and PFOS

		Parameter (units)										
Reference	Species	BW (kg)	Cardiac output[a] (L/hour/kg$^{0.74}$)	K_a (hour^{-1})	V_{cc} (L/kg)	k_{12} (hour^{-1})	$R_{V2:V1}$ (unitless)	T_{maxc} (μmole/hour)	K_T (μM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOA[b]						
Lou et al. (2009)	Mouse: CD1 (F)	0.02	8.68	290 (0.6–73,000)	0.18 (0.16–2.0)	0.021 (3.1x10^{-10} to 3.8x10^{4})	1.07 (0.26–5.84)	4.91 (1.75–2.96)	0.037 (0.0057–0.17)	0.011 (0.0026–0.051	0.077 (0.015–0.58)	9.7x10^{-4} (3.34x10^{-9}–7.21)
Dewitt et al. (unpublished)	Mouse: C57Bl/6 (F)	0.02	8.68	340 (0.53–69,000)	0.17 (0.13–2.3)	0.35 (0.058–52)	53 (11–97)	2.7 (0.95–22)	0.12 (0.033–0.24)	0.034 (0.014–0.17)	0.017 (0.010–0.081)	7.6x10^{-5} (2.7x10^{-10}–6.4)
Kemper (2003)	Rat: Sprague-Dawley (F)	0.20 (0.16–0.23)[c]	12.39	1.7 (1.1–3.1)	0.14 (0.11–0.17)	0.098 (0.039–0.27)	9.2 (3.4–28)	1.1 (0.25–9.6)	1.1 (0.27–4.5)	0.086 (0.031–0.23)	0.039 (0.014–0.13)	2.6x10^{-5} (2.9x10^{-10}–28)
Kemper (2003)	Rat: Sprague-Dawley (M)	0.24 (0.21–0.28)[c]	12.39	1.1 (0.83–1.3)	0.15 (0.13–0.16)	0.028 (0.0096–0.08)	8.4 (3.1–23)	190 (5.5–50,000)	0.092 (3.4x10^{-4}–1.6)	0.08 (0.03–0.22)	0.22 (0.011–58)	0.0082 (1.3x10^{-8}–7.6)
Butenhoff et al. (2004b)	Monkey: Cynomolgus (M/F)	7 (m), 4.5 (f)	19.8	230 (0.27–73,000)	0.4 (0.29–0.55)	0.0011 (2.4x10^{-10} to 3.5x104)	0.98 (0.25–3.8)	3.9 (0.65–9,700)	0.043 (4.3x10^{-5}–0.29	0.01 (0.0026–0.038)	0.15 (0.02–24)	0.0021 (3.3x10^{-9}–6.9)
		Parameter (units)										
Reference	Species	BW[d] (kg)	Cardiac output[e] (L/hour/kg$^{0.74}$)	K_a (hour^{-1})	V_{cc} (L/kg)	k_{12} (hour^{-1})	$R_{V2:V1}$ (unitless)	T_{maxc} (μmol/hour)	K_T (μM)	Free (unitless)	Q_{filc} (L/hour)	V_{filc} (L/kg)
						PFOS						
Chang et al. (2012)	Mouse: CD1 (F)	0.02	8.68	1.16 (0.617–42,400)	0.264 (0.24–0.286)	0.0093 (2.63e-10–38,900)	1.01 (0.251–4.06)	57.9 (0.671–32,000)	0.0109 (1.44x10^{-5}–1.45)	0.00963 (0.00238–0.0372)	0.439 (0.0125–307)	0.00142 (4.4x10^{-10}–6.2)
Chang et al. (2012)	Mouse: CD1 (M)	0.02	8.68	433.4 (0.51–803.8	0.292 (0.268–0.317	2,976 (2.8e-10–4.2e4)	1.29 (0.24–4.09)	1.1e4 (2.1–7.9e4)	381 (2.6x10^{-5}–2,900)	0.012 (0.0024–0.038)	27.59 (0.012–283)	0.51 (3.5x10^{-10}–6.09)
Chang et al. (2012)	Rat: Sprague-Dawley (F)	0.203	12.39	4.65 (3.02–1,980)	0.535 (0.49–0.581)	0.0124 (3.1e-10–46 800)	0.957 (0.238–3.62)	1,930 (4.11–83,400)	9.49 (0.00626–11,100)	0.00807 (0.00203–0.0291)	0.0666 (0.0107–8.95)	0.0185 (8.2x10^{-7}–7.34)
Chang et al. (2012)	Rat: Sprague-Dawley (M)	0.222	12.39	0.836 (0.522–1.51)	0.637 (0.593–0.68)	0.00524 (2.86e-10–43,200)	1.04 (0.256–4.01)	1.34e-06 (1.65e-10–44)	2.45 (4.88x10^{-10}–60 300)	0.00193 (0.000954–0.00249)	0.0122 (0.0101–0.025)	0.000194 (1.48x10^{-9}–5.51)
Seacat et al. (2002) and Chang et al. (2012)	Monkey: Cynomolgus (M/F)	3.42	19.8	132 (0.225–72,100)	0.303 (0.289–0.314)	0.00292 (2.59e-10–34,500)	1.03 (0.256–4.05)	15.5 (0.764–4,680)	0.00594 (2.34 x10^{-5}–0.0941)	0.0101 (0.00265–0.04)	0.198 (0.012–50.5)	0.0534 (1.1x10^{-7}–8.52)

[a]Cardiac outputs obtained from Davies and Morris (1993).
[b]Means and posterior distributions from the Bayesian Markov Chain Monte Carlo (MCMC) analysis (95% credible interval in parentheses) are reported.
[c]Estimated average body weight (BW) for species used except with Kemper (2003) study where individual rat weights were available and assumed to be constant.
[d]Average BW for species: individual-specific BWs.
[e]Cardiac outputs obtained from Davies and Morris (1993).

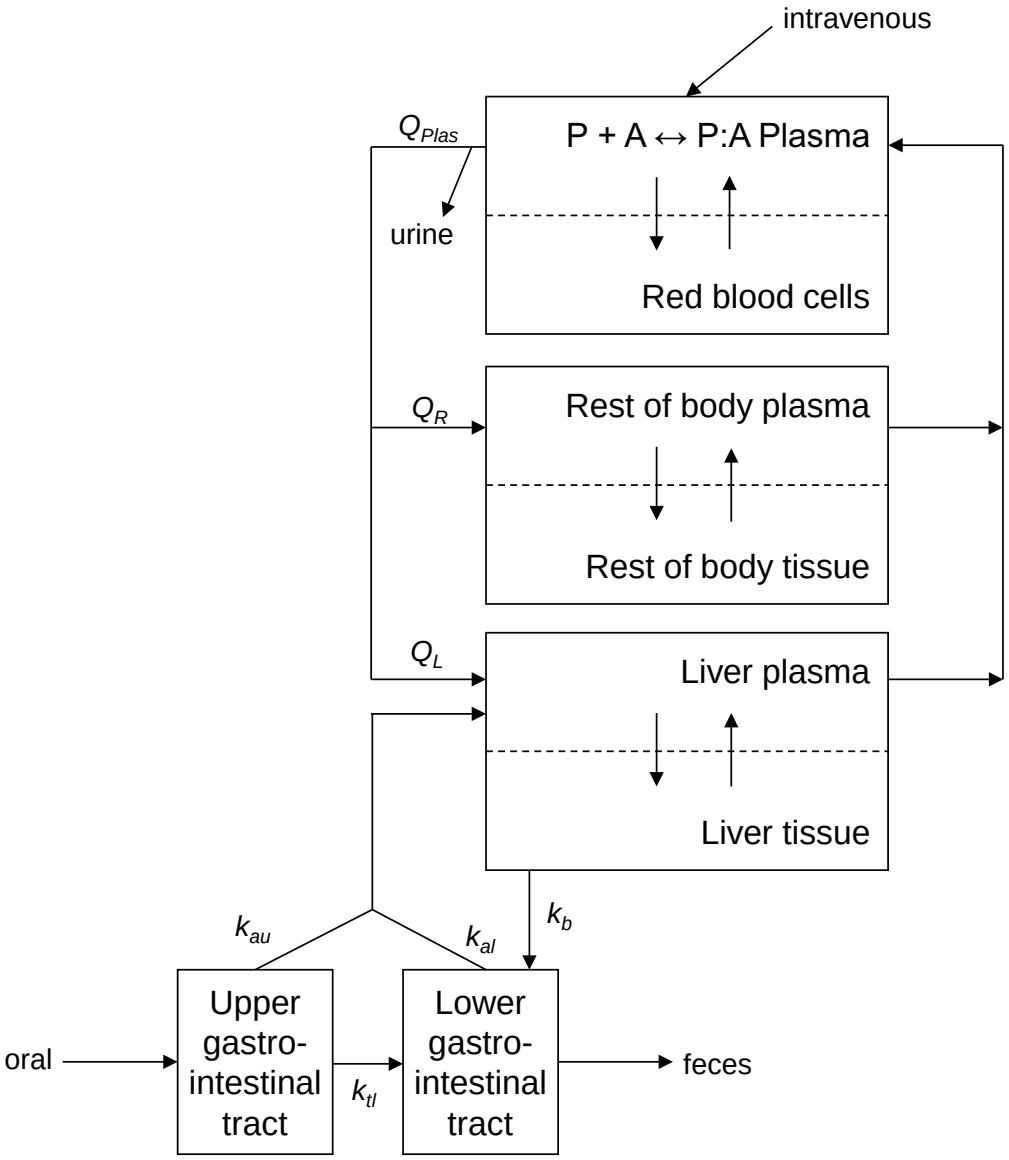
Source: Wambaugh et al. (2013)

3.1.5.5 Harris and Barton (2008) Rat Model

Harris and Barton (2008) developed a model for simulating PFOS kinetics in adult rats. The general structure of the model is depicted in Figure 3-9. Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Harris and Barton (2008). The model includes systemic compartments representing blood (including a bound and free fraction of plasma and red blood cells), liver, and a lumped compartment representing all other tissues. The gastrointestinal tract is simulated as separate compartments representing the upper and lower tracts. Absorption occurs from both the upper and lower tracts, with distinct first-order rate constants assigned to each. Biliary PFOS is transferred from liver to the lower tract. Absorbed PFOS is delivered to the liver where it enters plasma to be distributed to other tissues. Exchanges between PFOS in plasma and all tissues are assumed to be diffusion-limited, with the free pool in plasma participating in the exchange with red blood cells, and the total plasma pool exchanging with liver and all other tissues. Binding of PFOA to plasma albumin is assumed to be saturable, with a dissociation constant 10^{-7} M and a maximum capacity 4.1x 10^{-4} M. This is implemented by assigning bound PFOA to a subcompartment of plasma in which PFOA enters (binds) or exits (unbinds) at rates governed by binding *on* and *off* rates, respectively, that yield a dissociation constant of 10^{-7} M. Elimination of absorbed chemical occurs by biliary excretion and urinary excretion. Transfer from liver to the lower gastrointestinal tract (representing excretion following biliary transfer) is represented as a first-order process acting on the total amount of PFOS in liver. PFOA is transferred to urine from the free fraction of plasma at a rate governed by a urinary clearance parameter, which is assigned a value of 28% of renal plasma flow.

In evaluating performance of the model for simulating PFOS concentrations in a chronic rat feeding study, Harris and Barton (2008) found that the model predicted plasma and liver concentrations measured at 4 and 16 weeks, but over-predicted both at 104 weeks. Performance of the model was improved by having renal clearance increase and the liver/plasma partition coefficient decrease as a function of time (i.e., study duration). These results suggest the possibility that clearance of PFOS may be dependent on age and/or a metric of dose (e.g., cumulative internal dose). This may reflect age- or dose-related changes in kidney function, including tubular reabsorption or secretion of PFOS.

Figure 3-9. Conceptual Representation of a Physiologically Based Pharmacokinetic Model for PFOS Exposure in Rats



k_{al} = rate of absorption from the lower gastrointestinal tract; k_{au} = rate of absorption from the upper gastrointestinal tract; k_b = maximum rate of biliary elimination; k_{tl} = rate of transfer from upper-lower gastrointestinal tract; P:A = PFOS-bound albumin in plasma; PFOS = perfluorooctane sulfonic acid Q_L = plasma flow rate to the liver; Q_{Plas} = plasma flow rate by the heart; Q_R = plasma flow rate to the rest of body

Source: Harris and Barton 2008 (reproduced with permission of Elsevier Ireland Ltd. in the format reuse in a government report via Copyright Clearance Center; Toxicology Letters by European Societies of Toxicology)

Applications for Dosimetry Extrapolation and Risk Assessment. The model simulates kinetics of PFOS following oral or intravenous dosing in adult rats and includes several features that are different from other PBPK models of perfluoroalkyls. The Harris and Barton (2008) model includes a red cell compartment that allows predictions of whole-blood concentrations. The utility of this feature remains to be determined, since PFOS does not appreciably concentrate in red blood cells and PFOS (and other perfluoroalkyls) is typically monitored in the central compartment with measurements of plasma or serum concentrations. The model assumes that the total concentration of PFOS (not just the free concentration) in plasma is available for distribution to liver and other tissues, whereas other models assume that only the free pool in plasma exchanges with tissues. The practical consequence of this difference may not be significant in terms of the toxicokinetics of PFOS if the tissue/plasma partition coefficients in the various models were estimated based on the relevant perfluoroalkyl pool in plasma. However, without basing distribution kinetics on the free concentration, it is not possible for concentration-dependent free fraction to be modeled. The model assumes time-dependence in the liver uptake and urinary excretion of PFOS, which were needed to improve predictions of plasma and liver concentrations of PFOS during chronic exposures. Other rat models (Loccisano et al. 2012a) have not been similarly evaluated. A mechanistic understanding of the time-dependent changes in PFOS kinetics will be important for applications of these models for dosimetry extrapolation across exposure durations.

3.1.5.6 Worley and Fisher (2015a, 2015b) Rat Model

Worley and Fisher (2015a, 2015b) expanded the Loccisano et al. (2012a) adult rat model to include simulation of renal proximal tubule apical (tubule-lumen) and basolateral (tubule-plasma) PFOA transport. This configuration allowed the use of data from *in vitro* studies of kinetics of specific transporters thought to be involved in proximal tubular transport of PFOA in the parametrization of the model. The kidney compartment was expanded to include compartments representing the proximal tubule lumen (glomerular filtrate) and proximal tubule cells. In the model, transfer of PFOA to the tubule lumen is governed by the glomerular filtration rate, represented by a clearance parameter (L/hour/kg kidney). PFOA in the tubule lumen can undergo first-order transfer to urine or saturable transport into the tubule cell (Km, Vmax). PFOA in the tubule exchanges with PFOA in plasma by three mechanisms: saturable transport from plasma into the cell (Km, Vmax), first-order transport from the cell to plasma (kefflux), or bidirectional diffusion between the cell and plasma (kdif). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OATP1a1 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates

of Vmax were adjusted with relative activity factors, which were calibrated to *in vivo* observations of plasma PFOA elimination kinetics in rats (Kemper 2003). Values for kefflux (proximal tubule cell to kidney plasma) and kdif (diffusion between kidney plasma and the tubule cell) were also calibrated with *in vivo* data (Kemper 2003; Kudo et al. 2007).

Calibration of the relative activity factor for apical and basolateral membrane transport of PFOA to serum observations made in male and female rats resulted in lower values for activity of both transporters in females compared to males. This resulted in the model predicting lower rates of reabsorptive transfer of filtered PFOA to plasma, and higher renal and systemic (plasma) clearance in females compared to males. Because proximal tubule transporters were assumed to be saturable, the model predicts an increase in clearance with increasing PFOA dose, with larger increases in clearance at lower doses in females compared to males. The model simulated the observed dose-dependent increase in serum clearance (decreasing serum $t_{1/2}$) and higher serum clearance of PFOA (lower $t_{1/2}$) in female rats compared to males (Kemper 2003).

3.1.5.7 Worley et al. (2017b) Human Model

Worley et al. (2017b) scaled and calibrated the Worley and Fisher (2015a, 2015b) rat model to simulate PFOA kinetics in humans exposed to PFOA in drinking water. Physiological parameters were allometrically scaled to the human. Tissue-plasma partition coefficients were derived from human autopsy data (kidney, liver) or studies of distribution of PFOA in rats (Fabrega et al. 2014; Kudo et al. 2007; Perez et al. 2013). Parameter values (Km, Vmax) for apical and basolateral transport of PFOA were derived from *in vitro* estimates for OAT4 (apical) and OAT1 and OAT3 (basolateral) (Nakagawa et al. 2008; Weaver et al. 2010; Yang et al. 2010; Yamada et al. 2007). These estimates were scaled to kidney proximal tubule cell mass (Hsu et al. 2014) and the mass-scaled estimates of Vmax were adjusted with relative activity factors. Parameters that control apical and basolateral transfers of PFOA in the proximal tubule and absorption in the gastrointestinal tract were calibrated against data on serum PFOA concentrations measured in people who drank water from a municipal water supply (Worley et al. 2017b). Model parameter values were adjusted to achieve agreement with geometric mean serum PFOA concentrations measured at two times separated by 6 years. The model was evaluated by comparing predicted and observed serum PFOA concentrations in populations exposed to PFOA in drinking water (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). A sensitivity analysis of the model identified that following biokinetic parameters that had standardized sensitivity coefficients >0.1: parameters controlling proximal tubule transport and urinary excretion, plasma-liver partition coefficient,

biliary excretion and protein binding. These parameters, along with drinking water consumption, were assigned probability distributions to conduct a Monte Carlo analysis of predicted serum PFOA predictions associated with exposures to PFOA in drinking water. The probabilistic model simulated interindividual variability in serum PFOA concentrations observed in exposed populations (Bartell et al. 2010; Emmett et al. 2006b; Steenland et al. 2009a, 2009b). These results suggest that that biokinetic variability, as well as exposure variability, may contribute to variability in serum PFOA concentrations observed in populations.

3.1.5.8 Fàbrega et al. (2014, 2016) Human Model

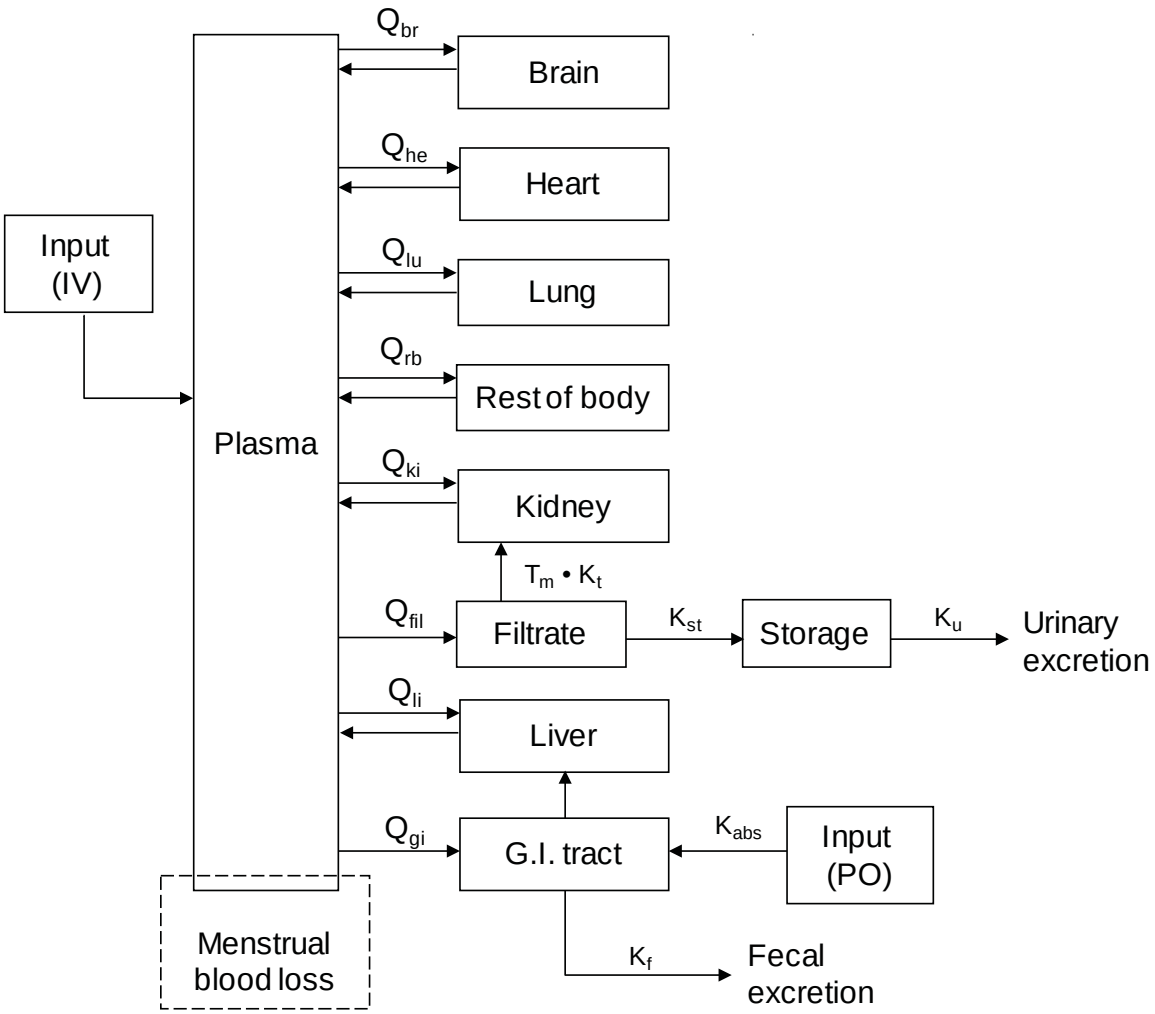
Fàbrega et al. (2014, 2016) modified the Loccisano et al. (2011, 2013) human models for PFOA and PFOS with inclusion of brain and lung compartments and removal of the skin compartment. Tissue-plasma partition coefficients were re-estimated using data from human cadavers (Maestri et al. 2006) in place of estimates based on rat data (Loccisano et al. 2011). The major differences in the partition coefficients for PFOA were lower values for liver in humans (1.03) compared to rats (2.20), higher values for fat in humans (0.47) compared to rats (0.04), and inclusion of partition coefficients for brain (0.17) and lung (1.27). For PFOS, the major differences in the partition coefficients were lower values for liver in humans (2.67) compared to rats (3.72) and higher values for fat in humans (0.33) compared to rats (0.14). Values for parameters that control urinary excretion (Tm and Km for reabsorptive transport from glomerular filtrate to kidney tissue) were recalibrated based on plasma concentration data (Ericson et al. 2007). Fàbrega et al. (2014) compared predictions to observed concentrations of PFOA and PFOS in cadaver samples (from Tarragona County, Spain) for constant intakes of 0.11 μg/day for PFOA or 0.13μg/day for PFOS. Better agreement with observations was achieved with partition coefficients based on cadaver data. Fàbrega et al. (2016) performed a quantitative uncertainty analysis of predictions of tissue PFOA and PFOS concentrations by assigning lognormal probability distributions to renal transport parameters, the unbound fraction in plasma, and intake. Probability distributions for PFOA and PFOS intakes were based on data from Domingo et al. (2012a, 2012b). Distributions for biokinetic parameters were established to achieve a coefficient of variation of 0.3 (Allen et al. 1996; Brochot et al. 2007; Sweeney et al. 2001). Observations of tissue PFOA and PFOS were within uncertainty bounds on predictions.

3.1.5.9 Kim et al. (2018) Rat and Human Model

Kim et al. (2018) developed a model for simulating the kinetics of PFHxS in rats and humans. The structures of the rat and human models are identical (Figure 3-10). Complete lists of parameters and parameter values and the bases for parameter values and evaluations of model predictions in comparison to observations are reported in Kim et al. (2018). The model includes compartments representing plasma (including a bound and free fraction), brain, gastrointestinal tract, heart, lung, kidney and renal glomerular filtrate, liver, and a lumped compartment representing all other tissues. A storage compartment receives PFHxS from the glomerular filtrate and is included in the model to simulate the time delay between elimination from plasma and appearance of PFHxS urine. Absorption from the gastrointestinal tract is simulated as the balance between first-order absorption and fecal excretion of unabsorbed PFHxS. Absorbed PFHxS is assumed to be delivered to the liver. Exchanges between PFHxS in tissues with the free pool in plasma are assumed to be flow-limited (governed by blood flow) with equilibrium determined by the tissue:plasma partition coefficient. Partition coefficients were estimated from the tissue:plasma concentration ratios measured in female and male rats 14 days after a single intravenous dose of PFHxS (0.5–10 mg/kg). Values for each sex were significantly different for brain, lung, liver, spleen, gastrointestinal tract, adipose, and skeletal muscle; in each case, male>female. The highest partition coefficient was in male liver (approximately 0.13), with the value for female being approximately half of the male value. PFHxS in plasma is simulated as instantaneous distributions into free and bound fractions. The free fraction was estimated from ultrafiltration studies of rat and human plasma. The free fraction was assigned a constant of 0.069% in female and 0.076% in male rats.

Elimination of absorbed PFHxS in the rat model occurs by fecal and urinary excretion. Fecal excretion of absorbed PFHxS is represented as flow-limited transfer from plasma to the gastrointestinal tract and first order transfer from the gastrointestinal tract to feces. Excretion in urine is simulated as the balance between transfer from the free fraction of plasma to the glomerular filtrate and renal tubular reabsorption, which removes PFHxS from the glomerular filtrate and returns it to kidney tissue. Renal tubular reabsorption is simulated as a capacity-limited process with parameters T_m (µg/hour), representing the maximum rate of transport, and K_T (µg/L), representing affinity for the transporter (the concentration in the glomerular filtrate at which reabsorptive transport rate is half of maximum). This representation of renal tubular reabsorption is used to simulate observed sex differences in elimination of PFHxS from

Figure 3-10. Structure of the PBPK Model for PFHxS in Rats and Humans*



*PFHxS can be resorbed into the kidney with transporter maximum (T_m) and transporter affinity constant (K_t). Ks indicates a rate constant; K_{st}, the rate constant to the storage compartment; K_u, the urinary elimination rate constant; K_f, the transfer rate constant from the G.I. tract to fecal elimination; and K_{abs}, the oral absorption rate constant. Q_s refers to the blood flows between plasma and tissues, except for Q_{fil}, which is a clearance from the plasma to the filtrate compartment. Menstrual blood loss (dotted square) is only applicable to female humans.

G.I. = gastrointestinal; IV = intravenous; PBPK = physiologically based pharmacokinetic; PFHxS = perfluorohexane sulfonic acid; PO = *per os*

Source: Reprinted by permission from Springer Nature, Kim et al. 2018

plasma, which have been attributed to higher reabsorptive capacity in male rats (see Section 3.1.4). Values for the maximum and affinity parameters for PFHxS result in higher reabsorptive clearances from the glomerular filtrate (T_m/K_T=5.2) in male rats compared to female rats (T_m/K_T=0.057), and correspondingly lower urinary clearance of PFHxS from plasma in male rats. Values for T_m and K_t in humans were assumed to be the same as those in rats. Tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983). Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2012a).

The rat model was calibrated and evaluated against data on plasma and tissue levels of PFHxS measured following a single intravenous (0.5–10 mg/kg) or gavage dose (1 or 4 mg/kg) of PFHxS (Kim et al. 2018). Temporal profiles of plasma PFHxS and cumulative urinary excretion following intravenous or oral dosing were within ±1 SD of observations. Predicted cumulative urinary excretion of PFHxS reproduced the observed sex differences in urinary excretion with slower excretion and higher plasma levels in males compared to females. Terminal levels of PFHxS in heart, kidney, liver, and lung predicted for 14 days following oral dosing were within the range of observed values.

The human model was developed from the rat model with the following attributes:

- Human tissue volumes and blood flows were assigned values based on various sources (Davies and Morris 1993; Igari et al. 1983).
- Glomerular filtrate volume and flow were assigned values from Loccisano et al. (2011).
- Values for the free fraction in human plasma were 0.023% in females and 0.025% in males, based on results from ultrafiltration studies.
- Sex-specific values for renal tubular reabsorption parameters, T_m and K_t, were assumed to be the same in rats and humans.
- First order rate constants were scaled by 0.25 power of body weight ($BW^{0.25}$).
- Loss of PFHxS in menstrual blood was included in the human female model. This is represented as a direct loss of loss of 42.5 mL blood (25 mL plasma) per month (Verner and Longnecker 2015).

Kim et al. (2018) does not report an evaluation of the human model.

Applications for Dosimetry Extrapolation and Risk Assessment. The rat and human models were applied interspecies dosimetry extrapolation of a rat NOAEL for PFHxS (1 mg/kg/day). The rationale for the rat NOAEL is described in Kim et al. (2018). The dosimetry extrapolation was applied to the rat

model to predict a steady-state plasma concentration of PFHxS corresponding to a chronic oral dose of 1 mg/kg/day (value not reported). The equivalent human dose was predicted from the human PBPK model as the daily dose required to achieve the same steady state concentration in the human.

3.1.6 Animal-to-Human Extrapolations

Interspecies differences in the toxicokinetics of perfluoroalkyls and possible differences in the mechanisms of toxicity have been found. The elimination rate for PFOA in female rats is approximately 45 times faster than in male rat, 150 times faster than in Cynomolgus monkeys, and approximately 5,000–9,000 times faster than in humans (Bartell et al. 2010; Butenhoff et al. 2004c; Kemper 2003; Olsen et al. 2007a). Elimination of PFOS in male rats is approximately 3 times faster than in Cynomolgus monkeys and approximately 40 times faster than in humans (Chang et al. 2012; De Silva et al. 2009; Olsen et al. 2007a; Seacat et al. 2002). These large differences in elimination rates imply that similar external PFOA or PFOS dosages (i.e., mg/kg/day) in rats, monkeys, or humans would be expected to result in substantially different steady-state internal doses (i.e., body burdens, serum concentrations) of these compounds in each species. In addition, exposure durations required to achieve steady state would be expected to be much longer in humans than in monkeys or rats. Assuming a terminal elimination $t_{1/2}$ of 1,400 days for PFOA in humans (Olsen et al. 2007a), a constant rate of intake for 17 years would be required to achieve 95% of steady state. Steady state (i.e., 95%) would be achieved in approximately 110 days in monkeys ($t_{1/2}$=25 days, Butenhoff et al. 2004c), 30 days in male rats ($t_{1/2}$=7 days; Kemper 2003), and 1 day in female rats ($t_{1/2}$=0.2 days; Kemper 2003). Using an internal dose metric such as serum perfluoroalkyl concentration and PBPK models that can account for these differences in elimination rates can decrease the uncertainty in extrapolating from animals to humans.

The mode of action for most health outcomes associated with perfluoroalkyl exposure has not been fully characterized in humans or laboratory animals. Some perfluoroalkyl-induced effects observed in rats and mice appear to be mediated through the PPARα-dependent and -independent mechanisms (see Section 2.20 for additional information). Interpretation of the relevance of the effects observed in laboratory animals is complicated since it is generally agreed that humans and nonhuman primates are refractory, or at least less responsive than rodents, to PPARα-mediated effects (Corton et al. 2014; Klaunig et al. 2003; Maloney and Waxman 1999). While studies in mice have identified specific effects that require PPARα activation, for example, postnatal viability (Abbott et al. 2007) and some immunological effects (Yang et al. 2002b), other effects such as hepatomegaly and antigen-specific antibody response (DeWitt et al. 2016) were reported to be PPARα-independent (Yang et al.

2002b). Therefore, further studies are needed to expand the knowledge regarding PPARα-dependent and -independent effects that would allow selection of an appropriate animal model for perfluoroalkyls toxicity. In the absence of data to the contrary, ATSDR assumes that the health effects observed in laboratory animals are relevant to humans. The exception is some of the hepatic effects observed in rodents; increases in liver weight and hepatocellular hypertrophy observed in rats and mice were considered adaptive and not relevant to humans (see Section 2.9 for details).

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at greater exposure risk to unusually high exposure levels to perfluoroalkyls are discussed in Section 5.7, Populations with Potentially High Exposures.

The possible association between serum perfluoroalkyl levels in children and health effects has been examined in participants of the C8 Health Project and in the general population. The studies examined a number of health effects including alterations in serum lipid levels, adverse renal outcomes, neurodevelopmental alterations, and reproductive development. Immunotoxicity has been examined in children in several general population studies. Additionally, a large number of studies have examined the possible association of elevated serum perfluoroalkyl levels and adverse birth outcomes.

Similar to adults, associations between serum PFOA and PFOS and serum cholesterol levels were observed in a study of over 12,000 children (Frisbee et al. 2010); an increased risk of high cholesterol was also observed in children with higher serum PFOA and PFOS levels. A smaller study of children (n=43)

living in the Mid-Ohio Valley did not find associations between serum PFOA levels and hematology parameters, total cholesterol and liver enzymes, indices of kidney function, or serum TSH levels (Emmett et al. 2006b). Another study of highly-exposed residents did not find any associations between serum PFOA levels in children aged 6–12 years and IQ, reading and math skills, language, memory, learning, or attention (Stein et al. 2013). Similarly, no association between serum PFOA, PFOS, or PFNA levels in children 5–18 years old and the likelihood of ADHD diagnosis was observed in a study of highly-exposed residents, although the study did find an increased risk associated with higher PFHxS levels (Stein and Savitz 2011). A general population study that utilized the NHANES data found an association between serum PFOA, PFOS, and PFHxS levels and the risk of ADHD diagnosis (as reported by the parent) (Hoffman et al. 2010). Another smaller-scale study found associations between serum PFOS, PFNA, PFDA, PFHxS, and FOSA and impulsivity; no association with PFOA was found (Gump et al. 2011). A study of children 8–18 years of age participating in the C8 studies found reduced odds of reaching puberty at higher serum PFOA levels (Lopez-Espinosa et al. 2011); however, the biological significance of the short delay (4–5 months) is not known.

Several studies have evaluated immunotoxicity in children and adolescents. These studies have found impaired antibody responses associated with serum PFOA, PFOS, PFHxS, and PFDA (Grandjean et al. 2012, 2017; Granum et al. 2013; Mogensen et al. 2015a; Stein et al. 2016a). An increased asthma diagnosis was also associated with serum PFOA levels (Dong et al. 2013; Humblet et al. 2014; Zhu et al. 2016). Marginal evidence of an association with asthma diagnosis was also found for PFOS, PFHxS, PFNA, PFDA, PFBS, and PFDoDA (Dong et al. 2013; Zhu et al. 2016), although some studies found no associations for these compounds (Humblet et al. 2014; Smit et al. 2015; Stein et al. 2016a).

Hines et al. (2009) showed that *in utero* exposure (GDs 1–17) to low levels of PFOA (0.01–0.3 mg/kg/day) resulted in increases in body weight gain in 10–40-week-old mice; by 18 months of age, the body weights in these mice were similar to controls. Increases in serum insulin and leptin levels were also observed in the mice exposed to 0.01 and 0.1 mg/kg/day. The study also compared body weight and body composition of *in utero* exposed mice (exposed on GDs 1–17) and adult exposed mice (exposed for 17 days starting at 8 weeks of age) and found that *in utero* exposure to 1 mg/kg/day resulted in significantly higher body weight, brown fat weight, and white fat weight; this was not observed in mice exposed to 5 mg/kg/day. The results of the study suggest that gestational exposure to low doses of PFOA may result in increased susceptibility to PFOA toxicity.

A number of studies of highly exposed residents and the general population have examined the potential associations between serum perfluoroalkyl levels and alterations in birth weight. Decreases in birth weight have been found to be associated with higher PFOA (Fei et al. 2007; Lee et al. 2013; Maisonet et al. 2012; Savitz et al. 2012b) or PFOS levels (Maisonet et al. 2012), but not with lower levels of perfluoroalkyls (Fei et al. 2007; Hamm et al. 2010; Inoue et al. 2004; Kim et al. 2011; Monroy et al. 2008; Washino et al. 2009; Whitworth et al. 2012b). The decreases in birth weight were small (<20 g or 0.7 ounces per 1 ng/mL). Additionally, no increases in the risk of low birth weight infants were found in highly exposed populations (Darrow et al. 2013; Nolan et al. 2009; Savitz et al. 2012b; Stein et al. 2009). No apparent alterations in the risk of birth defects were found in C8 Health Studies (Darrow et al. 2013; Savitz et al. 2012b; Stein et al. 2009) or in another study of these communities (Nolan et al. 2009).

The developmental toxicity of PFOA and PFOS has been investigated in a number of rat and mouse studies. The observed effects include PFOA- and PFOS-induced increases in prenatal losses and decreases in pup survival, decreases in pup body weight, and neurodevelopmental toxicity (Abbott et al. 2007; Albrecht et al. 2013; Case et al. 2001; Chen et al. 2012b; Era et al. 2009; Fuentes et al. 2006, 2007a, 2007b; Grasty et al. 2003; Hu et al. 2010; Johansson et al. 2008; Lau et al. 2003, 2006; Luebker et al. 2005a, 2005b; Onishchenko et al. 2011; Thibodeaux et al. 2003; White et al. 2007, 2009, 2011; Wolf et al. 2007; Xia et al. 2011; Yahia et al. 2008, 2010). Additionally, delays in mammary gland development were observed in mice exposed to PFOA (Macon et al. 2011; White et al. 2007, 2009, 2011). A limited number of developmental endpoints have been examined in rats and mice exposed to PFDA, PFHxS, or PFBA (Butenhoff et al. 2009a; Das et al. 2008; Harris and Birnbaum 1989; Johansson et al. 2008; Viberg et al. 2013). A more in-depth discussion of the developmental toxicity of perfluoroalkyls in animals is included in Section 2.17.

PFOA and PFOS, as well as other perfluoroalkyls, are valid biomarkers of exposure to these compounds in children, as they are in adults. No relevant studies were located regarding interactions of perfluoroalkyls with other chemicals in children or adults.

No studies examining increased susceptibility to the toxicity of perfluoroalkyls were identified. The available epidemiological data identify several potential targets of toxicity of perfluoroalkyls, and individuals with pre-existing conditions may be unusually susceptible. For example, it appears that exposure to PFOA or PFOS can result in increases in serum lipid levels, particularly cholesterol levels. Thus, an increase in serum cholesterol may result in a greater health impact in individuals with high levels of cholesterol or with other existing cardiovascular risk factors. Associations have been found between

PFOA and PFOS levels and an increased risk of hypertension/pre-eclampsia in pregnant women. The liver has been shown to be a sensitive target in a number of animal species and there is some indication that it is also a target in humans. Therefore, individuals with compromised liver function may represent a susceptible population. Likewise, individuals with a compromised immune system may have an increased risk of perfluoroalkyl-induced immunotoxicity.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to perfluoroalkyls are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for perfluoroalkyls from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effect caused by perfluoroalkyls are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the

biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Measurement of serum or whole-blood perfluoroalkyl concentrations is the standard accepted biomarker of perfluoroalkyl exposure in humans. Perfluoroalkyls have been detected in the serum of workers, residents living near perfluoroalkyl facilities, and the general population. As part of NHANES, CDC has been measuring serum levels of perfluoroalkyls in the U.S. general population since 1999. Of the 12 perfluoroalkyls examined in this toxicological profile, blood concentrations of 7 compounds (PFOA, PFOS, PFDA, PFHxS, PFNA, and PFUnA) were detected in enough subjects to allow for estimation of the geometric mean. As compared to the general population, serum PFOA and PFOS levels are much higher in individuals with occupational exposure to these compounds (Olsen et al. 2003a; Sakr et al. 2007a) and PFOA levels are much higher in individuals living near a PFOA manufacturing facility (Emmett et al. 2006a; Steenland et al. 2009a), suggesting that serum levels are a good biomarker of exposure. Due to the long half-life of some perfluoroalkyls, particularly PFOA and PFOS, elevated serum levels may not be indicative of recent exposure. Although elevated serum levels are likely to be indicative of exposure to the parent compound, their presence in blood can also indicate exposure to other perfluoroalkyls. For example, PFOS can be derived from metabolism of FOSA (Olsen et al. 2005; Seacat and Luebker 2000). PFOA can be derived from metabolism of 8-2 fluorotelomer alcohol (Fasano et al. 2006; Henderson and Smith 2007; Kudo et al. 2005; Nabb et al. 2007). Exposure of mice to 8–2 telomer alcohol also generated PFNA as a metabolite (Kudo et al. 2005). Most epidemiological studies measured serum perfluoroalkyl levels as a biomarker of exposure. In general, these studies provided a one-time serum perfluoroalkyl level, but lacked information on actual environmental exposure concentrations or doses, route of exposure, and exposure duration. The differences in elimination half-lives between perfluoroalkyls also confounds the interpretation of one-time measurements; the relative concentration of the perfluoroalkyls measured in serum may not be reflective of the actual mixture to which the individual was exposed.

Two studies have also evaluated the use of perfluoroalkyl levels in hair as a biomarker of exposure. In rats administered PFOA, PFOS, or PFNA in the drinking water for 90 days, significant correlations between hair perfluoroalkyl levels and serum and tissue (liver, heart, lung, kidney) levels were found, suggesting that hair perfluoroalkyl levels may be a reliable biomarker of exposure (Gao et al. 2015). A study in humans (Alves et al. 2015) has also found detectable levels of PFBA, PFHxA, PFOA, PFBS, and

PFHxS in hair samples, but PFHpA, PFNA, and PFOS were not detected in hair samples. The study did not evaluate the potential relationship between serum perfluoroalkyl levels and hair levels, which does not allow for an assessment of whether hair is a viable biomarker of exposure.

Urinary perfluoroalkyl levels have also been evaluated as a biomarker of exposure (Worley et al. 2017a). A study of highly exposed residents measured urinary PFOA, PFOS, PFNA, and PFHxS levels. With the exception of PFOA, the proportion of values below the detection limit was too high to calculate mean or median values. The study found a strong linear correlation between serum PFOA levels and urinary PFOA levels in men and a nonsignificant weak correlation between serum and urinary PFOA levels in women.

3.3.2 Biomarkers of Effect

There are no specific biomarkers of effect caused by perfluoroalkyls.

3.4 INTERACTIONS WITH OTHER CHEMICALS

There are limited data on the interactions of perfluoroalkyls with other chemicals. Particularly absent are studies examining toxicological and toxicokinetic interactions of a perfluoroalkyl with other perfluoroalkyls. Olestra decreased the absorption of PFOA from the gastrointestinal tract of mice (Jandacek et al. 2010). No additional information was located regarding interactions among chemicals of this class or between perfluoroalkyls and other chemicals. Both PFOA and PFOS (and many other diverse chemicals) can activate the PPARα, as well as other PPARs to a lesser extent (Takacs and Abbott 2007; Vanden Heuvel et al. 2006). Therefore, it is not unreasonable to speculate that interactions at the receptor level might occur; however, there are no experimental data to support or rule out this presumption. PPARα-independent mechanism are also involved in the toxicity of perfluoroalkyls and interactions between compounds are also likely to influence these mechanisms.

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of perfluoroalkyls is located in Table 4-1. This information includes synonyms, chemical formulas and structures, and identification numbers. The perfluoroalkyls discussed in this profile exist as linear and branched isomers depending upon the method of production (see Chapter 5) and the reported values for the physical-chemical properties are typically reflective of the mixtures rather than a single specific isomer.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of perfluoroalkyls is located in Table 4-2.

Perfluoroalkyls are very stable, owing to the strength of the carbon-fluorine bonds, the presence of the three electron pairs surrounding each fluorine atom, and the shielding of the carbon atoms by the fluorine atoms (3M 1999; Kissa 2001; Schultz et al. 2003). Perfluoroalkyl carboxylates and sulfonates are resistant to direct photolysis and reaction with acids, bases, oxidants, and reductants (3M 2000; EPA 2008a; OECD 2002, 2006a, 2007; Schultz et al. 2003).

APFO was shown to decompose starting at 196°C (Krusic and Roe 2004) and PFOA was shown to decompose rapidly in the presence of crushed borosilicate glass at 307°C (Krusic et al. 2005). 1-H perfluoroheptane and perfluoroheptene are noted degradation products.

Perfluoroalkyl carboxylates and sulfonates consist of a perfluorocarbon tail that is both hydrophobic and oleophobic and a charged end that is hydrophilic (3M 1999; de Vos et al. 2008; Kissa 2001; Schultz et al. 2003). This combination of hydrophobic and oleophobic characteristics makes these substances very useful as surfactants. The ability of these substances to repel oil, fat, and water has resulted in their use in surface protectants (Kissa 2001). Their ability to reduce the surface tension of aqueous systems to <20 mN/m has resulted in their use as wetting agents (Kissa 2001). Neutral or uncharged perfluoroalkyls or very long chain constituents are expected to form separate layers when mixed with hydrocarbons and water. Conversely, charged species, salts, and ionized species at relevant pH (i.e., PFOS, PFOA, PFHpA, PFNA) and short-chain species (i.e., PFBA, PFBS) have relatively good solubility in water and alcohol.

Table 4-1. Chemical Identity of Perfluoroalkyls

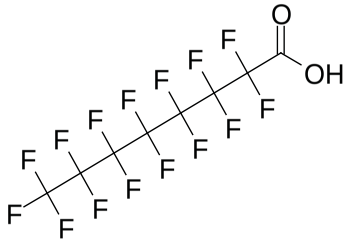
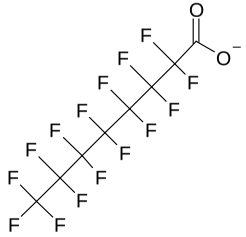
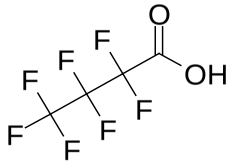
Characteristic	Information		
Chemical name	Perfluorooctanoic acid	Ammonium perfluorooctanoate	Perfluorobutanoic acid
Synonym(s)	PFOA; pentadecafluoro-1-octanoic acid; pentadecafluoro-n-octanoic acid; pentadecafluorooctanoic acid; perfluorocaprylic acid; perfluorooctanoic acid; perfluoroheptanecarboxylic acid; octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8, 8-pentadecafluoro-	APFO; ammonium pentadecafluorooctanoate; octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-ammonium salt (1:1)	PFBA; heptafluoro-1-butanoic acid; heptafluorobutanoic acid; heptafluorobutyric acid; perfluorobutyric acid; perfluoropropanecarboxylic acid; 2,2,3,3,4,4,4-heptafluorobutanoic acid
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_8HF_{15}O_2$	$C_8H_4F_{15}NO_2$	$C_4HF_7O_2$
Chemical structure			
CAS Registry Number	335-67-1	3825-26-1	375-22-4

Table 4-1. Chemical Identity of Perfluoroalkyls

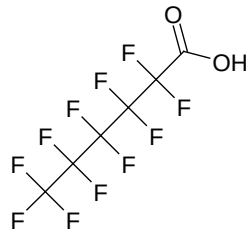
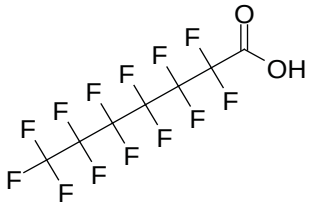
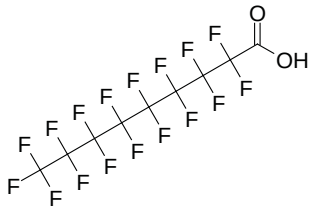
Characteristic	Information		
Chemical name	Perfluorohexanoic acid	Perfluoroheptanoic acid	Perfluorononanoic acid
Synonym(s)	PFHxA; undecafluoro-1-hexanoic acid; hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-	PFHpA; perfluoro-n-heptanoic acid; tridecafluoro-1-heptanoic acid; heptanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,7-tridecafluoro-	PFNA; perfluoro-n-nonanoic acid; perfluorononan-1-oic acid; heptadecafluoro-nonanoic acid; nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_6HF_{11}O_2$	$C_7HF_{13}O_2$	$C_9HF_{17}O_2$
Chemical structure			
CAS Registry Number	307-24-4	375-85-9	375-95-1

Table 4-1. Chemical Identity of Perfluoroalkyls

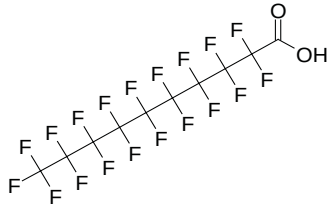
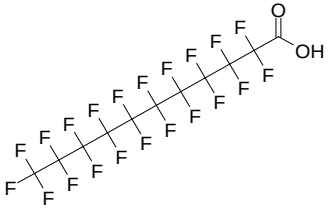
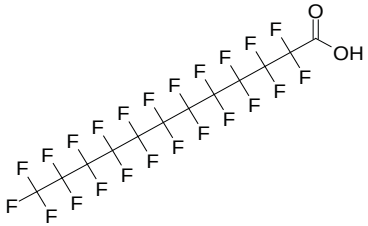
Characteristic	Information		
Chemical name	Perfluorodecanoic acid	Perfluoroundecanoic acid	Perfluorododecanoic acid
Synonym(s)	PFDA; PFDeA; Ndfda; nonadecafluoro-n-decanoic acid; nonadecafluorodecanoic acid; perfluoro-n-decanoic acid; decanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-nonadecafluoro-	PFUnA; perfluoro-n-undecanoic acid; henicosafluoroundecanoic acid; 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heneicosafluoroundecanoic acid	PFDoDA; tricosafluorododecanoic acid; dodecanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-tricosafluoro-
Registered trade name(s)	No data	No data	No data
Chemical formula	$C_{10}HF_{19}O_2$	$C_{11}HF_{21}O_2$	$C_{12}HF_{23}O_2$
Chemical structure			
CAS Registry Number	335-76-2	2058-94-8	307-55-1

Table 4-1. Chemical Identity of Perfluoroalkyls

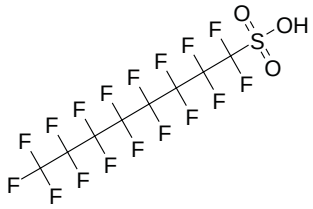
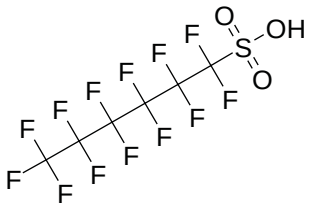
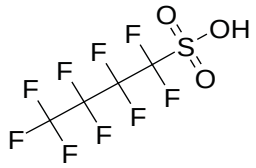
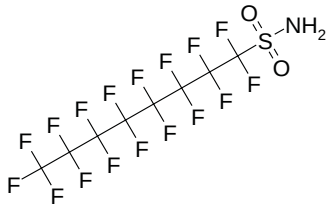
Characteristic	Information	
Chemical name	Perfluorooctane sulfonic acid	Perfluorohexane sulfonic acid
Synonym(s)	PFOS; 1-perfluorooctanesulfonic acid; heptadecafluoro-1-octanesulfonic acid; heptadecafluorooctan-1-sulphonic acid; perfluorooctane sulfonate; perfluorooctylsulfonic acid; 1-octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-	PFHxS; perfluorohexane-1-sulphonic acid; 1-hexanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-; 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane-1-sulfonic
Registered trade name(s)	No data	No data
Chemical formula	$C_8HF_{17}O_3S$	$C_6HF_{13}O_3S$
Chemical structure		
CAS Registry Number	1763-23-1	355-46-4

Table 4-1. Chemical Identity of Perfluoroalkyls

Characteristic	Information	
Chemical name	Perfluorobutane sulfonic acid	Perfluorooctanesulfonamide
Synonym(s)	PFBS; 1-perfluorobutanesulfonic acid; nonafluoro-1-butanesulfonic acid; nonafluorobutanesulfonic acid; pentyl perfluorobutanoate; 1,1,2,2,3,3,4,4,4-nonafluoro-1-butanesulfonic acid; 1,1,2,2,3,3,4,4,4-nonafluoro-butane-1-sulphonic acid; 1-butanesulfonic acid, nonafluoro- (6Cl,7Cl,8Cl)	FOSA; perfluorooctylsulfonamide; perfluoro-octanesulfonic acid amide; heptadecafluorooctane-sulphonamide; 1-octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-
Registered trade name(s)	No data	No data
Chemical formula	$C_4HF_9O_3S$	$C_8H_2F_{17}NO_2S$
Chemical structure		
CAS Registry Number	375-73-5	754-91-6

CAS = Chemical Abstracts Services

Sources: Calafat et al. 2007a, 2007b; CAS 2008; ChemIDplus 2008, 2017; RTECS 2008

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFOA	APFO	PFBA	PFHxA
Molecular weight	414.069[a]	431.1[b]	214.039[a]	314.06[c]
Color	White to off-white[d]	No data	No data	Colorless[d]
Physical state	Solid[e]	Solid[b]	Liquid[a]	Liquid[d]
Melting point	54.3°C[a]	Decomposition starts above 105°C[b]	-17.5°C[a]	No data
Boiling point	188°C[a]	No data	121°C[a]	168°C at 742 mm Hg[f]
Density at 20°C	1.8 g/cm^3[g]	No data	1.651 g/cm^3[a]	1.789[f]
Odor	No data	No data	No data	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	9.5x10^3 mg/L at 25°C[h] 2.29 x10^3 mg/L at 24°C[i] 3.3x10^3 mg/L at 25°C[j] 4.34x10^3 mg/L at 24.1°C[k]	>500 g/L[b]	2.14x10^5 mg/L at 25°C[l]	15,700 mg/L[m]
Organic solvents	No data	No data	Soluble in ethanol and toluene; insoluble in petroleum ether[a]	No data
Partition coefficients:				
Log K_{ow}	Not applicable[n]	No data	Not applicable[n]	Not applicable[n]
Log K_{oc}	1.69–2.36[o] 2.06[p]	K_{OC} 49–230[q]	2.17, average (n=7)[o]	2.06, average (n=7)[o]
pKa	-0.5[r] 0.5[s]	No data	0.08 (estimated)[t]	-0.16[m]
Vapor pressure	0.017 mm Hg at 20°C (extrapolated); 0.962 mm Hg at 59.25°C (measured)[u] 0.0316 mm Hg at 25°C[i]	0.0081 Pa at 20°C[b]	44 mm Hg at 56°C[h]	No data
Henry's law constant	0.362 Pa-m^3/mol[l]	No data	1.24Pa-m^3/mol[l]	No data
Autoignition temperature	Not applicable[v]	No data	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	No data	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[u]	No data	Not applicable[u]	Not applicable[u]
Conversion factors	1 ppm=16.94 mg/m^3; 1 mg/m^3=0.059 ppm[w]	1 ppm=17.63 mg/m^3; 1 mg/m^3=0.057 ppm[w]	1 ppm=8.75 mg/m^3; 1 mg/m^3=0.11 ppm[w]	1 ppm=12.84 mg/m^3; 1 mg/m^3=0.078 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFHpA	PFNA	PFDA	PFUnA
Molecular weight	364.06[u]	464.08[u]	514.084[t]	564.085[w]
Color	Beige[d]	No data	No data	No data
Physical state	Crystalline solid[d]	No data	No data	No data
Melting point	24–30°C[x]	No data	No data	97.9–100.3°C[x]
Boiling point	175°C at 742 mm Hg[h]	No data	219°C	No data
Density at 20°C	1.792 g/cm^3 [y]	No data	No data	No data
Odor	No data	No data	No data	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	4.37x10^5 mg/L at 25°C[l]	No data	No data	No data
Organic solvents	No data	No data	No data	No data
Partition coefficients:				
Log K_{ow}	Not applicable[n]	Not applicable[n]	Not applicable[n]	Not applicable[n]
Log K_{oc}	2.04, average (n=7)[o]	2.39[p]	2.79[p]	3.30[p]
pKa	-0.15 (estimated)[t] -2.29 (estimated)	-0.21 (estimated)[d]	-0.17 (estimated)[t]	-0.17 (estimated)[t]
Vapor pressure	4.6 mm Hg at 25°C[h] 0.133 at 25°C[d]	4.83x10^{-3} mm Hg at 20°C (extrapolated); 8.4 mm Hg at 99.63°C (measured)[t]	7.62x10^{-4} mm Hg at 20°C (extrapolated); 23.5 mm Hg at 129.56°C (measured)[aa]	3.44x10^{-4} mm Hg at 20°C (extrapolated); 4.62 mm Hg at 112.04°C (measured)[aa]
Henry's law constant at 25°C	0.573 Pa-m^3/mol[h]	No data	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]
Conversion factors	1 ppm=14.89 mg/m^3; 1 mg/m^3=0.067 ppm[w]	1 ppm=18.98 mg/m^3; 1 mg/m^3=0.053 ppm[w]	1 ppm=21.03 mg/m^3; 1 mg/m^3=0.048 ppm[w]	1 ppm=23.07 mg/m^3; 1 mg/m^3=0.043 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFDoDA	PFOS	PFHxS
Molecular weight	614.1[c]	500.03[c]	400.12[c]
Color	No data	No data	No data
Physical state	No data	No data	No data
Melting point	No data	≥400°C (potassium salt)[z]	No data
Boiling point	No data	No data	No data
Density at 20°C	No data	No data	No data
Odor	No data	No data	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No data	No data
Solubility:			
Water	No data	570 mg/L (potassium salt in pure water)[z]	No data
Organic solvents	No data	No data	No data
Partition coefficients:			
Log K_{ow}	Not applicable[j]	Not applicable[j]	Not applicable[j]
Log K_{oc}	No data	3.14, average (n=7)[o] 2.57[p]	2.28, average (n=7)[o]
pKa	-0.17 (estimated)[t]	0.14 (estimated)[t]	0.14 (estimated)[t]
Vapor pressure	5.11×10^{-60} mm Hg at 20°C (extrapolated)[z]	2.48×10^{-6} mm Hg at 20°C (potassium salt)[e]	No data
Henry's law constant at 25°C	No data	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]	Not applicable[v]
Conversion factors	1 ppm=25.12 mg/m^3; 1 mg/m^3=0.04 ppm[w]	1 ppm=20.45 mg/m^3; 1 mg/m^3=0.049 ppm[w]	1 ppm=16.36 mg/m^3; 1 mg/m^3=0.061 ppm[w]
Explosive limits	Not applicable[v]	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Property	PFBS	FOSA
Molecular weight	300.1[c]	499.15[c]
Color	No data	No data
Physical state	No data	No data
Melting point	No data	No data
Boiling point	No data	No data
Density at 20°C	No data	No data
Odor	No data	No data
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water	No data	No data
Organic solvents	No data	No data
Partition coefficients:		
Log K_{ow}	Not applicable[n]	Not applicable[n]
Log K_{oc}	2.06 avg (n=7)[o]	No data
pKa	0.14 (estimated)[t]	6.24 (estimated)[t]
Vapor pressure	No data	No data
Henry's law constant	No data	No data
Autoignition temperature	Not applicable[v]	Not applicable[v]
Flashpoint	Not applicable[v]	Not applicable[v]
Flammability limits	Not applicable[v]	Not applicable[v]

Table 4-2. Physical and Chemical Properties of Perfluoroalkyls

Conversion factors	1 ppm=12.27 mg/m^3; 1 mg/m^3=0.081 ppm[u]	1 ppm=20.42 mg/m^3; 1 mg/m^3=0.049 ppm[w]
Explosive limits	Not applicable[u]	Not applicable[u]

[a]Lide 2005.
[b]EPA 2014.
[c]EPA 2008c.
[d]HSDB 2019.
[e]3M 2008c.
[f]Savu 1994a.
[g]Kroschwitz and Howe-Grant 1994.
[h]Kauck and Diesslin 1951.
[i]Bhhatarai and Gramatica 2011.
[j]Inoue et al. 2012.
[k]Rahman et al. 2014.
[l]Kwan 2001.
[m]Zhao et al. 2014.
[n]The log K_{ow} is not measurable since these substances are expected to form multiple layers in an octanol-water mixture (3M 1999, 2008c; EPA 2005a).
[o]McGuire et al. 2014.
[p]Higgins and Luthy 2006.
[q]Prevedouros et al. 2006.
[r]Goss 2008.
[s]Vierke et al. 2013.
[t]SPARC 2008.
[u]Kaiser et al. 2005.
[v]Perfluorocarboxylates and perfluorosulfonates are nonflammable (3M 1999, Kissa 2001, OECD 2007). However, they readily degrade via incineration (Krusic and Roe 2004; Krusic et al. 2005; Yamada et al. 2005).
[w]Calculated using molecular weight and the equation ppm=(X mg/m^3) x (24.45/molecular weight); mg/m^3=(X.ppm) x (molecular weight/24.45).
[x]ChemID Plus 2008.
[y]Kunleda and Shinoda 1976.
[z]Siegemund et al. 2015.
[aa]3M 2000.

APFO= ammonium perfluorooctanoate; FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The formation of an emulsified layer between the octanol and water interface makes the accurate measurement of properties such as log K_{ow} very difficult using conventional experimental techniques like the shake flask method or the slow stir technique (EPA 2005a, 3M 1999, 2008c). Xiang et al. (2018) used reverse-phase high-performance liquid chromatography (HPLC) to study the partitioning between the stationary column and mobile phase as a means of simulating the octanol-water partitioning process. As discussed in this study, acidic ionizable compounds are difficult to measure with this method since the pH of the mobile phase should be 2 log units lower than the pK_a of the chemical; however, extremely low pHs damage the stationary column. Therefore, a mobile phase over a range of pHs (1.09–5.00) was used to estimate the log D (sum of the ionized and unionized species in octanol/sum of the ionized and unionized species in water) over this pH range for 11 perfluoroalkylcarboxylic acids, including PFOA, and then the log D was converted to the neutral species log K_{ow}. The estimated log K_{ow} values of the perfluoroalkylcarboxylic acids (C4–C14) were in the range of 1.05 (PFBA) to 7.19 (perfluorotetra-decanoic acid).

Both the potential to form separate layers when mixed with hydrocarbons and water and the propensity for charged or ionized perfluoroalkyls to concentrate at interfaces make the measurement of the n-octanol water partition coefficient impractical (3M 1999; EPA 2005a).

The pKa range (Table 4-2) indicates that perfluoroalkyls will exist in anion form when in contact with water at environmental and physiologically relevant pHs. An estimated pKa of 6.24 indicates that FOSA will exist as both the anion and the neutral species (SPARC 2008). Perfluoroalkyl salts, such as APFO, will form the corresponding anions when dissolved in water. Prevedouros et al. (2006) reported a Krafft point of 22°C and critical micelle concentration of $3.7x10^3$ mg/L for the perfluorooctanoate anion (PFO). At temperatures above the Krafft point, the solubility of PFO is expected to increase abruptly due to the formation of micelles.

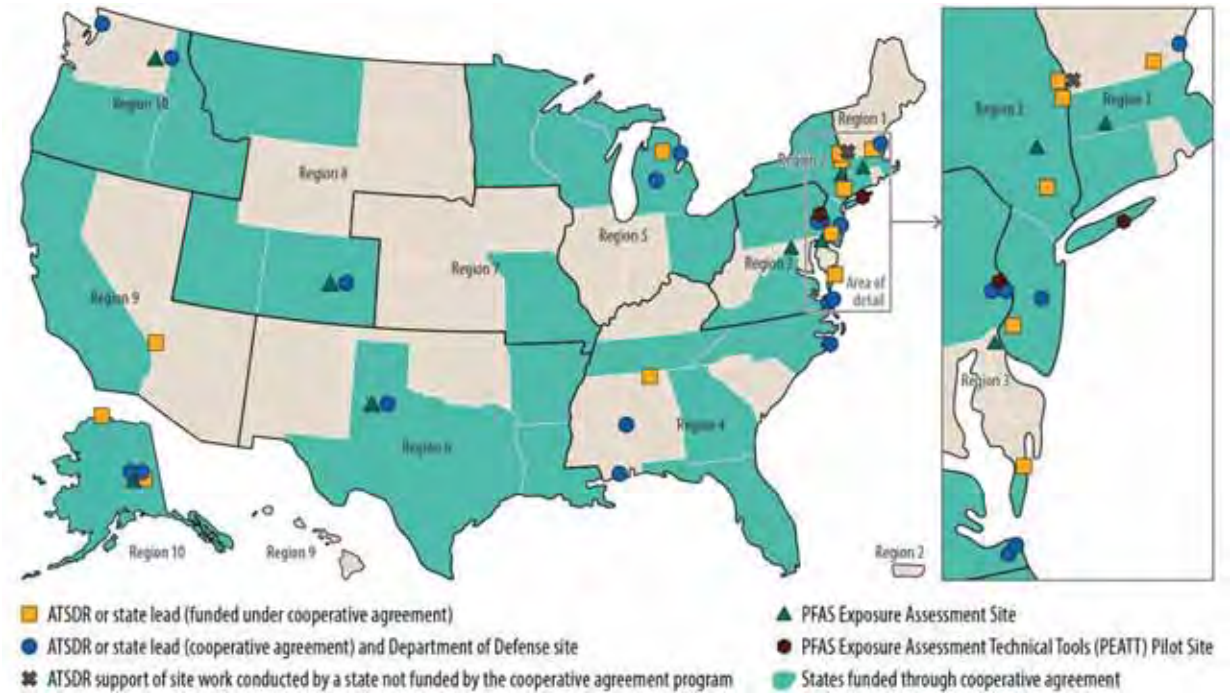
Vapor pressures at 25°C were extrapolated for PFOA, PFNA, PFDA, PFUnA, and PFDoDA using Antoine coefficients. Experimental vapor pressures were as follows: 0.962–724 mm Hg (59.25–190.80°C) for PFOA; 8.40–750 mm Hg (99.63–203.12°C) for PFNA; 23.5–750 mm Hg (129.56–218.88°C) for PFDA; 4.62–750 mm Hg (112.04–237.65°C) for PFUnA; and 6.42–750 mm Hg (127.58–247.36°C) for PFDoDA (Kaiser et al. 2005).

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Perfluoroalkyls have been identified in at least 4 of the 1,854 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2017). However, the number of sites in which substance perfluoroalkyls have been evaluated is not known. Figure 5-1 illustrates perfluorinated compound (PFAS) sites with ATSDR, state health department, EPA, or Department of Defense involvement.

Figure 5-1. Perfluorinated Compound (PFAS) Sites with ATSDR, State Health Department, U.S. Environmental Protection Agency, or Department of Defense Involvement



Source: ATSDR 2018

- The general population is exposed to the perfluoroalkyls through food and water ingestion, dust ingestion, inhalation exposure, and hand-to-mouth transfer of materials containing these substances.
- PFOA, PFOS, and their precursor substances are no longer produced or used in the United States or most other industrialized nations; however, these substances are persistent in the environment and exposure near highly contaminated sites may continue to occur.
- Serum levels of PFOA and PFOS in the general population of the United States have declined dramatically since 2000.

Perfluoroalkyls have been released to air, water, and soil in and around fluorochemical facilities located within the United States (3M 2007b, 2008a, 2008b; Barton et al. 2007; Davis et al. 2007; DuPont 2008; EPA 2008a; Post et al. 2013). Since the early 2000s, eight companies in the fluorochemical industry have been working in concert with the EPA to phase out the production and use of long-chain perfluoroalkyls and their precursors (3M 2008a; DuPont 2008; EPA 2007a, 2008a, 2016a). Perfluorocarboxylic acids containing seven or more perfluorinated carbon groups and perfluoroalkyl sulfonic acids containing six or more perfluorinated carbon units are considered long-chain substances. Perfluorinated carboxylic acids and sulfonic acids containing less than seven and six perfluorinated carbons, respectively, are considered short-chain substances. PFOA, PFOA precursors, and higher homologues have been phased out by the eight corporations in the perfluorotelomer/fluorotelomer industry (Arkema, Asahi, BASF [successor to Ciba], Clariant, Daikin, 3M/Dyneon, DuPont, and Solvay Solexis) as part of the EPA's PFOA Stewardship Program (DuPont 2008; EPA 2008a, 2016a). Industrial releases of these compounds in the United States have declined or have been totally eliminated based on company reports submitted to EPA (EPA 2008a, 2016a). It is noted that PFOA and PFOS may still be produced domestically, imported, and used by companies not participating in the PFOA Stewardship Program. PFOA and PFOS may also be present in imported articles. Although the United States and most industrialized nations have stopped producing PFOA and PFOS, China remains a major producer and user of both substances, and its production has increased as production in the rest of the world has declined (HAES 2017; Li et al. 2015; Lim et al. 2011).

Perfluoroalkyl carboxylic acids and sulfonic acids are expected to dissociate in the environment based on their low pKa values (Kissa 2001; SPARC 2008), and anions will not volatilize from water or soil surfaces (Prevedouros et al. 2006). The unique surfactant properties of these substances may prevent total dissociation of perfluoroalkyls in water (EPA 2005a; Kissa 2001; Prevedouros et al. 2006); therefore, some volatilization of perfluoroalkyls may occur since the neutral forms of these substances are considered to be volatile (Barton et al. 2007; EPA 2005a; Kim and Kannan 2007). Perfluoroalkyls have been detected in air both in the vapor phase and as adsorbed to particulates (Kim and Kannan 2007). Perfluoroalkyls are very stable compounds and are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). Perfluoroalkyls released to the atmosphere are expected to adsorb to particles and settle to the ground through wet or dry deposition (Barton et al. 2007; Hurley et al. 2004; Prevedouros et al. 2006). The chemical stability of perfluoroalkyls and the low volatility of these substances in ionic form indicate that perfluoroalkyls will be persistent in water and soil (3M 2000; Prevedouros et al. 2006). Soil

adsorption coefficient data as well as monitoring studies suggest that perfluoroalkyls such as PFOA are mobile in soil and can leach into groundwater (Davis et al. 2007; Prevedouros et al. 2006).

Perfluoroalkyls have been detected in environmental media and biota of the Arctic region and in other remote locations such as open ocean waters (Barber et al. 2007; Brown et al. 2018; Prevedouros et al. 2006; Wei et al. 2007a; Yamashita et al. 2005, 2008). Proposed source pathways include long-range atmospheric transport of precursor compounds followed by photooxidation to form perfluoroalkyls, direct long-range transport of perfluoroalkyls via oceanic currents, and transport of perfluoroalkyls in the form of marine aerosols (Armitage et al. 2006; Barber et al. 2007; Brown et al. 2018; Prevedouros et al. 2006; Wania 2007). Direct transport of perfluoroalkyls in the atmosphere has also been proposed as a source pathway since these substances have been detected in the vapor phase in outdoor air samples (CEMN 2008; Prevedouros et al. 2006). The actual source of perfluoroalkyls in remote locations is likely to be a combination of these pathways.

The highest concentrations of PFOA and PFOS were in apex predators, such as polar bears, which indicates that these substances biomagnify in food webs (de Vos et al. 2008; Houde et al. 2006b; Kannan et al. 2005; Kelly et al. 2007; Smithwick et al. 2005a, 2005b, 2006). The bioaccumulation potential of perfluoroalkyls is reported to increase with increasing chain length (de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue (de Vos et al. 2008; Kissa 2001).

The levels of PFOA and PFOS in serum samples of U.S. residents have decreased appreciably since the phase out of these substances in the United States. The geometric mean serum levels of PFOS have declined over 84% from NHANES survey years 1999–2000 (30.4 ng/mL) to 2013–2014 (4.72 ng/mL) and the geometric mean serum levels of PFOA have declined 70% over the same temporal period, decreasing from 5.2 ng/mL in years 1999–2000 to 1.56 ng/mL for 2015–2016 (CDC 2018).

Mean concentrations of PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFBA, and FOSA are generally <1 ng/mL (Calafat et al. 2006b, 2007a, 2007b; CDC 2015; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003a, 2003b, 2004c, 2005, 2007a). Major PFOS exposure pathways proposed for the general population include food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill- or home-treated carpets (Trudel et al. 2008). For PFOA, the major exposure pathways are proposed to be oral exposure resulting from general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. While migration of residual PFOA in paper packaging and

wrapping into food is also a potential route of exposure (Trudel et al. 2008), polyfluoroalkyl phosphoric acids in food packaging can also be metabolized in the body to PFOA (D'eon and Mabury 2007; D'eon et al. 2009). Polyfluoroalkyl phosphoric acids are fluorinated surfactant substances used to greaseproof food-containing paper products. Biotransformation of the 8:2 polyfluoroalkyl phosphoric acid and the 8:2 fluorotelomer alcohol into PFOA has been demonstrated (D'eon et al. 2009). Based on these proposed exposure pathways, Trudel et al. (2008) estimated that adult uptake doses for high-exposure scenarios were approximately 30 and 47 ng/kg body weight/day for PFOS and PFOA, respectively. The estimated dosage for children under the age of 12 under a high-exposure scenario were estimated to be 101–219 and 65.2–128 ng/kg body weight/day for PFOS and PFOA, respectively (Trudel et al. 2008). It is noted that the Trudel et al. (2008) study used older monitoring data and thus, may not be an accurate reflection of current intakes. Estimated daily doses for the general population were also estimated by Vestergren et al. (2008) to range from 3.9 to 520 ng/kg body weight/day for PFOS and from 0.3 to 150 ng/kg body weight/day for PFOA. Infants and toddlers had the highest estimated dosages due to greater hand-to-mouth contact with treated carpeting, mouthing activities of clothes, and greater dust ingestion. While conversion of precursor compounds to PFOA and PFOS was generally considered as a minor contribution to the total exposure, under certain scenarios, it was estimated that up to 80% of the intake could be attributable to exposure to precursor substances followed by subsequent metabolism to PFOS or PFOA (Vestergren et al. 2008).

Perfluoroalkyls have been detected in human breast milk and umbilical cord blood. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples were 0.360–0.685 and 0.210–0.609 ng/mL, respectively (Kärrman et al. 2007; Llorca et al. 2010; So et al. 2006b; Völkel et al. 2008). Maximum concentrations of other perfluoroalkyls were <0.18 ng/mL (Kärrman et al. 2007). PFOS and PFOA have been detected in most umbilical cord blood samples with reported concentrations of 4.9–11.0 and 1.6–3.7 ng/mL, respectively (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007). Other perfluoroalkyls have been detected less frequently, with maximum concentrations of <2.6 ng/mL.

Individuals who perform jobs that require frequent contact with perfluoroalkyl-containing products, such as individuals who install and treat carpets or firefighters, are expected to have occupational exposure to these substances. Individuals who work at fluorochemical facilities generally have had higher perfluoroalkyl serum levels than the general population based on exposures in the work environment (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007). Studies of individuals living near fluorochemical facilities indicate that drinking water is the major exposure pathway (Emmett et al. 2006a;

Hölzer et al. 2008; Wilhelm et al. 2009). 3M conducted an exposure assessment to estimate the cumulative exposure to PFOA due to activities at the Decatur, Alabama facility. On-site exposure to groundskeepers, maintenance workers, construction workers, and on-site trespassers were considered. Off-site exposures to anglers, boaters, and residential individuals were also estimated. Various plausible exposure scenarios were considered, and the highest PFOA exposure doses by receptor and pathway occurred for local residents from groundwater followed by residents consuming drinking water from the West Morgan/East Lawrence (WM/EL) public drinking water supply (3M 2008c).

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

Perfluoroalkyls have been manufactured industrially by electrochemical fluorination (ECF), fluorotelomer iodide oxidation, fluorotelomer olefin oxidation, and fluorotelomer iodide carboxylation (Prevedouros et al. 2006; Schultz et al. 2003). During the ECF process, an organic acyl or sulfonyl fluoride backbone structure is dissolved in a solution of aqueous hydrogen fluoride (Savu 1994b; Siegemund et al. 2005). A direct electrical current is then passed through the solution, which replaces all of the hydrogens on the molecule with fluorines. Perfluoroacyl fluorides produced by ECF are hydrolyzed to form the perfluorocarboxylic acid, which is then separated via distillation. This method was used extensively by 3M in the production of perfluoroalkylsulfonates such as PFOS (3M 1999; Hekster et al. 2003; Schultz et al. 2003).

$$R_hCOF + HF \longrightarrow R_fCOF + H_2 + \text{byproducts}$$

$$R_fCOF + H_2O \longrightarrow R_fCOOH + HF$$

Perfluoroalkanesulfonyl fluorides produced by ECF are hydrolyzed under alkaline conditions to form the corresponding salt (Savu 1994b; Siegemund et al. 2005). Acidification followed by distillation yields the anhydrous perfluoroalkanesulfonic acid.

$$R_hSO_2F + HF \longrightarrow R_fSO_2F + H_2$$

$$R_fSO_2F + KOH \longrightarrow R_fSO_3K + HF$$

$$R_fSO_3K + H_2SO_4 \longrightarrow R_fSO_3H + KHSO_4$$

Perfluorosulfonamide compounds, such as FOSA, can be formed by reacting the perfluoroalkanesulfonyl fluoride with a primary or secondary amine (3M 1999; Hekster et al. 2003; Siegemund et al. 2005).

The fluorotelomer iodide oxidation process was developed by DuPont and has served as the basis for their fluoropolymer production chemistry (Buck et al. 2011; Hekster et al. 2003; Savu 1994a; Siegemund et al. 2005). It begins with the preparation of pentafluoroiodoethane from tetrafluoroethene. Tetrafluoroethene is then added to this product at a molar ratio that gives a product of desired chain length. Finally, the product is oxidized to form the carboxylic acid. The process produces linear perfluorocarboxylic acids of even carbon numbers as illustrated below.

$$5C_2F_4 + IF_5 + 2I_2 \xrightarrow{\text{catalyst}} 5C_2F_5I$$

$$C_2F_5I + nC_2F_4 \longrightarrow C_2F_5(C_2F_4)_nI$$

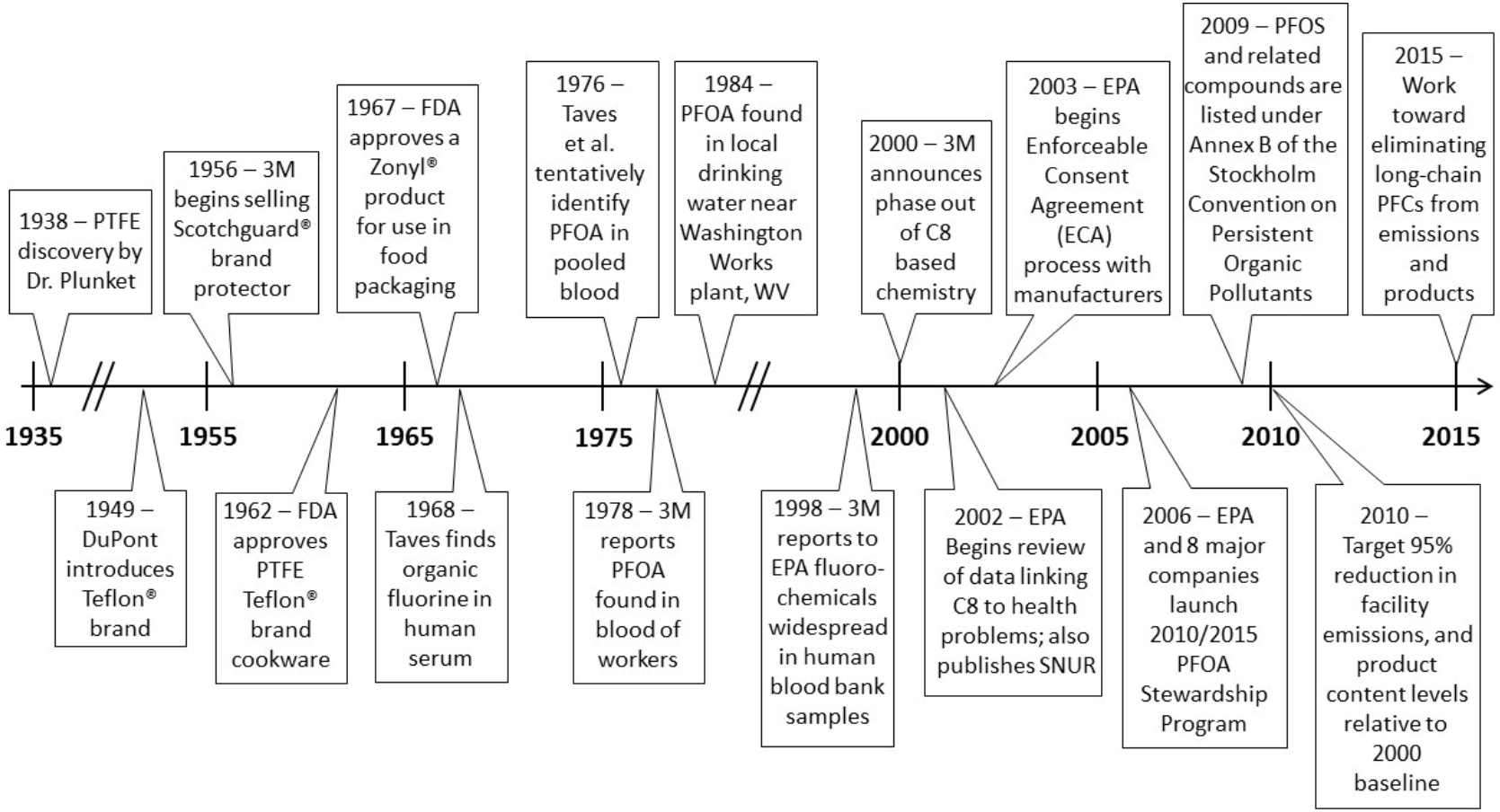
$$C_2F_5(C_2F_4)_nI \xrightarrow{SO_3} C_2F_5(C_2F_4)_{n-1}CF_2COOH$$

Fluorotelomer alcohols are created similarly, but the perfluoroalkyl iodide (telomer A) is reacted with ethylene to create $F(CF_2)_nCH_2CH_2I$ (telomer B), which is converted to the alcohol. The ECF process resulted in a mixture of linear and branched isomers, whereas the telomerization processes yielded predominantly linear products. It has been reported that the 3M ECF process resulted in approximately 70% linear and 30% branched isomers for PFOS and 78% linear and 22% branched isomers for PFOA (Benskin et al. 2009).

No information is available in the TRI database on facilities that manufacture, process, or otherwise use perfluoroalkyls because this class of substances is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005a, 2016g). The Chemical Data Reporting (CDR) rule, under the Toxic Substances Control Act (TSCA), requires manufacturers (including importers) to provide EPA with information on the production and use of chemicals in commerce in large quantities. Information on perfluoroalkyls can be found at (https://www.epa.gov/chemical-data-reporting/basic-information-chemical-data-reporting#what).

Perfluoroalkyls have been manufactured for their direct use in commercial products as well as for their use in industrial process streams. Two important chemicals that have resulted from manufacturing involving perfluoroalkyls, namely PFOS and PFOA, are of worldwide interest given their detection in multiple media in the environment. However, these substances and related long-chain perfluoroalkyls have been essentially phased out as a joint effort by EPA and industry (Lindstrom et al. 2011). The timeline for history of perfluorinated compound production, use, and phase out is presented in Figure 5-2.

Figure 5-2. Timeline of Important Events in the History of Polyfluorinated Compounds



EPA = U.S. Environmental Protection Agency; PFC = perfluorinated compound; PFOA = perfluorooctanoic acid; PTFE = polytetrafluoroethylene; SNUR = significant new use rule; WV = West Virginia

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Given their unique properties, certain narrow exceptions exist for specific applications. Additionally, many of the substances that were used in the production of stain resistant or anti-sticking products that could break down into PFOA and PFOS have also been replaced.

The 3M Company was the principal worldwide manufacturer of PFOS and related chemicals. As a result of its phase-out decision in May 2000, 3M no longer manufactures perfluorooctanyl compounds (PFOA and PFOS). The company ceased manufacturing and using the vast majority of these compounds within approximately 2 years of the phase-out announcement, and ceased all manufacturing and the last significant use of this chemistry by the end of 2008 (3M 2008a; EPA 2007a). In 2000, EPA finalized the SNUR for 88 perfluoroalkyl sulfonate compounds, which requires manufacturers to notify EPA 90 days prior to commencing manufacture or import of these substances for a significant new use to allow time for evaluation (EPA 2002, 2007a, 2008a). The purpose of this rule was to limit future manufacturing and importation of these substances. According to EPA, the rule allowed for the continuation of a few limited, highly technical uses for which no alternatives are available, and which are characterized by very low volume, low exposure, and low releases. The SNUR was amended in 2007 to include 183 additional perfluoroalkyl sulfonate compounds (EPA 2007a, 2008a).

Included on the current list are PFOS, PFHxS, and FOSA. EPA believed that the perfluoroalkyl sulfonate compounds listed under the SNUR were no longer manufactured in the United States; however, during the comment period of the 2007 amendment, EPA learned of the ongoing use of tetraethylammonium perfluorooctanesulfonate as a fume/mist suppressant in metal finishing and plating baths (EPA 2007a). EPA has since excluded this from the list of significant uses. This rule has been amended again by the EPA to designate the processing, use, or importation of long-chain perfluoroalkyls as a significant new use if there are no current ongoing uses, or for uses that were scheduled to end December 31, 2015 (EPA 2015). As part of this amendment, EPA proposed to amend a SNUR for perfluoroalkyl sulfonate chemical substances that would make the exemption inapplicable for persons who import perfluoroalkyl sulfonate chemical substances as part of carpets or any articles that contain long-chain perfluoroalkyls.

In 2006, the eight major companies of the perfluoropolymer/fluorotelomer industry agreed to participate in EPA's PFOA Stewardship Program (EPA 2008a). All public documents and reports from the PFOA Stewardship Program may be reviewed at the EPA docket (EPA-HQ-OPPT-2006-0621). This program included voluntary commitments from these companies to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95% no later than 2010, and to work toward elimination of these substances in products by 2015. Progress reports have been submitted annually

beginning in 2007 and are available from the EPA PFOA Stewardship Program website. Data from these reports that list the content and percent reduction of PFOA, PFOA precursors, and higher PFOA homologues in products are listed in Table 5-1. Nonconfidential emission reports from 2014 and 2015 obtained from the EPA docket indicate that the companies have met the goals of the program. According to its 2015 emission report to EPA, Solvay Specialty Polymers (formerly Solvay Solexis) ceased using PFOA and related higher homologs in 2010 and ceased importation of products containing PFOA residuals in December 2015 (Jones 2016). DuPont does not use PFOA any longer in the emulsion polymerization of polytetrafluoroethylene (PTFE), replacing it with a processing aid consisting of the ammonium salt of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate, also known as GenX or hexafluoropropylene oxide dimer acid (HFPO-DA) (Wang et al. 2013b). (In 2013, DuPont separated its performance chemicals, titanium technologies, fluoroproducts division, and chemical solutions into a separate company named Chemours). Similarly, 3M and other manufacturers are using various poly- and perfluoroether acid salts in fluoropolymer manufacturing and have reformulated surface treatment products to employ short-chain substances that are not as bioaccumulative as the long-chain perfluoroalkyls. Wang et al. (2013b) provide a comprehensive review of the newer substances that manufacturers are using as replacements for legacy perfluoroalkyls in fluoropolymer manufacturing, metal plating, firefighting foams, and other miscellaneous uses such as food packaging materials. Although expected to be less, bioaccumulative modeling studies have suggested that short-chain homologues and other replacements are likely to be persistent and mobile in the environment (Gomis et al. 2015)

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
2006 (EPA 2008a)							
Arkema, Inc.	PFOA and higher homologues	>500–1,000	0%	>70–150	30	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	500–1,570	12%	0.12	Not applicable	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Ciba Specialty Chemicals Corporation	PFOA	0.05 kg	>99%	0.05 kg	>99%	0.05 kg	>99%
	Higher homologues	0.05 kg	>99%	0.05 kg	>99%	0.05 kg	>99%
	Precursors	0	>99%	0	>99%	0	>99%
Clariant International Ltd.	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	280	34%	2; 300	0%	0.28	72%
	Precursors and higher homologues	Not applicable	Not applicable	Not applicable	Not applicable	2,500	78%
E.I. DuPont de Nemours and Company	PFOA, PFOA salts	547	44%	69	80%	246 kg	50%
	Direct precursors	Not applicable	Not applicable	Not applicable	Not applicable	57 kg	14%
3M/Dyneon	PFOA	0	100%	Not reported	Not reported	Not applicable	Not applicable
Solvay Solexis	PFOA and PFOA salts	600–700	59%	Not applicable	Not applicable	Not applicable	Not applicable
	Higher homologues	Not applicable	Not applicable	170–200	0%	Not applicable	Not applicable
	Precursors	0	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
				2013 (EPA 2016a)			
Arkema, Inc.	PFOA and higher homologues	Not applicable	100%	>25–75	96%	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	0	100%	18	100%	Negligible	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
BASF (Ciba Specialty Chemicals Corporation)	PFOA and higher homologues	Not applicable	100%	Not applicable	100%	Not applicable	100%
	Precursors	Not applicable	100%	Not applicable	100%	Not applicable	100%
Clariant International Ltd.	PFOA, PFOA salts, and higher homologues	None reported	Not applicable	None reported	Not applicable	<1 kg	Not applicable
	Precursors	None reported	Not applicable	None reported	Not applicable	<7 kg	Not applicable
Daikin America, Inc.	PFOA	None reported	100%	None reported	100%	None reported	100%
	Precursors and higher homologues	None reported	Not applicable	None reported	Not applicable	None reported	>99%
E.I. DuPont de Nemours and Company	PFOA and PFOA salts	1	99%	0	99%	9 kg	>99%
	Higher homologues	1	99%	0	99%	None reported	99%
	Precursors	None reported	None reported	None reported	None reported	None reported	98%
3M/Dyneon	PFOA, PFOA salts, and higher homologues	0	100%	0	Not applicable	0	No telomere production
	Precursors	No precursor production	No precursor production	No precursor production	No precursor production	No precursor production	No precursor production

Table 5-1. Content (ppm) and Percent Reduction of PFOA, PFOA Homologues, or PFOA Precursors in Products from 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Dispersions		Other fluoropolymers		Telomers	
Company	Chemicals	Content	Percent reduction[a]	Content	Percent reduction[a]	Content	Percent reduction[a]
Solvay Solexis	PFOA, PFOA salts, and higher homologues	Not applicable	>99.999%	Not applicable	>99.999%	Not applicable	>99.999%
	Precursors	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable

[a]Percent reduction in product content of these compounds from baseline year levels. The baseline year is the year nearest to the year 2000 for which company data are available.

PFOA precursors include: octane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7, 8,8-heptadecafluoro-8-iodo- (CAS 507-63-1); 1-decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-(CAS 678-39-7); 1-decene, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 21652-58-4); 2-propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 27905-45-9); 2-propenoic acid, 2-methy 1-, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 1996-88-9); 2-decenoic acid, 3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-hexadecafluoro- (CAS 70887-84-2); and decanoic acid, 3,3,4,4,5,5,6,6,7, 7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 27854-31-5).

Higher homologues include: dodecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12-pentacosafluoro-12-iodo (CAS 307-60-8); decane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heneicosafluoro-10-iodo- (CAS 423-62-1); nonanoic acid, heptadecafluoro- (CAS 375-95-1); decanoic acid, nonadecafluoro- (CAS 335-76-2); 1-Decanol, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro- (CAS 678-39-7); decane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluoro-10-iodo- (CAS 2043-53-0); dodecane, 1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10-heneicosafluoro-12-iodo- (CAS 2043-54-1); 2-propenoic acid, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl ester (CAS 4980-53-4); and 2-propenoic acid, 2-methyl-, 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-heneicosafluorododecyl ester (CAS 17741-60-5).

CAS = Chemical Abstracts Service; PFOA = perfluorooctanoic acid

China is one of the few remaining producers and consumers of PFOA and its salts, with a total of 480 metric tons produced from 2004 to 2012 (Li et al. 2015). China also continues to be a producer of PFOS. Growth in production volumes in China have coincided with decreases in production in the west. For example, China produced approximately 30 metric tons of PFOS in 2001; however, as 3M ceased production, China's production of PFOS increased to 91, 165, and 247 metric tons in 2004, 2005, and 2006, respectively (Lim et al. 2011). PFOA production in China was approximately 30 metric tons in 2004, but increased to approximately 90 metric tons in 2012 (Li et al. 2015). According to a report from the Hubei Academy of Environmental Sciences (HAES), China is planning to gradually phase out the production of some PFOS uses before 2019 and conduct a best available technology (BAT)/best environmental practice (BEP) analysis with the ultimate goal of completely phasing out the production and use of PFOS and potential precursors (HAES 2017).

Historical U.S. production volume data for PFOA, PFBA, and PFOS reported by manufacturers under the EPA Inventory Update Rule (IUR) are provided in Table 5-2. Production volume ranges for the ammonium salt of PFOA, APFO, are also listed. During the reporting year 2002, manufacturers reported that the production volumes were within the range of 10,000–500,000 pounds (6–227 metric tons) for PFOS and PFOA and within the range of 500,000–1,000,000 pounds (227–454 metric tons) for APFO (EPA 2008b). PFBA was reported as having a production volume within the range of 10,000–500,000 pounds (6–227 metric tons) during 1986; however, PFBA production volumes were not reported for subsequent years (EPA 2008b). None of the other perfluoroalkyls were listed in EPA's IUR database. Current U.S. production volume data for perfluoroalkyls are limited. The IUR database has been superseded by the Chemical Data Reporting (CDR) database. Data for 2012 indicated that PFOA was not imported into the United States, but any use or production volume data were reported as confidential business information. No data were located in the CDR for the other substances listed in Table 5-2. Nonconfidential emission reports from 2015 obtained from the EPA docket indicate that there is no current production of PFOA or PFOS in the United States.

Table 5-2. U.S. Production Volume Ranges for Perfluoroalkyls (1986–2002) Reported under the EPA Inventory Update Rule

Perfluoro-alkyl	Reporting year production volume range (pounds)				
	1986	1990	1994	1998	2002
PFOA	10,000–500,000	Not reported	10,000–500,000	10,000–500,000	10,000–500,000
APFO	10,000–500,000	10,000–500,000	10,000–500,000	10,000–500,000	500,000–1,000,000
PFBA	10,000–500,000	Not reported	Not reported	Not reported	Not reported
PFOS	Not reported	Not reported	10,000–500,000	Not reported	10,000–500,000

APFO = ammonium perfluorooctanoate; EPA = Environmental Protection Agency; PFBA = perfluorobutanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: EPA 2008b

5.2.2 Import/Export

The SNURs cited in Section 5.2.1 severely limits the production, import, or export of the long-chain perfluoroalkyls. There were no reported imports of chemicals listed in Table 4-1 in 2012 from the CDR database. Production volumes and import volumes of the 8:2 fluorotelomer alcohol were listed as confidential business information by DuPont. Shaw Industries Group reported that they imported 37,478 pounds of 6:2 fluorotelomer alcohol into the United States in 2012, but DuPont declared both production volume and import volumes as confidential business information (EPA 2016d).

5.2.3 Use

Applications of perfluoroalkyls have made use of their unique surfactant properties (Schultz et al. 2003). The alkyl tails of perfluoroalkyls make these substances both hydrophobic (water-repelling) and oleophobic (oil-repelling) (3M 1999; Kissa 2001; Schultz et al. 2003). Because of these properties, perfluoroalkyls have been used extensively in surface coating and protectant formulations (Kissa 2001). Major applications have included protectants for paper and cardboard packaging products, carpets, leather products, and textiles that enhance water, grease, and soil repellency (Hekster et al. 2003; Schultz et al. 2003). These compounds have been widely used in industrial surfactants, emulsifiers, wetting agents, additives, and coatings as well (3M 1999; Schultz et al. 2003). Perfluoroalkyls have been used in fire-fighting foams since they are effective in extinguishing hydrocarbon fueled fires (Schultz et al. 2003). Perfluoroalkyls have also been used as processing aids in the manufacture of fluoropolymers such as nonstick coatings on cookware, membranes for clothing that are both waterproof and breathable, personal care products (such as dental floss, cosmetics, sunscreens), electrical wire casing, fire and chemical resistant tubing, and plumbing thread seal tape (DuPont 2008; EPA 2008a).

5.2.4 Disposal

Information concerning disposal of individual perfluoroalkyl products may be found on Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) from the manufacturers of the chemicals. Two methods are generally recommended for the disposal of fluoropolymer dispersions. The first method involves precipitation, decanting, or filtering to separate solids from liquid waste. The dry solids are then disposed of in an approved industrial solid waste landfill or incinerated, while the liquid waste is discharged to a waste water treatment facility (Plastics Europe 2012). The second method involves incineration at temperatures >800°C using a scrubber to remove hydrogen fluoride (Plastics Europe 2012). According to perfluorochemical facility assessment reports, historical disposal of perfluoroalkyl containing waste has been through on- and off-site landfills, through sludge incorporation (subsurface injection), and through incineration (3M 2007b, 2008a; ATSDR 2005). Pilot scale studies in which carpet samples were incinerated using a rotary kiln incinerator indicated that most perfluoroalkyls were effectively destroyed in combustors (Lemieux et al. 2007). Similar conclusions were reached by Yamada et al. (2005) when studying the incineration of textiles and paper treated with fluorotelomer-based acrylic polymers. Incineration at conventional temperatures is a proven technology for treating wastes containing perfluoroalkyls.

5.3 RELEASES TO THE ENVIRONMENT

There is no information listed in EPA's Toxic Release Inventory (TRI) on releases of perfluoroalkyls to the environment from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported within this program (EPA 2005b, 2016g).

Perfluoroalkyls are man-made compounds that are not naturally occurring in the environment. Perfluoroalkyls such as PFOS and PFOA have been widely used in the manufacturing of many consumer products (Hekster et al. 2003; Schultz et al. 2003). These substances are still detected in both environmental and biological media around the world as well as in serum samples collected from the general population (Calafat et al. 2006b, 2007a, 2007b; CDC 2018, 2019; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003b, 2003c, 2004b, 2004c, 2005, 2007a; Prevedouros et al. 2006).

In 2006, the eight major companies of the perfluoropolymer/perfluorotelomer industry agreed to participate in EPA's PFOA Stewardship Program (EPA 2008a). This included voluntary commitments from these companies to reduce facility emissions and product content of PFOA and related chemicals on a global basis by 95% no later than 2010, and to work toward elimination of these substances by 2015 (EPA 2008a). Data from 2007 and 2013 progress reports regarding releases of PFOA, PFOA precursors, and higher PFOA homologues to all media as well as percent reduction in releases are listed in Table 5-3.

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluoro-telomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
		2006 Data (EPA 2008a)		
Arkema, Inc.	PFOA and higher homologues	>1,000–10,000	For fluorotelomer production: >0.1–1	22%
	Precursors	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	4,922	For fluorotelomer production: <1	6%
	Precursors	Not applicable	Not applicable	Not applicable
BASF (Ciba Specialty Chemicals Corporation)	PFOA	0.05[b]		>99%
	Higher homologues	0.05[b]		>99%
	Precursors	0[b]		>99%

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluoro-telomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
Clariant International Ltd.	Not applicable	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	Confidential business information	For fluorotelomer production: $8.0x10^{-3}$; for telomer production: $6.4x10^{-7}$	94% for FP production; 92% for telomer production
	Precursors and higher homologues	Confidential business information	For production: $6.4x10^{-7}$	22% for telomer production
E.I. DuPont de Nemours and Company	PFOA, PFOA salts	1,100	Not reported	98%
	Direct precursors	Confidential business information	Not reported	Confidential business information
3M/Dyneon	PFOA	0	0	100%
Solvay Solexis	PFOA and PFOA salts	Not applicable	Not applicable	Not applicable
	Higher homologues	>1,000–10,000	For fluorotelomer production: 0.161	28%
	Precursors	Not applicable	Not applicable	Not applicable
	2013 data (EPA 2016a)			
Arkema, Inc.	PFOA and higher homologues	>500–2,000	>0.001–0.005	91%
	Precursors	Not applicable	Not applicable	Not applicable
Asahi Glass Company	PFOA, PFOA salts, and higher homologues	0	Not applicable	100%
	Precursors	Not applicable	Not applicable	Not applicable
BASF (Ciba Specialty Chemicals Corporation)	PFOA	Not applicable	Not applicable	100%
	Precursors	Not applicable	Not applicable	100%
Clariant International Ltd.	PFOA, PFOA salts	Not applicable	Not applicable	Not applicable
	Precursors	Not applicable	Not applicable	Not applicable
Daikin America, Inc.	PFOA	0	0	100%

Table 5-3. Reported Emissions of PFOA, PFOA Homologues, or PFOA Precursors in Products from the 2006 and 2013 U.S. Operations of Fluoropolymer/Fluorotelomer Companies

		Releases to all media from fluoro-telomer and telomer manufacturing		
Company	Chemicals	kg	kg of release/100 kg of product produced	Percent reduction in emissions[a]
E.I. DuPont de Nemours and Company	Precursors and higher homologues	0	0	100%
	PFOA, PFOA salts	90	None reported	99.8%
	Higher homologues	None reported	None reported	None reported
	Precursors	Confidential business information	None reported	Confidential business information
3M/Dyneon	PFOA, PFOA salts, and higher homologues	0	0	100%
	Precursors	0	0	Not applicable
Solvay Solexis	PFOA, PFOA salts, and higher homologues	0	0	>99.999%
	Precursors	0	0	Not applicable

[a]Percent reduction in product content of these compounds from baseline year levels. The baseline year is the year nearest to the year 2000 for which company data are available.
[b]Total for emissions and product content

PFOA = perfluorooctanoic acid

While the United States and most industrialized countries around the world have ceased production of PFOS and PFOA, China is still a major producer of both substances (Li et al. 2015; Lim et al. 2011). Over the period from 2004 to 2012, it was estimated that 250 metric tons of PFOA were released to the environment from production in China (Li et al. 2015). Fluoropolymer manufacturing and processing was considered the dominant source of environmental releases, accounting for >80% of the total, while PFOA releases related to end use consumer products accounted for 6% of the total. Brazil produced 379 metric tons of N-EtFOSA for use in the pesticide, Sulfuramid, from 2004 and 2015, which contribute to PFOS releases to the environment (Wang et al. 2017).

Prevedouros et al. (2006) estimated the total global historical emissions of perfluoroalkyl carboxylates into the environment from both direct and indirect sources from the time period of 1951–2004. These data are provided in Table 5-4. Based on these estimations, direct emissions (3,200–6,900 metric tons) have far exceeded indirect emissions (30–350 metric tons). The largest direct emissions identified are

from industrial processes such as the manufacture of perfluoroalkyl carboxylates (470–900 metric tons), fluoropolymer manufacture (2,400–5,400 metric tons), and fluoropolymer processing (210–320 metric tons). Direct release of perfluoroalkyl carboxylates from use of aqueous firefighting foams and consumer and industrial products were estimated to be 50–100 and 40–200 metric tons, respectively. The largest indirect emissions identified were from perfluoroalkyl carboxylate residual impurities in perfluorooctyl-sulfonyl fluoride products (20–130 metric tons) and fluorotelomer-based precursor degradation (6–130 metric tons). Wang et al. (2014) expanded upon the work of Prevedouros et al. (2006) by considering additional emission sources of these substances and estimating emissions from 2003 to 2015 and projecting future emissions. These authors estimated emissions of 820–7,180 metric tons for 2003–2015 and projected between 20 and 6,420 metric tons for years 2016–2030. The estimates by Prevedouros et al. (2006) and Wang et al. (2014) contain a great degree of uncertainty as demonstrated by the wide range of values presented in the data. Wang et al. (2014) stated that uncertainty analysis using Monte Carlo methods is not possible because there is insufficient information available with respect to the range or distribution of the emissions. Instead, they introduced a scoring system to provide a qualitative description of the accuracy of the estimates that ranged from 0–1 (low uncertainty) to 2–3 (high uncertainty where estimates were based on crude assumptions or extrapolations).

Table 5-4. Global Historical PFCA Production and Emissions Estimates from 1951 to 2004[a]

Environmental input source	Historical time period (years)	Estimated total global historical PFCA emissions (tonnes)
Direct PFCA sources		
PFCA manufacture		
PFO/APFO	1951–2004	400–700
PFN/APFN	1975–2004	70–200
Total manufactured		470–900
Industrial and consumer uses		
Fluoropolymer manufacture (APFO)	1951–2004	2,000–4,000
Fluoropolymer dispersion processing (APFO)	1951–2004	200–300
Fluoropolymer manufacture (APFN)	1975–2004	400–1,400
Fluoropolymer processing (APFN)	1975–2004	10–20
Aqueous firefighting foams (AFFF)	1965–1974	50–100
Consumer and industrial products	1960–2000	40–200

Table 5-4. Global Historical PFCA Production and Emissions Estimates from 1951 to 2004[a]

Environmental input source	Historical time period (years)	Estimated total global historical PFCA emissions (tonnes)
Total direct		3,200–6,900
Indirect PFCA sources		
POSF-based products		
PFCA residual impurities[b]	1960–2002	20–130
POSF-based precursor degradation	1960–2002	1–30
POSF-based AFFF	1970–2002	3–30
Fluorotelomer-based products		
PFCA residual impurities[b]	1974–2004	0.3–30
Fluorotelomer-based precursor degradation	1974–2004	6–130
Fluorotelomer-based AFFF	1975–2004	<1
Total indirect		30–350
Total source emissions (direct and indirect)		3,200–7,300

[a]Low and high estimated values as well as the period of use/production for each source are based upon publicly available information cited in the text.
[b]Some authors classify residual impurities as a direct emission source rather than indirect emission source (Buck et al. 2011).

AFFF = aqueous firefighting foams; APFN = ammonium perfluorononanoate; APFO = ammonium perfluoro-octanoate; PFCA = perfluorinated carboxylic acid; PFN = perfluorononanoate; PFO = perfluorooctanoate; POSF = perfluorooctanesulfonyl fluoride

Source: Prevedouros et al. 2006

Wang et al. (2017) estimated the global emissions in 1958–2015 as 1,228–4,930 tonnes of PFOS and 1,230–8,738 tonnes of PFOS precursors, with most emissions occurring from 1958 to 2002. It was estimated that PFOS emissions from 2016 to 2030 will be 8–153 tonnes.

5.3.1 Air

There is no information listed in the TRI on releases of perfluoroalkyls to the atmosphere from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

According to 3M, low levels of PFOA were released to air during manufacturing processes at the Decatur, Alabama facility until use of this substance ceased in 2004 (3M 2008b). This company states that there are currently no process-related air emissions of PFOA at this facility (3M 2008b). PFOA concentrations as high as 75,000–900,000 pg/m^3 were measured at the fence line of the DuPont Washington Works

facility near Parkersburg, West Virginia in 2004 (Barton et al. 2006; Davis et al. 2007; Prevedouros et al. 2006). High volume air samples collected at several monitoring stations near the Washington Works facility during nine events between August and October of 2005 contained PFOA at reported concentrations ranging from 10 to 75,900 pg/m^3 (EPA 2007b). The mean and median of these reported concentrations are 5,500 and 240 pg/m^3, respectively.

The presence of perfluoroalkyls in indoor air and dust indicates that perfluoroalkyl-containing consumer products such as treated carpets and textiles may be sources of release to air (Barber et al. 2007; Jahnke et al. 2007b; Kubwabo et al. 2005; Moriwaki et al. 2003; Prevedouros et al. 2006; Shoeib et al. 2004; Strynar and Lindstrom 2008). Perfluoroalkyls have also been identified on both indoor and outdoor window films (Gewurtz et al. 2009). Disposal of perfluoroalkyl-containing consumer products is also expected to be a source of release to air (Prevedouros et al. 2006). Harada et al. (2005a, 2006) proposed that automobiles may be a source of PFOA in urban air based on elevated levels measured near heavy traffic areas and the widespread use of this substance in automobile materials.

Perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids are formed by the atmospheric photooxidation of precursor compounds such as fluorotelomer alcohols and perfluoroalkyl sulfonamides (D'eon et al. 2006; Ellis et al. 2004; Martin et al. 2006; Wallington et al. 2006; Wania 2007). Perfluoroalkyl carboxylic acids including PFOA, PFNA, PFHpA, and PFBA were observed as products during a laboratory study involving the photooxidation of 4:2, 6:2, and 8:2 fluorotelomer alcohols (Ellis et al. 2003, 2004). D'eon et al. (2006) observed both perfluoroalkyl carboxylic acids and perfluorobutane sulfonate among products of the photooxidation of N-methyl perfluorobutane sulfonamidoethanol.

5.3.2 Water

There is no information listed in the TRI on releases of perfluoroalkyls to water from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

There are a number of sources of perfluoroalkyl release to surface water and groundwater, including release from manufacturing sites, industrial use, use and disposal of perfluoroalkyl-containing consumer products, fire/crash training areas, waste water treatment facilities, and from the use of contaminated biosolids (3M 2008b; Clara et al. 2009; Davis et al. 2007; Eggen et al. 2010; EPA 2009a; Kelly and

Solem 2009; Moody and Field 1999; Moody et al. 2003; Sinclair and Kannan 2006; Prevedouros et al. 2006).

Waste water discharge was identified as a release pathway for APFO from the DuPont Washington Works facility in West Virginia (Davis et al. 2007). The average monthly concentrations of APFO measured in surface water from three outlets at the facility during 2007 and early 2008 ranged from 3.65 to 377 µg/L (EPA 2008d). Reported concentrations of APFO and PFOA measured in surface water from four separate outlets at this facility during the same period were 3–64 and 2.3–61 µg/L, respectively. During perfluorochemical operations at the 3M Cottage Grove facility in Minnesota, waste water treatment plant effluent containing perfluoroalkyls was discharged to the Mississippi River. Discharge into Bakers Creek from the waste water treatment plant at the 3M Decatur facility was a principal source of PFOA release from this facility (3M 2008b).

Elevated levels of perfluoroalkyls, such as PFOA, PFOS, and PFHxS, measured in groundwater near fire-training areas are attributed to the use of these substances in aqueous firefighting foams (Moody and Field 1999; Moody et al. 2003). The concentrations of these three perfluoroalkyls in groundwater near a military fire-training site in Michigan were 8–105, 4.0–110, and 9–120 µg/L, respectively (Moody et al. 2003). A study of landfill leachates showed that perfluoroalkyls were primarily distributed to the water phase of leachates, which could eventually contaminate ground water (Eggen et al. 2010). Lang et al. (2017) estimated that between 563 and 638 kg of perfluoroalkyl substances were released from landfill leachate in the United States in 2013.

Waste water treatment plants have been shown to be significant contributors to perfluoroalkyls contamination of surface and ground water (Clara et al. 2009; EPA 2009a; Kelly and Solem 2009; Loganathan et al. 2007; Sinclair and Kannan 2006; Yu et al. 2009c). Influent, effluent, and sludge samples from 28 public and private waste water treatment plants in Minnesota were analyzed for 13 perfluoroalkyls; detectable concentrations of perfluoroalkyls were found in several facilities, primarily urban treatment plants (Kelly and Solem 2009). Elevated levels of PFOS at one facility (1.51 µg/L in effluent) were attributed to a chrome plating facility using a surfactant containing fluorosulfonate to control hexavalent chromium emissions. Another study of chromium electroplating facilities in Chicago, Illinois and Cleveland, Ohio also found them to be significant sources of PFOS and other perfluoroalkyls in the environment (EPA 2009a). It was determined that perfluoroalkyls were being discharged from all 11 facilities at quantifiable levels and that PFOS was detected in waste water from 10 out of 11 facilities at levels of 0.0314–39 µg/L. PFOA and PFOS were detected in effluents of six waste water treatment

plants located in New York at levels of 0.058–1.05 and 0.003–0.068 µg/L, respectively (Sinclair and Kannan 2006). PFOS and PFOA were detected in effluents of two waste water treatment plants located in Singapore at levels of 0.0053–0.5609 and 0.0112–1.057 µg/L, respectively (Yu et al. 2009c). PFOA, PFOS, and several other perfluoroalkyls were detected in effluent samples of 21 waste water treatment plants and 9 industrial point sources; PFOA and PFOS were reportedly identified in the effluents of all of the facilities monitored at an average level of 0.060 µg/L for both substances (Clara et al. 2009).

Studies comparing perfluoroalkyl levels in influent and effluent from municipal waste water treatment facilities have found higher levels of some perfluoroalkyls, such as PFNA, PFOA, PFOS, and FOSA, in the effluent, as compared to the influent (Loganathan et al. 2007; Schultz et al. 2006b). For others, such as PFHxS and PFDA, waste water treatment resulted in lower concentrations or no change in the concentrations. Increases in perfluoroalkyl concentrations are likely due to the breakdown of perfluoroalkyl precursors such as polyfluoroalkyl phosphoric acids or fluorotelomer alcohols (D'eon et al. 2009; Gauthier and Mabury 2005; Wang et al. 2005a, 2005b).

A study of eight waste water treatment plants that discharge effluent to the San Francisco Bay found increases in PFBA and PFHxA levels between 2009 and 2014 and declines in longer-chain legacy substances (Houtz et al. 2016). Average concentrations of PFBA were 7.4±4.7 ng/L in 2009 and 16±5.8 ng/L in 2014 and the average concentration of PFHxA rose from 17±4.0 ng/L in 2009 to 25±5.1 ng/L in 2014. The average PFOA concentration decreased from 32±30 ng/L in 2009 to 21±13 ng/L in 2014 and the average PFOS concentration decreased from 24±32 ng/L in 2009 to 13±4.4 ng/L in 2014.

Land application of biosolids (treated sewage sludge) can also result in the release of perfluoroalkyls to surface and groundwater (Clark and Smith 2011; Lindstrom et al. 2011; Sepulvado et al. 2011). There appears to be some differences in the distribution of PFOA and PFOS in waste water effluent and biosolids, with higher levels of PFOA in waste water and higher PFOS levels in biosolids (Guo et al. 2010).

5.3.3 Soil

There is no information listed in the TRI on releases of perfluoroalkyls to soil from facilities manufacturing, processing, or otherwise using perfluoroalkyls because these releases are not required to be reported (EPA 2005b, 2016g).

Perfluoroalkyls can be inadvertently released to soils through the use of biosolids applied as fertilizer to help maintain productive agricultural soils and stimulate plant growth. PFOA and PFOS were detected in both biosolids and biosolid-amended soils (Sepulvado et al. 2011). Six samples of biosolids obtained from the Metropolitan Water Reclamation District of Greater Chicago had levels of PFOS and PFOA of 80–219 and 8–68 ng/g, respectively (Sepulvado et al. 2011). The mean sum (±SD) of all perfluoroalkyls in the biosolids was 433±121 ng/g, with PFOS being most prominent. Perfluoroalkyls can also be released into soil due to atmospheric transport and wet/dry deposition (Rankin et al. 2016; Strynar et al. 2012).

Liu et al. (2007) measured PFOA as a product of the biodegradation of 8:2 fluorotelomer alcohol in soil. This result, along with similar findings in activated sludge tests, indicates that biodegradation of fluorotelomer alcohols may result in the formation of perfluoroalkyl carboxylic acids in soil (Liu et al. 2007; Rankin et al. 2014; Wang et al. 2005a, 2005b).

5.4 ENVIRONMENTAL FATE

5.4.1 Transport and Partitioning

Air. Barton et al. (2007) investigated the atmospheric partitioning of PFOA during rain events near an industrial facility and concluded that this substance will be primarily adsorbed to particles in the air since PFOA was not detected in the vapor phase (detection limit of 0.2 ng/m^3). Concentrations of PFOA in raindrops and as particulates were 11.3–1,660 ng/L and 0.09–12.40 ng/m^3, respectively. The authors proposed that PFOA or APFO released into air from industrial facilities will be scavenged by atmospheric particles (including aqueous aerosols and raindrops) and dissociate to form the perfluorooctanoate anion. Although Barton et al. (2007) did not detect PFOA in the vapor phase during rain events, low concentrations (<0.12–3.16 pg/m^3) of vapor-phase perfluoroalkyls measured by Kim and Kannan (2007) in urban air provide evidence of a partitioning equilibrium. Wet and dry deposition are expected to be the principal removal mechanisms for perfluoroalkyl carboxylic acids and sulfonic acids in particulate form from the atmosphere. Residence times with respect to these processes are expected to be days to weeks (Barton et al. 2007; Hurley et al. 2004; Kim and Kannan 2007).

Long-range atmospheric transport of precursor compounds such as fluorotelomer alcohols and perfluoroalkyl sulfonamides followed by the atmospheric photooxidation of these substances to form perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids resulted in PFOA and PFOS

contamination in remote locations with no direct point sources for these compounds (Barber et al. 2007; D'eon et al. 2006; Dinglasan-Panlilio and Mabury 2006; Ellis et al. 2004; Martin et al. 2006; Simcik 2005; Small 2009; Wallington et al. 2006; Wania 2007). Fluorotelomer alcohols and perfluoroalkyl sulfonamides are volatile and possess long enough atmospheric residence times for long-range transport to occur (Barber et al. 2007; Yarwood et al. 2007). The presence of fluorotelomer alcohols and perfluoroalkyl sulfonamides in urban and Arctic air offers evidence of long-range atmospheric transport (Loewen et al. 2005; Shoeib et al. 2006; Stock et al. 2004). Photooxidation studies have demonstrated the conversion of these substances to perfluoroalkyl carboxylic acids and sulfonates. According to Young et al. (2007), the presence of perfluorodecanoic acid and perfluoroundecanoic acid in an Arctic ice cap indicates atmospheric oxidation of precursors as a source. Yeung et al. (2017) collected samples of snow and water from the Central Arctic region in 2012 and observed that perfluorinated alkyl substances were only detectable in ocean waters above a depth of 150 m. Atmospheric deposition from precursors was estimated to account for approximately 34–59% of the PFOA input to the ocean. Gawor et al. (2014) studied the distribution of fluorotelomer alcohols, fluorinated sulfonamides, and fluorinated sulfonamidoethanols in polar, remote, and urban regions of the world during four sampling campaigns covering the years 2005–2011. They observed higher levels of fluorotelomer alcohols as compared to the fluorinated sulfonamides and sulfonamidoethanols, with the 8:2 fluorotelomer alcohol being the predominant species in most samples.

Water. The pKa range of perfluoroalkyls indicates that these substances will exist primarily as the dissociated conjugate base (anion) when in contact with water at environmental pH (pH 5–9). Volatilization will not be an important environmental fate process when the substances exist as anions; however, under acidic conditions, undissociated perfluoroalkyls may volatilize into the atmosphere (Martin et al. 2006). Perfluoroalkyls may be transported to remote areas by direct oceanic advection of these substances (Armitage et al. 2006; Barber et al. 2007; Simcik 2005; Wania 2007; Yamashita et al. 2005, 2008). Perfluoroalkyls may also be transported over long distances in the form of marine aerosols (Barber et al. 2007; CEMN 2008; Prevedouros et al. 2006). This transport mechanism may be especially relevant since surfactants have been shown to accumulate in upper sea layers and at water surfaces (Prevedouros et al. 2006).

Perfluoroalkyls have been measured in invertebrates, fish, amphibians, reptiles, birds, bird eggs, and mammals located around the world (Brown et al. 2018; Dai et al. 2006; Giesy and Kannan 2001; Houde et al. 2005, 2006a, 2006b; Keller et al. 2005; Kannan et al. 2001a, 2001b, 2002a, 2002b, 2002c, 2002d, 2005, 2006; Sinclair et al. 2006; So et al. 2006a; Wang et al. 2008). The highest concentrations of PFOA

and PFOS in animals are measured in apex predators, such as polar bears, which indicates that these substances biomagnify in food webs (de Vos et al. 2008; Houde et al. 2006b; Kannan et al. 2005; Kelly et al. 2007). Loi et al. (2011) also provided evidence of the biomagnification potential of several perfluoroalkyl substances in tropical food webs. Evidence for biomagnification of chlorinated polyfluoroalkyl ether sulfonic acids, which have been used in the plating industry, has recently been reported (Liu et al. 2017; Shi et al. 2015). Table 5-5 shows levels of PFOA and PFOS measured in Arctic organisms. The bioaccumulation potential of perfluoroalkyls increases with increasing chain length from 4 to 8 carbon units and then declines with further increases in chain length (Conder et al. 2008; de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue, which may explain why bioconcentration factors (BCFs) are lower than expected in aquatic organisms (de Vos et al. 2008; Kissa 2001). The Arctic Monitoring and Assessment Programme December 2017 report contains a large summary of environmental monitoring data for perfluoroalkyl substances in the Arctic (AMAP 2017). Temporal trends indicated declining residues of PFOA in the liver of ringed seals at several Arctic monitoring sites; however, PFNA and PFDA showed increasing levels at some locations and declining levels at others (AMPA 2017). Declining levels of PFOA and PFOS were observed for polar bears in Canadian Arctic and Greenland; however, total perfluoroalkyl levels remained relatively constant as precursor substances appear to continue to be transported and degraded in the Arctic food web. Moreover, newer perfluorinated substances such as perfluoroethylcyclohexane sulfonate (used in hydraulic fluids) and perfluorobutane sulfonamide (a PFBS precursor) have been detected in some environmental media.

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

	Concentration (ng/g)		
Location and organism	PFOA	PFOS	Reference
Northeastern Canada, 1996–2002; wet weight[a]			Tomy et al. 2004
Zooplankton (n=5)	2.6	1.8	
Clams (n=5)	ND	0.28	
Shrimp (n=7)	0.17	0.35	
Arctic cod (n=6)	0.16	1.3	
Redfish (n=7)	1.2	1.4	
Walrus (n=5)	0.34	2.4	
Narwhal (n=5)	0.9	10.9	
Beluga (n=5)	1.6	12.6	
Black-legged kittiwake (n=4)	ND	10.0	
Glaucous gulls (n=5)	0.14	20.2	

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

Location and organism	Concentration (ng/g) PFOA	PFOS	Reference
Northern Canada, 1992–2002[a]			Martin et al. 2004a
Polar bear (n=7)	8.6	3,100	
Arctic fox (n=10)	<2	250	
Ringed seal (n=9)	<2	16	
Mink (n=10)	<2	8.7	
Common loon (n=5)	<2	20	
Northern fulmar (n=5)	<2	1.3	
Black guillemot (n=5)	<2	ND	
White sucker (n=3)	<2	7.6	
Brook trout (n=2)	<2	39	
Lake whitefish (n=2)	<2	12	
Lake trout (n=1)	<2	31	
Northern pike (n=1)	<2	5.7	
Arctic sculpin (n=1)	<2	12	
Northwestern Canada, 2004			Powley et al. 2008
Zooplankton (n=3)	ND	ND–0.2	
Arctic cod (n=5)	ND	0.3–0.7	
Ringed seal (n=5)		2.5–8.6	
Bearded seal (n=1)	ND	1.3	
Northern Norway; ng/g wet weight[a]			Verreault et al. 2005, 2007
Herring gull eggs	<0.091–0.652	21.4–42.2	
Glaucous gulls			
Eggs (n=10)	<0.70	104	
Plasma (n=20)	<0.70–0.74	134	
Nanavut, Canada			Butt et al. 2007a, 2007b
Thick-billed murres	<MDL[b]–0.16	<0.40–0.76	
Northern fulmars	<MDL[b]–0.09	<0.40–0.60	
Ringed seals	<0.85–6.2	2–20	
Northern Canada, 2002–2005			Butt et al. 2008
Ringed seal livers (n=110)	<0.7–13.9	0.89–189	
Greenland			Bossi et al. 2005
Ringed seals	<1.2	12.5–95.6	
North American and European Arctic, 1999–2002			Smithwick et al. 2005a
Polar bears (n>72)	<2.3–57.1	263–6,340	
Greenland, 1999–2001			Smithwick et al. 2005b
Polar bears (n=29)[a]	10	2,470	
Greenland, 1972–2002			Smithwick et al. 2006
Polar bears	1.6–4.4	120–1,400	
Arviat, 2010–2012			AMAP 2017
Ringed seal liver (n=28)	0.62±0.6	No data	

Table 5-5. Biological Monitoring of PFOA and PFOS in the Arctic

Location and organism	Concentration (ng/g) PFOA	PFOS	Reference
Pangnirtung, 2009–2011			AMAP 2017
Ringed seal liver (n=7)	0.22±0.15	No data	
Resolute, 2010–2013			AMAP 2017
Ringed seal liver (n=46)	0.23±0.16	No data	
Sachs Harbor, 2011–2013			AMAP 2017
Ringed seal liver (n=29)	0.54±0.36	No data	
Ulukhaktok, 2010–2013			AMAP 2017
Ringed seal liver (n=9)	1.4±0.11	No data	

[a]Reported as mean values
[b]Minimum detection limits for study analytes ranged from 0.03 to 2.3 ng/g. To calculate means, concentrations less than the MDL were replaced with a random value that was less than half the MDL.

MDL = minimum detection limit; ND = not detected; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Sediment and Soil. K_{oc} values of 17–230 measured for PFOA in soils of various organic carbon content indicate that PFOA will be mobile in soil and will not adsorb to suspended solids and sediment in the water column (Davis et al. 2007; Prevedouros et al. 2006); rather, it tends to remain in solution and migrate with groundwater (Davis et al. 2007). This is supported by the presence of PFOA in groundwater at the Decatur, Cottage Grove, and Washington Works fluorochemical industrial facilities (3M 2007b, 2008b; Davis et al. 2007). Other sources of perfluoroalkyls in soil include air emissions followed by atmospheric deposition to soils and subsequent leaching (Davis et al. 2007). Low volatility, high water solubility (9,500 mg/L at 25°C), and low sorption to solids indicate that the perfluorooctanoate anion will accumulate in surface waters, especially oceans (Armitage et al. 2006; Kauck and Diesslin 1951; Prevedouros et al. 2006; Wania 2007). McGuire et al. (2014) reported field-based K_{oc} values for several perfluoroalkyl substances (see Table 4-2).

Perfluoroalkyls can be taken up by plants in contaminated soils. Laboratory studies have suggested that short-chain perfluoroalkyls such as PFBA are more concentrated in edible portions of plants when compared to longer carbon chain substances such as PFOA or PFOS (Blaine et al. 2013, 2014a, 2014b; MDH 2014). Yoo et al. (2011) studied the accumulation of perfluoroalkyl carboxylic acids, perfluorosulfonic acids, and fluorotelomer alcohols in grass samples collected near Decatur, Alabama and calculated the grass-soil accumulation factor (GSAF), which is the concentration of perfluoroalkyl in grass divided by the concentration of perfluoroalkyl in soil. The shortest chain compounds had the largest GSAFs, and accumulation factors decreased rapidly with chain length. The mean (±SD) GSAF values

were 3.4±2.6, 0.90±0.66, 0.25±0.23, 0.12±0.08, 0.10±0.08, 0.11±0.09, and 0.10±0.09 for PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, and PFDoDA, respectively. The GSAF for PFOS was 0.07±0.04. Increasing salinity and temperature was shown to increase uptake and transport from the roots into the shoots by the wheat plants grown in hydroponic systems spiked with perfluorocarboxylic acids (Zhao et al. 2016). Transport into the shoots also increased with decreasing carbon chain length. Concentrations in the shoots of the wheat plants increased in the following order: PFBA > PFHpA > PFOA > PFDoDA. Stahl et al. (2009) demonstrated that both PFOA and PFOS were taken up from soil by five cultivated plants (spring wheat, oats, potatoes, maize, and perennial ryegrass) and the amount of uptake was generally linearly dependent upon the concentration of PFOA or PFOS spiked to the soil. Lysimeter studies suggested that PFOA and short-chain perfluoroalkyls such as PFBA pass through the soil and get taken up by plants more quickly as compared to PFOS (Stahl et al. 2013).

Krippner et al. (2014) analyzed the uptake of perfluoroalkyl substances with chain length of C4–C10 over a pH range of 5–7. They observed that short-chain perfluoroalkyls partition predominantly and at higher concentrations to the shoot. Longer-chain substances such as PFOA, PFNA, and PFDA, as well as PFHxS and PFOS, accumulated at higher concentrations in the roots of maize plants. Maize grown in soil pots containing perfluoroalkyls only accumulated compounds with a chain length ≤C8 in the kernels (Krippner et al. 2015).

Other Media. Data are not available regarding the transport and partitioning of perfluoroalkyls in other media.

5.4.2 Transformation and Degradation

Perfluoroalkyls are considered to be environmentally persistent chemicals (EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The carbon atoms of the perfluoroalkyl chain are protected from attack by the shielding effect of the fluorine atoms; furthermore, environmental degradation processes generally do not possess the energy needed to break apart the strong fluorine-carbon bonds (3M 2000; Hekster et al. 2003; Schultz et al. 2003). Perfluoroalkyls are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (OECD 2002, 2007; Prevedouros et al. 2006).

Air. Although transport and partitioning information indicates that air will not be a sink for perfluoroalkyls in the environment, low concentrations of perfluoroalkyl carboxylic acids, sulfonic acids, and sulfonamides have been measured in air both in the vapor phase and as bound to particulates (Barton

et al. 2007; Kim and Kannan 2007). Available information indicates that photodegradation will not compete with wet deposition as an atmospheric removal process for perfluoroalkyls (Barton et al. 2007; Hurley et al. 2004; Prevedouros et al. 2006). However, photooxidation may be an important degradation mechanism for perfluoroalkyl sulfonamides (D'eon et al. 2006; Martin et al. 2006).

PFOA does not absorb UV light at environmentally relevant wavelengths (>290 nm); Hori et al. (2004a) reported a weak absorption band for PFOA that ranged from 220 to 270 nm. Based on the measured absorption wavelength of PFOA, perfluoroalkyl carboxylic acids are not expected to undergo direct photolysis. Following irradiation of the potassium salt of PFOS with light of wavelength 290–800 nm for 67–167 hours, it was concluded that there was no evidence of direct photolysis of PFOS under any of the test conditions (OECD 2002). Based on these test results for PFOS, perfluoroalkyl sulfonic acids are not expected to undergo direct photolysis in the atmosphere.

A measured photooxidation rate constant is not available for PFOA. Hurley et al. (2004) measured the reaction of short-chain (C1–C4) perfluoroalkyl carboxylic acids with photochemically generated hydroxyl radicals. The proposed mechanism begins with abstraction of the carboxyl hydrogen, which is followed by the removal of the carboxyl group and generation of a perfluoroalkyl radical. Finally, the perfluoroalkyl chain is broken down one carbon atom at a time through an unzipping sequence. The same rate constant, 1.69×10^{-13} cm^3/molecule-second, was measured for the photooxidation of the C2, C3, and C4 molecules, indicating that the chain length may have little effect on the reactivity of perfluoroalkyls with hydroxyl radical. According to the authors, this rate constant corresponds to a half-life of 130 days. Based on the data for the short-chain structures, the authors concluded that atmospheric photooxidation of perfluoroalkyl carboxylic acids is not expected to compete with wet and dry deposition, which is predicted to occur on a time scale of the order of 10 days.

Atmospheric photooxidation data are not available for perfluoroalkyl sulfonic acids. Atmospheric photooxidation studies involving n-methyl perfluorobutane sulfonamidoethanol (Me-FBSE) and n-ethyl perfluorobutanesulfonamide (Et-FBSA) indicate possible mechanisms for the reaction of these substances with atmospheric hydroxyl radicals (D'eon et al. 2006; Martin et al. 2006). Products observed from the photooxidation of these compounds indicate the following pathways: removal of an alkyl from the amide (cleavage of the N-C bond); removal of the amido group (cleavage of the S-N bond); and removal of the sulfonamido group (cleavage of the S-C bond) (D'eon et al. 2006; Martin et al. 2006). The last two pathways indicate that FOSA may be photooxidized through removal of the amido or sulfonamido group. The third pathway, cleavage of the S-C bond, also indicates a photooxidation mechanism for

perfluoroalkyl sulfonic acids. Martin et al. (2006) proposes an unzipping sequence for the perfluoroalkyl chain following removal of the sulfonyl group.

Measured rate constants for the reaction of Me-FBSE and Et-FBSA with atmospheric hydroxyl radicals are $5.8x10^{-12}$ and $3.74x10^{-13}$ cm^3/molecule-second, respectively (D'eon et al. 2006; Martin et al. 2006). Atmospheric half-lives calculated using these rate constants were 2 days for Me-FBSE and 20–50 days for Et-FBSA.

Water. PFOS and PFOA are expected to be stable to hydrolysis in the environment based on half-lives of 41 and 92 years, respectively, calculated from experimental hydrolysis data that were measured at pH 5, 7, and 9 (OECD 2002, 2006b). Based on the data for PFOS and PFOA, hydrolysis is not expected to be an important degradation process for perfluorinated carboxylates and sulfonates in the environment. Hydrolysis data were not located for perfluoroalkyl sulfonamides.

Available information indicates that perfluoroalkyls are resistant to aerobic biodegradation. PFOA and PFNA were not biodegraded using an Organisation for Economic Co-operation and Development (OECD) guideline (301F) manometric respirometry screening test for ready biodegradability; 0% of the theoretical oxygen demand was reached after 28 days (Stasinakis et al. 2008). Meesters and Schröder (2004) reported that PFOA and PFOS were not degraded from an initial concentration of 5 mg/L in aerobic sewage sludge in a laboratory scale reactor.

Substances such as fluorotelomer alcohols and perfluoroalkyl sulfonamides are degraded to other substances such as PFOA and PFOS in water and can be considered a source of these substances in the environment (Liu et al. 2007).

Sediment and Soil. Data are not available regarding the transformation and degradation of perfluoroalkyls in sediment and soil. Based on the chemical stability of these substances and their resistance to biodegradation in screening tests, environmental degradation processes are not expected to be important removal mechanisms for perfluoroalkyls in sediment and soil (3M 2000; EPA 2008a; Hekster et al. 2003; OECD 2002, 2007; Prevedouros et al. 2006; Schultz et al. 2003).

Substances such as fluorotelomer alcohols and perfluoroalkyl sulfonamides are degraded to other substances such as PFOA and PFOS in soil and sediment and can be considered a source of these substances in the environment (Liu et al. 2007; Washington and Jenkins 2015; Washington et al. 2015).

Other Media. Data are not available regarding the transformation and degradation of perfluoroalkyls in other media.

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to perfluoroalkyls depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of perfluoroalkyls in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on perfluoroalkyls levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-6 shows the limits of detection typically achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-7.

Detections of perfluoroalkyls in air, water, and soil at NPL sites are summarized in Table 5-8.

Table 5-6. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.1 pg/m^3	Harada et al. 2006
Drinking water	0.5–6.5 ng/L	EPA 2009c (Method 537)
Surface water and groundwater	0.2 ng/L	Nakayama et al. 2007
Soil	0.11–0.75 µg/kg (median reporting limits)	Anderson et al. 2016
Sediment	0.21–1.2 µg/kg (median reporting limits)	Anderson et al. 2016
Whole blood Serum	0.1–2 ng/mL 0.082–0.2 ng/mL	Kärrman et al. 2005 CDC 2015

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

Table 5-7. Summary of Environmental Levels of Perfluoroalkyls[a]

Media	Low	High[b]	For more information
Outdoor air (pg/m^3)			Table 5-9
PFOA	1.22	900,000	
PFOS	0.46	9.8	
Indoor air (pg/m^3)			Table 5-10
PFOA		4.4 (mean)	
PFOS		<47.4 (mean)	
Dust (ng/g)			Table 5-11
PFOA	<2.29	9,818	
PFOS	<4.56	18,071	
Surface water (ppb)			Tables 5-12, 5-14, 5-17
PFOA	0.00051	598	
PFOS	<0.00008	8,970	
Groundwater (ppb)			Tables 5-14, 5-17
PFOA	0.083	619	
PFOS	0.0404	4,300	
Drinking water (ppb)			EPA 2010
PFOA	<0.0023	~100	
Ocean water (pg/L)			Table 5-15
PFOA	88	192,000	
PFOS	8	57,700	
Food (ppb)			Fromme et al. 2007b
PFOA	0.025	118.29	
PFOS	0.025	1.03	
Soil (ppb)			Tables 5-16, 5-17
PFOA	<0.00017	140	
PFOS	0.0002	9700	

[a]For PFOA or PFOS only.
[b]High levels are representative of monitoring data at localized contaminated sites and are not reflective of background environmental levels.

Table 5-8. Perfluoroalkyls Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
PFOA					
Water (ppb)	0.35	0.25	6,064	5	4
Soil (ppb)	18,050	18,050	1,000	2	2
Air (ppbv)			No data		
PFOS					
Water (ppb)	0.91	0.35	9,089	4	3
Soil (ppb)	108,000	108,000	1,000	2	2
Air (ppbv)			No data		

Table 5-8. Perfluoroalkyls Levels in Water, Soil, and Air of National Priorities List (NPL) Sites

Medium	Median[a]	Geometric mean[a]	Geometric standard deviation[a]	Number of quantitative measurements	NPL sites
PFBA					
Water (ppb)	2.15	1.03	28,192	3	3
Soil (ppb)	1,600	1,600	1,000	2	2
Air (ppbv)			No data		
PFBS					
Water (ppb)	0.05	0.02	6,770	2	2
Soil (ppb)	224	224	1,000	2	2
Air (ppbv)			No data		
PFHpA					
Water (ppb)	0.07	0.04	3,169	3	2
Soil (ppb)	1,275	1,275	1,000	2	2
Air (ppbv)			No data		
PFHxA					
Water (ppb)	0.25	0.10	8,444	2	2
Soil (ppb)	1,175	1,175	1,000	2	2
Air (ppbv)			No data		
PFHxS					
Water (ppb)	0.26	1.12	52,496	4	3
Soil (ppb)	5,585	5,585	1,000	2	2
Air (ppbv)			No data		
PFNA					
Water (ppb)			No data		
Soil (ppb)	27.2	27.2	1,000	2	2
Air (ppbv)			No data		
PFPeA					
Water (ppb)	0.18	0.11	3,465	3	3
Soil (ppb)	178	178	1,000	2	2
Air (ppbv)			No data		

[a]Concentrations found in ATSDR site documents from 1981 to 2017 for 1,854 NPL sites (ATSDR 2017). Maximum concentrations were abstracted for types of environmental media for which exposure is likely. Pathways do not necessarily involve exposure or levels of concern.

5.5.1 Air

Perfluoroalkyl levels have been measured in outdoor air at locations in the United States, Europe, Japan, and over the Atlantic Ocean (Barber et al. 2007; Barton et al. 2006; Harada et al. 2005a, 2006; Kim and Kannan 2007). Concentrations reported in these studies are provided in Table 5-9.

Mean PFOA levels ranged from 1.54 to 15.2 pg/m^3 in air samples collected in the urban locations in Albany, New York; Fukuchiyama, Japan; and Morioka, Japan and in the rural locations in Kjeller, Norway and Mace Head, Ireland. Higher mean concentrations (101–552 pg/m^3) were measured at the urban locations in Oyamazaki, Japan and Manchester, United Kingdom, and semirural locations in Hazelrigg, United Kingdom. Maximum reported concentrations at Oyamazaki and Hazelrigg were 919 and 828 pg/m^3, respectively. The authors attributed the elevated concentrations at the Hazelrigg location to emissions from a fluoropolymer production plant located 20 km upwind of this semirural community.

Elevated levels of PFOA were observed in air samples collected along the fence line of the DuPont Washington Works fluoropolymer manufacturing facility, which is located near Parkersburg, West Virginia, in the Ohio River valley (Barton et al. 2006).

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Location	Mean (range) concentration (pg/m^3)			Reference
	PFOA	PFHpA	PFNA	
Urban				
Albany, New York				
Gas phase (n=8)	3.16 (1.89–6.53)	0.26 (0.13–0.42)	0.21 (0.16–0.31)	Kim and Kannan 2007
Particulate phase (n=8)	2.03 (0.76–4.19)	0.37 (<0.12–0.81)	0.13 (<0.12–0.40)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	262.7 (72–919); 3,412.8 ng/g in dust	—	—	Harada et al. 2005b
Fukuchiyama, Japan	15.2; 314 ng/g in dust	—	—	Harada et al. 2006
Morioka, Japan (n=8)	2.0 (1.59–2.58)	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	341, 15.7	8.2, 0.2	<26.6, 0.8	Barber et al. 2007

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Rural				
Kjeller, Norway (n=2)	1.54	0.87	0.12	Barber et al. 2007
Mace Head, Ireland (n=4)	8.9	<0.001	<3.3	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	101, 552[b,c]	1.6, 14.4[b]	0.9	Barber et al. 2007
Marine air				
Near Europe (northwest) (n=3)	1.22 (0.5–2.0)	<0.6 (ND–<0.6)	0.3 (ND–0.5)	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	<0.5 (ND–0.7)	ND	<0.2 (ND–0.3)	Jahnke et al. 2007a
Source dominated				
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	430,000 (75,000–900,000)[d]	—	—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	5,500 (10–75,900)	—	—	EPA 2007b
	Mean (range) concentration (pg/m^3)			
Location	PFDA	PFUnA	PFDoDA	Reference
Urban				
Albany, New York				
Gas phase (n=8)	0.63 (0.24–1.56)	<0.12 (ND–0.16)	0.27 (0.14–0.43)	Kim and Kannan 2007
Particulate phase (n=8)	0.27 (0.13–0.49)	ND	0.12 (<0.12–0.38)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	—	—	—	Harada et al. 2005b
Fukuchiyama, Japan	—	—	—	Harada et al. 2006
Morioka, Japan (n=8)	—	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	5.4, <0.8	<0.01, <0.4	<0.01, <0.01	Barber et al. 2007
Rural				
Kjeller, Norway (n=2)	<0.15	<0.12	<0.12	Barber et al. 2007
Mace Head, Ireland (n=4)	<2.8	<0.002	<0.003	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	1.0, 8.3[b]	0.7	<0.01	Barber et al. 2007

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Marine air					
Near Europe (northwest) (n=3)	<0.6 (ND–0.6)	ND		<0.14 (ND–0.17)	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	ND	0.03 (ND–0.2)		ND	Jahnke et al. 2007a
Source dominated					
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	—	—		—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	—	—		—	EPA 2007b
	Mean (range) concentration (pg/m^3)				
Location	PFOS	PFBS	PFHxS	FOSA	Reference
Urban					
Albany, New York					
Gas phase (n=8)	1.70 (0.94–3.0)	—	0.31 (0.13–0.44)	0.67 (0.22–2.26)	Kim and Kannan 2007
Particulate phase (n=8)	0.64 (0.35–1.16)	—	<0.12	0.29 (<0.12–0.79)	Kim and Kannan 2007
Oyamazaki, Japan (n=12)	5.2 (2.51–-9.80); 72.2 ng/g in dust	—	—	—	Harada et al. 2005b
Fukuchiyama, Japan	2.2; 46.0 ng/g in dust	—	—	—	Harada et al. 2006
Morioka, Japan (n=8)	0.7 (0.46–1.19)	—	—	—	Harada et al. 2005b
Manchester, United Kingdom (n=2,1)[a]	46, 7.1	2.2, <1.6	1.0, 0.1	<1.6, <0.2	Barber et al. 2007
Rural					
Kjeller, Norway (n=2)	1.0	<0.09	0.05	0.78	Barber et al. 2007
Mace Head, Ireland (n=4)	<1.8	<1.0	0.07	<0.56	Barber et al. 2007
Hazelrigg, United Kingdom (semi-rural) (n=10)	1.6	2.6	0.04	0.2	Barber et al. 2007
Marine air					
Near Europe (north west) (n=3)	1.36 (0.4–2.5)	ND	0.12 (0.02–0.3)	ND	Jahnke et al. 2007a
Near Africa (east coast) (n=5)	0.544 (0.05–1.9)	ND	0.013 (ND–0.05)	ND	Jahnke et al. 2007a

Table 5-9. Concentrations of Perfluoroalkyl in Outdoor Air

Source dominated					
DuPont Washington Works Facility; Parkersburg, West Virginia (n=28)	—	—	—	—	Barton et al. 2006
DuPont Washington Works Facility; Parkersburg, West Virginia (n=90)	—	—	—	—	EPA 2007b

[a]Mean values were reported for separate sampling sessions.
[b]The second concentration reported was measured during an earlier sampling session (n=2).
[c]A maximum PFOA concentration of 828 pg/m^3 was measured in air at Hazelrigg, United Kingdom.
[d]Average and range of concentrations in 6 out of 28 samples that contained PFOA.

"—" indicates no available data; FOSA = perfluorooctane sulfonamide; ND = not detected; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

The reported concentrations in these samples ranged from 75,000 to 900,000 pg/m^3. The highest concentrations were measured at locations downwind of the facility. High volume air samples collected at several monitoring stations near the Washington Works facility contained PFOA at reported concentrations ranging from 10 to 75,900 pg/m^3 (EPA 2007b). The mean and median of these reported concentrations are 5,500 and 240 pg/m^3, respectively.

PFOS was detected above quantitation limits in most of the studies, but concentrations were generally below 5 pg/m^3. A concentration of 46 pg/m^3 was reported in samples from Manchester, United Kingdom. Reported concentrations of other perfluoroalkyls (PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, PFHxS, and FOSA) were generally <1 pg/m^3 in these studies. PFHpA was detected at slightly higher concentrations (8.2 and 14.4 pg/m^3) at Manchester and Hazelrigg, United Kingdom, respectively.

Jahnke et al. (2007a) collected eight marine air samples during a cruise between Germany and South Africa (53°N to 33°S). Perfluoroalkyl concentrations steadily declined as the sampling moved further from Europe and toward less industrialized regions. Only PFOS was detected in the two samples collected over the Atlantic Ocean east of southern Africa.

Measurements of perfluoroalkyls in snow samples collected from Canadian Arctic ice caps suggest that volatile precursors, such as fluorinated telomer alcohols, may oxidize in the atmosphere at these locations (Young et al. 2007). Reported concentrations in these snow samples were 2.6–86 pg/L for PFOS, 12–147 pg/L for PFOA, 5.0–246 pg/L for PFNA, <8–22 pg/L for PFDA, and <6–27 pg/L for PFUnA.

The concentration of PFOS measured in rainwater collected during a rain event in Winnipeg, Manitoba was 0.59 ng/L (Loewen et al. 2005). PFOA, PFNA, PFDA, PFUnA, and PFDoDA were not detected in the rainwater. Reported method detection limits for these compounds were 7.2, 3.7, 1.7, 1.2, and 1.1 ng/L, respectively.

Studies of perfluoroalkyl concentrations in indoor environments are available. The reported mean concentrations of perfluoroalkyls measured in four indoor air samples collected from Tromso, Norway (Barber et al. 2007) are presented in Table 5-10.

Table 5-10. Concentrations of Perfluoroalkyl in Indoor Air[a]

	Mean concentration (pg/m^3)
PFOA	4.4
PFHpA	0.8
PFNA	2.7
PFDA	3.4
PFUnA	<1.3
PFDoDA	1.2
PFOS	<47.4
PFBS	<0.5
PFHxS	<4.1
FOSA	2.8

[a]Samples (n=4) collected in Tromso, Norway in May–June 2005.

FOSA = perfluorooctane sulfonamide; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Source: Barber et al. 2007

Several studies have measured perfluoroalkyl levels in indoor dust samples (Table 5-11). Kubwabo et al. (2005) measured the concentrations of selected perfluoroalkyls in dust samples from 67 Canadian homes. PFOA, PFOS, and PFHxS were each detected in 37, 33, and 15% of these samples, respectively (detection limits of 2.29, 4.56, and 4.56 ng/g, respectively). FOSA was only detected above 0.99 ng/g in 10% of the samples and PFBS was not detected in any of the samples. Moriwaki et al. (2003) measured PFOS and PFOA concentrations in vacuum cleaner dust samples collected from 16 Japanese homes. PFOS and PFOA were detected in every sample with reported concentrations of 11–140 and 69–380 ng/g, respectively, in 15 of the 16 samples. One of the samples contained 2,500 ng/g PFOS and 3,700 ng/g PFOA. The geometric means of PFOA and PFOS in pooled indoor air sample meta data analysis were

reported as 37.34 and 38.91 ng/g, respectively (Mitro et al. 2016). Precursor substances present in dust can be biotransformed to perfluoroalkyl substances and may be a further source of human exposure. Fluorotelomer alcohols were detected in hotel dust in China at levels ranging from 24.8 to 678 ng/g (Yao et al. 2018). Makey et al. (2017) reported precursor substances such as dipolyfluoroalkyl phosphates (diPAPs), fluorotelomer alcohols, perfluorooctyl sulfonamides, and sulfonamidoethanols in airborne and dust samples.

Table 5-11. Concentrations of Perfluoroalkyls in Indoor Dust

	Concentration (ng/g): mean (range); median			
Location	PFOA	PFHpA	PFNA	Reference
Ottawa, Canada (n=67)	106.00 (<2.29–1,234); 19.72[a]	—	—	Kubwabo et al. 2005
Japan (n=16)	380 (70–3,700); 165	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	296 (<10.2–1,960); 142[b]	109 (<12.5–1,150); 50.2[b]	22.1 (<11.3–263); 7.99[b]	Strynar and Lindstrom 2008
United Kingdom, Australia, German, and United States (n=39)[c]	96.5 (9,818)	97.3 (5,195)	<LOQ (832)	Kato et al. 2009a
Stockholm, Sweden[d]		—	—	Björklund et al. 2009
Houses (n=10)	54 (15–98)			
Apartments (n=38)	93 (17–850)			
Daycare centers (n=10)	41 (31–110)			
Offices (n=10)	70 (14–510)			
Cars (n=5)	33 (12–96)			

	Concentration (ng/g): mean (range) median			
Location	PFDA	PFUnA	PFDoDA	Reference
Ottawa, Canada (n=67)	—	—	—	Kubwabo et al. 2005
Japan (n=16)	—	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	15.5 (<9.40–267); 6.65[b]	30.4 (<10.7–588); 7.57[b]	18.0 (<11.0–520); 7.78[b]	Strynar and Lindstrom 2008
United Kingdom, Australia, German, and United States (n=39)[c]	<LOQ (1,965)	<LOQ (732)	<LOQ (1,048)	Kato et al. 2009a
Stockholm, Sweden[d]	—	—	—	

	Concentration (ng/g): mean (range); median				
Location	PFOS	PFBS	PFHxS	FOSA	Reference
Ottawa, Canada (n=67)	443.68 (<4.56–5,065); 37.8[a]	ND[a]	391.96 (<4.56–4,305); 23.1[a]	<0.99[a]	Kubwabo et al. 2005
Japan (n=16)	200 (11–2,500); 24.5	—	—	—	Moriwaki et al. 2003
North Carolina and Ohio (n=112)	761 (<8.93–12,100); 201[b]	41.7 (<12.5–1,150); 9.11[b]	874 (<12.9–35,700); 45.5[b]	—	Strynar and Lindstrom 2008

Table 5-11. Concentrations of Perfluoroalkyls in Indoor Dust

United Kingdom, Australia, German, and United States (n=39)[c]	479.6 (18,071)	359.0 (7,718)	185.5 (43,765)	<LOQ (18.4)	Kato et al. 2009a
Stockholm, Sweden[d]		—	—	—	Björklund et al. 2009
Houses (n=10)	39 (15–120)				
Apartments (n=38)	85 (8–1,100)				
Daycare centers (n=10)	31 (23–65)				
Offices (n=10)	110 (29–490)				
Cars (n=5)	12 (8–33)				

[a]Method detection limits (MDL) and percent below MDL are as follows: PFOA (2.29 ng/g, 37%), PFOS (4.56 ng/g, 33%), PFBS (1.38 ng/g, 100%), PFHxS (4.56, 15%), and FOSA (0.99 ng/g, 90%).
[b]LOQ and percent above LOQ are as follows: PFHpA (12.5 ng/g, 74.1%), PFOA (10.2 ng/g, 96.4%), PFNA (11.3 ng/g, 42.9%), PFDA (9.40 ng/g, 30.4%), PFUnA (10.7 ng/g, 36.6%), PFDoDA (11.0 ng/g, 18.7%), PFOS (8.93 ng/g, 94.6%), PFHxS (12.9 ng/g, 77.7%), PFBS (12.5 ng/g, 33.0%). Values below the LOQ were assigned a value of LOQ/1.412 when calculating the median and mean.
[c]Reported values are the 50th percentile and (maximum) values; all minimum values were <LOQ.
[d]Reported values are the median and range.

"—" indicates no available data; FOSA = perfluorooctane sulfonamide; LOQ = limit of quantification; ND = not detected; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluorundecanoic acid

Strynar and Lindstrom (2008) measured perfluoroalkyl levels in 112 indoor dust samples collected from homes and daycare centers in North Carolina and Ohio. These authors detected PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoDA, PFOS, PFHxS, and PFBS. PFOS and PFOA were detected in 94.6 and 96.4% of the samples, respectively. Maximum detections in the samples were as high as 12,100 ng/g for PFOS and 35,700 ng/g for PFHxS. Household dust samples collected from the United Kingdom, Australia, Germany, and the United States showed the presence of perfluoroalkyls (Kato et al. 2009a). PFOS, PFBS, and PFHxS were detected in 74.4, 92.3, and 79.5% of the samples, respectively, whereas PFOA, PFNA, and PFDA were detected in 64.1, 25.6, and 38.5% of the samples, respectively. Björklund et al. (2009) measured PFOA and PFOS in dust samples collected in houses, apartments, daycare centers, offices, and cars in Sweden. PFOA and PFOS were detected in 100 and 79% of the apartment samples and in 100 and 60% of the car samples. The authors concluded that while dietary intake was the major PFOA/PFOS exposure pathway for adults and toddlers in the general population, dust ingestion could become an important pathway under a worst-case scenario (e.g., high dust ingestion and maximum dust levels).

Perfluoroalkyls in indoor and outdoor environments in urban, suburban, and rural locations near Toronto, Ontario, Canada were assessed using window films for passive sampling. The sum of perfluoroalkyls concentrations on outdoor window films ranged from 0.04 to 0.75 pg/cm^2 in winter and from 0.04 to

0.92 pg/cm^2 in summer, with higher values found in urban and suburban locations than in rural locations. Indoors, concentrations on window film ranged from less than the detection limit (which ranged from 0.033 to 0.06 pg/cm^2) to 2.1 pg/cm^2 in winter and from 0.08 to 4.3 pg/cm^2 in summer, although there were no distinct trends between urban and rural for indoor concentrations (Gewurtz et al. 2009).

5.5.2 Water

PFOS and PFOA have been widely detected in surface water samples collected from various rivers, lakes, and streams in the United States (Boulanger et al. 2004; DRBC 2013; Kannan et al. 2005; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005; Sinclair et al. 2004, 2006). Levels of these substances in surface water appear to be declining since the phase out of these two substances. Zhang et al. (2016) measured surface water levels of perfluoroalkyls in 2014 at 37 sites across the northeastern United States. Detectable levels of PFOA and PFNA were found at all sites, and PFHxS, PFOS, and PFDA were detectable at >90% of the sites. The respective maximum concentrations of PFOA, PFOS, PFHxS, and PFNA were 56 ng/L measured in the Passaic River, New Jersey, 27.5 ng/L measured in the Woonasquatucket River, Rhode Island, 43 ng/L measured at Mill Cove, Rhode Island, and 14 ng/L measured at Mill Cove, Rhode Island (Zhang et al. 2016).

Less data are available regarding the concentrations of other perfluoroalkyls in surface water. PFHpA and PFHxS were commonly detected in the few studies that analyzed surface water for these compounds (DRBC 2013; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005). Concentrations of PFOA and PFOS measured in surface water are presented in Table 5-12 and other perfluoroalkyls are summarized in Table 5-13. Maximum concentrations of PFOS, PFOA, PFHpA, PFNA, PFDA, PFUnA, PFDoDA, PFBS, and PFHxS measured in surface water collected from the Cape Fear Basin, North Carolina were 287, 132, 329, 194, 120, 52.1, 4.46, 9.41, and 35.1 ng/L, respectively (Nakayama et al. 2007). Much higher concentrations of PFOS (198–1,090 ng/L) have been measured in Onondaga Lake in Syracuse, New York (Sinclair et al. 2006). Onondaga Lake is a Superfund site that has become contaminated through industrial activity along its banks.

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

	Concentration		
Location	PFOA	PFOS	Reference
Great Lakes			Boulanger et al. 2004
Lake Ontario (n=8)	15–70	6–121	

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

Location	Concentration		Reference
	PFOA	PFOS	
Lake Erie (n=8)	21–47	11–39	
New York State waters			Sinclair et al. 2006
Lake Ontario (n=13)	18–34	2.9–30	
Niagara River (n=3)	18–22	3.3–6.7	
Lake Erie (n=3)	13–27	2.8–5.5	
Finger Lakes (n=13)	11–20	1.3–2.6	
Onondaga Lake (n=3)	39–64	198–1,090 (median=756)	
Oneida Lake (n=1)	19	3.5	
Erie Canal (n=3)	25–59	5.7–13	
Hudson River (n=8)	22–173 (median=35)	1.5–3.4	
Lake Champlain (n=4)	10–46	0.8–7.7	
Albany, New York			Kim and Kannan 2007
Lake water (n=11)	3.27–15.8 (median=7.20)	ND–9.30 (median=2.88)	
Surface water runoff (n=14)	0.51–29.3 (median=3.80)	<0.25–14.6 (median=0.81)	
New York/New Jersey/Rhode Island metropolitan region	56 (maximum) 3.5 (median)	27.5 (maximum) 0.96 (median)	Zhang et al. 2016
Michigan water regions			Sinclair et al. 2004
Detroit (n=10)	<8–16.14	<0.08–6.13	
Flint (n=4)	<8–23.01	1.50–12.31	
Saginaw Bay (n=5)	<8–24.08	3.10–12.69	
Northeastern Michigan (n=2)	<8	0.87–6.34	
Upper Peninsula (n=7)	<8–13.77	<0.8–3.09	
Northwestern Michigan (n=2)	11.96	<0.8–4.48	
Western Michigan (n=6)	<8–15.17	<0.8–5.32	
Southwestern Michigan (n=5)	8.74–35.86	7.22–29.26	
Lansing (n=3)	<8–13.37	1.04–4.96	
Minnesota Waters and Lake Michigan			Simcik and Dorweiler 2005
Remote (n=4)			
Loiten	0.7	ND	
Little Trout	0.3	1.2	
Nipisiquit	0.1	ND	
Tettegouche	0.5	0.2	
Urban (n=4)			
Calhoun	20	47	
Lake Harriet	3.5	21	
Lake of the Isles	0.5	2.4	

Table 5-12. Concentrations of PFOA and PFOS in Surface Water (ng/L)

Location	Concentration		Reference
	PFOA	PFOS	
Minnesota River	1.2	9	
Lake Michigan (n=4)	<0.6–0.5	1–3.2	
Cape Fear Basin, North Carolina			Nakayama et al. 2007
80 Sites (n=100)			
Mean	43.4	31.2	
Median	12.6	28.9	
Minimum	ND	<1	
Maximum	287	132	
Percent not detected[a]	7.6	0	
Raisin and St. Clair Rivers, Michigan			Kannan et al. 2005
Raisin River	14.7	3.5	
St. Clair River (n=3)	4.0–5.0	1.9–3.9	
Conasauga River, Georgia	253–1,150	192–318	Konwick et al. 2008
Dalton, Georgia	49.9–299	15.8–120	Konwick et al. 2008
Tidal Delaware River (six locations)			DRBC 2013
2007	3.54–75.40	2.70–8.42	
2008	3.99–48	3.53–11.7	
2009	3.29–27.7	2.86–7.97	
Several rivers in Japan	0.1–67,000	0.3–59	Harada and Koizumi 2009
Lake Victoria Gulf, Kenya	0.4–96.4 (rivers) 0.4–11.6 (lakes)	<0.4–13.23 (rivers); <0.4–2.53 (lakes)	Orata et al. 2009
River Po, Italy	1–1,270	1–25	Loos et al. 2008

[a]Detection limit is 0.05 ng/L

ND = not detected; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Great Lakes (Boulanger et al. 2004)								
Lake Ontario (n=8)	—	—	—	—	—	—	—	—
Lake Erie (n=8)	—	—	—	—	—	—	—	—
New York State waters (Sinclair et al. 2006)								
Onondaga Lake (n=3)	—	—	—	—	—	—	4.2–8.5	—

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Erie Canal (n=3)	—	—	—	—	—	—	2.5–5.6	—
Other lakes and rivers	—	—	—	—	—	—	0.5–2.8	—
Albany, New York (Kim and Kannan 2007)								
Lake water (n=11)	1.15–12.7	ND–3.51	0.25–3.58	ND–1.45	ND–<0.25	—	<0.25–4.05	ND–0.47
Surface water runoff (n=14)	<0.25–6.44	<0.25–5.90	ND–8.39	ND–1.99	ND–1.60	—	ND–13.5	ND–2.14
New York/New Jersey/Rhode Island metropolitan region (Zhang et al. 2016)								
	48.2 (max); 0.9 (median)	14 (max); 0.4 (median)	5.8 (max); 0.2 (median)	1.9 (max); 0.1 (median)	2.6 (max); 0.0 (median)	6.2 (max); 0.4 (median)	43 (max); 0.7 (median)	—
Minnesota waters and Lake Michigan (Simcik and Dorweiler 2005)								
Remote (n=4)								
Loiten	10	ND	ND	—	—	—	—	—
Little Trout	4.8	ND	ND	—	—	—	—	—
Nipisiquit	0.9	<0.3	ND	—	—	—	—	—
Tettegouche	3.1	ND	ND	—	—	—	—	—
Urban (n=4)					—	—	—	—
Calhoun	11	0.6	0.5	—	—	—	—	—
Lake Harriet	2.6	ND	ND	—	—	—	—	—
Lake of the Isles	0.4	ND	ND	—	—	—	—	—
Minnesota River	0.7	1.9	ND	—	—	—	—	—
Lake Michigan (n=4)	<0.6–4.1	<0.6–3.1	ND	—	—	—	—	—
Cape Fear Basin, North Carolina (Nakayama et al. 2007)								
80 Sites (n=100)								
Mean	38.7	33.6	22.1	10.4	2.17	2.58	7.29	—
Median	14.8	5.70	13.2	5.67	1.95	2.46	5.66	—
Maximum	329	194	120	52.1	4.46	9.41	35.1	—
Percent not detected[b]	32.9	10.1	15.2	17.7	53.2	38.0	45.6	—
Raisin and St. Clair Rivers, Michigan (Kannan et al. 2005)								
Raisin River	—	—	—	—	—	—	<1	<10
St. Clair River (n=3)	—	—	—	—	—	—	<1	<10

Table 5-13. Concentrations of Other Perfluoroalkyls in Surface Water

Location (reference)[a]	Concentration (ng/L)							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Delaware River (6 locations) (DRBC 2013)								
2007	ND–24.30	1.71–976	ND-9.97	ND-26	ND	—	ND-4.48	ND
2008	2.03–16.3	3.24–650	ND-5.25	ND-11.8	ND	—	BD-3.55	ND
2009	1.14–47.4	1.65–546	ND-1.75	ND-8.3	ND	—	ND-3.62	ND

[a]See Table 5-12 for numbers of samples collected at these locations.
[b]Detection limit = 0.05 ng/L.

"—" indicates no available data; ND = not detected; FOSA = perfluorooctane sulfonamide; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

Higher levels of perfluoroalkyls are expected in surface water and groundwater surrounding perfluorochemical industrial facilities. Paustenbach et al. (2007) estimated PFOA concentrations in environmental media for communities located near the DuPont Washington Works chemical manufacturing facility. From this analysis, the authors concluded that much of the PFOA detected in groundwater near the facility was attributed to deposition to soil surfaces following atmospheric emissions from the plant followed by subsequent leaching into groundwater. DuPont entered into an agreement with the EPA to collect monitoring data for PFOA in the Ohio River around the DuPont Washington Works facility. In its final phase III assessment, it was reported that levels of PFOA downstream from the facility in 2011 were about an order of magnitude lower than when monitoring began in 2002, while levels upstream essentially remained unchanged (URS 2012). The maximum concentration measured in 2011 was 200 ng/L and was obtained from a monitoring location adjacent to the site.

PFOA, PFOS, PFBA, PFHxS, and PFBS have been detected in the municipal drinking water of communities located near the 3M Cottage Grove fluorochemical facility (ATSDR 2008). Xiao et al. (2015) summarized soil and groundwater monitoring data of select perfluoroalkyls near this facility. Groundwater samples for PFBA, PFHxA, PFOA, PFHxS, and PFOS in wells surrounding the site obtained from 2009 to 2013 showed little or no change in concentration over this time period, even though 3M had stopped producing perfluoroalkyls at this facility in 2002. Levels in groundwater were shown to decrease exponentially with distance from the source. A measured PFOA concentration of approximately 20,000 ng/L was observed from a well near the historical unlined disposal site of this

facility, but levels decreased to <100 ng/L measured at a distance of 1.4 km away. Some other examples are provided in Table 5-14.

Table 5-14. Concentrations of Perfluoroalkyls in Surface Water and Groundwater at Fluorochemical Industrial Facilities

Location	Percent detection and concentration (µg/L)					Reference
	PFOA	PFBA	PFOS	PFHxS	PFBS	
DuPont Washington Works Facility, West Virginia						
Groundwater						
Borings (n=18)						Davis et al. 2007
Percent detected	89%[a]	—	—	—	—	
Minimum	0.0912[a]	—	—	—	—	
Maximum	78[a]	—	—	—	—	
Wells (n=14)			—	—	—	Davis et al. 2007
Percent detected	100%[a]	—	—	—	—	
Minimum	0.081[a]	—	—	—	—	
Maximum	37.1[a]	—	—	—	—	
Wells (n=3)			—	—	—	EPA 2008d
Percent detected	100%	—	—	—	—	
Minimum	2.8	—	—	—	—	
Maximum	100	—	—	—	—	
Surface water						
Outlets (n=4)						EPA 2008d
Percent detected	100%	—	—	—	—	
Minimum	2.3	—	—	—	—	
Maximum	61	—	—	—	—	
3M Cottage Grove Facility, Minnesota						
Groundwater						
Wells (n=1–7)						3M 2007b
Percent detected	100%	100%	100%	100%	100%	
Minimum	24.6	23.3	26.0	6.47	2.11	
Maximum	619	318	26.0	40.0	26.1	
Surface water						
East and West Cove (n=3–9)						3M 2007b
Percent detected	100%	100%	100%	100%	78%	
Minimum	0.172	0.803	0.227	0.0936	0.304	
Maximum	2.79	1.01	3.12	4.58	9.69	
Mississippi River Shoreline (n=52–80)						3M 2007b
Percent detected	60%	52%	43%	28%	56%	
Maximum	0.760	6.92	0.539	1.04	3.05	

Table 5-14. Concentrations of Perfluoroalkyls in Surface Water and Groundwater at Fluorochemical Industrial Facilities

Location	Percent detection and concentration (µg/L)					Reference
	PFOA	PFBA	PFOS	PFHxS	PFBS	
Mississippi River Transect (n=34–44)						3M 2007b
Percent detected	14%	12%	0%	0%	0%	
Maximum	0.0501	0.0530	ND	ND	ND	
3M Decatur Facility, Alabama						
Groundwater						
Off-site groundwater (n=18)						3M 2008c
Percent detected	94%	—	—	—	—	
Mean	1.87	—	—	—	—	
Range	0.083–19.8	—	—	—	—	
Off-site groundwater						3M 2010
Range			0.0404–2.41	0.0396–0.622	0.0615–0.480	
Groundwater range (n=51)	0.149–6.41	0.0104–1.26	0.012–0.151	0.0127–0.0875	0.0101–0.0766	Lindstrom et al. 2011
Surface water						
On-site surface water (n=7)						3M 2008c
Percent detected	100%	—	—	—	—	
Median	2.66	—	—	—	—	
Range	0.32–127	—	—	—	—	
Off-site surface water (n=60)						3M 2008c
Percent detected	98%	—	—	—	—	
Range	0.026–27.7	—	—	—	—	
Tennessee River						Hansen et al. 2002
Upstream of facility (n=19)						
Percent detected	0%	—	100%	—	—	
Range	<25	—	16.8–52.6	—	—	
Downstream of facility (n=21)				—	—	
Percent detected	0%	—	100%	—	—	
Median	355	—	107	—	—	
Range	<25–598	—	30.3–144	—	—	

[a]Analyte was reported as APFO.

"—" indicates no available data; APFO = ammonium perfluorooctanoate; ND = not detected; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Groundwater sampling of PFOA and PFOS was conducted at 401 military installations (DoD 2018). The PFOS/PFOA levels exceeded EPA's lifetime health advisories of 70 ppt at 22% of the installations (9/64 Army, 40/127 Navy/U.S. Marine Corp, 39/203 Air Force, and 2/7 Defense Logistics Agency

installations). The PFOS/PFOA levels exceeded the EPA lifetime health advisories in 1,621 of the 2,668 groundwater wells sampled (61%).

Yamashita et al. (2005) measured PFOA, PFOS, PFNA, and PFHxS concentrations in ocean water collected from locations in the Atlantic Ocean, Pacific Ocean, and areas near China, Korea, and Japan. These concentrations are listed in Table 5-15. Wei et al. (2007a) measured perfluoroalkyl concentrations in surface seawaters from the western Pacific Ocean, Indian Ocean, and near-Antarctic region. PFOS and PFOA were detected in 60 and 40% of the samples, respectively, with maximum concentrations of 71.7 and 441.6 pg/L, respectively. Concentrations of other perfluoroalkyls (PFHxS, PFBS, PFDoDA, PFDA, PFNA, PFHpA) were generally below detection in most samples, with the exceptions being in samples collected near Shanghai, the Philippines, and Indonesia. Maximum concentrations of these perfluoroalkyls ranged from 3.1 to 70.2 pg/L near Shanghai. PFOA, PFOS, and other perfluoroalkyl species were monitored in waste water effluents and 20 rivers located in Japan (Murakami et al. 2008). Perfluoroalkyls were ubiquitous in the river water samples, with concentrations of PFOA as large as 0.054–0.192 μg/L in seven of the river samples with low waste water effluent sources.

Table 5-15. Concentrations of PFOA and PFOS in Ocean Water[a]

	Concentration (pg/L)			
Location	PFOA	PFOS	PFNA	PFHxS
North Atlantic (n=9)	160–338	8.6–36	15–36	4.1–6.1
Mid Atlantic (n=7)	100–439	37–73	—	2.6–12
Central to Eastern Pacific (n=14)	15–62	1.1–20	1.0–16	0.1–1.6
Western Pacific (n=2)	136–142	54–78	—	2.2–[a]2.8
Tokyo Bay (n=8)	1,800–192,000	338–57,700	163–71,000	17–5,600
Offshore Japan (n=4)	137–1,060	40–75	—	3.0–6.1
Coastal Hong Kong (n=12)	673–5,450	70–2,600	22–207	<5–311
Coastal China (n=14)	243–15,300	23–9,680	2.0–692	<5–1,360
Coastal Korea (n=10)	239–11,350	39–2,530	15–518	<5–1,390
Sulu Sea (n=5)	88–510	<17–109	—	<0.2
South China Sea (n=2)	160–420	8–113	—	<0.2

[a]Includes samples of coastal and open ocean water.

"—" indicates no available data; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Yamashita et al. 2005

The presence of PFOA and PFOS in surface water in the United States indicates that drinking water taken from these sources may contain detectable levels of these substances. The most vulnerable drinking water systems are those in close proximity to sites that are contaminated with perfluoroalkyls. PFOA was detected in 12 out of 13 samples collected from four municipal drinking water treatment plants that draw water from the Tennessee River and are located downstream from the 3M Decatur Facility in Alabama. Reported concentrations ranged from 0.025 to 0.16 μg/L (3M 2008c). PFOA was not detected in any samples collected from a fifth plant located upstream of the 3M Decatur facility (3M 2008c). Sampling conducted in October 2015 continued to show a presence of perfluoroalkyls in water samples collected at nine sites along the Tennessee River near Decatur, with maximum PFOS, PFBS, and PFOA levels reported as 0.220, 0.160, and 0.120 μg/L, respectively (Newton et al. 2017). Waterways adjacent to areas where aqueous film forming foams (AFFFs) used to fight fires have been shown to have high levels of perfluoroalkyls. For example, a PFOS concentration of 2,210 μg/L was detected in the Etobicoke Creek, Canada following the use of AFFF at the Toronto Pearson Airport (D'agostino and Mabury 2017). The vertical distribution of 15 perfluoroalkyl substances was studied in concrete samples obtained from a location in which AFFFs were used in firefighter training (Baduel et al. 2015). At the surface of the concrete pad, PFOS was observed to be the dominant substance measured; however, shorter-chain compounds were observed to a depth of 12 cm from the surface, suggesting vertical transport of the shorter-chain compounds and the potential for movement into groundwater.

Hu et al. (2016) presented geospatial monitoring data for six perfluoroalkyls (PFBS, PFHxS, PFHpA, PFOA, PFOS, and PFNA) in U.S. drinking water from information contained in the EPA third Unregulated Contaminant Monitoring Rule (UCMR 3) program. Frequency of detection and concentrations in drinking water was correlated with proximity to industrial facilities using perfluoroalkyls, military fire training areas, and the number of waste water treatment plants. Note that UCMR 3 required all large water systems (4,120 PWSs, serving >10,000 people) and a representative sample of 800 small water systems (serving ≤10,000 people) to monitor for PFOA and PFOS from 2013 through 2015.

It was reported that 66 public drinking water systems that serve 6 million U.S. residents had at least one sample that exceeded the current EPA health advisory level of 0.07 μg/L for PFOA and PFOS (Hu et al. 2016). The dataset used by Hu et al. (2016) has since been updated by the EPA (EPA 2017). The most recent report dated January 2017 showed that PFOS, PFOA, PFNA, PFHxS, PFHpA, and PFBS were detected above their respective minimal reporting level in 95, 117, 14, 55, 86, and 8 out of 4,920 public water systems (EPA 2017). PFOA and PFOS were identified above the health advisory level of

0.07 µg/L in 13 and 46 out of the 4,920 public water supplies, respectively (EPA 2017). It is possible that combined concentrations of PFOA and PFOS may exceed the advisory level while individual concentrations may still be below 0.07 µg/L.

Based on a memorandum of understanding with the EPA, DuPont began collecting water monitoring data of both public and private wells near the Washington Works chemical plant. The quarterly reports and monitoring data affiliated with these reports may be obtained from the regulations.gov portal (http://www.regulations.gov). In samples of water collected at 17 public water facilities from 2002 to 2009 in West Virginia and Ohio, PFOA levels ranged from below the detection limit (0.0023 µg/L) to nearly 100 µg/L in a few test wells in Little Hocking, Ohio (EPA 2010). In the final phase III summary report, PFOA concentrations were reported to range from below the detection limit to 0.79 µg/L in 34 wells located approximately 3 miles upstream and 82 miles downstream from the facility (URS 2012).

Rumsby et al. (2009) reviewed the presence of PFOS and PFOA in drinking waters worldwide and discussed treatment methods for removing these substances from public water supplies. Conventional waste water treatment does not always efficiently remove perfluoroalkyls, and effluent may contain higher levels of some perfluoroalkyls than influent due to degradation of precursor substances during the treatment process (Schultz et al. 2006a, 2006b). While granulated activated carbon and reverse osmosis followed by nanofiltration have been shown to be effective methods of removing perfluoroalkyls, conventional methods such as chlorination, ozonolysis, and slow sand filtration may not be as effective. As a consequence, public drinking water systems impacted by effluent from waste water treatment plants often contain higher levels of perfluoroalkyls than systems that are not impacted by waste water treatment plant effluent. Quinones and Snyder (2009) analyzed raw and finished water at seven different public water systems in the United States for the presence of perfluoroalkyls. Water systems that were heavily impacted by waste water treatment plant effluents had greater frequency and higher levels of perfluoroalkyls when compared to water systems that were not highly impacted by waste water treatment plants. For example, no perfluoroalkyls were detected in either influent or finished water from a public water system in Aurora, Colorado with no impact from waste water treatment plant effluent; however, PFHxA, PFOA, PFNA, PFDA PFUnA, PFHxS, and PFOS were detected in all samples of a Los Angeles, California public water system that was highly impacted by waste water effluent. Perfluoroalkyls were commonly detected in the influent and effluent of 10 waste water treatment plants across the United States (Schultz et al. 2006a). PFBS was detected in 100% of both influent and effluent samples of the 10 plants, while other perfluoroalkyls like PFOA, PFOS, PFNA, PFHxA, PFHpA, and PFHxS were detected in 80% of the influent and effluent samples at the 10 plants. In a national study of

10 perfluoroalkyls in raw and treated drinking water of France, Boiteux et al. (2012) observed that several perfluoroalkyl carboxylic acids had greater concentrations in treated water than the raw water. In eight drinking water treatment plants, PFBA, PFHxA, and PFHpA were not detected in raw water, but were detected in treated water, indicating that these substances were released from saturated activated carbon used to treat raw waters or were formed by the degradation of precursor substances. Perfluoroalkyl sulfonates appeared to be removed more efficiently than the carboxylates. PFHxS, PFBS, and PFOS were detected less frequently in treated water as compared to raw water influent. These three compounds comprised 53% of the total concentration of perfluoroalkyls in the raw water samples, but only 37% of the total concentration of the perfluoroalkyls in the treated water. The summed concentration of 10 perfluoroalkyls was analyzed in the raw water and treated water of two drinking water treatment plants downstream from a fluoropolymer manufacturing facility located in France (Dauchy et al. 2012). The total concentration of perfluoroalkyls in the raw water at four sampling locations of the first plant ranged from 0.140 to 0.287 μg/L, while the summed concentration in the treated water was 0.179 μg/L. The total concentration of the 10 perfluoroalkyls in raw water at the second plant was 0.132 μg/L, while the total concentration in the treated water was 0.130 μg/L. Levels of PFHxA were greater in the treated water than the raw water at three of the four raw water sampling points, and levels of PFNA were greater in treated water than raw water at all sampling points of the first plant, but were slightly lower in the treated water of the second drinking water plant even though both systems used simple chlorination to treat the water.

PFOA was detected in 65% of the public drinking water systems tested in New Jersey in 2006 at concentrations ranging from 0.005 to 0.039 μg/L (Post et al. 2009). In a follow-up study conducted in 2009, PFOA was detected in 57% of raw water samples from 29 additional public drinking water systems in New Jersey at a maximum concentration of 0.100 μg/L (Post et al. 2013). Nine other perfluoroalkyls were also tested for, with PFOS and PFNA being the most frequently detected compounds (30% detection frequency each) after PFOA. PFOA and PFOS were detected in tap water from 21 cities located in China at concentrations of <0.0001–0.0459 and <0.0001–0.0148 μg/L, respectively (Jin et al. 2009). Mak et al. (2009) published a study comparing detections of perfluoroalkyls including PFOA and PFOS in tap water collected in China, Japan, India, Canada, and the United States. PFOA and PFOS were the predominant species measured, accounting for 40–50% of the total perfluoroalkyls present in water, with the exception of certain location of India where PFOS or PFOA may not have been present or were present at low levels.

Gellrich et al. (2013) analyzed 119 samples of mineral water, 26 samples of tap water, 18 spring water samples, and 14 raw water samples from Germany for the presence of perfluoroalkyls. Perfluoroalkyls were detected in 58% of all of the samples tested, with the greatest summed total concentration observed in tap water at 0.0427 μg/L. The maximum concentration of individual perfluoroalkyls occurring in bottled water, spring water, untreated water, and tap water were observed for PFBS (0.0133 μg/L), PFOA (0.0074 μg/L), PFBS (0.010 μg/L), and PFHxS (0.0121 μg/L), respectively. Perfluoroalkyls were widely detected in drinking water samples collected in 2008 at 40 different locations of Catalonia, Spain (Ericson et al. 2009). Median concentrations ranged from 0.00002 μg/L (FOSA) to 0.00098 μg/L (PFOA). The most frequently detected compounds were PFOS and PFHxS, which were detected in 35 and 31 samples, respectively. PFOS, PFOA, and PFHxS were detected in all samples collected in a study of drinking water contamination of perfluoroalkyls in Rio de Janeiro, Brazil (Quinete et al. 2009). Concentration ranges were 0.00058–0.00670 μg/L (PFOS), 0.00035–0.00282 μg/L (PFOA), and 0.00015–0.001 μg/L (PFHxS) respectively.

Perfluoropolyethers (PFPEs) such as perfluoroether carboxylic and sulfonic acids containing one or more ether oxygens in the carbon backbone are expected to be less persistent than the legacy substances. However, hexafluoropropylene oxide trimer and dimer acid (HFPO-TA and HFPO-DA) were detected in downstream water samples near a fluoropolymer facility in China at levels of 5.2–68.5 μg/L (Pan et al. 2017). Pan et al. (2018) also provided data of HFPO-DA and HFPO-TA in surface waters in China, United Kingdom, United States, Sweden, Germany, Netherlands, and South Korea. The substances were frequently detected in surface waters in all countries with median levels of 0.00095 μg/L (HFPO-DA) and 0.00021 μg/L (HFPO-TA). Gebbink et al. (2017) also reported that the GenX (HFPO-DA) was detected in all sampling sites downstream from a fluoropolymer facility in the Netherlands and at three out of four drinking water facilities located near the facility. Short-chain perfluoroalkyl compounds and legacy perfluoroalkyls were analyzed for in 97 drinking water samples from Canada and other nations in 2015–2016 (Kabore et al. 2018). PFOA and PFOS levels did not exceed 0.005 μg/L in any of the 97 samples; however, high detection frequencies ranging from 64 to 92% were observed in tap water for some short-chain perfluoroalkyls.

5.5.3 Sediment and Soil

Concentrations of perfluoroalkyls in soils are expected to be greater in the vicinity of fluorochemical plants that produced or used these substances as processing aids in the manufacture of fluoropolymers than in the environment at large. Levels of some perfluoroalkyls measured in soil and sediment

surrounding perfluorochemical industrial facilities are listed in Table 5-16. PFOA was detected in most soil and sediment samples collected on- and off-site at the 3M Decatur facility in Alabama in monitoring studies conducted between October 2004 and December 2006. Maximum soil concentrations were as high as 14,750 ng/g on-site and 7.85 ng/g off-site, and maximum sediment concentrations were as high as 347 ng/g on-site and 2,385 ng/g off-site (3M 2008c). The highest levels of PFOA were measured in soil from on-site fields formerly amended with PFOA-containing sludge. In its final project report for this location, six on-site soil samples were analyzed in December 2012 for the presence of PFOA, PFOS, PFBS, and PFHxS. Average levels were 3.86–3,890 ng/g (PFOS), 3.56–270 ng/g (PFHxS), 0.423–64.8 ng/g (PFBS), and 17.0–1,410 ng/g (PFOA) (3M 2012).

Table 5-16. Concentrations of Perfluoroalkyls in Soil and Sediment at Fluorochemical Industrial Facilities

	Percent detection and concentration (ng/g)					
Location	PFOA	PFBA	PFOS	PFHxS	PFBS	Reference
DuPont Washington Works Facility, West Virginia						
Soil						
Boring samples (n=22)						Davis et al. 2007
Percent detected	36%[a]	—	—	—	—	
Minimum	<0.17[a]	—	—	—	—	
Maximum	170[a]	—	—	—	—	
3M Cottage Grove Facility, Minnesota						
Soil						
Boring samples (n=50–108)						3M 2007b
Percent detected	100%	—	95%	90%	60%	
Maximum	21,800	—	104,000	3,470	139	
Fire training area (n=8–11)						3M 2007b
Percent detected	91%	82%	100%	100%	73%	
Maximum	262	11.5	2,948	62.2	24.6	
Sediment						
East and West Cove (n=21–28)						3M 2007b
Percent detected	100%	93%	100%	96%	65%	
Minimum	0.764	ND	40.0	ND	ND	
Maximum	1,845	94.6	65,450	126	9.14	
Mississippi River shoreline (n=84–92)						3M 2007b
Percent detected	70%	44%	80%	28%	29%	
Maximum	341	124	79.0	11.5	29.4	
Mississippi River transect (n=38–40)						3M 2007b
Percent detected	18%	0%	82%	0%	0%	
Maximum	1.09	ND	3.16	ND	ND	

Table 5-16. Concentrations of Perfluoroalkyls in Soil and Sediment at Fluorochemical Industrial Facilities

	Percent detection and concentration (ng/g)					
Location	PFOA	PFBA	PFOS	PFHxS	PFBS	Reference
3M Decatur Facility, Alabama						
Soil						
On-site former sludge incorporation area (n=357)						3M 2008c
Percent detected	99%	—	—	—	—	
Mean	885–929					
Range	2.91–14,750	—	—	—	—	
On-site background (n=18)						3M 2008c
Percent detected	100%	—	—	—	—	
Mean	3.53–4.1					
Range	1.61–6.03	—	—	—	—	
Off-site soil (n=23)						3M 2008c
Percent detected	100%	—	—	—	—	
Mean	3.68–4.6					
Range	0.72–7.85	—	—	—	—	
Sediment						
On-site sediment (n=8)						3M 2008c
Percent detected	88%	—	—	—	—	
Median	16.8					
Range	1.64–347	—	—	—	—	
Off-site sediment (n=30)						3M 2008c
Percent detected	93%	—	—	—	—	
Range	0.39–2,385	—	—	—	—	
3M Decatur Facility, Alabama December 2012						
On-site former sludge incorporation area (n=6)						3M 2012
Percent detected	100%	—	100%	86%	86%	
Mean	17.0–1,410	—	3.86–3,890	3.56–270	0.423–64.8	
3M Cottage Grove Facility, Minnesota						
Surface soil along U.S. Highway 10 near facility						Xiao et al. 2015
Percent detected	100%	—	100%	—	—	
Median	8.0		12.2			
Range	5.5–125.7		0.2–28.2			

[a]Analyte was reported as APFO.

"—" indicates no available data; APFO = ammonium perfluorooctanoate; ND = not detected; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

PFOA, PFOS, and PFHxS were detected in 90–100% of soil samples collected from a former tar neutralization area, a former sludge disposal area, a former solids burn pit area, a former waste water treatment plant area, and a former fire training area at the 3M Cottage Grove facility in Minnesota (3M 2007b). PFBS was detected in 60–73% of these samples. Maximum concentrations for these substances were 21,800, 104,000, 3,470, and 139 ng/g, respectively. Levels of PFBA were only reported for soil in the fire training area; it was detected in 9 out of 11 samples from this location at 0.306–11.5 ng/g. The percent detection of these compounds in sediment from the East and West Cove sites was similar to that in soil. Maximum concentrations of PFOA and PFOS were 1,845 and 65,450 ng/g, respectively. These perfluoroalkyls were also analyzed in Mississippi River sediment near the Cottage Grove Facility. Levels of these compounds were much greater along the facility shoreline compared to levels in transect samples collected at points crossing the river. Maximum shoreline concentrations for PFOA, PFBA, PFOS, PFHxS, and PFBS were 341, 124, 79.0, 11.5, and 29.4 ng/g, respectively. PFHxS, PFBS, and PFBA were not detected in any of the transect samples, and PFOA was found in only 18%. Although the maximum concentration of PFOS was 3.16 ng/g, it was still detected in 82% of the transect samples.

PFOA and PFOS were detected in all surface soils (top 10 cm) samples collected at 28 sites in September and October of 2012 along U.S. Highway 10 running from Cottage Grove, Minnesota (where the former 3M perfluoroalkyl manufacturing facility was located) to Big Lake, Minnesota (Xiao et al. 2015). Measured levels of PFOS and PFOA ranged from 0.2 to 28.2 and from 5.5 to 125.7 ng/g, respectively. Subsurface soils up to a depth of 65 cm were collected at four sites as well. Levels of PFOA and PFOS generally increased with increasing depth at each of the locations, suggesting a downward movement of the contaminants and the potential to contaminate groundwater.

The use of aqueous firefighting foams at fire training areas of military installations has resulted in widespread contamination of perfluoroalkyls in the soil and groundwater at these facilities. Monitoring data obtained from 40 sites at 10 U.S. military installations in the continental United States and Alaska were collected for several perfluoroalkyls (Anderson et al. 2016). These data are summarized in Table 5-17.

Table 5-17. Summary of Perfluoroalkyls Detected in Soil, Sediment, Surface Water, and Groundwater at 10 Military Installations[a]

Compound	Parameter	Surface soil (µg/kg)	Subsurface soil (µg/kg)	Sediment (µg/kg)	Surface water (µg/L)	Groundwater (µg/L)
PFBA	DF	38.46	29.81	24.24	84.00	85.51
	Median	1.00	0.960	1.70	0.076	0.180
	Maximum	31.0	14.0	140	110	64.0
PFBS	DF	35.16	34.62	39.39	80.00	78.26
	Median	0.775	1.30	0.710	0.106	0.200
	Maximum	52.0	79.0	340	317	110
PFHxA	DF	70.33	65.38	63.64	96.00	94.20
	Median	1.75	1.04	1.70	0.320	0.820
	Maximum	51.0	140	710	292	120
PFHxS	DF	76.92	59.62	72.73	88.00	94.93
	Median	5.70	4.40	9.10	0.710	0.870
	Maximum	1,300	520	2,700	815	290
PFHpA	DF	59.34	45.19	48.48	84.00	85.51
	Median	0.705	0.660	1.07	0.099	0.235
	Maximum	11.4	17.0	130	57.0	75.0
PFOA	DF	79.12	48.08	66.67	88.00	89.86
	Median	1.45	1.55	2.45	0.382	0.405
	Maximum	58.0	140	950	210	250
FOSA	DF	64.84	29.81	75.76	52.00	48.55
	Median	1.20	0.470	1.30	0.014	0.032
	Maximum	620	160	380	15.0	12.0
PFOS	DF	98.90	78.85	93.94	96.00	84.06
	Median	52.5	11.5	31.0	2.17	4.22
	Maximum	9,700	1,700	190,000	8,970	4,300
PFNA	DF	71.43	14.42	12.12	36.00	46.38
	Median	1.30	1.50	1.10	0.096	0.105
	Maximum	23.0	6.49	59.0	10.0	3.00
PFDA	DF	67.03	12.50	48.48	52.00	34.78
	Median	0.980	1.40	1.90	0.067	0.023
	Maximum	15.0	9.40	59.0	3.20	1.80
PFUnA	DF	45.05	9.62	24.24	20.00	8.70
	Median	0.798	1.15	160	0.021	0.025
	Maximum	10.0	2.00	14.0	0.210	0.086

Table 5-17. Summary of Perfluoroalkyls Detected in Soil, Sediment, Surface Water, and Groundwater at 10 Military Installations[a]

Compound	Parameter	Surface soil (µg/kg)	Subsurface soil (µg/kg)	Sediment (µg/kg)	Surface water (µg/L)	Groundwater (µg/L)
PFDoDA	DF	21.98	6.73	45.45	20.00	4.35
	Median	1.95	2.40	2.80	0.058	0.022
	Maximum	18.0	5.10	84.0	0.071	0.062

[a]Water concentrations are ppb (µg/L); soil and sediment levels are ppb (µg/kg).

DF = detection frequency as a percentage; FOSA = perfluorooctane sulfonamide; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFDA = perfluoro-n-decanoic acid; PFHpA = perfluoroheptanoic acid; PFHxA = perfluorohexanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Source: Anderson et al. (2016)

Perfluoroalkyls have been detected in soils that were amended with biosolids (Sepulvado et al. 2011). Several perfluoroalkyls were detected in biosolid-amended soils, with PFOS being the predominant compound with levels ranging from 5.5 to 483 ng/g, depending upon the loading rate.

5.5.4 Other Media

In a study conducted by the Food and Drug Administration (FDA) of 91 food samples collected during the 2017 Total Diet Study, PFOS was detected in 10 meat/seafood samples; the levels ranged from 0.134 ng/g in a boiled frankfurter to 0.865 ng/g in baked tilapia (FDA 2019). PFBA was detected in one sample of raw/frozen pineapple (0.068 ng/g). PFOA, PFHxS, PFNA, PFDA, PFBS, and PFHxA levels were below the lower limit of quantitation. It is important to note that FDA states that the sample size is limited and cannot be used to draw definitive conclusions. Levels of PFOS, PFOA, PFBS, PFHxS, PFHxA, PFHpA, PFDA, PFNA, and PFDoDA were analyzed in 31 food items collected from 5 grocery stores located in Texas in 2009 (Schecter et al. 2010). PFOA was the most frequently detected item (detected in 17 of 31 of the food samples), with levels ranging from 0.07 ng/g in potatoes to 1.80 ng/g in olive oil. PFOS, PFHxA, PFHpA, PFNA, PFDA, and PFDoDA were not detected in any samples. PFBS and PFHxS were detected in cod at 0.12 and 0.07 ng/g, respectively. The data for PFOA are summarized in Table 5-18. Several studies have evaluated the levels of perfluoroalkyls in fish from lakes and rivers in the United States; these data are summarized in Table 5-19.

Table 5-18. Detections of PFOA in 31 U.S. Food Items

Food	PFOA concentration in ng/g (LOD for non-detects)
Hamburger	0.15
Bacon	0.24
Sliced turkey	ND (0.02)
Sausage	0.09
Ham	0.02
Sliced chicken breast	0.02
Roast beef	ND (0.02)
Canned chili	0.02
Salmon	0.23
Canned tuna	ND (0.05)
Fresh catfish fillet	0.30
Tilapia	0.10
Cod	0.10
Canned sardines	0.19
Frozen fish sticks	0.21
Butter	1.07
American cheese	ND (0.04)
Other cheese	ND (0.04)
Whole milk	ND (0.02)
Ice cream	ND (0.03)
Frozen yogurt	ND (0.02)
Whole milk yogurt	ND (0.02)
Cream cheese	ND (0.03)
Eggs	ND (0.04)
Olive oil	1.80
Canola oil	ND (0.05)
Margarine	0.19
Cereals	ND (0.04)
Apples	ND (0.02)
Potatoes	0.07
Peanut butter	0.10

LOD = limit of detection; ND = not detected; PFOA = perfluorooctanoic acid

Source: Schecter et al. 2010

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Lake trout (Lake Superior)	1.1	4.8	<0.01–0.43	1.0	0.72	0.90	<0.02–0.87		0.37	0.25	Furdui et al. 2007
Lake trout (Lake Superior)	<0.42	2.3	<0.10	0.70	0.39	1.1			0.97		DeSilva et al. 2011
Lake trout (Lake Michigan)	4.4	16	<0.01–0.87	0.57	0.76	0.74	<0.02–0.97		0.41	0.99	Furdui et al. 2007
Lake trout (Lake Huron)	1.6	39	<0.01–6.2	2.8	2.2	2.7	<0.02–1.43		0.88	1.6	Furdui et al. 2007
Lake trout (Lake Huron)	<0.42	17	<0.10	1.4	1.3	1.8	NE		0.74		DeSilva et al. 2011
Lake trout (Lake Erie)	1.6	121	<0.01–1.2	2.9	4.9	3.5	<0.02–0.71		0.97	2.1	Furdui et al. 2007
Lake trout (Eastern Lake Erie)	<0.42	96	1.4	2.6	6.1	5.7	NE		2.0		DeSilva et al. 2011
Walleye (Western Lake Erie)	0.50	1.1	<0.10	1.2	3.6	3.1	NE		1.1		DeSilva et al. 2011
Lake trout (Lake Ontario)	1.5	46	0.65	1.1	1.8	1.6	<0.02–1.39		0.70	0.82	Furdui et al. 2007
Lake trout (Lake Ontario)	0.88	2.5	0.70	0.90	1.4	2.	0.64		0.32		DeSilva et al. 2011
Mixture of whole fish (Missouri River)	<1.00	84.7	1.89	0.43	0.25	<1.00	1.53	<0.40	0.49		Ye et al. 2008
Mixture of whole fish (Mississippi River)	<0.20	83.1	0.42	0.78	1.24	3.38	0.27	<0.20	<0.40		Ye et al. 2008
Mixture of whole fish (Ohio River)	<1.00	147	0.52	1.03	3.88	6.57	<4.00	<0.40	1.72		Ye et al. 2008
Smallmouth bass (Raisin River)	<2	2.0–41.3	<1							<1–4.1	Kannan e al. 2005

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Smallmouth bass (St Clair River)	<2	<2–2.7	<1							1.1–6.3	Kannan e al. 2005
Smallmouth bass (Calumet River)	<2	2.5–7.6	<1							<1	Kannan e al. 2005
Carp[a] (Saginaw Bay)	<36	124	<34							<19	Kannan e al. 2005
Carp[a] (Saginaw Bay)		120									Giesy and Kannan 2001
Lake whitefish[a] (Michigan waters)		130									Giesy and Kannan 2001
Chinook salmon[a] (Michigan waters)		110									Giesy and Kannan 2001
Brown trout[a] (Michigan waters)		<6–46									Giesy and Kannan 2001
Bluegill (St Croix River, Minnesota)		2.87			<LOQ	<LOQ			<LOQ		Delinsky et al. 2009
Bluegill (Lake Calhoun, Minnesota)		272			5.82	4.18			4.72		Delinsky et al. 2009
Bluegill (Haw River, North Carolina)		29.8			10.3	26.9			7.25		Delinsky et al. 2009
Mixture of fish fillet (Zumbrol Lake, Minnesota)		52.4			3.24						Delinsky et al. 2010
Mixture of fish fillet (McCarrons Lake, Minnesota)		47.3			1.97						Delinsky et al. 2010

Table 5-19. Detections of Perfluoroalkyls in Fish from U.S. Lakes and Rivers

Type of seafood (location)	Perfluoroalkyl concentration in ng/g										Reference
	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnA	PFHpA	PFBS	PFDoDA	FOSA	
Mixture of fish fillet (Pickerel Lake, Minnesota)		10.0			1.23						Delinsky et al. 2010
Mixture of fish fillet (Carlos Lake, Minnesota)		12.3									Delinsky et al. 2010
Mixture of fish fillet (other Minnesota lakes)		1.08–5.13									Delinsky et al. 2010
Mixture of fish fillet (Mississippi River sites, Minnesota)		3.06–20.00	0.47		2.94–15.0	2.13–6.72			3.74–4.42		Delinsky et al. 2010

[a]Perfluoroalkyl levels measured in muscle.

Grayed cells indicate that study did not evaluate compound; FOSA = perfluorooctane sulfonamide; LOQ = limit of quantification; NE = not evaluated; PFBS = perfluorobutane sulfonic acid; PFDoDA = perfluorododecanoic acid; PFDA = perfluorodecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOS = perfluorooctane sulfonic acid; PFUnA = perfluoroundecanoic acid

Concentrations of perfluoroalkyls have been reported in foods sampled in Canada, the United Kingdom, and Germany (Food Standards Agency 2006; Fromme et al. 2007b; Tittlemier et al. 2007). Perfluoroalkyls were detected in only 9 out of 54 food composites collected during Canadian Total Diet studies from 1992 to 2004 (Tittlemier et al. 2007). PFOS was detected in beef steak, ground beef, luncheon meats, marine fish, freshwater fish, and microwave popcorn at concentrations ranging from 0.98 to 2.7 ng/g, wet weight. PFOA was detected in roast beef, pizza, and microwave popcorn at 0.74–3.6 ng/g, wet weight. PFHpA was detected in pizza and microwave popcorn at 1.5–2.0 ng/g, wet weight. PFNA was detected only in beef steak at 4.5 ng/g, wet weight. PFDA, PFUnA, and PFDoDA were analyzed for but not detected in any of the food composites. During the U.K. Food Standards Agency Total Diet Study, PFOS was detected in eggs, sugars and preserves, potatoes, and canned vegetables at 1, 1, 10, and 2 μg/kg, respectively (Food Standards Agency 2006). PFOA was detected only in potatoes at 1 μg/kg. Neither substance was detected in the bread, miscellaneous cereals, carcass meats, offal, meat products, poultry, fish, oils and fats, green vegetables, other vegetables, fresh fruit, fruit products, beverages, milk, dairy products, or nuts categories. Fromme et al. (2007b) detected PFOS, PFOA, and PFHxS in 33, 45, and 3%, respectively, of 214 daily duplicate food portions for 31 adults in the city of Munich, Germany. Concentrations were 0.025–1.03 ng/g fresh weight for PFOS, 0.025–118.29 ng/g fresh weight for PFOA, and 0.05–3.03 ng/g fresh weight for PFHxS. Reported 90th percentile values were 0.11 and 0.21 ng/g fresh weight for PFOS and PFOA, respectively (Fromme et al. 2007b).

The temporal trend of perfluoroalkyl residues in eggs, milk, and farmed rainbow trout from Sweden were studied from 1999 to 2010 (Johansson et al. 2014). Over this period, the mean annual decreases in levels of PFOS were 18 and 31% in rainbow trout and eggs, respectively. The mean annual decreases of PFOA and PFHxS were 12 and 11%, respectively, in eggs. The detection frequency of PFOA and PFHxS was too low in milk samples to assess changes in levels over the time period, and decreases in the levels of PFOS were found to be not statistically significant over the temporal period. The mean annual decrease in levels of PFHxS in rainbow trout was 4.3% annually.

Elevated levels of PFOS were measured in water and fish samples obtained from 2009 and 2012 at six sampling locations along the Welland River and Lake Niapenco in Ontario, Canada (Gewurtz et al. 2014). These locations were downstream from the Hamilton International airport where PFOS containing AFFF was used until the mid-1990s at a firefighting training facility at the airport. PFOS concentrations were generally highest in benthic feeding fish collected at the sampling locations nearest to the airport. The maximum level of PFOS was observed in common carp collected at a site near the airport at a concentration of 2,300 ng/g. Maximum levels of PFOS in smallmouth bass, largemouth bass, and

channel catfish were 640, 450, and 430 ng/g, respectively. Freshwater fish and seafood obtained from China had detectable levels of nine perfluoroalkyls, with PFOS being detected in 62% of the samples at a concentration range of <0.10–26.2 ng/g and PFOA detected in 70% of the samples at levels of <0.10–1.99 ng/g (Zhang et al. 2011).

PFOA was detected in the packaging paper of two microwave popcorn bags at 0.3–4.7 ng/cm^2 uncooked and 0.5–4.3 ng/cm^2 cooked (Sinclair et al. 2007). The mean mass of PFOA in the gas phase of popcorn vapors following popping was 16–17 ng/cm^2. PFHpA, PFNA, PFDA, PFUnA, and PFDoDA were detected in one of the bags at 0.4–3.2 ng/cm^2 uncooked and 0.5–4.3 ng/cm^2 cooked; however, these perfluoroalkyls were not detected (<0.2 ng/cm^2) in the second bag. Begley et al. (2005) measured PFOA concentrations of 6–290 µg/kg in microwave popcorn bags. These authors also tested a hamburger wrapper, sandwich wrapper, French fry box, and soak-proof paper plates and did not find PFOA above the detection limit in these products. The concentration of PFOA measured in undiluted perfluoro paper coating formulations ranged from 88,000 to 160,000 µg/kg (Begley et al. 2005).

A study of perfluorinated and polyfluorinated substances in food packaging from U.S. fast food restaurants found perfluoroalkyl carboxylates (such as PFOA and PFHxA), perfluoroalkyl sulfonates (such as PFBS), fluorotelomer sulfonates, and unknown polyfluorinated compounds (Schaider et al. 2017). PFOA was detected in 6 of the 20 samples collected in 2014–2015. Fluorotelomer alcohols that can degrade or metabolize to perfluoroalkyl carboxylic acids such as PFOA continue to be detected in food packaging materials. Yuan et al. (2016) analyzed 69 food contact materials (paper tableware, paper cups, cupcake cups, paper boxes, paper bags, and microwave popcorn bags) produced in China and 25 materials (paper tableware, microwave popcorn bags, and paper cups) produced in the United States. The median concentration of total fluorotelomer alcohols in food contact materials produced in China (sum of 6:2, 8:2, 10:2, 12:2, 14:2, 16:2, and 18:2 fluorotelomer alcohol) ranged from 2 to 18,200 ng/g, with the highest levels observed in microwave popcorn bags. The detection frequencies of 6:2, 8:2, 10:2, 12:2, 14:2, 16:2, and 18:2 fluorotelomer alcohol in all food contact materials were reported as 38, 65, 77, 70, 58, 35, and 30%, respectively. The only fluorotelomer detected in paper tableware produced in the United States was 6:2 fluorotelomer alcohol, with a detection frequency of 11%; however, all fluorotelomer alcohols, with the exception of 18:2 fluorotelomer alcohol, were detected in microwave popcorn bags from the United States, although the levels were much lower than those produced in China. Table 5-20 shows the median concentration of the fluorotelomer alcohols in microwave popcorn bags produced in the United States versus those produced in China. In contrast to the products produced in China, the predominant fluorotelomer alcohol currently detected in food contact materials produced in the

United States appears to be 6:2 fluorotelomer alcohol. The authors concluded that the results of the PFOA Stewardship Program had effectively reduced the usage of long-chain fluorotelomer alcohols in the United States; however, they were still widely present in products produced in China.

Table 5-20. Fluorotelomer Alcohols Detected in Microwaveable Popcorn Bags Produced in China and the United States

Fluorotelomer alcohol	Median concentration of FTOH in microwave popcorn bag produced in China (ng/g)	Median concentration of FTOH in microwave popcorn bag produced in the United States (ng/g)
6:2 FTOH	80	485
8:2 FTOH	4,810	1.36
10:2 FTOH	6,700	0.73
12:2 FTOH	5,650	0.55
14:2 FTOH	384	0.12
16:2 FTOH	61	<MQL
18:2 FTOH	7.5	Not detected

FTOH = fluorotelomer alcohol; MQL = method quantitation limit

Source: Yuan et al. 2016

Washburn et al. (2005) measured the concentration of the perfluorooctanoate anion in fluorotelomer-treated consumer articles as well as the fluorotelomer formulations used for the treatments. PFOA was detected in mill-treated carpeting (0.2–0.6 mg/kg), carpet-care solution-treated carpeting (0.2–2 mg/kg), treated apparel (<0.02–1.4 mg/kg), treated home textiles (<0.02–1.4 mg/kg), industrial floor waxes and wax removers (0.0005–0.06 mg/kg), latex paint (0.02–0.08 mg/kg), and home and office cleaners (0.005–0.05 mg/kg). The concentrations of PFOA measured in the formulations used for these applications were 30–80, 1–50, <1–40, <1–40, 5–120, 50–150, and 50–150 mg/L, respectively. PFOA was not detected in treated upholstery (<0.034 mg/kg), treated technical textiles (<0.034 mg/kg), treated nonwoven medical garments (<0.034 mg/kg), or stone, tile, and wood sealants (<0.1 mg/kg).

Liu et al. (2014) measured levels of perfluoroalkyls in 35 consumer products that are typically used indoors, such as treated home textiles, food contact paper, carpet care products, and floor waxes. All products were obtained from retail stores in the United States between March 2007 and September 2011. The general trend was that these products contained decreasing quantities of perfluorocarboxylic acids, including PFOA, over the temporal period studied; however, there was an increase in use of PFBS, presumably as a replacement of PFOS in consumer products. Levels of perfluoroalkyls were analyzed in 115 random samples of consumer products obtained in Germany in 2010, including textiles, carpets,

cleaning and impregnating agents, leathers, food contact materials, baking and sandwich paper, and ski waxes (Kotthoff et al. 2015). Cleaning agents and some baking and sandwich papers had nondetectable or negligible amounts of perfluoroalkyls; however, PFOA and PFOS were frequently detected in outdoor textiles, ski wax, leather products, gloves, awning cloths, nanosprays, impregnation sprays, and food contact materials. Cleaning agents, nanosprays, and impregnation sprays tended to have the highest level of fluorotelomer alcohols. EPA (2009b) analyzed 116 articles of commerce obtained from retail outlets in the United States from March 2007 and May 2008 for the presence of perfluorocarboxylic acids ranging in carbon number from C5 (perfluoropentanoic acid) to C12 (perfluorododecanoic acid). Total C5–C12 perfluorocarboxylic acid levels ranged from below the detection limit (1.5 ng/g) to 47,100 ng/g, with levels of PFOA ranging from below the detection limit to 6,750 ng/g.

PTFE is a fluoropolymer used in applications such as nonstick cookware coatings and plumbing sealant tape. In the past, PFOA had been used as a processing aid in the emulsion polymerization of PTFE (DuPont 2008). PFOA was largely removed from the fluoropolymer material during the baking and curing step of nonstick cookware coatings in a high temperature oven; however, residual PFOA could be found in the final coatings (DuPont 2008). Begley et al. (2005) measured PFOA concentrations of 4–75 μg/kg in PTFE cookware, 3 μg/kg in PTFE-based dental floss, 4 μg/kg in PTFE-based dental tape, and 1,800 μg/kg in PTFE film/sealant tape. PFOA was not detected in tubing made of a fluoro-ethylene-propene copolymer (Begley et al. 2005).

Studies have been conducted that investigated the release of PFOA from PTFE cookware when heated. Sinclair et al. (2007) reported PFOA release concentrations ranging from 19 to 287 pg/cm^2 measured using four new nonstick frying pans. These concentrations were measured at normal cooking temperatures within the range of 180–229°C. PFOA was detected in water (7 and 75 ng) boiled for 10 minutes in two out of five non-stick pans (Sinclair et al. 2007). PFOA was not found above the detection limit (0.1 ng/cm^2) during 40 extraction tests on PTFE cookware using an ethanol/water mixture (Washburn et al. 2005). Likewise, Powley et al. (2005) conducted extraction texts on commercial fluoropolymer-treated cookware using water and water/ethanol mixtures at 100 and 125°C. Under simulated cooking conditions, PFOA was not identified above the detection limit of 100 pg/cm^2. Begley et al. (2005) reported that additional PFOA was not generated in the PTFE coating of three empty pans heated to 320°C (DuPont 2008). According to DuPont, the non-stick coating on a pan may begin to deteriorate if the pan is accidentally heated above 348°C, which is well above the maximum recommended cooking temperature of 260°C (DuPont 2008). Although it is possible for an unattended

empty pan to reach these high temperatures, overheating non-stick cookware is expected to be prevented in most cases because food oils begin to generate smoke around 190°C (Begley et al. 2005).

A comprehensive study that examined 116 articles of commerce (AOC) found perfluorocarboxylic acids, including PFOA, in many commercially available substances, such as carpet care products and waxes (EPA 2009b). Levels of PFOA ranged from nondetectable to 6,750 ng/g, and levels of total perfluorocarboxylic acids (the sum of C5–C12 acids) ranged from nondetectable to 47,100 ng/g. Perfluoroalkyls, including PFOA, have been detected at low levels in personal care products such as cosmetics and sunscreens (Fujii et al. 2013).

5.6 GENERAL POPULATION EXPOSURE

Levels of perfluoroalkyls have been measured in indoor air, outdoor air, dust, food, surface water, and various consumer products. Possible exposure pathways have been proposed; however, the relative importance of these pathways, including their association with the accumulation of perfluoroalkyls in blood, remains unclear (Apelberg et al. 2007b; Begley et al. 2005; Calafat et al. 2006b; Trudel et al. 2008; Washburn et al. 2005). For populations that have elevated levels of perfluoroalkyls in water supplies, the primary route of exposure is expected to be ingestion of contaminated drinking water.

Trudel et al. (2008) provides a thorough analysis of general population exposure to PFOS and PFOA based on the available information and proposes the following possible exposure pathways: food and water consumption, ingestion of house dust, hand-to-mouth transfer from treated carpets, migration into food from PFOA-containing paper or cardboard, inhalation of indoor and ambient air, and inhalation of impregnation spray aerosols. Other pathways proposed to be less significant included oral exposure from hand-to-mouth contact with clothes and upholstery, migration into food prepared with PTFE-coated cookware, dermal exposure from wearing treated clothes, deposition of spray droplets on skin while using impregnation sprays, skin contact with treated carpet and upholstery, and deposition of dust onto skin (Trudel et al. 2008). The strong correlation between PFOA and PFOS concentrations in human serum samples indicates that common exposure pathways for these two substances are possible (Calafat et al. 2007a).

In order to estimate human uptake and the major pathways for human exposure to PFOS and PFOA, reported levels of these compounds in various environmental media, including food and consumer products, were analyzed with respect to product use patterns, personal activity patterns, and personal

intake rates (Trudel et al. 2008). For PFOS, the major exposure pathways in a high-exposure scenario were proposed to be food and water ingestion, dust ingestion, and hand-to-mouth transfer from mill-treated carpets. Relative contributions of these pathways to the total uptake of PFOS in adults were estimated to be approximately 80, 15, and 5%, respectively (Trudel et al. 2008). For PFOA, the major exposure pathways in a high-exposure scenario were proposed to be oral exposure resulting from migration from paper packaging and wrapping into food, general food and water ingestion, inhalation from impregnated clothes, and dust ingestion. Relative contributions of these pathways to the total uptake of PFOA in adults were estimated to be approximately 60, 15, 15, and 10%, respectively (Trudel et al. 2008). Major exposure pathways for the intermediate and low exposure scenarios were proposed to be through food and drinking water (PFOA and PFOS) and ingestion of house dust (PFOA only). Based on these proposed exposure pathways, adult uptake doses estimated for low, medium, and high exposure scenarios were approximately 7, 15, and 30 ng/kg body weight/day, respectively, for PFOS and approximately 0.4, 2.5, and 41–47 ng/kg body weight/day, respectively, for PFOA (Trudel et al. 2008). The estimated uptake values were similar for men and women. Trudel et al. (2008) used older monitoring data and the estimated intakes may not be reflective of current exposure since there has been a downward trend in PFOA and PFOS exposure.

Fromme et al. (2009) assessed human exposure to perfluoroalkyls for adults in the general population of western countries. Based on measurements of indoor and outdoor air, house dust, drinking water, and dietary PFOS and PFOA levels, the investigators estimated average daily exposure levels of 1.6 ng/kg body weight/day for PFOS and 2.9 ng/kg body weight/day for PFOA. Upper daily exposure levels were determined to be 8.8 ng/kg body weight/day for PFOS and 12.6 ng/kg body weight/day for PFOA. The investigators concluded that the oral route, especially diet, was the primary route of exposure to perfluoroalkyls (Fromme et al. 2007a, 2007b, 2009).

As a group of compounds, perfluoroalkyls appear to be ubiquitous in human blood based on the widespread detection of these substances in human serum samples (Byrne et al. 2017; Calafat et al. 2006b, 2007a, 2007b; De Silva and Mabury 2006; Kuklenyik et al. 2004; Olsen et al. 2003b, 2003c, 2004b, 2004c, 2005, 2007a). Tables 5-21 and 5-22 list concentrations of perfluoroalkyls measured in serum samples collected from a representative sample of the general population in the United States. Most studies have reported that PFOA and PFOS levels have been detected in over 90% of subjects (Calafat et al. 2006b, 2007a, 2007b; Olsen et al. 2003b, 2007b, 2004c, 2005, 2008). PFHxS, PFNA, and PFDA are also typically detected in over 90% of the subjects (Calafat et al. 2006b, 2007a, 2017a, 2007b; Kuklenyik et al. 2004; Olsen et al. 2017a, 2017b).

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
U.S. Residents—NHANES			
1999–2000 (n=1,562)			Calafat et al. 2007a
Percent >LOD	100%	100%	
LOD	0.1	0.2	
Geometric mean	5.2	30.4	
95th percentile	11.9	75.6	
2003–2004 (n=2,094)			Calafat et al. 2007b
Percent >LOD	99.7%	99.9%	
LOD	0.1	0.4	
Geometric mean	3.95	20.7	
95th percentile	9.80	54.6	
2005–2006 (n=2,120)			
Percent >LOD	NR	NR	CDC 2018
LOD	0.1	0.2	
Geometric mean	3.92	17.1	
95th percentile	11.3	47.5	
2007–2008 (n=2,100)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	4.12	13.2	
95th percentile	9.60	40.5	
2009–2010 (n=2,233)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.07	9.32	
95th percentile	7.50	32.0	
2011–2012 (n=1,904)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	2.08	6.31	
95th percentile	5.68	21.7	
2013–2014 (n=2,165)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.94	4.99	
95th percentile	5.57	18.5	

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
2015–2016 (n=1,993)			CDC 2019
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.56	4.72	
95th percentile	4.17	18.3	
U.S. blood donors			
2000–2001 (n=645)			Olsen et al. 2003b
Percent >LLOQ[b]	92%	99.8%	
Geometric mean	4.6	34.9	
95th percentile[c]	12.1	88.5	
Maximum	52.3	1,656.0	
2006 (n=600)			Olsen et al. 2008, 2017b
Geometric mean	3.44	14.5	
95th percentile	7.9	31.5	
2010 (n=600)			Olsen et al. 2017b
Geometric mean	2.44	8.3	
95th percentile	5.6	21.8	
2015 (n=616)			Olsen et al. 2017b
Geometric mean	1.09	4.3	
95th percentile	3.2	8.6	
U.S. Regional			
Minneapolis-St. Paul blood donors (plasma)			
2005 (n=40)			Olsen et al. 2007b
Percent >LLOQ	95%	100%	
LLOQ	NR	3.4	
Geometric mean	2.2	15.1	
75th percentile	3.5	20.2	
Maximum	4.7	36.9	
Atlanta, Georgia			
2003 (n=20)			Kuklenyik et al. 2004
Percent >LOD	100%	100%	
LOD	0.1	0.4	
Mean	4.9	55.8	
Minimum	0.2	3.6	
Maximum	10.4	164.0	

Table 5-21. Concentrations of PFOA and PFOS in Human Serum Collected in the United States

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Seattle, Washington elderly individuals			
No date reported (n=238)			Olsen et al. 2004c
Percent >LLOQ[b]	99.2%	99.5%	
Geometric mean	4.2	31.0	
95th percentile[b]	9.7	84.1	
Maximum	16.7	175.0	
Washington County, Maryland			
1974 (n=178)			Olsen et al. 2005
Percent >LLOQ	71%	100%	
LLOQ	1.9	3.9	
Geometric mean	2.1	30.1	
75th percentile	3.0	40.2	
1989 (n=178)			Olsen et al. 2005
Percent >LLOQ	99%[o]	100%[p]	
Geometric mean	5.5	33.3	
LLOQ	1.9	3.9	
75th percentile	6.7	44.0	
Pease Tradeport, Portsmouth, New Hampshire			
2015–2016 (n=1,578)			NH HHS 2016
Percent >LLOQ	99.2	99.8	
LOD	0.1	0.1	
Geometric mean	8.59	3.09	
95th percentile	8.28–8.91	2.99–3.19	
St. Lawrence Island, Alaska			
2013–2014 (n=85)			Byrne et al. 2017
Percent >LOD	92	99	
50th percentile	1.01	4.55	
95th percentile	2.14	12.32	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the limit of detection or lower limit of quantification, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Experimental LLOQs not determined.
[c]Reported as bias-corrected estimates.
[d]LLOQ, LOQ, or LOD not reported.

CI = confidence interval; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
U.S. Residents NHANES								
1999–2000 (n=1,562) (Calafat et al. 2007a)								
Percent >LOD	10%	95%	25%	12%	<1%		100%	100%
LOD	0.4	0.1	0.2	0.2	0.2		0.1	0.05
Geometric mean	<0.4	0.5	<0.2	<0.2	<0.2		2.1	0.4
95th percentile	NR	1.7	0.5	NR	NR		8.7	1.4
2003–2004 (n=2,094) (Calafat et al. 2007b)								
Percent >LOD	6.2%	98.8%	31.3%	9.7%	<0.1%	<0.4%	98.3%	22.2%
LOD	0.3	0.1	0.3	0.3	1.0	0.4	0.3	0.2
Geometric mean	<0.3	1.0	<0.3	<0.3	<1.0	<0.4	1.9	<0.2
95th percentile	0.4	3.2	0.8	0.6	<1.0	<0.4	8.3	0.2
2005–2006 (n=2,120) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.4	0.1	0.2	0.2	0.2	0.1	0.1	0.1
Geometric mean	—	1.09	0.355	—	—	—	1.67	—
95th percentile	0.700	3.60	1.50	0.700	<LOD	0.1	8.30	0.300
2007–2008 (n=2,100) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.4	0.082	0.2	0.2	0.2	0.1	0.1	0.1
Geometric mean	—	1.22	0.286	—	—	—	1.95	—
95th percentile	0.500	3.28	0.900	0.600	<LOD	<LOD	9.80	<LOD
2009–2010 (n=2,233) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.082	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	1.26	0.279	0.172	—	—	1.66	—
95th percentile	0.200	3.77	0.900	0.900	<LOD	<LOD	6.90	<LOD
2011–2012 (n=1,904) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	0.881	0.199	—	—	—	1.28	—
95th percentile	0.220	2.54	0.690	0.620	0.140	<LOD	5.44	<LOD

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
2013–2014(n=2,168) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	—	0.675	0.185	—	—	—	1.35	NR
95th percentile	0.200	2.00	0.700	0.500	0.200	<LOD	5.60	NR
2015–2016(n=1,993) (CDC 2019)								
Percent >LOD		NR	NR	NR	NR		NR	
LOD		0.1	0.1	0.1	0.1		0.1	
Geometric mean		0.577	0.154	—	—		1.18	
95th percentile		1.90	0.700	0.400	<LOD		4.90	
U.S. blood donors								
2000–2001 (n=645) (Olsen et al. 2003b, 2017a, 2017b)								
Percent >LLOQ[c]	62%	100%	97.7%	93.8%	22.6%		52%	2%
Geometric mean	0.13	0.57	0.16	0.10	NR		1.9	NR
95th percentile[b]	0.5	1.4	0.4	0.3	0.04		9.5	NR
2006 (n=600) (Olsen et al. 2008, 2017a, 2017b)								
Percent>LLOQ	62%	100%	99.8%	99.8%	68%	1.2%	95.7%	
LLOQ[d]	<0.5	NR	<0.05	<0.05	<0.05	<0.5	<0.5	
Geometric mean	0.09	0.97	0.34	0.18	0.04	LLOQ	1.52	
95th percentile	0.4	2.2	0.8	0.5	0.07	LLOQ	5.7	
2010 (n=600) (Olsen et al. 2017a, 2017b)								
Percent>LLOQ	79.7%	100%	100%	99.8%	46.2%	22.5%	95.5%	
LLOQ[d]	<0.05	NR	NR	<0.025	<0.025	<1.0	<0.05	
Geometric mean	0.05	0.83	0.27	0.14	0.03	LLOQ	1.34	
95th percentile	0.2	2.3	0.8	0.5	0.06	0.3	5.3	
2015 (n=616) (Olsen et al. 2017a, 2017b)								
Percent>LLOQ	3.3%	100%	96.9%	47.9%	0.6%	8.4%	99.7%	0%
LLOQ[d]	<0.09	<0.093	<0.093	<0.466	<0.932	<0.047	<0.04	<0.093
Geometric mean	NR	0.43	0.15	NR	NR	LLOQ	0.87	LLOQ
95th percentile	0.16	1.1	0.49	0.25	LLOQ	0.02	3.5	LLOQ

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
U.S. Regional								
Midwestern United States (De Silva and Mabury 2006)								
2004–2005 (n=16)								
Percent detected[e]		100%	100%	13%	0%			
Mean		0.77	0.17	NR	NA			
Maximum		1.2	0.25	0.067	NA			
Atlanta, Georgia (Kuklenyik et al. 2004)								
2003 (n=20)								
Percent >LOD	10%	100%	75%	85%	10%		100%	75%
LOD	0.3	0.1	0.3	0.3	1		0.3	0.2
Mean[b]	<0.3	2.6	0.7	0.8	<1		3.9	0.34
Maximum	8.5	3.9	1.2	1.4	1.6		11.2	0.7
Seattle, Washington; elderly population (Olsen et al. 2004c)								
No date reported (n=238)								
Percent >LLOQ[c]							76%	"Few"
Geometric mean							2.2	NR
95th percentile[b]							8.3	NR
Maximum							40.3	NR
Washington County, Maryland (Olsen et al. 2005)								
1974 (n=178)								
Percent >LLOQ							63%	
LLOQ								
Geometric mean							1.5	
75th percentile							2.5	
1989 (n=178)								
Percent >LLOQ							82%	
LLOQ							1.4	
Geometric mean							2.5	
75th percentile							1.6	

Table 5-22. Concentrations of Other Perfluoroalkyls in Human Serum Collected in the United States

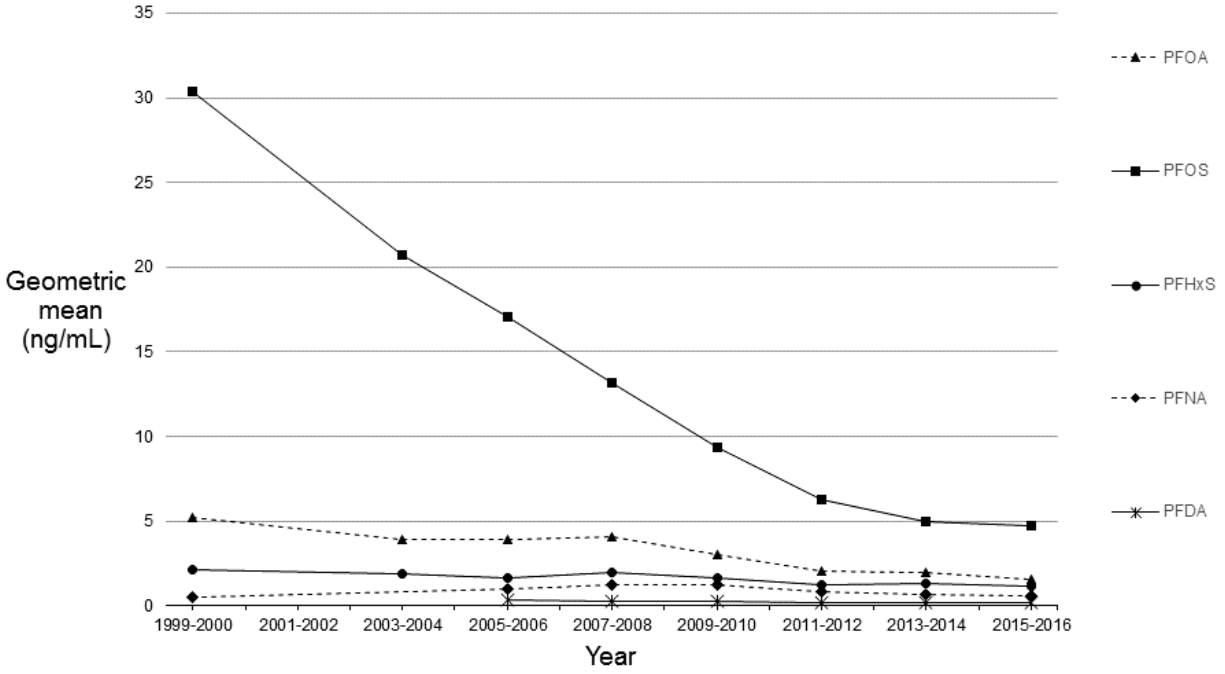
	Detection and concentration (ng/mL [ppb])[a]							
Sample population	PFHpA	PFNA	PFDA	PFUnA	PDoDA	PFBS	PFHxS	FOSA
Pease Tradeport, Portsmouth, New Hampshire (NH HHS 2016)								
2015–2016 (n=1,578)								
Percent >LOD	0.9	85.2	42.1	30.0	4.7	20.0	94.2	2.0
LOD	NA	0.1	0.1	0.1	NA	NA	0.1	0.1
Geometric mean	0.07	0.73	0.22	0.19	0.08	0.04	4.12	0.13
95th percentile	0.07	0.70–0.75	0.21–0.23	0.18–0.19	0.08	0.04	3.92–4.33	0.12–0.14
St. Lawrence Island, Alaska (Byrne et al. 2017)								
2013–2014 (n=85)								
Percent >LOD		99	39	72			32	
50th percentile		2.21	<LOD	0.72			<LOD	
95th percentile		7.35	1.06	1.72			2.74	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Reported as bias-corrected estimates.
[c]Experimental LLOQs not determined.
[d]Highest LLOQ listed.
[e]LOD not reported.

Grayed cells indicate that study did not evaluate compound; "—" indicates no available data;
FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; NA = not applicable; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

As illustrated in Figure 5-3, there has been a clear trend in decreasing serum levels of both PFOS and PFOA in the general population of the United States since 2000 as these substances were phased out; from 1999–2000 to 2015–2015 (CDC 2018, 2019), the geometric mean serum PFOS and PFOA levels in the general population have declined approximately 84 and 70%, respectively. Serum concentrations for PFHxS and PFDA (Figure 5-3) have also been declining over time, whereas serum PFNA concentrations increased from 1999–2000 to 2009–2010 and then started to decrease (Figure 5-3).

Figure 5-3. Geometric Mean Concentrations of PFOA, PFOS, PFHxS, PFNA, and PFDA in U.S. Residents from 1999 to 2016



NHANES = National Health and Nutrition Examination Survey; PFDA = perfluorodecanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: CDC 2018

Serial studies of American Red Cross blood donors also allow for temporal evaluations of serum perfluoroalkyl levels. Olsen et al. (2008) reported a nearly 60% decline in PFOS blood levels when comparing data from 2001 to 2006; from 2006 to 2015, the serum PFOS levels dropped another 70% (Olsen et al. 2017b). From 2000 to 2015, the PFOS levels dropped 88%. Serum PFOA levels among the blood donors decreased 76% from 2000 to 2015 (Olsen et al. 2003b, 2017b). Geometric mean serum PFHxS levels went from 1.9 ng/mL in 2000 to 0.87 ng/mL in 2015, a 54% decrease (Olsen et al. 2003b, 2017a, 2017b). From 2006 to 2015, both serum PFNA and PFDA levels decreased by 56% (Olsen et al. 2017a, 2017b).

Several studies have evaluated sex- and age-related differences in serum perfluoroalkyl levels in the general population. In an analysis of NHANES data from 1999 to 2008, Kato et al. (2011) found that males had significantly higher levels of PFOA, PFOS, and PFNA than females and that PFOS levels increased with age, especially in females. Fu et al. (2014b) analyzed the effects of sex and age on levels of perfluoroalkyls in a study of 133 (79 male, 54 female) participants. In general, higher levels of PFOA,

PFOS, PFNA, and PFDA were observed in male subjects; however, differences were only statistically significant for PFOA and PFDA. Sex differences in other perfluoroalkyls were not observed. For both male and female subjects, increasing levels of PFOA, PFNA, and PFOS were positively correlated with increasing age. Age- and sex-specific differences have been incorporated into a regression model developed by Jain (2015), which uses measured serum PFOA and PFOS levels to predict total serum perfluoroalkyl levels.

A small number of studies have measured serum perfluoroalkyl levels in U.S. children (Tables 5-23 and 5-24). NHANES has included adolescents (ages 12–19 years) in the 1999–2000 through 2013–2014 surveys (Calafat et al. 2007a, 2007b; CDC 2018). Analysis of NHANES data from younger children were conducted for the 2001–2002 survey (ages 6–11 years) (Kato et al. 2009b) and 2013–2014 survey (ages 3–5 and 6–11 years) (CDC 2018). Pinney et al. (2014) measured serum PFOA, PFOS, PFHxS, PFNA, and PFDA levels in girls (ages 6–8 years) living in Cincinnati, Ohio and San Francisco, California; and Olsen et al. (2004b) measured PFOA, PFOS, PFHxS, and FOSA in the serum of children ages 2–12 years from various locations in the United States who were diagnosed with group A streptococcal infections. Serum levels of several perfluoroalkyls were examined in children exposed to the World Trade Center (WTC) disaster (n=123) and a sociodemographically-matched comparison group (n=185) (Trasande et al. 2017). Children exposed during the WTC disaster were identified from the WTC Health Registry (WTCHR). All participants were ≤8 years of age on September 11, 2001. Blood serum levels of PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnA were collected during 2014–2016. For all perfluoroalkyls, serum levels in WTCHR children were significantly ($p<0.01$) higher than in matched controls, with percentage increases above control as follows: PFOA 29%; PFOS 34%; PFHxS 26%; PFNA 24%; PFDA 27%; and 200% PFUnA.

Geometric mean serum perfluoroalkyl levels in adolescents (ages 12–19 years) included in NHANES are similar to geometric mean concentrations reported for adults (Calafat et al. 2007a, 2007b; CDC 2018). For example, geometric mean concentrations of PFOA and PFOS measured during the 1999–2000 and 2003–2004 NHANES surveys were 3.9–5.5 and 19.3–29.1 ng/mL, respectively, in adolescent serum and 3.9–5.2 and 20.7–30.4 ng/mL, respectively, in serum of the total population. The most recent NHANES survey (2013–2014) also included serum levels for children aged 3–5 and 6–11 years; the geometric mean concentrations in the younger children were similar to those in adolescents and the total population.

Olsen et al. (2004b) also found that the geometric mean serum PFOA, PFOS, PFHxS, and PFNA levels in older children (12–19 years of age) were similar to those measured in adults (Olsen et al. 2003b).

However, estimated 95th percentile values of PFHxS measured in children were noted to be higher than values estimated for adults. Olsen et al. (2004b) reported bias-corrected 95th percentile estimates of 65 ng/mL for PFHxS in the serum of children ages 2–12 years. This value is higher than bias-corrected 95th percentile estimates of 9.5 and 8.3 ng/mL based on PFHxS measurements in the serum of adult blood donors (Olsen et al. 2003b) and elderly individuals (Olsen et al. 2004c), respectively (see Table 5-22). The difference is less extreme in the NHANES data, with PFHxS 95th percentile values of 12.9–13.1 ng/mL reported for children compared to values of 8.3–8.7 ng/mL reported for the total population. Reasons for the observed differences of PFHxS levels in childhood serum samples compared to adult samples have not been determined. Olsen et al. (2004b) stated that different exposure and activity patterns between children and adults should be considered. For example, children may have a higher exposure than adults to PFHxS, a substance that has been used in carpet treatment applications, since they are lower to the ground and have increased contact with carpeted floors (Calafat et al. 2007a; Olsen et al. 2004b).

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
U.S. Adolescents—NHANES (ages 12–19)			
1999–2000 (n=543)			Calafat et al. 2007a
Percent >LOD	100%	100%	
LOD	0.1	0.2	
Geometric mean	5.5	29.1	
95th percentile	11.2	56.8	
2003–2004 (n=640)			Calafat et al. 2007b
Percent >LOD	99.7%[b]	99.9%[b]	
LOD	0.1	0.4	
Geometric mean	3.9	19.3	
95th percentile	8.6	42.2	
2005–2006 (n=640)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.59	15.0	
95th percentile	8.40	38.5	
2007–2009 (n=357)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	3.91	11.3	
95th percentile	7.30	28.0	

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
2009–2010 (n=364)			CDC 2013
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	2.74	6.84	
95th percentile	5.00	18.1	
2011–2012 (n=344)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.80	4.16	
95th percentile	3.59	10.8	
2013–2014 (n=401)			CDC 2018
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.66	3.54	
95th percentile	3.47	9.30	
2015–2016 (n=353)			CDC 2019
Percent >LOD	NR	NR	
LOD	0.1	0.2	
Geometric mean	1.25	2.94	
95th percentile	2.47	6.60	
U.S. Children—NHANES			
2001–2002; ages 6–11 (n=936)			Kato et al. 2009b
Least square mean	6.1–7.6	30.45–42.45	
2013–2014; ages 3–5 (n=181)			
Percent >LOD	100%	100%	Ye et al. 2018a; CDC 2018
LOD	0.1	0.1	
Geometric mean	2.00	3.38	
95th percentile	5.58	8.82	
2013–2014; ages 6–11 (n=458)			
Percent >LOD	100%	100%	Ye et al. 2018a; CDC 2018
LOD	0.1	0.1	
Geometric mean	1.89	4.15	
95th percentile	3.84	12.4	
U.S. Regional			
Girls ages 6–8; Cincinnati, Ohio (n=353)			Pinney et al. 2014
Percent >LOD[c]	99.7%	99.7%	
Geometric mean	7.8	13.2	
Median	7.3	13.6	

Table 5-23. Percent Detection and Levels of PFOA and PFOS in Children's Serum

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Girls ages 6–8; San Francisco, California (n=351)			Pinney et al. 2014
Percent >LOD[c]	100%	100%	
Geometric mean	5.7	13.2	
Median	5.8	12.5	
Children ages 6–10; Project Viva, Boston Massachusetts 2007–2010 (n=653)			Harris et al. 2017
Percent >LOD	99.5%	99.5%	
Geometric mean	4.2	6.2	
90th percentile	7.9	13.7	
Children ages 2–12; 23 states and District of Columbia 1994–1995 (n=598)			Olsen et al. 2004b
Percent >LLOQ[c]	96%	100%	
Geometric mean	4.9	37.5	
95th percentile[d]	10	89	
Children ages 1–19; West Virginia and Ohio 2005–2006 (n=4,943)			Mondal et al. 2012
Geometric mean	31.2	19.2	
90th percentile	201	36.8	
Children (DOB: 9/11/1993–9/10/2001); New York City, WTCHR 2014–2016 (n=123)			Trasande et al. 2017
Percent >LOD	100%	100%	
Median	1.81	3.72	
Children (DOB: 9/11/1993–9/10/2001); New York City, not eligible for WTCHR 2014–2016 (n=185)			Trasande et al. 2017
Percent >LOD	100%	100%	
Median	1.39	2.78	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Percent detection for the adolescent age group was not specified for the 2003–2004 NHANES samples. Percentages listed here are for the total sample population.
[c]LOD or LLOQ not reported.

"—" indicates no available data; DOB = date of birth; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; WTCHR = World Trade Center Health Registry

Table 5-24. Percent Detection and Levels of Other Perfluoroalkyls in Children's Serum

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
U.S. Adolescents NHANES (ages 12–19)								
1999–2000 (n=543) (Calafat et al. 2007a)								
Percent >LOD	10%[b]	96%	15%	12%[b]	<1%[b]		100%	100%
Geometric mean	—	0.5	<0.2	—	—		2.7	0.4
95th percentile	—	1.1	0.5	—	—		12.9	1.5
2003–2004 (n=640) (Calafat et al. 2007b)								
Percent >LOD	6.2%[b]	98.8%[b]	31.3%[b]	9.7%[b]	<0.1%[b]	<0.4%[b]	98.3%[a]	22.2%[b]
Geometric mean	<0.3	0.9	<0.3	<0.3	<1.0	<0.4	2.4	<0.2
95th percentile	0.5	2.7	0.7	<0.3	<1.0	<0.4	13.1	0.3
2005–2006 (n=640) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.929	0.295	—	—	—	2.09	—
95th percentile	1.10	2.70	0.800	0.500	<LOD	0.100	14.1	0.300
2007–2008 (n=357) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	1.16	0.231	—	—	—	2.40	—
95th percentile	0.600	2.54	0.800	0.300	<LOD	<LOD	15.9	0.300
2009–2010 (n=364) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	1.10	0.220	—	—	—	2.03	—
95th percentile	0.400	2.62	0.600	0.400	<LOD	<LOD	12.3	<LOD
2011–2012 (n=344) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.741	0.146	—	—	—	1.28	—
95th percentile	0.190	2.06	0.360	0.250	<LOD	<LOD	6.45	<LOD
2013–2014 (n=402) (CDC 2018)								
Percent >LOD	NR	NR	NR	NR	NR	NR	NR	NR
Geometric mean	—	0.599	0.136	—	—	—	1.27	—
95th percentile	0.200	2.00	0.400	0.200	0.200	<LOD	6.30	<LOD
2015–2016 (n=353) (CDC 2019)								
Percent >LOD		NR	NR	NR	NR		NR	
Geometric mean		0.481	—	—	—		0.918	
95th percentile		1.20	0.300	0.200	<LOD		3.10	
U.S. Children NHANES								
2001–2002; ages 6–11 (n=936) (Kato et al. 2009b)								
Least square mean		0.7-1.2						
2013–2014; ages 3–5 (n=181) (Ye et al. 2018a, 2018b; CDC 2018)								
Percent >LOD	27%	100%	50%	27%	0%	1%	100%	7%
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	<LOD	0.764	<LOD	<LOD	<LOD	<LOD	0.715	<LOD
95th percentile	0.310	3.49	0.370	0.370	<LOD	<LOD	1.62	0.110

Table 5-24. Percent Detection and Levels of Other Perfluoroalkyls in Children's Serum

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
2013–2014; ages 6–11 (n=458) (Ye et al. 2018a, 2018b; CDC 2018)								
Percent >LOD	15%	100%	46%	28%	0%	7%	100%	2%
LOD	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Geometric mean	<LOD	0.809	<LOD	<LOD	<LOD	<LOD	0.913	<LOD
95th percentile	0.170	3.19	0.350	0.250	<LOD	0.130	4.14	<LOD
U.S. Regional								
Girls ages 6–8; Cincinnati, Ohio (n=353) (Pinney et al. 2014)								
Percent >LOD[c]		99.9	75.8				99.7	19
Geometric mean		1.4	0.3				5.1	<LOD
Median		1.4	0.3				5.2	<LOD
Girls ages 6–8; San Francisco, California (n=351) (Pinney et al. 2014)								
Percent >LOD[c]		100	78.7				100	10
Geometric mean		1.7	0.3				3.0	<LOD
Median		1.6	0.3				2.3	<LOD
Children ages 6–10; Project Viva, Boston Massachusetts 2007–2010 (n=653) (Harris et al. 2017)								
Percent >LOD[c]		99.5%	88.2%				99.5%	0.9%
Geometric mean		1.7	0.3				2.2	<LOD
90th percentile		3.8	0.6				7.0	<LOD
Children ages 2–12; 23 states and District of Columbia 1994–1995 (n=598) (Olsen et al. 2004b)								
Percent >LLOQ							78%	14%
LLOQ							NR	1.0
Geometric mean							4.5	<2.0
95th percentile[b]							65	<2.0
Children (DOB: 9/11/1993–9/10/2001); New York City, WTCHR 2014–2016 (n=123) (Trasande et al. 2017)								
Percent >LOD	99.7[d]	75[d]	53[d]				100	
Median	0.61	0.14	0.12				0.67	
Children (DOB: 9/11/1993–9/10/2001); New York City, not eligible for WTCHR 2014-2015 (n=185) (Trasande et al. 2017)								
Percent >LOD	99.7[d]	75[d]	53[d]				100	
Median	0.49	0.11	0.04				0.53	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]Percent detection for the adolescent age group was not specified for these samples. Percentages listed here are for the total sample population.
[c]LOD or LLOQ not reported.
[d]%>LOD reported for combined WTCHR and non-WTCHR groups.

Gray cells indicate that chemical was not evaluated; "—" indicates no available data; DOB = date of birth; FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NC = not calculated; ND = no data; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

When estimating PFOS and PFOA uptake doses for children, Trudel et al. (2008) assumed the same exposure pathways for children as were proposed for adults, but considered exposure from hand-to-mouth transfer from treated carpets to be much larger in children. This pathway was estimated to contribute 40–60% of the total uptake of both PFOS and PFOA in infants (0–1 years), toddlers (1–4 years), and children (5–11 years) in the high-exposure scenario. Exposure via human breast milk was included in the food consumption pathway for infants. Exposure via mouthing of clothes, carpet, and upholstery was also considered for children <12 years old; however, this was considered to be a minor pathway of exposure. PFOS uptake doses estimated for the low-, medium-, and high-exposure scenarios were 18.1–219 ng/kg body weight/day for infants, 14.8–201 ng/kg body weight/day for toddlers, and 9.7–101 ng/kg body weight/day for children. PFOA uptake doses estimated for the low-, medium-, and high-exposure scenarios were 2.2–121 ng/kg body weight/day for infants, 1.2–128 ng/kg body weight/day for toddlers, and 0.8–65.2 ng/kg body weight/day for children. In contrast to the estimates for children under age 12, relative exposure pathways and uptake doses estimated for teenagers (12–20 years old) were approximately the same as for adults.

Perfluoroalkyls have been measured human breast milk and umbilical cord blood; reported concentrations are listed in Tables 5-25 and 5-26. Measurements of perfluoroalkyls in amniotic fluid, meconium, neonatal blood, or other tissues have not been located.

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a]		Reference
	PFOA	PFOS	
Umbilical cord blood			
San Francisco, California			Morello-Frosch et al. 2016
Percent >LOD[c]	56%	100%	
Geometric mean	–	2.27	
95th percentile	1.68	4.35	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a]		Reference
	PFOA	PFOS	
Baltimore THREE Study			Apelberg et al. 2007a, 2007b
Cord serum (n=299)			
Percent >LOD	100%	99%	
LOD	0.1–0.2	0.2	
Geometric mean	1.6	4.9	
Minimum	0.3	<0.2	
Maximum	7.1	34.8	
Maternal serum (n=293)			
Median	1.4–1.6	4.1–5.0	
Germany			Midasch et al. 2007
Cord plasma (n=11)			
Percent detected	100%	100%	
LOQ	0.5	0.5	
Median	3.4	7.3	
Maternal plasma (n=11)			
Percent detected	100%	100%	
LOQ	0.5	0.5	
Median	2.6	13.0	
Spain			Manzano-Salgado et al. 2015
Cord serum (n=66)			
Percent >LOD	100%	100%	
Minimum	0.60	0.53	
Maximum	10.56	4.71	
Maternal serum (n=53)			
Percent >LOD	100%	100%	
Minimum	0.86	1.17	
Maximum	14.54	23.14	
Danish National Birth Cohort			Fei et al. 2007
Cord blood (n=50)			
Percent >LLOQ	98%	100%	
LLOQ	1.0	1.0	
Mean	3.7	11.0	
Maternal blood (n=200)			
Percent >LLOQ	98%	100%	
LLOQ	1.0	1.0	
Mean	4.5	29.9	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Japan			Inoue et al. 2004
Cord serum (n=15)			
Percent >LOD	0%	100%	
LOD	0.5	0.5	
Range		1.6–5.3	
Maternal serum (n=15)			
Percent >LOD	20%	100%	
LOD	0.5	0.5	
Range	0.5–2.3	4.9–17.6	
Breast milk			
Massachusetts (n=45)			Tao et al. 2008b
Milk			
Percent >LOQ[b]	89%	96%	
Mean	0.0438	0.131	
Minimum	<0.0301	<0.032	
Maximum	0.161	0.617	
Sweden (n=12)			Kärrman et al. 2007
Milk			
Percent >LOD	8%[c]	100%	
LOD	0.01	0.005	
Mean	–	0.201	
Range	<0.209–0.492	0.060–0.470	
Maternal serum			
Percent >LOD	100%	100%	
LOD	0.01	0.005	
Mean	3.8	20.7	
Range	2.4–5.3	8.2–48.0	
Sweden (n=20)			Sundstrom et al. 2011
Milk			
LOD	0.005	0.005	
1997 Mean	0.138	0.237	
2007 Mean	0.086	0.122	
Norway (n=9)			Thomsen et al. 2010
Milk			
LOD	0.008	0.003	
Median	0.05	0.11	
Range	0.016–0.19	0.028–0.36	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
China (n=19)			So et al. 2006b
Percent >LOD	100%	100%	
LOD	0.021–0.027	0.001–0.0036	
Range	0.047–0.210	0.045–0.360	
Middle East, including Jordan (n=19)			Al-sheyab et al. 2015
Percent >LOQ	100%	94%	
LOQ	0.01	0.01	
Mean	0.14	0.035	
Range	0.024–1.22	0.006–0.18	
France (n=48)			Antignac et al. 2013
Percent >LOD	90%	98%	
LOD	0.05	0.05	
Mean	0.082	0.092	
Range	<LOD-0.22	<LOD-0.33	
France (n=61)			Cariou et al. 2015
Percent >LOD	77%	82%	
LOD	0.05	0.04	
Mean	0.041	0.04	
Range	<LOD-0.31	<LOD-0.376	
Belgium			Croes et al. 2012
Percent >LOD	100%	100%	
LOD	0.01	0.01	
Mean	0.08	0.13	
Italy (n=49)			Guerranti et al. 2013
Percent >LOD	2% (1 sample)	27%	
LOD	0.5[e]	0.5[d]	
Mean	8.04[e]	0.85[e]	
Italy			Barbarossa et al. 2013
Primiparous (n=21)			
Percent >LOQ	81%	90%	
LOQ	0.024	0.015	
Mean	0.076	0.057	
Range	<LOQ–-0.24	<LOQ–0.29	
Multiparous (n=16)			
Percent >LOQ	69%	62%	
LOQ	0.024	0.015	
Mean	0.043	0.036	
Range	<LOQ–0.1	<LOQ–0.12	

Table 5-25. Percent Detection and Levels of PFOA and PFOS in Umbilical Cord Blood and Breast Milk

Location	Detection and concentration (ng/mL [ppb])[a] PFOA	PFOS	Reference
Korea			Kang et al. 2016
Percent >LOD	98%	98%	
LOD	0.019	0.007	
Median	0.072	0.05	
Czech Republic (n=50)			Lankova et al. 2013
Percent >LOQ	100%	100%	
LOQ	0.006	0.005	
Mean	0.05	0.033	
Range	0.012–0.13	0.007–0.11	
Germany/Hungary (n=70)			Völkel et al. 2008
Percent >LOQ	16%	100%	
Minimum	<0.200	0.028	
Maximum	0.460	0.639	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]LOD or LLOQ not reported.
[c]Reported as bias-corrected estimates.
[d]ng/g wet weight.

"—" indicates no available data; DOB = date of birth; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; NR = not reported; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; WTCHR = World Trade Center Health Registry

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a] PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Umbilical cord blood								
San Francisco, California (Morello-Frosch et al. 2016) (n=65)								
Percent >LOD[c]	56%	97%	9%	84%	0%	25%		91%
Geometric mean	—	0.29	—	0.03	—	—		0.02
95th percentile	0.23	0.93	0.49	0.16	—	0.03		0.10
Baltimore THREE Study (Apelberg et al. 2007a, 2007b)								
Cord serum (n=299)								
Percent >LOD	2%		24%	34%	5%	3%		26%
LOD	0.4		0.2	0.2	0.2	0.1		0.05
Minimum	<0.4		<0.2	<0.2	<0.2	<0.1		<0.05
Maximum	2.6		1.1	1.9	1.7	0.2		0.8

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Japan (Inoue et al. 2004)								
Cord serum (n=15)								
Percent >LOD								0%
LOD								1.0
Maternal serum (n=15)								
Percent >LOD								0%
LOD								1.0
Spain (Manzano-Salgado et al. 2015)								
Cord blood (n=66)								
Percent >LOD		100%					88%	
Minimum		0.13					0.05	
Maximum		2.24					1.93	
Maternal serum (n=53)								
Percent >LOD		100%					96%	
Minimum		0.20					0.05	
Maximum		5.37					2.53	
Breast milk								
Massachusetts (n=45) (Tao et al. 2008b)								
Milk								
Percent >LOQ[b]	<1%	64%	<1%	<1%	<1%	<1%	51%	
Mean	NR	0.01726	NR	NR	NR	NR	0.0145	
Minimum	<0.010	<0.0052	<0.00772	<0.00499	<0.00440	<0.0100	<0.0120	
Maximum	0.0234	0.0184	0.0111	0.00884	0.00974	0.0198	63.8	
France (n=49) (Antignac et al. 2013)								
Percent >LOD	2%	2%					100%	
LOD	0.05	0.05					NR	
Mean	—	—					0.049	
Minimum	<LOD	<LOD					0.040	
Maximum	0.074	0.064					0.066	
France (n=61) (Cariou et al. 2015)								
Percent >LOD		0%					15%	
LOD		0.05					0.03	
Mean		0.014					0.026	
Minimum		<LOD					<LOD	
Maximum		<LOD					0.217	
Belgium (n=40) (Croes et al. 2012)								
Percent >LOD		42%					20%	
LOD		0.01					0.01	
Mean		—					—	

Table 5-26. Percent Detection and Levels of Other Perfluoroalkyls in Children's Umbilical Cord Blood and Breast Milk

Sample population	Detection and concentration (ng/mL [ppb])[a]							
	PFHpA	PFNA	PFDA	PFUnA	PFDoDA	PFBS	PFHxS	FOSA
Sweden (n=12) (Kärrman et al. 2007a)								
Milk								
Percent >LOD		17%	0%	0%			100%	67%
LOD		0.005	0.008	0.005			0.01	0.007
Mean		0.017	—	—			0.085	0.013
Range		<0.005–0.020	—	—			0.031–0.172	<0.007–0.030
Maternal serum								
Percent >LOD		100%	100%	100%			100%	75%
LOD		0.005	0.008	0.005			0.01	0.007
Mean		0.80	0.53	0.40			4.7	0.24
Range		0.43–2.5	0.27–1.8	0.20–1.5			1.8–11.8	0.16–0.19
China (n=19) (So et al. 2006b)								
Percent >LOD	37%	100%	100%	100%		11%	100%	
LOD	0.005–0.010	0.001–0.010	0.0011–0.0025	0.0022–0.0050		0.001–0.005	0.001–0.010	
Range	<0.005–0.0067	0.01–0.062	0.0038–0.011	0.0091–0.056		<0.001–0.0025	0.004–0.10	

[a]"Less than" values indicate that the concentration was reported as below the LOD or LLOQ. For cases where samples had concentrations below the LOD or LLOQ, a value between zero and the LOD or LLOQ was assigned when calculating the mean concentration.
[b]LOD or LLOQ not reported.

Gray cells indicate that chemical was not evaluated; "—" indicates no available data; DOB = date of birth; FOSA = perfluorooctane sulfonamide; LLOQ = lower limit of quantification; LOD = limit of detection; LOQ = limit of quantification; ND = no data; NR = not reported; PFBS = perfluorobutane sulfonic acid; PFDA = perfluorodecanoic acid; PFDoDA = perfluorododecanoic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFUnA = perfluoroundecanoic acid

Tao et al. (2008b) measured perfluoroalkyl concentrations in 45 human breast milk samples collected from Massachusetts. PFOS, PFOA, PFHxS, and PFNA were each detected in 96, 89, 51, and 64% of the samples, respectively, with median concentrations of 106, 36.1, 12.1, and 6.97 pg/mL, respectively. PFHpA, PFDA, PFUnA, PFDoDA, and PFBS were each detected in <1% of the samples. Perfluoroalkyls have also been measured in the human breast milk of individuals from Sweden, China, and Germany/ Hungary (Kärrman et al. 2007; So et al. 2006b; Völkel et al. 2008). PFOS was detected in all samples, while detection of PFOA ranged from 8 to 100% in these studies. The reported maximum concentrations of PFOS and PFOA measured in human breast milk samples collected during these studies were 0.360–0.639 and 0.210–0.490 ng/mL, respectively (Kärrman et al. 2007; So et al. 2006b; Völkel et al. 2008). Other perfluoroalkyls detected in human breast milk included PFHpA, PFNA, PFDA, PFUnA, PFBS,

PFHxS, and FOSA. Maximum concentrations of these compounds were reported to be <0.18 ng/mL (Kärrman et al. 2007).

The presence of perfluoroalkyls in umbilical cord blood indicates that these substances can cross the placental barrier resulting in the exposure of babies *in utero* (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007). In most studies, PFOS and PFOA have been detected in most umbilical cord blood samples with reported maximum (or 95th percentile) concentrations of 5.3–34.8 and 1.68–7.1 ng/mL, respectively (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Midasch et al. 2007; Morello-Frosch et al. 2016). Inoue et al. (2004) did not detect PFOA in 15 cord blood samples from Japan; however, this compound was only detected in the maternal serum of three mothers. Apelberg et al. (2007a) also reported concentrations of other perfluoroalkyls measured in 299 cord serum samples collected during the Baltimore THREE Study. Of these compounds, PFDA, PFUnA, and FOSA were detected most frequently (24, 34, and 26%, respectively). Maximum concentrations in these samples ranged from 1.1 to 1.9 ng/mL. PFHpA, PFDoDA, and PFBS were each detected in <6% of the samples, with maximum concentrations ranging from 0.2 to 2.6 ng/mL. Manzano-Salgado et al. (2015) studied the potential transfer of perfluoroalkyls from mothers to their children during pregnancy. Maternal blood and cord serum were collected from 66 mother-child pairs and analyzed for the presence of perfluoroalkyls. A positive correlation was found between maternal plasma and maternal serum with cord serum levels, and the authors concluded that either maternal plasma or maternal serum could be used as a method to estimate fetal exposure to perfluoroalkyls. Median concentrations of PFOS and PFOA were 6.18 and 2.85 ng/mL, respectively, in maternal plasma and 6.99 and 2.97 ng/mL, respectively, in maternal serum. PFOS and PFOA levels in cord serum were 1.86 and 1.90 ng/mL, respectively. A biomonitoring survey of 1,533 pregnant females in Denmark from 2008 to 2013 showed decreasing levels of most perfluoroalkyls in the females' blood during this time period (Bjerregaard-Olesen et al. 2016). The results of this study showed that serum levels of PFHxS, PFOS, PFOA, PFNA, and PFDA decreased at a rate of 7.0, 9.3, 9.1, 6.2, and 6.3 per year, respectively. Morello-Frosch et al. (2016) measured the levels of perfluoroalkyls in 77 maternal and 65 paired umbilical cord blood samples from pregnant females and newborn children in San Francisco, California. Perfluoroalkyls, including PFOA and PFOS, were widely detected; however, concentrations in cord blood or serum were typically equal to or lower than maternal blood levels.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Potentially high exposures to perfluoroalkyls can occur in the following population categories: perfluoroalkyl production and manufacturing workers, communities located near fluorochemical facilities, and individuals with prolonged use of perfluoroalkyl-containing products. Workers at perfluoroalkyl production and manufacturing facilities and community members living near these facilities may have higher exposure to perfluoroalkyls than the general population based on elevated concentrations of these substances measured in air, soil, sediment, surface water, groundwater, and vegetation surrounding these facilities (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007; Olsen 2015). Additionally, children may be at risk for higher potential exposure due to hand-to-mouth transfer of chemicals from dust and the ingestion of dirt (Shoeib et al. 2004; Trudel et al. 2008). Levels of perfluoroalkyls measured in the blood of production and manufacturing workers at several facilities are listed in Table 5-27. The serum PFOA, PFOS, and PFHxS levels in workers were frequently 100–1,000 times higher than in the general population. 3M estimated PFOA doses for various on-site exposure scenarios based on monitoring information collected at the Decatur Facility in Alabama (3M 2008c). Occupational exposure scenarios included groundskeeper/maintenance worker and construction/utility worker exposed to on-site soils, surface water, and sediment. According to 3M, estimated on-site exposure to PFOA ranged from $3.2x10^{-6}$ to 2.4 ng/kg/day, with the highest estimated exposure corresponding to construction/utility workers engaged in projects involving contact with soil from an on-site field. Chang et al. (2008a) measured concentrations of PFBA in the serum of 127 former employees and 50 current employees of the 3M Cottage Grove Facility in Minnesota. PFBA serum concentrations were below the detection limit in 73.2% of the former employees and 68.0% of the current employees. Only 4% of the serum samples contained PFBA above 2 ng/mL, with maximum concentrations of 6.2 ng/mL for the former employees and 2.2 ng/mL for the current employees.

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

	Mean concentration (ng/mL [ppb])			
Location	PFOA	PFOS	PFHxS	Reference
3M Decatur, Alabama				
1993 (n=111)	0.00–80,000 (range)			Olsen et al. 1998b
1995 (n=80)	0.00–114,100 (range)			Olsen et al. 1998b
1995 (n=90)		2,440		Olsen et al. 1999
1997 (n=84)		1,960		Olsen et al. 1999
1998 (n=126)	1,536	1,505	345	Olsen 2015
2000 (n=215)	11,900	1,400		Olsen et al. 2003a

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

Location	Mean concentration (ng/mL [ppb])			Reference
	PFOA	PFOS	PFHxS	
2000 (n=263)	1,780	1,320	250	Olsen 2015
1999–2004 (n=26 retired workers)				Olsen et al. 2007a
Initial	691	799	290	
Final	262	403	182	
3M Cottage Grove, Minnesota				
1993 (n=111)	5,000 (1,100 geometric mean)			Olsen et al. 2000; Olsen 2015
1995 (n=80)	6,800 (1,200 geometric mean)			Olsen et al. 2000; Olsen 2015
1997 (n=74)	6,400 (1,300 geometric mean)			Olsen et al. 2000; Olsen 2015
2000 (n=122)	4,630 (810 geometric mean)	860		Olsen and Zobel 2007; Olsen 2015
DuPont Washington Works, Ohio				
1979-2004				Woskie et al. 2012
All workers (n=1308 workers, 2125 samples)	2,050			
Direct PFOA exposure (n=170 workers; 541 samples)	5,470			
Direct PFOA exposure (n=96 workers; 208 samples)	2,530			
2004–2005				Emmett et al. 2006a
Substantial occupational exposure (n=18)	824			
2004				Sakr et al. 2007b
Current occupational exposure (n=259)	494 (median)			
Intermittent current occupational exposure (n=160)	176 (median)			
Past occupational exposure (n=264)	195 (median)			
3M Antwerp, Belgium				
1995 (n=88)		1,930		Olsen et al. 1999
1997 (n=65)		1,480		Olsen et al. 1999
2000 (n=206)	1,030	960		Olsen et al. 2003a
2000 (n=258)	840	800	170	Olsen 2015

Table 5-27. Concentrations of PFOA, PFOS, and PFHxS in Human Serum for Occupationally Exposed Individuals

Location	Mean concentration (ng/mL [ppb]) PFOA	PFOS	PFHxS	Reference
Miteni, Trissino, Italy				
2007				
Current occupational exposure (n=39)	5,710[c] (200–47,040)			Costa et al. 2009
Former occupational exposure (n=11)	4,430[c] (530–18,660)			Costa et al. 2009
2000 (n=25)	18,800			Costa et al. 2009
2001 (n=42)	19,700			Costa et al. 2009
2002 (n=46)	19,300			Costa et al. 2009
2003 (n=41)	13,700			Costa et al. 2009
2004 (n=34)	11,400			Costa et al. 2009
2006 (n=49)	10,800			Costa et al. 2009
2007 (n=50)	11,600			Costa et al. 2009

[a]Data include results from three retirees from the 3M plant in Cottage Grove, Minnesota.

Gray cells indicate that chemical was not evaluated; PFHxS = perfluorohexane sulfonic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Individuals who performed jobs that require frequent and/or prolonged contact with perfluoroalkyl containing products, such as firefighters, waste handlers, individuals who install and treat carpets, or individuals with prolonged use of ski wax may also have higher occupational exposure to perfluoroalkyls. Some firefighting foams contain perfluoroalkyls, and firefighters who use these products have been shown to have greater exposures as compared to the general population. Dobraca et al. (2015) compared perfluoroalkyl serum levels of a group of firefighters in California to an adult population from the NHANES survey. Levels of PFOA and PFOS were only slightly higher in the firefighter group (geometric means 3.75 and 12.50 ng/mL, respectively) when compared to adult males in the 2009–2010 NHANES general population survey (3.61 and 12.13 ng/mL, respectively); however, PFDA serum concentrations of firefighters were up to 3 times greater than the NHANES comparison group for the 25th–95th percentiles (50th percentile in firefighters; 0.72 ng/mL compared to 0.30 ng/mL) and the geometric mean (0.90 ng/mL compared to 0.30 ng/mL). In a small-scale study of 37 firefighters participating in the C8 Health Project, significantly (adjusted for age, water district, household income, and smoking) higher levels of PFOA and PFHxS were found in the firefighters compared to 5,373 male participants with other jobs (Jin et al. 2011). Geometric mean PFOA and PFHxS levels were 37.59 and 4.77 ng/mL, respectively, in the firefighters and 31.59 and 3.62 ng/mL, respectively, in the other

participants. No significant differences in PFOS or PFNA levels were found between the groups. A biomonitoring study of 149 firefighters in Australia showed that 100% of serum samples collected had detectable levels of PFOA, PFOS, PFHxS, and PFNA (Rotander et al. 2015). Serum levels of PFHxS were found to be approximately 10–15 times higher than levels found in the general population of Australia and Canada, while PFOS levels in the firefighters were approximately 6–10 times greater than the general population of these nations.

Elevated serum levels of PFOA, PFNA, PFHxA, PFHpA, PFDA, and PFUnA have been found in professional ski waxes containing fluorotelomers; the perfluoroalkyls were likely formed via fluorotelomer metabolism (Olsen 2015). Christensen et al. (2016) conducted a biomonitoring study of perfluoroalkyls on male fishermen from Wisconsin ≥50 years old with a history of sport fish consumption. Increasing age and lower BMI were generally associated with higher levels of the perfluoroalkyls; however, there were only weak correlations observed between amounts of fish consumption and perfluoroalkyl levels, with the exception of PFDA. Levels of PFOA, PFNA, and PFHxS in the blood of the male anglers were similar to the levels for a subset of the NHANES 2011–2012 survey (non-Hispanic white males ≥50 years old); however, levels of PFOS and PFDA were approximately 2 times greater in the anglers as compared to the NHANES survey subgroup. The median and 95th percentile concentrations of PFOS in the anglers were 19.00 and 54.00 ng/mL, respectively, as compared to 10.33 and 25.83 ng/mL, respectively, in the NHANES study group. The median and 95th percentile concentrations of PFDA in the anglers were 0.52 and 1.90 ng/mL, respectively, as compared to 0.23 and 0.53 ng/mL in the NHANES study group. Family members of occupationally exposed workers have been shown to have higher exposure to perfluoroalkyls via dust transfer as compared to family members of nonoccupationally exposed workers (Fu et al. 2015).

PFOA, PFOS, PFBA, PFBS, PFNA, and PFHxS have been detected in the municipal drinking water and private wells of some communities located near fluorochemical facilities (3M 2008c; ATSDR 2008; Emmett et al. 2006a; Hoffman et al. 2011; Hölzer et al. 2008; Post et al. 2013; Steenland et al. 2009a; Wilhelm et al. 2009). Emmett et al. (2006a) compared PFOA serum levels to various types of exposure for individuals living in the Little Hocking community (near DuPont's Washington Works facility) and concluded that residential water source was the primary determinant of serum PFOA at this location. These authors reported that the mean human serum PFOA level was 105 times higher than the residential drinking water level. Median serum PFOA levels were 371 ng/mL in residents for whom this was the only residential water source and 71 ng/mL in those who used bottled, cistern, or spring water. Increased serum PFOA was associated with increasing number of drinks of tap water daily and also with increasing

use of water for making soups and stews and in-home canning of fruits and vegetables. Use of a carbon water filter reduced PFOA levels by about 25%. In a follow-up study, 231 study participants in the Little Hocking Water District were evaluated 15 months later with 88% using bottled water exclusively; 8% had made other changes to their ingestion of residential water including use of activated carbon water filters. PFOA levels had decreased an average of 26% from the initial levels (Emmett et al. 2009). Similarly, Bartell et al. (2010) found that serum levels of PFOA declined significantly following the implementation of GAC filtration of the public water supply. The average decrease in serum PFOA levels for Lubeck, West Virginia residents primarily consuming public water at home (n=130) was 26% 1 year after treatment began. Similar trends were reported for residents of Little Hocking, Ohio. The average decrease in PFOA serum levels for residents primarily consuming public water (n=39) was about 11% 6 months after treatment began.

Median PFOA serum levels for residents currently residing in six water districts located in the mid-Ohio Valley near the Washington Works facility ranged from 12.1 to 224.1 ng/mL, while the median concentration ranged from 10.5 to 33.7 ng/mL for residents who previously worked or resided in these districts (Steenland et al. 2009a). Former employees at the chemical plant had much higher levels (median=75 ng/mL) than people who had not worked at the plant (median=24 ng/mL), but lower levels than those who continued to be employed at the plant during the monitoring period (median=148 ng/mL). Another study of this community reported a median serum PFOA concentration of 24.3 ng/mL for 45,276 non-occupationally exposed individuals in 2005–2006 (Shin et al. 2011a). This was about 8 times greater than the median concentration (3.20 ng/mL) of 2,120 residents of the general population taken from the NHANES data for 2005–2006 (CDC 2018).

A study of residents consuming drinking water from the Ohio River and the Ohio River Aquifer reported elevated median PFOA levels of 13.8 ng/mL in blood samples collected in 1991–1993 from 139 residents (Herrick et al. 2017). In samples collected in 2011–2013 (n=133), the serum PFOA levels dropped to 4.3 ng/mL. The serum PFOS levels in samples collected in 1994–1996 (median of 32.2 ng/mL, n=189) were similar to levels in the general population (Herrick et al. 2017); similar to the decline observed in the general population, median serum PFOS levels were 6.3 ng/mL in 2011–2013.

The Minnesota Department of Health conducted a biomonitoring study of 196 residents living in Washington county, east of the Minneapolis-St. Paul metropolitan area; private and municipal drinking water wells were shown to be contaminated with perfluoroalkyls (MDH 2009). In 2008–2009, geometric mean serum PFOA, PFOS, and PFHxS levels were 15.4, 35.9, and 8.4 ng/mL, respectively; these values

were higher than levels reported in NHANES (Landsteiner et al. 2014; MDH 2009). The investigators noted that the geometric mean serum concentrations of these three substances were greater in residents obtaining drinking water from municipal water supplies as compared to residents with private wells (Landsteiner et al. 2014). Monitoring efforts also conducted in Washington County, Minnesota near the 3M Cottage Grove Facility revealed widespread contamination of PFBA in the groundwater in 2006 (ATSDR 2008). PFBA was detected in 28% of the community members' serum samples; the 75th percentile serum concentration for PFBA was 0.135 ng/mL and the maximum concentration was 8.5 ng/mL (MDH 2000).

ATSDR performed an exposure investigation for residents of Decatur, Alabama following an accidental release of perfluoroalkyls into the Decatur waste water treatment plant (ATSDR 2013). A group of 155 residents had their blood tested for levels of eight perfluoroalkyls. Serum levels for PFNA, PFDA, and FOSA were lower or similar to levels of the general population when compared to the NHANES results. Elevated serum PFOA, PFOS, and PFHxS levels were observed; geometric mean values were 16.3, 39.8, and 6.4 ng/mL, respectively. Residents who used the West Morgan/East Lawrence public water supply had significantly higher geometric mean serum levels of PFOA (17.59 ng/mL) and PFHxS (6.68 ng/mL) as compared to the geometric mean for a similar demographic group from the NHANES survey. Serum perfluoroalkyl levels in the residents were shown to be much lower than levels found in occupationally exposed individuals who regularly worked with these substances (ATSDR 2013).

Additional blood serum levels of PFOA and PFOS for residents in selected areas of Ohio, West Virginia, New Jersey, and Minnesota whose residential source of drinking water may have been contaminated are available from the EPA docket on PFOA and related perfluoroalkyls (EPA-HQ-OPPT-2003-0012) (Bilott 2004, 2005a, 2005b, 2007).

The Emmett et al. (2006a) and Steenland et al. (2009a) studies of the community near the Washington Works facility and the 3M (2008c) study of the Decatur Facility in Alabama found age-related differences in serum PFOA levels. In a comparison of serum PFOA levels in various age groups of residents serviced by the Little Hocking Water Association district, Emmett et al. (2006a) found that 2–5-year-old children had a higher serum PFOA (median 600 ng/mL) compared with residents in all other age groups (median 321 ng/mL) except for the group aged >60 years, whose levels were similar to those in young children. Several factors may have contributed to the observed high levels in children: infants and young children proportionally drink more water per kg of body weight than adults; children (and also the elderly) tend to spend more time at home with exclusive use of residential water than other age groups; and trans-

placental and breast milk exposures could also contribute to levels in children. In the Steenland et al. (2009a) study, PFOA serum levels tended to be highest for children aged 0–9 years and persons >50 years old. The serum levels of the 69,030 residents participating in this study categorized by age are provided in Table 5-28. 3M (2008c) estimated doses for various off-site exposure scenarios based on monitoring information collected at the Decatur Facility. Exposure scenarios include local children and adult residents exposed to PFOA in off-site soils, groundwater, municipal water, fish from the Tennessee River, and surface water and sediments in the Tennessee River. According to 3M, estimated off-site exposure of local residents to PFOA ranged from 0.011 to 260 ng/kg/day, with the highest estimated exposure corresponding to children whose source of drinking water was groundwater adjacent to the southern side of the facility.

Table 5-28. Blood Serum Levels for 69,030 Current and Former Residents of Six Water Districts in the Mid-Ohio Valley (2005–2006)

Age (years)	Number (percentage of total)	Median perfluorooctanoic acid (PFOA) level (ng/mL)
0–9	4,915 (7.1)	32.8
10–19	9,658 (14.0)	26.6
20–29	10,073 (14.6)	21.0
30–39	10,547 (15.3)	22.7
40–49	12,113 (17.6)	28.0
50–59	10,515 (15.2)	33.6
60–69	6,881 (10)	42.9
≥70	4,328 (6.3)	40.1

Source: Steenland et al. 2009a

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of perfluoroalkyls is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of perfluoroalkyls.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.1 Existing Information on Health Effects

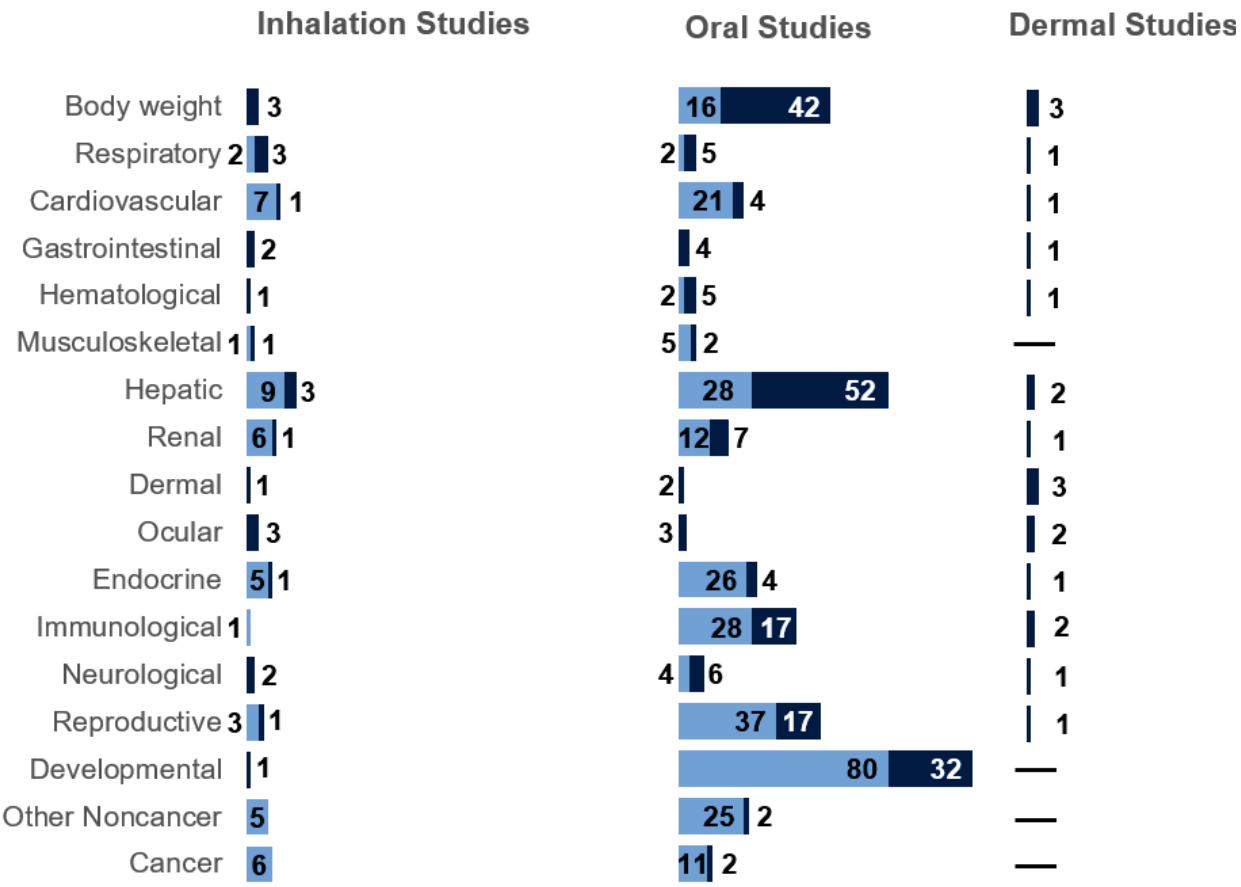
Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to PFOA, PFOS, and other perfluoroalkyls that are discussed in Chapter 2 are summarized in Figures 6-1, 6-2, and 6-3, respectively. The purpose of these figures is to illustrate the information concerning the health effects of perfluoroalkyls. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

As illustrated in Figures 6-1, 6-2, and 6-3, most of the data on the toxicity of PFOA, PFOS, and other perfluoroalkyls come from epidemiological studies in humans; oral exposure is the assumed route of exposure for the epidemiological studies. The epidemiology database consists of health evaluations of subjects exposed in occupational settings (primarily PFOA and PFOS), highly exposed residents living near a PFOA facility, and studies of the general population. The most commonly examined endpoints in the epidemiological studies were developmental, hepatic, reproductive, and immunological effects.

Figure 6-1. Summary of Existing Health Effects Studies on PFOA by Route and Endpoint*

Potential body weight, hepatic, and developmental effects were the most studied endpoints

The majority of the studies examined oral exposure in **humans** (versus **animals**)

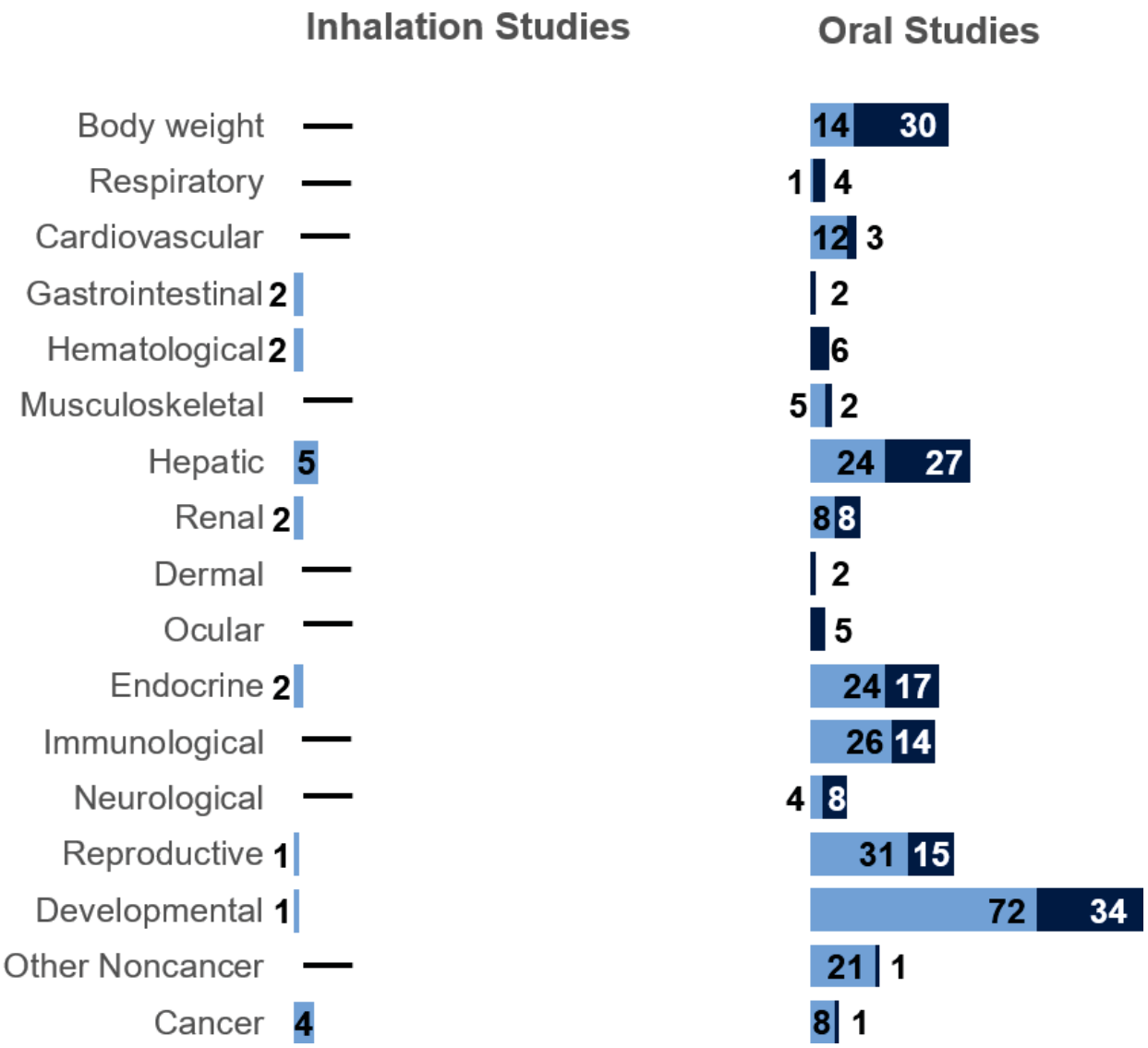


*Oral exposure was the presumed route of exposure for human studies involving environmental exposure. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available.

Figure 6-2. Summary of Existing Health Effects Studies on PFOS by Route and Endpoint*

Potential developmental, hepatic, and reproductive effects were the most studied endpoints

The studies examined oral exposure in **humans** (versus **animals**)

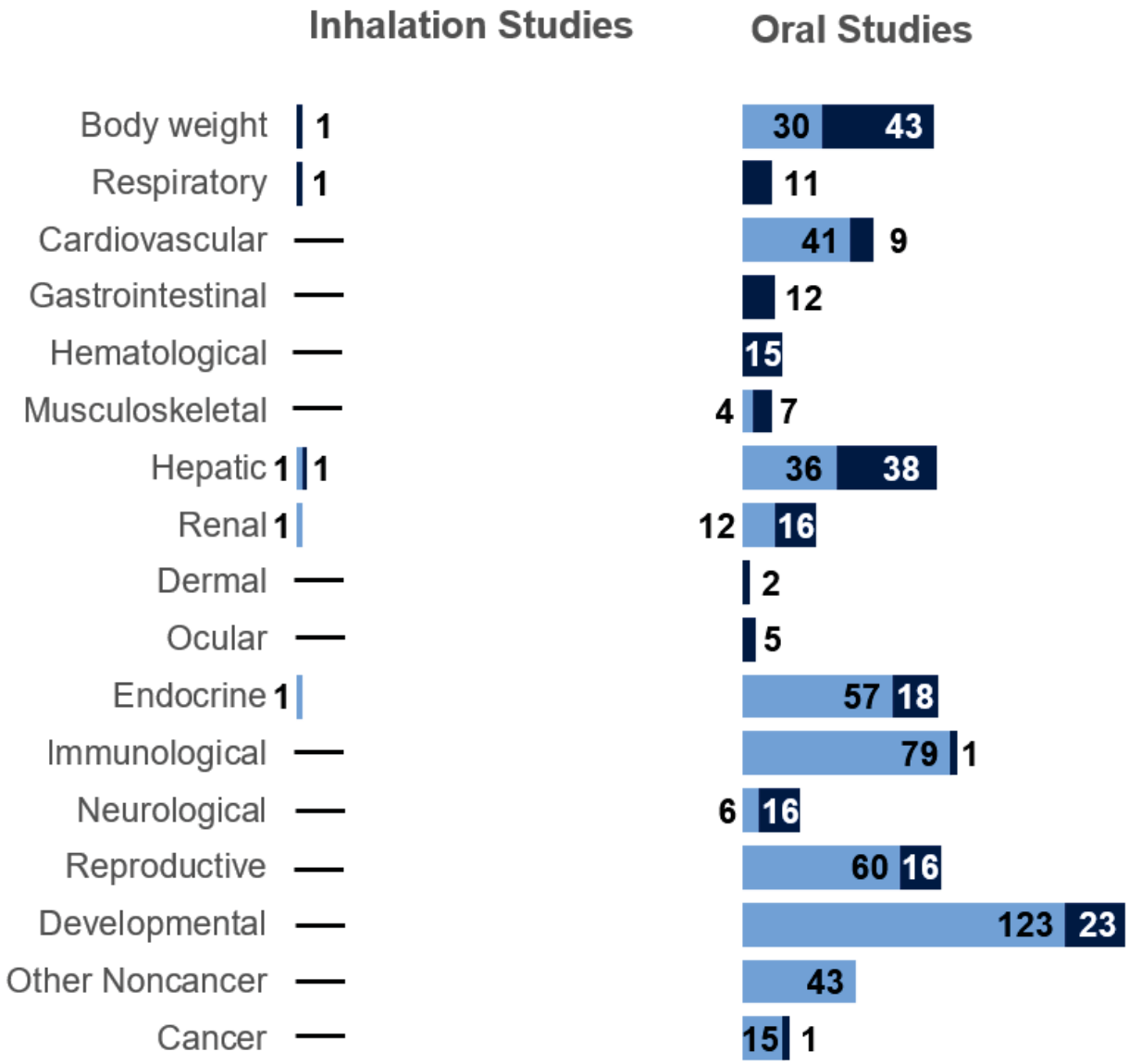


*Oral exposure was the presumed route of exposure for human studies involving environmental exposure. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available.

Figure 6-3. Summary of Existing Health Effects Studies on Other Perfluoroalkyls by Route and Endpoint*

Potential hepatic, immunological, and developmental effects were the most studied endpoints

The majority of the studies examined oral exposure in **humans** (versus **animals**)



*Oral exposure is the presumed route of exposure for human studies involving environmental exposure. Most human studies examined multiple perfluoroalkyls. Human and animal studies may have examined more than one endpoint. A "—" indicates that no studies are available. Includes data for PFBA, PFHxA, PFHpA, PFNA, PFDA, PFUnA, PFBS, PFHxS, PFDoDA, and FOSA.

Most of the information regarding the effects of perfluoroalkyls in animals has been derived from oral studies; considerably less information is available from inhalation and dermal exposure studies. PFOA and PFOS have been the most extensively studied members of this class of chemicals, and oral administration has been the preferred route of exposure in animal studies. Information regarding other perfluoroalkyls covered in this profile is limited to acute-duration oral studies with PFHxS, PFNA, PFDA, PFBA, PFDoDA, PFHxA, and FOSA; intermediate-duration oral studies with PFHxS, PFNA, PFDA, PFUnA, PFBS, PFBA, PFDoDA, and PFHxA; and a chronic-duration oral study with PFHxA. An acute-duration-inhalation study with PFNA is also available. The most commonly examined endpoints were hepatic, body weight, developmental, reproductive, and immunological effects.

6.2 Identification of Data Needs

Missing information in Figures 6-1, 6-2, and 6-3 should not be interpreted as a "data need." A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Acute-Duration MRLs. The available acute inhalation database for PFOA was considered inadequate for derivation of an MRL due to lack of measured serum PFOA levels in the available animal studies and the lack of PBPK model parameters that could be used to predict serum levels. The inhalation database for PFNA was not considered adequate due to the limited endpoints examined and the short exposure duration of the only available study. No inhalation data were available for PFOS or the other perfluoroalkyls. A number of studies have evaluated the acute toxicity of PFOA and PFOS following oral exposure and have identified several sensitive targets of toxicity. Smaller numbers of studies evaluated potential sensitive targets of acute toxicity for PFNA and PFDA. However, toxicokinetic differences between humans and laboratory animals, particularly the relative short half-life in rodents compared to humans, preclude derivation of an acute MRL for these compounds. For other perfluoroalkyls (PFHxS, PFBA, PFDoDA, FOSA), the available studies were not considered adequate for identification of critical targets; did not examine sensitive targets that were identified for other perfluoroalkyls, such as developmental and immunological endpoints; or involved a single exposure. No acute oral data were identified for PFUnA, PFHpA, or PFBS. Research is needed to develop a PBPK model that would allow for extrapolation from rodents to humans. Additionally, toxicity studies are needed for most perfluoroalkyls to identify critical targets of toxicity and/or establish dose-response relationships. These

studies should examine developmental and reproductive endpoints that have been established as the most sensitive targets of toxicity for PFOA and PFOS.

Intermediate-Duration MRLs. No intermediate-duration inhalation studies were identified for perfluoroalkyls. Oral studies suggest that developmental and immune effects are the most sensitive targets of toxicity; similar effects are likely to occur following inhalation exposure because perfluoroalkyls are not metabolized. Inhalation studies are needed to establish dose-response relationships and to establish whether the respiratory tract is a sensitive target of toxicity. The intermediate-duration oral databases were considered adequate for derivation of MRLs for PFOA and PFOS. The MRL for PFOA is based on altered bone development measured in mature mice exposed *in utero*. The principal study only tested one PFOA dose level and only examined long bones; additional studies utilizing several dose levels, examining other types of bone, and testing a second species would provide support for the MRL. A modifying factor was used for PFOS due to the lack of PBPK modeling parameters; additional studies are needed that would allow for predicting steady-state serum PFOS levels for immunotoxicity studies in laboratory animals. An important decision made in the derivation of intermediate oral MRLs was to use the time-weighted average serum concentrations (C_{TWA}) of PFOA or PFOS as the basis for extrapolations of the dose-response PODs from animal studies to human equivalent doses (HEDs). However, the available data on the toxicity of PFOA and PFOS do not provide convincing evidence that toxicity outcomes are more likely to be determined by C_{TWA} rather than C_{max}. Since the PBPK model used for dosimetry modeling of the animal studies predicted that C_{TWA} is lower than C_{max} in the principal studies for the MRLs, selection of C_{TWA} as the internal dose metric results in lower values for MRLs. Therefore, use of C_{TWA}, rather than C_{max} is a health-protective decision that might be more adequately evaluated with additional studies that evaluate associations between C_{max}, C_{TWA}, and toxicity outcome responses.

Intermediate-duration MRLs were also derived for PFHxS and PFNA; however, these were based on marginal databases and additional dose-response studies are needed to support the basis of the MRL. The databases were not considered adequate for PFUnA, PFBS, PFBA, or PFDoDA due to the lack of studies examining potential sensitive targets (developmental and/or immune effects). No intermediate-duration oral studies are available for PFDA, PFHpA, or FOSA. Intermediate-duration oral studies are needed for these seven perfluoroalkyls to provide information on sensitive targets and establish dose-response relationships. These studies should include measurement of serum perfluoroalkyl levels, which would allow for estimating HEDs.

An additional uncertainty in the MRLs was the confidence in the elimination half-lives for perfluoroalkyls used in the empirical model for calculating HEDs. Estimated half-lives for PFOA and PFOS vary and contributors to this variability have not been completely characterized. The empirical model used to calculate HEDs is linear; therefore, the change in the HED is approximately proportional to the change in the half-life. A halving of the half-life would result in a doubling of the HED. Studies that can improve confidence in the half-life estimates would increase confidence in MRLs.

Chronic-Duration MRLs. The lack of chronic-duration inhalation studies for perfluoroalkyls precluded derivation of chronic MRLs. Chronic toxicity studies examining a wide range of endpoints are needed to identify the most sensitive target and establish concentration-response relationships. A small number of chronic duration oral studies have been identified in laboratory animals. Four studies examined the chronic toxicity of PFOA, PFOS, or PFHxA. These studies were not considered suitable for derivation of MRLs because they did not evaluate immunotoxicity which was a sensitive target following shorter term exposures. Studies examining this potentially sensitive endpoint are needed to identify the most sensitive target following chronic exposure.

Health Effects. Over 600 studies have evaluated the toxicity of perfluoroalkyls; epidemiological studies account for over 400 of the toxicity studies. Evidence from epidemiological studies suggest associations between perfluoroalkyl exposure and several health outcomes including liver damage, increases in serum lipids, thyroid disease, immune effects, reproductive toxicity, and developmental toxicity. The primary health effects observed in laboratory animals are liver, developmental, and immune toxicity. Although a large number of studies evaluating health effects are available, there is a need for additional studies to address data gaps. Future laboratory animal studies should include measurement of serum perfluoroalkyl levels, as this would provide valuable information for comparing effects observed in laboratory animals to effects observed in humans.

Hepatic Effects. Evidence from acute, intermediate, and/or chronic oral studies in rats, mice, and monkeys indicates that the liver is a sensitive target of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, PFBA, PFBS, PFDoDA, and PFHpA toxicity. The effects observed in rodents differ from those observed in humans. In humans, exposure to PFOA, PFOS, PFNA, and PFDA appear to result in increases in serum lipid levels, particularly total cholesterol levels. However, animal studies have found decreases in serum lipid levels associated with exposure to most perfluoroalkyls. It is not known if the species differences are due to different mechanisms of toxicity or differences in

exposure levels (serum levels observed in animal studies are orders of magnitude higher than those in human studies).

Immune Effects: Epidemiological data suggest an association between PFOA, PFOS, PFHxS, and PFDA and decreased antibody response to vaccines. This is supported by acute- and intermediate-duration studies of PFOA and PFOS in laboratory animals. There is also evidence of immunotoxicity following a single injection of PFNA; some of the immune effects persisted 4 weeks post-exposure. Shorter-term studies are needed for other perfluoroalkyls. In addition, chronic-duration studies evaluating immune endpoints, particularly immunosuppression, for all perfluoroalkyls would allow for identification of the critical targets of toxicity.

Reproductive Effects. Decreases in mammary gland development have been demonstrated in several PFOA mouse studies. The effect levels observed in these studies are very low, although there is some indication that at lower doses, the changes in mammary gland development do not affect lactation. Additional studies are needed to evaluate the adversity of these alterations. This endpoint has not been evaluated for other perfluoroalkyls and studies are needed to determine whether it is also a sensitive effect for these compounds.

Developmental Effects. Based on the results of laboratory animal studies, developmental endpoints are targets of PFOA, PFOS, PFHxS, PFNA, PFDA, PFUnA, and PFBA toxicity following acute- and/or intermediate-duration oral exposure. Studies are needed to evaluate potential developmental effects for PFHxS following intermediate-duration oral exposure. Additionally, cross-fostering studies would provide information that could be used to evaluate the health impact of lactational exposure to perfluoroalkyls. Epidemiological studies in children suggest altered responses to vaccination; two animal studies have evaluated immune effects following perinatal exposure to PFOA and PFOS, but data are lacking for other perfluoroalkyls.

Potential Interactions between Perfluoroalkyls. A common limitation of the epidemiological data is co-exposure to multiple perfluoroalkyls. There are limited data on possible interactions between perfluoroalkyls and possible effects on toxicity and toxicokinetics. Animal studies examining the possible interactions between perfluoroalkyls would be useful for interpreting the epidemiological study results; this is especially important since humans are typically exposed to multiple perfluoroalkyls and many of them are likely to have similar mechanisms of action.

Mechanisms of Toxicity. Many of the effects observed in rodents, particularly liver and developmental effects, involve the activation of PPARα; humans and nonhuman primates are less responsive to PPARα agonists than rats and mice. However, the results of studies in PPARα-null mice suggest that PPARα-independent mechanisms also play a role in the liver, immunological, and developmental toxicity. Additional studies are needed on the mechanisms of toxicity to assess whether the effects observed in laboratory animals are relevant to humans. Mechanistic studies would also provide support for the critical effects used to derive the MRLs for PFOA, PFOS, PFHxS, and PFNA.

Epidemiology and Human Dosimetry Studies. As previously mentioned, information is available regarding the effects of exposure to perfluoroalkyls in humans derived from health evaluations of subjects exposed in occupational settings, residents living near a PFOA manufacturing facility with high levels of PFOA in the drinking water, and the general population. Although many studies found statistically significant associations between serum perfluoroalkyl levels and the occurrence of an adverse health effect, the findings were not consistent across studies. Interpretation of the human data is limited by the reliance of cross-sectional studies, which do not establish causality, and the lack of exposure data. Studies on serum lipids suggest that the dose-response curve is steeper at lower concentrations and flattens out at higher serum perfluoroalkyl concentrations (Steenland et al. 2010a); additional studies that could be used to establish dose-response relationships would be valuable. Mechanistic studies examining the association between perfluoroalkyl exposure and serum lipid levels would also provide valuable insight. Clarification of the significance and dose-response relationships for other observed effects is also needed. Longitudinal studies examining a wide range of endpoints would be useful for identifying critical targets of toxicity in humans exposed to perfluoroalkyls. The available human studies have identified some potential targets of toxicity; however, cause-and-effect relationships have not been established for any of the effects, and the effects have not been consistently found in all studies. Mechanistic studies would be useful for establishing causality. When possible, health assessments should include subjects of different race/ethnicity and age to determine potential race/ethnicity- and age-based susceptibilities. Another limitation of the epidemiological studies is co-exposure to other perfluoroalkyls; studies that statistically controlled for co-exposure to other pollutants would decrease this uncertainty. As noted previously, there is a need for studies evaluating potential interactions between perfluoroalkyls.

Biomarkers of Exposure and Effect. Data are available regarding levels of perfluoroalkyls in serum from the general population, highly exposed residents, and perfluoroalkyl workers. Information is needed regarding the toxicokinetics (see also below) of perfluoroalkyls in humans to be able to relate levels of

these compounds in serum to exposure to specific perfluoroalkyls; data on matched serum and urine samples would be valuable. Also needed is further information on the relationship between serum and liver concentrations of perfluoroalkyls in humans.

Absorption, Distribution, Metabolism, and Excretion. Several epidemiological studies have examined the kinetics of serum perfluoroalkyl concentrations following a change in environmental or occupational exposure, from which estimates of terminal elimination half-lives in adults are available for PFOA, PFOS, PFHxS, PFBA, and PFBS. Other studies provide data on the renal clearances of PFOA and PFOS, binding of PFOA, PFOS, and PFHxS to human plasma protein, tissue levels (primarily blood, maternal and fetal cord serum, and breast milk). Data on other aspects of the toxicokinetics of perfluoroalkyls in humans are not available and could serve to improve predictions of internal dosimetry associated with exposures to perfluoroalkyls (bioavailability, kinetics of tissue distribution and elimination, binding in tissues, external-internal dose relationships, all aspects of toxicokinetics in children and aging populations).

Toxicokinetics of perfluoroalkyls have been studied much more extensively in rodents (rats and mice) and less extensively in Cynomolgus monkeys; however, a number of data gaps have been identified:

- Absorption studies; oral absorption data are available for PFOA, PFOS, and PFBA, but are more limited for other perfluoroalkyls and for other exposure routes. Studies elucidating the mechanisms of pulmonary and gastrointestinal absorption are also needed.

- Studies have shown that elimination kinetics, and therefore, internal dose-external dose relationships, are dependent on structure, including the terminal acid group (carboxylate or sulfonate), carbon chain length, and carbon chain branching. These structural features affect plasma and tissue protein binding, renal and biliary clearances, tissue levels, maternal-fetal transfer, and lactational transfer of perfluoroalkyls. Studies examining differences between perfluoroalkyls would be useful for extrapolating health effects and toxicokinetic data across compounds.

- Toxicokinetic studies have found sex- and dose-dependent subcellular distribution of PFOA in rats. Further studies on the mechanisms for dose-dependency, characterization of subcellular binding proteins, and mechanistic linkages between subcellular distribution and toxicity of perfluoroalkyls are needed.

- The distribution and elimination of PFOA and PFOS are greatly influenced by binding interactions with albumin and other high molecular weight plasma proteins; available data suggest that binding to plasma proteins, as well as the volume of distribution, may be sex- and species-specific. Interactions with albumin have been partially characterized to the extent that binding capacity and affinity constants have been estimated, but the rates of association and dissociation have not been reported.

- Liver uptake and renal clearance of PFOS also appeared to be time-dependent in a PBPK model used to predict plasma and liver in concentrations of PFOS in a chronic rat study (Harris and Barton et al. 2008). Mechanisms underlying these time dependencies have not been elucidated.

Comparative Toxicokinetics. Toxicokinetic studies conducted in various rodent species (mice, rats, hamsters, rabbits) and in Cynomolgus monkeys have revealed profound species and sex differences as well as dose dependencies in the tissue distribution and elimination kinetics of PFOA and PFOS. Studies conducted in rats have revealed contributing mechanisms for sex differences in elimination of PFOA; slower elimination of PFOA in male rats compared to female rats has been attributed to sex hormone-modulated renal tubular transport of PFOA that results in markedly lower renal clearance of PFOA in the sexually mature male rat (see Section 3.5.1, Excretion). Sex differences in elimination of PFOA have also been observed in hamsters; unlike the rat, male hamsters excreted absorbed PFOA more rapidly than female hamsters. Sex differences in elimination of PFOA have not been observed in other rodent species, in Cynomolgus monkeys, or in limited observations made in humans. Sex differences in elimination rates of perfluoroalkyls in humans have not been demonstrated in population studies of serum elimination kinetics or renal clearance. Although the few studies that estimated elimination half-lives or renal clearances in male and female humans have not found significant sex differences, these outcomes may reflect the relatively low serum concentrations in these subjects compared with studies that were conducted in nonhuman primates and rodents (i.e., sex differences in elimination may vary with dose and/or plasma concentration). Additionally, the failure to account for the influence of reduced estrogen levels (in postmenopausal women) and reduced testosterone levels (in older males) in occupational and/or site-related epidemiological studies may also account for the lack of findings of sex-related differences.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in the Health Effects subsection above. It is not known whether children are more or less susceptible than adults to the effects of exposure to perfluoroalkyls because there are no studies that specifically addressed this question. Several studies have examined the possible associations between perfluoroalkyl exposure and health outcomes in children living in an area with high PFOA contamination and in the general population. Although some studies have found statistically significant associations, they are not adequate for establishing causality. Follow-up studies of the C8 population could allow for a longitudinal assessment of health effects in children and would be useful in determining whether the observed effects are due to perfluoroalkyl exposure. Toxicokinetics information in children is needed. Half-life studies have been conducted in adults; there is the need to understand if these are applicable to children. There are no

studies that have examined whether young animals are more or less susceptible than adults to perfluoroalkyls toxicity. Additional information on this issue would be useful.

Physical and Chemical Properties. Perfluoroalkyls have unique and complex physical and chemical properties (Kissa 2001; Schultz et al. 2003). Sources are available that provide helpful insights into the structural aspects and surfactant nature of these substances; however, many of the properties are still not well understood (CEMN 2008; Kissa 2001; Schultz et al. 2003). In general, specific properties such as physical state, melting point, boiling point, density, solubility, vapor pressure, micelle formation, and acid dissociation in water have not been determined or are not well described for these compounds. Measurements of these endpoints are needed. Information regarding the potential association of these species in water would be useful. Where determination of a particular endpoint is not possible, a thorough description of the physical and chemical properties as they relate to that endpoint would be helpful. Perfluoroalkyls discussed in this profile exist as a mixture of linear and branched isomers. Isomer-specific data would also be useful for the various physical-chemical properties. Wang et al. (2013b) identified several of the fluorinated compounds that are currently being used by major manufacturers as alternatives to PFOA and PFOS. These compounds are being used as processing aids in the emulsion polymerization of PTFE and other polymers as well as surface treatment uses, metal plating uses, firefighting foams, and other miscellaneous uses such as food contact materials. A data need exists to determine the physical and chemical properties of these replacement substances.

The production, use, import, and export of perfluoroalkyls have changed dramatically since 2000. Most nations no longer produce or use PFOS or PFOA (China is a notable exception). Major fluoropolymer manufacturers in the United States have altered their chemical processes to use alternative fluorinated substances in their production processes. Information regarding the production, import, and export volumes of these substances is needed.

Recommended methods for the disposal of perfluoroalkyls have not been located. In the past, perfluoroalkyl-containing waste has been disposed of in on- and off-site landfills, through sludge incorporation, and through incineration (3M 2007b, 2008b; ATSDR 2005). New disposal methods that avoid release of these substances into the open environment and prevent contamination of nearby soil, sediment, and groundwater should be developed. The eventual breakdown of fluorotelomer-based polymers with the eventual release of substances such as PFOA is not well understood. Early researchers have concluded that the half-life for this process is >1,000 years; however, more recent data suggest much shorter time scale of 1–2 decades (Rankin et al. 2014; Washington and Jenkins 2015; Washington et al.

2009, 2014, 2015). Additional studies on the potential release of perfluoroalkyls from the eventual degradation of fluoropolymers in landfills would be useful.

Environmental Fate. Perfluoroalkyls are very stable compounds and are resistant to biodegradation, direct photolysis, atmospheric photooxidation, and hydrolysis (3M 2000; EPA 2008a; OECD 2002, 2007; Schultz et al. 2003). The chemical stability of perfluoroalkyls and the low volatility of these substances in ionic form indicate that perfluoroalkyls will be persistent in water and soil (3M 2000; Prevedouros et al. 2006). K_{oc} values ranging from 17 to 230 indicate that PFOA will be mobile in soil and can leach into groundwater (Davis et al. 2007; Prevedouros et al. 2006). Environmental fate and potential pathways of PFOA exposure at and near the DuPont Washington Works site have been discussed (Small 2009). Wang et al. (2013b) identified several of the fluorinated compounds that are currently being used by major manufacturers as alternatives to PFOA and PFOS. Environmental fate and toxicity research of newer replacement substances is ongoing (De Silva et al. 2016; Gomis et al. 2018; Kabore et al. 2018).

Bioavailability from Environmental Media. Perfluoroalkyls are widely detected in humans and animals, indicating that several of these substances are bioavailable. The bioaccumulation potential of perfluoroalkyls is reported to increase with increasing chain length (de Vos et al. 2008; Furdui et al. 2007; Martin et al. 2004b). In living organisms, perfluoroalkyls bind to protein albumin in blood, liver, and eggs and do not accumulate in fat tissue (de Vos et al. 2008; Kissa 2001). The mechanism of perfluoroalkyl uptake in animals is not fully understood; additional studies would be helpful (de Vos et al. 2008). Perfluoroalkyls discussed in this profile exist as a mixture of linear and branched isomers. Data regarding the bioavailability of branched versus linear substances would be useful. A data need exists to determine the bioavailability of the replacement substances identified in Wang et al. (2013b) used in place of PFOA and PFOS.

Food Chain Bioaccumulation. High levels of certain perfluoroalkyls in animals have been measured in apex predators, such as polar bears, which indicates that some perfluoroalkyls possess the ability to bioaccumulate (de Vos et al. 2008; Houde et al. 2006a; Kannan et al. 2005; Smithwick et al. 2005a, 2005b, 2006). Perfluoroalkyl sulfonates with carbon chain length lower than 8 tend to bioaccumulate less than PFOS. Ongoing monitoring of perfluoroalkyl levels in animals may help to determine whether efforts to phase out these substances will have had an effect on their biomagnification. A data need exists to determine the bioaccumulation potential of the new replacement substances used in place of PFOA and PFOS.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of perfluoroalkyls in contaminated media at hazardous waste sites are needed so that the information obtained on levels of perfluoroalkyls in the environment can be used in combination with the known body burden of perfluoroalkyls to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Concentrations of perfluoroalkyls have been measured in surface water from several locations across the United States (Boulanger et al. 2004; Kannan et al. 2005; Kim and Kannan 2007; Nakayama et al. 2007; Simcik and Dorweiler 2005; Sinclair et al. 2004, 2006). Continued monitoring for perfluoroalkyls in surface water would be useful. Data are available regarding levels of perfluoroalkyls in outdoor air, indoor air, indoor dust, food, food packaging, and consumer products (3M 2001; Barber et al. 2007; Begley et al. 2005; Food Standards Agency 2006; Fromme et al. 2007b; Harada et al. 2005b, 2006; Jogsten et al. 2009; Kim and Kannan 2007; Kubwabo et al. 2005; Moriwaki et al. 2003; Tittlemier et al. 2007; Washburn et al. 2005). Comprehensive studies monitoring for perfluoroalkyls in these matrices within the United States are needed. Elevated concentrations of perfluoroalkyls have been measured in air, water, soil, and sediment near fluorochemical industrial facilities (3M 2007b, 2008b, 2008c; Barton et al. 2006; Davis et al. 2007; Hansen et al. 2002). Additional research is needed to evaluate how soil physical and chemical properties influence the bioavailability of perfluoroalkyls. Continued monitoring for perfluoroalkyls in these matrices are needed to assess exposure of individuals working at these locations and individuals who live near these facilities. A data need also exists to perform environmental monitoring of the replacement substances identified in Wang et al. (2013b) used in place of PFOA and PFOS, particularly near manufacturing locations.

Exposure Levels in Humans. Trudel et al. (2008) provided a thorough assessment of the exposure of the general population to PFOS and PFOA. 3M (2008b) provided an assessment of exposure of individuals to PFOA on-site at a fluoropolymer facility. Uptake values and exposure pathways determined in these studies should be examined further. Conclusions made in these assessments are expected to be adjusted as future monitoring data are made available. Large-scale monitoring of perfluoroalkyls in human serum in the United States is ongoing (Calafat et al. 2006a). Future results of human monitoring studies would be useful for assessing human exposure to these substances over time. The results of these studies can be examined for correlations between human perfluoroalkyl levels and the phasing out of perfluoroalkyls by companies of the fluorochemical industry. Levels of perfluoroalkyls in human urine have been reported (Jurado-Sanchez et al. 2014). Higher exposure levels for individuals who reside in areas where substances such as PFOA contaminated both public and private water supplies

have been documented (Emmett et al. 2006a, 2009). Continued biomonitoring of legacy compounds such as PFOA and PFOS as well as other perfluoroalkyls is needed.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. Trudel et al. (2008) provided a thorough assessment of the exposure of children to PFOS and PFOA. These conclusions should be reexamined with respect to future biomonitoring data when they become available. Data are available regarding the levels of perfluoroalkyls in young children (Kato et al. 2009b; Olsen et al. 2004b; Toms et al. 2009). NHANES monitoring data for 2013–2014 for children of ages 3–11 years have recently been released (CDC 2018; Ye et al. 2018a). Data provided from these efforts will be useful in assessing the exposure of young children to perfluoroalkyls.

Concentrations of perfluoroalkyls have been measured in human breast milk and cord blood (Apelberg et al. 2007a, 2007b; Fei et al. 2007; Inoue et al. 2004; Kärrman et al. 2007; Midasch et al. 2007; So et al. 2006b; Völkel et al. 2008). Additional monitoring for perfluoroalkyls in these media would be useful. Continued biomonitoring of legacy compounds such as PFOA and PFOS as well as replacement substances is needed.

6.3 Ongoing Studies

A number of federal agencies are sponsoring ongoing studies; a list of these studies are available at https://www.atsdr.cdc.gov/pfas/PFAS-health-effects.html.

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding perfluoroalkyls in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency. A list of some select state drinking water regulations/guidelines or health-based values are summarized in Table 7-2.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the MRLs for perfluoroalkyls.

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference
	Air		
EPA	RfC	No data	IRIS 2018
WHO	Air quality guidelines	No data	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2018
	DWEL		
	PFOA	0.00037 mg/L	EPA 2016e
	PFOS	0.00037 mg/L	EPA 2016f
	Lifetime Health Advisory		
	PFOA	0.07 µg/L	EPA 2016e
	PFOS	0.07 µg/L	EPA 2016f
	National primary drinking water regulations	No data	EPA 2009d
	RfD	No data	IRIS 2018
	PFOA	$2x10^{-5}$ mg/kg/day	EPA 2016e
	PFOS	$2x10^{-5}$ mg/kg/day	EPA 2016f
WHO	Drinking water quality guidelines	No data	WHO 2017
FDA	Substances added to food	No data[a]	FDA 2018
	Cancer		
ACGIH	Carcinogenicity classification		
	APFO	A3[b]	ACGIH 2001
HHS	Carcinogenicity classification	No data	NTP 2016a
EPA	Carcinogenicity classification	No data	IRIS 2018
	PFOA	Suggestive evidence for carcinogenic potential	EPA 2016e

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference
	PFOS	Suggestive evidence for carcinogenic potential	EPA 2016f
	10^{-6} Cancer risk		
	PFOA	0.5 µg/L	EPA 2016e
IARC	Carcinogenicity classification		IARC 2017
	PFOA	Group 2B[c]	
	Occupational		
ACGIH	TLV-TWA		
	APFO	0.01 mg/m^3 [d]	ACGIH 2001
OSHA	PEL (8-hour TWA) for general industry, shipyards and construction	No data	OSHA 2018b 29 CFR 1910.1000, Table Z-1
	PEL (8-hour TWA) for shipyards and construction	No data	OSHA 2018a 29 CFR 1915.1000, Table Z
	PEL (8-hour TWA) for construction	No data	OSHA 2018c 29 CFR 1926.55, Appendix A
NIOSH	REL (up to 10-hour TWA)	No data	NIOSH 2016
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2016b
DOE	PACs-air		DOE 2018b
	PFOA		
	PAC-1[c]	1.1 mg/m^3	
	PAC-2[c]	12 mg/m^3	
	PAC-3[c]	75 mg/m^3	
	PFBA		
	PAC-1[c]	0.5 mg/m^3	
	PAC-2[c]	5.5 mg/m^3	
	PAC-3[c]	33 mg/m^3	

[a]The Substances Added to Food inventory replaces EAFUS and contains the following types of ingredients: food and color additives listed in FDA regulations, flavoring substances evaluated by FEMA or JECFA, GRAS substances listed in FDA regulations, substances approved for specific uses in food prior to September 6, 1958, substances that are listed in FDA regulations as prohibited in food, delisted color additives, and some substances "no longer FEMA GRAS".
[b]A3: confirmed animal carcinogen with unknown relevance to humans.
[c]Group 2B: possibly carcinogenic to humans.
[d]Skin notation.
[e]Definitions of PAC terminology are available from DOE (2018a).

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline level; AIHA = American Industrial Hygiene Association; APFO = ammonium perfluorooctanoate; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = Drinking Water Equivalent Level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FAO = Food and Agriculture Organization of the United Nations; FDA = Food and Drug Administration; FEMA = Federal Emergency Management Agency; GRAS = generally recognized as safe; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; JEFCA = Joint FAO/WHO Expert Committee on Food Additives; NIOSH = National Institute for Occupational Safety and Health; NTP = National

Table 7-1. Regulations and Guidelines Applicable to Perfluoroalkyls

Agency	Description	Information	Reference

Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; PFBA = perfluorobutanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TWA = time-weighted average; WHO = World Health Organization

Table 7-2. Select State Drinking Water Guidelines for Perfluoroalkyls[a]

	Value (ppb or µg/L)						
Value type	PFOA	PFOS	PFBS	PFBA	PFNA	PFHxS	Reference
Connecticut							
Drinking water action level	0.07[b]	0.07[b]	ND	ND	0.07[b]	0.07[b]	Connecticut DPH 2016
Maine							
Maximum exposure guideline for drinking water	0.07[c]	0.07[c]	ND	ND	ND	ND	MECDC 2016
Massachusetts							
Drinking water guidelines	0.07[b]	0.07b[a]	ND	ND	0.07[b]	0.07[b]	MassDEP 2018
Michigan							
Residential and nonresidential drinking water criteria	0.07[d]	0.07[d]	ND	ND	ND	ND	Michigan DEQ 2018a
Human noncancer drinking water value	0.42	0.011	ND	ND	ND	ND	Michigan DEQ 2016
Minnesota							
Health risk limit[e]							MDH 2019
Short-term	0.035	ND	ND	7	ND	ND	
Subchronic	0.035	ND	9	7	ND	ND	
Chronic	0.035	0.3	7	7	ND	ND	
Health-based value[e]							
Short-term	ND	0.015	3	ND	ND	0.047	
Subchronic	ND	0.015	3	ND	ND	0.047	
Chronic	ND	0.015	2	ND	ND	0.047	
Nevada							
Basic comparison level	0.667	0.667	667	ND	ND	ND	NDEP 2017
New Jersey							
Health-based chronic maximum contaminant level	0.014 (recommend-ation)	0.013 (recommend-ation)	ND	ND	0.013	ND	DWQI 2018a, 2017, 2018b, 2015

Table 7-2. Select State Drinking Water Guidelines for Perfluoroalkyls[a]

	Value (ppb or µg/L)						
Value type	PFOA	PFOS	PFBS	PFBA	PFNA	PFHxS	Reference
North Carolina							
Interim maximum allowable concentration in groundwater	2	ND	ND	ND	ND	ND	NC DEQ 2013, NCDENR 2012
Vermont							
Drinking water health advisory	0.02[b]	0.02[b]	ND	ND	0.02[b]	0.02[b]	Vermont DOH 2018

[a]Current as of September 2018.
[b]Value applies to the sum of PFOA, PFOS, PFHxS, PFHpA, and PFNA concentrations.
[c]MECDC notes that according to the EPA lifetime health advisory for PFOA and PFOS, when both PFOS and PFOA are present in drinking water, the combined levels are not to exceed 0.07 ppb.
[d]Value applies to the sum of PFOA and PFOS groundwater concentrations (Michigan DEQ 2018b).
[e]Health risk limits are rule values and health-based values are guidance values. Guidance is developed in-between rulemaking and may update an older rule. As a result, both rules and guidance values may be available for a contaminant (dual guidance) and the two values may be different (MDH 2014).

DEQ = Department of Environmental Quality; DOH = Department of Health; DPH = Department of Public Health; DWQI = Drinking Water Quality Institute; MassDEP = Massachusetts Department of Environmental Protection; MECDC = Maine Center for Disease Control and Prevention; MDH = Minnesota Department of Health; NCDENR = North Carolina Department of Environment and Natural Resources; ND = no data; NDEP = Nevada Division of Environmental Protection; PFBA = perfluorobutanoic acid; PFBS = perfluorobutane sulfonic acid; PFHpA = perfluoroheptanoic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

ATSDR uses the POD/uncertainty factor approach to derive MRLs. Potential PODs are NOAELs, LOAELs, or the BMDL. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals. ATSDR utilizes uncertainty factors to account for uncertainties associated with extrapolating from: (1) a LOAEL to a NOAEL; (2) extrapolating from animals to humans; and (3) to account for human variability (Chou et al. 1998; Pohl and Abadin 1995).

Default values of 10 are used for each of these categories of uncertainty factors; a value of 1 can be used if complete certainty exists for a particular uncertainty factor category. A partial uncertainty factor of 3 can be used when chemical-specific data decreases the uncertainty. On a case-by-case basis, ATSDR also utilizes modifying factors to account for MRL-specific database deficiencies.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

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INTRODUCTION

Overview of Epidemiological Studies

A large number of epidemiological studies have evaluated a wide range of potential health outcomes resulting from exposure to perfluoroalkyls, particularly PFOA and PFOS. The epidemiological studies fall into three broad categories: occupational exposure primarily to airborne PFOA and PFOS, exposure to PFOA-contaminated drinking water by residents living near a PFOA production facility, and general population exposure to background levels of perfluoroalkyls. Most of the occupational exposure studies were conducted in workers at four facilities in Minnesota, Alabama, West Virginia, and the Netherlands. Studies of the highly-exposed residents primarily come from several large-scale studies (C8 Health Project, C8 Health Study) of Mid-Ohio Valley residents living near the Washington Works facility in West Virginia who were exposed to high levels of PFOA in the drinking water. General population studies primarily utilized data collected in NHANES in the United States and several large-scale health studies conducted in Europe.

Most of the epidemiological studies lack environmental monitoring data and there is a potential for multiple sources of exposure (inhalation and oral). However, the majority of the epidemiological studies used serum perfluoroalkyl levels as a biomarker of exposure. One limitation of the C8 Health Studies is that they used blood samples collected in 2005–2006. However, the facility started using PFOA in the 1950s and peak usage was in the 1990s and by 2003, there was an 87% decline in PFOA emissions, as compared to 1999 levels (Emmett et al. 2006a). Therefore, serum PFOA levels measured in 2005–2006 likely do not represent earlier higher exposures, which may have contributed to observed health outcomes. As an alternative to using older serum PFOA levels, several C8 Health Studies estimated serum levels based on data on the release of PFOA from the facility and pharmacokinetic modeling. Of the three categories of subjects examined in the epidemiological studies, workers have the highest potential exposure to perfluoroalkyls, followed by the highly-exposed residents in the Mid-Ohio Valley (referred to as community exposure), and then the general population. In one study of workers at the Washington Works facility in West Virginia, the average serum PFOA level in 2001–2004 was 1,000 ng/mL (Sakr et al. 2007a); the mean PFOA level in community residents (without occupational exposure) near this facility was 423 ng/mL in 2004–2005 (Emmett et al. 2006a). By comparison, the geometric mean concentration of PFOA in the U.S. population was 3.92 ng/mL in 2005–2006 (CDC 2013).

Identification of Adverse Health Effects Based on Epidemiological Studies. Although a large number of epidemiological studies have examined the potential of perfluoroalkyls to induce adverse health effects, most of the studies were cross-sectional in design and do not establish causality. Epidemiological studies have found statistically significant associations between serum perfluoroalkyl levels and several health effects, although the results were not consistent across studies. Many of the studies reported dose-related trends, but these trends were not as apparent when comparing across studies; some effects were observed in populations with background PFOA levels but not in populations with high serum PFOA levels. Given the inconsistencies, ATSDR evaluated whether the preponderance of the data supported an association between perfluoroalkyl exposure and a particular health effect, taking into consideration the consistency of the findings across studies, the quality of the studies, dose-response, and plausibility. It should be noted that although the data may provide strong evidence for an association, it does not imply that the observed effect is biologically relevant because the magnitude of the change is within the normal limits or not indicative of an adverse health outcome. Plausibility depends primarily on experimental toxicology studies that establish a biological mechanism for the observed effects.

Using this approach, the available epidemiological data identify several potential health hazards of PFOA, PFOS, PFHxS, PFNA, and PFDA in humans as listed below.

PFOA

- Pregnancy-induced hypertension/pre-eclampsia
- Increases in serum hepatic enzymes, particularly alanine aminotransferase, and decreases in serum bilirubin levels
- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines
- Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight

PFOS

- Pregnancy-induced hypertension/pre-eclampsia
- Liver damage, as evidenced by increases in serum enzymes and decreases in serum bilirubin levels
- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines
- Small (<20 g or 0.7 ounces per 1 ng/mL increase in blood perfluoroalkyl level) decreases in birth weight

PFHxS

- Liver damage, as evidenced by increases in serum enzymes and decreases in serum bilirubin levels
- Decreased antibody response to vaccines

PFNA

- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines (based on limited evidence)

PFDA

- Increases in serum lipids, particularly total cholesterol and LDL cholesterol
- Decreased antibody response to vaccines

Limitations of Epidemiological Data. There are sufficient epidemiological data to identify possible sensitive targets for many of the perfluoroalkyls; however, there are two major limitations to establishing dose-response relationships for these effects and using the epidemiological studies to derive MRLs: accurate identification of environmental exposure levels producing increased risk for adverse effects (exposure estimates and routes of exposure) and likely co-exposure to mixtures of perfluoroalkyls. Other limitations include the cross-sectional design of the majority of epidemiological studies and the potential that reverse causality contributes to the observed associations.

Uncertainty in Exposure Estimates. In general, epidemiological studies provide a one-time serum perfluoroalkyl concentration, but lack information on actual environmental exposure concentration or doses, routes of exposure, and exposure duration. Although serum perfluoroalkyl levels provide reliable information on recent exposure (weeks to years, depending on the elimination $t_{1/2}$ for the perfluoroalkyl), they likely do not reflect historical exposure levels or exposure levels at the onset of the effect. This is especially true for occupational exposure cohorts where past exposure levels were higher before industrial hygiene improved and in the C8 community studies since peak PFOA levels in drinking water occurred at least 10 years prior to the onset of the studies. Additionally, data from NHANES suggest that some

perfluoroalkyl (PFOA, PFOS, PFHxS, and PFDA) levels are declining in the general population; for example, the geometric mean serum levels of PFOA and PFOS declined from 5.2 and 30.4 ng/mL, respectively, in 1999–2000 to 1.56 and 4.72 ng/mL in 2015–2016. In contrast, levels of PFNA have increased during that time frame; the geometric mean went from 0.5 ng/mL in 1999–2000 to 1.26 ng/mL in 2009–2010 and then decreased to 0.675 ng/mL in 2015–2016. Most studies do not provide adequate information to determine whether perfluoroalkyl levels reflect a steady state and relatively constant exposure, since most designs only include a single measurement. An added uncertainty occurs in studies that used maternal serum levels as the biomarker of exposure for effects in children or for effects on fertility.

It is assumed that workers were primarily exposed via inhalation; however, oral exposure may have also contributed to the total perfluoroalkyl body burden, particularly since workers frequently lived in communities with elevated levels of PFOA in the drinking water. It has been determined that drinking water was the primary source of perfluoroalkyls in residents living near a PFOA facility (Emmett et al. 2006a); however, it is likely that airborne PFOA contributed to overall body burden. Drinking water is the likely primary route of exposure for the general population.

Uncertainty due to Co-Exposure to Other Perfluoroalkyls. Based on NHANES data, the U.S. general population is exposed to a variety of perfluoroalkyls. A number of studies reported a high degree of correlation between different perfluoroalkyls; however, most studies did not control for exposure to other perfluoroalkyls. Given that many of the perfluoroalkyls have similar targets of toxicity and possible mechanisms of action, it is likely that several perfluoroalkyls contributed to the observed effects. The potential interactions between different perfluoroalkyls have not been fully elucidated.

In summary, the epidemiological databases for several perfluoroalkyls provide valuable information on hazard identification; however, uncertainties regarding doses associated with adverse effects and possible interactions between compounds preclude use of these data to derive MRLs.

Overview of Laboratory Animal Studies

Laboratory animal studies are available for 11 perfluoroalkyls (no data were located for PFHpA); however, more than 70% of the studies examined PFOA and/or PFOS. The laboratory animal studies primarily involved oral exposure and examined a wide range of potential health outcomes. The primary health effects observed in laboratory animals were liver toxicity, developmental toxicity, and immune toxicity. Other effects typically observed at higher doses included weight loss, histological alterations in reproductive tissues, and histological alterations in the thyroid gland. The sensitive targets of toxicity identified in the laboratory animals are similar to those observed in epidemiological studies.

Limitations of Laboratory Animal Studies for Derivation of MRLs. Use of controlled animal studies eliminates the uncertainties regarding effective doses and co-exposure to other perfluoroalkyls. However, there are uncertainties associated with derivation of MRLs based on animal studies, in part, because of large interspecies differences in the toxicokinetics of perfluoroalkyls for which mechanisms are not completely understood. Available information on the toxicokinetics of perfluoroalkyls in humans, nonhuman primates, and various rodent species indicate that elimination rates (and very likely elimination mechanisms and hormonal regulation of these mechanisms) vary substantially across chemical species (i.e., carbon chain length) and animal species (i.e., slower in humans compared to nonhuman primates and rodents), and show pronounced sex differences within certain species (e.g., faster elimination in female rats). As a result, there is some uncertainty associated with extrapolation of external dose-response relationships from animals to humans. Several PBPK models of PFOA and PFOS have been reported that simulate the substantial differences in pharmacokinetics of these compounds between humans and nonhuman primates or between humans and rats. These include human models for PFOA and PFOS (see

Section 3.1.5). An additional uncertainty in the animal data is the relevance of effects associated with activation of PPARα. Many of the effects observed in rodents, particularly liver and developmental effects, involve the activation of PPARα; humans and nonhuman primates are less responsive to PPARα agonists than rats and mice. However, studies in PPARα-null mice suggest that PPARα-independent mechanisms also play a role in the liver, immunological, and developmental toxicity.

MRL Approach

The following approach was used for derivation of MRLs:

- Identify sensitive endpoints from epidemiological studies
- Identify laboratory animal studies that have evaluated dose-response relationships for toxicity targets identified in epidemiological studies
- Estimate a POD using animal serum perfluoroalkyl levels for sensitive endpoints
- Calculate HEDs using the assumption that a serum concentration resulting in an effect in a laboratory animal would also result in an effect in humans. An empirical pharmacokinetic model was used to estimate a human dose associated with this serum concentration for PFOA and PFOS. Measured serum concentrations in laboratory animal studies were used to calculate the HEDs for PFHxS and PFNA.
- Apply appropriate uncertainty factors informed by comparison of the POD to serum perfluoroalkyl levels reported in epidemiological studies

Rationale for Internal Dose Metric Used in Dosimetry Extrapolation. The time-weighted average serum concentration (C_{TWA}) was selected as the internal dose metric for dose-response modeling and dosimetry extrapolation. The C_{TWA} was used rather than the maximum concentration (C_{max}) for the following reasons:

- C_{TWA} provides a better representation of the history of exposure in the principal studies selected for the MRLs for PFOA (Koskela et al. 2016) and PFOS (Luebker et al. 2005a). The relatively slow elimination of PFOA and PFOS predicted in mice and rats results in a build-up of serum concentrations during the exposure duration. As a result, the C_{max} is predicted to occur soon after the last dose in these studies (based on the Wambaugh et al. 2013 model).

- The assumption that must be accepted to justify using the C_{max} is that only the last dose of PFOA or PFOS, which results in the C_{max}, determines the toxicity outcome, and the earlier exposure history contributes only by building up the levels to the C_{max}.

- The available data on the toxicity of PFOA and PFOS do not provide convincing evidence that toxicity outcomes are more likely to be determined by C_{max} rather than the exposure history, represented by C_{TWA}.

- Given that C_{max} is predicted to exceed C_{TWA} in the principal studies (based on the Wambaugh et al. 2013 model), the resulting HED that achieves a steady-state serum concentration equal to the C_{max} would be larger than the corresponding HED based on C_{TWA}. In the absence of strong evidence for C_{max} being a more appropriate dose metric than C_{TWA}, use of C_{TWA} for dosimetry extrapolation is an appropriate health-protective assumption in the derivation of the MRL.

Predicting Mean Serum PFOA and PFOS Concentrations in Laboratory Animals. TWA serum concentrations corresponding to external doses (mg/kg/day) and exposure durations (days) were predicted with a pharmacokinetic (PK) model for the animal species, strain, and sex used in the studies (Wambaugh et al. 2013). The Wambaugh et al. (2013) model was selected over other available

pharmacokinetic models for the following reasons: (1) it provided a single model structure (parameters) for simulating kinetics of PFOA and PFOS; (2) Wambaugh et al. (2013) derived parameter values for sex-specific species and strains used in the candidate principal studies for the oral MRLs (female and male CD1 mouse, C57BL/6 mouse, Sprague-Dawley rat, cynomolgus monkey); and (3) models were calibrated using Bayesian parameter estimation based on multiple PFOA (n=6) and PFOS (n=2) pharmacokinetics studies for specific species, strains, and sexes; and were then evaluated by comparing predicted and observed serum concentrations from toxicology studies performed on the same species, strains, and sex. Predicted and observed terminal serum concentrations for PFOA and PFOS agreed within a factor of 2, showed strong linear correlation, and distributed symmetrically along the line of identity, suggesting minimal bias in predictions across species, strains, and sexes.

The TWA serum concentration was calculated as follows (Equation A-1):

$$C_{TWA} = \frac{C_{AUC}}{ED} \qquad \text{Eq. (A-1)}$$

where C_{TWA} is the predicted TWA serum concentration (mg/L), C_{AUC} is the predicted area under the curve (AUC) of the serum concentration-time profile for the exposure (mg hour/L), and *ED* is the exposure duration (hour). Gavage studies were simulated as a single dose (e.g., gavage) given once every 24 hours. Daily drinking water exposures were simulated as a 12-hour period of dosing followed by 12 hours with no dosing. This assumes that the animals consumed water during a 12-hour active period and received the total daily dose during this 12-hour period. The dosing interval was 0.1 hour.

The Wambaugh et al. (2013) model was originally implemented in R (v2.10.0) and was migrated to MATLAB (vR2016) for calculations of MRLs. Wambaugh et al. (2013) reported mean and confidence limits for parameter values estimated from a Bayesian Markov Chain Monte Carlo (MCMC) analysis. The posterior means were used as point estimates for parameters in the MATLAB version. Function of the point estimate implementation in MATLAB was verified by comparing predictions of C_{AUC} obtained from the MATLAB version with predictions from the MCMC analysis reported in EPA (2016e, 2016f). This comparison for PFOA included a total of 18 predictions of C_{AUC} for female CD-1 mice (Lau et al. 2006; Wolf et al. 2007), female C57Bl6 mice (DeWitt et al. 2008), and male Sprague-Dawley rats (Butenhoff et al. 2004b). The r^2 for MATLAB vs R predictions of C_{AUC} was 0.99 and the average relative percent difference (MATLAB-R) was 2.8% (range: -6.6–13.5). The comparison for PFOS included a total of 28 predictions of C_{AUC} for female CD-1 mice (Lau et al. 2003), female Sprague-Dawley rats (Butenhoff et al. 2009b; Lau et al. 2003; Luebker et al. 2005a, 2005b), and male and female Cynomolgus monkeys (Seacat et al. 2002). The r^2 for MATLAB vs R predictions of C_{AUC} was 1.00 and the average relative percent difference (MATLAB-R) was 4.6% (range: -11–20).

Estimating TWA Serum PFHxS and PFNA Concentrations in Laboratory Animals. Because a PK model for predicting the TWA serum concentrations was not identified for PFHxS and PFNA, a TWA serum concentration was estimated from measured serum concentrations. ATSDR estimated the TWA values from the areas under the curve calculated using the trapezoid rule. Since most studies did not report pre-exposure levels, serum concentrations in the control group were used as the baseline concentration.

Estimating HEDs for Perfluoroalkyls. The serum concentration PODs identified from the laboratory animal data were converted to an equivalent dose in humans, which is defined as the continuous ingestion dose (mg/kg/day) that would result in steady-state serum concentrations of perfluoroalkyl equal to the serum concentration (µg/mL) selected as the POD. Although human PBPK models for PFOA and PFOS have been reported, the simpler empirical model was selected for deriving

HEDs for the following reasons. Human PBPK models have not been validated with observations made in humans for which individual exposures were known with sufficient certainty to evaluate confidence in predicting dose-serum concentration relationships. Calibration and validation of human PBPK models have relied on comparing predicted and observed declines in serum concentrations following declines or cessation of exposure (Fàbrega et al. 2014, 2016; Loccisano et al. 2011; Worley et al. 2017b). This approach validates the ability of the models to predict serum concentration half-lives, which can be estimated directly from the observation data and represented in the empirical model. However, it does not validate the ability of the models to predict serum concentration in association with known exposures. Worley et al. (2017b) reported good agreement between the distribution of observed and predicted serum concentrations of PFOA within a study population, when assumptions about variability in exposure and biokinetics were incorporated into the simulations.

The relationship between perfluoroalkyl external dosage (mg/kg/day) and steady-state serum concentration (C_{ss}, mg/L) in humans was estimated assuming a single-compartment first-order model in which elimination kinetics are adequately represented by observed serum elimination $t_{1/2}$ values for the specific perfluoroalkyl, an assumed apparent volume of distribution (V_d, L/kg) and gastrointestinal absorption fraction. In the first-order single-compartment model, continuous exposure will result in a steady-state body burden *(BB_{SS}*, mg/kg) for PFOA or PFOS, which will be distributed in a single volume of distribution to yield a steady-state serum concentration (Equation A-2):

$$C_{SS} = \frac{BB_{SS}}{V_d} \quad \text{Eq. (A-2)}$$

At steady state, the rate of first-order elimination rate (a constant fraction of the body burden, k_e per day) will equal the absorbed dosage (D_{ss}, mg/kg/day) adjusted for gastrointestinal absorption (AF) (Equation A-3):

$$D_{SS} \cdot AF = BB_{SS} \cdot k_e \quad \text{Eq. (A-3)}$$

Rearrangement of Equation A-3 allows calculation of the steady-state body burden corresponding to a given external dosage (Equation A-4):

$$BB_{SS} = \frac{D_{SS} \cdot AF}{k_e} \quad \text{Eq. (A-4)}$$

The relationship between the elimination rate constant *(k_e*, day^{-1}) and the elimination half-life ($t_{1/2}$, day), is given in Equation A-5:

$$k_e = \frac{\ln(2)}{t_{1/2}} \quad \text{Eq. (A-5)}$$

Combining Equations A-2 and A-3 yields an expression relating the external steady-state dosage and steady-state serum concentration (Equation A-6):

$$D_{SS} = \frac{C_{SS} \cdot k_e \cdot V_d}{AF} \quad \text{Eq. (A-6)}$$

The above estimates of C_{SS}/D_{SS} are sensitive to the input parameters, $t_{1/2}$, AF, and V_d. The empirical model used to calculate HEDs is linear; therefore, the change in the HED is approximately proportional to the change in the half-life. A halving of the half-life would result in a doubling of the HED.

PFOA and PFOS. Several studies have estimated PFOA and PFOS half-lives ($t_{1/2}$) in workers (Costa et al. 2009; Olsen et al. 2007a) or highly exposed residents (Bartell et al. 2010). Estimates of the half-lives based on Olsen et al. (2007a) were derived from longitudinal measurements of serum concentrations of PFOA and PFOS in a group of fluorochemical production workers (24 males, 2 females); the estimated half-lives were 3.8 years (95% confidence limit [CL] 3.1–4.4) and 5.4 years (95% CL 3.9–6.9), respectively. Costa et al. (2009) reported a half-life for PFOA of 5.1 years (SD 1.7) for a group of workers (n=16) following their cessation of PFOA production work. A longitudinal study by Bartell et al. (2010) followed serum PFOA concentrations in 200 subjects recruited from the Lubeck Public Service District and Little Hocking Water Association and followed for a period of 6–12 months after mitigation of exposures from drinking water. The estimated half-life for PFOA was 2.3 years (95% CL 2.1–2.4). A fourth study estimated half-lives in a cross-sectional study of residents served by the Lubeck Public Service District and Little Hocking Water Association (Seals et al. 2011). The estimated half-lives ranged from 2.9 to 10.1 years (1,059–3,687 days) for PFOA. Results from the longitudinal studies are shown in Table A-1. For the MRL calculations, the PFOA half-life estimated by Olsen et al. (2007a) was selected over the half-life estimated by Bartell et al. (2010) because the Olsen et al. (2007a) study had a longer follow-up time (>5 years compared to 6–12 months) and estimates of the terminal half-life appear to increase with longer follow-ups because slower kinetics make a larger contribution to the terminal half-life (Seals et al. 2011). This may reflect a larger contribution of slower kinetics or ongoing exposure to the terminal half-life observable with longer follow-ups (Worley et al. 2017a) or other factors such as differences in the age-distribution of the populations studied. The decision to use the longer half-life from Olsen et al. (2007a) is also health protective in that a longer half-life would result in higher predicted serum concentrations for a given intake and, therefore, lower HEDs for a given serum concentration POD. Estimates of the half-life for PFOA and PFOS are most applicable to serum concentrations within the above ranges and would be less certain if applied to serum concentrations substantially below or above these ranges. Serum concentrations during the 5-year observation period in the Olsen et al. (2007a) study are provided in Table A-2.

Table A-1. Half-Life PFOA and PFOS Levels in Humans

PFOA $t_{1/2}$ (days)	PFOS $t_{1/2}$ (days)	Exposure type	Number	Source
1,378	1,976	Occupational	26	Olsen et al. (2007a)
1,862	NA	Occupational	16	Costa et al. (2009)
840	NA	Environmental	200	Bartell et al. (2010)

NA = not available; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Table A-2. Serum PFOA and PFOS Concentrations Measured in Fluorochemical Production Workers

	PFOA (ppb)	PFOS (ppb)
Initial	408 (72, 5,100)	626 (145, 3,490)
Final	148 (17, 2,435)	295 (37, 1,740)

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Source: Olsen et al. 2007a

Estimates of volume of distribution (V_d) are based on non-compartmental modeling of serum concentration kinetics in monkeys and are assumed to be applicable to humans at the above serum concentrations (Table A-3).

Table A-3. Apparent Volume of Distribution for PFOA and PFOS

PFOA V_d (L/kg)	PFOS V_d (L/kg)	Source
0.18 (male) 0.20 (female)	NA NA	Butenhoff et al. (2004c)
NA NA	0.20 (male) 0.27 (female)	Chang et al. (2012)
0.3	0.3	Harada et al. (2005a)

NA = not applicable; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid

Numerous studies conducted in various animal models provide evidence for approximately complete absorption of oral doses of PFOA and PFOS (i.e., AF≈1, see Section 3.1.1).

PFHxS. For PFHxS, the estimate of the elimination t½ was derived from longitudinal measurements of serum concentrations of PFHxS in a group of retired fluorochemical production workers (24 males and 2 females) observed for a 5-year period; the estimated half-life was 8.5 years (3,109 days) (Olsen et al. 2007a). The range of initial serum concentrations was 16–1,295 ng/mL (mean of 290 ng/mL), and the final concentrations ranged from 10 to 791 ng/mL (mean of 182 ng/mL). Estimates of the t½ for PFHxS are most applicable to serum concentrations within the above ranges and would be less certain if applied to serum concentrations substantially below or above these ranges.

Estimates of volume of distribution (V_d) are based on non-compartmental modeling of serum concentration kinetics in monkeys and are assumed to be applicable to humans at the above serum concentrations. Sundström et al. (2012) estimated the apparent Vd for PFHxS at 0.287 L/kg for male Cynomolgus monkeys and at 0.213 L/kg for female Cynomolgus monkeys.

Few studies have been conducted in animals that provide estimates for a gastrointestinal absorption factor of oral doses of PFHxS. Sundström et al. (2012), based on comparison of the AUC for oral and intravenous administration, estimated an oral absorption fraction for PFHxS (administered as a single 10 mg/kg dose) of 50% in female rats. However, as the authors point out, this estimate may not be reliable due to the short (24 hours) observation period (Sundström et al. 2012) and that "female C_{max} values did not differ significantly between the oral and IV doses, and T_{max} after oral dosing was estimated to be at approximately 30 min." These latter observations suggest approximately complete bioavailability. The AUC for male rats following oral exposure was not available, and the AUC after

intravenous administration was done in only one male rat (Sundström et al. 2012). A study conducted by Kim et al. (2016) in rats estimated an approximately 100% oral bioavailability based on the C_{max} value and AUC comparison between oral and intravenous doses. Therefore, an absorption fraction (AF) of 1 was used for PFHxS.

PFNA. For PFNA, the elimination half-life estimates were derived by paired blood and urine samples (n=86) from Chinese adults in a study that measured the concentrations of a number of perfluoroalkyls, including PFNA (Zhang et al. 2013). The participants were first divided into four groups; young females (age ≤50 years, n=20), older females (>50 years, n=19), young males (≤50 years, n=32), and older males (>50 years, n=15). The group of young females had significantly lower levels of perfluoroalkyls than the other groups; therefore, the three other groups were combined. The lower perfluoroalkyl levels were likely due to the elimination via menstrual bleeding, pregnancy, and lactation. The estimated arithmetic mean elimination half-lives for the young female group and the combined male and older female group for PFNA were 2.5 and 4.3 years (913 and 1,570 days), respectively.

Toxicokinetics parameters for perfluorocarboxylic acids, among them PFOA and PFNA analogs, were investigated in rats by Ohmori et al. (2003). The authors estimated that the V_d values in steady state were not much different between the perfluorocarboxylic acids and between the sexes. Based on this, the estimated volume of distribution for PFNA in humans will be assumed to be the same for PFOA, 0.2 L/kg.

There are no studies on absorption of PFNA in humans. In rodents, oral absorption occurs rapidly as indicated by its presence in the serum of rodents soon after oral administration (Tatum-Gibbs et al. 2011). Therefore, based on animal studies of PFNA and other perfluorocarboxylic acid analogs, as well as sufficient findings of PFNA and other perfluorocarboxylic acids in human blood, it can be assumed that PFNA is well absorbed after oral exposure; therefore, an AF of 1 was used.

Model Input Parameters. The first-order one-compartment model input parameters ($t_{1/2}$, V_d, and AF) are provided in Table A-4.

Table A-4. First Order One-Compartment Model Parameters

Parameter	PFOA	PFOS	PFHxS	PFNA
Serum elimination half-life[a]; $t_{1/2}$ (day)	1,400[a]	2,000[a]	3,100[a]	900[b]
Serum elimination rate constant[c], k_e (day^{-1})	4.95×10^{-4}	3.47×10^{-4}	2.23×10^{-4}	7.59×10^{-4}
Gastrointestinal absorption fraction[d], AF	1	1	1	1
Apparent volume of distribution, V_d (L/kg)	0.2[e]	0.2[e]	0.287[f]	0.2[e]

[a]Estimates from Olsen et al. (2007a).
[b]Estimates from Zhang et al. (2013) for young females.
[c]Calculated using Equation 5.
[d]Based on studies in rodents and nonhuman primates.
[e]Estimates based on studies in nonhuman primates (Butenhoff et al. 2004c; Chang et al. 2012; Harada et al. 2005a).
[f]Estimates based on studies in nonhuman male primates (Sundström et al. 2012).

PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonic acid; PFHxS = perfluorohexane sulfonic acid; PFNA = perfluorononanoic acid

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: Derivation of an inhalation MRL was precluded because inhalation-specific PBPK/pharmacokinetic model parameters are not available for PFOA and none of the studies reported serum PFOA concentrations.

Four studies have examined the acute toxicity of airborne PFOA in laboratory animals (Griffith and Long 1980; Kennedy et al. 1986; Staples et al. 1984). The observed effects included excessive salivation and eye and nose irritation in rats exposed to 18,600 mg/m^3 for 1 hour (Griffith and Long 1980), weight loss and pulmonary edema in rats exposed to 380 mg/m^3 for 4 hours (Kennedy et al. 1986), weight loss in rats exposed nose-only to 84 mg/m^3 6 hours/day, 5 days/week for 2 weeks (Kennedy et al. 1986), and decreases in maternal weight gain at 10 mg/m^3 and maternal deaths and decreases in neonatal body weight at 25 mg/m^3 in rats exposed 6 hours/day on GDs 6–15 (Staples et al. 1984). The 2-week study also reported increases in liver weight and hepatocellular hypertrophy in rats exposed to 7.6 mg/m^3.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFOA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFOA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFOA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFOA.

Rationale for Not Deriving an MRL: An acute-duration oral MRL cannot be derived for PFOA because the modeling approach used for estimating HEDs cannot be used to estimate acute human exposure where the exposure duration of 14 days is 1% of the elimination half-life in humans.

Acute-duration oral studies are available in rats and mice and provide information on body weight, hepatic, immunological, reproductive, and developmental effects. The liver effects consisted of increases in liver weight, hepatocellular hypertrophy, and/or decreases in serum cholesterol and triglycerides in rats and mice exposed to ≥1 mg/kg/day (Cook et al. 1992; Elcombe et al. 2010; Haughom and Spydevold 1992; Ikeda et al. 1985; Kawashima et al. 1995; Kennedy 1987; Liu et al. 1996; Pastoor et al. 1987; Iwai and Yamashita 2006; Permadi et al. 1992, 1993; Vetvicka and Vetvickova 2013; White et al. 2009; Wolf et al. 2007; Xie et al. 2003; Yang et al. 2000, 2001, 2002b). Consistent with the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria), the liver weight increases and hypertrophy observed in rats and mice were not considered relevant to human risk assessment. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

The immunological effects consisted of impaired responses to T-dependent antigens, such as sRBCs, altered antibody response, and decreases in spleen and thymus weights at 11.5 mg/kg/day and higher (DeWitt et al. 2009; Vetvicka and Vetvickova 2013; Yang et al. 2001, 2002a). Information on the potential reproductive toxicity of PFOA is limited to three studies that reported increases in serum estradiol levels in rats exposed to ≥2 mg/kg/day for 14 days (Biegel et al. 1995; Cook et al. 1992; Liu et al. 1996). A number of studies have evaluated the developmental toxicity of PFOA. In the only acute-duration developmental toxicity study in rats, no alterations in fetal body weight or malformations were observed at 100 mg/kg/day (Staples et al. 1984). Mice appear to be more sensitive to PFOA's developmental toxicity; observed effects include decreases in litter weight (Hu et al. 2010), decreases in pup body weight (White et al. 2007, 2009; Wolf et al. 2007), alterations in spontaneous activity (Johansson et al. 2008), increases in resorbed embryos (Chen et al. 2017b), and delays in mammary gland development (White et al. 2007, 2009; Wolf et al. 2007). The lowest LOAEL for developmental effects in mice was 0.5 mg/kg/day for decreased litter weight. A list of the NOAEL and LOAEL values for the immunological, reproductive, and developmental effects is presented in Table A-5.

Table A-5. Summary of the Adverse Effects Observed in Laboratory Animals Following Acute-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 10 days		11.5	Decreased spleen and thymus weights	Yang et al. 2001
Mouse 10 days	7.5	15	Altered response to sRBC	DeWitt et al. 2009
Mouse 7 days		20	Altered response to sRBC, decreased antibody formation	Vetvicka and Vetvickova 2013
Mouse 7 days		24	Decreased response to horse red blood cells	Yang et al. 2002a
Reproductive				
Rat 14 days	0.2	2	2-Fold increase in serum estradiol levels	Liu et al. 1996
Mouse GDs 1–7		2.5	Decrease in the number of corpora lutea	Chen et al. 2017b
Rat 14 days	1	10	63% increase in serum estradiol levels	Cook et al. 1992
Rat 14 days		25	184% increase in serum estradiol levels	Biegel et al. 1995
Developmental				
Mouse GDs 6–17		0.5	Decreased litter weight on PND 2	Hu et al. 2010
Mouse PND 10		0.58	Decreased spontaneous behavior and altered response to cholinergic stimulant	Johansson et al. 2008
Mouse GDs 8–17 or 12–17		5	Altered mammary gland development, decreased pup body weight on PND 20	White et al. 2007
Mouse GDs 8–17		5	Delayed mammary gland development, decreased pup body weight on PND 20	White et al. 2007
Mouse Various GDs		5	Delayed mammary gland development; decreased pup body weight at weaning	White et al. 2009 Wolf et al. 2007

[a]LOAELs are for less serious effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; sRBC = sheep red blood cell

The lowest LOAEL values were identified for developmental effects; Hu et al. (2010) identified a LOAEL of 0.5 mg/kg/day for decreases in litter weight on PND 2 and Johansson et al. (2008) identified a LOAEL of 0.58 mg/kg for decreases in spontaneous behavior (locomotion and total activity) and decreased response to a cholinergic stimulant in adult mice exposed to PFOA on PND 10. Neither study identified NOAEL values.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:**	Perfluorooctanoic acid (PFOA)
CAS Numbers:	335-67-1
Date:	March 2020
Profile Status:	Final
Route:	Oral
Duration:	Intermediate
***MRL*:**	$3x10^{-6}$ mg/kg/day
Critical Effect:	Skeletal alterations in adult offspring
Reference:	Koskela et al. 2016
Point of Departure:	0.000821 mg/kg/day
Uncertainty Factor:	300
LSE Graph Key:	63
Species:	Mouse

MRL Summary: An intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day was derived for PFOA based on skeletal alterations at 13 and 17 months of age in the offspring of mice fed a diet containing PFOA on GD 1 through GD 21 (Koskela et al. 2016). The MRL is based on a HED LOAEL of 0.000821 mg/kg/day and a total uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability).

Selection of the Critical Effect: Intermediate-duration oral studies of PFOA in animals indicate that the liver, immune system, reproductive system, and the developing organism are the primary targets of toxicity because adverse outcomes were observed at lower doses than other effects and have been consistently observed across studies. A summary of the lower LOAEL values (and associated NOAEL values) for these tissues/systems is presented in Table A-6; given the large number of studies, this table is limited to studies that identified LOAEL values of ≤4 mg/kg/day. Although these studies identified the lowest LOAEL values, not all were considered suitable as the basis of an intermediate-duration oral MRL.

Exposure to low levels of PFOA results in increases in liver weight, hepatocellular hypertrophy, and decreases in serum lipids in rats, mice, and monkeys exposed to PFOA for intermediate durations. The increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in the rodents are likely adaptive responses to peroxisome proliferation and are not considered relevant for human risk assessment (Hall et al. 2012). Consistent with the Hall et al. (2012) criteria, the increases in liver weight and hepatocellular hypertrophy, in the absence of other degenerative alterations, were not considered adverse. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

A small number of animal studies have reported degenerative lesions, lesions to specialty cells, bile duct lesions, or inflammation; these endpoints were considered relevant for human risk assessment (Butenhoff et al. 2004b; Cui et al. 2009; Loveless et al. 2008). The lowest LOAEL for adverse liver effects was 0.96 mg/kg/day for increased liver weight, hepatocellular hypertrophy, and focal necrosis in mice exposed for 28 days (Loveless et al. 2008). *In utero* exposure has also resulted in liver effects in offspring (Filgo et al. 2015a; Quist et al. 2015a); the lowest maternal LOAEL identified in these studies was

0.01 mg/kg/day (Quist et al. 2015a). Because the Quist et al. (2015a) study did not provide incidence data for the reported inflammation, this study was not considered suitable for derivation of an MRL. Hepatic effects consisting of increases in absolute liver weight at ≥3 mg/kg/day and increases in serum triglyceride levels at 30/20 mg/kg/day have also been observed in monkeys administered capsules containing PFOA (Butenhoff et al. 2002); no histological alterations were observed in surviving animals, but hepatocellular degeneration and necrosis was noted in a monkey sacrificed early due to morbidity. The small number of animals examined and early deaths at several dose levels precludes using this study as the basis of an MRL.

Two studies examining the immunotoxicity of PFOA following intermediate-duration oral exposure found decreases in antigen-specific antibody responses in mice exposed for 15 days (DeWitt et al. 2008, 2016); the lowest LOAEL was 1.88 mg/kg/day (DeWitt et al. 2016). Reproductive and developmental toxicity studies have identified very low LOAELs of ≥0.0024 mg/kg/day for delays in mammary gland development in dams and offspring (Macon et al. 2011; Tucker et al. 2015; White et al. 2011). However, the mammary gland effect did not result in an adverse effect on lactational support at maternal doses as high as 1 mg/kg/day, based on normal growth and survival in F2 pups (White et al. 2011). Given that milk production was adequate to support growth, the biological significance of the delayed development of the mammary gland observed at very low doses is uncertain and was not considered a suitable basis for the MRL. Other developmental effects include increases in locomotor activity (Cheng et al. 2013; Goulding et al. 2017; Onishchenko et al. 2011; Sobolewski et al. 2014) at ≥0.1 mg/kg/day, reduced ossification of proximal phalanges and early preputial separation and delayed vaginal opening at ≥1 mg/kg/day (Lau et al. 2006; Yang et al. 2009), altered long bone morphology and decreased bone mineral density in 13- and 17-month-old mice following *in utero* exposure to 0.3 mg/kg/day (Koskela et al. 2016), decreases in pup survival at ≥0.6 mg/kg/day (Abbott et al. 2007; Albrecht et al. 2013), decreases in the number of successful births at ≥3 mg/kg/day (Ngo et al. 2014), and reduced neonatal weight gain and delayed eye opening at ≥3 mg/kg/day (Wolf et al. 2007).

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Hepatic				
Mouse GDs 1–17		0.01	Hepatocellular hypertrophy and periportal inflammation in offspring	Quist et al. 2015a, 2015b
Mouse 28 days	0.29	0.96	Moderate to severe hepatocellular hypertrophy and focal necrosis	Loveless et al. 2008
Mouse GDs 1–17 (examined at 18 months of age)	0.3	1	Increased severity of chronic inflammation in liver	Filgo et al. 2015a, 2015b
Rat 70–90 days	1	3	Increased liver weight, hepatocellular hypertrophy and necrosis	Butenhoff et al. 2004b
Monkey 26 weeks		3	Increased absolute liver weight	Butenhoff et al. 2002

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 15 days	0.94	1.88	Reduced antibody response	DeWitt et al. 2016
Mouse 15 days	1.88	3.75	Reduced sRBC response	DeWitt et al. 2008
Reproductive				
Mouse GDs 1–17		0.0024	Delayed mammary gland development in dams (3-generation study)	White et al. 2011
Mouse GDs 1–17		1	Delayed mammary gland development in dams (single-generation study)	White et al. 2011
Developmental				
Mouse GD 7–PND 22		0.0024	Impaired development of mammary glands	White et al. 2011
Mouse GDs 10–17		0.01	Impaired development of mammary glands	Macon et al. 2011
Mouse GDs 1–17		0.01	Impaired development of mammary glands	Tucker et al. 2015
Mouse GD 7–PND 21		0.1	Neurodevelopmental	Sobolewski et al. 2014
Mouse GDs 1–21		0.3	Altered exploratory behavior in adult offspring; increased global activity in males	Onishchenko et al. 2011
Mouse GDs 1–21		0.3	Skeletal alterations in mature offspring	Koskela et al. 2016
Mouse GDs 1–17		0.3	Impaired development of mammary glands	Macon et al. 2011
Mouse GDs 1–17	0.3	0.6 (SLOAEL)	Decreased pup survival	Abbott et al. 2007
Mouse GDs 1–17		1	Reduced ossification of proximal phalanges and advanced preputial separation	Lau et al. 2006
Mouse 4 weeks starting at PND 21		1	Delayed vaginal opening	Yang et al. 2009
Mouse GDs 1–17	0.3	1	Increased ambulatory activity	Goulding et al. 2017
Rat GD 1–PND 21		1.6	Neurodevelopmental	Cheng et al. 2013
Mouse GDs 1–17	0.1	3	Decreased number of successful births	Ngo et al. 2014

Table A-6. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Mouse GDs 1–17		3	Reduced pups per litter on PND 20	Albrecht et al. 2013
Mouse GDs 1–17		3	Reduced weight gain, delayed eye opening	Wolf et al. 2007

[a]Unless otherwise noted, LOAELs are for less serious effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; SLOAEL = serious LOAEL; sRBC = sheep red blood cell

Selection of the Principal Study: As outlined in the MRL approach section, serum PFOA levels were predicted for the administered doses for most of the studies listed in Table A-6. Mean serum PFOA levels could not be predicted for four studies because pharmacokinetic model parameters were not available for Wistar rats (Cheng et al. 2013), male CD-1 mice (Loveless et al. 2008), or 129S1/SvlmJ wild-type mice (Abbott et al. 2007; Albrecht et al. 2013). A summary of the predicted serum PFOA levels is presented in Table A-7.

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Hepatic				
CD Mouse 28 days	0.29 0.96 9.6	Not calculated	Moderate to severe hepatocellular hypertrophy and focal necrosis at 0.96 mg/kg/day	Loveless et al. 2008
129/Sv Mouse GDs 1–17	0.01 0.1 0.3 1 5	0.423 4.21 12.5 39.2 102	Increased severity of chronic inflammation in liver of offspring aged 18 months at 1 mg/kg/day	Filgo et al. 2015a, 2015b
Sprague-Dawley Rat 70–90 days	1 3 10 30	60.4 136 222 242	Increased liver weight, hepatocellular hypertrophy and necrosis at 10 mg/kg/day	Butenhoff et al. 2004b
Cynomolgus Monkey 6 months	3 10 20/30	68.5 93.8 113	Increased liver weight at 3 mg/kg/day	Butenhoff et al. 2002

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Immunological				
C58BL/6N Mouse 15 days	0.94	21.4	Reduced antibody response at 1.88 mg/kg/day	DeWitt et al. 2016
	1.88	42.5		
	3.75	58.4		
	7.5	83.5		
C57BL/6N Mouse 15 days	0.94	21.4	Reduced sRBC response at 3.75 mg/kg/day	DeWitt et al. 2008
	1.88	42.5		
	3.75	58.4		
	7.5	83.5		
	15	109		
	30	149		
Developmental				
C57BL/6 Mouse GD 7–PND 21	0.1	2.23	Neurodevelopmental effects (increased horizontal and vertical activity and decreased resting activity) at 0.1 mg/kg/day	Sobolewski et al. 2014
C57BL/6 Mouse GDs 1–21	0.3	8.29	Skeletal alterations at 0.3 mg/kg/day	Koskela et al. 2016
C57BL/6 Mouse GDs 1–21	0.3	8.29	Neurodevelopmental (decreased number of inactive periods, altered novelty induced activity) at 0.3 mg/kg/day	Onishchenko et al. 2011
129S1/SvlmJ Mouse GDs 1–17	0.1	Not calculated[a]	Decreased pup survival at 0.6 mg/kg/day	Abbott et al. 2007
	0.3			
	0.6			
	1			
	3			
	5			
	10			
	20			
CD-1 Mouse GDs 1–17	1	39.2	Reduced ossification of proximal phalanges and advanced preputial separation at 1 mg/kg/day	Lau et al. 2006
	3	83.6		
	5	102		
	10	125		
	20	155		
	40	205		

Table A-7. Summary of the Predicted TWA Serum PFOA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOA (µg/mL)	Effect	Reference
Mouse 4 weeks starting at PND 21	1 5 10	Not calculated	Delayed vaginal opening	Yang et al. 2009
Mouse GDs 1–17	0.1 0.3 1	4.21 12.5 39.2	Increased ambulatory activity at 1 mg/kg/day	Goulding et al. 2017
Wistar Rat GD 1–PND 21	1.6	Not calculated	Neurodevelopmental effects (increased locomotor activity in males and decreased activity in females) at 1.6 mg/kg/day	Cheng et al. 2013
C57BL/6J Mouse GDs 1–17	0.1 3	2.43 62.0	Decreased number of successful births at 3 mg/kg/day	Ngo et al. 2014
SV/129 Mouse GDs 1–17	3	Not calculated[b]	Reduced pups per litter on PND 20 at 3 mg/kg/day	Albrecht et al. 2013
CD-1 Mouse GDs 1–17	3 5	84.8 102	Reduced weight gain, delayed eye opening at 3 mg/kg/day	Wolf et al. 2007

[a]Reported serum PFOA concentrations at weaning for the dams that did not have pups which survived to weaning were 4.4, 10.4, 17.4, 26.3, 76.6, 72.4, and 68.2 µg/mL in the 0.1, 0.3, 0.5, 1, 5, 10, and 20 mg/kg/day group, respectively.
[b]Reported serum PFOA concentration was 17 µg/mL for dams treated with 1.6 mg/kg/day.

GD = gestation day; PFOA = perfluorooctanoic acid; PND = postnatal day; sRBC = sheep red blood cell; TWA = time-weighted average

Selection of the Point of Departure for the MRL: The NOAEL/LOAEL and the benchmark dose (BMD) approaches were utilized to identify potential PODs for derivation of the intermediate-duration oral MRL for PFOA. The only datasets with predicted TWA serum PFOA levels amenable to BMD modeling were from the DeWitt et al. (2008, 2016) immunotoxicity studies and Lau et al. (2006) developmental toxicity study. The Sobolewski et al. (2014), Onishchenko et al. (2011), Koskela et al. (2016), Ngo et al. (2014), and Wolf et al. (2007) studies were not considered for BMD modeling because only one or two PFOA doses were tested. No adequate BMD models adequately fit the data from the Lau et al. (2006) study. Adequate fit was found for the DeWitt et al. (2008, 2016) studies; the BMD modeling results are presented at the end of this section.

HEDs were calculated for each potential PODs (NOAEL, LOAEL, or BMD value) identified in laboratory animal studies using the first order single-compartment model previously discussed and the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. The HEDs for each POD are presented in Table A-8. The potential POD_{HED} values were divided by an uncertainty factor to calculate candidate MRLs; these values are also presented in Table A-8. The candidate MRLs range from $7.4x10^{-7}$ mg/kg/day for neurodevelopmental effects in mice

(Sobolewski et al. 2014) to $4.5x10^{-4}$ mg/kg/day for liver effects in male rats (Butenhoff et al. 2004b). The lowest LOAEL (expressed as predicted serum concentration) was identified in the Sobolewski et al. (2014) study, which found neurodevelopmental effects in mouse offspring at predicted serum PFOA concentration of 2.23 µg/mL. However, this study was not considered suitable as the basis of the MRL because the subroute and vehicle used for the controls (peanut oil with anisole administered via gavage) were different from the PFOA group (PFOA dissolved in water and added to diet). Rather, the Onishchenko et al. (2011) and Koskela et al. (2016) studies, which identified the second lowest LOAEL (serum PFOA concentration) of 8.29 µg/mL were considered. In the Onishchenko et al. (2011) study, circadian activity was assessed using a TrafficCage in which all animals in the group were placed in a single cage and activity was measured. Thus, activity was only measured on a group basis and it is possible that one animal could skew the results. Thus, this study was not considered a suitable basis for an MRL.

Table A-8. Summary of Potential Points of Departures (PODs) and Human Equivalent Doses (HEDs) for Intermediate-Duration Oral MRL for PFOA

Endpoint (reference)	Predicted serum concentrations (µg/mL) NOAEL or BMDL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Neurodevelopmental effects (increased horizontal and vertical activity and decreased resting activity) in mice (Sobolewski et al. 2014)		2.23	0.000221	300[b]	$7.4x10^{-7}$
Neurodevelopmental effects (decreased number of inactive periods, altered novelty induced activity) in mice (Onishchenko et al. 2011)		8.29	0.000821	300[b]	$2.7x10^{-6}$
Skeletal alterations in mice (Koskela et al. 2016)		8.29	0.000821	300[b]	$2.7x10^{-6}$
Decreased number of successful births in mice (Ngo et al. 2014)	2.43	62.0	0.000241	30[c]	$8.0x10^{-6}$
Reduced ossification of proximal phalanges and advanced preputial separation in mice (Lau et al. 2006)		39.2	0.00388	300[b]	$1.3x10^{-5}$
Increased ambulatory activity (Goulding et al. 2017)	12.5	39.2	0.00124	30[c]	$4.1x10^{-5}$
Reduced weight gain and delayed eye opening (Wolf et al. 2007)		84.8	0.00840	300[b]	$2.8x10^{-5}$
Reduced response to dinitrophenyl-ficoll (DNP) antigen in female mice (DeWitt et al. 2016)	12.23 ($BMDL_{1SD}$)		0.00121	30[c]	$4.0x10^{-5}$
Increased severity of chronic inflammation in liver of offspring aged 18 months (Filgo et al. 2015a, 2015b)	12.5	39.2	0.00124	30[c]	$4.1x10^{-5}$

Table A-8. Summary of Potential Points of Departures (PODs) and Human Equivalent Doses (HEDs) for Intermediate-Duration Oral MRL for PFOA

Endpoint (reference)	Predicted serum concentrations (µg/mL) NOAEL or BMDL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Reduced response to sRBC in female mice (DeWitt et al. 2008)	33.49 ($BMDL_{1SD}$)		0.00332	30[c]	$1.1x10^{-4}$
Increased liver weight, hepatocellular hypertrophy, and necrosis in male rats (Butenhoff et al. 2004b)	136	222	0.0135	30[c]	$4.5x10^{-4}$

[a]HED calculated using Equation A-6 where C_{ss} is the serum concentration associated with the NOAEL or BMDL or the LOAEL if there was no NOAEL or BMDL, K_e=4.95x10^{-4}; V_d=0.2, and AF=1.
[b]UF of 10 for extrapolation from a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and UF of 10 for human variability.
[c]UF of 3 for extrapolation from animals to humans with dosimetric adjustments, and 10 for human variability.

BMDL = lower confidence limit on the BMD; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; POD = point of departure; sRBC = sheep red blood cell; UF = uncertainty factor

Summary of the Principal Study:

Koskela A, Finnila MA, Korkalainen M, et al. 2016. Effects of developmental exposure to perfluorooctanoic acid (PFOA) on long bone morphology and bone cell differentiation. Toxicol Appl Pharmacol 301:14-21.

Pregnant C57BL/6/Bk1 mice were exposed to PFOA (96% pure) in food at dose levels of 0 mg/kg/day (n=10) or 0.3 mg/kg/day (n=6) from GD 1 throughout pregnancy (presumed GD 21). PFOA was dissolved in ethanol and applied to palatable food in volumes adjusted according to individual body weights to provide 0.3 mg/kg/day, followed by evaporation of ethanol; controls received food with ethanol applied and then evaporated. It is noted that litter mates of these offspring were examined for neurobehavioral effects in a study conducted by Onishchenko et al. (2011). Groups of five female offspring were sacrificed at either 13 or 17 months of age. The following parameters were used to assess toxicity: body weight and morphometric/biochemical properties in bone (femurs and tibias) of offspring.

As reported in Onishchenko et al. (2011), no differences in dams weight gain, litter size or sex ratio, or pup body weight or brain weight at birth were observed; significant increases in pup liver weight was observed in the PFOA group. Offspring body weight was significantly higher in comparison with controls at 13 and 17 months of age (9.9 and 7.8%, respectively). In 17-month-old offspring, there was a 6.8% increase in periosteal area of the femoral cortical bone and increases in the peri- and endosteal perimeters (3.2 and 5.2%, respectively) and the marrow area (10.0%); an increase in medullary area was also observed. There were no differences in femoral cortical bone area or femoral mineral density. In the tibia, the total area inside the periosteal envelope and the periosteal perimeter were increased (4.9 and 3.5%, respectively). Although the investigators noted in the text that tibial medullary areas were "essentially the same between groups," data in Figure 2 of the paper show a significant increase at 17 months. Significant decreases in tibial mineral density were observed at 13 and 17 months. There

were no significant differences in the tibial medullary area or the endosteal perimeter. There was a trend for increasing maximum force (Fmax); however, the effect was not statistically significant. There were no significant effects on any other measured biochemical parameter in the femur or tibia (stiffness, maximum energy, absorption). Concentrations of PFOA in the femurs and tibias of treated animals were significantly higher (4–5 times) than controls at 13 and 17 months. Koskela et al. (2016) suggested that the PFOA-induced increase in body weight gain may have indirectly affected bone homeostasis, but noted that *in vitro* data provide evidence of a direct effect on osteoblasts and osteoclasts.

Strengths and Weaknesses: The Koskela et al. (2016) study has a number of strengths including examination of several measures of bone status tested at different ages, measurement of bone PFOA levels, and tests to evaluate potential mechanisms of action. To evaluate whether developmental exposure resulted in bone damage in mature animals, the study evaluated bone morphology (periosteal, cortical, and medullary areas and bone mineral density) and bone biomechanical properties (stiffness, maximum force, and maximum energy); all tests were conducted on femur and tibia bone. Measurement at two ages (13 and 17 months) allowed for an evaluation of whether the effect of PFOA on bone changed as the animals aged. The companion *in vitro* study of osteoclasts and osteoblasts provided mechanistic support for the *in vivo* findings. Additionally, the *in vitro* study evaluated four PFOA concentrations and found concentration-related differences.

There are several study limitations that affect the interpretation of the study results; these include the small number of animals tested, use of only one PFOA dose level, inadequate reporting of dietary PFOA levels, and lack of measured serum PFOA levels. Tests of potential alterations in bone mineral density and bone biomechanical properties were only evaluated in 5–6 female offspring per group; however, support for the finding comes from the consistency of the findings at 13 and 17 months of age. The use of only one PFOA dose level does not allow for the establishment of dose-response relationships. This study limitation is mitigated by the extensive intermediate-duration oral exposure database, which allows for an overall assessment of dose-response. The dams were exposed to PFOA dissolved in alcohol and sprayed onto the food pellets. The study did not measure the amount of residual alcohol or the actual amount of PFOA on the food pellets. Koskela et al. (2016) measured PFOA levels in the tibias and femurs but did not measure serum PFOA levels. ATSDR estimated the TWA serum PFOA concentrations using the Wambaugh et al. (2013) model. The lack of measured serum PFOA levels did not allow for validation of whether the model accurately predicted serum levels; the model was validated using data from other intermediate-duration PFOA studies in rats and mice.

Calculation of Internal Dosimetric: TWA serum PFOA concentrations corresponding to external doses and exposure durations were predicted from a pharmacokinetic model (Wambaugh et al. 2013) using animal species-, strain-, and sex-specific parameters (see MRL approach section for details).

Human Equivalent Dose: HEDs were calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFOA equal to the serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details).

Uncertainty Factor: The $LOAEL_{HED}$ is divided by a total uncertainty factor of 300:

- 10 for the use of a LOAEL
- 3 for extrapolation from animals to humans with dosimetric adjustment
- 10 for human variability

$MRL = LOAEL_{HED} \div UFs$
$0.000821 \text{ mg/kg/day} \div (10 \times 3 \times 10) = 3 \times 10^{-6} \text{ mg/kg/day}$

Other Additional Studies or Pertinent Information that Lend Support to this MRL: *In vitro* studies conducted by Kosela et al. (2016) found that at lower concentrations (0.1–10 μM), PFOA stimulated osteoblast differentiation, as evidenced by increased osteocalcin mRNA expression and increased calcium secretion. At higher PFOA levels (>100 μM), osteocalcin expression and calcium secretion were decreased. Lower concentrations of PFOA (0.1–1.0 μM) also increased the number of osteoclasts and increased resorption activity; as with osteoblasts, decreased activity was observed at >100 μM PFOA concentrations.

Epidemiological studies have not evaluated the potential association between serum PFOA levels and impaired development of bone. A small number of studies in adults have examined potential associations with osteoarthritis risk. Innes et al. (2011) reported an elevated risk of physician diagnosed osteoarthritis among adults under 55 years of age with serum PFOA concentrations >13.6 ng/mL; the OR (95% CI) was 1.22 (1.02–1.45) among participants with serum PFOA in the second quartile (13.6–28.0 ng/mL). In a study of NHANES participants, Khalil et al. (2016) found an elevated risk of osteoarthritis among women, OR of 1.84 (1.17–2.90); the mean serum PFOA concentration was 3.7 ng/mL. This study also found in inverse association between serum PFOA and femur neck mineral density in women, but not in men. A second study of NHANES participants also found an elevated risk of osteoarthritis in women with serum PFOA levels of >5.89 ng/mL, OR of 1.98 (1.24–3.19) (Uhl et al. 2013). When segregated by age, an association was found in younger women (20–49 years of age), OR of 4.95 (1.27–19.4), but not among older women (50–84 years of age), OR of 1.33 (0.82–1.16) (Uhl et al. 2013). No association between estimated cumulative exposure to PFOA and the risk of osteoarthritis was observed in an occupational study in which 80% of the cohort was male (Steenland et al. 2015). A discussion of the other findings from epidemiological studies is presented in the MRL introduction section.

Benchmark Dose Modeling: BMD modeling was conducted for the DeWitt et al. (2008) and DeWitt et al. (2016) immunotoxicity studies. Using predicted TWA serum PFOA levels as the internal dosimetric, the IgM response data (summarized in Tables A-9 and A-10) were fit to all available continuous models in EPA's Benchmark Dose Software (BMDS, version 2.6.0). The following procedure for fitting continuous data was used: the simplest model (linear) was first applied to the data while assuming constant variance; if the data were consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data while assuming constant variance. Adequate model fit was judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark dose response (BMR). Among all of the models providing adequate fit to the data, the lowest BMDL (the lower limit of a one-sided 95% CI on the BMD) was selected as a reasonably conservative POD when differences between the BMDLs estimated from these models are >2–3-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was chosen. If the test for constant variance was negative, the linear model was run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provided an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means was evaluated and the polynomial, power, and Hill models were fit to the data and evaluated while the variance model was applied. Model fit and POD selection proceeded as described earlier. For both datasets, a BMR of 1 SD change from the control was used.

Table A-9. T-Cell Independent IgM Antibody Response in C57BL/6N Female Mice Immunized with Sheep Red Blood Cells

Number of animals per group	Administered dose (mg/kg/day)	Predicted TWA serum PFOA concentration (µg/mL)	IgM antibody titers[a] [mean serum IgM titer (log_2) to reach 0.5 OD]	SE[a]
8	0	0	7.28	0.13
8	0.94	21.4	7.39	0.07
8	1.88	42.5	7.08	0.10
8	3.75	58.4	6.75[b]	0.09
8	7.5	83.5	6.61[b]	0.12

[a]Data taken from Figure 3-C using GrabIt.
[b]Statistically different from controls ($p<0.05$).

PFOA = perfluorooctanoic acid; SE = standard error; TWA = time-weighted-average

Source: DeWitt et al. 2008

Table A-10. T-Cell Independent IgM Antibody Response In C57BL/6 Female Mice Immunized with Dinitrophenyl-Ficoll

Number of animals per group	Administered dose (mg/kg/day)	Predicted TWA serum PFOA concentration (µg/mL)	T-cell independent IgM antibody response[a] [mean serum titer (log_2) to reach 0.5 OD]	SD[a]
8	0	0	11.38	0.56
8	0.94	21.4	11.01	1.11
8	1.88	42.5	9.67[b]	1.34
8	3.75	58.4	9.81[b]	1.46
8	7.5	83.5	9.62[b]	0.97

[a]Data taken from Figure 3b using GrabIt.
[b]Statistically different from controls ($p<0.05$).

PFOA = perfluorooctanoic acid; SE = standard error; TWA = time-weighted-average

Source: DeWitt et al. 2016

The results of the BMD analysis of the DeWitt et al. (2008) and DeWitt et al. (2016) datasets are presented in Tables A-11 and A-12. For the DeWitt et al. (2008) data, the Hill model with constant variance provided the best fit to the IgM response data, as judged by the model with the lowest AIC since the range of BMDL values were sufficiently close; the fit of this model is presented in Figure A-1. For the DeWitt et al. (2016) IgM response data, constant variance models provided adequate fit; since the estimated BMDL values were not sufficiently close, the model with the lowest BMDL (Exponential 4) was selected; the fit of this model is presented in Figure A-2.

Table A-11. T-Cell Independent IgM Antibody Response in C57BL/6N Female Mice Immunized With Sheep Red Blood Cells Using Predicted TWA Serum PFOA as the Dose Metric (DeWitt et al. 2008)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMC	Dose above BMC	Overall largest	AIC	BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)
Constant variance									
Exponential (model 2)[d]	<0.0001	0.46	0.08	1.73	0.72	1.73	-50.41	ND	ND
Exponential (model 3)[d]	<0.0001	0.46	0.10	0.16	-1.30	1.33	-50.61	42.55	26.37
Exponential (model 4)[d]	<0.0001	0.46	0.08	1.73	0.72	1.73	-50.41	ND	ND
Exponential (model 5)[d]	<0.0001	0.46	0.36	-0.14	0.09	0.67	-52.34	44.11	33.33
Hill[d,e]	**<0.0001**	**0.46**	**0.41**	**-0.05**	**0.06**	**0.60**	**-52.47**	**43.57**	**33.49**
Linear[f]	<0.0001	0.46	0.09	1.69	0.67	1.69	-50.66	ND	ND
Polynomial (2-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Polynomial (3-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Polynomial (4-degree)[f]	<0.0001	0.46	0.08	0.10	-1.41	1.43	-50.00	ND	ND
Power[d]	<0.0001	0.46	0.10	0.16	-1.32	1.35	-50.49	42.62	26.23

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, the only models that provided adequate fit to the means were the Exponential 3 and 5, Hill, and Power models. BMDLs for models providing adequate fit were considered to be sufficiently close (differed by <2–3-fold); therefore, the model with the lowest AIC was selected (Hill).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); ND = not determined, model did not provide adequate fit; PFOA = perfluorooctanoic acid; SD = standard deviation; TWA = time-weighted average

Figure A-1. Predicted (Hill Model with Constant Variance, 1 Standard Deviation Benchmark Response) and Observed IgM Response Using Predicted TWA Serum PFOA as the Dose Metric

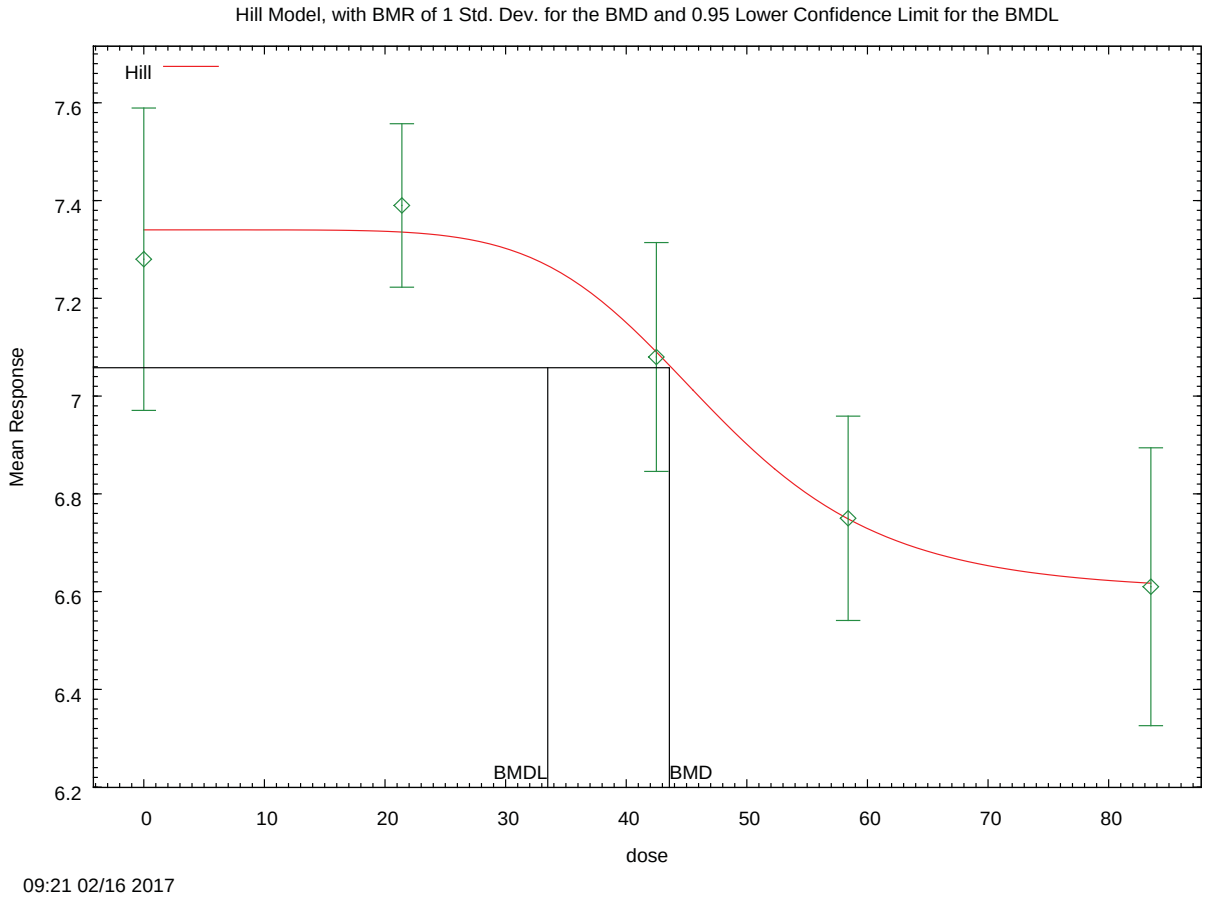


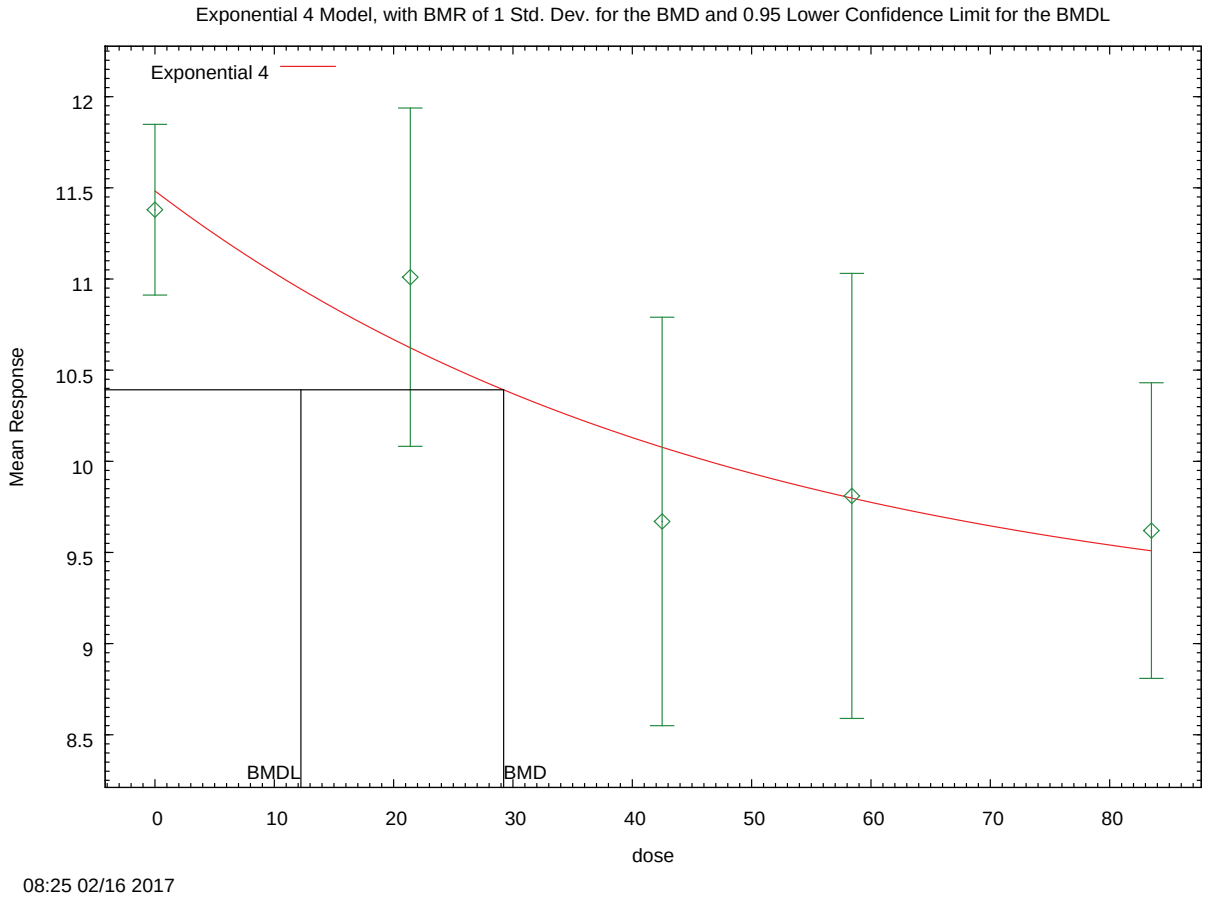
Table A-12. T-Cell Independent IgM Antibody Response In C57BL/6 Female Mice Immunized With Dinitrophenyl-Ficoll Using Predicted TWA Serum PFOA as the Dose Metric (DeWitt et al. 2016)

Model	Test for significant difference p-value[a]	Variance p-value[b]	Means p-value[b]	Scaled residuals[c] Dose below BMC	Scaled residuals[c] Dose above BMC	Scaled residuals[c] Overall largest	AIC	BMD_{1SD} (ng/mL)	$BMDL_{1SD}$ (ng/mL)
Constant variance									
Exponential (model 2)[d]	0.0029	0.12	0.34	-1.47	-0.17	-1.47	54.00	45.00	29.56
Exponential (model 3)[d]	0.0029	0.12	0.34	-1.47	-0.17	-1.47	54.00	45.00	29.56
Exponential (model 4)[d,e]	**0.0029**	**0.12**	**0.31**	**1.01**	**-1.06**	**-1.06**	**54.97**	**29.22**	**12.23**
Exponential (model 5)[d]	0.0029	0.12	0.71	0.00	-0.08	0.29	54.76	26.62	18.75
Hill[d]	0.0029	0.12	0.93	0.00	-0.08	0.29	52.76	23.66	19.11
Linear[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (2-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (3-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Polynomial (4-degree)[f]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63
Power[d]	0.0029	0.12	0.30	-1.52	-0.23	-1.52	54.27	47.96	32.63

[a]Values >0.05 fail to meet conventional goodness-of-fit criteria.
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Scaled residuals at doses immediately below and above the benchmark dose; also the largest residual at any dose.
[d]Power restricted to ≥1.
[e]Selected model. Constant variance model provided adequate fit to variance data. With constant variance model applied, all models provided adequate fit to the means. BMDLs for models providing adequate fit were not considered to be sufficiently close (differed by >2-fold, but <3-fold). In order to remain conservative, the model with the lowest BMDL was selected (Exponential 4).
[f]Coefficients restricted to be negative.

AIC = Akaike Information Criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected benchmark response; BMDL = 95% lower confidence limit on the BMD (subscripts denote benchmark response: i.e., $_{10}$ = exposure concentration associated with 10% extra risk); NA = not applicable (BMDL computation failed); SD = standard deviation; TWA = time-weighted average

Figure A-2. Predicted (Exponential 4 Model with Constant Variance, 1 Standard Deviation Benchmark Response) and Observed IgM Response Using Predicted TWA Serum PFOA as the Dose Metric



Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanoic acid (PFOA)
CAS Numbers: 335-67-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: ATSDR did not identify an adequate study with an exposure duration of ≥365 days. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: The chronic oral animal database for PFOA is limited to dietary exposure studies in male and female rats (3M 1983; Butenhoff et al. 2012c) or male rats (Biegel et al. 2001). The lowest LOAEL identified in the Butenhoff et al. (2012c; 3M 1983) study was 1.5 mg/kg/day for inflammation of salivary gland in male rats exposed to PFOA in the diet for 2 years. At 15 mg/kg/day, hepatocellular necrosis was observed after 1 year of exposure and vascular mineralization was observed in the testes. In the Biegel et al. (2001) study, exposure to 13.6 mg/kg/day PFOA in the diet for 2 years resulted in decreases in body weight gain, increases in Leydig cell hyperplasia, and pancreatic acinar cell hyperplasia in male rats.

The chronic-duration database for PFOA was not considered adequate for MRL derivation due to uncertainty in the selection of the critical effect. The Butenhoff et al. (2012c) study identified the salivary gland as the most sensitive target, but these alterations were only observed in males and may have been due to an antemortem viral infection. Intermediate-duration oral studies have suggested that the immune system is a sensitive target of toxicity in mice; however, potential alterations in immune function have not been investigated in chronic-duration studies; the Butenhoff et al. (2012c) study did conduct histological examinations of immune tissues.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFOS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFOS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFOS.

Rationale for Not Deriving an MRL: An acute-duration oral MRL for PFOS cannot be derived because the modeling approach used for estimating HEDs cannot be used to estimate acute human exposure where the exposure duration of 14 days is <1% of the PFOS elimination half-life in humans.

A number of studies have examined the toxicity of PFOS in laboratory animals following acute-duration exposure. The available data suggest that the liver, developing organism, and immune system are sensitive targets. The liver effects consisted of decreases in serum lipids, increases in liver weight, and hepatocellular hypertrophy (Elcombe et al. 2012a, 2012b; Era et al. 2009; Fuentes et al. 2006; Haughom and Spydevold 1992; Vetvicka and Vetvickova 2013; Wan et al. 2011); using the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria) for assessing the adversity of liver alterations for peroxisome proliferators, these effects were not considered relevant for human risk assessment. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs. A decrease in serum HDL cholesterol levels was also observed in male and female monkeys receiving three doses of 13.3 or 14 mg/kg/day (TWA dose in males and females, respectively) over 315 days (Chang et al. 2017).

Immunological effects included altered responses to sRBC and decreased IgM antibody formation in response to antigen exposure in mice (Vetvicka and Vetvickova 2013; Zheng et al. 2009). Developmental effects consisted of decreases in neonatal survival (Abbott et al. 2009; Grasty et al. 2003), increases in post-implantation losses (Lee et al. 2015a), decreases in fetal body weight (Case et al. 2001; Era et al. 2009; Fuentes et al. 2007b; Lee et al. 2015a), increases in malformations (Era et al. 2009), and alterations in motor activity (Hallgren et al. 2015; Johansson et al. 2008). The lowest LOAEL identified was 0.5 mg/kg/day for increased post-implantation losses in mice (Lee et al. 2015a). A summary of the adverse effect levels for the immunological and developmental effects are presented in Table A-13.

Table A-13. Summary of the Adverse Effects Observed in Laboratory Animals Following Acute-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Immunological				
Mouse 7 days		5	Impaired response to T-cell mitogens; suppressed response to sRBC	Zheng et al. 2009
Mouse 7 days		20	Inhibition of T lymphocyte proliferation in response to sRBC; decreased phagocytosis by peripheral blood cells and NK cell activity; decreased IgM antibody formation in response to OVA	Vetvicka and Vetvickova 2013
Developmental				
Mouse GDs 11–16		0.5	Increased post-implantation losses	Lee et al. 2015a
Mouse Once		0.75	Decreased motor activity	Johansson et al. 2008
Rabbit GDs 6–20	1	2.5	Decreased fetal body weight	Case et al. 2001
Mouse GDs 15–18		4.5	Decreased number of live pups per litter on PND 15	Abbott et al. 2009
Mouse GDs 12–18		6	Reduced pup body weight on PNDs 4 and 8	Fuentes et al. 2007b
Mouse Once		11.3	Altered spontaneous behavior in pups	Hallgren et al. 2015
Rat GDs 19–20		25 (SLOAEL)	Decreased neonatal survival	Grasty et al. 2003
Rat GDs 2–5, 6–9, 10–13, 14–17, or 17–20		25 (SLOAEL)	Decreased neonatal survival	Grasty et al. 2003
Mouse GDs 11–15		50	Cleft palate and reduced fetal body weight	Era et al. 2009

[a]Unless otherwise noted, the LOAEL is for a less serious effect.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NK = natural killer; NOAEL = no-observed-adverse-effect level; perfluorooctane sulfonic acid; PND = postnatal day; SLOAEL = LOAEL for a serious effect; sRBC = sheep red blood cell

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:**	Perfluorooctane sulfonic acid (PFOS)
CAS Numbers:	1763-23-1
Date:	March 2020
Profile Status:	Final
Route:	Oral
Duration:	Intermediate
MRL:	$2x10^{-6}$ mg/kg/day
Critical Effect:	Delayed eye opening and decreased pup body weight
Reference:	Luebker et al. 2005a
Point of Departure:	0.000515 mg/kg/day
Uncertainty Factor:	30
Modifying Factor:	10
LSE Graph Key:	35
Species:	Rat

MRL Summary: An intermediate-duration oral MRL of $2x10^{-6}$ mg/kg/day was derived for PFOS based on delayed eye opening and transient decrease in F2 body weight during lactation in the offspring of rats administered PFOS via gavage in a 2-generation study (Luebker et al. 2005a). The MRL is based on a HED NOAEL of 0.000515 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

Selection of the Critical Effect: Intermediate-duration studies in monkeys, rats, and mice have identified several sensitive targets of PFOS toxicity including the liver, nervous system, immune system, and the developing organism; adverse outcomes occurred in these tissues at lower doses than other effects. The lowest LOAEL and NOAEL values for these outcomes are presented in Table A-14; given the large number of intermediate-duration studies, this table was limited to studies which identified LOAEL values of ≤3 mg/kg/day. The liver effects observed in monkeys, rats, and mice included increases in liver weight, decreases in serum lipids, hepatocellular degeneration, and focal necrosis (Cui et al. 2009; Curran et al. 2008; Elcombe et al. 2012a; Lefebvre et al. 2008; Seacat et al. 2002, 2003; Thibodeaux et al. 2003; Wan et al. 2011; Yahia et al. 2008). In the absence of degenerative changes such as necrosis, the liver hypertrophy observed in rodent studies was not considered relevant to human risk assessment (Hall et al. 2012). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight, hepatocellular hypertrophy, and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

Several studies have examined potential neurological endpoints and found overt signs of neurotoxicity (cachexia, lethargy, and tonic convulsions in response to stimuli) in rats exposed to 5 or 8.5 mg/kg/day (Cui et al. 2009; Kawamoto et al. 2011) and impaired spatial learning and memory in mice exposed to 2.15 mg/kg/day (Long et al. 2013). Four studies have evaluated the immune response of PFOS exposed mice following exposure to an antigen (sRBC) or a virus (Dong et al. 2009, 2011; Guruge et al. 2009; Peden-Adams et al. 2008). Although the studies have consistently reported adverse effects, there is considerable overlap in LOAEL values. Peden-Adams et al. (2008) identified the lowest LOAEL of 0.00166 mg/kg/day with a NOAEL of 0.000166 mg/kg/day for a suppressed response to sRBC in mice administered PFOS for 28 days. Longer duration studies (Dong et al. 2009, 2011) have identified NOAEL values (0.0083 and 0.0167 mg/kg/day) in mice exposed to PFOS for 60 days that are higher than

the LOAELs identified in the Peden-Adams et al. (2008) study. It is noted that the studies used different mouse strains (B6C3F1 in the Peden-Adams study and C57BL/6N in the Dong studies), which may account for this difference. A variety of developmental effects have been observed in rats and mice; these include increases in postnatal mortality (Chen et al. 2012b; Lau et al. 2003; Luebker et al. 2005a, 2005b; Xia et al. 2011; Yahia et al. 2008), neurodevelopmental alterations (locomotor activity and impaired learning) (Butenhoff et al. 2009b; Onishchenko et al. 2011; Wang et al. 2015c), developmental delays (Lau et al. 2003; Luebker et al. 2005a), and malformations and anomalies (sternal defects and cleft palate) (Era et al. 2009; Thibodeaux et al. 2003; Yahia et al. 2008). The Wang et al. (2015c) study showed that decreases in spatial learning were observed in rats exposed *in utero* and in rat pups exposed postnatally (PND 7). Other effects that occur at similar doses include decreases in body weight (Lefebvre et al. 2008; Luebker et al. 2005a, 2005b; Seacat et al. 2002) and alterations in thyroid hormone levels (decreases in T3 and T4 levels and increases in TSH levels) (Curran et al. 2008; Luebker et al. 2005b; Thibodeaux et al. 2003).

The most sensitive targets of PFOS toxicity in laboratory animals are similar to those identified in longer term epidemiological studies. These effects include liver damage and increases in serum lipids, decreased antibody response to vaccines, and small decreases in birth weight; epidemiological studies have not consistently found neurological effects to be associated with serum PFOS levels.

Table A-14. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Hepatic				
Monkey 26 weeks	0.15	0.75	Increased liver weight, decreased serum cholesterol, hepatocellular hypertrophy, lipid vacuolation	Seacat et al. 2002
Neurological				
Mouse 3 months	0.43	2.15	Impaired spatial learning and memory	Long et al. 2013
Immunological				
Mouse 28 days	0.00016	0.00166	Suppressed response to sRBC	Peden-Adams et al. 2008
Mouse 21 days	0.005	0.025	Decreased resistance to influenza virus	Guruge et al. 2009
Mouse 60 days	0.0083	0.083	Impaired response to sRBC	Dong et al. 2009
Mouse 60 days	0.0167	0.083	Impaired response to sRBC	Dong et al. 2011
Developmental				
Mouse GDs 1–21		0.3	Decreased locomotion, muscle strength, motor coordination in adult offspring	Onishchenko et al. 2011
Rat 84 days	0.1	0.4	Delayed eye opening	Luebker et al. 2005a
Rat 67 days		0.4	Decreased pup weight	Luebker et al. 2005b

Table A-14. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFOS

Species and exposure duration	NOAEL (mg/kg/day)	LOAEL[a] (mg/kg/day)	Effect	Reference
Rat GD 1–PND 1		0.8	Decreased spatial learning	Wang et al. 2015c
Rat GD 0–PND 20	0.3	1	Increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17	Butenhoff et al. 2009b
Mouse GDs 1–17		1	Delayed eye opening	Lau et al. 2003
Mouse GDs 0–17		1	Increased sternal defects	Yahia et al. 2008
Rat GDs 1–21	0.1	2	Increased postnatal mortality and severe lung histopathology	Chen et al. 2012b
Rat GDs 2–21	0.6	2	Increased neonatal mortality	Xia et al. 2011

[a]LOAELs for less serious health effects.

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NK = natural killer; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; sRBC = sheep red blood cell

Selection of the Principal Study: Using the Wambaugh et al. (2013) pharmacokinetic model, TWA serum concentrations corresponding to external doses (mg/kg/day) and exposure durations (days) were predicted for the studies listed in Table A-14. Pharmacokinetic model parameters were not available for C57BL/6N mice, B6C3F1 mice, or Wistar rats, which precluded predicting TWA serum concentrations for the Long et al. (2013), Dong et al. (2009, 2011), Guruge et al. (2009), Peden-Adams et al. (2008), Wang et al. (2015c), Onishchenko et al. (2011), and Yahia et al. (2008) studies. The predicted serum PFOS levels for each administered dose is presented in Table A-15.

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Hepatic				
Cynomolgus Monkey 26 weeks	0.03 (males)	7.81	Increased liver weight, decreased serum cholesterol, hepatocellular hypertrophy, mild bile stasis, lipid vacuolation at 0.75 mg/kg/day	Seacat et al. 2002
	0.15 (males)	37.8		
	0.75 (males)	150		
	0.03 (females)	7.72		
	0.15 (females)	37.6		
	0.75 (females)	146		

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Neurological				
C57BL/6 Mouse 3 months	0.43 2.15 10.75	Not calculated	Impaired spatial learning and memory at 2.15 mg/kg/day	Long et al. 2013
Immunological				
B6C3F1 Mouse 28 days	0.00016 0.00166 0.00331 0.0166 0.0331	Not calculated[a]	Suppressed response to sRBC at 0.00166 mg/kg/day	Peden-Adams et al. 2008
B6C3F1 Mouse 21 days	0.005 0.025	Not calculated[a]	Decreased resistance to influenza virus at 0.025 mg/kg/day	Guruge et al. 2009
C57BL/6N Mouse 60 days	0.0083 0.083 0.41667 0.8333 2.0833	Not calculated[a]	Impaired response to sRBC at 0.083 mg/kg/day	Dong et al. 2009
C57BL/6N Mouse 60 days	0.0083 0.0167 0.083 0.41667 0.8333	Not calculated[a]	Impaired response to sRBC at 0.083 mg/kg/day	Dong et al. 2011
Developmental				
C57BL/6 Mouse GDs 1–21	0.3	Not calculated	Decreased locomotion, muscle strength, motor coordination in adult offspring at 0.3 mg/kg/day	Onishchenko et al. 2011
Sprague-Dawley Rat 84 days	0.1 0.4 1.6 3.2	7.43 29.7 119 238	Delayed eye opening in F1 pups and transient decrease in F2 pup body weight during lactation at 0.4 mg/kg/day	Luebker et al. 2005a
Sprague-Dawley Rat 67 days	0.4 0.8 1 1.2 1.6 3.2	24.1 48.1 60.1 72.2 96.2 120	Decreased pup weight per litter at birth and on LD 5 at 0.4 mg/kg/day	Luebker et al. 2005b

Table A-15. Summary of the Predicted TWA Serum PFOS levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Predicted TWA serum PFOS (µg/mL)	Effect	Reference
Wistar Rat GD 1–PND 1	0.8	Not calculated[b]	Decreased spatial learning at 0.8 mg/kg/day	Wang et al. 2015c
Sprague-Dawley Rat GD 0–PND 20	0.1 0.3 1	3.75 11.3 37.5	Increased locomotor activity and concurrent failure to habituate to test environment in male pups on PND 17 at 1 mg/kg/day	Butenhoff et al. 2009b
CD-1 Mouse GDs 1–17	1 5 10 15 20	31.9 146 216 244 260	Delayed eye opening at 1 mg/kg/day	Lau et al. 2003
ICR Mouse GDs 0–17	1	Not calculated	Increased sternal defects at 1 mg/kg/day	Yahia et al. 2008
Sprague-Dawley Rat GDs 1–21	0.1 2	2.01 40.1	Increased postnatal mortality and severe lung histopathology at 2 mg/kg/day	Chen et al. 2012b
Sprague-Dawley Rat GDs 2–21	0.1 0.6 2	1.92 11.5 38.3	Increased neonatal mortality at 2 mg/kg/day	Xia et al. 2011

[a]See Table A-17 for measured serum PFOS concentrations.
[b]Reported serum PFOS concentrations of 25.7 and 64.3 µg/mL in dams on PND 7 and 35, respectively.

GD = gestation day; LD = lactation day; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; sRBC = sheep red blood cell; TWA = time-weighted average

Selection of the Point of Departure for the MRL: None of the studies with predicted serum PFOS levels had datasets that were amenable for BMD modeling; thus, the NOAEL/LOAEL approach was used to identify PODs for derivation of the intermediate-duration MRL for PFOS. A summary of the PODs is presented in Table A-16. HEDs were calculated for each potential POD (NOAEL or LOAEL) identified in laboratory animal studies using the first-order single-compartment model previously discussed and the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. The HEDs for each POD are presented in Table A-16. The potential POD_{HED} values were divided by a total uncertainty factor to calculate candidate MRLs; these values are also presented in Table A-16. The lowest administered doses associated with adverse effects were found in the immunotoxicity studies conducted by Dong et al. (2009, 2011), Guruge et al. (2009), and Peden-Adams et al. (2008). These data could not be considered as PODs because TWA serum PFOS values could not be predicted due to the lack of pharmacokinetic model parameters for the two mouse strains tested. Although there is considerable overlap between the LOAEL for IgM response to sRBC (0.00166 mg/kg/day) identified in the Peden-Adams et al. (2008) 28-day study and the NOAELs for IgM

response to sRBC (0.0083 and 0.0167 mg/kg/day) identified in the Dong et al. (2009, 2011) 60-day studies, the data do suggest that immunotoxicity could occur at <0.3 mg/kg/day (the lowest LOAEL identified in developmental toxicity studies).

Table A-16. Summary of Potential Points of Departures Human Equivalent Doses (POD_{HED}) for Intermediate-Duration Oral MRL for PFOS

Endpoint (reference)	Predicted serum concentrations (µg/mL) NOAEL	LOAEL	POD_{HED}[a] (mg/kg/day)	Total UF	Candidate MRLs (mg/kg/day)
Increased rat pup mortality and lung histopathology (Chen et al. 2012b)	2.01	40.1	0.000139	300[b]	$4.6x10^{-7}$
Decreased rat pup weight at birth and on PND 4 (Luebker et al. 2005b)		24.1	0.00167	3,000[c]	$5.6x10^{-7}$
Delayed eye opening in mouse pups (Lau et al. 2003)		31.9	0.00221	3,000[c]	$7.4x10^{-7}$
Neurodevelopmental effects in male rat pups (Butenhoff et al. 2009b)	11.3	37.5	0.000780	300[b]	$2.6x10^{-6}$
Delayed eye opening and decreased F2 rat pup body weight (Luebker et al. 2005a)	7.43	29.7	0.000515	300[b]	$1.7x10^{-6}$
Increased neonatal mortality in rat pups (Xia et al. 2011)	11.5	38.3	0.000797	300[b]	$2.7x10^{-6}$
Hepatic effects in monkeys (Seacat et al. 2002)	37.8	150	0.00262	300[b]	$8.7x10^{-6}$

[a]HED calculated using Equation A-6 where C_{ss} is the serum concentration associated with the NOAEL or BMDL or the LOAEL if there was no NOAEL or BMDL, $K_e=3.74x10^{-4}$; $V_d=0.2$, and AF=1.
[b]UFs of 3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability and modifying factor (MF) of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.
[c]UF of 10 for extrapolation from a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustments, and UF of 10 for human variability and MF of 10 for concern that immunotoxicity may be a more sensitive endpoint than developmental toxicity.

BMDL = lower limit on the benchmark dose; LOAEL = lowest-observed-adverse-effect level; MRL = Minimal Risk Level; NOAEL = no-observed-adverse-effect level; PFOS = perfluorooctane sulfonic acid; PND = postnatal day; UF = uncertainty factor

The serum PFOS concentrations predicted to occur at the lowest LOAEL values were 24.1, 29.7, and 31.9 µg/mL identified in the Luebker et al. (2005b), Luebker et al. (2005a), and Lau et al. (2003) studies; decreases in pup body weight and delays in eye opening were observed at these levels. Luebker et al. (2005a) was the only study that identified a NOAEL for these effects. The predicted serum concentration for this NOAEL dose was selected as the basis for the MRL.

Summary of the Principal Study:

Luebker DJ, Case MT, York RG, et al. 2005a. Two-generation reproduction and cross-foster studies of perfluorooctanesulfonate (PFOS) in rats. Toxicol 215:126-148.

Groups of Sprague-Dawley rats (P generation) (35/sex/dose level) were administered PFOS (86.9% pure) by gavage in deionized water with 2% Tween-80 at doses of 0, 0.1, 0.4, 1.6, or 3.2 mg/kg/day for 6 weeks before mating and until sacrifice (after mating for males, GD 10 for some females, and PND 21 for the remaining females). Body weight and feed consumption were evaluated during the dosing period. Prior to mating, 15 females per dose group were evaluated for estrous cycling. Ten females/dose group were sacrificed on GD 10 and the remaining females were allowed to give birth (F1 generation). Parental rats sacrificed on GD 10 were examined for number of corpora lutea, implantations, and viable and non-viable fetuses. Body weight of F1 was evaluated during lactation; also, F1 rats were assessed for developmental landmarks during lactation. On PND 22, the F1 rats were started on the same diet as the parental rats. At approximately PND 90, F1 were mated to produce the F2 generation. F1 males and females were killed as the P generation. F1 females and males were evaluated for vaginal patency and preputial separation, respectively. At age 24 days, F1 rats were administered three neurobehavioral tests (learning, memory retention, and avoidance memory). At the age of 70 days, F1 were administered three different neurobehavioral tests (neuromuscular coordination, swimming ability, learning, and memory). PFOS was analyzed in liver and blood from parental females and in liver from F1on PND 21; and in liver and serum from parental males after mating and after 42–56 days of dosing.

There were no deaths in parental males or females and no clinical signs in parental males. High-dose parental males had significantly reduced terminal body weight (11% reduction). Absolute and relative food consumption was reduced during treatment in males by less than 10%. Parental females at 0.4 mg/kg/day and higher had localized areas of partial alopecia. Body weight of parental females in the 3.2 mg/kg/day group was significantly lower during cohabitation and gestation (11% reduced). Absolute and relative food consumption were significantly reduced in 3.2 mg/kg/day parental females during premating and gestation (>15%) and in 1.6 mg/kg/day parental females during lactation. Administration of PFOS did not affect any mating or fertility parameter. Estrous cycling was not affected. Examination of parental females sacrificed on GD 10 showed no significant effect on numbers of corpora lutea or implantations or viable and non-viable fetuses. Significant delivery observations for 3.2 mg/kg/day parental females included reduced number of implantations per delivered litter, decreased gestational length, increased number of dams with all pups dying on PNDs 1–4 (also at 1.6 mg/kg/day). Observation of F1 pups during PNDs 1–21 showed significantly reduced weight and decreased viability (≥1.6 mg/kg/day). Examination of dead F1 pups did not reveal a cause of death; no labored breathing was noted in pups at birth. Developmental delays were noted at 1.6 mg/kg/day (pinna unfolding, surface righting, and air righting), and 0.4 mg/kg/day (eye opening). The investigators noted that the delay in eye opening was not considered an adverse outcome but did not provide a rationale for this conclusion. Follow-up observations of 0.1 and 0.4 mg/kg/day offspring showed no alterations in body weight or food consumption, including F1 females during gestation and lactation. Sexual maturation was not affected in F1 males or females; no effects were noted in the neurobehavioral tests. Reproductive performance of F1 were not affected. Viability of F2 pups during PNDs 1–21 was not affected. F2 pup weight was significantly reduced at 0.4 mg/kg/day on PND 7 (13%) and PND 14 (9.6%). The investigators noted that the decrease in pup weight was not considered toxicologically relevant and may have been due to minimally larger live litter sizes, as compared to the control group, and that there were no differences on PND 21. ATSDR notes that there were no significant differences in pup body weight between the control group and the 0.4 mg/kg/day group on PND 4 prior to culling and after culling, and considers the delay in eye opening to be toxicologically relevant. Serum and liver PFOS increased with dose.

Strengths and Weaknesses: The Luebker et al. (2005a) study is a well-designed 2-generation study evaluating a number of reproductive and developmental endpoints in adequate number of animals. The study was designed to evaluate four PFOS dose levels administered prior to mating and during mating, gestation, and lactation across two generations. The test included a number of parameters to assess reproductive performance (mating, estrous cycling, and fertility), reproductive outcomes (gestation length, number of implantation sites, stillbirths), and neonatal toxicity (survival and body weight). The experiment also included a cross-foster study, which allowed for the evaluation of whether neonatal effects were due to maternal care/maternal toxicity or to a direct effect on the pups. An additional strength of the study is that it evaluated several endpoints (e.g., lung morphology and lung glycogen stores) that could elucidate the mechanisms of action for fetal deaths. Luebker et al. (2005a) measured serum and liver PFOS levels, which allowed for validation of the Wambaugh et al. (2013) model's predicted serum TWA PFOS level. Although the study was designed to evaluate four PFOS dose levels, high mortality in the F1 offspring at the two highest dose levels resulted in a discontinuation of these dose levels, which limits the amount of data that can be used to establish dose-response relationships.

Calculations of Internal Dosimetric: TWA serum PFOS concentrations corresponding to external doses and exposure durations were predicted from a pharmacokinetic model (Wambaugh et al. 2013) using animal species-, strain-, and sex-specific parameters (see MRL approach section for details).

Human Equivalent Dose: HEDs were calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFOS equal to the serum concentration selected as the POD were calculated using the first order single-compartment model (see MRL approach section for details).

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability
- 10 MF for concern that immunotoxicity may be a more sensitive endpoint of PFOS toxicity than developmental toxicity

$MRL = NOAEL_{HED} \div (UFs \times MF)$
$0.000515 \text{ mg/kg/day} \div ((3 \times 10) \times 10) = 2x10^{-6} \text{ mg/kg/day}$

Although pharmacokinetic model parameters were not available for the strain/sex of the animals tested in the immunotoxicity studies, most of the studies did provide measured serum PFOS levels. The serum PFOS levels at the NOAEL and LOAEL doses are presented in Table A-17. The measured serum PFOS levels associated with altered immune responses are approximately 1–10 times lower than the serum concentration predicted to occur at the NOAEL dose. These data suggest that immunotoxicity may be a more sensitive effect than developmental toxicity.

Table A-17. Measured Serum PFOS Levels at the NOAEL and LOAEL Doses for Immunological Effects

Effect, species and exposure duration		Dose (mg/kg/day)	Measured mean serum PFOS (µg/mL)	Reference
Impaired response to sRBC in mice exposed for 60 days	NOAEL	0.0083	0.674	Dong et al. 2009
	LOAEL	0.083	7.132	

Table A-17. Measured Serum PFOS Levels at the NOAEL and LOAEL Doses for Immunological Effects

Effect, species and exposure duration		Dose (mg/kg/day)	Measured mean serum PFOS (µg/mL)	Reference
Impaired response to sRBC in mice exposed for 60 days	NOAEL	0.0167	2.36	Dong et al. 2011
	LOAEL	0.083	10.75	
Decreased resistance to influenza virus in mice exposed for 21 days	NOAEL	0.005	0.189	Guruge et al. 2009
	LOAEL	0.025	0.670	
Suppressed response to sRBC in mice exposed for 28 days	NOAEL	0.00016	0.0178	Peden-Adams et al. 2008
	LOAEL	0.00166	0.0915	

PFOS = perfluorooctane sulfonic acid; sRBC = sheep red blood cell

A candidate MRL was calculated using the NOAEL of 0.0167 mg/kg/day identified in the Dong et al. (2011). This study was selected over the other immunotoxicity studies because it identified the highest NOAEL for immunotoxicity and it had the longest exposure duration; the Peden-Adams et al. (2008) was not selected because the LOAEL of 0.00166 mg/kg/day is not supported by the other three studies. A TWA concentration was estimated using a similar approach described for PFHxS and PFNA in the MRL approach section. The estimated TWA concentration was 1.2 µg/mL for the 0.0167 mg/kg/day; this estimated TWA concentration was used to calculate a HED of 0.000083 mg/kg/day. A candidate MRL of $3x10^{-6}$ was calculated using an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustments and 10 for human variability). This MRL is similar to the MRL calculated from the Luebker et al. (2005a) study and lends support to using the additional modifying factor of 10 to account for the lack of pharmacokinetic modeling parameters for the mouse strains tested for immunotoxicity.

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctane sulfonic acid (PFOS)
CAS Numbers: 1763-23-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: ATSDR did not identify an adequate study with an exposure duration of ≥365 days. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: Immune function was not examined following chronic-duration oral exposure in laboratory animal studies; the only chronic-duration oral study (Butenhoff et al. 2012b; Thomford 2002b), did not find histological alterations in immune tissues (lymph nodes, spleen, and thymus) in rats at doses as high as 1.04 mg/kg/day. Impaired immune function was the most sensitive endpoint in intermediate-duration mouse studies. Given the concern that immunotoxicity may occur at lower doses than liver toxicity, a chronic-duration oral MRL for PFOS is not recommended at this time.

One study has evaluated the chronic toxicity of PFOS in laboratory animals. Histological alterations in the liver were the primary effects observed in rats exposed to PFOS in the diet for 2 years (Butenhoff et al. 2012b; Thomford 2002b). Centrilobular hepatocellular hypertrophy was observed in rats exposed to ≥0.1 mg/kg/day. At 1.04 mg/kg/day, increases in the incidence of single cell necrosis and cystic degeneration were observed in the liver. Decreases in body weight were observed at 1.04 mg/kg/day in female rats. Thus, the 1.04 mg/kg/day dose was identified as the lowest LOAEL for this study. Epidemiological data (Dalsager et al. 2016; Dong et al. 2013; Fei et al. 2010; Grandjean et al. 2012, 2016; Granum et al. 2013; Kielsen et al. 2016; Mogensen et al. 2015a; Stein et al. 2016a; Zhu et al. 2016) suggest that the immune system is a sensitive target of PFOS toxicity following long-term exposures, which is supported by intermediate-duration PFOS laboratory animal studies (Dong et al. 2009, 2011; Guruge et al. 2009; Peden-Adams et al. 2008).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHxS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHxS.

Rationale for Not Deriving an MRL: The acute oral database for PFHxS was not considered adequate for derivation of an MRL due to the short duration of the only available study and the lack of pharmacokinetic model parameters for calculating an HED.

In the only available study of PFHxS in laboratory animals, Viberg et al. (2013) reported altered spontaneous behavior and habituation in adult mice administered a single gavage dose of 9.2 mg/kg/day PFHxS on PND 10; no alterations were observed at 6.1 mg/kg/day. This single exposure study was not considered adequate as the basis of an acute-duration MRL for PFHxS due to the uncertainty of whether an MRL based on this study would be protective for repeated exposures or for other potential sensitive endpoints, such as immunotoxicity.

For perfluoroalkyls, ATSDR has used the approach of predicting TWA serum perfluoroalkyl levels in laboratory animals and calculating HEDs for these serum concentrations. For PFOA and PFOS, the Wambaugh et al. (2013) pharmacokinetic model was utilized for predicting the TWA serum perfluoroalkyl concentrations. However, strain-, sex-, and compound-specific model parameters are not available for other perfluoroalkyls, thus precluding deriving MRLs for other perfluoroalkyls. Other approaches such as “read across” (i.e., using data for a particular endpoint from one chemical to predict the same endpoint for another chemical that has similar chemical structure or mechanisms of action) or equivalency factors were considered for the other perfluoroalkyls; however, there are limited data available that would allow for comparison of the toxicity and toxicokinetic properties of different perfluoroalkyls. Peters and Gonzalez (2011) noted that the toxic equivalency factor approach would not be suitable for perfluoroalkyls because the current data suggest that the toxicity of these compounds appear to be mediated by multiple receptors, including PPARα, CAR, and PXR, and that there may be species differences in the response mediated by different receptors. Additionally, available data suggest that there are qualitative differences in the toxicities of various perfluoroalkyls.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:**	Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers:	355-46-4
Date:	March 2020
Profile Status:	Final
Route:	Oral
Duration:	Intermediate
MRL:	$2x10^{-5}$ mg/kg/day
Critical Effect:	Thyroid follicular epithelial hypertrophy/hyperplasia
Reference:	Butenhoff et al. 2009a
Point of Departure:	0.0047 mg/kg/day
Uncertainty Factor:	30
Modifying Factor:	10
LSE Graph Key:	33
Species:	Rat

MRL Summary: An intermediate-duration oral MRL of $2x10^{-5}$ mg/kg/day was derived for PFHxS based on thyroid follicular epithelial hypertrophy/hyperplasia in adult male rats administered via gavage PFHxS for a minimum of 42 days (Butenhoff et al. 2009a). The MRL is based on a HED NOAEL of 0.0047 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability) and a modifying factor of 10 for database limitations.

Selection of the Critical Effect: Four intermediate-duration studies in laboratory animals have been identified for PFHxS. In a developmental toxicity study, increased incidences of thyroid follicular cells hypertrophy/hyperplasia were observed in F0 male rats administered ≥3 mg/kg/day (Butenhoff et al. 2009a). Increased liver weight and centrilobular hepatocellular hypertrophy were also observed in the males at ≥3 mg/kg/day. Consistent with the Hall et al. (2012), the liver effects were not considered a relevant endpoint for humans. Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

No reproductive or developmental effects were reported in the Butenhoff et al. (2009a) study. A second developmental toxicity study reported decreases in serum thyroxine levels in rat dams and pups administered 5 mg/kg/day PFHxS on GDs 7–22 (Ramhøj et al. 2018); no alterations in pup birth weight or weight gain were observed. In a 1-generation reproductive/developmental toxicity study, decreases in the number of pups per litter were observed in the offspring of mice administered 1 mg/kg/day (Chang et al. 2018). At 3 mg/kg/day, single cell necrosis and microvascular fatty changes were observed at 3 mg/kg/day. Liver effects (decreases in serum lipids, increases in hepatic triglyceride levels, and increases in liver weight) were also observed in mice exposed to 6 mg/kg/day PFHxS in the diet for 4–6 weeks (Bijland et al. 2011) and mice administered 0.3 mg/kg/day (Chang et al. 2018). Using the Hall et al. (2012) criteria (see Section 2.9 for a discussion of the criteria), the liver effects were not considered relevant for human risk assessment. Thus, the lowest LOAEL identified in intermediate-duration studies was 3 mg/kg/day for thyroid effects.

There is some uncertainty regarding the selection of thyroid alterations as the critical effect. Butenhoff et al. (2009a) suggested that the histological alterations in the thyroid may be secondary to the liver effects

(hepatocellular hypertrophy). The alteration may be due to binding competition between PFHxS and thyroid hormones and possible induction of thyroid hormone metabolism by the liver. Ramhøj et al. (2018) reported decreases in serum T4 levels but did not evaluate possible thyroid gland histological alterations. The Chang et al. (2018) 1-generation reproduction study did not find alterations in serum TSH levels in mice.

A limited number of epidemiological studies have examined potential thyroid effects. Two epidemiological studies have examined thyroid disease associated with PFHxS exposure (Chan et al. 2011; Wen et al. 2013); one study found increased risk of subclinical hypothyroidism and subclinical hyperthyroidism among women (Wen et al. 2013) and the second study did not find an increased risk of hypothyroxinemia (Chan et al. 2011). The small number of studies precludes evaluating the possible association between PFHxS exposure and thyroid disease in humans. A meta-analysis of epidemiological data (Kim et al. 2018) found an inverse correlation between serum PFHxS levels and total T4 levels in the general population; there was no correlation among pregnant women. No associations were found for free T4, total T3, or TSH.

Species-related differences in thyroid parameters between rats and humans also add to the uncertainty. Some differences include higher rate of T4 production in rats than in humans, and very low levels of thyroxine binding globulin compared to high levels in humans, and sex-related differences in serum TSH levels (higher levels in males compared to females) in rats, but not in humans (Choksi et al. 2003). It is not known if these species differences would influence the relative toxicity of PFHxS.

Selection of the Principal Study: Since the liver effects were not considered relevant to humans, the lowest LOAEL identified for PFHxS was 1 mg/kg/day for decreases in the number of pups per litter identified in the Chang et al. (2018) study. The investigators noted that the toxicological significance of this alteration was uncertain because there was no clear dose-response and no alterations in the number of implantation sites, number of viable pups, or pup to implant ratios. Thus, the Butenhoff et al. (2009a) study, which reported thyroid effects in male rats at LOAEL of 3 mg/kg/day, with a NOAEL of 1 mg/kg/day, was selected as the principal study.

Summary of the Principal Study:

Butenhoff JL, Chang SC, Ehresman DJ, et al. 2009a. Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. Reprod Toxicol 27:331-341. (Results from this study are also reported in Hoberman and York 2003.)

The reproductive/developmental effects of PFHxS were studied in Sprague-Dawley rats (15/sex/group). Doses of 0, 0.3, 1, 3, or 10 mg/kg/day PFHxS were administered by gavage in an aqueous vehicle. Male rats were dosed beginning 14 days before cohabitation and continued until 1 day before sacrifice (a minimum of 42 days). Females were dosed beginning 14 days before cohabitation and continued until 1 day before sacrifice on PND 21 or GD 25 (rats that did not deliver a litter). Endpoints evaluated included: body weight, food consumption, estrous cycling, functional observational battery (FOB; tests of autonomic function, reactivity and sensitivity, excitability, gait and sensorimotor coordination, grip strength, and clinical signs), hematology and clinical chemistry, gross necropsy, organ weights, histopathology, and sperm evaluations. At parturition, litters were evaluated for size and viability; weight of the pups was also recorded. Pups were sacrificed on PND 22.

The following are findings for male F0 rats. Treatment with PFHxS did not affect survival and did not induce clinical signs that could be attributed to the chemical. Terminal body weight in the 10 mg/kg/day groups was approximately 6% lower than controls. Food consumption was not affected. Necropsy did not reveal any treatment-related changes. Histopathological effects were restricted to the liver and

thyroid of males treated with 3 and 10 mg/kg/day. Liver effects consisted of minimal to moderate hypertrophy of centrilobular hepatocytes. The affected hepatocytes were enlarged with an increased amount of dense eosinophilic granular cytoplasm. In the thyroid, the changes consisted of hypertrophy and/or hyperplasia of follicular cells. These effects could have been associated with the liver effects.

Significant organ weight changes consisted of increased absolute and relative liver weight at 3 and 10 mg/kg/day and decreased heart/brain weight at 10 mg/kg/day. Significant hematology changes consisted of decreased hemoglobin at 1 mg/kg/day, decreased red cell count and hematocrit at 3 mg/kg/day, and increased prothrombin time at 0.3 mg/kg/day. Increases in albumin, BUN, alkaline phosphatase, calcium, and albumin/globulin ratio were seen at 10 mg/kg/day. The investigators noted that the alterations in prothrombin time were slight and did not follow a specific trend and the values were within the normal range. There were no significant effects on the FOB or on motor activity and no significant effects on sperm parameters. There were no significant effects in any parameter monitored in F0 females or in pups. Treatment with PFHxS had no significant effect on the gross or microscopic morphology of the spleen, thymus, or lymph nodes. There were no significant effects on sex organ weights or gross or microscopic lesions in the reproductive organs of males and females. Fertility was not affected by treatment with PFHxS and there were no significant effects on sperm parameters. Estrous cycling was not affected by dosing with PFHxS. Treatment with PFHxS did not significantly affect any of the developmental parameters evaluated including gestation length, number of dams delivering litters, averages for implantation sites per delivered litter, number of dams with stillborn pups, number of dams with no live pups, dams with all pups dying, number of pups surviving per litter, sex ratios, litter size, or pup weight. Also, necropsy of the pups showed no treatment-related effects, and pup liver weight was not affected. Treatment with PFHxS had no significant effect on the FOB or motor activity. The battery tested autonomic functions, reactivity and sensitivity to stimuli, excitability, gait and sensorimotor coordination, limb grip strength, and abnormal clinical signs.

Strengths and Weaknesses: The Butenhoff et al. (2009a) study is a well-designed study evaluating male and female reproductive endpoints and developmental endpoints. An adequate number of animals were exposed to three PFHxS dose levels. An additional strength of the study is the inclusion of parameters that evaluated potential neurobehavioral effects, hematological and clinical chemistry parameters, and histopathological examination of the liver and thyroid. Measurement of serum PFHxS levels allowed for estimation of a TWA serum concentration that could be used to calculate a HED. One weakness of the study is that thyroid hormone levels were not measured; these data could have been useful in evaluating the observed histological alterations in the thyroid gland.

Selection of the Point of Departure for the MRL: The HED of the NOAEL of 1 mg/kg/day identified in the Butenhoff et al. (2009a) developmental toxicity study was selected as the POD for the MRL. A TWA serum PFHxS concentration of 73.22 µg/mL was estimated for the adult males exposed to 1 mg/kg/day (Butenhoff et al. 2009a).

Human Equivalent Dose: The HED was calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFHxS equal to the estimated TWA serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details). The HED was calculated using Equation A-6 where C_{ss} is 73.22 µg/mL, K_e=2.23x10^{-4}; V_d=0.287, and AF=1. The $NOAEL_{HED}$ is 0.0047 mg/kg/day

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and a modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability

- 10 MF for database limitations to account for small number of studies examining the toxicity of PFHxS following intermediate-duration exposure and the limited scope of these studies in particular studies examining immunotoxicity, a sensitive endpoint for other perfluoroalkyls.

  MRL = $NOAEL_{HED}$ ÷ (UFs x MF)
  0.0047 mg/kg/day ÷ ((10 x 3) x 10) = $2x10^{-5}$ mg/kg/day

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section. An empirical steady state model was used to estimate the HED from a POD based on a 42-day exposure of adult rats. The resulting HED is lower than the daily 42-day human dose that would be expected to achieve the POD serum concentration.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexane sulfonic acid (PFHxS)
CAS Numbers: 355-46-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: No chronic duration studies were identified for PFHxS. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFHxS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: The only available inhalation exposure study for PFNA (Kinney et al. 1989) was not considered suitable for derivation of an inhalation MRL due to its lack of histopathological examination and short exposure duration.

In the only available inhalation exposure study for PFNA, Kinney et al. (1989) noted labored breathing in rats during and after a 4-hour nose-only exposure to 590 mg/m^3 exposure; the study also reported an increase in relative liver weight 5 days after exposure to $\geq$67 mg/m^3.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFNA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFNA.

Rationale for Not Deriving an MRL: An acute-duration oral MRL cannot be derived for PFNA because the study identifying the lowest dose for a non-hepatic effect (Fang et al. 2009) did not measure serum PFNA levels, which are needed for estimating an HED.

A number of studies examined the toxicity of PFNA in rats and mice exposed for acute durations. These studies reported immune, liver, and body weight effects. Immune effects included increases in thymus weight in rats at 1 mg/kg/day (Fang et al. 2009), decreases in thymus and spleen weights in rats at 3 mg/kg/day (Fang et al. 2009, 2010), and an alteration in splenic lymphocyte phenotypes in mice at 1 mg/kg/day (Fang et al. 2008). In the only study examining immune function, no alterations in splenic lymphocyte response to ConA were observed at doses as high as 5 mg/kg/day in mice (Fang et al. 2008). Liver effects included increases in hepatic lipid levels at ≥0.2 mg/kg/day (Wang et al. 2015a), increases in liver weights at ≥0.2 mg/kg/day (Wang et al. 2015a; Kennedy 1987), serum lipid levels at ≥1 mg/kg/day (Fang et al. 2012a, 2012b), hepatocellular vacuolation at 5 mg/kg/day (Fang et al. 2012b), and increases in serum aminotransferases at 5 mg/kg/day (Wang et al. 2015a). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

Decreases in body weight were observed in rats and mice administered 5 mg/kg/day (Hadrup et al. 2016; Wang et al. 2015a).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate
MRL: $3x10^{-6}$ mg/kg/day
Critical Effect: Decreased body weight and developmental delays
Reference: Das et al. 2015
Point of Departure: 0.001 mg/kg/day
Uncertainty Factor: 30
Modifying Factor: 10
LSE Graph Key: 39
Species: Mouse

MRL Summary: An intermediate-duration oral MRL of $3x10^{-6}$ mg/kg/day was derived for PFNA based on decreased body weight gain and developmental delays in the offspring of mice administered via gavage PFNA on GDs 1–17 (Das et al. 2015). The MRL is based on a HED NOAEL of 0.001 mg/kg/day and a total uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustments and 10 for human variability), and a modifying factor of 10 for database limitations.

Selection of the Critical Effect: The intermediate-duration database consists of three developmental toxicity studies in rats and mice and a 90-day study in mice. The lowest LOAEL for developmental toxicity was 1.1 mg/kg/day in mice administered PFNA on GDs 1–18; at this dose, decreases in litter size and pup survival were observed (Wolf et al. 2010). At higher doses (2–5 mg/kg/day), decreases in pup body weight, delays in postnatal development (Das et al. 2015; Rogers et al. 2014; Wolf et al. 2010), increases in pup systolic blood pressure (Rogers et al. 2014), and reduced nephron endowment (Rogers et al. 2014) were observed. A study of PPARα knockout mice did not find alterations pup body weight or postnatal development at 2 mg/kg/day (Wolf et al. 2010). In the 90-day study, decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; and decreased litter size (males mated to unexposed females) were observed at 0.5 mg/kg/day; no changes were observed at 0.2 mg/kg/day (Singh and Singh 2018). A summary of the observed effects is presented in Table A-18.

Table A-18. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFNA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Parkes mouse 90 days	0.2	No effects reported	Singh and Singh 2018
	0.5	Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size	

Table A-18. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFNA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
129S1/svlm mouse GDs 1–18 (offspring followed until PND 21)	0.83	No effects reported	Wolf et al. 2010
	1.1	Decreased litter size and pup survival	
	1.5	No effects reported	
	2.0	Decreased number of live pups per litter and decreased pup body weight gain	
CD-1 mouse GDs 1–17 (offspring followed until PND 287)	1	No effects reported	Das et al. 2015
	3	Decreased body weight gain and delayed eye opening, preputial separation, and vaginal opening	
	5	Decreased postnatal survival, 80% mortality between PND 2 and 10	
	10	Full litter resorption	
Sprague-Dawley rat GDs 1–20 offspring followed through PND 434)	5	Decreased birth weight, increased blood pressure at 10 weeks of age; reduced nephron endowment	Rogers et al. 2014

GD = gestation day; PFNA = perfluorononanoic acid; PND = postnatal day

Selection of the Principal Study: The lowest LOAEL was 0.5 mg/kg/day identified in the Singh and Singh (2018) reproductive toxicity study. However, this study could not be used to derive an MRL for PFNA because the investigators did not measure serum PFNA levels. Developmental toxicity, including decreases in pup survival, developmental delays, and decreases in birth weight have been observed in three studies. A comparison of the estimated TWA serum PFNA levels (Table A-19) for the Wolf et al. (2010) and Das et al. (2015) studies (measured serum levels were not available from the Rogers et al. 2014 study) showed that the lowest LOAEL for developmental effects was 10.9 µg/mL (Das et al. 2015); this study reported a NOAEL of 6.8 µg/mL. Thus, the Das et al. (2015) study was selected as the principal study for the MRL.

Table A-19. Summary of Estimated TWA Serum PFNA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Estimated TWA serum PFNA (µg/mL)	Effect	Reference
Parkes mouse 90 days	0.2	Not calculated	No effects reported	Singh and Singh 2018
	0.5		Decreased sperm motility, viability, and number; degenerative changes in seminiferous tubules; decreased litter size	

Table A-19. Summary of Estimated TWA Serum PFNA levels in Laboratory Animals Following Intermediate-Duration Oral Exposure

Species and exposure duration	Dose (mg/kg/day)	Estimated TWA serum PFNA (µg/mL)	Effect	Reference
129S1/svlm mouse GDs 1–18 (offspring followed until PND 21)	0.83	4.47	No effects reported	Wolf et al. 2010
	1.1	11.6	Decreased litter size and pup survival	
	1.5	10.5	No effects reported	
	2.0	17.6	Decreased number of live pups per litter and decreased pup body weight gain	
CD-1 mouse GDs 1–17 (offspring followed until PND 287	1	6.8	No effects reported	Das et al. 2015
	3	10.9	Decreased body weight gain and delayed eye opening, preputial separation, and vaginal opening	
	5	39.7	Decreased postnatal survival, 80% mortality between PND 2 and 10	
	10	NA	Full litter resorption	
Sprague-Dawley rat GDs 1–20 (offspring followed through PND 434)	5	Not calculated	Decreased birth weight, increased blood pressure at 10 weeks of age; reduced nephron endowment	Rogers et al. 2014

GD = gestation day; PFNA perfluorononanoic acid; PND = postnatal day

Summary of the Principal Study:

Das KP, Grey BE, Rosen MB, et al. 2015. Developmental toxicity of perfluorononanoic acid in mice. Reprod Toxicol 51:133-144.

Groups of 8–10 timed-pregnant female CD-1 mice were administered via gavage 0, 1, 3, 5, or 10 mg/kg/day PNFA at a dosing volume of 10 ml/kg body weight in deionized water on GDs 1–17. On GD 17, selected mice from each group were sacrificed for maternal and fetal examination, while the remaining mice were allowed to give birth. Pups were observed for postnatal survival up to PND 24 as well as growth and development up to PND 287. The following parameters were used to assess toxicity: clinical observations, maternal body weight, pup body weight (pre- and postnatal), organ weights (liver, gravid uterus weight), number of implantation sites, percent of live fetuses, percent of prenatal loss per litter, and morphological changes (eye opening, vaginal opening, preputial separation).

Maternal weight loss beginning on GD 8 was observed at 10 mg/kg/day; on GD 13, the 10 mg/kg/day group weighed approximately 30% less than controls. The 10 mg/kg/day group was terminated on GD 13. Significant increases in full litter resorptions occurred at 10 mg/kg/day (7/7 compared to 2/8 in controls). There were no adverse effects on pregnancy outcome following *in utero* exposure to 5 mg/kg. Statistically significant dose-related increases in absolute and relative liver weights were observed in dams in the 1, 3, and 5 mg/kg/day groups examined on GD 17, as well as in dams examined on post-weaning day 28. There were no effects on the number of implants, number of live fetuses, or fetal body weight. Relative and absolute fetal liver weight were significantly increased; however, the increase did not appear to be dose-related. Visceral and skeletal examination of fetuses revealed no treatment-related

effects. Increases in postnatal deaths were observed in the 5 mg/kg/day offspring between PND 2 and 10; postnatal survival was approximately 20% on PND 10. Weight gain was significantly reduced in pups from the 3 and 5 mg/kg dose groups from PND 1 to 24. The changes in males were dose-related and persisted from PND 25 to 287. Weight reduction in females was less substantial in comparison with males and returned to control levels by 7 weeks of age. Relative pup liver weights were significantly increased at all doses up to PND 24 and at 3 and 5 mg/kg/day on PND 42. No significant effects on liver weight were detectable by PND 70. Postnatal development (eye opening, preputial separation, and vaginal opening) was significantly delayed (by 2–7 days) at 3 and 5 mg/kg/day.

The serum PFNA levels (means±standard error of the mean) in the pregnant dams (measured at term) were 0.015±0.003, 13.67±1.45, 21.85±3.17, and 79.48±22.69 μg/mL in the 0. 1, 3, and 5 mg/kg/day groups (serum concentrations were provided to ATSDR by C. Lau).

Strengths and Weaknesses: Das et al. (2015) is a well-designed developmental toxicity study in mice. One strength of the study is that it included evaluation of potential anomalies in fetuses, as well as monitoring postnatal growth and development through PND 70. An additional strength of the study is the inclusion of serum and liver PFNA measurements, which allow for cross-species evaluations. Inclusion of measurement of the expression of genes related to PPARα, CAR, and PXR provides valuable mechanisms-of-action data. Although the study tested four PFNA dose levels, increases in maternal morbidity at 10 mg/kg/day and pup lethality at 5 mg/kg/day limited the data available to establish dose-response relationships.

Selection of the Point of Departure for the MRL: The HED of the NOAEL of 1 mg/kg/day identified in the Das et al. (2015) developmental toxicity study was selected as the POD for the MRL. A TWA serum PFNA concentration was estimated for dams using the serum concentration in the control group (0.015 μg/mL) as the baseline concentrations and the terminal concentration for the 1 mg/kg/day group (13.67 μg/mL) resulting in an estimated TWA serum concentration of 6.8 μg/mL.

Human Equivalent Dose: The HED was calculated based on the assumption that humans would have similar effects as the laboratory animal at a given serum concentration. HEDs that would result in steady-state serum concentrations of PFNA equal to the estimated TWA serum concentration selected as the POD were calculated using the first-order single-compartment model (see MRL approach section for details). The HED was calculated using Equation A-6 where C_{ss} is 6.8 μg/mL, K_e=7.59x10^{-4}; V_d=0.2, and AF=1. The K_e was calculated using the 2.5-year elimination half-life in young women; this value was selected over the 4.3-year value for the combined group of males and older females because the MRL is based on a developmental toxicity study. The $NOAEL_{HED}$ is 0.001 mg/kg/day.

Uncertainty Factor and Modifying Factor: The $NOAEL_{HED}$ is divided by a total uncertainty factor (UF) of 30 and modifying factor (MF) of 10:

- 3 UF for extrapolation from animals to humans with dosimetric adjustment
- 10 UF for human variability
- 10 MF for database limitations to account for small number of studies examining the toxicity of PFNA following intermediate-duration exposure and the limited scope of these studies. The available data suggest that reproductive toxicity may be a more sensitive endpoint than developmental toxicity; however, this endpoint could not be used to derive the MRL because the Singh and Singh (2018) study did not measure serum PFNA levels. Additionally, intermediate-duration studies for other perfluoroalkyls suggest that immune function is a sensitive target of toxicity; however, this potential endpoint has not been examined in intermediate-duration PFNA studies.

$$MRL = NOAEL_{HED} \div (UFs \times MF)$$

0.001 mg/kg/day ÷ ((10 x 3) x 10) = $3x10^{-6}$ mg/kg/day

Other Additional Studies or Pertinent Information that Lend Support to this MRL: A discussion of the findings from epidemiological studies is presented in the MRL introduction section.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorononanoic acid (PFNA)
CAS Numbers: 375-95-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: No chronic-duration studies were identified for PFNA. Although adequate data are available for intermediate-duration exposure, ATSDR does not extrapolate across exposure duration.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFNA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: The available acute oral database for PFDA was not considered adequate for derivation of an MRL because the study identifying the lowest adverse effect level did not measure serum PFDA levels, which are needed to estimate HEDs.

Several laboratory animal studies have examined the acute oral toxicity of PFDA; most were limited in scope. The lowest LOAEL was 1 mg/kg/day for decreases in fetal weight in mice administered PFDA on GDs 6–15 (Harris and Birnbaum 1989). At 12.8 mg/kg/day, decreases in the number of live fetuses per litter were observed; maternal weight loss was also observed at this dose level (Harris and Birnbaum 1989). Another developmental toxicity study did not report alterations in performance on neurobehavioral tests in 2–4-month-old mice administered 10.8 mg/kg/day PFDA on PND 10 (Johansson et al. 2008). Other effects observed in acute exposure studies include decreases in maternal weight gain at 6.4 mg/kg/day (Harris and Birnbaum 1989), weight loss at ≥9.5 mg/kg/day in rats (Kawashima et al. 1995) and mice (Harris and Birnbaum 1989; Permadi et al. 1992, 1993), increases in T3 and T4 levels in mice at 80 mg/kg/day (Harris et al. 1989), decreases in spleen weight in mice at 80 mg/kg/day (Harris et al. 1989), and atrophy and lymphoid depletion in thymus and spleen in mice at 160 mg/kg/day (Harris et al. 1989). Liver effects included increases in liver weight at ≥2.4 mg/kg/day (Brewster and Birnbaum 1989; Harris et al. 1989; Kawashima et al. 1995; Permadi et al. 1992, 1993), increases in hepatic lipid levels at ≥9.5 mg/kg/day (Brewster and Birnbaum 1989; Kawashima et al. 1995), and hepatocellular hypertrophy at ≥20 mg/kg/day (Harris et al. 1989). Although there is uncertainty regarding the exact, and possibly multiple, mechanism(s) for these liver effects, peroxisome proliferation is a likely contributor, a mechanism that cannot be reliably extrapolated to humans (Hall et al. 2012). Therefore, increases in liver weight and hepatic lipid levels and alterations in serum lipid levels observed in rats and mice, in the absence of other degenerative lesions, were not considered appropriate endpoints for deriving MRLs.

To derive MRLs for perfluoroalkyls, ATSDR used the approach of predicting TWA serum perfluoroalkyl levels in laboratory animals or measured serum perfluoroalkyl levels and calculating HEDs for these serum concentrations. For PFOA and PFOS, the Wambaugh et al. (2013) pharmacokinetic model was utilized for predicting the TWA serum perfluoroalkyl concentrations. However, strain-, sex-, and compound-specific model parameters are not available for other perfluoroalkyls. The Harris and Birnbaum (1989) study, which identified the lowest adverse effect level, did not measure maternal serum PFDA levels. Thus, HEDs could not be calculated using animal serum PFDA levels. Other approaches such as “read across” or equivalency factors were considered; however, there are limited data available that would allow for comparison of the toxicity and toxicokinetic properties of different perfluoroalkyls. Peters and Gonzalez (2011) noted that the toxic equivalency factor approach would not be suitable for perfluoroalkyls because the current data suggest that the toxicity of these compounds appear to be mediated by multiple receptors, including PPARα, CAR, and PXR, and that there may be species differences in the response mediated by different receptors. Additionally, available data suggest that there are qualitative differences in the toxicities of various perfluoroalkyls.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: Two studies conducted by Frawley et al. (2018) evaluated the intermediate-duration toxicity of PFDA. In a 28-day study in rats, increases in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and decreases in phagocytosis by fixed tissue macrophages in the liver were observed in female rats administered 0.25 mg/kg/day PFDA. At 0.5 mg/kg/day, single cell necrosis was observed. In the second study, decreases in splenic T-cells and macrophages were observed in mice administered 1.25 mg/kg PFDA once a week for 4 weeks.

The Frawley et al. (2018) study was not considered for the principal study since it did not measure serum PFDA levels.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorodecanoic acid (PFDA)
CAS Numbers: 335-76-2
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFDA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFUnA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: No acute-duration oral studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: Intermediate-duration oral database was considered inadequate for derivation of an MRL for PFUnA because the only available study did not measure serum PFUnA levels, which are needed to calculated HEDs (see MRL approach in Appendix A introduction).

One study was identified that examined the oral toxicity of PFUnA in laboratory animals. In this study, decreases in body weight, hematological alterations, increases in liver weight, and centrilobular hypertrophy were observed in rat dams administered 1.0 mg/kg/day for 41–46 days (Takahashi et al. 2014). The study also found decreases in pup body weight on PNDs 0 and 4 at 1.0 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroundecanoic acid (PFUnA)
CAS Numbers: 2058-94-8
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFUnA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFUnA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHpA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluoroheptanoic acid (PFHpA)
CAS Numbers: 375-85-9
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFHpA.

Rationale for Not Deriving an MRL: No oral studies in laboratory animals were identified for PFHpA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFBS.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: No acute-duration oral studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: Several studies have evaluated the toxicity of PFBS following intermediate-duration oral exposure and have identified several targets of toxicity. However, none of these studies included measurement of serum PFBS levels that are needed to calculate a HED and MRL derivation.

Limited data available on the toxicity of PFBS in laboratory animals have identified the liver, kidneys, stomach, and hematological systems and the developing organism as targets of toxicity. Decreases in hemoglobin and hematocrit levels were observed in male rats administered 200 mg/kg/day PFBS for 90 days (Lieder et al. 2009a); decreases in erythrocyte levels were observed at 600 mg/kg/day. Administration of 600 mg/kg/day for 90 days also resulted in tubular and ductal papillary epithelial hyperplasia in the kidneys and necrosis and hyperplasia/hyperkeratosis in the forestomach (Lieder et al. 2009a). Effects in the liver consisted of decreases in plasma triglyceride levels in mice exposed to 30 mg/kg/day for 4–6 weeks (Bijland et al. 2011), increases in absolute and relative liver weight in male rats administered 300 mg/kg/day for at least 70 days (Lieder et al. 2009b) or 900 mg/kg/day for 28 days (3M 2001), and hepatocellular hypertrophy in rats administered 1,000 mg/kg/day in a 2-generation study (Lieder et al. 2009b). In general, no biologically relevant alterations in performance on FOB tests or motor activity tests were observed in rats administered 900 mg/kg/day PFBS for 28 days (3M 2001) or 600 mg/kg/day for 90 days (Lieder et al. 2009a).

Decreases in fetal body weight were observed in two studies involving administration of PFBS to rats on GDs 6–20 (York 2002, 2003a); one study reported a LOAEL of 1,000 mg/kg/day (York 2002) and the other a LOAEL of 2,000 mg/kg/day with a NOAEL of 1,000 mg/kg/day (York 2003a). In a developmental toxicity mouse study, decreases in pup body weight, developmental delays (eye opening and vaginal opening), impaired development of the reproductive system (delay in first estrous, decreases in ovarian follicles, decreases in uterine endometrial and myometrial thickness), and decreases in total T4 and T3 and increases in TSH were observed in the offspring of mice administered 200 mg/kg/day PFBS (Feng et al. 2017). Decreases in maternal total T4, free T4, and total T3 and increases in TSH were observed at 200 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutane sulfonic acid (PFBS)
CAS Numbers: 375-73-5
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFBS.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFBS.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFBA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: Laboratory animal studies for other perfluoroalkyls have identified immunotoxicity and developmental toxicity as sensitive endpoints following acute-duration oral exposure; these potential targets have not been investigated for PFBA. Thus, the database was considered inadequate for identifying a critical endpoint and evaluating dose-response relationships.

Three studies have examined the acute toxicity of PFBA in laboratory animals for a limited number of potential endpoints. Ikeda et al. (1985) reported that administration of approximately 20 mg/kg/day PFBA in the diet to male rats for 2 weeks did not significantly affect relative liver weight, but increased catalase activity in liver homogenates by 42% and induced peroxisome proliferation, as assessed by electron microscopy. In a similar study, dietary administration of approximately 78 mg/kg/day PFBA to male mice for 10 days induced a 63% increase in absolute liver weight (Permadi et al. 1992). The increase in liver weight was accompanied by changes in enzymes involved in drug metabolism and/or in deactivation of reactive oxygen species; however, PFBA did not have a significant effect on parameters of peroxisomal fatty acid β-oxidation (Permadi et al. 1993). In a more comprehensive study, no significant effect on a wide range of endpoints including body and organ weights, hematology and clinical chemistry, and histopathology were observed in rats administered 184 mg/kg/day for 5 days (3M 2007a).

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: The available intermediate-duration database was not considered adequate for derivation of an MRL. Although the available studies have examined potentially sensitive endpoints and developmental toxicity and both studies measured serum PFBA levels, the database is missing a reliable estimate of elimination half-life in humans. Chang et al. (2008b) reported serum half-lives in small groups of subjects (<10 subjects); only 2 of the subjects were females. Because developmental toxicity is one of the more sensitive endpoints, data from females is needed in order to estimate the HED.

The intermediate-duration oral database for PFBA consists of a developmental study in mice (Das et al. 2008) and 28- and 90-day gavage studies in rats (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). In the developmental study, PFBA administered to pregnant mice on GDs 1–17 did not affect newborn weight gain or viability (Das et al. 2008). The most sensitive response was a delay in eye opening in the pups at maternal doses of PFBA of 35 mg/kg/day. In the 28- and 90-day studies, hyperplasia/hypertrophy of the follicular epithelium of the thyroid and hepatocellular hypertrophy were observed at ≥30 mg/kg/day (Butenhoff et al. 2012a; van Otterdijk 2007a, 2007b). In addition, the 90-day study reported hematological alterations in male rats dosed with 30 mg/kg/day PFBA. The NOAEL for these effects was 6 mg/kg/day.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorobutanoic acid (PFBA)
CAS Numbers: 375-22-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFBA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFBA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFDoDA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: The database was considered inadequate for derivation of an MRL.

Two studies have examined the acute-oral toxicity of PFDoDA. Shi et al. (2007) reported decreases in body weight and decreases in serum testosterone and estradiol levels in rats following a 14-day gavage administration of 5 mg/kg/day (Shi et al. 2007). The study also reported an increase in serum cholesterol levels at 10 mg/kg/day. In the second study, Zhang et al. (2008) found increases in liver weight and hepatic triglyceride and cholesterol levels in rats administered via gavage ≥5 mg/kg/day for 14 days; these liver effects were not considered relevant to humans. Given the limited number of endpoints examined in Shi et al. (2007) this study, including the lack of histopathological examination, this study was not considered suitable for the derivation of an MRL.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: Three intermediate-duration studies examined the oral toxicity of PFDoDA. Decreases in serum estradiol and increases in serum cholesterol were observed in pubertal females exposed to 3 mg/kg/day PFDoDA on PNDs 24–72 (Shi et al. 2009b). In a second study by this group, decreases in serum testosterone levels were observed in male rats administered 0.2 mg/kg/day PFDoDA for 110 days (Shi et al. 2009a). In a one-generation reproductive/developmental toxicity study, increases in maternal deaths were observed in rats administered 2.5 mg/kg/day PFDoDA prior to mating and throughout gestation, and lactation days 1–5 (Kato et al. 2015). Other effects observed in the male and female parental animals administered 2.5 mg/kg/day included decreases in body weight, decreases in mean corpuscular volume and reticulocytes, single cell hepatocellular necrosis (females only), pancreatic interstitial edema (females only), atrophy of the thymic cortex (females only), atrophy of the adrenal cortex (males only), decrease forelimb grip strength (males only), hemorrhage at the implantation site, and continuous diestrus in unmated females (Kato et al. 2015). The study also reported decreases in pup body weight in the only litter with live pups.

The Shi et al. (2009a) study identified the lowest LOAEL of 0.5 mg/kg/day. However, this study was not considered suitable for MRL derivation because it examined a limited number of endpoints (body weight and reproductive toxicity in males). The Kato et al. (2015) study examined a wide range of endpoints, but effects were only observed at a lethal dose.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorododecanoic acid (PFDoDA)
CAS Numbers: 307-55-1
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFDoDA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for PFDoDA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for FOSA.

Rationale for Not Deriving an MRL: No inhalation studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: The acute-duration database for FOSA was not considered adequate for identifying critical targets of toxicity because the Seacat and Luebker (2000) study only examined a limited number of potential endpoints and the potential developmental and immunological effects (sensitive targets for other perfluoroalkyls) were not examined.

One laboratory animal study evaluated the acute oral toxicity of FOSA. Seacat and Luebker (2000) did not find alterations in body weight or liver weight in rats administered a single dose of 5 mg/kg/day FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: No intermediate-duration oral studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorooctanesulfonamide (FOSA)
CAS Numbers: 754-91-6
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for FOSA.

Rationale for Not Deriving an MRL: No chronic-duration oral studies in laboratory animals were identified for FOSA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No acute-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Inhalation
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL for PFHxA.

Rationale for Not Deriving an MRL: No chronic-duration inhalation studies in laboratory animals were identified for PFHxA.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Acute

MRL Summary: There are insufficient data for derivation of an acute-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: The acute database for PFHxA was not considered adequate for derivation of an MRL because serum PFHxA levels at the lowest LOAEL were below the detection limit and an elimination half-life has not been estimated for humans; both of these toxicokinetic parameters are needed to estimate HEDs.

Two developmental toxicity studies conducted by Iwai and Hoberman (2014) examined the acute toxicity of PFHxA following gavage administration. Increases in stillborn pups and decreases in pup body weight were observed at 175 mg/kg/day; no effects were observed at 35 mg/kg/day. In the second study, decreases in birth weight and delayed eye opening was observed at 350 mg/kg/day; the NOAEL was 100 mg/kg/day. These studies were not considered adequate for derivation of an MRL because the measured serum PFHxA levels at 35 and 175 mg/kg/day groups were below the limit of detection. Additionally, the elimination half-life has not been estimated in humans.

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: Five studies have evaluated the intermediate-duration toxicity of PFHxA in rats. A summary of the adverse effects observed in these studies is presented in Table A-20. These studies identify several targets of toxicity including the respiratory tract, erythrocytes, thyroid, thymus, and developing organism; the lowest LOAEL is 100 mg/kg/day for nasal lesions and decrease in body weight gain in males. None of the available studies evaluated immune function, which has been identified as a sensitive target in intermediate oral studies for other perfluoroalkyls.

The data are considered inadequate for MRL derivation because an elimination half-life has not been estimated in humans. Thus, a HED cannot be calculated and an MRL cannot be derived.

Table A-20. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFHxA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Rat 92–93 days (GW) 30 M, 30 F	20	No effects observed	Loveless et al. 2009
	100	Degeneration/atrophy of nasal olfactory epithelium	
	500	Respiratory metaplasia, decreased RBC, hemoglobin, and hematocrit; increased reticulocytes; thyroid follicular epithelial hypertrophy	
Rat 110–120 days (premating, gestation, lactation) (GW) 20 M, 20 F	20	No effect observed	Loveless et al. 2009
	100	Decreased weight gain in males	
	500	Decreased maternal weight gain, decreased pup body weight during lactation period	
Rat 90 days (GW) 10 M, 10 F	10	No effects observed	Chengelis et al. 2009b
	50	No effects observed	
	200	Slight decrease in RBC, hemoglobin, and hematocrit and increase in reticulocytes	

Table A-20. Summary of the Adverse Effects Observed in Laboratory Animals Following Intermediate-Duration Oral Exposure to PFHxA

Species and exposure duration	Dose (mg/kg/day)	Effect	Reference
Rat 32–44 days (GW) 10–15 M, 10–15 F	50	No effects observed	Kirkpatrick 2005
	150	Decreased hemoglobin levels (males only)	
	315 (TWA dose)	Decreased hemoglobin levels (males only), increased reticulocyte levels (males only), thymic atrophy (females only)	
Rat GDs 1–20 (GW) 22 F	20	No effect observed	Loveless et al. 2009
	100	No effect observed	
	500	Decreased maternal weight gain; decreased fetal body weight	

F = female(s); GD = gestation day; (GW) = gavage in water; M = male(s); RBC = red blood cell; TWA = time-weighted average

Agency Contacts (Chemical Managers): Melanie Buser

MINIMAL RISK LEVEL (MRL) WORKSHEET

***Chemical Name*:** Perfluorohexanoic acid (PFHxA)
CAS Numbers: 307-24-4
Date: March 2020
Profile Status: Final
Route: Oral
Duration: Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration oral MRL for PFHxA.

Rationale for Not Deriving an MRL: The chronic duration oral database for PFHxA is not considered adequate for derivation of a chronic MRL because the only study available did not measure serum PFHxA levels and elimination half-life data are not available for humans. These toxicokinetic data are needed to derive HEDs.

One study has evaluated the chronic oral toxicity of PFHxA in laboratory animals (Klaunig et al. 2015). Exposure to female rats to 200 mg/kg/day resulted in hematological alterations (decreases in red blood cells and hemoglobin levels and increases in reticulocyte counts), renal effects (tubular degeneration, necrosis, increased urine volume and reduced specific gravity), and liver effects (necrosis); no adverse alterations were observed at 30 mg/kg/day or at 100 mg/kg/day in males. This study was not considered suitable for derivation of an MRL because serum PFHxA levels were not measured. Additionally, an elimination half-life has not been estimated in humans.

Agency Contacts (Chemical Managers): Melanie Buser

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR PERFLUOROALKYLS

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to perfluoroalkyls.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for perfluoroalkyls. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of perfluoroalkyls have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of perfluoroalkyls are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

- Health Effects
  - Species
    - Human
    - Laboratory mammals
  - Route of exposure
    - Inhalation
    - Oral
    - Dermal (or ocular)
    - Parenteral (these studies will be considered supporting data)
  - Health outcome
    - Death
    - Systemic effects
    - Body weight effects
    - Respiratory effects
    - Cardiovascular effects
    - Gastrointestinal effects
    - Hematological effects
    - Musculoskeletal effects
    - Hepatic effects
    - Renal effects
    - Dermal effects
    - Ocular effects
    - Endocrine effects
    - Immunological effects
    - Neurological effects
    - Reproductive effects
    - Developmental effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Other noncancer effects
Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The current literature search was intended to update the draft toxicological profile for perfluoroalkyls released for public comment in 2015. The following main databases were searched in March 2008, September/October 2013, May 2016, and September 2018:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, and Medical Subject Headings (MeSH) terms for perfluoroalkyls. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance priority list (SPL) resource page, and other items as needed. Regulations applicable to perfluoroalkyls were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
PubMed	
9/11/2018	((1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR "perfluorododecanoic acid"[nm] OR "perfluorobutanesulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw]) AND (2015/05/01:3000[dp] OR 2016/05/01:3000[mhda] OR 2016/05/01:3000[crdat] OR 2016/05/01:3000[edat])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR "pharmacology"[sh:noexp] OR toxicokinetics[mh:noexp] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))) (("FC-143 surfactant"[nm] OR 307-24-4[rn] OR 3825-26-1[rn] OR "Hexanoic acid, 2,2,3,3,4,4,5,5,6,6,6-undecafluoro-"[tw] OR "Hexanoic acid, undecafluoro-"[tw] OR "Perfluorohexanoic acid"[tw] OR "Undecafluoro-1-hexanoic acid"[tw] OR "Undecafluorohexanoic acid"[tw] OR "Ammonium pentadecafluorooctanoate"[tw] OR "Ammonium perfluorocaprilate"[tw] OR "Ammonium perfluorocaprylate"[tw] OR "Ammonium perfluorooctanoate"[tw] OR "FC 143"[tw] OR "Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-, ammonium salt"[tw] OR "Octanoic acid, pentadecafluoro-, ammonium salt"[tw] OR "Pentadecafluoro-1-octanoic acid, ammonium salt"[tw] OR "Pentadecafluorooctanoic acid, ammonium salt"[tw] OR "Perfluoroammonium octanoate"[tw] OR "Perfluorooctanoic acid, ammonium salt"[tw]) NOT (1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR "perfluorododecanoic acid"[nm] OR "perfluorobutanesulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	"endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR "pharmacology"[sh:noexp] OR toxicokinetics[mh:noexp] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))))
05/24/2016	(((((("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw]) AND (2013/09/01:3000[crdat] OR 2013/09/01:3000[edat])) NOT medline[sb]))))) OR ((((("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "C11-PFA"[tw] OR "et-pfosa-acoh"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluorooctane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "me-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Ndfda"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentadecafluoro-1-octanoic

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Database search date	Query string
	acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorodecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "perfluorooctane sulphonic acid"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw] OR "Perfluoroundecanoic acid"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "pfdoa"[tw] OR "Pfhpa"[tw] OR "PFHS cpd"[tw] OR "pfhxs"[tw] OR "pfna"[tw] OR "PFOA"[tw] OR "PFOS"[tw] OR "pfsoa"[tw] OR "Pfua"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw] OR 1763-23-1[rn] OR 2058-94-8[rn] OR 2355-31-9[rn] OR 2991-50-6[rn] OR 307-55-1[rn] OR 335-67-1[rn] OR 335-76-2[rn] OR 355-46-4[rn] OR 375-22-4[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn]) AND (2013/09/01:3000[mhda] OR 2013/09/01:3000[crdat] OR 2013/09/01:3000[edat])) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR ai[sh] OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "pharmacology"[sh:noexp] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR "Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh] OR cancer[sb] OR (me[sh] AND ("humans"[mh] OR "animals"[mh]))))
10/03/2013	("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR " RNA, Messenger "[mh] OR " RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh]) AND ((335-67-1[rn] OR 1763-23-1[rn] OR 355-46-4[rn] OR 2991-50-6[rn] OR 2355-31-9[rn] OR 335-76-2[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR 2058-94-8[rn] OR 307-55-1[rn] OR 375-22-4[rn] OR 80AM718FML[rn]) AND 2007/05/01:2013/10/03[dp])
09/19/2013	(((335-67-1[rn] OR 1763-23-1[rn] OR 355-46-4[rn] OR 2991-50-6[rn] OR 2355-31-9[rn] OR 335-76-2[rn] OR 375-73-5[rn] OR 375-85-9[rn] OR 375-95-1[rn] OR 754-91-6[rn] OR 2058-94-8[rn] OR 307-55-1[rn] OR 375-22-4[rn] OR 80AM718FML[rn]) AND 2007/05/01:2013/09/19[dp]) AND (((Caprylates/metabolism[MeSH Terms] OR Fluorocarbons/metabolism[MeSH Terms] OR "Alkanesulfonic Acids/metabolism"[MeSH Terms] OR "Sulfonic Acids/metabolism"[MeSH Terms] OR "Decanoic Acids/metabolism"[MeSH Terms] OR "Heptanoic Acids/metabolism"[MeSH Terms] OR "Hydrocarbons, Fluorinated/metabolism"[MeSH Terms] OR "Fatty Acids/metabolism"[MeSH Terms]

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Database search date	Query string
	OR Sulfonamides/metabolism[MeSH Terms]) AND ("humans"[MeSH Terms] OR "animals"[MeSH Terms])) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND (Endocrine System[mh] OR Hormones[mh] OR Endocrine disruptors[mh])) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND "environmental exposure"[MeSH Terms]) OR ((Caprylates[MeSH Terms] OR Fluorocarbons[MeSH Terms] OR "Alkanesulfonic Acids"[MeSH Terms] OR "Sulfonic Acids"[MeSH Terms] OR "Decanoic Acids"[MeSH Terms] OR "Heptanoic Acids"[MeSH Terms] OR "Hydrocarbons, Fluorinated"[MeSH Terms] OR "Fatty Acids"[MeSH Terms] OR Sulfonamides[MeSH Terms]) AND "chemically induced"[MeSH Subheading]) OR ((((((((((("caprylates/adverse effects"[MeSH Terms] OR "caprylates/antagonists and inhibitors"[MeSH Terms] OR "caprylates/blood"[MeSH Terms] OR "caprylates/cerebrospinal fluid"[MeSH Terms] OR "caprylates/pharmacokinetics"[MeSH Terms] OR "caprylates/poisoning"[MeSH Terms] OR "caprylates/toxicity"[MeSH Terms] OR "caprylates/urine"[MeSH Terms])))) OR (("fluorocarbons/adverse effects"[MeSH Terms] OR "fluorocarbons/antagonists and inhibitors"[MeSH Terms] OR "fluorocarbons/blood"[MeSH Terms] OR "fluorocarbons/pharmacokinetics"[MeSH Terms] OR "fluorocarbons/poisoning"[MeSH Terms] OR "fluorocarbons/toxicity"[MeSH Terms] OR "fluorocarbons/urine"[MeSH Terms]))) OR (("alkanesulfonic acids/adverse effects"[MeSH Terms] OR "alkanesulfonic acids/antagonists and inhibitors"[MeSH Terms] OR "alkanesulfonic acids/blood"[MeSH Terms] OR "alkanesulfonic acids/cerebrospinal fluid"[MeSH Terms] OR "alkanesulfonic acids/pharmacokinetics"[MeSH Terms] OR "alkanesulfonic acids/poisoning"[MeSH Terms] OR "alkanesulfonic acids/toxicity"[MeSH Terms] OR "alkanesulfonic acids/urine"[MeSH Terms]))) OR (("sulfonic acids/adverse effects"[MeSH Terms] OR "sulfonic acids/antagonists and inhibitors"[MeSH Terms] OR "sulfonic acids/blood"[MeSH Terms] OR "sulfonic acids/cerebrospinal fluid"[MeSH Terms] OR "sulfonic acids/pharmacokinetics"[MeSH Terms] OR "sulfonic acids/poisoning"[MeSH Terms] OR "sulfonic acids/toxicity"[MeSH Terms] OR "sulfonic acids/urine"[MeSH Terms]))) OR (("decanoic acids/adverse effects"[MeSH Terms] OR "decanoic acids/antagonists and inhibitors"[MeSH Terms] OR "decanoic acids/blood"[MeSH Terms] OR "decanoic acids/pharmacokinetics"[MeSH Terms] OR "decanoic acids/poisoning"[MeSH Terms] OR "decanoic acids/toxicity"[MeSH Terms] OR "decanoic acids/urine"[MeSH Terms]))) OR (("heptanoic acids/adverse effects"[MeSH Terms] OR "heptanoic acids/antagonists and inhibitors"[MeSH Terms] OR "heptanoic acids/blood"[MeSH Terms] OR "heptanoic acids/cerebrospinal fluid"[MeSH Terms] OR "heptanoic acids/pharmacokinetics"[MeSH Terms] OR "heptanoic acids/poisoning"[MeSH Terms] OR "heptanoic acids/toxicity"[MeSH Terms] OR "heptanoic acids/urine"[MeSH Terms]))) OR (("hydrocarbons, fluorinated/adverse effects"[MeSH Terms] OR "hydrocarbons, fluorinated/antagonists and inhibitors"[MeSH Terms] OR "hydrocarbons, fluorinated/blood"[MeSH Terms] OR "hydrocarbons, fluorinated/cerebrospinal fluid"[MeSH Terms] OR "hydrocarbons, fluorinated/pharmacokinetics"[MeSH Terms] OR "hydrocarbons, fluorinated/toxicity"[MeSH Terms] OR "hydrocarbons, fluorinated/urine"[MeSH Terms]))) OR (("fatty acids/adverse effects"[MeSH Terms] OR "fatty acids/antagonists and inhibitors"[MeSH Terms] OR "fatty acids/blood"[MeSH Terms] OR "fatty acids/cerebrospinal fluid"[MeSH Terms] OR "fatty acids/pharmacokinetics"[MeSH Terms] OR "fatty acids/poisoning"[MeSH Terms] OR "fatty acids/toxicity"[MeSH Terms] OR "fatty acids/urine"[MeSH Terms]))) OR (("sulfonamides/adverse effects"[MeSH Terms] OR "sulfonamides/antagonists and inhibitors"[MeSH Terms] OR "sulfonamides/blood"[MeSH Terms] OR "sulfonamides/cerebrospinal fluid"[MeSH Terms] OR "sulfonamides/pharmacokinetics"[MeSH Terms] OR "sulfonamides/poisoning"[MeSH Terms] OR "sulfonamides/toxicity"[MeSH Terms] OR "sulfonamides/urine"[MeSH Terms])))))) OR (("Perfluorooctanoic acid"[tw] OR "Pentadecafluoro-1-octanoic acid"[tw] OR "Pentadecafluoro-n-octanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorocaprylic acid"[tw] OR "Perfluoroctanoic acid"[tw] OR "Perfluoroheptanecarboxylic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Pentadecafluorooctanoic acid"[tw] OR "Perfluorooctanoic acid"[tw] OR "Perfluorooctane sulfonic acid "[tw] OR "Heptadecafluoro-1-octanesulfonic acid"[tw] OR "heptadecafluoro-1-octane sulfonic acid"[tw] OR "Heptadecafluorooctane-1-sulphonic acid"[tw] OR "heptadecafluorooctane sulfonic

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	acid"[tw] OR "Perfluorooctane sulfonate"[tw] OR "Perfluorooctylsulfonic acid"[tw] OR "perfluorooctane sulphonic acid"[tw] OR "perfluorooctanesulfonic acid"[tw] OR "Perfluorooctanesulfonate"[tw] OR "Heptadecafluorooctane-1-sulphonic acid1-Perfluorooctanesulfonic acid"[tw] OR "Perfluorohexane sulfonic acid"[tw] OR "pfhxs"[tw] OR "perfluorohexanesulfonic acid"[tw] OR "perfluorohexanesulfonate"[tw] OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid"[tw] OR "Perfluorohexane-1-sulphonic acid"[tw] OR "PFHS cpd"[tw] OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "et-pfosa-acoh"[tw] OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine"[tw] OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-"[tw] OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid"[tw] OR "me-pfosa-acoh"[tw] OR "Perfluorodecanoic acid"[tw] OR "Nonadecafluoro-n-decanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-N-decanoic acid"[tw] OR "Perfluoro-n-decanoic acid"[tw] OR "Perfluorodecanoic acid"[tw] OR "Nonadecafluorodecanoic acid"[tw] OR "Perfluoro-N-decanoic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Pentyl perfluorobutanoate"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Tridecafluoro-1-heptanoic acid"[tw] OR "Perfluoro-n-heptanoic acid"[tw] OR "Perfluoroheptanoic acid"[tw] OR "Tridecafluoroheptanoic acid"[tw] OR "Perfluorononanoic acid"[tw] OR "Perfluoro-n-nonanoic acid"[tw] OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid"[tw] OR "Perfluorononan-1-oic acid"[tw] OR "Perfluorooctane sulfonamide"[tw] OR "Perfluorooctanesulfonamide"[tw] OR "Perfluoroctylsulfonamide"[tw] OR "Perfluorooctanesulfonic acid amide"[tw] OR "Heptadecafluorooctanesulphonamide"[tw] OR "Perfluoroundecanoic acid"[tw] OR "Perfluoro-n-undecanoic acid"[tw] OR "Henicosafluoroundecanoic acid"[tw] OR "Perfluorododecanoic acid "[tw] OR "Perfluorododecanoic acid"[tw] OR "Tricosafluorododecanoic acid"[tw] OR "Perfluorolauric acid"[tw] OR "Perfluorobutyric acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Heptafluoro-1-butanoic acid"[tw] OR "Heptafluorobutanoic acid"[tw] OR "Heptafluorobutyric acid"[tw] OR "Perfluorobutanoic acid"[tw] OR "Perfluoropropanecarboxylic acid"[tw]) NOT medline[sb]) OR (("PFOA"[tw] OR "PFOS"[tw] OR "Pfua"[tw] OR "pfdoa"[tw] OR "C11-PFA"[tw] OR "pfsoa"[tw] OR "pfna"[tw] OR "Pfhpa"[tw] OR "pfbus"[tw] OR "PFDA"[tw] OR "pfdea"[tw] OR "Ndfda"[tw]) NOT medline[sb])
Toxcenter	
9/11/2018	FILE 'TOXCENTER' ENTERED AT 15:57:25 ON 11 SEP 2018 CHARGED TO COST=EH011.10.LB.01.05 L1 7667 SEA FILE=TOXCENTER 1763-23-1 OR 2058-94-8 OR 2355-31-9 OR 2991-50-6 OR 307-55-1 OR 335-67-1 OR 335-76-2 OR 355-46-4 OR 375-22-4 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 L2 1413 SEA FILE=TOXCENTER 307-24-4 OR 3825-26-1 L4 189 SEA FILE=TOXCENTER L2 NOT L1 L5 7856 SEA FILE=TOXCENTER L1 OR L2 L6 7800 SEA FILE=TOXCENTER L5 NOT TSCATS/FS L7 7460 SEA FILE=TOXCENTER L6 NOT PATENT/DT ACTIVATE TOXQUERY/Q --------- L8 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L9 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L10 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L11 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L12 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L13 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L14 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L15 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L16 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L17 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L18 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L19 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L20 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L21 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L22 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L23 QUE (ENDOCRIN? AND DISRUPT?)
	L24 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L25 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L26 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L27 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L28 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L29 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L30 QUE (NEPHROTOX? OR HEPATOTOX?)
	L31 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L32 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L33 QUE L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32
	L34 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L35 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L36 QUE L33 OR L34 OR L35
	L37 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L38 QUE L36 OR L37

	L39 5371 SEA FILE=TOXCENTER L7 AND L38
	L40 1566 SEA FILE=TOXCENTER L39 AND MEDLINE/FS
	L41 1116 SEA FILE=TOXCENTER L39 AND BIOSIS/FS
	L42 2649 SEA FILE=TOXCENTER L39 AND CAPLUS/FS
	L43 40 SEA FILE=TOXCENTER L39 NOT (L40 OR L41 OR L42)
	L44 3543 DUP REM L40 L41 L43 L42 (1828 DUPLICATES REMOVED) ANSWERS '1-3543' FROM FILE TOXCENTER
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L45 1566 SEA FILE=TOXCENTER L44
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L46 594 SEA FILE=TOXCENTER L44
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L47 1350 SEA FILE=TOXCENTER L44
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L48 33 SEA FILE=TOXCENTER L44

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L49 865 SEA FILE=TOXCENTER (L45 OR L46 OR L47 OR L48) AND (ED>20160401 OR PY>2015)
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L*** DEL 1566 S L39 AND MEDLINE/FS
	L50 1566 SEA FILE=TOXCENTER L44
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L*** DEL 1116 S L39 AND BIOSIS/FS
	L51 594 SEA FILE=TOXCENTER L44
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L*** DEL 2649 S L39 AND CAPLUS/FS
	L52 1350 SEA FILE=TOXCENTER L44
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L*** DEL 40 S L39 NOT (L40 OR L41 OR L42)
	L53 33 SEA FILE=TOXCENTER L44
	L54 2678 SEA FILE=TOXCENTER (L50 OR L51 OR L52 OR L53) NOT L49
	L55 43 SEA FILE=TOXCENTER L54 AND L4
	L56 908 SEA FILE=TOXCENTER L49 OR L55
	SAVE TEMP L56 PFOA/Q
	D SCAN L56
05/25/2016	FILE 'TOXCENTER' ENTERED AT 08:37:55 ON 25 MAY 2016
	L1 5994 SEA 335-67-1 OR 1763-23-1 OR 355-46-4 OR 2991-50-6 OR 2355-31-9 OR 335-76-2 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 OR 2058-94-8 OR 307-55-1 OR 375-22-4
	L2 5967 SEA L1 NOT TSCATS/FS
	L3 5731 SEA L2 NOT PATENT/DT
	L4 1847 SEA L3 AND ED>=20130701
	ACT TOXQUERY/Q
	L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)
	L7 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L10 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L11 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L12 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L13 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L14 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L15 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L16 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L17 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L18 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L19 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L20 QUE (ENDOCRIN? AND DISRUPT?)
	L21 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L22 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L23 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L24 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L25 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L26 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L27 QUE (NEPHROTOX? OR HEPATOTOX?)
	L28 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L29 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L30 QUE L5 OR L6 OR L7 OR L8 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29
	L31 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L32 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L33 QUE L30 OR L31 OR L32
	L34 QUE (NONHUMAN MAMMALS)/ORGN
	L35 QUE L33 OR L34
	L36 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L37 QUE L35 OR L36
	L38 1318 SEA L4 AND L37
	L39 1148 SEA L4 AND L30
	L40 356 SEA L38 AND MEDLINE/FS
	L41 297 SEA L38 AND BIOSIS/FS
	L42 664 SEA L38 AND CAPLUS/FS
	L43 1 SEA L38 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L44 931 DUP REM L40 L41 L43 L42 (387 DUPLICATES REMOVED)
	L*** DEL 356 S L38 AND MEDLINE/FS
	L*** DEL 356 S L38 AND MEDLINE/FS
	L45 356 SEA L44
	L*** DEL 297 S L38 AND BIOSIS/FS
	L*** DEL 297 S L38 AND BIOSIS/FS
	L46 190 SEA L44
	L*** DEL 664 S L38 AND CAPLUS/FS
	L*** DEL 664 S L38 AND CAPLUS/FS
	L47 385 SEA L44
	L48 575 SEA (L45 OR L46 OR L47) NOT MEDLINE/FS
	D SCAN L48
09/19/2013	FILE 'TOXCENTER' ENTERED AT 09:10:51 ON 19 SEP 2013
	L1 3993 SEA 335-67-1 OR 1763-23-1 OR 355-46-4 OR 2991-50-6 OR 2355-31-9 OR 335-76-2 OR 375-73-5 OR 375-85-9 OR 375-95-1 OR 754-91-6 OR 2058-94-8 OR 307-55-1 OR 375-22-4
	L2 3966 SEA L1 NOT TSCATS/FS
	L3 3782 SEA L2 NOT PATENT/DT
	L4 2796 SEA L3 AND PY>2006
	ACTIVATE TOXBROAD/Q
	L5 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L6 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT)
	L7 QUE (ACUTE OR SUBACUTE OR LD50 OR LC50)
	L8 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT
	L9 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L10 QUE (VAPOR? OR VAPOUR? OR AEROSOL?)
	L11 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L12 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET? OR DRINKING(W)WATER?)
	L13 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	L14 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L15 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L16 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L17 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L18 QUE (SPERM? OR NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMEN TAL?)
	L19 QUE (ENDOCRIN? AND DISRUPT?)
	L20 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L21 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L22 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L23 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L24 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L25 QUE (GENETOX? OR GENOTOX? OR MUTAGEN?)
	L26 QUE GENETIC(W)TOXIC?
	L27 QUE L5 OR L6 OR L7 OR L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21
	L28 QUE L27 OR L22 OR L23 OR L24 OR L25 OR L26
	L29 QUE L28 OR L8
	L30 QUE NEPHROTOX? OR HEPATOTOX? OR ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?
	L31 QUE L29 OR L30
	L32 QUE RAT OR RATS OR MOUSE OR MICE OR GUINEA PIG OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR GOAT OR GOATS OR SHEEP OR MONKEY? OR MACAQUE?
	L33 QUE MARMOSET? OR FERRET? OR GERBIL? OR HAMSTER? OR RODENT? OR LAGOMORPHA OR BABOON? OR BOVINE OR CANINE OR CAT OR CATS OR FELINE OR PIGEON?
	L34 QUE OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?
	L35 QUE L31 OR L32 OR L33 OR L34
	L36 QUE NONHUMAN MAMMALS/ORGN
	L37 QUE L35 OR L36
	L38 QUE HUMAN? OR HOMINIDAE OR MAMMAL? OR PRIMATE?
	L39 QUE L37 OR L38
	L40 2012 SEA L4 AND L39
	L41 619 SEA L40 AND MEDLINE/FS
	L42 417 SEA L40 AND BIOSIS/FS
	L43 975 SEA L40 AND CAPLUS/FS
	L44 1 SEA L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L45 1308 DUP REM L41 L42 L44 L43 (704 DUPLICATES REMOVED)
	L*** DEL 619 S L40 AND MEDLINE/FS
	L*** DEL 619 S L40 AND MEDLINE/FS
	L46 619 SEA L45
	L*** DEL 417 S L40 AND BIOSIS/FS
	L*** DEL 417 S L40 AND BIOSIS/FS
	L47 217 SEA L45
	L*** DEL 975 S L40 AND CAPLUS/FS
	L*** DEL 975 S L40 AND CAPLUS/FS
	L48 471 SEA L45
	L*** DEL 1 S L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L*** DEL 1 S L40 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L49 1 SEA L45
	L50 689 SEA (L46 OR L47 OR L48 OR L49) NOT MEDLINE/FS

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	SAVE TEMP L50 PERFLUOROALKYLS/A PFOA/A
	L51 217 SEA L50 AND BIOSIS/FS
	L52 471 SEA L50 AND CAPLUS/FS
	L53 220 SEA L52 AND 4-?/CC
	L54 1 SEA L50 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L55 438 SEA L51 OR L53 OR L54
	L56 689 SEA L55 OR L52
	D SCAN L55
ToxLine	
9/11/2018	(1763-23-1 [rn] OR 2058-94-8 [rn] OR 2355-31-9 [rn] OR 2991-50-6 [rn] OR 307-55-1 [rn] OR 335-67-1 [rn] OR 335-76-2 [rn] OR 355-46-4 [rn] OR 375-22-4 [rn] OR 375-73-5 [rn] OR 375-85-9 [rn] OR 375-95-1 [rn] OR 754-91-6 [rn]) AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("perfluoro n heptanoic acid" OR "perfluoro n nonanoic acid" OR "perfluoro n undecanoic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonic acid" OR "perfluorobutanoic acid" OR "perfluorobutyric acid" OR "perfluorocaprylic acid" OR "perfluorooctanoic acid" OR "perfluorooctylsulfonamide" OR "perfluorodecanoic acid" OR "perfluorododecanoic acid" OR "perfluorododecanoic acid " OR "perfluoroheptanecarboxylic acid" OR "perfluoroheptanoic acid" OR "perfluorohexane sulfonic acid" OR "perfluorohexane 1 sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "perfluorolauric acid" OR "perfluorononan 1 oic acid" OR "perfluorononanoic acid" OR "perfluorooctane sulfonamide" OR "perfluorooctane sulfonate" OR "perfluorooctane sulfonic acid " OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonamide" OR "perfluorooctanesulfonate" OR "perfluorooctanesulfonic acid" OR "perfluorooctanesulfonic acid amide" OR "perfluorooctanoic acid" OR "perfluorooctylsulfonic acid" OR "perfluoropropanecarboxylic acid" OR "perfluoroundecanoic acid" OR "pfbus" OR "pfda" OR "pfdea" OR "pfdoa" OR "pfhpa" OR "pfhs cpd" OR "pfhxs" OR "pfna" OR "pfoa" OR "pfos" OR "pfsoa" OR "pfua" OR "tricosafluorododecanoic acid" OR "tridecafluoro 1 heptanoic acid" OR "tridecafluoroheptanoic acid") AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("1 1 2 2 3 3 4 4 4 nonafluoro 1 butanesulfonic acid" OR "1 1 2 2 3 3 4 4 4 nonafluorobutane 1 sulphonic acid" OR "1 1 2 2 3 3 4 4 5 5 6 6 6 tridecafluorohexane 1 sulfonic acid" OR "1 perfluorobutanesulfonic acid" OR "2 2 3 3 4 4 5 5 6 6 7 7 8 8 9 9 9 heptadecafluoro nonanoic acid" OR "2 (n ethyl perfluorooctane sulfonamido) acetic acid" OR "2 (n methyl perfluorooctane sulfonamido) acetic acid" OR "c11 pfa" OR "et pfosa acoh" OR "glycine n ethyl n ((1 1 2 2 3 3 4 4 5 5 6 6 7 7 8 8 8 heptadecafluorooctyl) sulfonyl) " OR "henicosafluoroundecanoic acid" OR "heptadecafluoro 1 octane sulfonic acid" OR "heptadecafluoro 1 octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "heptadecafluorooctane 1 sulphonic acid" OR "heptadecafluorooctanesulphonamide" OR "heptafluoro 1 butanoic acid" OR "heptafluorobutanoic acid" OR "heptafluorobutyric acid" OR "me pfosa acoh" OR "n ethyl n ((heptadecafluorooctyl) sulphonyl) glycine" OR "ndfda" OR "nonadecafluoro n decanoic acid" OR "nonadecafluorodecanoic acid" OR "nonafluoro 1 butanesulfonic acid" OR "nonafluorobutanesulfonic acid" OR "pentadecafluoro 1 octanoic acid" OR "pentadecafluoro n octanoic acid" OR "pentadecafluorooctanoic acid" OR "pentyl perfluorobutanoate" OR "perfluoro n decanoic acid") AND 2015:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	(307-24-4 [rn] OR 3825-26-1 [rn] OR "hexanoic acid 2 2 3 3 4 4 5 5 6 6 6-undecafluoro-" OR "hexanoic acid undecafluoro-" OR "perfluorohexanoic acid" OR "undecafluoro-1-hexanoic acid" OR "undecafluorohexanoic acid" OR "ammonium pentadecafluorooctanoate" OR "ammonium

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	perfluorocaprilate" OR "ammonium perfluorocaprylate" OR "ammonium perfluorooctanoate" OR "fc 143" OR "octanoic acid 2 2 3 3 4 4 5 5 6 6 7 7 8 8 8-pentadecafluoro- ammonium salt" OR "octanoic acid pentadecafluoro- ammonium salt" OR "pentadecafluoro-1-octanoic acid ammonium salt" OR "pentadecafluorooctanoic acid ammonium salt" OR "perfluoroammonium octanoate" OR "perfluorooctanoic acid ammonium salt") AND 1900:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
05/24/2016	((("C11-PFA" OR "et-pfosa-acoh" OR "Henicosafluoroundecanoic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "Heptadecafluorooctane-1-sulphonic acid1-Perfluorooctanesulfonic acid" OR "Heptadecafluorooctanesulphonamide" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Heptafluorobutyric acid" OR "me-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Ndfda" OR "Nonadecafluoro-n-decanoic acid" OR "Nonadecafluorodecanoic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluoro-n-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Pentyl perfluorobutanoate" OR "Perfluoro-n-decanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" OR "Perfluorocaprylic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonamide" OR "Perfluorodecanoic acid" OR "Perfluorododecanoic acid " OR "Perfluorododecanoic acid" OR "Perfluoroheptanecarboxylic acid" OR "Perfluoroheptanoic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "Perfluorolauric acid" OR "Perfluorononan-1-oic acid" OR "Perfluorononanoic acid" OR "Perfluorooctane sulfonamide" OR "Perfluorooctane sulfonate" OR "Perfluorooctane sulfonic acid " OR "perfluorooctane sulphonic acid" OR "Perfluorooctanesulfonamide" OR "Perfluorooctanesulfonate" OR "Perfluorooctanesulfonic acid amide" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonic acid" OR "Perfluoropropanecarboxylic acid" OR "Perfluoroundecanoic acid" OR "pfbus" OR "PFDA" OR "pfdea" OR "pfdoa" OR "Pfhpa" OR "PFHS cpd" OR "pfhxs" OR "pfna" OR "PFOA" OR "PFOS" OR "pfsoa" OR "Pfua" OR "Tricosafluorododecanoic acid" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid") AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) OR ("1763-23-1" OR "2058-94-8" OR "2355-31-9" OR "2991-50-6" OR "307-55-1" OR "335-67-1" OR "335-76-2" OR "355-46-4" OR "375-22-4" OR "375-73-5" OR "375-85-9" OR "375-95-1" OR "754-91-6") AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]))) AND 2013:2016 [yr] ("1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1-Perfluorobutanesulfonic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-") AND 2013:2016 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org])
09/18/2013	("perfluorooctanoic acid" OR "pentadecafluoro 1 octanoic acid" OR "pentadecafluoro n octanoic acid" OR "pentadecafluorooctanoic acid" OR "perfluorocaprylic acid" OR "perfluoroctanoic acid" OR "perfluoroheptanecarboxylic acid" OR "perfluorooctanoic acid" OR "pentadecafluorooctanoic acid" OR "perfluorooctanoic acid" OR "perfluorooctane sulfonic acid " OR "heptadecafluoro 1 octanesulfonic acid" OR "heptadecafluoro 1 octane sulfonic acid" OR "heptadecafluorooctane 1 sulphonic acid" OR "heptadecafluorooctane sulfonic acid" OR "perfluorooctane sulfonate" OR "perfluorooctylsulfonic acid" OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonic acid"

Table B-2. Database Query Strings Post Public Comment Searches

Database search date	Query string
	OR "perfluorooctanesulfonate" OR "heptadecafluorooctane 1 sulphonic acid1 perfluorooctanesulfonic acid" OR "perfluorohexane sulfonic acid pfhxs " OR "perfluorohexanesulfonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexane 1 sulphonic acid" OR "pfhs cpd" OR "2 (n ethyl perfluorooctane sulfonamido) acetic acid" OR "et pfosa acoh" OR "n ethyl n ((heptadecafluorooctyl) sulphonyl) glycine" OR "2 (n methyl perfluorooctane sulfonamido) acetic acid" OR "me pfosa acoh" OR "perfluorodecanoic acid" OR "nonadecafluoro n decanoic acid" OR "nonadecafluorodecanoic acid" OR "perfluoro n decanoic acid" OR "perfluoro n decanoic acid" OR "perfluorodecanoic acid" OR "nonadecafluorodecanoic acid" OR "perfluoro n decanoic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonic acid" OR OR "pentyl perfluorobutanoate" OR "nonafluoro 1 butanesulfonic acid" OR "perfluoroheptanoic acid" OR "tridecafluoro 1 heptanoic acid" OR "perfluoro n heptanoic acid" OR "perfluoroheptanoic acid" OR "tridecafluoroheptanoic acid" OR "perfluorononanoic acid" OR "perfluoro n nonanoic acid" OR "perfluorononan 1 oic acid" OR "perfluorooctane sulfonamide" OR "perfluorooctanesulfonamide" OR "perfluoroctylsulfonamide" OR "perfluorooctanesulfonic acid amide" OR "heptadecafluorooctanesulphonamide" OR "perfluoroundecanoic acid" OR "perfluoro n undecanoic acid" OR "hennone) AND 2007:2013 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR NIH RePORTER [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) NOT PubMed [org] NOT pubdart [org]

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS[a]	
9/11/2018 5/23/2016 9/18/2013	Compounds searched: 1763-23-1; 2058-94-8; 2355-31-9; 2991-50-6; 307-55-1; 335-67-1; 335-76-2; 355-46-4; 375-22-4; 375-73-5; 375-85-9; 375-95-1; 754-91-6; 307-24-4; 3825-26-1
NTP	
9/11/2018	Content types: Reports & Publications; Systematic Reviews; ROC Profiles, Reviews or Candidates; Testing Status "1763-23-1" "2058-94-8" "2355-31-9" "2991-50-6" "307-55-1" "335-67-1" "335-76-2" "355-46-4" "375-22-4" "375-73-5" "375-85-9" "375-95-1" "754-91-6" "Henicosafluoroundecanoic acid" "heptadecafluoro-1-octane sulfonic acid" "Heptadecafluoro-1-octanesulfonic acid" "heptadecafluorooctane sulfonic acid" "Heptadecafluorooctane-1-sulphonic acid" "Heptadecafluorooctanesulphonamide" "Heptafluoro-1-butanoic acid" "Heptafluorobutanoic acid" "Heptafluorobutyric acid" "me-pfosa-acoh" "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" "Ndfda" "Nonadecafluoro-n-decanoic acid" "Nonadecafluorodecanoic acid" "Nonafluoro-1-butanesulfonic acid" "Nonafluorobutanesulfonic acid" "Pentadecafluoro-1-octanoic acid" "Pentadecafluoro-n-octanoic acid" "Pentadecafluorooctanoic acid" "Pentyl perfluorobutanoate" "Perfluoro-n-decanoic acid" "Perfluoro-n-heptanoic acid" "Perfluoro-n-nonanoic acid" "Perfluoro-n-undecanoic acid" "Perfluorobutane sulfonic acid" "Perfluorobutanesulfonic acid" "Perfluorobutanoic acid" "Perfluorobutyric acid" "Perfluorocaprylic acid" "Perfluoroctanoic acid" "Perfluoroctylsulfonamide" "Perfluorodecanoic acid" "Perfluorododecanoic acid" "Perfluorododecanoic acid " "Perfluoroheptanecarboxylic acid" "Perfluoroheptanoic acid" "Perfluorohexane sulfonic acid" "Perfluorohexane-1-sulphonic acid" "perfluorohexanesulfonate" "perfluorohexanesulfonic acid" "Perfluorolauric acid" "Perfluorononan-1-oic acid" "Perfluorononanoic acid" "Perfluorooctane sulfonamide" "Perfluorooctane sulfonate" "Perfluorooctane sulfonic acid " "perfluorooctane sulphonic acid" "Perfluorooctanesulfonamide" "Perfluorooctanesulfonate" "perfluorooctanesulfonic acid" "Perfluorooctanesulfonic acid amide" "Perfluorooctanoic acid" "Perfluorooctylsulfonic acid" "Perfluoropropanecarboxylic acid" "Perfluoroundecanoic acid" "pfbus" "PFDA" "pfdea" "pfdoa" "Pfhpa" "PFHS cpd" "pfhxs" "pfna" "PFOA" "PFOS" "pfsoa"

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"Pfua" "Tricosafluorododecanoic acid" "Tridecafluoro-1-heptanoic acid" "Tridecafluoroheptanoic acid" "307-24-4" "3825-26-1" "Perfluorohexanoic acid" "Undecafluoro-1-hexanoic acid" "Undecafluorohexanoic acid" "Ammonium pentadecafluorooctanoate" "Ammonium perfluorocaprilate" "Ammonium perfluorocaprylate" "Ammonium perfluorooctanoate" "FC 143" "Octanoic acid, pentadecafluoro-, ammonium salt" "Pentadecafluoro-1-octanoic acid, ammonium salt" "Pentadecafluorooctanoic acid, ammonium salt" "Perfluoroammonium octanoate" "Perfluorooctanoic acid, ammonium salt"
5/23/2016	"335-67-1" OR "1763-23-1" OR "355-46-4" OR "2991-50-6" OR "2355-31-9" OR "335-76-2" OR "375-73-5" OR "375-85-9" OR "375-95-1" OR "754-91-6" OR "2058-94-8" OR "307-55-1" OR "375-22-4" OR "Perfluorooctanoic acid" OR "PFOA" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Perfluoroctanoic acid" OR "Perfluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "PFOS" OR "Perfluorooctane sulfonate" OR "Perfluorooctylsulfonic acid" OR "perfluorooctane sulphonic acid" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanesulfonate" OR "Heptadecafluorooctane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "pfhxs" OR "Ndfda" OR "Nonadecafluorodecanoic acid" OR "Nonadecafluoro-n-decanoic acid" OR "Perfluorodecanoic acid" OR "Perfluoro-n-decanoic acid" OR "PFDA" OR "pfdea" OR "1-Perfluorobutanesulfonic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "pfbus" OR "Perfluorononanoic acid" OR "pfna" OR "Perfluorooctanesulfonamide" OR "Perfluorododecanoic acid" OR "pfdoa" OR "Heptafluorobutyric acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" Screened: 146 hits
	"1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentyl perfluorobutanoate" OR "Pentadecafluoro-n-octanoic acid" OR "Perfluorocaprylic acid" OR "Perfluoroheptanecarboxylic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "1-Perfluorooctanesulfonic acid" OR "PFHS cpd" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "et-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "me-pfosa-acoh" OR "Perfluoroheptanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Pfhpa" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluorononan-1-oic acid" OR "Heptadecafluorooctanesulphonamide" OR "Perfluoroctylsulfonamide" OR "Perfluorooctane sulfonamide" OR "Perfluorooctanesulfonic acid amide" OR "Pfsoa" OR "C11-PFA" OR "Henicosafluoroundecanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluoroundecanoic acid" OR "Pfua" OR "Perfluorolauric acid" OR "Tricosafluorododecanoic acid" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Perfluoropropanecarboxylic acid" Screened: 0 hits

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
NIH RePORTER	
2/28/2017	Text Search: "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR "1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluorohexane-1-sulfonic acid" OR "1-Perfluorobutanesulfonic acid" OR "2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9,9-heptadecafluoro-Nonanoic acid" OR "2-(N-Ethyl-perfluorooctane sulfonamido) acetic acid" OR "2-(N-Methyl-perfluorooctane sulfonamido) acetic acid" OR "C11-PFA" OR "et-pfosa-acoh" OR "Glycine, N-ethyl-N-((1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctyl)sulfonyl)-" OR "Henicosafluoroundecanoic acid" OR "heptadecafluoro-1-octane sulfonic acid" OR "Heptadecafluoro-1-octanesulfonic acid" OR "heptadecafluorooctane sulfonic acid" OR "Heptadecafluorooctane-1-sulphonic acid" OR "Heptadecafluorooctanesulphonamide" OR "Heptafluoro-1-butanoic acid" OR "Heptafluorobutanoic acid" OR "Heptafluorobutyric acid" OR "me-pfosa-acoh" OR "N-Ethyl-N-((heptadecafluorooctyl)sulphonyl)glycine" OR "Ndfda" OR "Nonadecafluoro-n-decanoic acid" OR "Nonadecafluorodecanoic acid" OR "Nonafluoro-1-butanesulfonic acid" OR "Nonafluorobutanesulfonic acid" OR "Pentadecafluoro-1-octanoic acid" OR "Pentadecafluoro-n-octanoic acid" OR "Pentadecafluorooctanoic acid" OR "Pentyl perfluorobutanoate" OR "Perfluoro-n-decanoic acid" OR "Perfluoro-n-heptanoic acid" OR "Perfluoro-n-nonanoic acid" OR "Perfluoro-n-undecanoic acid" OR "Perfluorobutane sulfonic acid" OR "Perfluorobutanesulfonic acid" OR "Perfluorobutanoic acid" OR "Perfluorobutyric acid" OR "Perfluorocaprylic acid" OR "Perfluoroctanoic acid" OR "Perfluoroctylsulfonamide" OR "Perfluorodecanoic acid" OR "Perfluorododecanoic acid" OR "Perfluorododecanoic acid" OR "Perfluoroheptanecarboxylic acid" OR "Perfluoroheptanoic acid" OR "Perfluorohexane sulfonic acid" OR "Perfluorohexane-1-sulphonic acid" OR "perfluorohexanesulfonate" OR "perfluorohexanesulfonic acid" OR "Perfluorolauric acid" OR "Perfluorononan-1-oic acid" OR "Perfluorononanoic acid" OR "Perfluorooctane sulfonamide" OR "Perfluorooctane sulfonate" OR "Perfluorooctane sulfonic acid" OR "perfluorooctane sulphonic acid" OR "Perfluorooctanesulfonamide" OR "Perfluorooctanesulfonate" OR "Perfluorooctanesulfonic acid amide" OR "perfluorooctanesulfonic acid" OR "Perfluorooctanoic acid" OR "Perfluorooctylsulfonic acid" OR "Perfluoropropanecarboxylic acid" OR "Perfluoroundecanoic acid" OR "pfbus" OR "PFDA" OR "pfdea" OR "pfdoa" OR "Pfhpa" OR "PFHS cpd" OR "pfhxs" OR "pfna" OR "PFOA" OR "PFOS" OR "pfsoa" OR "Pfua" OR "Tricosafluorododecanoic acid" OR "Tridecafluoro-1-heptanoic acid" OR "Tridecafluoroheptanoic acid" (Advanced), Search in: Projects Admin IC: All, Fiscal Year: Active Projects, 2017, 2016, 2015, 2014, 2013, 2012 Screened: 80
4/7/2014	Compounds searched: 335-67-1; 1763-23-1; 355-46-4; 2991-50-6; 2355-31-9; 335-76-2; 375-73-5; 375-85-9; 375-95-1; 754-91-6; 2058-94-8; 307-55-1; 375-22-4 Screened: 82 hits
Other	Identified throughout the assessment process

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The September 2018 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 941
- Number of records identified from other strategies: 153
- Total number of records to undergo literature screening: 1,094

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on perfluoroalkyls:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

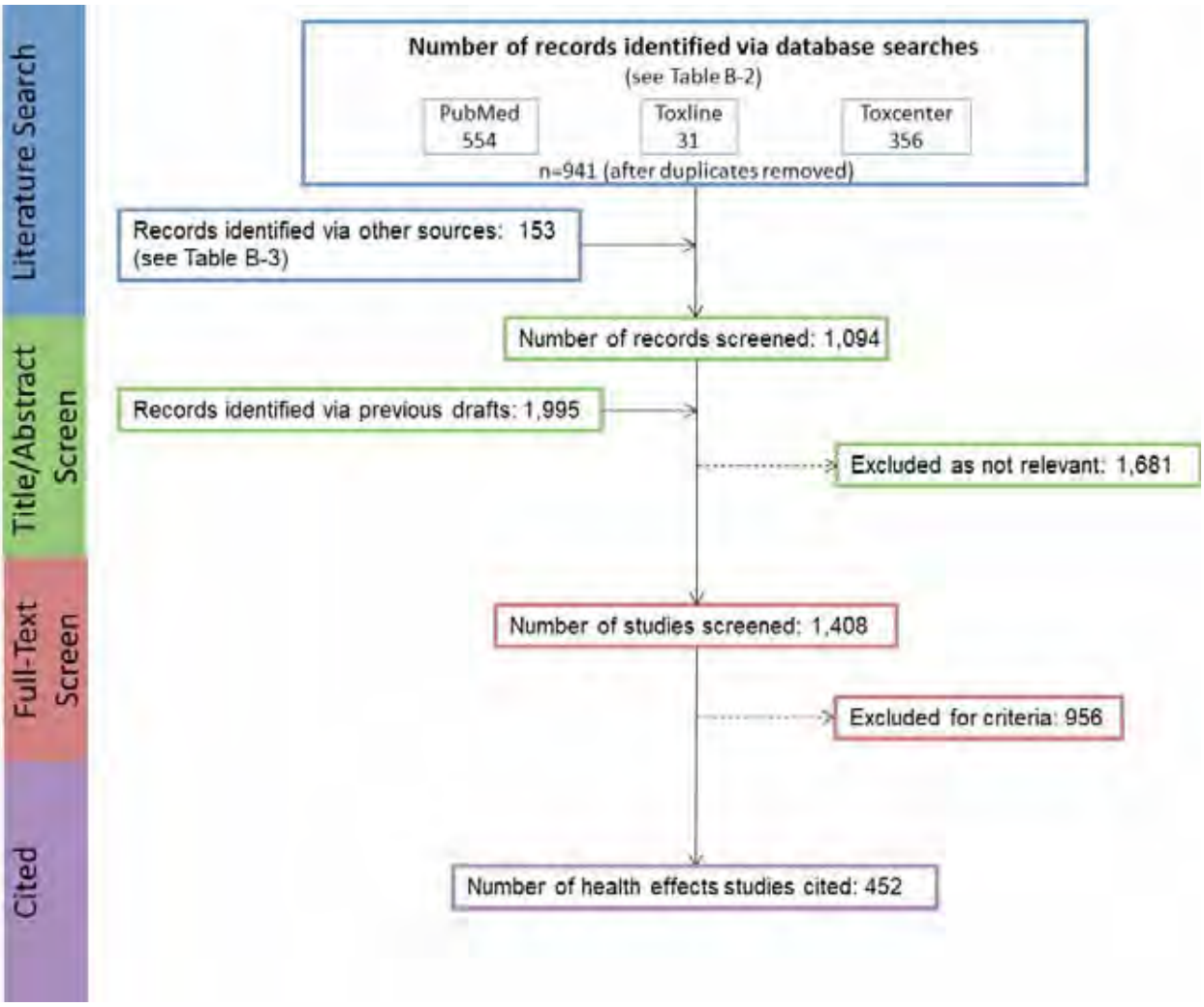
- Number of titles and abstracts screened: 1,094
- Number of studies considered relevant and moved to the next step: 1,408

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 1,408
- Total number of health effects studies cited in the profile: 452

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. September 2018 Literature Search Results and Screen for Perfluoroalkyls



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (<15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to “Chemical X” via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

APPENDIX B

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral (1)

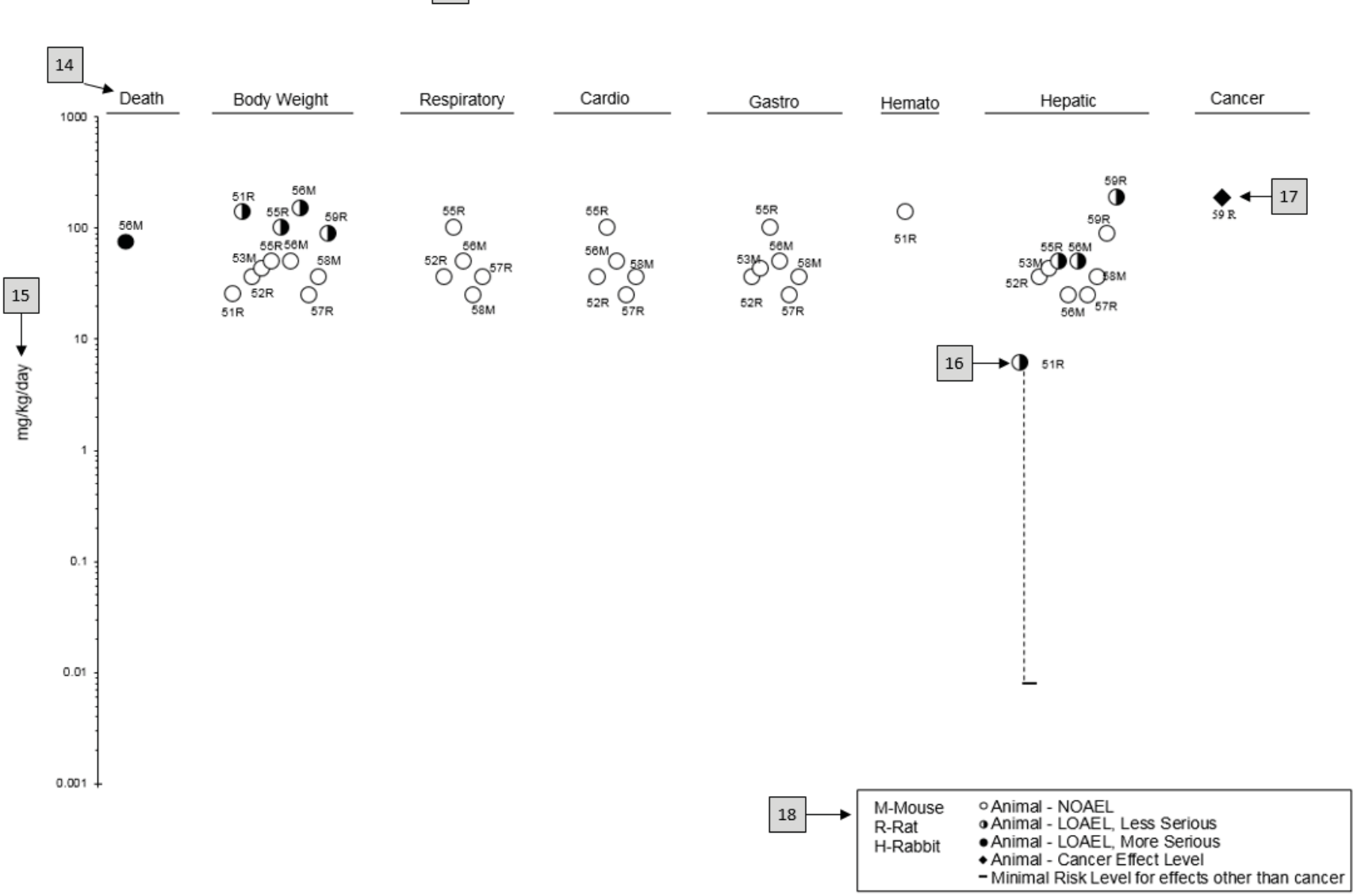
Figure key[a] (3)	Species (strain) No./group (4)	Exposure parameters (5)	Doses (mg/kg/day) (5)	Parameters monitored (6)	Endpoint (7)	NOAEL (mg/kg/day) (8)	Less serious LOAEL (mg/kg/day) (9)	Serious LOAEL (mg/kg/day) (9)	Effect
CHRONIC EXPOSURE (2)									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992 (10)									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

[a]The number corresponds to entries in Figure 2-x.

(11) [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:
Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) ≥1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Reverse Causation—Describes an association where the outcome results in a change in the biomarker of exposure.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APFO	ammonium perfluorooctanoate
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEHSU	Pediatric Environmental Health Specialty Unit
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
PFBA	perfluorobutanoic acid
PFBS	Perfluorobutane sulfonic acid
PFDA	perfluorodecanoic acid
PFDoDA	perfluorododecanoic acid
PFHpA	perfluoroheptanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
FOSA	perfluorooctane sulfonamide
PFOS	perfluorooctane sulfonic acid
PFUnA	perfluoroundecanoic acid
pg	picogram
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification

SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
$T_{1/2}$	Half-life
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

Attachment

1D

6

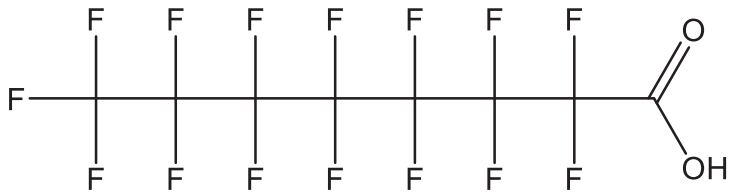
Notification Level Recommendations

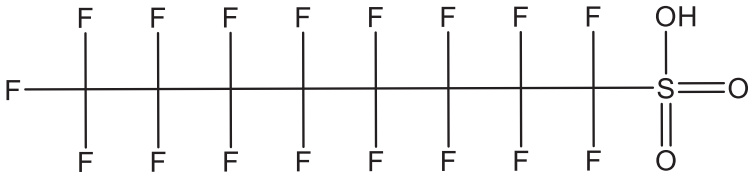
Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Drinking Water

August 2019

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

Notification Level Recommendations for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) in Drinking Water





Prepared by

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August 2019

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SUMMARY

This document presents final notification level (NL) recommendations by the Office of Environmental Health Hazard Assessment (OEHHA) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in drinking water. This supersedes the underlying scientific analysis (OEHHA, 2018) that supported the interim NLs that were adopted in 2018 (SWRCB, 2018a,b).

Based on the current evaluation of recent human and animal toxicity data, and applying OEHHA's risk assessment methodology and the US Environmental Protection Agency's (US EPA) human clearance factors (US EPA, 2016a,b) to account for the chemical half-life differences between rodents and humans, OEHHA developed PFOA and PFOS reference levels (RLs) for cancer effects. These levels represent concentrations of the chemicals in drinking water that would not pose more than a one in one million cancer risk over a lifetime:

- 0.1 ng/L (nanogram/liter) or parts per trillion (ppt) for PFOA, based on pancreatic and liver tumors in male rats (NTP, 2018c);
- 0.4 ng/L (or ppt) for PFOS, based on liver tumors in male rats (Butenhoff et al. 2012a) and the structural and biological similarity of PFOS to PFOA.

OEHHA also developed RLs for noncancer effects as follows:

- 2 ng/L (or ppt) for PFOA, based on liver toxicity in female mice (Li et al., 2017);
- 7 ng/L (or ppt) for PFOS, based on immunotoxicity in male mice (Dong et al., 2009).

The cancer RLs cited above are lower than the levels of PFOA and PFOS that can be reliably detected in drinking water using currently available technologies. In light of this, OEHHA recommends that the State Water Resources Control Board (SWRCB) set the NLs at the lowest levels at which PFOA and PFOS can be reliably detected in drinking water using available and appropriate technologies.

INTRODUCTION

At SWRCB's request, OEHHA has developed recommendations for drinking water NLs for PFOA and PFOS. Health and Safety Code Section 116455 defines an NL as the level of a drinking water contaminant that SWRCB has determined, based on available scientific information, does not pose a significant health risk but, when exceeded, warrants notification to a water system's governing body and other specified entities. NLs are nonregulatory, health-based advisory levels that SWRCB establishes as a precautionary measure for contaminants for which regulatory standards have not been set but that may be considered candidates for the establishment of maximum contaminant levels.

As a first step, OEHHA in June 2018 presented recommended interim NLs for PFOA and PFOS to SWRCB. OEHHA performed an expedited review of health-based values developed by several federal and state government agencies (US EPA, 2016a; US EPA, 2016b; New Jersey DWQI, 2017; ATSDR, 2018; New Jersey DWQI, 2018) and found the process used by New Jersey to be sufficient for establishing the interim NLs for PFOA and PFOS. Thus, OEHHA recommended that SWRCB adopt the following interim NLs based on New Jersey's evaluation:

- 14 ng/L (or ppt) for PFOA, based on liver toxicity in mice (Loveless et al., 2006) and carcinogenicity in rats (Butenhoff et al., 2012b);
- 13 ng/L (or ppt) for PFOS, based on immunotoxicity in mice (Dong et al., 2009).

In July 2018, SWRCB adopted these interim NLs for PFOA and PFOS, based on OEHHA's recommendations.

OEHHA has now completed a focused review, primarily evaluating studies that have been published since the above-cited reviews. This review evaluated human and animal toxicity studies published since 2016 and focused on hepatotoxicity, immunotoxicity, thyroid toxicity, reproductive toxicity, and cancer. These endpoints are known hazards of PFOA and PFOS exposure, and were readily observed in recent studies.

OEHHA recommends that SWRCB set the final NLs at the lowest levels at which PFOA and PFOS can be reliably detected in drinking water using currently available and appropriate technologies. OEHHA has developed RLs of 0.1 ppt for PFOA and 0.4 ppt for PFOS based on cancer endpoints, which are below levels that can be reliably detected with current technologies. RLs for noncancer endpoints are 2 ppt for PFOA based on liver toxicity and 7 ppt for PFOS based on immunotoxicity.

While OEHHA reviewed human epidemiology studies focusing on liver toxicity, immunotoxicity, and thyroid toxicity, an epidemiological analysis is not presented in this document because there were no studies that could be used for point of departure (POD) determination and dose-response assessment. Nonetheless, the epidemiology data suggest that there are associations between PFOA and/or PFOS and suppressed antibody response and increased liver enzymes. These epidemiological data are supportive of the animal toxicology data used to derive the RLs for noncancer effects. Use of data on immunotoxicity for noncancer RLs is further supported by the National Toxicology Program's (NTP) immunotoxicity review of PFOA and PFOS, which concluded that these chemicals are presumed to be an immune hazard to humans (NTP, 2016). The epidemiology data on thyroid hormone levels are inconsistent and, at times, contradictory.

TOXICOLOGICAL REVIEW

Liver Toxicity – PFOA

In vivo studies

PFOA exposure has consistently induced liver toxicity in experimental animals, and as with PFOS, a thorough examination of the literature was previously conducted by other agencies (US EPA, 2016a; New Jersey DWQI, 2017; ATSDR, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathology, increased biomarkers of liver damage, and changes in liver lipid content were observed.

OEHHA's review of recent animal studies that were not included in the above-cited reviews by other agencies is summarized in Table 1. Notable studies are described in greater detail below.

Table 1. Summary of recent animal toxicity studies of PFOA reporting liver effects

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Balb/c mice (n not specified)	0, 1, or 5 mg/kg-day orally for 7 days	↑ absolute liver weight; hepatocyte cytoplasmic vacuolization; ↑ serum ALT; changes in serum and liver lipid levels	LOAEL: 1 mg/kg-day for ↑ serum ALT levels	Hui et al. (2017)
Male Balb/c mice (n=20/dose)	0 or 1.25 mg/kg-day for 28 days	↑ relative liver weight; altered glucose metabolism	NA[a]	Zheng et al. (2017)
Male Balb/c mice (n=5/dose)	0, 0.5, or 2.5 mg/kg-day via oral infusion for 28 days	↑ absolute and relative liver weight; changes in lipid metabolism; altered glucose metabolism	NOAEL: 0.5 mg/kg-day for increased liver weight	Yu et al. (2016)
Male Balb/c mice (n not specified)	0, 0.08, 0.31, 1.25, 5, or 20 mg/kg-day via gavage for 28 days	hepatocyte swelling; lipid deposits	Not provided[b]	Yan et al. (2017)
C57BL/6 mice (n=4/dose, sex not specified)	0 or 20 mg/kg-day i.p. for 1 or 3 days	↑ relative liver weight	NA[a]	Abe et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=5/dose)	Single dose of 0 or 150 mg/kg intragastrically	↑ relative liver weight	NA[a]	Cavallini et al. (2017)
Male Kunming mice (n not specified)	Single oral dose of 0 or 5 mg/kg	↑ hepatic cytoplasmic vesicles; ↑ inflammatory cells around the hepatic portal area; changes in hepatic cholesterol level	NA[a]	Wu et al. (2017)
Male and female Balb/c mice (n=30/sex/ group)	0, 0.05, 0.5, or 2.5 mg/kg-day via oral gavage for 28 days	↑ absolute liver weight; hepatocellular hypertrophy and apoptosis; lipid accumulation in cytoplasm of hepatocytes; mitochondrial morphology changes; changes in mitochondrial membrane potential; oxidative DNA damage (ROS generation)	LOAEL: 0.05 mg/kg-day for hepatic mito-chondrial membrane potential changes, apoptosis, oxidative DNA damage	Li et al. (2017)
Male Kunming mice (n=8/dose)	0, 1, or 5 mg/kg-day intragastrically for 21 days	↑ absolute and relative liver weight; ↑ serum ALT and AST; elevated blood insulin; ↓serum triglycerides and H-LDL; elevated triglycerides in liver; ↑ L-LDL in serum; ↑ hepatic vacuoles	NOAEL: 1 mg/kg-day for ↑ liver enzymes and triglyceride levels	Wu et al. (2018)
Pregnant Kunming mice (n=8/dose)	0 or 5 mg/kg-day intragastrically throughout gestation	↑ ALT, AST, triglycerides, and cholesterol in pup serum on PND 21 (although the changes were not statistically significant)	NA[a]	Qin et al. (2018)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male and female Sprague Dawley rats (n=10/sex/ dose)	0, 0.625, 1.25, 2.5, 5 or 10 mg/kg-day via oral gavage for males and females for 28 days	Hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, ↑ absolute and relative liver weight, changes in cholesterol and triglyceride levels, ↑ serum ALT and ALP **Males**: ↑ serum AST and bilirubin	LOAEL: 0.625 mg/kg-day for hepatocyte cytoplasmic alteration and ↑ liver weight in males	NTP (2018b)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day, calculated by OEHHA) in feed for 16 weeks	↑ absolute and relative liver weight, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, ↑ serum ALT and ALP	NOAEL: 300 ppm (27.7 mg/kg-day) for all liver endpoints	NTP (2018c)
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↑ relative liver weight, liver necrosis, liver pigment, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, hepatocyte single cell death, ↑ serum ALT and ALP, ↑ bile salts	LOAEL: 150 ppm (14.7 mg/kg-day) for all liver endpoints	NTP (2018c)
Male Sprague Dawley rats (n=10/dose)	0, 20, 40, or 80 ppm (0, 1.8, 3.7, or 7.5 mg/kg-day) in feed for 16 weeks	↑ absolute and relative liver weight, liver necrosis, liver pigment, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, hepatocyte single cell death, ↑ serum ALT and ALP	LOAEL: 20 ppm (1.8 mg/kg-day) for ↑ liver weight, ↑ ALT and ALP, and hepatocyte necrosis, cytoplasmic alteration, and single cell death	NTP (2018c)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Female Sprague Dawley rats (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Liver necrosis, liver pigment, bile duct hyperplasia, hepatocyte cytoplasmic alteration, hepatocyte hypertrophy, hepatocyte single cell death, hepatocyte ↑ mitoses	LOAEL: 300 ppm (18 mg/kg-day) for hepatocyte cytoplasmic alteration and hepatocyte hypertrophy	NTP (2018c)
Male Sprague Dawley rats (n=50/dose)	0, 20, 40, or 80 ppm (0, 1, 2.2, or 4.5 mg/kg-day) in feed for 107 weeks	Liver cystic degeneration, liver eosinophilic and mixed cell focus, liver focal inflammation, liver necrosis, liver pigment, hepatocyte hypertrophy, hepatocyte cytoplasmic alteration, hepatocyte single cell death	LOAEL: 20 ppm (1 mg/kg-day) for liver necrosis, hepatocyte hypertrophy, and hepatocyte cytoplasmic alteration	NTP (2018c)

[a] LOAEL/NOAEL not applicable for single dose studies.
[b] Histology data are presented in the supplementary materials, but specific doses for which hepatocyte swelling and lipid deposits become significant are not provided.
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GD, gestation day; i.p., intraperitoneal; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; PND, postnatal day

Recently, the NTP released toxicity data from subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female rats. Animals were given PFOA in feed (concentrations are provided in Table 1). For the chronic studies, an additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure; 150 or 300 parts per million [ppm] for males and females). The toxicity data obtained from this additional cohort were examined to provide supportive evidence of toxicity (when compared with non-perinatally exposed animals), but were not evaluated specifically for NL development. Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 milligrams per kilogram of bodyweight per day [mg/kg-day]) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving these doses were examined at 16 weeks, and the study was repeated with lower doses. Liver toxicity was observed in all of the studies, regardless of sex or duration. Common liver effects include increased weight, increased alanine aminotransferase (ALT) and alkaline phosphatase (ALP), necrosis, liver pigment, hepatocyte cytoplasmic alteration and hypertrophy, and hepatocyte single

cell death (NTP, 2018b,c). Liver toxicity lowest-observed-adverse-effect levels (LOAELs) of 0.625 mg/kg-day and 1 mg/kg-day for the 28-day and 107-week studies in male rats, respectively, were identified. This corresponds to plasma concentrations of 50.7 and 81.4 milligrams per liter (mg/L) respectively. Plasma/serum concentration is the most appropriate dose metric for extrapolating toxicity data from rodent studies to humans because of the large difference in the chemical's half-life between rodents (1-3 weeks) and humans (2-3 years). This accounts for the accumulation of PFOA in humans due to the chemical's long half-life. Plasma concentration in the chronic male rat study was determined at 16 weeks, but because the serum half-life of PFOA is estimated to be 4-6 days in male rats (New Jersey, 2017; Lau et al., 2006), it is anticipated that by 16 weeks, a steady-state concentration would have been reached. Thus, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing.

Male Kunming mice administered 0, 1, or 5 mg/kg-day PFOA intragastrically for 21 days displayed increased absolute and relative liver weight, increased serum ALT and aspartate aminotransferase (AST), elevated hepatic triglycerides, decreased serum triglycerides, increased hepatic vacuoles, changes in serum cholesterol levels, and increased blood insulin (Wu et al., 2018). OEHHA identified a no-observed-adverse-effect level (NOAEL) of 1 mg/kg-day based on these effects.

Li et al. (2017) administered 0, 0.05, 0.5 or 2.5 mg/kg-day PFOA via oral gavage to male and female Balb/c mice for 28 days. The authors reported decreased body weight, increased absolute liver weight, hepatocellular hypertrophy and apoptosis, lipid accumulation in hepatocyte cytoplasm, changes to mitochondrial morphology and membrane potential, and oxidative DNA damage (increased 8-hydroxydeoxyguanosine formation) in the liver. Toxicity endpoint data and PFOA serum concentrations were quantified using GetData graph digitizer software (version 2.26), and are presented in Table 2. Female mice were more sensitive to apoptosis than male mice. The administered dose of 0.05 mg/kg-day corresponds to a serum concentration of approximately 1 microgram per milliliter (µg/ml) (for both sexes), which was measured at the end of the exposure period. OEHHA identified a LOAEL of 0.05 mg/kg-day (serum concentration of 1 µg/ml) for changes in mitochondrial membrane potential, increases in biomarkers of apoptosis (caspase-9 and p53), and increased oxidative DNA damage (Li et al., 2017).

Table 2. Dose metrics and endpoints in female mice from Li et al. (2017)

Administered dose (mg/kg-day)	Reported serum concentration (mg/L)	Cells with mitochondrial membrane potential changes (%)	Caspase-9 levels (iU/g)	p53 levels (iU/g)	8-OHdG (ng/g)
0	0	1.2 ± 0.5	71.3 ± 4.2	28.9 ± 3.5	22.9 ± 7.3
0.05	0.97	12.3 ± 1.2**	130.2 ± 9.0**	46.8 ± 5.1**	68.6 ± 6.2**
0.5	2.7	17.6 ± 1.1**	157.9 ± 3.5**	58.3 ± 4.5**	87.9 ± 9.3**
2.5	9.5	39.3 ± 14.6**	220.9 ± 1.1**	69.0 ± 3.2**	96.8 ± 2.6**

8-OHdG, 8-hydroxydeoxyguanosine; iU/g, international units/gram
**p<0.01, statistical analysis by OEHHA

Reduced body weight and increased absolute and/or relative liver weight were also reported in several other studies using Balb/c mice and Sprague Dawley rats with higher doses (ranging from 0 to 10 mg/kg-day) for 7-28 days (Du et al., 2018; Hui et al., 2017; Zheng et al., 2017; Yu et al., 2016; NTP, 2018b). Sprague Dawley rats also displayed hepatocyte hypertrophy and cytoplasmic alteration, changes in cholesterol and triglyceride levels, and increased serum ALT, AST, ALP, and bilirubin levels following oral exposure to PFOA for 28 days (NTP, 2018b).

Hepatotoxicity was also observed in frogs (Tang et al., 2018).

In vitro studies

Human liver HL-7702 cells treated with 0, 1, 2.5, or 7.5 micromoles/L (μM) PFOA had elevated levels of apoptosis and oxidative DNA damage (Li et al., 2017). Increased apoptosis was also observed in the mouse liver AML12 cell line (Wu et al., 2017). PFOA increased apoptosis, decreased mitochondrial membrane integrity, and increased anti-inflammatory interleukin 10 (IL-10) levels in rat and/or human organotypic multi-culture hepatocellular models (Orbach et al., 2018). Impaired proteolysis and autophagosome accumulation were observed in HepG2 cells treated with 0, 50, 100, or 200 μM PFOA (Yan et al., 2017). Liu et al. (2017) reported increased oxidative stress in primary rat hepatocytes treated with ≥6.25 μM PFOA.

Mechanistic studies

Mechanisms of hepatotoxicity have been previously reviewed (US EPA, 2016a; New Jersey DWQI, 2017). It has been established that PFOA can induce toxicity via activation of the nuclear receptor peroxisome proliferator-activated receptor alpha

(PPARα). However, PPARα activation does not explain all of the observed toxicity, and studies in PPARα knockout mice clearly demonstrate PPARα-independent toxicity (reviewed by US EPA, 2016a; New Jersey DWQI, 2017). Furthermore, there is evidence that PFOA activates other nuclear receptors, including constitutive androstane receptor (CAR), pregnane X receptor (PXR), and estrogen receptor alpha (ERα) (New Jersey DWQI, 2017). Recently, it was demonstrated that PFOA indirectly activates CAR, differently from the prototypical CAR activator phenobarbital (Abe et al., 2017).

Additional recent studies examining mechanisms of hepatotoxicity are briefly summarized here.

In mouse liver and human hepatocytes, PFOA administration decreased hepatocellular hepatocyte nuclear factor 4 alpha (HNF4α), which has an important role in hepatocyte differentiation (Beggs et al., 2016). Additionally, PFOA increased levels of the anti-inflammatory cytokine iterleukin-10 (IL-10) in human and rat organotypic cell culture models (Orbach et al., 2018).

Several studies have examined hepatic transcriptomic/proteomic changes in mice (Hui et al., 2017; Li et al., 2017; Abe et al., 2017; Zheng et al., 2017), and in mammalian liver cells (Beggs et al., 2016; Song et al., 2016; Zhang et al., 2016a; Liu et al., 2017; Yan et al., 2017) following PFOA administration. In general, PFOA exposure altered the levels of mRNA transcripts and/or proteins involved with apoptosis, lipid metabolism, cell proliferation, autophagy and vesicular trafficking, and the Krebs cycle. These data suggest that PFOA induces significant gene expression changes in the liver, and are supportive of the observed hepatotoxicity in animals.

Critical Study Selection

Li et al. (2017) generated a LOAEL of 0.05 mg/kg-day (administered dose) for changes in mitochondrial membrane potential, increases in biomarkers of apoptosis, and increased oxidative DNA damage in the liver of female mice. This LOAEL corresponds to a serum concentration of 0.97 mg/L, which is lower than the POD of 4.35 mg/L based on increased relative liver weight in male mice (Loveless et al., 2006) that formed the basis for the interim NL. Therefore, the Li et al. (2017) study is more appropriate than the Loveless et al. (2006) study as a critical study for POD derivation.

Liver Toxicity – PFOS

In vivo studies

PFOS exposure has consistently induced liver toxicity in experimental animals, and a thorough examination of the literature was previously conducted by other agencies (US EPA, 2016b; New Jersey DWQI, 2018). In general, increases in absolute and/or relative liver weight, increased liver histopathology, increased biomarkers of liver damage, and changes in liver lipid content were observed.

Several animal studies published after 2016 reported various hepatotoxic endpoints following oral exposure to PFOS. These studies are summarized in Table 3.

Table 3. Summary of recent animal toxicity studies of PFOS reporting liver effects

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Pregnant mice (strain not specified) (n=3-5/ dose)	0, 1, 10, or 20 mg/kg-day orally from GD1 to GD14	fetal liver enlargement	Doses that caused effect were not specified	Mehri et al. (2016)
Male and female Cynomolgus monkeys (n=6/sex/ dose)	0 or 14 mg/kg orally on three separate occasions over 422 days; maximum PFOS serum concentrations of 165 µg/mL for females and 160.8 µg/mL for males on day 365	no toxicologically significant effects reported	NOAEL: 165 µg/mL serum PFOS	Chang et al. (2017)
Male and female Sprague Dawley rats (n=12/sex/ dose)	0 or 100 ppm in diet; equivalent to 6 mg/kg-day for males and 6.6 mg/kg-day for females	**Both sexes:** ↑ absolute and relative liver weight; hepatocellular hypertrophy **Males:** ↓ serum cholesterol and triglyceride levels; cytoplasmic vacuolization; ↑ lipid content **Females:** ↓ free fatty acids and triglycerides	NA[a]	Bagley et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; ↑ oxidative stress and apoptosis	LOAEL: 1 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis	Han et al. (2018a)
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	↑ absolute liver weight; hepatocyte degeneration; cytoplasmic vacuolization; ↑ serum ALT and AST	NOAEL: 1 mg/kg-day for increased liver enzymes	Han et al. (2018b)
Male Sprague Dawley rats (n=7/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks (males only)	↑ absolute and relative liver weight; hepatocellular hypertrophy; cytoplasmic vacuolization; ↑ serum ALT and AST; inflammatory cellular infiltration; ↑ apoptosis	LOAEL: 1 mg/kg-day for increased liver enzymes	Wan et al. (2016)
Male C57BL/6 mice (n=10/dose)	0, 2.5, 5, or 10 mg/kg-day via oral gavage for 30 days	↑ absolute liver weight; ↑ serum ALT and AST; hepatocyte vacuolization and necrosis; ↑ oxidative stress and apoptosis	LOAEL: 2.5 mg/kg-day for ↑ liver enzymes, oxidative stress, and apoptosis	Xing et al. (2016)
Male wild-type or ERβ knock-out mice (n=8/dose/ group)	0 or 5 mg/kg-day via oral gavage for 28 days	Hepatocyte degeneration and vacuolization; ↓ hepatic cholesterol and bile acids	NA[a]	Xu et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male C57BL/6 mice (n=5-6/dose)	0, 0.003, 0.006, or 0.012% (0, 30, 60 or 120 mg/kg-day) in diet for 21 or 23 days	↑ relative liver weight; ↑ ALT, bile acids and triglycerides; hepatocyte vacuolization and necrosis; altered lipid metabolism	LOAEL: 30 mg/kg-day for increased liver weight and triglycerides	Zhang et al. (2016b)
Male and female Sprague Dawley rats (n=10/sex/ dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day via oral gavage for males and females for 28 days	Hepatocyte hypertrophy, ↑ absolute/relative liver weight, ↓ cholesterol and triglycerides, ↑ ALT, ALP, bile salt/acid, albumin, and direct bilirubin; **Males**: ↑ AST, ↓ globulin, hepatocyte cytoplasmic vacuolization; **Females**: hepatocyte cytoplasmic alteration, ↑ total bilirubin	LOAEL: 0.312 mg/kg-day for ↑ relative liver weight in males and females	NTP (2018a)

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ERβ, estrogen receptor beta; GD, gestation day; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level

Briefly, several recent studies in Sprague Dawley rats reported various hepatotoxic endpoints following 3-4 weeks of oral exposure to PFOS, including increased absolute and/or relative liver weight (Han et al. 2018b; Bagley et al., 2017; Wan et al. 2016; NTP, 2018a), increased serum ALT and AST (Han et al., 2018a; Wan et al., 2016; NTP, 2018a), altered cholesterol and triglyceride levels (Bagley et al., 2017; NTP, 2018a), hepatocellular hypertrophy (Han et al., 2018a; Bagley et al., 2017; Wan et al., 2016; NTP, 2018a), cytoplasmic vacuolization (Han et al., 2018a; Han et al., 2018b; Wan et al., 2016; NTP, 2018a), and hepatocyte degeneration/necrosis (Han et al., 2018b; Bagley et al., 2017). Additionally, increased levels of oxidative stress markers (Han et al., 2018a), apoptosis (Han et al., 2018a; Wan et al., 2016), and hepatic cell proliferation

(Han et al., 2018b) were observed. OEHHA identified a LOAEL of 0.312 mg/kg-day, based on increased relative liver weight in rats (NTP, 2018a).

Similar endpoints (decreased body weight, increased liver weight, increased ALT, AST, and bile acids, hepatocyte vacuolization and necrosis, and increased oxidative stress) were observed in mice given PFOS orally (doses from 2.5-120 mg/kg-day) for 3-4 weeks (Xing et al., 2016; Xu et al., 2017; Zhang et al., 2016b). OEHHA identified a LOAEL of 2.5 mg/kg-day for increases in liver enzymes, markers of oxidative stress, and apoptosis in C57BL/6 mice (Xing et al., 2016).

Choline supplementation reduced PFOS-induced hepatic oxidative stress and changes in lipid metabolism in male C57BL/6 mice (Zhang et al., 2016b), but had no impact on steatosis in Sprague Dawley rats (Bagley et al., 2017). Additionally, ERβ (estrogen receptor beta) knockout mice did not show the hepatotoxic effects (hydropic degeneration and vacuolization of hepatocytes, decreased hepatic cholesterol and bile acid levels) that were present in wild-type mice (Xu et al., 2017). Furthermore, hepatocyte vacuolization, fatty degeneration, lipid accumulation, and ultrastructural changes in the liver were observed in zebrafish exposed to PFOS (Cheng et al., 2016; Cui et al., 2017).

In vitro studies

Cytotoxicity, oxidative stress, mitochondrial dysfunction, impaired proteolysis, autophagosome formation, and lysosomal membrane permeabilization were observed in HepG2 cells exposed to up to 200 µM PFOS (Wan et al., 2016; Yao et al., 2016; Yan et al., 2017). Primary hepatocytes from Sprague Dawley rats (that were depleted of glutathione prior to PFOS exposure) showed increased oxidative stress, decreased mitochondrial membrane potential, lysosomal membrane damage, and proteolysis following exposure to PFOS (Khansari et al., 2017).

Mechanistic studies

Mechanisms of hepatotoxicity have been previously reviewed (US EPA, 2016b; New Jersey DWQI, 2018). It has been established that PFOS can induce hepatotoxicity via activation of the nuclear receptor PPARα. However, PPARα activation does not explain all of the observed hepatotoxicity. It has been suggested that PFOS may interact with other nuclear receptors, including CAR, PXR, PPARβ/δ, PPARγ, HNF4α, and ERα (New Jersey DWQI, 2018). Recently, it was shown that PFOS-induced liver toxicity also appears to act via ERβ, as ERβ knockout mice did not display the adverse effects (hepatocyte vacuolization, hydropic degeneration, changes in levels of cholesterol and bile acids) observed in wild-type mice (Xu et al., 2017). PFOS also increased expression of ERβ in HepG2 cells (Xu et al., 2017). Beggs et al. (2016) demonstrated that PFOS decreased HNF4α levels in mouse liver and human hepatocytes.

Additional recent studies examining mechanisms of hepatotoxicity are briefly summarized below.

PFOS induced autophagosome formation and lysosome membrane permeabilization in HepG2 cells (Yao et al., 2016). Spinster-1, a sphingolipid transporter involved in cell death, was implicated in toxicity, as knocking out this protein attenuated lysosome membrane permeabilization. PFOS also inhibited activation of protein kinase B in HepG2 cells, which could lead to changes in cell proliferation and apoptosis (Qiu et al., 2016b).

Several studies have examined hepatic transcriptomic/proteomic changes in rats (Dong et al., 2016; Wan et al., 2016; Han et al., 2018a; Han et al., 2018b), mice (Lai et al., 2017; Xu et al., 2017), zebrafish (Cheng et al., 2016; Fai Tse et al., 2016; Cui et al., 2017), mammalian liver cells (Han et al., 2018b; Wan et al., 2016; Beggs et al., 2016; Song et al., 2016), and chicken eggs (Jacobsen et al., 2018) following PFOS administration. In general, PFOS exposure altered the levels of mRNA transcripts and/or proteins involved with apoptosis, lipid metabolism, cell proliferation, necrosis, and carcinogenesis. The transcriptomic/proteomic evidence is indicative of hepatotoxicity and supports the animal toxicity data.

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent PFOS studies showing liver toxicity are orders of magnitude higher than the NOAEL of 0.008 mg/kg-day (administered dose) for immunotoxicity from Dong et al. (2009) (discussed below in the PFOS immunotoxicity section), which was the basis for OEHHA's interim NL recommendation. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOS NL.

Immunotoxicity - PFOA

In a systematic review, NTP (2016) determined that PFOA is "presumed to be an immune hazard to humans" through suppression of antibody response as shown in animal and human studies. Assessments by US EPA (2016a), New Jersey Drinking Water Quality Institute (DWQI) (2017) and the US Agency for Toxic Substances and Disease Registry (ATSDR) (2018) have also described immune toxicity effects in humans and animals. Effects on spleen and thymus have been observed as well as the inability for the immune system to respond to a challenge.

In vivo studies

Since the publication of the above cited assessments, several recent studies reported similar effects on the immune system. These studies are summarized in Table 4.

Table 4. Summary of recent animal toxicity studies of PFOA reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
Female C57BL/6N PPARα KO and WT mice (n=6/dose/ group)	0, 7.5 or 30 mg/kg-day in drinking water for 15 days	↓ relative spleen and relative thymus weights in WT mice; ↓SRBC-specific IgM antibody responses in KO and WT mice.	LOAEL: 7.5 mg/kg-day for ↓ relative thymus weight in WT mice	Dewitt et al. (2016)
Female C57BL/6N WT mice (n=8/dose)	0, 0.94, 1,88, 3.75, or 7.5 mg/kg-day in drinking water for 15 days	↓ dinitrophenyl-ficoll (DNP)-specific IgM antibody response; ↓ relative spleen and thymus weight (high dose)	NOAEL: 0.94 mg/kg-day for ↓ antibody response	Dewitt et al. (2016)
Female C57BL/6N WT mice (n=4/dose/ group)	0, 3.75 or 7.5 mg/kg-day in drinking water for 10, 13 or 15 days	Changes in splenic lymphocyte subpopulations	LOAEL: 3.75 mg/kg-day for changes in splenic lymphocyte subpopulations	Dewitt et al. (2016)
Male ICR mice (n=5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on day 0 and 7. OVA + 100 or 150 mg/kg 3 times on days 9 and 13 orally. Control mice had 150 mg/kg PFOA only or OVA only.	↓ rectal temperature; ↑ serum histamine, TNF-α, IgG1 and IgE levels	LOAEL: 100 mg/kg for ↑ TNF-α and IgE levels in sensitized mice	Lee et al. (2017)

Sex/Species	Exposure	Endpoints	NOAEL/LOAEL	Reference
C57BL/6 mice (sex not specified) (n=4/group)	0 or 2 mg/kg via oral gavage for 25 days. Mice infected with *Citrobacter* at day 7.	↓weight gain; ↓ in pathogen clearance at late stage infection; induction of IL-22 from ILC3 and Th17 cells; ↓ mucin	NA[a]	Suo et al. (2017)
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↓ absolute and relative spleen weight; lymphoid follicle atrophy	LOAEL: 14.7 mg/kg-day for ↓ absolute and relative spleen weight	NTP (2018c)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day) in feed for 16 weeks	Pigment in spleen	LOAEL: 27.6 mg/kg-day for pigment in spleen	NTP (2018c)

[a] LOAEL/NOAEL not applicable for single dose studies.
GD, gestation day; IgE, immunoglobulin E; IgM, immunoglobulin M; IL-22, interleukin 22; KO, knockout; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; PND, postnatal day; PPARα, peroxisome proliferator-activated receptor alpha; SRBC, sheep red blood cells; TNF-α, tumor necrosis factor alpha; WT, wild-type

In vitro studies

Lee et al. (2016) investigated the effect of PFOA on mast cells and its association with allergic inflammation. Increased histamine and β-hexoaminidase release was observed in IgE-stimulated mast cells. The increased histamine release was the result of increased intracellular calcium induced by PFOA. Cytokine gene and protein expression were also increased. A decrease in IL-10 was also observed in PFOA-treated multicellular organotypic culture models of human or rat cells (Orbach et al., 2018).

Mechanistic studies

The database of studies on the mechanism for immune toxicity is limited. US EPA (2016a) and New Jersey DWQI (2017) suggested that the effects of PFOA on the immune system may have a mode of action that is both PPARα-dependent and independent.

Lee et al. (2016) found that the mechanism for cytokine induction observed in PFOA-treated mast cells was the result of activation of nuclear factor kappa B (NF-κB), a nuclear factor that helps regulate immune response in cells.

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent immunotoxicity studies are substantially higher than the LOAEL of 0.05 mg/kg-day for liver toxicity from the Li et al. (2017) study, which is selected as a critical study for development of a noncancer RL. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOA NL.

Immunotoxicity – PFOS

A systematic review by NTP (2016) determined that PFOS is presumed to be an immune hazard to humans. The database of studies investigating the immune toxicity of PFOS is limited and has been reviewed in recent assessments by US EPA (2016a), New Jersey DWQI (2018) and ATSDR (2018). Effects on immune organs as well as immune suppression have been observed.

In vivo studies

OEHHA conducted a literature search to find additional studies published after the above-cited reviews and these recent studies are summarized in Table 5.

Table 5. Summary of recent animal toxicity studies of PFOS reporting immune toxicity

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male and female Sprague Dawley rats (n=10/sex/dose)	0, 0.312, 0.625, 1.25, 2.5 or 5 mg/kg-day for 28 days via oral gavage	**Males**: ↓white blood cells, ↓neutrophils, ↓ eosinophils, ↓ relative thymus weight **Females**: ↓ relative thymus weight at 1.25 mg/kg-day (not statistically significant at higher doses)	NOAEL: 2.5 mg/kg-day for all endpoints in males	NTP (2018a)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male ICR mice (n=5/dose)	Treated mice were sensitized with OVA to induce active systemic anaphylaxis on day 0 and 7. OVA + 50, 100 or 150 mg/kg, 3 times on days 9, 11 and 13 orally. Control mice had 150 mg/kg PFOS only or OVA only.	↓ rectal temperature; ↑ histamine, TNF-α, IgG and IgE levels in sensitized mice	LOAEL: 50 mg/kg for ↑TNF-α and IgE levels	Lee et al. (2018)
Male Sprague Dawley rats (n=6/dose)	0, 1, or 10 mg/kg-day orally for 4 weeks	↑ serum TNF-α and IL-6 levels	LOAEL: 1 mg/kg-day for ↑ serum TNF-α and IL-6 levels	Han et al. (2018b)

IgE, immunoglobulin E; IgG, immunoglobulin G; IL-6, interleukin 6; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; OVA, ovalbumin; TNF-α, tumor necrosis factor alpha

There are no new studies that are more sensitive than the Dong et al. (2009) study for derivation of the noncancer RL for PFOS. In this study, 10 adult male C57BL/6 mice per dose group were administered 0, 0.008, 0.083, 0.417, 0.833, or 2.08 mg/kg-day PFOS via oral gavage for 60 days. Significant toxicity endpoints include: decreased body, spleen, thymus, and kidney weights; increased liver weight; decreased splenic and thymic cellularity, and T cell CD4/CD8 subpopulations; altered natural killer cell activity; decreased splenic lymphocyte proliferation; and decreased sheep red blood cell (SRBC)-specific IgM plaque forming cell response. Serum concentrations were reported for each dose. A NOAEL of 0.008 mg/kg-day (serum concentration of 0.674 mg/L) was identified for decreased plaque-forming cell response.

In vitro studies

Han et al. (2018b) compared changes in tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) levels between Kupffer cells and hepatocytes treated with PFOS. Exposure to 100 µM PFOS for 48 hours caused a transient but significant increase in TNF-α in Kupffer cells while levels remained unchanged in hepatocytes. Interleukin 7 (IL-7) was significantly elevated in Kupffer cells for the entire 48-hour exposure duration

while levels remained unchanged in hepatocytes. Blockage of TNF-α and IL-6 inhibited gadolinium chloride-induced hepatocyte proliferation. The authors suggest that cytokine expression in Kupffer cells is involved in hepatocyte proliferation through a NF-kB/TNF-α/IL-6 dependent pathway. In a study in primary human decidual stromal cells, PFOS inhibited cortisone induced reduction of the inflammatory cytokines, IL-6 and interleukin 1 beta (IL-1β) (Yang et al., 2016).

Mechanistic studies

In the risk assessment by New Jersey DWQI (2018), the authors summarized that the immunotoxicity of PFOS may be PPARα mediated, or it may be due to lipid imbalance or be a stress response, but the specific mechanism remains unclear.

Han et al. (2018b) found that hepatocyte proliferation observed in PFOS treated mice was influenced by PFOS-induced cytokine expression in Kupffer cells, and occurred through the NF-kB/TNF-α/IL-6 pathway. Blocking TNF-α and IL-6 inhibited hepatocyte proliferation.

Critical Study Selection

The recent immunotoxicity studies of PFOS are much less sensitive than the Dong et al. (2009) study, which was the basis for OEHHA's interim NL recommendation. Thus, these recent immunotoxicity studies are not considered as critical studies for POD derivation.

Thyroid Toxicity – PFOA

Thyroid effects have been reported in animals environmentally exposed to perfluoroalkyl and polyfluoroalkyl substances (PFAS). Levels of the thyroid hormone, T3 (triiodothyronine), were negatively associated with PFAS in polar bears and hooded seals (Bourgeon et al., 2017; Grønnestad et al., 2018).

Several recent mechanistic studies showed that PFOA, PFOS, and other medium-chain PFAS bind to the thyroxine transport protein transthyretin (Ren et al., 2016; Zhang et al., 2016b; Xin et al., 2018). Xin et al. (2018) also showed that PFOS can bind to thyroid hormone receptors.

NTP recently released subacute (28 days) and chronic (16 or 107 weeks) bioassays for PFOA conducted in male and female rats. Animals were given PFOA in feed (concentrations are provided in Table 6). For the chronic studies, an additional cohort of animals was exposed to PFOA during gestation and lactation (perinatal exposure). Although the initial chronic study in male rats with concentrations of 0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed was ended at 21 weeks due to overt toxicity, it appears a subset of animals receiving these doses were examined at 16 weeks, and

the study was repeated with lower doses. Results are summarized in Table 6. Thyroid follicular cell hypertrophy was observed in male and female rats in the 28-day studies, and in female rats in the 107-week study. Thyroid toxicity was not observed in female rats in the 16-week study and male rats in the 107-week study (NTP, 2018c). It should be noted, however, that male rats exposed perinatally in the 107-week study had higher incidences of thyroid follicular cell hypertrophy, although statistical significance was not reached (p=0.087, Fisher's exact test, done by NTP). OEHHA identified a LOAEL of 0.625 mg/kg-day (corresponding to a plasma concentrations of 50.7 and 0.49 mg/L in males and females, respectively) for changes in thyroid hormone levels in male and female rats for the 28-day studies, and a NOAEL of 14.7 mg/kg-day (plasma concentration of 193 mg/L) for thyroid follicular cell hypertrophy and changes in thyroid weight in male rats in the chronic studies.

Table 6. Thyroid toxicity from the NTP (2018b,c) subacute and chronic studies of PFOA in Sprague Dawley rats

Sex	Exposure	Endpoints	NOAEL/ LOAEL
Male (n=10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Thyroid follicular cell hypertrophy (trend), increased relative thyroid weight, decreased TSH, T3, fT4 and tT4	LOAEL: 0.625 mg/kg-day for changes in thyroid hormones
Female (n=10/dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day via oral gavage for 28 days	Thyroid follicular cell hypertrophy, increased TSH, decreased fT4 and tT4	LOAEL: 0.625 mg/kg-day for increased TSH
Male (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	Decreased relative and increased absolute thyroid weight, thyroid follicular cell hypertrophy	NOAEL: 14.7 mg/kg-day for all thyroid endpoints
Male (n=10/dose)	0, 20, 40, or 80 ppm (0, 1.8, 3.7, or 7.5 mg/kg-day) in feed for 16 weeks	Decreased absolute thyroid weight (not significant at the highest dose)	NOAEL: 1.8 mg/kg-day for decreased thyroid weight
Female (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Thyroid follicular cell hypertrophy	NOAEL: 18 mg/kg-day for thyroid follicular cell hypertrophy

LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; TSH, thyroid stimulating hormone; T3, triiodothyronine; fT4, free thyroxine; tT4, total thyroxine

PFOA was associated with hyperthyroidism in a case control study of 72 cats (Bost et al., 2016).

Critical Study Selection

Thyroid toxicity observed in the NTP (2018b,c) subacute and chronic studies of PFOA is not considered for POD derivation because this endpoint is much less sensitive than the hepatotoxicity endpoints reported by Li et al. (2017).

Thyroid Toxicity – PFOS

NTP (2018a) conducted subacute studies in male and female rats with PFOS. Animals were given 0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day PFOS via oral gavage for 28 days. Decreases in T3, fT4 (free thyroxine), and tT4 (total thyroxine) were observed in both sexes, while decreased absolute thyroid weight was reported in males only (NTP, 2018). A LOAEL of 0.312 mg/kg-day (corresponding to plasma concentrations of 23.7 and 30.5 mg/L for males and females, respectively) was identified based on decreases in fT4 and tT4 in both sexes.

A recent study in male and female cynomolgus monkeys given 14 mg/kg PFOS via oral gavage on three separate occasions over an observation period of 422 days showed a slight reduction in serum tT4 in both sexes (Chang et al., 2017b). There were no significant changes in TSH or fT4. The authors did not consider the reduction in tT4 to be toxicologically relevant because a sufficient reservoir of inactive (bound to protein) T4 remained available to maintain thyroid hormone homeostasis.

Critical Study Selection

Thyroid toxicity observed in the subacute NTP (2018a) studies is not considered for POD derivation because this endpoint is much less sensitive than the immunotoxicity reported by Dong et al. (2009).

Reproductive Toxicity – PFOA

In vivo studies

Reproductive effects of PFOA in animals were described in recent assessments by US EPA (2016a), New Jersey DWQI (New Jersey DWQI, 2017) and ATSDR (2018). Additionally, in 2017, PFOA was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity. Subchronic studies in mice showed reproductive toxicity effects such as decreased litter size, increased litter resorptions, and increased fetal death. Male mice exposed to PFOA had decreased testis weight, decreased sperm count and testicular damage. A two-generational study in rats showed no reproductive toxicity.

Studies of PFOA exposure reporting reproductive toxicity effects published after 2016 are summarized in Table 7.

Table 7. Summary of recent animal toxicity studies of PFOA reporting reproductive toxicity

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Kunning mice (n=6/dose)	0, 2.5, 5, or 10 mg/kg-day for 14 days orally	↓ absolute testis weight (high dose); ↓ sperm count (all doses); morphological changes in seminiferous tubules; ↓ SOD levels, CAT activity in testes (all doses); ↓ MDA and H_2O_2 levels in the testis (mid and high dose)	LOAEL: 2.5 mg/kg-day for ↓ sperm count	Liu et al. (2015)
Male BALB/C mice (n=11/dose)	0, 1.25, 5, or 20 mg/kg-day via oral gavage for 28 days	↓ triglyceride and cholesterol in epididymis (mid and high dose); ↓ in relative epididymis weight (low and high dose); changes in expression of genes and proteins related to triglyceride, cholesterol and fatty acid metabolism in the epididymis; changes in fatty acid composition in epididymis; ↑ MDA levels in epididymis (low and mid dose) ↓ GSH-Px levels in epididymis (mid and high dose)	LOAEL: 1.25 mg/kg-day for ↓ in relative epididymis weight	Lu et al. (2016)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Pregnant Kunming mice (n=10/dose), male offspring evaluated for effects on PND 21 and 70	0,1 ,2.5, or 5 mg/kg-day via oral gavage from GD 1-17	**Pups**: ↓ number of surviving mice at weaning (high dose); ↑ absolute testis weight (high dose) on PND 21, ↓ absolute testis weight (low dose) on PND 70; ↑ testosterone (low dose) on PND 70, ↓ testosterone in testis (all doses) on PND 21 and (mid and high dose) on PND 70; ↓ Leydig cells (mid and high dose, PND 21 and 70); vacuolization of Sertoli cells and ↓ spermatozoa at high dose	LOAEL: 1 mg/kg-day for ↑ testoster-one, ↓ in absolute testis weight	Song et al. (2018)
Pregnant Kunning mice (n=12/dose)	0, 2.5, 5, or 10 mg/kg-day via oral gavage from GD 1 to GD 7 or 13	**Dams:** ↑ number of resorbed embryos on GD 13 (high dose); ↑ serum estradiol on GD 7 (high dose); ↓ serum progesterone on GD 13 (mid and high dose); ↓ number of corpora lutea on GD 7 (low and mid dose); ↓ number of corpora lutea on GD 13 (mid and high dose); ↓ ratio of corpora lutea to ovarian areas on GD 7 and 13; ↑ CAT and SOD activity, H_2O_2, and MDA levels in ovary; ↑ apoptosis protein markers (p53 and Bax) in ovary	LOAEL: 2.5 mg/kg-day for oxidative stress, apoptosis markers and ↓ in number of corpora lutea	Chen et al. (2017b)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male BALB/c mice (n=15/dose)	0, 1.25, 5 or 20 mg/kg-day via oral gavage for 28 days	↑ CBG protein levels in testes (low and mid dose); ↓ CBG protein levels in testis (high dose); ↑ CBG (all doses) and corticosterone levels (mid and high dose) ↓ adrenocorticotropic hormone serum levels (ACTH) (high dose)	LOAEL: 1.25 mg/kg-day for ↑ CBG levels in testis and serum	Sun et al. (2018)
Pregnant C57BL/6J mice (n=6/dose for dams, 9/dose for pups)	Dietary exposure to 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day (targeted concentration). Exposure started 2 weeks before mating and continued during mating (1 week), gestation (3 weeks), and lactation (3 weeks). Pups organs evaluated at 26 weeks (males) or 28 weeks (females).	**Dams**: ↓ litter size at two highest doses **Pups (both sexes)**: ↓ body weight at PND 4; hepatocellular anisokary-osis and karyomegaly **Male pups**: ↑ absolute and relative liver weight; ↑ eosinophilic liver foci; lipid accumulation in liver **Female pups**: ↓ triglycerides and cholesterol	**Dams:** NOAEL: 0.3 mg/kg-day for ↓ litter size **Pups:** NOAEL: 0.003 mg/kg-day for ↓ body weight in females on PND 4	van Esterik et al. (2016)
Male and female Sprague Dawley rats (n=10/sex/ dose)	0, 0.625, 1.25, 2.5, 5, or 10 mg/kg-day for 28 days via oral gavage	**Males**: ↑ relative testis weight, ↓ absolute epididymis weight, ↓ cauda epididymis weight, ↓ cauda epididymis sperm count	NOAEL: 2.5 mg/kg-day for ↑ relative testis weight and ↓ cauda epididymis weight	NTP (2018b)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male Sprague Dawley rats (n=10/dose)	0, 150, or 300 ppm (0, 14.7, or 29.5 mg/kg-day) in feed for 16 weeks	↓ absolute testis weight	NOAEL: 14.7 mg/kg-day for ↓ absolute testis weight	NTP (2018c)
Female Sprague Dawley rats (n=10/dose)	0, 300, or 1,000 ppm (0, 27.7, or 92.7 mg/kg-day) in feed for 16 weeks	Ovarian cysts	NOAEL: 27.7 mg/kg-day for ovarian cysts	NTP (2018c)
Female Sprague Dawley rats (n=50/dose)	0, 300, or 1,000 ppm (0, 18, or 63 mg/kg-day) in feed for 107 weeks	Squamous metaplasia in the endometrium	LOAEL: 18 mg/kg-day for endometrial squamous metaplasia	NTP (2018c)

ACTH, adrenocorticotropic hormone; CAT, catalase; CBG, corticosteroid binding globulin; GD, gestation day; GSH-Px, glutathione peroxidase; H_2O_2, hydrogen peroxide; LOAEL, lowest-observed-adverse-effect level; MDA, malondialdehyde; NOAEL, no-observed-adverse-effect level; PND, postnatal day; SOD, superoxide dismutase

As seen in studies cited in previous risk assessments by other agencies, a number of studies in mice reported reproductive toxicity following exposure to PFOA for 1-4 weeks. In male mice, studies reported decreased testis and epididymis weights and sperm count (Lu et al., 2016; Liu et al., 2015; Song et al., 2018). In females, studies reported decreases in litter size, changes in estrous cycle and changes in hormone levels (van Esterik et al, 2016; Chen et al; 2017b). A study by NTP (2018b) was the only study found in rats. Decreased absolute cauda epididymis weight and sperm count and increased relative testis weight were observed after a 28-day gavage study. In a 16-week oral gavage study, decreased absolute testis weight was observed in male rats (NTP, 2018c).

van Esterik et al. (2016) administered 0, 0.003, 0.01, 0.03, 0.1, 0.3, 1, or 3 mg/kg-day PFOA in the diet to 6 pregnant C57BL/6J mice per dose group. Exposure started 2 weeks before mating and continued through mating (1 week), gestation (3 weeks), and lactation (3 weeks). Toxicity in the F_1 generation was monitored in 6-9 pups from 2-3 litters in each dose group. Decreased litter sizes were reported at the two highest doses. Additionally, several developmental disorders were reported in pups, including the following: increased liver weight, increased eosinophilic liver foci and lipid

accumulation in liver in males; decreased femur length and femur weight, decreased quadriceps femoris muscle weight, decreased adipocyte cell size, and decreased serum triglycerides and cholesterol in females; and decreased body weight at postnatal day (PND) 4, decreased tibia length, and hepatocellular anisokaryosis and karyomegaly in pups of both sexes. OEHHA determined a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4 ($p<0.001$; student's T-test determined by OEHHA).

In vitro studies

A number of in vitro studies investigated the effects of PFOA treatment on Leydig cells. Mouse Leydig tumor cell lines showed an increase in gene and protein expression of cortisol binding protein (CBG) (Sun et al., 2018). Decreased mitochondrial membrane potential and increases in reactive oxygen species (ROS) were observed at PFOA concentrations of 50 µM and greater in mouse Leydig tumor cells (Zhao et al., 2017).

PFOA did not induce cell death or ROS production in male human embryonic stem cells; however, cells showed a decrease in spermatid production (Steves et al., 2018). Cytotoxicity was observed at concentrations greater than 250 µM PFOA in the human cell lines HEK293T, MCF-7, LNCaP, and H295R while no cytotoxicity was observed in MDA-kb2 cells at concentrations up to 500 µM (Behr et al., 2018). In the same study, a concentration of 100 µM PFOA co-incubated with estradiol (E2) increased ERβ activity in HEK293T cells. An increase in the production of estrone was measured in H295R cells treated with 100 µM PFOA.

Mechanistic studies

An earlier assessment by New Jersey DWQI (2017) reviewed possible mechanisms for reproductive toxicity, specifically in male mice. Possible modes of action described were PPARα activation and disruption of the blood-testis barrier leading to oxidative stress and estrogenic effects of PFOA. A recent study by Zhao et al. (2017) showed impairment of mitochondrial function and increase in ROS in mouse Leydig tumor cells.

Mice treated with 5 mg/kg-day PFOA for 28 days showed alterations in gene and protein expression related to endocytosis and the blood-testis barrier that are supportive of studies showing toxicity in the male testis (Lu et al., 2017).

A possible mechanism for male reproductive toxicity involves changes in cholesterol and fatty acid metabolism. Lu et al. (2016) showed PFOA activated Akt/AMPK signaling, a pathway that regulates lipid metabolism.

Critical Study Selection

van Esterik et al. (2016) generated a NOAEL of 0.003 mg/kg-day based on decreased body weight in female pups on PND 4. This NOAEL is two orders of magnitude lower than the NOAEL of 0.3 mg/kg-day from Loveless et al. (2006), which is associated with increased relative liver weight in male mice and was the basis for the interim NL. Although the van Esterik et al. (2016) study may provide a lower POD than the Loveless et al. (2006) study, the most appropriate dose metric, serum levels of PFOA, is not available for RL derivation.

Reproductive Toxicity – PFOS

In vivo studies

Reproductive effects of PFOS were described in recent assessments by US EPA (2016b), New Jersey DWQI (2018) and ATSDR (2018). Additionally, in 2017, PFOS was listed under Proposition 65 as a chemical known to the state of California to cause reproductive toxicity. More recent studies identified effects such as decreases in testis and/or epididymis weights, decreases in sperm count, increases in apoptosis and apoptosis markers in the ovary or testis, decreases in litter size, changes in hormone levels, and changes in estrous cycle. These studies are summarized in Table 8.

Table 8. Summary of recent animal toxicity studies of PFOS reporting reproductive toxicity

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Female ICR mice (n=136/dose)	0 or 0.1 mg/kg-day in drinking water for 4 months[a]	Prolongation of estrous cycle; ↓ estrous cycles per month; increase in atretic follicles; ↓ number of corpora luteum; changes in hormone levels at each estrous cycle	NA	Feng et al. (2015)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Pregnant Sprague Dawley rats (n=4/dose)	0, 5, or 20 mg/kg-day via oral gavage from GD 11-19	**Dams**: ↓ body weight, serum cholesterol levels **Pups**: ↓ body weight, body length, absolute testis weight, anogenital distance of male pups, testosterone in testis, Leydig cell number, testosterone biosynthetic enzyme levels, HDL levels in liver and testis; apoptosis in fetal Leydig cells	NOAEL: 5 mg/kg-day for all pup endpoints	Zhao et al. (2014)
Male ICR mice (n=20/dose)	0, 0.25, 2.5, 25, or 50 mg/kg-day via oral gavage for 28 days	↓sperm count, ↑ Sertoli cell vacuolization and derangement of cells layers; damage in blood-testis barrier between Sertoli cells	NOAEL: 0.25 mg/kg-day for all endpoints	Qiu et al. (2013)
Female ICR mice (n=20/dose)	0 or 10 mg/kg-day orally for 30 days	Prolongation of duration of diestrus; ↓ number of corpora lutea; ↓ serum levels of P4, LH and GnRH on day 7, ↓ serum levels of GnRH, E2, T4 and T3 on day 14; ↑ serum levels of CORT on day 14	NA	Wang et al. (2018)
Male C57 mice (n=12/dose)	0, 0.5 or 10 mg/kg-day via oral gavage for 5 weeks	↓ body weight, absolute and relative testis weight, sperm count, serum testosterone levels; vacuolization in spermatogonia, spermatocytes and Leydig cells; ↑ apoptotic cells in testes, apoptosis related proteins, ↑ ERα and ERβ protein expression	LOAEL: 0.5 mg/kg-day for ↑ ERβ protein expression	Qu et al. (2016)

Sex/Species	Exposure	Endpoints	NOAEL/ LOAEL	Reference
Male ICR mice (n=10/dose)	0, 0.5, 5 or 10 mg/kg-day via oral gavage for 4 weeks	↓ sperm count	NOAEL: 0.5 mg/kg-day for ↓ sperm count	Qiu et al. (2016a)
Male and female Sprague Dawley Rats (n=10/sex/ dose)	0, 0.312, 0.625, 1.25, 2.5, or 5 mg/kg-day for 28 days via oral gavage	**Females**: ↑ testosterone	NOAEL: 0.625 mg/kg-day for ↑ testosterone	NTP (2018a)

[a] Animals were exposed up to 6 months. Due to a significant decrease in body weight of PFOS exposed animals at 6 months, only animals treated for 4 months were used for subsequent endpoints. It is unclear how many animals were exposed to PFOS for 4 months or 6 months.
CORT, corticosterone; ERα,β, estrogen receptor α,β; E2, estradiol; GnRH, gonadotrophin-releasing hormone; HDL, high density lipoprotein; LH, luteinizing hormone; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; P4, progesterone; T3, triiodothyronine; T4, thyroxine

In vitro studies

Studies in Sertoli cells isolated from mice, rats and humans reported effects such as perturbation of tight junction proteins resulting in inhibition of the tight junction permeability barrier (Li et al., 2016; Qiu et al., 2016a; Chen et al., 2017a; Gao et al., 2017). Decreases in mitochondrial membrane potential and increases in ROS generation were observed at PFOS concentrations of 50 µM and greater in mouse Leydig tumor cells (Zhao et al., 2017).

Cytotoxicity was observed at concentrations greater than 250 µM in human HEK293T, MCF-7, LNCaP, H295R and MDA-kb2 cell lines (Behr et al., 2018). In the same study, concentrations greater than 50 µM PFOS co-incubated with E2 increased ERβ activity in HEK293T cells. In MDA-kb2 cells, PFOS co-incubated with dihydrotestosterone (DHT) increased androgen receptor (AR) activity. An increase in production of estrone and progesterone was measured in H295R cells treated with 100 µM PFOS. In porcine ovarian theca and granulosa cells, PFOS at 1.2 µM caused a decrease in basal secretion of progesterone, androstenedione and estradiol (Chaparro-Ortega et al., 2018).

Mechanistic studies

An earlier assessment by US EPA (2016b) reviewed possible mechanisms for reproductive toxicity. Male reproductive toxicity may be caused by disruptions in gap junction intercellular communication by PFOS, compromising the blood-testis barrier in Sertoli cells. Recent in vitro studies assessed effects of PFOS on the blood-testis barrier and showed that PFOS perturbs tight junction proteins and function, and causes

microfilament disruption resulting in Sertoli cell injury (Gao et al., 2017; Li et al., 2016; Qiu et al., 2016a; Chen et al., 2017a).

Gene and protein expression were analyzed in the testes of rats exposed to PFOS for 28 days (Lopez-Doval et al., 2016). These investigators showed that PFOS inhibits the expression of follicle stimulating hormone receptor and AR, while inducing the expression of gonadotropin-releasing hormone receptor and luteinizing hormone receptor.

PFOS has been suggested to interact with estrogen receptors. Qu et al. (2016) found that PFOS altered expression of ERα and ERβ in mouse testis after exposure to at least 0.5 mg/kg-day for 5 weeks. ERα-induced anteroventral periventricular nucleus (AVPV)-kisspeptin neuron activation was suppressed by PFOS, causing alterations in the estrous cycle in female ICR mice (Wang et al., 2018).

Critical Study Selection

The NOAELs/LOAELs (based on administered dose) determined from these recent studies reporting reproductive effects are orders of magnitude larger than the NOAEL of 0.008 mg/kg-day (administered dose) for immunotoxicity from Dong et al. (2009), which was the basis for OEHHA's interim NL recommendation. Therefore, these studies are not considered for POD derivation in support of a final recommendation on the PFOS NL.

Cancer – PFOA

Cancer bioassays in laboratory animals conducted prior to 2016 have been thoroughly described previously (US EPA, 2016; New Jersey, 2018; IARC, 2017). These studies are briefly described below, and significant cancer incidences are reported in Table 9.

Table 9. Significant tumor incidences following exposure to PFOA

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence	Reference
Male Sprague Dawley rats (n=50/dose)	Oral in diet for 106 weeks	Leydig Cell Adenoma	0, 1.3, or 14.2	0/33, 2/36, 7/44*	Butenhoff et al. (2012b), data from Sibinsky (1987)
Male Sprague Dawley rats (n=76-79/ dose)	Oral in diet for 104 weeks	Hepatocellular adenoma or carcinoma	0[a] or 13.6	3/79, 10/76*	Biegel et al. (2001)

Sex/Species	Exposure	Tumor type	Dose (mg/kg-day)	Incidence	Reference
Male Sprague Dawley rats (n=76-79/ dose)	Oral in diet for 104 weeks	Pancreatic acinar cell adenoma or carcinoma	0[a] or 13.6	1/79, 8/76*	Biegel et al. (2001)
Male Sprague Dawley rats (n=76-79/ dose)	Oral in diet for 104 weeks	Leydig cell adenoma	0[a] or 13.6	2/78, 8/76*	Biegel et al. (2001)
Pregnant CD-1 mice (n=6-14 dams/dose or 21-37 female pups/dose)	Oral in drinking water from GD 1 to GD 17, pups followed for 18 months	Hepatocellular adenoma	0, 0.01, 0.1, 0.3, 1, or 5	0/29, 1/29, 1/37, 4/26*, 0/31, 1/21	Filgo et al. (2015)

[a] Pair-fed control, fed same amount of food as treated group
*p<0.05, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA
GD, gestation day

Sibinsky (1987), as reported by Butenhoff et al. (2012), administered 0, 30, or 300 ppm PFOA to rats (0, 1.3, or 14.2 mg/kg-day for males; 0, 1.6 or 16.1 mg/kg-day for females) in the diet for 105-106 weeks. In male animals, a significant increase in Leydig cell adenomas and preneoplastic pancreatic acinar cell hyperplasia was observed in the high dose group. In females, a significant increase in preneoplastic ovarian tubular hyperplasia was observed (later reclassified as gonadal stromal hyperplasia in a pathology review by Mann and Frame, 2004). An increase in mammary gland fibroadenoma was initially reported, but a follow-up examination of the pathology revealed no significant increase over controls (Hardisty et al., 2010).

Biegel et al. (2001) administered 0 or 300 ppm (0 or 13.6 mg/kg-day) to male rats in the diet for 24 months. A significant increase in several tumor types was reported, including hepatocellular adenomas or carcinomas, pancreatic acinar cell adenomas or carcinomas, and Leydig cell adenomas. Additionally, preneoplastic pancreatic acinar cell hyperplasia and Leydig cell hyperplasia were increased.

Filgo et al. (2015) exposed three different strains of pregnant mice (CD-1, 129/SV wild-type, and 129-SV PPARα knock-out) to doses of PFOA in drinking water ranging from 0 to 5 mg/kg-day from gestation day (GD) 1 to GD 17. Offspring were observed for 18

months. A significant increase in hepatocellular adenomas, and a significant trend for hepatic hemangiosarcomas, were observed in the CD-1 F_1 generation. Liver tumors were not significantly increased in wild-type or knock-out 129/SV mice. It should be noted that the liver was the only organ evaluated in these studies.

Recently, NTP (2018c) released carcinogenicity data from chronic bioassays of PFOA in male and female rats (see Liver Toxicity section for study details). Significant increases in hepatocellular adenomas/carcinomas and pancreatic acinar cell adenomas/carcinomas were observed in male rats, shown in Table 10. Female rats had an increase in uterine adenomas/carcinomas, shown in Table 11. Animals that died before the first observed tumor incidence were not included in the dose-response analysis. Furthermore, carcinogenicity data from animals exposed perinatally were not considered for derivation of the RL because the tumor incidences in these animals were comparable to animals that were not exposed perinatally, suggesting that exposure during gestation and lactation had minimal impact on tumor development later in life.

Table 10. Hepatocellular and pancreatic tumor incidences in male rats exposed to PFOA in the diet for 107 weeks (NTP, 2018c)

Conc. (ppm)	Dose (mg/kg-day)	Plasma conc. (mg/L)	Human Equivalent Dose (mg/kg-day)	Hepatocellular adenoma or carcinoma	Pancreatic acinar cell adenoma or carcinoma
0	0	BD[a]	0	0/36[b]	3/43
20	1.0	81.4	0.011	0/42	29/49***
40	2.3	131	0.018	7/35**	26/41***
80	4.8	160	0.022	11/37***	32/40***

[a] BD = below the limit of detection. Values were considered 0 for the dose-response analysis

[b] Incidence/effective number of animals

**p<0.01, ***p<0.001, pairwise comparison with Fisher's exact test, statistical analysis by OEHHA

Table 11. Uterine tumor incidences in female rats exposed to PFOA in the diet for 107 weeks (NTP, 2018c)

Concentration (ppm)	Dose (mg/kg-day)	Plasma Concentration (mg/L)	Uterine adenoma or carcinoma
0	0	BD[a]	2/32
300	18	20.4	5/40
100	63	72.3	8/35

[a] BD = below the limit of detection. Values were considered 0 for the dose-response analysis

[b] Incidence/effective number of animals

[#]p<0.05 for trend test, statistical analysis by OEHHA

Plasma concentrations in the chronic male rat study were determined at 16 weeks, but because the serum half-life of PFOA is estimated to be 4-6 days in male rats, it is

anticipated that by 16 weeks, a steady-state concentration would have been reached. Therefore, the plasma concentration would remain relatively stable over the 107-week period of continuous dosing. Plasma concentrations are converted to human equivalent doses (HEDs) using the human clearance factor of 1.4×10^{-4} L/kg-day for PFOA, determined by US EPA (2016a). The formula is shown below:

Serum concentration (mg/L) × clearance factor (L/kg-day) = HED (mg/kg-day)

The resulting HEDs are presented in Table 10. Hepatic adenomas/carcinomas and pancreatic acinar cell adenomas/carcinomas are critically evaluated for RL development.

Cancer – PFOS

Summaries of the sole report of carcinogenicity bioassays (Butenhoff et al., 2012a) for PFOS have been previously published (US EPA, 2016b; New Jersey DWQI, 2018). The study design and significant results are briefly described below.

Butenhoff et al. (2012a) published a report of carcinogenicity studies from 2002 by 3M, a former PFOS manufacturer (Thomford, 2002). In these studies, male and female Sprague Dawley rats were administered 0, 0.5, 2, 5, or 20 ppm PFOS (0, 0.024, 0.098, 0.242, or 0.984 mg/kg-day for males; 0, 0.029, 0.120, 0.299, or 1.251 mg/kg-day for females) in the diet for two years. An additional group was administered 20 ppm for one year, and then control diet for the next year (data not shown). An increase in hepatocellular adenoma incidence was observed in both male and female animals at the highest dose. Combined hepatocellular adenoma/carcinoma incidence was also increased in female rats. Positive trends for hepatocellular adenomas were reported in both sexes. Tumor incidence data are summarized in Tables 12 and 13. It should be noted that the relatively low effective number of female rats was not due to high levels of premature mortality (mortality in treated groups was comparable to controls), but due to the fact that the first incidence of hepatocellular adenoma/carcinoma appeared quite late in the bioassay (day 653).

Table 12. Hepatocellular tumor incidences in male rats exposed to PFOS in the diet for 2 years (Butenhoff et al., 2012a)

Conc. (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Human equivalent dose (mg/kg-day)	Hepatocellular adenoma
0	0	0.014	1.2×10^{-6}	0/41[b]
0.5	0.024	2.64	2.1×10^{-4}	3/42
2.5	0.098	12.1	9.8×10^{-4}	3/47
5	0.242	32.3	2.6×10^{-3}	1/44
20	0.984	121	9.8×10^{-3}	7/43*

[a] Calculated by OEHHA

[b] Incidence/effective number of animals. Animals that died before the first tumor incidence were not considered in the dose-response assessment.

*p<0.05, reported by study authors

Table 13. Hepatocellular tumor incidences in female rats exposed to PFOS in the diet for 2 years (Butenhoff et al., 2012a)

Conc. (ppm)	Dose (mg/kg-day)	Serum conc. (mg/L)[a]	Human equivalent dose (mg/kg-day)	Hepatocellular adenoma or carcinoma
0	0	0.841	6.8×10^{-5}	0/28[b]
0.5	0.029	5.49	4.5×10^{-4}	1/29
2.5	0.120	23.0	1.9×10^{-3}	1/16
5	0.299	66.4	5.4×10^{-3}	1/31
20	1.251	215	1.7×10^{-2}	6/32*

[a] Calculated by OEHHA

[b] Incidence/effective number of animals. Animals that died before the first tumor incidence were not considered in the dose-response assessment.

*p<0.05, reported by study authors

Because the biological half-life of PFOS differs greatly between rats (9-10 weeks) and humans (4-5 years), administered dose is not the appropriate dose metric for toxicity assessment. Serum PFOS concentration is a more suitable dose metric for extrapolating toxicity in rodents to toxicity in humans. Serum concentrations at various time points were measured, and the results are reported in Table 14.

Table 14. Mean serum PFOS concentrations (in mg/L) in rats from Butenhoff et al. (2012)

Week	Sex	0 ppm	0.5 ppm	2.5 ppm	5 ppm	20 ppm
4	Male	0	0.907	4.33	7.57	41.8
4	Female	0.026	1.61	6.62	12.6	54
14	Male	0	4.04	17.1	43.9	148

Week	Sex	0 ppm	0.5 ppm	2.5 ppm	5 ppm	20 ppm
14	Female	2.67	6.96	27.3	64.4	223
53	Male	0.025	-	-	-	146
53	Female	0.395	-	-	-	220
103	Male	-	-	-	-	-
103	Female	-	-	20.2	-	-
105	Male	0.012	1.31	7.6	22.5	69.3
105	Female	0.084	4.35	-	75	233

A dash (-) indicates that data were not collected at that time point

In males, the maximum serum concentration was reached at 14 weeks. At 105 weeks, serum concentrations in all dose groups were typically 2- to 3-fold less than at 14 weeks, indicating that PFOS is more rapidly eliminated at later time points. The authors attributed this increased urinary elimination of PFOS to chronic progressive nephritis. In females, serum concentrations measured at 105 weeks were typically comparable to the values measured at 14 weeks. Because serum concentrations in males declined after 14 weeks, the PFOS serum concentration values at terminal sacrifice would underestimate the serum concentrations at earlier time points. Therefore, OEHHA calculated the area under the curve (AUC) for each dose group using a simple linear interpolation. The time-weighted average serum concentration at each dose is determined by dividing the AUC by the duration of the study (103 or 105 weeks). These time-weighted average serum concentrations are presented in Tables 12 and 13.

Serum concentrations in rats are converted to HEDs using the human clearance factor of 8.1×10^{-5} L/kg-day for PFOS, determined by US EPA (US EPA, 2016). These values are presented in Tables 12 and 13, and the conversion formula is shown below:

Serum concentration (mg/L) × clearance factor (L/kg-day) = HED (mg/kg-day)

There is sufficient evidence to consider and critically evaluate the liver tumors in male and female rats for RL development. First, the two chronic bioassays reported in Butenhoff et al. (2012) are of sufficient quality (appropriate length, suitable number of animals per dose, adequate reporting, etc.) to warrant consideration as critical studies. Second, recent studies of PFOA by NTP (2018c) provide additional support for considering carcinogenicity as a critical endpoint for PFOS. In their assessment, NTP (2018c) showed that chronic exposure to PFOA led to a significant increase in hepatocellular adenomas and/or carcinomas in male rats (data presented in Table 10), which is similar to the carcinogenic effects of PFOS reported by Butenhoff et al. (2012). The similarity in molecular structure between PFOS and PFOA suggests that these two chemicals may have comparable biological activities, and in fact, the noncancer toxicology profiles of these two chemicals are similar. The International Agency for Research on Cancer (IARC, 2017) designated PFOA possibly carcinogenic to humans (Group 2B). PFOS also produced a positive trend for pancreatic carcinomas in male

rats (data not shown), which is a critical tumor type in PFOA-exposed male rats in the NTP (2018c) bioassay (Table 10). It should be noted that the highest administered dose in the Butenhoff et al. (2012) PFOS bioassay (0.984 mg/kg-day) was essentially the same as the lowest administered dose in the NTP (2018c) PFOA bioassay (1.0 mg/kg-day). This suggests that the Butenhoff et al. (2012) studies are less sensitive than the NTP (2018c) studies, and that the modest, but significant, tumor incidences observed (when compared against the NTP (2018c) PFOA data) are the result of overall lower administered doses. Third, although there is minimal evidence to indicate PFOS is genotoxic or mutagenic (US EPA, 2016b; New Jersey DWQI, 2018), increases in hepatic oxidative stress (Xing et al., 2016; Han et al., 2018a), hepatocellular hypertrophy (Han et al., 2018a; Bagley et al., 2017; Wan et al., 2016; NTP, 2018a), and cell proliferation (Han et al., 2018b) in rodents have been observed in recent short-term studies of PFOS. Additional data regarding the modes of action of PFOS are needed to clarify whether or not these effects are precursors of liver tumors, but at present, there is not enough evidence to rule out the possibility.

DOSE-RESPONSE ASSESSMENT AND REFERENCE LEVEL CALCULATIONS

Noncancer – PFOA

From Table 1, the lowest LOAEL is 0.05 mg/kg-day, which corresponds to a serum concentration of 0.97 mg/L, for hepatic mitochondrial membrane potential changes and increased apoptosis and oxidative DNA damage (Li et al., 2017). These endpoints were also frequently observed in in vitro studies. OEHHA selected the data from this study as the basis of a POD for calculating an RL for noncancer effects. For the purpose of comparison, the recommended interim NL was based on increased relative liver weight in a mouse study, from which a NOAEL of 0.3 mg/kg-day (based on administered dose) was determined (Loveless et al., 2006).

A NOAEL of 0.003 mg/kg-day was identified from the van Esterik et al. (2016) study, based on reduced female pup body weight on PND 4 in animals exposed to PFOA during gestation and lactation. However, serum concentrations were not reported in this study, and due to the complexity of the dosing scheme (PFOA was administered to dams during pregnancy and lactation), kinetic modeling was not conducted to predict serum concentrations. Therefore, this study is not considered for derivation of a noncancer RL. However, OEHHA acknowledges the potential for developmental toxicity at PFOA levels below the selected POD, and an additional uncertainty factor is added to account for this (discussed below).

The most sensitive endpoints from the Li et al. (2017) study (increased oxidative DNA damage, changes in mitochondrial membrane potential, and increased biomarkers of apoptosis in the liver of female mice) were analyzed with benchmark dose (BMD) software (BMDS version 2.6, US EPA). BMD modeling of the endpoints in Table 2 did

not generate any models with an acceptable goodness-of-fit. Therefore, the LOAEL of 0.97 mg/L is selected as the POD for noncancer effects.

Pharmacokinetic (PK) modeling

Administered dose in rodent studies of PFOA is not the optimal dose metric for toxicity evaluation because of the great difference in the chemical's half-life between humans (2-3 years) and rodents (1-3 weeks). The preferred dose metric is PFOA serum concentration. OEHHA evaluated available PK models to predict PFOA serum concentrations from administered doses. However, after critical evaluation, OEHHA found several shortcomings with the available models that lowered overall confidence in these models' ability to adequately predict serum concentrations. Therefore, OEHHA is using reported serum concentrations for RL derivation.

Acceptable Daily Dose Calculation

To calculate the acceptable daily dose (ADD), which is an estimated maximum daily dose of a chemical that can be consumed by humans for an entire lifetime without toxic effects, the POD is divided by the total uncertainty factor (UF). Because the dose metric for the POD is serum concentration, the ADD is first expressed as a target human serum concentration rather than the typical mg/kg-day dose.

A total uncertainty factor (UF) of 300 is applied in calculating the ADD for PFOA: 3 for interspecies extrapolation, 10 for intraspecies variability, 3 for LOAEL to NOAEL extrapolation, and 3 for the potential for developmental toxicity at the point of departure. When developing a health-protective RL of a chemical in drinking water, the adverse effect or an upstream physiological change that leads to an adverse effect occuring at the lowest dose is selected as the critical effect. Because the critical endpoints here are upstream physiological changes that can lead to adverse effects in a known target organ of PFOA toxicity, the liver, OEHHA is applying a LOAEL to NOAEL UF of 3 rather than 10. OEHHA also is applying a subchronic to chronic extrapolation UF of 1, consistent with the New Jersey DWQI (2017) assessment for PFOA, in which the critical endpoint was increased liver weight from a 14-day study, and a subchronic to chronic UF of 1 was used. New Jersey DWQI's rationale was that, based on evaluation of multiple studies, early manifestations of liver toxicity do not appear to increase in magnitude with chronic exposures. This rationale would also apply to the upstream endpoints used as the basis of the POD from the Li et al. (2017) critical study. For animal studies, OEHHA typically uses a UF of 10 for interspecies extrapolation ($\sqrt{10}$ for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics) and a UF of 30 for intraspecies variability (10 for pharmacokinetics and $\sqrt{10}$ for pharmacodynamics). Since PFOA is not known to be metabolized in animals or humans, and because PFOA serum concentration is the dose metric used in the dose-response analysis, the pharmacokinetic components of the interspecies and intraspecies uncertainty factors

are reduced. An intraspecies pharmacokinetics UF of √10 (rather than 10) is kept to account for potential PK differences in infants and children. Thus,

ADD = POD ÷ UF = 0.97 mg/L ÷ 300 = 0.0032 mg/L (target human serum concentration).

A NOAEL (based on administered dose) of 0.003 mg/kg-day was determined by OEHHA from the van Esterik et al. (2016) study, based on decreased female pup body weight in the F_1 generation of dams administered PFOA throughout gestation and lactation. By comparison, the administered dose LOAEL from the Li et al. (2017) study is 0.05 mg/kg-day. Although it is unknown what the serum concentrations are in the van Esterik et al. (2016) study, it is possible that developmental toxicity occurred at a lower concentration than the hepatotoxicity in the Li et al. (2017) study. Therefore, an additional uncertainty factor of 3 is included to account for this possibility.

The ADD, expressed as a target human serum concentration of 3.2 µg/L, is slightly higher than average PFOA serum levels nationally and in California. Biomonitoring data from California[1] reported a geometric mean of 1.49 µg/L PFOA in the serum of 337 people in 2013 (the 95th percentile was 4.57 ng/mL). This is comparable to the geometric mean of 1.94 µg/L PFOA in the serum of the general US population, as determined from the National Health and Nutrition Examination Survey (NHANES) data (ATSDR, 2018).

As noted above, in order to account for PFOA's long half-life in humans relative to rodents, the ADD is expressed as a target serum concentration. To calculate a noncancer RL, the target serum concentration must be converted to an HED expressed as a dose in mg/kg-day. This is done by multiplying the ADD by a daily clearance factor, which reflects the clearance of PFOA from the body, of 1.4×10^{-4} L/kg-day for PFOA (US EPA, 2016a), as shown below.

ADD = 3.2 µg/L × 1.4×10^{-4} L/kg-day = 4.5×10^{-4} µg/kg-day or 0.45 ng/kg-day

The relative source contribution (RSC) is the proportion of exposures to a chemical attributed to tap water (including inhalation and dermal exposures, e.g., during showering), as part of total exposure from all sources (including food and air pollution). The RSC values typically range from 20 to 80 percent (expressed as 0.20 to 0.80), and are determined based on available exposure data. The default RSC of 0.2 is selected because there is not enough data to determine specific exposure patterns for PFOA. In addition to drinking water, there are several other sources of PFOA that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOA released to air may adsorb to airborne particles and travel long distances (US EPA, 2016a). Additionally, the use of PFOA in many consumer

[1] From Biomonitoring California, Results for Perfluorochemicals (PFCs) (last accessed February 1, 2019)

products and its environmental persistence has led to the presence of PFOA in indoor air and dust. In fact, US EPA (2016a) reports that the most common exposure routes of PFOA are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOA in drinking water. PFOA is not very volatile in its ionized form (its predominant form in water) (Johansson et al., 2017), so inhalation of PFOA directly from drinking water is not anticipated to be a major route of exposure. Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water. Ionized PFOA penetrates skin poorly compared to the neutral form, and PFOA should remain ionized in the stratum corneum due to its buffering capacity (Franko et al., 2012).

PFOA can permeate mouse and human skin in vitro, and be absorbed following dermal application in mice in vivo (Franko et al., 2012). However, a time-course of >5 hours is needed for PFOA to penetrate full-thickness human skin, and this exposure scenario is unlikely to occur from typical household uses of tap water. Additionally, solid PFOA and 1% PFOA in acetone were determined to be non-corrosive in an in vitro epidermal cell viability assay following three minutes of exposure. It should be noted, however, that solid PFOA was corrosive following one hour of exposure, whereas 1% PFOA in acetone was not.

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking water intake rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer RL, which is calculated using the following formula:

RL = ADD × RSC ÷ DWI, where

ADD = acceptable daily dose of 0.45 ng/kg-day,
RSC = relative source contribution of 0.2, and
DWI = daily water intake rate of 0.053 L/kg-day

RL = (0.45 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 2 ng/L or 2 ppt

Thus, the reference drinking water level for the noncancer effects of PFOA is 2 ng/L or 2 ppt based on a recent hepatotoxicity study in mice (Li et al., 2017).

Noncancer – PFOS

OEHHA did not identify any new studies to replace the Dong et al. (2009) study as the critical toxicity study for the noncancer effects of PFOS. Decreased plaque forming cell response was the most sensitive endpoint, and a NOAEL of 0.008 mg/kg-day was identified. The data are summarized in Table 15.

Table 15. Plaque forming cell response in male mice exposed to PFOS (Dong et al., 2009)

Dose (mg/kg-day)	Serum Concentration (mg/L)	Human Equivalent Dose (mg/kg-day)	Plaque Forming Response[a] (PFC/10^6 spleen cells)
0	0.048 ± 0.014[b]	3.9×10^{-6}	597 ± 64[b]
0.008	0.674 ± 0.166	5.5×10^{-5}	538 ± 52
0.083	7.132 ± 1.039	5.8×10^{-4}	416 ± 43*
0.417	21.638 ± 4.410	1.8×10^{-3}	309 ± 27*
0.833	65.426 ± 11.726	5.3×10^{-3}	253 ± 21*
2.08	120.670 ± 21.759	9.8×10^{-3}	137 ± 16*

[a] Data taken from New Jersey DWQI (2018). Authors state they received numerical data via personal communication with GH Dong.
[b] Mean ± SEM (n = 10/dose)
* p<0.05, reported by study authors

Using the equations shown above for PFOA, serum concentrations are converted to HEDs using the human clearance factor of 8.1×10^{-5} L/kg-day for PFOS, determined by US EPA (US EPA, 2016b). The formula is shown below:

Serum concentration (mg/L) × clearance factor (L/kg-day) = HED (mg/kg-day)

The resulting HEDs are presented in Table 15.

BMD modeling was performed using both serum concentrations and HEDs as the dose metric. However, an adequate model fit was not attained in either case. Therefore, the NOAEL of 0.008 mg/kg-day (corresponding to a serum concentration of 0.674 mg/L, Table 15) is selected as the POD.

A total UF of 30 is applied in calculating the ADD for PFOS: 3 for interspecies extrapolation and 10 for intraspecies variability. For animal studies, OEHHA typically uses a UF of 10 for interspecies extrapolation (√10 for pharmacokinetics and √10 for pharmacodynamics) and a UF of 30 for intraspecies variability (10 for pharmacokinetics and √10 for pharmacodynamics). However, because PFOS is not known to be metabolized in animals or humans, and because PFOS serum concentration is the dose metric used in the dose-response analysis, the pharmacokinetic components of the interspecies and intraspecies uncertainty factors are reduced. A subchronic to chronic UF of 3 is typically applied when the study duration is 8-12% of the animal's lifetime (OEHHA, 2008), in order to account for the potential exacerbation of toxicity following chronic exposure. However, New Jersey DWQI (2018) argues that the subchronic to chronic uncertainty factor is not necessary because the maximum decrease in plaque forming cell response remained relatively constant (~70-85%) across studies with different exposure durations (ranging from 7 to 60 days), thus increased exposure

duration does not lead to increased toxicity. OEHHA agrees and is applying a subchronic to chronic UF of 1 rather than 3.

To determine the ADD, expressed as a target human serum concentration, the POD is divided by the total UF, as shown below.

ADD = 0.674 mg/L ÷ 30 = 0.022 mg/L or 22 µg/L (target human serum concentration)

The target serum concentration of 22 µg/L is higher than average PFOS serum levels nationally and in California. Biomonitoring data from California[2] report a geometric mean of 5.21 µg/L PFOS in the serum of 337 people in 2013 (the 95th percentile was 17.6 ng/mL). This is comparable to the geometric mean of 4.99 µg/L PFOS in serum of the general US population, as determined from NHANES data (ATSDR, 2018).

The ADD is converted to an HED by multiplying the target human serum concentration by a daily clearance factor of 8.1×10^{-5} L/kg-day (US EPA, 2016b), as shown below.

ADD = 22 µg/L × 8.1×10^{-5} L/kg-day = 1.8×10^{-3} µg/kg-day or 1.8 ng/kg-day

The default RSC of 0.2 is selected because there is not enough specific data to determine specific exposure patterns to PFOS. In addition to drinking water, there are several other sources of PFOS that may contribute to exposure in the general population, including air, soil, food, and consumer and industrial products. PFOS released to air may adsorb to airborne particles and travel long distances (US EPA, 2016b). Additionally, the use of PFOS in many consumer products and its environmental persistence has led to the presence of PFOS in indoor air and dust. As with PFOA, US EPA (2016b) reports that the most common exposure routes of PFOS are diet and indoor dust. Thus, an RSC of 0.2 is appropriate, and consistent with RSCs used by other agencies, including US EPA and the State of New Jersey.

Oral ingestion is the primary route of exposure for PFOS in drinking water. Volatilization of the predominant anionic form in water (pKA <1.0) is not expected to occur (HSDB, 2018).

Dermal absorption is also not anticipated to be a significant route of exposure from typical household uses of tap water, based on its physicochemical similarities to PFOA. However, no specific studies could be identified that addressed absorption of PFOS following dermal exposure. ATSDR (2018) reports the results of an unpublished single-dose dermal absorption study in rabbits, where potassium PFOS or its diethanolamine salt (at doses up to 20 µg/kg) was applied to clipped, intact skin (Johnson et al., 1995a,b, as reported by ATSDR, 2018). Compared to controls, no increase in organic fluoride in the liver was detected, suggesting that PFOS was not absorbed.

[2] From Biomonitoring California, Results for Perfluorochemicals (PFCs) (last accessed February 1, 2019)

Because oral ingestion is considered to be the only significant route of drinking water exposure, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the noncancer RL, which is calculated using the following formula:

RL = ADD × RSC ÷ DWI, where

ADD = acceptable daily dose of 1.8 ng/kg-day,
RSC = relative source contribution of 0.2, and
DWI = daily water intake of 0.053 L/kg-day

RL = (1.8 ng/kg-day × 0.2) ÷ 0.053 L/kg-day = 7 ng/L or 7 ppt

Thus, the reference drinking water level for the noncancer effects of PFOS is 7 ng/L or 7 ppt based on immunotoxicity in mice.

Cancer – PFOA

Hepatocellular adenoma/carcinoma and pancreatic acinar cell adenoma/carcinoma in male rats were evaluated for RL derivation. For individual tumor sites, OEHHA uses the linear multistage cancer model from US EPA's BMD software (BMDS version 2.6, US EPA) to determine the dose associated with a benchmark response (BMR) of 5% increased risk of developing a tumor and the lower 95% confidence limit of that dose, the $BMDL_{05}$. For carcinogens that induce tumors at multiple sites and/or in different cell types at the same site in a particular species and sex, BMDS can be used to derive maximum likelihood estimates (MLEs) for the parameters of the multisite carcinogenicity model by summing the MLEs for the individual multistage models from the different sites and/or cell types. This multisite model is then used to provide a basis for estimating the cancer potency of a chemical that causes tumors at multiple sites. Using the HEDs as the dose metric, multisite benchmark dose modeling was performed to determine the cancer slope factor (CSF) for the hepatic and pancreatic tumors in male rats.

A multisite $BMDL_{05}$ of 0.000648 mg/kg-day was determined from the animal bioassay data (Table 10). To estimate from animal data an HED that would result in an equal lifetime risk of cancer, OEHHA uses body weight (BW) scaling to the ¾ power (OEHHA, 2009). This adjustment accounts for interspecies differences in pharmacokinetics and pharmacodynamics. Because pharmacokinetic differences have already been accounted for by using serum concentration as the dose metric instead of administered dose, the $BMDL_{05}$ only needs modification for pharmacodynamic differences ($BW^{1/8}$ adjustment). The equation is provided below.

$$BMDL_{05(human)} = BMDL_{05(animal)} \times (BW_{animal}/BW_{human})^{1/8}$$

where BW_{animal} is 0.509 kg, the time-weighted average body weight of control male rats from the NTP (2018c) 2-year bioassay, and BW_{human} is the default value of 70 kg. Thus,

the $BMDL_{05(human)}$ is 3.5×10^{-4} mg/kg-day. The human CSF is determined using the following equation:

$$CSF = BMR \div BMDL_{05} = 0.05 \div 3.5 \times 10^{-4} \text{ mg/kg-day} = 143 \text{ (mg/kg-day)}^{-1}$$

As described in the noncancer reference level derivation, oral ingestion is the primary route of exposure to PFOA in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies age sensitivity factors (ASFs, unitless) to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2 years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. These factors are typically applied unless chemical-specific data exist to better guide the risk assessment.

NTP (2018c) administered 300 ppm PFOA from GD 6 through PND 21 to a concurrent cohort of animals. There were no significant differences in tumor incidences between animals with and without perinatal exposure in the 20, 40 and 80 ppm dose groups (Table 16). This suggests that early-life exposures to PFOA do not substantially increase the likelihood of tumor formation later in life. Therefore, OEHHA is not applying ASFs for derivation of the cancer RL.

Table 16. Comparison of tumors in perinatally and non-perinatally exposed male rats (NTP, 2018c)

Tumor type	Exposure	0 ppm	20 ppm	40 ppm	80 ppm
Hepatocellular adenoma or carcinoma	No perinatal exposure	0/36[b]	0/42	7/35	11/37
Hepatocellular adenoma or carcinoma	300 ppm perinatal exposure	0/35	1/38	5/38	12/39
Pancreatic acinar cell adenoma or carcinoma	No perinatal exposure	3/43	29/49	26/41	32/40
Pancreatic acinar cell adenoma or carcinoma	300 ppm perinatal exposure	7/41	20/44	30/44	30/43

Perinatal exposure – GD 6 through PND 21

[b] Incidence/effective number of animals

Because oral ingestion is considered to be the only significant route of drinking water exposure to the compound, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to determine the RL. The RL for carcinogenic effects can be calculated using the following equation:

RL = R ÷ (CSF × DWI), where

R = default risk level of one in one million, or 10^{-6}
CSF = cancer slope factor in $(mg/kg\text{-}day)^{-1}$
DWI = daily water intake rate of 0.053 L/kg-day

Using the total lifetime drinking water exposure estimate of 0.053 L/kg-day, a RL for a one in one million cancer risk from PFOA in tap water is:

$RL = 10^{-6} \div (143\ (mg/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 1.3 \times 10^{-7}$ mg/L

RL = 0.1 ng/L or 0.1 ppt (rounded)

The cancer RL of 0.1 ppt should protect against the noncancer effects of PFOA since it is lower than the 2 ppt level for noncancer effects.

Cancer – PFOS

Hepatocellular adenomas in male rats, and hepatocellular adenomas/carcinomas in female rats were evaluated for RL derivation. As noted above, PFOS is being evaluated as a carcinogen because of the positive animal carcinogenicity bioassay data from Butenhoff et al. (2012), and because of the similarities in chemical structure and biologic activity between PFOS and PFOA. Calculation of the PFOS RL for cancer uses the same methods as used above for PFOA. Using the HEDs as the dose metric, BMD modeling produces a $BMDL_{05}$ of 0.0020 mg/kg-day for male rats and a $BMDL_{05}$ of 0.0027 mg/kg-day for female rats.

Applying the $BW^{1/8}$ adjustment for pharmacodynamics differences between animals, where the time-weighted average male body weight is 0.690 kg (from Thomford 2002), the time-weighted average female body weight is 0.414 kg (from Thomford 2002), and the body weight of humans is the default of 70 kg, the human $BMDL_{05}$ is 0.0011 mg/kg-day for males and 0.0014 mg/kg-day for females. These BMDLs result in human CSFs of 45.5 $(mg/kg\text{-}day)^{-1}$ for males and 35.7 $(mg/kg\text{-}day)^{-1}$ for females. The higher CSF from male animals is used to derive the RL for PFOS. As described in the noncancer RL derivation, oral ingestion is the primary route of exposure to PFOS in drinking water, and inhalation and dermal exposures are considered negligible.

When determining cancer risk, OEHHA typically applies ASFs to account for the increased susceptibility of infants and children to carcinogens (OEHHA, 2009). A weighting factor of 10 is applied for exposures that occur from the 3rd trimester to <2

years of age, and a factor of 3 is applied for exposures that occur from 2 through 15 years of age. These factors are typically applied unless chemical-specific data exist to better guide the risk assessment.

However, ASFs are not included when deriving the cancer RL for PFOA because the NTP (2018c) study provided evidence that early life exposure did not increase tumor incidences later in life (Table 16). Because it is anticipated that PFOS behaves in a similar manner as PFOA, OEHHA is excluding ASFs in the RL derivation for cancer.

Since oral ingestion is considered to be the only significant route of drinking water exposure to the compound, a lifetime average drinking rate of 0.053 L/kg-day (OEHHA, 2012) is used to calculate the RL for carcinogenic effects:

$$RL = 10^{-6} \div (45.5\ (mg/kg\text{-}day)^{-1} \times 0.053\ L/kg\text{-}day) = 4.2 \times 10^{-7}\ mg/L$$

RL = 0.4 ng/L or 0.4 ppt (rounded)

The cancer RL of 0.4 ppt should protect against the noncancer effects of PFOS since it is lower than the 7 ppt level for noncancer effects.

RECOMMENDED NOTIFICATION LEVELS

The cancer RLs for PFOA and PFOS should protect against both cancer and noncancer effects of these chemicals. However, these levels are below concentrations of PFOA and PFOS that can be reliably detected in drinking water, which limits the utility of setting the NLs at these levels.

OEHHA recommends that SWRCB establish the NLs at the lowest levels that can be reliably detected in drinking water using currently available and appropriate technologies.

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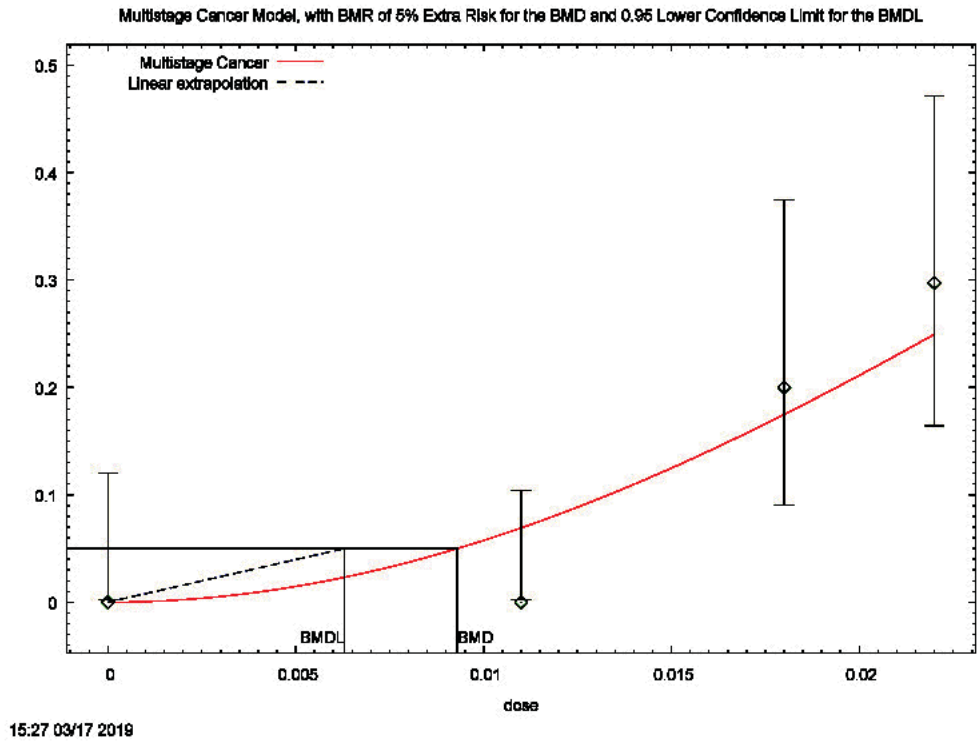
APPENDIX I. BENCHMARK DOSE MODELING RESULTS

Figure A1. Linear multistage cancer model output for liver adenoma/carcinoma in male rats exposed to PFOA (NTP, 2018c)



==========

MS_COMBO. (Version: 1.9; Date: 05/20/2014)
Input Data File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.(d)
Gnuplot Plotting File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.plt
Fri Jan 11 15:24:06 2019

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BMDS_Model_Run

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The form of the probability function is:
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = NTP2018livercancermaleeff.dax
~~~~~~~~~~

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 791.355

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -Beta(1) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Beta(2)
Beta(2)	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	592.678	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.0307	4			
Fitted model	-43.3357	1	6.61006	3	0.08542
Reduced model	-55.0387	1	30.0161	3	<.0001

AIC: 88.6714

Log-likelihood Constant 36.287873953351991

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0	0	0.000	36.000	0
0.011	0.0692	2.907	0.000	42.000	-1.767
0.018	0.1747	6.115	7.000	35.000	0.394
0.022	0.2494	9.227	11.000	37.000	0.674

Chi^2 = 3.73 d.f. = 3 P-value = 0.2919

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00930296

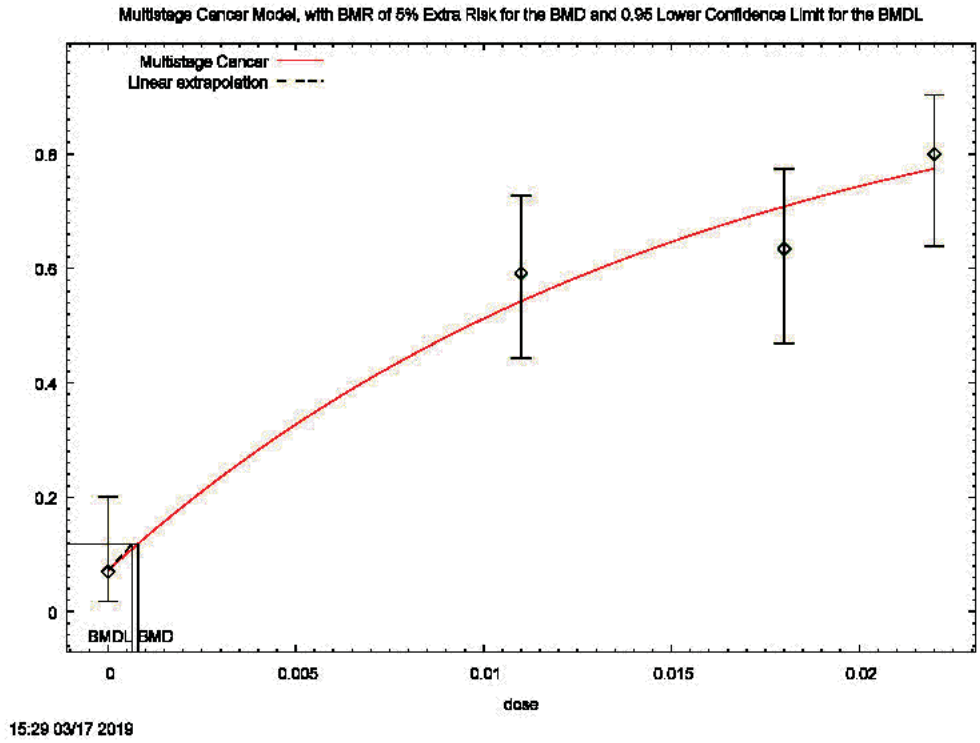
BMDL = 0.00629827

BMDU = 0.0114503

Taken together, (0.00629827, 0.0114503) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 7.93869

Figure A2. Linear multistage cancer model output for pancreatic acinar cell adenoma/carcinoma in male rats exposed to PFOA (NTP, 2018c)

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================================================================
MS_COMBO. (Version: 1.9;  Date: 05/20/2014)
Input Data File: K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.(d)
Gnuplot Plotting File:  K:\BMD saved files\Chemicals\PFOA\ntp2018 pfoa multi.plt
Fri Jan 11 15:24:06 2019
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BMDS_Model_Run
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose
Data file name = NTP2018panccancermaleeff.dax

Total number of observations = 4
```

Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0836357
Beta(1) = 63.4118

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.7
Beta(1)	-0.7	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.0721712	*	*	*
Beta(1)	64.3322	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-90.9548	4			
Fitted model	-91.7978	2	1.68581	2	0.4305
Reduced model	-119.773	1	57.6359	3	<.0001

AIC: 187.596

Log-likelihood Constant 83.425086842392645

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.0722	3.103	3.000	43.000	-0.061
0.011	0.5428	26.596	29.000	49.000	0.69
0.018	0.7086	29.051	26.000	41.000	-1.048
0.022	0.7747	30.987	32.000	40.000	0.383

Chi^2 = 1.73 d.f. = 2 P-value = 0.4221

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00079732

BMDL = 0.000651028

BMDU = 0.00100245

Taken together, (0.000651028, 0.00100245) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 76.8016

Multisite liver adenoma/carcinoma and pancreatic acinar cell adenoma/carcinoma in male rats (NTP, 2018c)

**** Start of combined BMD and BMDL Calculations.****

Combined Log-Likelihood -135.13347144919277

Combined Log-likelihood Constant 119.71296079574464

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

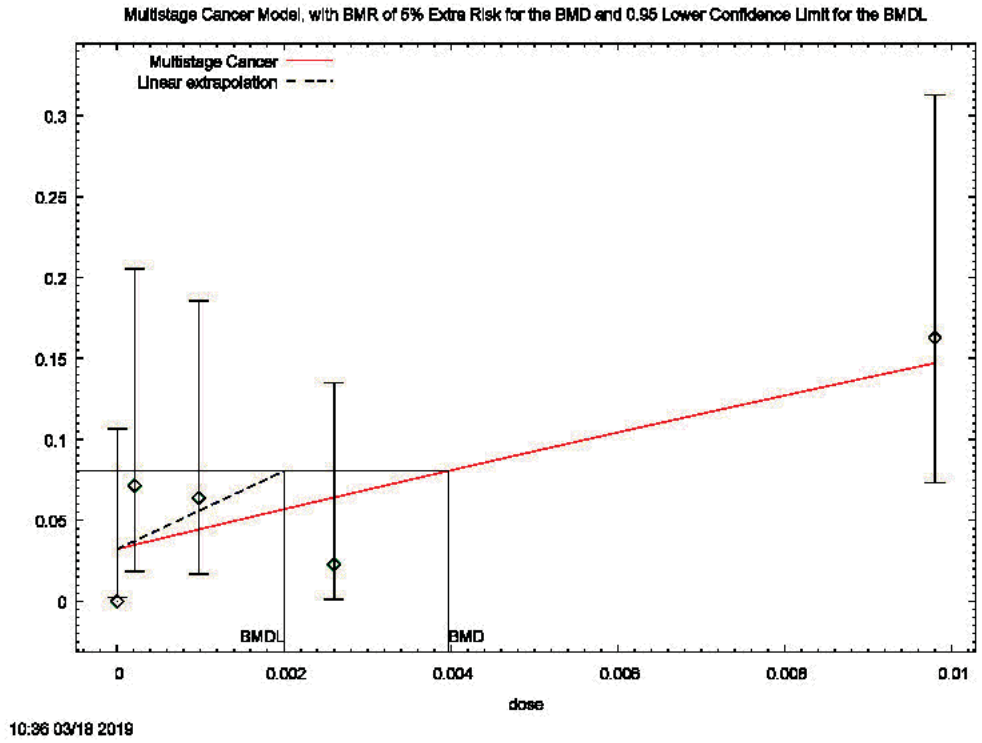
Confidence level = 0.95

BMD = 0.000791547

BMDL = 0.000647865

Multistage Cancer Slope Factor = 77.1766

Figure A3. Linear multistage cancer model output for hepatocellular adenomas in male rats exposed to PFOS (Butenhoff et al., 2012)

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=============================================================================
Multistage Model. (Version: 3.4;  Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 male hep
ad_Opt.(d)
Gnuplot Plotting File:  K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 male
hep ad_Opt.plt
Mon Mar 18 10:36:34 2019
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BMDS_Model_Run
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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

The parameter betas are restricted to be positive


Dependent variable = Effect
Independent variable = Dose
```

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0288938
Beta(1) = 14.2842

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.46
Beta(1)	-0.46	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.0321681	0.016529	-0.000228122	0.0645644
Beta(1)	12.9208	6.5633	0.0569607	25.7846

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-45.8404	5			
Fitted model	-48.8812	2	6.08161	3	0.1077
Reduced model	-51.9101	1	2.1394	4	0.01634

AIC: 101.762

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0	0.0322	1.32	0.000	41.000	-1.168
0.0002	0.0348	1.461	3.000	42.000	1.296
0.001	0.0443	2.084	3.000	47.000	0.649
0.0026	0.0641	2.822	1.000	44.000	-1.121
0.0098	0.1473	6.333	7.000	43.000	0.287

Chi^2 = 4.80 d.f. = 3 P-value = 0.1868

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00396983

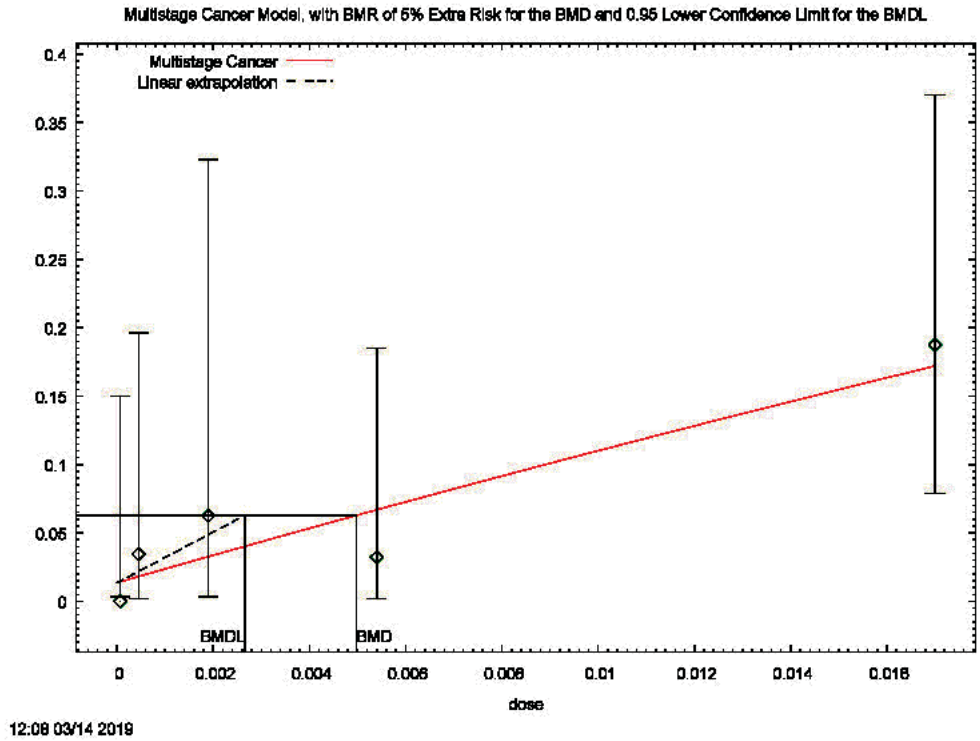
BMDL = 0.00200609

BMDU = 0.0138674

Taken together, (0.00200609, 0.0138674) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 24.9241

Figure A4. Linear multistage cancer model output for hepatocellular adenoma/carcinoma in female rats exposed to PFOS (Butenhoff et al., 2012)



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Multistage Model. (Version: 3.4; Date: 05/02/2014)
Input Data File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 female hep ad car_Opt.(d)
Gnuplot Plotting File: K:/BMD saved files/Chemicals/PFOS/msc_Butenhoff 2012 female hep ad car_Opt.plt
Thu Mar 14 12:08:12 2019

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BMDS_Model_Run

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The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(-beta1*dose^1)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 5
Total number of records with missing values = 0
Total number of parameters in model = 2
~~~~~~~~~~

Total number of specified parameters = 0
Degree of polynomial = 1

Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0.0140434
Beta(1) = 10.8528

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.45
Beta(1)	-0.45	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.0134513	0.0167796	-0.0194362	0.0463387
Beta(1)	10.3116	4.61895	1.25862	19.3646

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-27.951	5			
Fitted model	-29.095	2	2.28895	3	0.5146
Reduced model	-33.134	1	10.3672	4	0.03468

AIC: 62.1903

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0001	0.0141	0.396	0.000	28.000	-0.634
0.0004	0.018	0.523	1.000	29.000	0.667
0.0019	0.0326	0.521	1.000	16.000	0.674
0.0054	0.0669	2.073	1.000	31.000	-0.772
0.017	0.1721	5.507	6.000	32.000	0.231

Chi^2 = 1.95 d.f. = 3 P-value = 0.5831

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.00497433

BMDL = 0.00265944

BMDU = 0.0133689

Taken together, (0.00265944, 0.0133689) is a 90% two-sided confidence interval for the BMD

Cancer Slope Factor = 18.801

Attachment

1D

7

EPA

EPA-Final

Human Health Toxicity Values for

Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3)

Also Known as “GenX Chemicals”

Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3)
Also Known as "GenX Chemicals"

Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

EPA Document Number: 822R-21-010

October 2021

Disclaimer

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Acknowledgments

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This document was provided for review by staff in the following EPA Program Offices and Regions:

- Office of Water
- Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs
- Office of Chemical Safety and Pollution Prevention, Office of Pollution Prevention and Toxics
- Office of Chemical Safety and Pollution Prevention, Office of Science Coordination and Policy
- Office of Land and Emergency Management
- Office of Air and Radiation, Office of Transportation and Air Quality
- Office of Air and Radiation, Office of Air Quality Planning and Standards
- Office of Policy
- Office of Children’s Health Protection
- Office of Research and Development
- Regions 1–10

Contents

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Tables

Acronyms and Abbreviations

ºC	degree Celsius
3D	three dimensional
A/G	albumin-to-globulin
AAALAC	American Association for Accreditation of Laboratory Animal Care
ADME	absorption, distribution, metabolism, and excretion
AGD	anogenital distance
AIC	Akaike information criterion
ALD	approximate lethal dose
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AOP	adverse outcome pathway
AR	androgen receptor
AST	aspartate aminotransferase
atm-m^3/mol	atmosphere cubic meter per mole
ATP	adenosine triphosphate
BAF	bioaccumulation factor
BBDR	biologically based dose-response
BCF	bioconcentration factor
BCRP	breast cancer resistance protein
BMD	benchmark dose
BMD_{10}	dose level corresponding to the 95% lower confidence limit for a 10% response level
BMDL	benchmark dose lower limit
$BMDL_{10}$	lower bound on the BMD_{10}
BMDS	Benchmark Dose Software
BMR	benchmark response
BOD	biochemical oxygen demand
BUN	blood urea nitrogen
BW	body weight
BW_a	animal body weight
BW_h	human body weight
CASRN	Chemical Abstracts Service Registry Number
CFR	Code of Federal Regulations
cm/hr	centimeter per hour
CoA	coenzyme A
COV	coefficient of variation
Crl:CD(SD)	Sprague Dawley
DAF	dosimetric adjustment factor
DMEM/F-12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DWEL	drinking water equivalent level
DWTP	drinking water treatment plant
E	embryonic day
E1	heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether
E2	estradiol
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
F_0	parent generation
F_1	offspring of the F_0 generation
FABP	fatty acid-binding protein

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FRD-902	synonym for HFPO dimer acid ammonium salt
FRD-903	synonym for HFPO dimer acid
g	gram
g/L	gram per liter
g/mol	gram per mole
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
GD	gestation day
GLP	Good Laboratory Practices
GWG	gestational weight gain
H_3O+	hydronium ion
H&E	hematoxylin and eosin
HAWC	Health Assessment Workspace Collaborative
HDL	high-density lipoprotein
HED	human equivalent dose
HERO	Health & Environmental Research Online
HFPO	hexafluoropropylene oxide
HFPO-DA	HFPO dimer acid
HFPO dimer acid	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid
HFPO-TA	HFPO trimer acid
HFPO-TeA	hexafluoropropylene oxide tetramer acid
hL-FABP	human liver fatty acid-binding protein
HPLC	high-performance liquid chromatography
HPLC/MS/MS	high-performance liquid chromatography-tandem mass spectrometry
IC_{50}	concentration at which 50% inhibition is observed
ICR	Institute of Cancer Research
IgM	immunoglobulin M
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
K_{OC}	soil-water partition coefficient for organic compounds
K_{OW}	octanol-water partition coefficient
kPa	kilopascal
L/kg	liter per kilogram
LC_{50}	median lethal concentration
LD	lactation day
LD_{50}	median lethal dose
LDL	low-density lipoprotein
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
µg/g	microgram per gram
µg/L	microgram per liter
µg/mL	microgram per milliliter
µL	microliter
µM	micromolar
mg	milligram
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
mg/L	milligram per liter
mg/m^3	milligram per cubic meter

mg/mL	milligram per milliliter
mL	milliliter
mM	millimolar
mm Hg	millimeter of mercury
MOA	mode of action
MMAD	mass median aerodynamic diameter
mRNA	messenger ribonucleic acid
MRP2	multidrug resistance-associated protein 2
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N/A	not applicable
NAM	new approach methodology
NC DHHS	North Carolina Department of Health and Human Services
ND	not detected
ng/g	nanogram per gram
ng/mL	nanogram per milliliter
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NLM	National Library of Medicine
nM	nanomolar
nm	nanometer
NOAEL	no-observed-adverse-effect level
NQ	not quantified
NR	not rated
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
P-gp	P-glycoprotein
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCR	polymerase chain reaction
PECO	population, exposure, comparator, and outcome
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFO4DA	3,5,7,9-tetraoxadecanoic perfluoro acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PK	pharmacokinetic
Pk_a	acid dissociation constant
Pk_b	base dissociation constant
pM	picomolar
pmol	picomole
PMN	premanufacture notice
PMOH	ammonium perfluoro(2-methyl-3-oxahexanoate)
PMPP	3H-perfluoro-3-(3-methoxypropoxy) propanoic acid
PND	postnatal day
POD	point of departure
POD_{HED}	point of departure human equivalent dose
PPAR	peroxisome proliferator-activated receptor
PPARα	peroxisome proliferator-activated receptor alpha

PPAR-β/δ	peroxisome proliferator-activated receptor beta/delta
PPARγ	peroxisome proliferator-activated receptor gamma
ppm	parts per million
PWG	Pathology Working Group
RBC	red blood cell
RfD	Reference dose
RIVM	National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
RNA	ribonucleic acid
rT3	reverse triiodothyronine
SD	standard deviation
SDH	sorbitol dehydrogenase
TEM	transmission electron microscopy
TG	Test Guideline
TK	toxicokinetic
ToxRTool	Toxicological Data Reliability Assessment Tool
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
UF	uncertainty factor(s)
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
SE	standard error
SM	Standard Model
$T_{1/2}$	half-life
T3	triiodothyronine
T4	thyroxine
TDAR	T cell-dependent antibody response
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL extrapolation uncertainty factor
UF_S	extrapolation from subchronic to a chronic exposure duration uncertainty factor
UF_{TOT}	total uncertainty factor
VTG	vitellogenin
WOS	Web of Science

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final subchronic and chronic oral toxicity values (i.e., reference doses, or RfDs) for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6)—or hexafluoropropylene oxide (HFPO) dimer acid—and ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt. These chemicals are also known as "GenX chemicals" because they are the two major chemicals associated with GenX processing aid technology. The toxicity assessment for GenX chemicals is a scientific and technical report that provides an assessment of all available toxicity and carcinogenicity data and includes toxicity values associated with potential noncancer health effects following oral exposure (in this case, oral RfDs). This toxicity assessment evaluates human health hazards. It is not a risk assessment as it does not include an exposure assessment nor an overall risk characterization. Further, the toxicity assessment does not address the legal, political, social, economic, or technical considerations involved in risk management. The GenX chemicals toxicity assessment can be used by EPA, states, tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, if, and when, it is necessary to take action to address potential risk associated with human exposures to GenX chemicals.

These GenX chemicals are organic fluorinated ether chemicals that are part of a larger group of chemicals referred to as "per- and polyfluoroalkyl substances" or PFAS. In 2006, EPA initiated a stewardship program with the goal of eliminating chemical emissions of perfluorooctanoic acid (PFOA) and related chemicals by 2015. GenX chemicals are replacements for PFOA. Specifically, GenX is a trade name for a processing aid technology that enables the creation of fluoropolymers without the use of PFOA. Information on specific products containing these chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. Fluoropolymers are used in many applications, including the manufacture of nonstick coatings for cookware, water repellent garments, and other specialty agrochemical and pharmaceutical applications.

For HFPO dimer acid and its ammonium salt, acute, short-term, subchronic, chronic, and reproductive and developmental oral animal toxicity studies are available in rats and mice. Limited information identifying health effects in animals from inhalation of or dermal exposures to GenX chemicals is available. Repeated-dose toxicity data are available for oral exposure, but not for the other exposure routes (inhalation and dermal exposures). Thus, this assessment applies only to the oral route of exposure. These studies report liver toxicity (increased relative liver weight, hepatocellular hypertrophy, apoptosis, and single-cell/focal necrosis), kidney toxicity (increased relative kidney weight), immune effects (antibody suppression), hematological effects (decreased red blood cell count, hemoglobin, and hematocrit), reproductive/developmental effects (increased early deliveries, placental lesions, changes in maternal gestational weight gain, and delays in genital development in offspring), and cancer (liver and pancreatic tumors). Overall, the available toxicity studies demonstrate that the liver is particularly sensitive to HFPO dimer acid- and HFPO dimer acid ammonium salt-induced toxicity. Consistent with the *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a), EPA

concluded that there is *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans, based on the female hepatocellular adenomas and hepatocellular carcinomas and male combined pancreatic acinar adenomas and carcinomas observed in the chronic 2-year study in rats.

EPA followed the general guidelines for risk assessment set forth by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a) in determining the point of departure (POD) for the derivation of the RfDs for these chemicals. Consistent with the recommendations presented in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002), EPA applied uncertainty factors (UFs) to address intraspecies variability, interspecies variability, and extrapolation from a subchronic to a chronic exposure duration.

The critical study chosen for determining the subchronic and chronic RfDs for HFPO dimer acid and/or its ammonium salt was the oral reproductive/developmental toxicity study in mice with a no-observed-adverse-effect level (NOAEL) of 0.1 milligram per kilogram per day (mg/kg/day) based on liver effects (a constellation of lesions, including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) in females (DuPont-18405-1037, 2010; NTP, 2019). EPA determined that the constellation of liver lesions observed in the rodent are relevant to human health and not a result of PPARα-induced cell proliferation unique to rodents. Using EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012), EPA conducted benchmark dose modeling to empirically model the dose-response relationship in the range of observed data. Additionally, EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans. Allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UFs). The resulting POD human equivalent dose is 0.01 mg/kg/day. UFs applied include a 10 for intraspecies variability, 3 for interspecies differences, and 10 for database deficiencies, including immune effects and additional developmental studies, to yield a subchronic RfD of 0.00003 mg/kg/day or 0.03 µg/kg/day. In addition to those above, a UF of 10 was also applied for extrapolation from a subchronic to a chronic duration in the derivation of the chronic RfD of 0.000003 mg/kg/day or 0.003 µg/kg/day.

1.0 Introduction and Background

1.1 History of Assessment of GenX Chemicals

In 2008, DuPont de Nemours, Inc. (hereinafter DuPont) submitted premanufacture notices (PMNs) to the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) (Title 15 of the United States Code § 2601 *et seq*.) for two chemicals—2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6)—or hexafluoropropylene oxide (HFPO) dimer acid—and ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt—which are part of the GenX processing aid technology they developed.

Note: In July 2015, DuPont announced it had separated its Performance Chemicals segment through the creation of The Chemours Company. As a result, the GenX processing technology and associated chemicals are now products of The Chemours Company (Chemours, 2018). Because the submitted studies were conducted prior to the 2015 separation, however, the studies are referenced with DuPont identifiers.

Upon receipt, EPA assigned these PMNs case numbers P-08-0508 and P-08-0509, and they were reviewed by the New Chemicals Program in the Office of Pollution Prevention and Toxics (OPPT) and posted in the Federal Register (73 FR 46263, August 8, 2008) for public comment (EPA, 2008). A PMN assessment was completed and included a hazard assessment based on EPA review of test data submitted to the agency with the PMNs (including two 28-day oral (gavage) toxicity studies in mice (DuPont-24459, 2008) and rats (DuPont-24447, 2008)), as well as publicly available literature and TSCA confidential business information on other per- and polyfluoroalkyl substances (PFAS). Submitted test data on HFPO dimer acid and/or its ammonium salt were available for numerous endpoints such as acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity in mice and rats with dosing durations of up to 28 days.

EPA OPPT evaluated the methods and data submitted and deemed the studies acceptable to the agency. The studies submitted in 2008 with the PMNs formed the primary basis of EPA's hazard assessment at that time. The 28-day toxicity study in mice, from which EPA OPPT derived the point of departure (POD) of 0.1 milligrams per kilogram per day (mg/kg/day), was conducted according to Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) 407 (OECD, 2008a) and followed Good Laboratory Practices (GLP) (DuPont-24459, 2008; OECD, 2008a). The submitted studies were also used, in concert with information on other PFAS chemicals, to inform the decision to require further testing, as described in the Consent Order that concluded the PMN review (EPA, 2009).

The Consent Order included, among other things, additional testing pertaining to human health. The tests were identified in the Consent Order according to OECD TG numbers and/or EPA health effects TGs for pesticides and toxic substances numbers. Following are the studies included in the Consent Order relevant to human health and this assessment:

- Repeated dose metabolism and pharmacokinetics studies (OPPTS 870.7485) in mice and rats (Dupont-18405-1017, 2011)

- Modified Oral (Gavage) Reproduction/Developmental Toxicity Study in Mice (OECD TG 421) (Dupont-18405-1037, 2010; OECD, 2016a)
- 90 Day Oral (Gavage) Toxicity Study (OECD, 1998) (species not specified): Both mice (DuPont-18405-1307, 2010) and rats (Dupont-17751-1026, 2009) were submitted
- Combined Chronic Toxicity/Oncogenicity Study in Rats (OECD, 2009) (Dupont-18405-1238, 2013)

The OECD TGs are accepted internationally as standard methods for safety testing and:

> …are covered by the Mutual Acceptance of Data, implying that data generated in the testing of chemicals in an OECD member country, or a partner country having adhered to the Decision, in accordance with OECD Test Guidelines and Principles of GLP, be accepted in other OECD countries and partner countries having adhered to the Decision, for the purposes of assessment and other uses relating to the protection of human health and the environment (OECD, 2018a).

Specifically, for the required oral reproductive/developmental toxicity test, EPA OPPT included requirements for specific modifications to the test to increase the robustness of the study for this class of chemicals (DuPont-18405-1037, 2010; OECD, 2016a). These modifications are stated in the Consent Order (EPA, 2009) and were followed by the testing laboratory as outlined in the study report (DuPont-18405-1037, 2010). For the required combined chronic toxicity/oncogenicity study, EPA reviewed and concurred with protocols submitted to the agency prior to the study being conducted (DuPont-18405-1238, 2013). In addition, the submitter consulted with EPA on study findings to determine the need for additional data (e.g., further toxicokinetic testing based on results of the first tier OPPTS 870.7485 study). Finally, while not specifically required under the Consent Order, DuPont conducted and submitted results for additional OECD TG studies for Agency review (e.g., the prenatal and developmental toxicity study in rats (OECD, 2001b) (DuPont-18405-841, 2010).

1.2 Uses of GenX Chemicals under TSCA

GenX is a trade name for a processing aid technology developed by DuPont to make high-performance fluoropolymers without the use of perfluorooctanoic acid (PFOA) (Chemours, 2018). Transition to GenX processing aid technology began in 2009 as part of the company's commitment under the 2010/2015 PFOA Stewardship Program to work toward the elimination of these chemicals from emissions and products by 2015. Although production of most long-chain PFAS has been phased out in the United States and has been generally replaced by production of shorter chain PFAS, EPA is aware of ongoing use of long-chain PFAS by companies that did not participate in the PFOA Stewardship Program and ongoing use of the chemicals available in existing stocks or being newly introduced via imports.

Fluoropolymers are used in many applications because of their unique physical properties such as resistance to high and low temperatures, resistance to chemical and environmental degradation, and nonstick characteristics. Fluoropolymers also have dielectric and fire-resistant properties that have a wide range of electrical and electronic applications, including architecture, fabrics, automotive uses, cabling materials, food processing, electronics, pharmaceutical and biotech manufacturing, and semiconductor manufacturing (Gardiner, 2014).

One of the two PMNs EPA received in 2008, P-08-0508, was for HFPO dimer acid, a chemical used as an intermediate to make the polymerization aid HFPO dimer acid ammonium salt. The PMN for HFPO dimer acid ammonium salt was received by EPA under PMN P-08-0509 and is used as a replacement for PFOA in the manufacture of fluoropolymers. The GenX resin manufacturing process includes the thermal transformation of the HFPO dimer acid ammonium salt processing aid into a hydrophobic hydride. HFPO is used in the manufacture of HFPO dimer acid, HFPO dimer acid ammonium salt, other HFPO dimer acid derivatives, fluoropolymers (including polyethers), and other specialty agrochemical and pharmaceutical applications. Information on specific products containing GenX chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. GenX chemicals may also be generated as a byproduct of fluoromonomer production. When in water, both HFPO dimer acid and HFPO dimer acid ammonium salt dissociate to form the HFPO dimer acid anion ($HFPO^-$) as a common analyte. HFPO is manufactured from hexafluoropropene. HFPO dimer acid can react with additional HFPO to form the HFPO trimer acid and longer polymer fluorides. Other PFAS chemicals might be part of the GenX processing aid technology, but HFPO dimer acid and its ammonium salt are the major chemicals associated with this technology.

1.3 Occurrence

GenX chemicals were identified in North Carolina's Cape Fear River and its tributaries in the summer of 2012 (Strynar et al., 2015). Following this discovery, between June and December 2013, Sun et al. (2016) sampled source water at three drinking water treatment plants (DWTPs) (identified as DWTPs A, B, and C) treating surface water from the Cape Fear River watershed. The mean concentration of HFPO dimer acid in the finished drinking water treated by DWTP C was 0.631 microgram per liter (µg/L) (Sun et al., 2016). In a separate experiment to look at removal efficiency of DWTP C, water samples were taken during August 2014 from the raw water intake and after each treatment process step used by DWTP C (i.e., coagulation/ flocculation/sedimentation, raw and settled water ozonation, biological activated carbon filtration, and disinfection by medium-pressure ultraviolet lamps and free chlorine). GenX chemicals were found at concentrations of 0.4–0.5 µg/L at all steps of the treatment process, indicating that the concentrations of HFPO dimer acid were only slightly decreased by the conventional and advanced water treatment processes used at this DWTP.

The publication of these data prompted the North Carolina Department of Environmental Quality to sample sites for GenX chemicals along the Cape Fear River and in private wells close to the Chemours facility. In certain samples of surface water, groundwater, and finished drinking water, GenX chemicals were detected above 0.140 µg/L, which is North Carolina's drinking water health goal for GenX chemicals (NCDEQ, 2018c). Chemours has indicated that GenX chemicals have been discharged into the Cape Fear River for several decades as a byproduct of other manufacturing processes (NCDEQ, 2017). Petre et al. (2021) quantified the mass transfer of PFAS from contaminated groundwater to five tributaries of the Cape Fear River, including GenX chemicals. HFPO dimer acid and another fluoroether accounted for 61% of the total quantified PFAS. The study authors calculated that 32 kg/year of PFAS discharges from the groundwater to the five tributaries and the movement of these fluoroethers from the groundwater through the subsurface and into the streams occurred in less than the past 50 years. These data indicate that

the discharge of contaminated groundwater has led to long-term contamination of surface water and could lead to subsequent impacts on downstream drinking water (Petre et al, 2021).

Community concern over the detection of GenX chemicals in the Cape Fear Watershed led to the initiation of the GenX exposure study in Wilmington, North Carolina[1]. Blood samples from 344 Wilmington residents were collected between November 2017 and May 2018 and repeated blood samples from 44 of the participants were collected 6 months after the first sample collection. The blood sampling coincided with source control of GenX chemicals, and it is unknown whether study participants were drinking tap water at the time of collection. GenX chemicals were not detected above the analytical reporting limit of 2 ng/mL in any of the blood samples collected (Kotlarz et al., 2020).

GenX chemicals and other PFAS were also analyzed in 2682 urine samples from 2013–2014 National Health and Nutrition Examination Survey (NHANES) participants ≥ 6 years of age (Calafat et al., 2019). GenX chemicals were one of the few tested PFAS to be detected in the urine and was detected in approximately 1.2% of the population. The limit of detection was 0.1 µg/L. Importantly, this study demonstrated that the urine does not appear to be a good biomarker for PFAS. For example, PFOA and PFOS were detected in serum samples for > 98% of this study population, yet PFOA and PFOS were only detected in paired urine samples for < 0.1% of the same population.

In a report submitted by The Chemours Company to EPA, 24 human plasma samples were analyzed for HFPO dimer acid and were found at concentrations ranging from 1.0 ng/mL – 51.2 ng/mL. In seven of the samples, HFPO dimer acid was not detected above the analytical reporting limit of less than 1.0 ng/mL. No additional information about the study participants was provided in the report (DuPont- C30031_516655, 2017). GenX chemicals have been identified in other media, including rainwater and air emissions. North Carolina Department of Environmental Quality estimates for the Chemours Fayetteville Works plant (in the North Carolina Cape Fear watershed) indicate that Chemours' annual emissions of GenX chemicals could have exceeded 2,700 pounds per year during the reporting period (2017–2018) (NCDEQ, 2018a). Additional details on air emissions of GenX chemicals at the Fayetteville Works plant can be found at https://files.nc.gov/ncdeq/GenX/2018_April6_Letter_to_Chemours_DAQ_FINAL_signed.pdf. Rainwater samples were collected between February 28 and March 2, 2018 up to 7 miles from the North Carolina plant (NCDEQ, 2018b). The highest concentration of GenX chemicals in a rainwater sample (0.810 µg/L) was detected 5 miles from the Fayetteville Works facility center. The three samples collected 7 miles from the plant ranged from 0.045 to 0.060 µg/L (NCDEQ, 2018b). GenX chemicals also have been detected in three on-site production wells and one on-site drinking water well at the Chemours Washington Works facility in Parkersburg, WV. EPA subsequently requested that Chemours test for GenX chemicals in both raw and finished water at four public drinking water systems and 10 private drinking water wells. Chemours agreed to the testing and completed sampling during February 2018. The results from these samples are available at https://www.epa.gov/sites/production/files/2018-04/documents/hfpo_chemours_wash_works_sampling_2018.pdf and range before treatment from less than 0.010–0.081 µg/L in the public drinking water systems and less than 0.010–0.052

[1] https://genxstudy.ncsu.edu/

µg/L in the private drinking water wells (EPA, 2018a). All samples were below the limit of detection (0.010 µg/L) after treatment (EPA, 2018a).

Additionally, between the summer of 2016 and March 2018, GenX chemicals were identified in surface water and some soil samples collected upstream and downwind of a fluoropolymer production facility in Parkersburg, WV (Galloway et al., 2020). The highest concentrations of HFPO dimer acid in surface water samples (37–227 ng/L) were found in the direction of prevailing winds, directly across the Ohio River to the north and upstream to the northeast of the plant on the East Fork of the Little Hocking River. HFPO dimer acid was found in surface water samples up to 24 kilometers north of the facility, close to Beverly, OH. HFPO dimer acid was also detected in soil samples from Drag Strip Road, Veto Lake, and the Little Hocking Water Association at concentrations ranging from 3.09 nanograms per gram (ng/g) to 8.14 ng/g. These data reveal the downwind atmospheric transport of HFPO dimer acid.

Low concentrations of HFPO dimer acid (0.003–0.004 µg/L) were detected in the Delaware River, as reported in the recent publication by Pan et al. (2018).

The Kentucky Department of Environmental Protection (2019) reported detecting HFPO dimer acid in 11 samples from DWTPs at concentrations ranging from more than 1.32 ng/L to 29.7 ng/L. The study analyzed DWTPs using both surface water and ground water as sources and found the most frequent and highest detections of HPFO dimer acid at plants that use the Ohio River and ground water from the Ohio River alluvial aquifer as sources. For HFPO dimer acid, 10 detections were from surface water DWTPs and one detection was from a ground water DWTP. The ground water DWTP reported the highest concentration of HFPO dimer acid of all detections.

Globally, GenX chemical occurrence has been reported in Germany (Heydebreck et al., 2015; Pan et al., 2018), China (Heydebreck et al., 2015; Pan et al., 2017, 2018; Song et al., 2018), the Netherlands (Heydebreck et al., 2015; Gebbink et al., 2017; Pan et al., 2018), the United Kingdom (Pan et al., 2018), South Korea (Pan et al., 2018), and Sweden (Pan et al., 2018). HFPO dimer acid was also detected with a mean concentration of 30 pg/L in Artic surface water samples, suggesting long range transport (Joerss et al., 2020).

1.4 Other Assessments of GenX Chemicals

1.4.1 North Carolina Assessment

The North Carolina Department of Health and Human Services (NC DHHS) released a health assessment and provisional drinking water health goal for GenX chemicals in July 2017, which was finalized in October 2018 (NCDEQ, 2018c). North Carolina defines “health goal” as a nonregulatory, non-enforceable level of contamination below which no adverse health effects would be expected over a lifetime of exposure. The provisional health goal for exposure to GenX chemicals in drinking water is 0.140 µg/L, which is intended to protect the most sensitive population, namely bottle-fed infants. The state selected bottle-fed infants as the most sensitive population because they drink the largest volume of water per body weight (BW).

North Carolina’s provisional health goal is based on a reference dose (RfD) derived from a NOAEL of 0.1 mg/kg/day for liver effects (single-cell necrosis) in mice (DuPont-24459, 2008; DuPont-18405-1037, 2010). The total UF applied was 1,000, including individual factors to

account for interspecies variability (10), intraspecies variability (10), and extrapolation from a subchronic to a chronic exposure duration (10). This RfD of 0.0001 mg/kg/day was used to derive a drinking water equivalent level (DWEL), which considers exposure. The DWEL was calculated using BW and drinking water intake for bottle-fed infants and a relative source contribution of 20% to account for potential exposure to GenX chemicals from other media and routes, including air, soil, dust, and food (NCDEQ, 2018c). Additional details are available at NC DHHS.

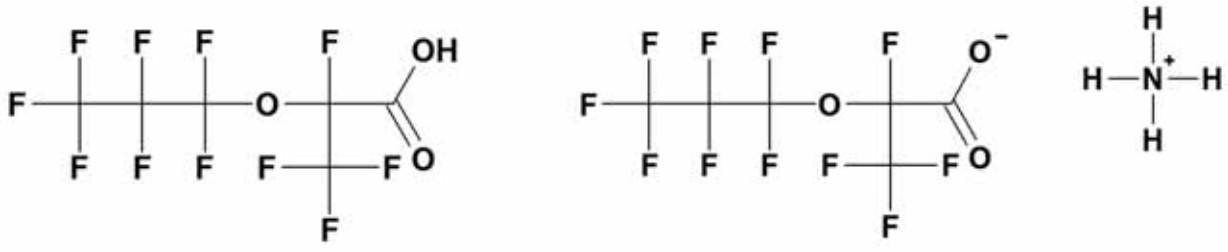
1.4.2 Report by the Netherlands National Institute for Public Health and the Environment

The National Institute for Public Health and the Environment (RIVM) in the Netherlands evaluated the data for GenX chemicals to set a safe limit for air. RIVM's assessment focused on the precursor 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903) (a synonym for HFPO dimer acid), the processing agent ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902) (a synonym for HFPO dimer acid ammonium salt), and the transformation product heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether (E1). Overall, RIVM concluded that there is no health risk expected for people living near plants from emissions of FRD-902 and FRD-903 at a limit of 73 nanograms per cubic meter (insufficient data are available to determine the toxicity of E1) (Beekman et al., 2016). RIVM used the oral carcinogenicity study in rats as the critical study (DuPont-18405-1238, 2013) and concluded that the study NOAEL was 0.1 mg/kg/day, based on increased albumin and the albumin-to-globulin (A/G) ratio observed at 12 months in males dosed with 1 mg/kg/day, an effect that indicates the potential for immunotoxicity. Using route-to-route extrapolation, RIVM converted this NOAEL to an air concentration to be used as the POD. UFs to account for intraspecies differences (10) and interspecies differences (1.8), and an additional factor to account for uncertainty in the human elimination of GenX chemicals (66) were applied to the POD to determine the chronic inhalation limit.

2.0 Nature of the Stressor

2.1 Chemical/Physical Properties

HFPO dimer acid and its ammonium salt are fluorinated organic compounds (Figure 1).



HFPO dimer acid
CASRN 13252-13-6

HFPO dimer acid ammonium salt
CASRN 62037-80-3

Figure 1. Structure of HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt

HFPO dimer acid is a liquid whereas its ammonium salt is a solid at room temperature. Both are highly soluble in water. Except in very acidic solvents (pH less than 3), the acid will dissolve and be present as the acid anion with a -1 charge. The associated cation ion will be a hydronium ion (H_3O^+) in water if other hydrogen ion acceptors are absent. Both compounds can volatilize from water to air, where they will dissolve in aerosolized water droplets or bind to suspended particulate matter. In soils, they will migrate with the aqueous phase or bind to the soil particle surfaces with areas of positive charge. The organic portions of HFPO dimer acid and its ammonium salt are stable to environmental degradation. Table 1 presents the chemical and physical properties of HFPO dimer acid and its ammonium salt.

Table 1. Chemical and Physical Properties of HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
CASRN	13252-13-6	62037-80-3	Chemical Abstracts Service.
CAS Index Name	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-ammonium salt (1:1)	Chemical Abstracts Service.
IUPAC Name	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	azanium;2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate	PubChem.
Synonyms	GenX Acid FRD 903 H-28307 C3 dimer acid 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid	GenX salt308 FRD 902 FDR 90208 H-21216 H-27529 H-28072 H-28397 H-28308 H-28548 HFPO dimer ammonium salt C3 dimer salt Ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate Ammonium perfluoro(2-methyl-3-oxahexanoate) PMOH	DuPont-24637, 2008; DuPont- 24698, 2008.
Chemical Formula	$C_6HF_{11}O_3$	$C_6H_4F_{11}NO_3$	
Molecular Weight	330.06 g/mol	347.08 g/mol	

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
Color/Physical State	Clear, colorless liquid (20 ˚C, 101.3 kPa)	Solid	DuPont-24637, 2008; DuPont-24698, 2008.
Boiling Point	129 ˚C	108 ˚C (as 86% salt solution in water) No measurement available for salt form	DuPont-24637, 2008; DuPont-24698, 2008.
Melting Point	< –40.0 ˚C	-21.0 ˚C (as 86% salt solution in water) No measurement available for salt form	DuPont-24637, 2008; DuPont-24698, 2008.
Vapor Pressure	306 Pa (2.7 mm Hg) (20 ˚C)	No measurement available	DuPont-24128, 2008; DuPont-24129, 2008.
Henry's Law Constant	< 2.5 x 10^{-4} atm-m^3/mol	No measurement available	Calculated from measured vapor pressure and highest measured solubility. Water solubility is reported to be "infinite" (DuPont-24128, 2008; DuPont-24129, 2008), so the actual K_h is expected to be much lower. These values should not be used to estimate partitioning between water and air.
Pk_a	2.84 (20 ˚C)	3.82	DuPont-26349, 2008.
Pk_b	8.1	8.1	DuPont-24198, 2008 (HFPO dimer acid ammonium salt).
K_{oc}		Soil–12 L/Kg (log 1.10) Sludge–12.6 L/Kg (log = 1.08)	DuPont-17568-1675, 2008.
K_{ow}	Not applicable[a]	Not applicable[a]	
Solubility in Water @ 20 ˚C	>751 g/L	>739 g/L	Highest tested values. Actual solubility not determined but described as "infinite" (DuPont-24128, 2008; DuPont-24129, 2008).

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
Half-life ($T_{1/2}$) in Water (25 ˚C)	Stable	Stable	Measured hydrolysis values for salt. No degradation in 5 days at 50 ˚C and pH 4, 7, and 9 (DuPont-24199, 2008).
Half-life ($T_{1/2}$) in Air	Stable	Stable	Ultraviolet-visible and visible spectrophotometry spectra for acid showed little absorption above 240 nm (DuPont-26349, 2008). EPA concurs with DuPont's assessment that the salt is assumed to be similar. Measured OH· reaction rate for E1 reaction product indicates T½ > 37 years.
Biodegradation	Biodegradation was not observed in ready biodegradation and inherent biodegradation tests	Biodegradation was not observed in ready biodegradation and inherent biodegradation tests	DuPont-A080558, 2009; DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010.
Bioconcentration (Fish BCF)	< 30 (log < 1)	< 30 (log < 1)	Measured BCF[b] < 30 at 0.02 mg/L and < 3 at 0.2 mg/L in Medaka 28 days (DuPont-A080560, 2009).
Bioaccumulation (Field BAF)	< 10	< 10	Pan et al., 2017.[c]

Notes: ºC = degrees Celsius; atm-m^3/mol = atmosphere cubic meters per mole; BAF = bioaccumulation factor; BCF = bioconcentration factor; g/L = grams per liter; g/mol = grams per mole; International Union of Pure and Applied Chemistry (IUPAC); K_{OC} = soil-water partition coefficient for organic compounds; K_{OW} = octanol-water partition coefficient; kPa = kilopascals; L/kg = liters per kilogram; mg/L = milligrams per liter; mm Hg = millimeters of mercury; nm = nanometer; Pk_a = acid dissociation constant; Pk_b = base dissociation constant; $T_{1/2}$ = half-life.

[a] Surfactants are surface acting agents that lower the interfacial tension between two liquids. Their amphiphilic nature (i.e., they contain both a hydrophilic part and a hydrophobic part) causes them to accumulate at interfaces such as the water-air interface, the water-food interface, and glass walls, which hampers the determination of their aqueous concentration. These surfactant properties present difficulties in applying existing methods for the experimental determination of log K_{ow} and produce unreliable results.

[b] The concentration of the propionate ion was not quantified in the BCF study, so the values are limits based on the limit of quantification for the analytical technique employed in the study.

[c] Pan et al. (2017) quantified the propionate ion and found that the concentrations were low in the tissues expected to most likely accumulate perfluorinated compounds (e.g., muscle, blood, and so forth). The tissue values indicate a BAF less than 10. Lipid tissue concentrations are not the basis for this BAF as is common for "traditional" organic compounds.

2.2 Environmental Fate

HFPO dimer acid and its ammonium salt are stable to photolysis, hydrolysis, and biodegradation. The degradation data suggest that the substances will be persistent (i.e., have a half-life ($T_{1/2}$) longer than 6 months) in air, water, soil, and sediments. Based on measured physical-chemical and sorption data, they are expected to run off into surface water and to rapidly leach to ground water from soil and landfills. As seen with PFOA and chemicals with similar properties, HFPO dimer acid and its ammonium salt might undergo long-range atmospheric transport in the vapor phase and associated with particulates. They are not expected to be removed during conventional wastewater treatment or conventional drinking water treatment.

When released to the freshwater environment, HFPO dimer acid will dissociate to the HFPO carboxylate anion and H_3O^+. The ammonium salt will dissolve to the HFPO carboxylate anion and the ammonium cation ($NH4^+$). It is expected that other salts of the HFPO dimer acid (e.g., potassium and sodium salts) will behave similarly. Both have high solubilities in water and are expected to remain in water with low sorption to sediment or soil. Given the vapor pressure, the acid can partition to air as well as to water. The salt can also be transported in air, although the mechanism of vapor phase transport is not understood (DuPont CCAS, 2009). In the vapor phase, the acid and salt are expected to be stable to direct photolysis and will undergo hydroxyl radical catalyzed indirect photolysis very slowly.

2.2.1 Water

Measured data for HFPO dimer acid and/or ammonium salt show that they are highly soluble in water (Table 1). The measured base dissociation constants (pK_b) indicate that the chemicals will exist primarily as the propionate ion at most environmental pH levels.

The chemicals are stable to hydrolysis. A hydrolysis study on the ammonium salt found no degradation at pH 4, 7, and 9 at 50 degrees Celsius (ºC) in 5 days, indicating a hydrolysis $T_{1/2}$ of more than 1 year at 20 ºC (DuPont-24199, 2008). Calculated Henry's Law constants (Table 1) suggest that partitioning from water to air might occur. Experimental data on the transfer of the acid and salt from water to air indicate that partitioning from surface water to the vapor phase might occur and some transfer from surface water to air is expected (DuPont CCAS, 2009). Water-air transport of these chemicals, however, is not well understood. Their surfactant properties, equilibrium between chemical forms as a function of pH, and interaction with dissolved cations make it difficult to accurately predict how the chemicals will behave in the aquatic environment.

2.2.2 Air

The acid was described as having "a significant vapor pressure" (DuPont CCAS, 2009). As observed with PFOA and other perfluorochemicals, these chemicals could be transported in the vapor phase or could associate with particulate material and be transported with the solids when released or partitioned into air.

When released to air or volatilized from water, the chemicals are stable and short- and long-range transport has occurred (D'Ambro et al., 2021; Galloway et al., 2020). For example, D'Ambro et al. (2021) demonstrated that just 2.5% of the total GenX concentrations (defined as the HFPO dimer acid and HFPO dimer acid fluoride) emitted from a fluoropolymer manufacturing facility in North Carolina were deposited within 150 kilometers of the facility. Removal from air is expected to occur through scavenging by water droplets and attachment to particulates followed by precipitation and settling. No studies of long-range transport or air removal rates are available.

2.2.3 Sediments and Soils

Organic carbon normalized sorption coefficients were measured by high-performance liquid chromatography (HPLC) (following OECD, 2001a). The sorption of the HFPO dimer acid ammonium salt to soil and sludge were 12.0 liters per kilogram (L/kg) (log = 1.10) and 12.6 L/kg (log = 1.08), respectively (DuPont-17568-1675, 2008; OECD, 2001a). Their high water solubility and low sorption potential indicate that the chemicals will tend to remain largely in water with little partitioning to soil or sediment. If applied or deposited to soil, they will run off or leach to ground water and, as indicated by the vapor pressure, could volatilize to air.

2.2.4 Biodegradation

GenX chemicals are resistant to biodegradation; no degradation was observed in standardized internationally recognized test methods for biodegradability. The aerobic aquatic biodegradation $T_{1/2}$ is on the order of years based on no measured inherent biodegradation of the acid or ammonium salt in OECD 302C, modified Ministry of International Trade and Industry studies (DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010; OECD, 2008b).[2] The HFPO dimer acid ammonium salt showed no inhibition of activated sludge respiration (OECD TG 209) (OECD, 2010a) at up to 1,000 milligrams per liter (mg/L) (DuPont-25938 RV1, 2008).

2.2.5 Incineration

A preliminary study submitted to EPA by DuPont/Chemours indicates that thermal degradation occurs (DuPont-PMN Attachment 119, 2008) and the potential for significant removal during incineration exists. Thermal degradation was reported to be rapid for HFPO dimer acid and/or its ammonium salt. The acid $T_{1/2}$ was reported to be about 2,500 seconds (about 42 minutes) at 150 ºC and about 1,900 seconds (about 32 minutes) at 200 ºC. The salt $T_{1/2}$ was 500 seconds (8.3 minutes) at 150 ºC and 200 seconds (3.3 minutes) at 200 ºC (DuPont-PMN Attachment 119, 2008).

2.2.6 Bioaccumulation

Measured steady-state fish BCFs in medaka (*Oryzias latipes*) exposed to the acid at 0.2 mg/L and 0.02 mg/L in a flow-through system for 28 days were less than 3 and less than 30, respectively (DuPont-A080560, 2009). These BCF results were observed—BCFs of less than 3

[2] HFPO dimer acid aerobic aquatic biodegradation $T_{1/2}$ = 0% by biochemical oxygen demand (BOD) and 1.5% by high-performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS); HFPO dimer acid ammonium salt aerobic aquatic biodegradation $T_{1/2}$ = < 1% by BOD and 0% by HPLC/MS/MS in 28 days (DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010).

and less than 30 when exposures were 0.2 mg/L and 0.02 mg/L of the acid, respectively—under the same conditions in common carp (*Cyprinus carpio*) (Hoke et al., 2016). A field-derived BAF was determined from a water body impacted by industrial perfluoroether releases. The log BAFs for specific tissues in the carp were 0.86 for blood, 0.50 for liver, and 0.61 for muscle. The tissue values indicate a BAF of less than 10 (Pan et al., 2017).

In a 4-day trout hepatocyte bioaccumulation screening test (non-TG) with the HFPO dimer acid ammonium salt, no metabolism was observed, suggesting that *in vivo* metabolism does not significantly affect potential bioaccumulation (DuPont-23459, 2007).

2.3 Toxicokinetics

In rats and mice, HFPO dimer acid and its ammonium salt are both absorbed from the gastrointestinal tract at levels that are proportional to dose following acute oral exposures. Transfer from plasma/serum to the liver, but not adipose tissue, was demonstrated in the few studies that conducted tissue analysis. The potential for maternal transfer to the fetus (Conley et al., 2019; Blake et al., 2020) during development and to the neonate during lactation (DuPont-18405-1037, 2010) was noted. Urine is the primary pathway for excretion. Based on data from studies of acute, single-dose, gavage, and intravenous exposures, $T_{1/2}$s in the beta (elimination) phase are longer in male rats and mice than in females. The male rats' $T_{1/2}$s in the beta (elimination) phase are relatively comparable to those for the male and female monkeys, whereas the female rats' $T_{1/2}$s are shorter.

HFPO dimer acid is a strong acid (acid dissociation constant (pK_a) = 2.84) and will be predominantly ionized in aqueous solutions with pH values higher than 4 and in both plasma and serum (DuPont-26349, 2008). Once in solution, the cation that counterbalances the HFPO dimer anion will vary with the salt used or the mineral ion composition of the solvent, plasma, serum, intercellular, and intracellular fluids. Based on the physical and chemical properties of HFPO dimer acid and its ammonium salt, once these chemicals enter physiologic compartments with pH values higher than 4 (e.g., most ambient water, serum, or blood), they will either dissociate (acid) or dissolve (ammonium salt) to yield the carboxylate anion. Thus, what is being measured in the studies outlined in this section is the HFPO dimer acid anion concentration regardless of whether animals are dosed with HFPO dimer acid or its ammonium salt.

2.3.1 Absorption

Oral. Sprague Dawley (Crl:CD(SD)) rats (five of each sex (5/sex)) were administered (via gavage) a single oral dose of 30 milligrams per kilogram (mg/kg) HFPO dimer acid ammonium salt in aqueous solution (purity 84%) in a study conducted according to EPA TG OPPTS 870.7485. Two animals of each sex served as controls. Urine and feces were collected at 0–6 hours, 6–12 hours, 12–24 hours, and every 24 hours until 168 hours post-dose. The 0–12-hour urine collections accounted for a mean of 95% to 97% of the dose, supporting a conclusion that these GenX chemicals are rapidly absorbed from the GI tract by male and female rats (DuPont-18405-1017 RV1, 2011).

In a similar guideline study with Crl/CD-1(ICR) mice (5/sex) (OPPTS 870.7485), the animals were administered a single oral dose of 3 mg/kg HFPO dimer acid ammonium salt (purity 84%) by gavage in aqueous solution (DuPont-18647-1017 RV1, 2011). Two animals of each sex served as controls. Urine and feces were collected at 0–6 hours, 6–12 hours, 12–24 hours, and

every 24 hours until 168 hours post-dose. In the 0–12-hour urine collections, 31% (mean) of the substance was found for the males and 39% (mean) for the females. By 168 hours post-dosing, the total accumulated urine values accounted for 90% and 92% of the total dose for male and female mice, respectively, indicating that both rats and mice extensively absorb the HFPO dimer acid anion. This study additionally shows mice either incompletely absorb HFPO dimer acid anions or eliminate it in urine at a slower rate than was seen in the rats (DuPont-18647-1017 RV1, 2011).

A 28-day gavage study by Rushing et al. (2017) indicates a potentially more complex toxicokinetic profile for HFPO dimer acid when dosing occurs over multiple days. Groups of six male and six female C57BL/6 mice were given doses of 1, 10, or 100 mg/kg/day of HFPO dimer acid daily for 28 days. Serum concentrations were measured at intervals of 1, 5, 14, and 28 days, and urine concentrations were measured on days 1, 2, 3, 5, 10, and 14. At each time point, serum levels reflected the magnitude of the dose, but not the exposure duration. The peak serum concentration occurred at day 5 for all but the high-dose males, where it occurred at day 14. Serum measurements for the 1- and 10-mg/kg/day doses were lower on days 14 and 28 than on day 5. The differences in serum concentration between days 5, 14, and 28 are not explained by the study authors, but could possibly indicate changes in absorption, tissue storage, or elimination after repeated dosing. The males exposed to 10 and 100 mg/kg/day had higher serum and urine concentrations than the females, as described in section 2.3.5 (Excretion). Based on the higher serum and urine concentrations, there appeared to be greater absorption in males than in females.

In a repeated-dose study following OECD TG 408 (OECD, 1998) guidelines, HFPO dimer acid ammonium salt (purity 84%) was administered to Crl:CD1(ICR) mice for 95 (males) or 96 (females) consecutive days via gavage at doses of 0, 0.1, 0.5, and 5 mg/kg/day (DuPont-18405-1307, 2010). Ten animals per sex per group (animals/sex/group) were included for toxicity evaluation, and an additional 15/sex/group were included for quantitation of the test substance plasma concentration 2 hours after dosing on day 0 (the first day of dosing) (5/sex/dose), providing a measure of post-dosing absorption (Table 2). Overall, plasma concentrations increased with increasing dose, indicating that absorption was not saturated, and displayed broad standard deviations indicative of considerable inter-animal variability in the absorption. The doses evaluated differ from those used by Rushing et al. (2017), limiting comparisons of the postexposure serum and plasma data. The sex difference seen by Rushing et al. (2017) (i.e., where male uptake to serum for the 1 and 10 mg/kg/day doses at the end of day 1 was greater than female uptake) is not as apparent at 2 hours post-dosing in this dataset.

Table 2. Plasma Concentration in Crl:CD1(ICR) Mice at 2 Hours after the First Gavage Exposure to HFPO Dimer Acid Ammonium Salt

Dose mg/kg/day	Males		Females	
	µg/mL	SD	µg/mL	SD
0	Not detected[a]	N/A	Not detected	N/A
0.1	0.736	0.099	0.824	0.072

Dose mg/kg/day	Males		Females	
0.5	3.806	1.197	3.606	1.308
5	42.58	5.214	35.34	9.262

Source: Dupont-18405-1307, 2010.
Notes: N/A = not applicable; µg/mL = micrograms per milliliter; SD = standard deviation.
[a] Detection limit of the method was 0.005 µg/mL in plasma.

Inhalation. There are no studies investigating HFPO dimer acid or its ammonium salt's uptake following inhalation exposures of aerosols. In a study conducted by Dupont (17751-723, 2009), one group of 5 male and 5 female Crl:CD(SD) rats were exposed to 5,200 milligrams per cubic meter (mg/m^3) and two groups of male and female Crl:CD(SD) rats (3/sex/group) were exposed to aerosols containing 0, 13, and 100 mg/m^3 of HFPO dimer acid ammonium salt (84% purity) for a single 4 hour period. One male and one female rat exposed to air only were used as the control. The rats in the 0, 13, and 100 mg/m^3 groups had a 2-day recovery period. The rats in the 5,200 mg/m^3 group recovered for 14-days. There were no measurements of the chemical in serum or plasma, however, to support an estimate of absorption by way of the respiratory tract.

Dermal. Absorption of HFPO dimer acid ammonium salt through the skin was determined *in vitro* with rat and human skin specimens (DuPont-25292, 2008). HFPO dimer acid ammonium salt (86% purity) was diluted with water to a concentration of 124 milligrams per milliliter (mg/mL). Serial receptor fluid samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours and analyzed for cumulative HFPO anion concentration.

Steady-state penetration rates were 70.3 ± 5.3 and 6.2 ± 5.3 micrograms per square centimeter per hour for rat and human skin, respectively, which yielded dermal permeability coefficients of 5.71E-4 ± 4.3E-5 centimeters per hour (cm/hr) for rats and 5.02E-5 ± 4.3E-5 cm/hr for humans. These dermal kinetic parameters demonstrate dermal absorption occurs, but at a relatively slower rate than chemicals that are well absorbed dermally.

2.3.2 Distribution

Crl:CD(SD) rats (3/sex/dose) were administered a single oral dose of 10 or 30 mg/kg by gavage in aqueous solution of either HFPO dimer acid ammonium salt (purity 84.5%) or HFPO dimer acid (purity 98%) (DuPont-24281, 2008; DuPont-24286, 2008). Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours, as described in section 2.3.6 (Clearance and Half-life Data). Liver and fat samples were presumed to be collected for analysis after the 168-hour plasma sample collection. In male rats dosed with HFPO dimer acid ammonium salt, the mean concentration in plasma at 168 hours post-dose was 0.036 ± 0.011 micrograms per milliliter (µg/mL) (36 ± 11 nanograms per milliliter (ng/mL)) for the low dose (10 mg/kg) and 0.057 ± 0.036 µg/mL (57 ± 36 ng/mL) for the high dose (30 mg/kg). In male rats dosed with HFPO dimer acid, the mean concentration in plasma at 168 hours post-dose was 0.041 ± 0.01 µg/mL (41 ± 10 ng/mL) for the low dose (10 mg/kg) and 0.128 ± 0.023 µg/mL (128 ± 23 ng/mL) for the high dose (30 mg/kg). In female rats, plasma concentrations of HFPO dimer acid anion were not above the limit of quantification (LOQ) in any sample at 168 hours post-dosing. In males dosed with HFPO dimer acid ammonium salt, the mean concentration of HFPO dimer acid anion in the liver 168 hours post-dose was 73 ± 25 ng/g for the low dose (10 mg/kg)

and 38 ± 15 ng/g for the high dose (30 mg/kg). In males dosed with HFPO dimer acid, the mean concentration of HFPO dimer acid anion in the liver 168 hours post-dose was 24 ± 6 ng/g for the low dose (10 mg/kg) and 89 ± 4 ng/g for the high dose (30 mg/kg). The mean liver tissue-to-plasma concentration ratio was higher in males for the ammonium salt (2.19) than for the acid (0.64) at the low dose (10 mg/kg). At the high 30 mg/kg dose, the liver tissue-plasma concentration ratio values in male rats were similar: 0.78 for the ammonium salt and 0.71 for the acid. Females at both doses, however, had a lower accumulation of HFPO dimer acid and its ammonium salt in the liver than in the male did. Overall, 10 out of 12 female rats dosed with HFPO dimer acid or its ammonium salt had undetectable concentrations of HFPO dimer acid anion in the liver (LOQ = 20 ng/g). Two females dosed with HFPO dimer acid ammonium salt at the low dose (10 mg/kg) had liver HFPO dimer acid anion concentrations above the LOQ, containing 20.6 and 54.1 ng/g of HFPO dimer acid anion. Females dosed with HFPO dimer acid did not have liver anion concentrations above the LOQ (20 ng/g). HFPO dimer acid anion concentrations in the fat tissue samples were below the LOQ of 20 ng/g in all the rats given HFPO dimer acid or HFPO dimer acid ammonium salt (DuPont-24281, 2008; DuPont-24286, 2008).

Crl:CD1(ICR) mice (3/sex/dose) were administered a single oral dose of 10 or 30 mg/kg by gavage in aqueous solution of HFPO dimer acid ammonium salt (purity 86%) (DuPont-25300, 2008). Unlike the rat studies, HFPO dimer acid was not evaluated in the mice. Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours, as described in section 2.3.6 (Clearance and Half-Life Data). Liver and fat samples were presumed to be collected for analysis after the 168 hour plasma sample collection. In males, the mean concentration of HFPO dimer acid anion in the liver was 384 ± 472 ng/g for the low dose (10 mg/kg) and 457 ± 337 ng/g for the high dose (30 mg/kg). The mean concentration in fat tissue was 31.6 ng/g in males for the high dose (30 mg/kg) and less than the LOQ (20 ng/g) for the low dose (10 mg/kg) and for both doses in females.

In male mice, the mean concentration in plasma at 168 hours post-dose was 0.759 ± 0.946 μg/mL (759 ± 946 ng/mL) for the low dose (10 mg/kg) and 0.83 ± 0.618 μg/mL (830 ± 618 ng/mL) for the high dose (30 mg/kg). In females, only one of three mice in each dose group had a plasma concentration above the LOQ at 168 hours post-dose, which was 0.0232 μg/mL (23.2 ng/mL) for the high dose (30 mg/kg) and 29.2 ng/g for the low dose (10 mg/kg). Based on the plasma and liver concentrations reported in the study, a liver-to-plasma ratio was calculated for males, but not for females because the females did not have liver concentrations above the LOQ. At the low dose (10 mg/kg), the average male liver-to-plasma ratio was 0.52, and at the high dose (30 mg/kg), it was 0.58.

Because the perfluorinated portion of the HFPO dimer acid ether is similar to that of the perfluorinated alkane acids (e.g., PFOA), HFPO dimer acid and its ammonium salt are anticipated to be transported in serum either freely dissolved or bound to serum protein (Gomis et al., 2018). Additionally, studies have demonstrated that the major serum protein interaction site for some PFAS, including PFOA and perfluorohexanoic acid (PFHxA), is albumin (D'eon et al., 2010; Han et al., 2003). Considering these points and that albumin is the major transport protein in the blood, it is likely that GenX chemicals are also distributed via serum albumin (Peters, 1995). Indeed, Allendorf et al. (2019) demonstrate that bovine serum albumin binds

HFPO dimer acid, and that the albumin/water partition coefficient is in the same range as other PFAS (e.g., perfluorobutanoic acid (PFBA) and perfluorohexane sulfonic acid (PFHxS)).

A study by Sheng et al. (2018) reported that the HFPO dimer acid anion also binds to fatty acid-binding protein (FABP). FABPs are intracellular lipid carrier proteins for long-chain fatty acids, phospholipids, and a variety of chemicals that induce peroxisome proliferation (Erol et al., 2003). They constitute 2%–5% of the cytosolic protein in the liver. FABPs can be synthesized in the gastrointestinal tract and act as a systemic carrier of long-chain fatty acids in plasma and serum (Storch and McDermott, 2009). Thus, FABPs likely play a role in the systemic distribution of HFPO dimer acid in both its neutral and ionized forms.

2.3.3 Distribution during Gestation and Lactation

HFPO dimer acid ammonium salt can be transferred (distributed) from a pregnant animal to the fetus, as demonstrated in multiple studies. In an OECD TG 421 (OECD, 2016a) reproduction/developmental toxicity study (DuPont-18405-1037, 2010), pregnant Crl:CD1(ICR) mice (25/sex/group) were administered, by gavage, 0, 0.1, 0.5, or 5 mg/kg/day HFPO dimer acid ammonium salt from premating day 14 to lactation day (LD) 20/21. Blood was collected from the dams 2 hours after dosing on LD/postnatal day (PND) 21 (scheduled termination) and pooled. The litters were normalized on PND4 to 8 pups per litter (4/sex). Blood was collected and pooled from the pups not randomly selected on PND4. The HFPO dimer acid anion was present in the pooled plasma of PND4 pups at concentrations approximately two to four times lower than the concentrations in the dams on LD21. These results indicate that there is transfer of HFPO dimer acid anion from maternal serum. The PND/LD21 plasma levels in both male and female pups, however, were forty- to sixtyfold lower than the maternal LD21 plasma concentrations, indicating that the majority of fetal transfer occurred during gestation (DuPont-18405-1037, 2010).

Blake et al. (2020) demonstrated that HFPO dimer acid can be transferred from the pregnant dam to the embryo during gestation. Pregnant CD-1 mouse dams were dosed from embryonic day (E) 1.5 to E11.5 or E17.5 with either deionized water (vehicle control), 1 or 5 mg/kg/day of PFOA, or 2 or 10 mg/kg/day of HFPO dimer acid. At E11.5 and E17.5, serum and a portion of the hepatic left lateral lobe were collected from pregnant dams after the final dose. Amniotic fluid was collected by needle aspiration from litters euthanized on E11.5 and whole embryos were collected on E11.5 and E17.5 to determine the concentration of HFPO dimer acid. HFPO dimer acid was detected in both the amniotic fluid and the whole embryo at 2 and 10 mg/kg/day and at both time points, demonstrating transfer of HFPO dimer acid from the pregnant dam to the fetus during gestation (Table 3).

Table 3. Concentrations of HFPO Dimer Acid in CD1 Pregnant Mice and Their Embryos at Embryonic Day 11.5 or 17.5[a]

Measurement [b]	Embryonic day	HFPO dimer acid	
		2 mg/kg/day	10 mg/kg/day
Maternal Serum (µg/mL)	11.5	33.5 ± 15.7	118.1 ± 10.4
	17.5 [c]	22.9 ± 17.1	58.5 ± 34.5

Measurement [b]	Embryonic day	HFPO dimer acid	
		2 mg/kg/day	10 mg/kg/day
Amniotic Fluid (µg/mL)	11.5	3.6 ± 2.2	9.3 ± 2.0
	17.5	NQ	NQ
Maternal Liver (µg/g)	11.5	5.45 ± 3.43	19.9 ± 4.2
	17.5	4.56 ± 2.80	14.2 ± 7.6
Whole Embryo (µg/g)	11.5	0.91 ± 0.22	3.21 ± 0.51
	17.5	3.23 ± 1.28	7.69 ± 2.92

Source: Blake et al., 2020.
Notes: µg/mL = micrograms per milliliter; µg/g = micrograms per gram embryo weight; SD = standard deviation; NQ = not quantified due to limited volume.
[a] For each reported measurement in this table, N = 6–8 per group.
[b] Limit of detection was 0.010 µg/mL; note all vehicle control samples were below the limit of detection.
[c] HFPO dimer acid was detected in the serum of vehicle control mice in the E17.5 group (0.211 ± 0.55 µg/mL).

HFPO dimer acid concentrations increased with increasing dose in all samples. The concentration of HFPO dimer acid in the whole embryo increased from E11.5 to E17.5 in both dose groups, indicating bioaccumulation in the embryo over the gestational period. Conversely, the concentration of HFPO dimer acid in the maternal serum decreases from E11.5 to E17.5 in both dose groups. The authors note that the decrease in maternal serum HFPO dimer acid could be the result of increased transfer to embryos over time or to dilution effect from blood volume expansion over the course of gestation.

In the DuPont-18405-1037 (2010) study, generally, the standard deviations were large in all dose groups, especially as compared to PND21. The male pups tended to have slightly higher plasma concentrations of HFPO dimer acid anion than the female pups at PND40. For example, at the 0.1 mg/kg/day-dose group, the concentration of HFPO dimer acid anion was 1.352 and 0.946 µg/mL (1,352 and 946 ng/mL) in male and female pups, respectively. Similarly, at the 0.5 mg/kg/day-dose group, the concentration of HFPO dimer acid anion was 6.282 and 4.074 µg/mL (6,282 and 4,074 ng/mL) in male and female pups, respectively, and it was 51.34 µg/mL (51,340 ng/mL) in male pups and 43.34 µg/mL (43,340 ng/mL) in female pups at 5 mg/kg/day (DuPont-18405-1037, 2010).

Transfer of HFPO dimer acid anion to the fetus was also demonstrated in groups of five Crl:CD(SD) rats exposed to doses of 0, 5, 10, 100, or 1,000 mg/kg/day from gestation day (GD)6 to GD20 (Dupont-18405-849 RV1, 2011). On GD20, blood was collected from individual dams 2 hours after dosing and trunk blood was collected from the fetuses and pooled by litter for analysis. The plasma concentration in the blood samples from the dams was three times higher than the plasma concentration in the pooled blood of their fetuses. The detection of HFPO dimer acid anion in the pooled fetus plasma demonstrates gestational transfer from dam to fetus.

Similarly, Conley et al. (2019) demonstrated transfer of HFPO dimer acid anion to the fetus by measuring serum concentrations of pregnant Crl:CD(SD) rats exposed to 0, 1, 3, 10, 30, 62.5, 125, 250, and 500 mg/kg/day HFPO dimer acid ammonium salt from GD14 through GD18.

Serum was collected from the dams in all dose groups and plasma was collected from the fetuses in the 0, 1, 3, 10 and 30 mg/kg/day groups. On GD18, trunk blood was collected from individual dams 2 hours after dosing and blood was collected from the fetuses' jugular vein and pooled per litter for analysis. HFPO dimer acid anion was detected in the pooled fetal plasma at all doses and the concentration increased with increasing maternal dose (Table 4). The study authors noted that, while the increases in maternal serum and fetal plasma were linear in the lower dose range (0–30 mg/kg/day), the maternal slope was significantly greater than the fetal slope. The maternal serum concentration of HFPO dimer acid anion increased 0.46 µg/mL (460 ng/mL) per 1 mg/kg increase in maternal dose while the fetal plasma concentration increased 0.12 µg/mL (120 ng/mL) per 1 mg/kg increase in maternal dose. Additionally, the study authors modeled uptake over the full maternal dose range (1–500 mg/kg) (Table 4) using exponential one-phase association and determined that a plateau was reached at 112 ± 15 µg/mL (112,000 ± 15,000 ng/mL), indicative of uptake saturation (Conley et al., 2019).

Table 4. Maternal Serum and Fetal Plasma Concentrations on GD18 in Crl:CD(SD) Rats Exposed to HFPO Dimer Acid Ammonium Salt from GD14-18

Oral dose mg/kg/day	Pregnant dam serum		Fetal plasma	
	µg/mL	SE	µg/mL	SE
0	0.027	0.008	0.018	0.01
1	0.68	0.08	0.13	0.06
3	1.2	0.3	0.49	0.04
10	4.6	1.1	1.9	0.2
30	13.9	3.1	3.5	0.4
62.5	30.7	2.9	N/A	N/A
125	46.0	10.3	N/A	N/A
250	81.8	21.6	N/A	N/A
500	100.7	26.4	N/A	N/A

Source: Conley et al., 2019, Table S10.
Notes: µg/mL = micrograms per milliliter; N/A = not applicable because no sample collected at that dose; SE = standard error.

Conley et al. (2021) also demonstrated transfer of HFPO dimer acid anion to the fetus and pup by measuring serum concentrations of pregnant Crl:CD(SD) rats exposed to 0, 1, 3, 10, 30, 62.5, or 125 mg/kg/day HFPO dimer acid ammonium salt from GD16 through GD20 or to 0, 10, 30, 62.5, 125, or 250 mg/kg/day from GD8 through PND2. Serum was collected from the dams and fetuses in all dose groups on GD20 in the GD16-20 experiment and from the dams on PND2 in the neonatal experiment. In the GD16–20 experiment, trunk blood and liver samples were collected from both dams and fetuses 2 to 4 hours after the final oral dose on GD20. Fetal serum was pooled per litter for analysis. On PND2 in the neonatal experiment, trunk blood and liver samples were collected from the dams 2 to 5.5 hours after the final oral dose and liver samples were collected from the pups. Maternal serum and liver HFPO dimer acid anion concentrations

increased as a function of dose during both experiments (Table 5). The study authors noted that there was no statistically significant difference in serum or liver concentration within a given dose group between the two experiments indicating that bioaccumulation did not occur after longer exposure. HFPO dimer acid anion was detected in the pooled fetal serum at all doses and the concentration generally increased with increasing maternal dose. Regression analyses showed that fetal and maternal serum concentrations increased log-linearly as a function of maternal oral dose, and maternal serum concentrations were approximately 2- to 3-fold greater than fetal serum concentrations. Liver concentrations of HFPO dimer acid anion in dams, fetuses, and pups also increased log-linearly. The fetal and maternal liver concentrations on GD20 were nearly identical for the 30–125 mg/kg/day dose levels. On PND2, male pup liver concentrations were significantly greater than female pup liver concentrations, which was most prominent at the 125 mg/kg/day dose level. PND2 liver concentrations for both sexes were approximately 10-fold lower than concentrations observed in GD20 fetal livers.

Table 5. Maternal and Offspring HFPO Dimer Acid Anion Concentrations in Serum and Liver Samples Collected on GD20 or PND2 from Crl:CD(SD) Rats Orally Exposed to HFPO Dimer Acid Ammonium Salt from GD16-20 or GD8-PND2

Oral dose mg/kg/day	Maternal serum GD20 (µg/mL)	Fetal serum GD20 (µg/mL)	Maternal serum PND2 (µg/mL)	Maternal liver GD20 (µg/g)	Fetal liver GD20 (µg/g)	Maternal liver PND 2 (µg/g)	Female pup liver PND 2 (µg/g)	Male pup liver PND 2 (µg/g)
0	0.016 ± 0.014	0.014 ± 0.008	<LOQ	0.29 ± 0.12	0.07 ± 0.04	0.14 ± 0.05	0.07 ± 0.021	<LOQ
1	0.54 ± 0.10	0.33 ± 0.03	NA	2.11 ± 0.78	0.23 ± 0.09	NA	NA	NA
3	1.15 ± 0.28	1.56 ± 0.84	NA	3.18 ± 1.01	0.46 ± 0.05	NA	NA	NA
10	3.05 ± 0.90	3.14 ± 0.71	1.76 ± 0.60	3.70 ± 0.92	2.07 ± 0.18	2.90 ± 0.91	0.21 ± 0.05	0.22 ± 0.02
30	7.46 ± 2.59	2.74 ± 1.88	4.22 ± 0.83	8.36 ± 2.35	9.09 ± 0.96	4.42 ± 1.21	0.64 ± 0.14	1.10 ± 0.26
62.5	13.81 ± 3.76	7.63 ± 1.16	16.09 ± 5.88	21.65 ± 3.81	22.30 ± 4.96	22.93 ± 7.23	1.64 ± 0.11	2.37 ± 0.60
125	31.96 ± 6.67	11.68 ± 2.77	28.39 ± 9.63	42.82 ± 9.05	44.08 ± 10.54	43.99 ± 15.57	1.83 ± 0.83	4.96 ± 1.36
250	NA	NA	41.57 ± 12.91	NA	NA	45.88 ± 14.43	NA	6.48 (n = 1)

Source: Conley et al., 2021, Table S11.
Notes: Values reported as mean ± standard error of the mean (SEM); sample size n = 2–6 except where noted; LOQ = limit of quantitation (0.005 µg/mL for serum, 0.1 µg/g for liver); µg/mL = micrograms per milliliter; µg/g = micrograms per gram; N/A = not applicable because no sample collected at that dose.

In the studies of rats dosed during pregnancy in which plasma concentrations in both the dams and fetuses were measured at GD20 (Dupont-18405-849 RV1, 2011) or GD18 (Conley et al., 2019), the HFPO dimer acid anion plasma concentration ratio for dams to fetuses is approximately two to four. In the study of mice dosed during pregnancy (Dupont 18405-1037, 2010), plasma concentrations were measured in dams on LD21 and in pups on PND4, PND21, and PND40. If the plasma concentrations in dams on LD21 are assumed to be representative of those on LD4, the comparison to pup plasma concentrations on PND4 indicate a dam-to-pup plasma concentration ratio of two to four. Together these data indicate the efficiency of transfer in rats and mice is of a similar magnitude.

2.3.4 Metabolism

In two *in vitro* studies, hepatocytes (1 x 10^6 cells/mL for clearance incubations and 5 x 10^6 cells/mL for biotransformation incubations) prepared from male and female Crl:CD(SD) rats were incubated with 2 micromolar (μM) (clearance) or 200 μM (biotransformation) solutions of HFPO dimer acid ammonium salt for a total of 120 minutes (DuPont-23460, 2007). Samples were analyzed for HFPO dimer acid ammonium salt and potential metabolites at 5, 15, 30, 45, 60, 90, and 120 minutes. Heat-inactivated hepatocytes were used as negative controls and 4-nonylphenol in live hepatocytes were used as a positive control. There was no difference in the concentration of HFPO dimer acid between the viable and heat-inactivated hepatocytes, indicating that HFPO dimer acid ammonium salt is not metabolized by rat hepatocytes. Additionally, no metabolites were detected in the biotransformation incubation samples (DuPont-23460, 2007). Similar *in vitro* studies were conducted in rat hepatocytes in Gannon et al. (2016). Hepatocytes (1 x 10^6 cells/mL for clearance incubations and 5 x 10^6 cells/mL for biotransformation incubations) prepared from male and female Crl:CD(SD)rats were incubated with 5 μM (clearance) or 50 μM (biotransformation) solutions of HFPO dimer acid ammonium salt for a total of 120 minutes. Heat-inactivated hepatocytes were used as negative controls and samples were collected at 0, 30, 45, 60, 90, and 120 minutes. Gannon et al. (2016) concluded that the test substance was not metabolized by rat hepatocytes because there was no difference in clearance rate between live and heat-inactivated hepatocytes and no metabolites were identified.

In the single oral (gavage) study of rats described in section 2.3.1 (Absorption), the total accumulated amount of HFPO dimer acid ammonium salt at 168 hours post-dosing in the collected urine accounted for 103% $\pm$ 2.73% and 99.8% $\pm$ 6.41% of the administered dose for male and female rats, respectively, and there was no detection of metabolites (DuPont-18405-1017 RV1, 2011).

Similarly, in the single oral (gavage) study of mice described in section 2.3.1 (Absorption), the total accumulated amount of HFPO dimer acid ammonium salt accounted for 89.5% ± 6.91% and 91.5% ± 6.04% of the total dose for male and female mice, respectively, and there was no detection of metabolites in the urine (DuPont-18647-1017 RV1, 2011).

2.3.5 Excretion

Urine. Studies in rats, mice, and monkeys indicate that urine is the primary excretory pathway for GenX chemicals. In the DuPont-18405-1017 RV1 (2011) study, Crl:CD(SD)rats (5/sex) administered a single oral (gavage) dose of 30 mg/kg HFPO dimer acid ammonium salt excreted 95% to 97% of the dose in urine within 12 hours. The pooled urine collections accounted for virtually all the substance administered with no evidence of metabolic alteration. Study authors calculated the elimination $T_{1/2}$ in the urine for male and female rats to be 3 hours and 8 hours, respectively. In a companion study, Crl/CD1(ICR) mice (5/sex) were administered a single oral (gavage) dose of 3 mg/kg HFPO dimer acid ammonium salt (purity 84%) (DuPont-18647-1017 RV1, 2011). Urinary elimination in mice appeared to be less efficient than in the rats given that only 31% (mean) and 39% (mean) of the dose material was found in the 12-hour pooled urine for the male and female mice, respectively. At 168 hours post-dosing, the mean values for the pooled urine samples accounted for 90% and 92% of the total dose for the male and female mice, respectively (DuPont-18647-1017 RV1, 2011). Study authors calculated the elimination $T_{1/2}$ in the urine for male and female mice to be 21 hours and 18 hours, respectively. Based on the amounts in urine and the clearance from blood (see section 2.3.6), mice appear to have less of an

ability than rats to clear the HFPO dimer acid anion by transferring it to urine in the early postexposure period. The differences in the results of these studies might have been influenced by the different doses given to the rats (30 mg/kg) and the mice (3 mg/kg) (DuPont-18647-1017 RV1, 2011; DuPont-18405-1017 RV1, 2011).

The dynamic relationship across dose and exposure duration observed in serum measurements from the Rushing et al. (2017) study is also reflected in their data on urinary excretion. Urine concentrations were monitored on exposure days 1, 2, 3, 5, 10, and 14. For the 1- and 10-mg/kg/day doses, urinary concentration peaked on day 3 and, thereafter, declined monotonically. Males had higher urine concentrations than females at each time point, consistent with their higher serum concentrations. For the 100-mg/kg/day-dose group, the concentrations in urine peaked at day 2 and again at day 14 in males while in females they appeared to peak at 5 days followed by a decline at 10 and 14 days.

Feces. Fecal elimination of HFPO dimer acid appears to be minor in rats and mice in the available single-dose studies (DuPont-18405-1017 RV1, 2011; DuPont-18647-1017 RV1, 2011). Specifically, feces + cage wash (dried fecal matter) from male and female rats had 2% and 6% of recovered compound, respectively, while feces + cage wash from male and female mice had 12% and 8% of recovered compound, respectively. The data for combined fecal matter and cage wash suggest that mice might lose slightly more HFPO dimer acid through fecal matter than rats. Low fecal excretion could reflect low levels of hepatic loss via biliary excretion.

2.3.6 Clearance and Half-Life Data

Clearance time. In multiple study reports, the study authors did not calculate pharmacokinetic parameters such as $T_{½}$ or area under the curve and instead defined the metric “clearance time” as the time when 98.4% of the anion from the HFPO dimer acid ammonium salt was cleared from the plasma.

A total of 12 Crl:CD(SD) rats, 3/sex/dose, received a single oral dose of 10 or 30 mg/kg/day HFPO dimer acid ammonium salt (84.5% purity) by gavage (Dupont-24281, 2008). Plasma samples were collected from animals serially at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. In males, plasma levels peaked within the first 1–2 hours after dosing for the low dose, and within the first 30 minutes to 1 hour for the high dose. By days 4 to 5, plasma concentrations were less than 1% of the peak level, although still above the LOQ (0.02 µg/mL (20 ng/mL)). In females, the plasma levels peaked at 1 hour for the low dose and had usually declined to the LOQ (0.02 µg/mL (20 ng/mL)) by 24 hours. At the 30-mg/kg dose, the plasma levels of female rats peaked at 30 minutes to 1-hour post-dosing and declined to the LOQ (0.02 µg/mL (20 ng/mL)) by 24 or 48 hours. In male rats, the authors identified 12 hours as the clearance time at the low dose and 22 hours at the high dose (Table 6). In female rats, the clearance values were 4 hours and 8 hours for the low dose and high dose, respectively.

Table 6. Clearance Times in Plasma for Male and Female Rats and Mice Following a Single Oral Dose[a]

Chemical	Male rat	Male mouse	Female rat	Female mouse
10 mg/kg				
HFPO dimer acid ammonium salt	12 hr	143 hr	4 hr	57 hr
HFPO dimer acid	28 hr	ND	8 hr	no data
30 mg/kg				
HFPO dimer acid ammonium salt	22 hr	139 hr	8 hr	62 hr
HFPO dimer acid	22 hr	ND	4 hr	no data

Sources: Dupont-24281, 2008; Dupont-24286, 2008; Dupont-25300, 2008.
Notes: hr = hour
[a] "Clearance time" is defined as the time when 98.4% of the HFPO dimer acid ammonium salt was cleared from the plasma.

The same protocol was followed using HFPO dimer acid (98% purity) (Dupont-24286, 2008). At the low dose, plasma concentrations peaked within 1 hour in both male and female rats, while at the high dose, the peak plasma concentrations occurred in males at 1 or 2 hours and in females at 15 minutes. The clearance times in males were 28 hours and 22 hours for the low dose and high dose, respectively. The clearance times in females were 8 hours and 4 hours for the low dose and high dose, respectively (Table 6).

The protocol outlined in this section was also followed for mice with a total of 12 Crl:CD(ICR) mice, 3/sex/dose, receiving a single oral dose of 10 or 30 mg/kg/day HFPO dimer acid ammonium salt (86% purity) by gavage (Dupont-25300, 2008). Plasma samples were collected from animals serially at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dosing. Peak plasma HFPO dimer acid anion concentrations were reached within 8 hours for the males and 4 hours for the females at the 10-mg/kg dose. At the 30-mg/kg dose, the peak HFPO dimer acid anion concentrations were reached within 2 hours for both males and females. The mean clearance time was slower in the males (143 hours and 139 hours at the low dose and high dose, respectively) than in the females (57 hours and 62 hours at the low dose and high dose, respectively) (Table 6).

In the oral toxicokinetic studies, the clearance times were shorter in rats than in mice and were shortest in female rats compared to male rats for both anions from HFPO dimer acid and its ammonium salt. In rats at the 10-mg/kg dose, HFPO dimer acid took longer to clear than its ammonium salt in both male and female rats. At the 30-mg/kg dose, however, both HFPO dimer acid and its ammonium salt had the same clearance times in male rats, but the HFPO dimer acid ammonium salt took longer to clear in female rats.

In a cross-species pharmacokinetic study, Crl:CD(SD) rats (3/sex) were administered a single intravenous bolus of 10 or 50 mg/kg of HFPO dimer acid ammonium salt and Cynomolgus monkeys (3/sex) were administered a single intravenous bolus of the HFPO dimer acid ammonium salt (10 mg/kg) (DuPont-17751-1579 RV1, 2009). Plasma samples were collected at intervals over the first 24 hours post-dosing and once per day for the subsequent 7 days in the

rats and 21 days in the monkeys. In the rats, the plasma concentrations were consistently higher for the males than for females by approximately one to two orders of magnitude, consistent with the indication that female rats have more rapid elimination. The clearance times for male rats were 22 hours and 17 hours in the 10- and 50-mg/kg dose groups, respectively. The clearance times for female rats were 3 hours and 4 hours in the 10- and 50-mg/kg dose groups, respectively. Notably, the calculated clearance time in the male rats was longer for the 10-mg/kg dose group (22 hours) than the clearance time calculated in Dupont-24281 (2008) for male rats in the 10-mg/kg dose group (12 hours). Female rats had similar clearance times. Additionally, the standard deviations on each serum mean were broad for the rats in the 50-mg/kg dose group, indicative of wide differences between the three males and three females evaluated at that dose. In the monkeys, the standard deviations on each serum mean were broad, especially for the female monkeys over the first 2 hours, which is indicative of wide differences between the three males and three females evaluated. The plasma levels were generally higher in females over the first 2 hours, were nearly identical at 4 hours, and were slightly higher in the males from 4 to 336 hours. The levels of the anion from HFPO dimer acid ammonium salt were very low at 168 hours in male (0.004 µg/mL (4 ng/mL)) and female (0.001 µg/mL (1 ng/mL)) monkeys. For 408 hours and beyond, concentrations were below the LOQ of 0.001 µg/mL (1 ng/mL). The clearance times calculated for the male and female monkeys were 11 hours and 10 hours, respectively.

Half-lives. In Gannon et al. (2016), the goodness of fit was calculated for the plasma concentrations after oral and intravenous dosing (DuPont studies outlined above) using one- and two-compartment models, and the two-compartment model had a better fit. Pharmacokinetic parameters identified by Gannon et al. (2016) are presented for the intravenous studies in Table 7 and for the oral studies in Table 8. The alpha phase $T_{1/2}$ represents the plasma concentration in the early post-injection period and is considered to reflect the plasma distribution phase (Klaassen, 1996). The beta phase $T_{1/2}$ represents the period during which the chemical in the plasma has established an equilibrium with the levels in the body tissues and represents the elimination phase. The two-compartment model is a refinement of the prior pharmacokinetic analysis in which the clearance time was calculated. The two-compartment model better fits the data and separates distribution and elimination phases; therefore, generally for comparisons across the datasets, the $T_{1/2}$s are preferred.

Table 7. $T_{1/2}$ Estimates from Intravenous Injection in Sprague Dawley Rats and Cynomolgus Monkeys

$T_{1/2}$	Intravenous Exposures (in hours)			
	Male rat	Male monkey	Female rat	Female monkey
Alpha (Plasma Distribution) Phase	3.6	2.3	0.4	1.9
Beta (Plasma Elimination) Phase	89.1	64.1	22.6	79.6

Source: Gannon et al., 2016.

In the intravenous injection studies, the $T_{1/2}$ of the alpha phase of distribution is similar (about 2 hours) for male and female monkeys, but the $T_{1/2}$ of the beta (elimination) phase is longer in female monkeys. The $T_{1/2}$ of the beta (elimination) phase in female monkeys is longer than it is in the female rats, which could be a result of female monkeys having higher tissue stores than

female rats or clearance of HFPO dimer acid anion from their tissues might be slower. There are no studies, however, to distinguish these explanations such as a study of tissue concentrations over time. In rats, both the alpha and beta phases are shorter in females than in males; the beta phase $T_{1/2}$ is about four times longer in males, suggesting higher levels in tissues of males or slower clearance of HFPO dimer acid anion from their tissues (Gannon et al., 2016).

Gannon et al. (2016) also used the data from the single oral dose studies in rats and mice to derive estimates of alpha and beta phase $T_{1/2}$s to represent the distribution and elimination phases. The oral exposure data are not ideal for this calculation because the chemical is not directly injected into the blood. However, because intestinal uptake of HFPO dimer acid anion from the ammonium salt is believed to be rapid and there appears to be no metabolism, the estimates are reasonable for a two-compartment model.

In rats, following oral exposure, the alpha (distribution) $T_{1/2}$ phase is shorter in females than in males and the beta (elimination) phase $T_{1/2}$ is comparable for both sexes (Table 8). In mice, the $T_{1/2}$ estimates for the alpha phase are similar for both sexes and the $T_{1/2}$ estimates for the beta phase are shorter for females than for males (Table 8). The $T_{1/2}$ estimated for the beta phase in female rats is shorter from the intravenous data (22.6 hours) than from the oral gavage data (67.4 hours), while the other estimates of $T_{1/2}$ from the intravenous and oral gavage data for males and females are similar.

Table 8. $T_{1/2}$ Estimates from Single Oral Dose in Sprague Dawley Rats and Crl/CD1(ICR) Mice

$T_{1/2}$	Oral Exposures (in hours)			
	Male rat	Female rat	Male mouse	Female mouse
Alpha (Plasma Distribution) Phase	2.8	0.2	5.8	4.6
Beta (Plasma Elimination) Phase	72.2	67.4	36.9	24.2

Source: Gannon et al., 2016.

The time it takes to achieve a balance between gastrointestinal uptake and excretion (i.e., steady state) following daily gavage exposures to the HFPO dimer acid anion is dependent on the $T_{1/2}$s of the alpha and beta phases. When the data are well described by a multicompartmental model, the steady state is a function of the multiple $T_{1/2}$s for the intercompartmental distribution (alpha phase) and elimination (beta phase); however, at later times, the elimination $T_{1/2}$ is expected to dominate the time to steady state and to be reached approximately within four $T_{1/2}$s, or 6.15 days, for male mice (Ito, 2011). This was calculated by multiplying the oral gavage beta phase $T_{1/2}$ (36.9 hours) for male mice by 4 and dividing that product by 24 hours. The data from Rushing et al. (2017) for male mice clearly demonstrate a lack of serum steady state for male mice after receiving doses of 1, 10, and 100 mg/kg/day for 28 days because the serum concentrations do not remain constant after the expected 6 days. In fact, HFPO dimer acid concentrations continue to change between 5 and 14 days and 14 and 28 days. These continual changes in plasma concentration after 6 days indicate dynamics over multiple days that are not represented by typical multicompartment models and, therefore, are not appropriate for modeling the complexity of the pharmacokinetics of HFPO dimer acid and its ammonium salt.

Repeated-dose study. In a repeated-dose study with Crl:CD1(ICR) mice dosed with 0, 0.1, 0.5, or 5 mg/kg/day for at least 90 days, plasma measurements were determined 2 hours post-dosing on days 0, 28, and 95 (Dupont 18405-1307, 2010). Plasma concentrations increased less than twofold between the 2 hour and the 28-day measurements for both the males and females in all dose groups (Table 9). Unfortunately, the study provides no measurements between the 2-hour and 28-day time points to allow for a determination regarding steady state. As mentioned above, however, the Rushing et al. (2017) study in mice provides measurements in serum at 1, 5, 14, and 28 days following daily gavage dosing of C57BL/6 mice that clearly establish the lack of steady-state conditions, which supports development of a more complex model to represent these data.

Table 9. Mean Plasma Concentrations with Standard Deviations of Dosing Crl:CD1(ICR) Mice with HFPO Dimer Acid Ammonium Salt for at Least 90 Days

Dose mg/kg/day	Day 0			Day 28			Day 95		
	µg/mL	SD	COV	µg/mL	SD	COV	µg/mL	SD	COV
Males									
0	ND[a]	N/A	N/A	ND	N/A	N/A	ND	N/A	N/A
0.1	0.736	0.099	13%	1.124	0.238	21%	1.276	0.309	24%
0.5	3.806	1.197	31%	7.182	3.055	43%	7.068	2.398	34%
5	42.58	5.214	12%	52.240	16.725	32%	67.98	13.717	20%
Females									
0	ND	N/A	N/A	N/D	N/A	N/A	ND	N/A	N/A
0.1	0.824	0.072	9%	0.704	0.35	50%	0.74	0.282	38%
0.5	3.606	1.308	36%	4.198	1.239	30%	5.438	1.696	31%
5	35.34	9.262	26%	46.58	16.842	36%	45.58	5.741	13%

Source: Dupont 18405-1307, 2010.
Notes: COV = coefficient of variation (SD / mean); µg/mL = micrograms per milliliter; N/A = not applicable; ND = not detected; SD = standard deviation.
[a] Limit of detection = 0.005 µg/mL

Plasma concentrations remained relatively constant between 28 days and 95 days for male and female mice administered the 0.1-mg/kg/day dose in the Dupont 18405-1307 (2010) study (Table 9). At the 0.5-mg/kg/day dose, plasma concentrations were relatively constant from day 28 to 95 days for the males, but the females' plasma concentrations increased from 4.198 to 5.438 µg/mL (4,198 ng/mL to 5,438 ng/mL) (a 30% increase). This indicates that the HFPO dimer acid anion does not appear to accumulate at 0.1 mg/kg/day; however, it might have accumulation potential at 0.5 mg/kg/day. Interestingly, this increase in female plasma concentrations from 28 days to 95 days is equal to the coefficient of variation (COV) in the 28-day measurement, thus the difference between days 28 and 95 could be the result of inter-animal

differences in response to the same dose. Also interesting is that, at the 5-mg/kg/day dose, female plasma levels returned to approximately the same levels at 28 and 95 days (46.58 and 45.58 µg/mL (46,580 and 45,580 ng/mL), respectively) (Table 9). In the males, the plasma levels at 28 days increased from 52.24 to 67.98 µg/mL (52,240 ng/mL to 67,980 ng/mL) at 95 days (a 30% increase), again equaling the COV in the 28-day measurement. Thus, the difference between days 28 and 95 could be the result of variability in these measurements as a result of inter-animal differences and might not necessarily reflect accumulation of HFPO dimer acid anion.

3.0 Problem Formulation

3.1 Conceptual Model

The conceptual model provides useful, publicly available information to characterize and communicate the potential health hazards related to oral exposure to HFPO dimer acid and its ammonium salt. Figure 2 depicts in a conceptual diagram the sources of these GenX chemicals, the routes of exposure to biological receptors of concern (e.g., human activities related to ingested tap water such as drinking, food preparation, and consumption), the potential assessment endpoints (e.g., effects such as liver toxicity), and populations at risk of exposure to HFPO dimer acid and its ammonium salt. As outlined in the legend for Figure 2, the green boxes indicate where there are limited data available for these GenX chemicals. This includes quantitative data for oral exposure to HFPO dimer acid and its ammonium salt, as well as the limited data available for some of the potential sources of exposure to these chemicals. The quantitative data for oral exposure to HFPO dimer acid and its ammonium salt includes animal toxicity and toxicokinetic studies; no epidemiological studies on health effects in humans are available. The white boxes indicate that no data are publicly available to allow for determining if GenX chemicals are found in certain sources and that no human toxicity data exist.

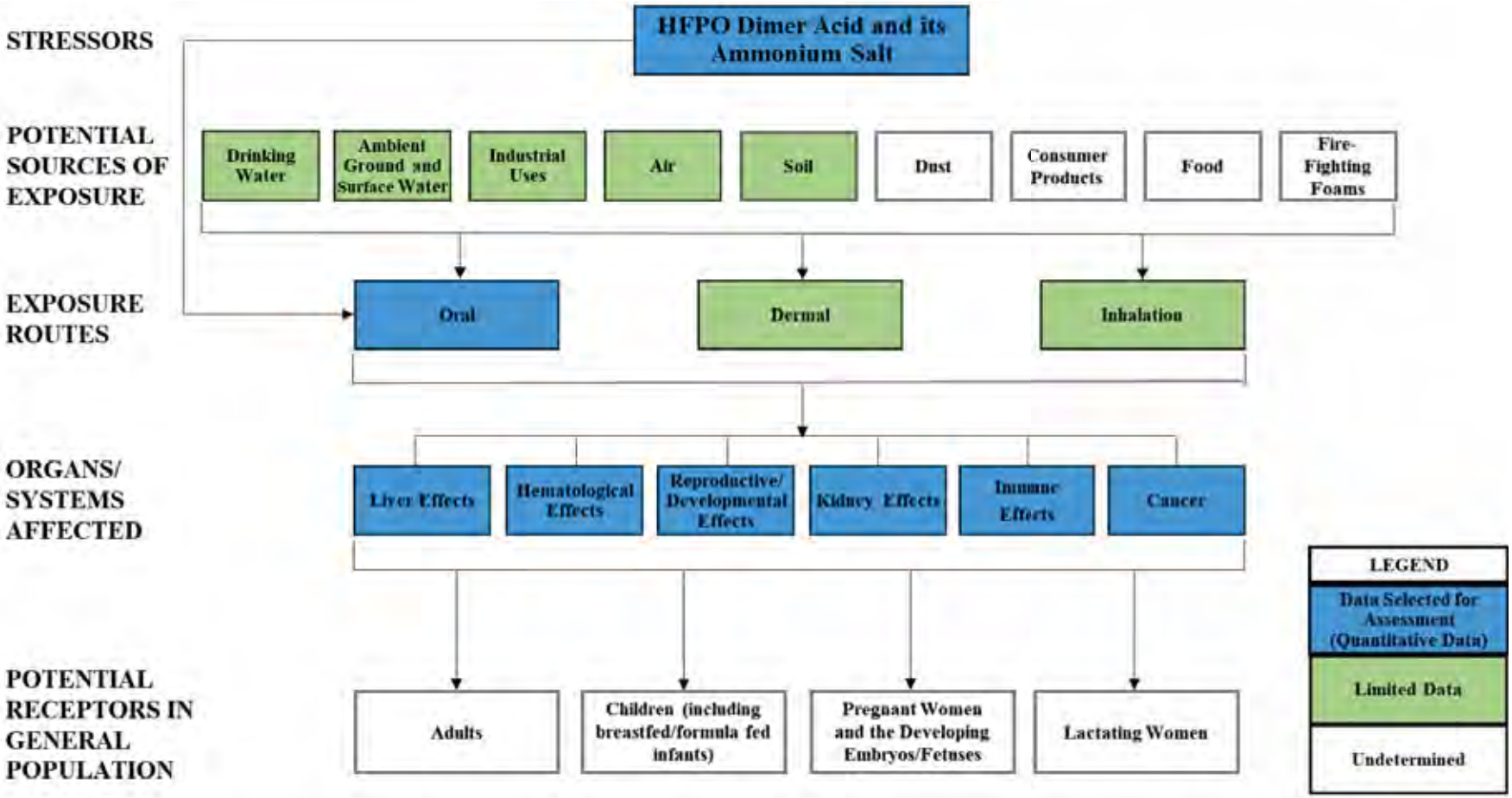


Figure 2. Conceptual Model for HFPO Dimer Acid and Its Ammonium Salt

3.2 Overall Scientific Objectives

This document provides the health effects basis for the development of oral RfDs for subchronic and chronic durations for GenX chemicals, including the science-based decisions providing the basis for estimating the POD. This section discusses the factors EPA considers in the process of developing a POD (depicted in Figure 2).

Stressors: This assessment addresses only HFPO dimer acid and its ammonium salt. It does not address any other chemicals used in the GenX processing technology or any other precursors, metabolites, or degradate of HFPO dimer acid and its ammonium salt. Uses of GenX chemicals include as intermediates and as polymerization aids in the production of fluoropolymers. These chemicals are two of several replacements for PFOA and its ammonium salt and could have many applications in consumer products (e.g., stain- and water-repellant textiles) and industrial processes (e.g., pharmaceutical and semiconductor manufacturing). Information on specific products containing GenX chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. GenX chemicals may also be generated as a byproduct of fluoromonomer production. Publicly available data, although limited, indicate that sources of exposure to GenX chemicals include both ground and surface waters used for drinking. Many other potentially important sources of exposure to GenX chemicals exist given their use as a replacement for PFOA, including foods; indoor dust in a home or work environment; indoor and outdoor air; soil; biosolids; and consumer products within the home, workplace, children's schools, and daycare centers. Very little quantitative information on these sources of exposure, however, is available.

Routes of Exposure: Nonoccupational exposure to GenX chemicals in water can occur through oral exposure (i.e., drinking water, cooking with water, and incidental ingestion from showering) and is expected to occur by dermal exposure (i.e., contact of exposed parts of the body with water containing GenX chemicals during bathing or showering, and dishwashing) and inhalation exposure (e.g., volatilization of the GenX chemicals from the water during bathing or showering, or while using a humidifier or vaporizer). There is limited information identifying health effects from inhalation or dermal exposures to GenX chemicals in animals. Specifically, two acute dermal toxicity tests (one in rats and one in rabbits), one dermal irritation study in rabbits, and one acute inhalation toxicity test in rats (see section 4.1) have been conducted. Repeated-dose toxicity data are available for oral exposure, but not for inhalation and dermal exposures. Since the only quantitative data available for HFPO dimer acid and its ammonium salt are for oral exposure, this assessment applies only to that route.

Receptors: The receptors are those in the general population who could be exposed to GenX chemicals in tap water through ingestion (i.e., adults, the elderly, women of childbearing age, pregnant women, fetuses, infants, and children). In the conceptual model in Figure 2, the box for adults includes sensitive life stages (e.g., women of childbearing age and the elderly). In this toxicity assessment, the first two steps (Step 1. Hazard Identification and Step 2. Dose Response) of the four-step risk assessment process developed by the National Academy of Sciences are addressed. This toxicity assessment summarizes potential health effects associated with exposure to GenX chemicals and identifies levels at which those health effects might occur. Potential exposure to receptors is not determined. Toxicity values from this assessment can be combined with specific exposure information (Step 3. Exposure Assessment) to help characterize the

potential public health risks associated with exposure to these chemicals (Step 4. Risk Characterization) to the receptors outlined here.

Endpoints: No human epidemiological studies for GenX chemicals are available. Oral exposure studies of acute, subchronic, and chronic duration are available in rodent species, including rats and mice. The recommended definitions of study duration were applied as outlined in *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002). Using this approach, the employed study durations are as follows:

- **Acute:** Exposure by the oral, dermal, or inhalation route for 24 hours or less.
- **Short-term:** Repeated exposure by the oral, dermal, or inhalation route for more than 24 hours, up to 30 days.
- **Subchronic**: Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the life span in humans (more than 30 days up to approximately 90 days in typically used laboratory animal species).
- **Chronic:** Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species).

Adverse effects observed following exposure to HFPO dimer acid and/or its ammonium salt include liver toxicity (e.g., hypertrophy, single-cell necrosis, focal necrosis and apoptosis), hematological effects (e.g., decreased red blood cell (RBC) count, hemoglobin, and hematocrit), kidney toxicity (e.g., increased kidney weight, necrosis, and hyperplasia), reproductive and developmental effects (e.g., placental lesions, changes in maternal gestational weight gain (GWG), and BW changes), immune effects (e.g., T cell-dependent antibody response (TDAR) suppression and lymphocyte increases), and *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans (e.g., liver and pancreatic acinar cell tumors).

In most of the available animal studies, hepatocellular hypertrophy and necrosis of the liver appear to be the most sensitive effects observed. The increases in relative liver weight, hepatocellular hypertrophy, and peroxisome activity (e.g., peroxisomal beta-oxidation induction) can be associated with activation of cellular peroxisome proliferator-activated receptor alpha (PPARα) receptors, making it difficult to determine if this change is a reflection of PPARα activation or an indication of GenX chemical toxicity. This is important because the PPARα response could be more relevant to rodents than humans. EPA evaluated liver effects resulting from exposure to GenX chemicals in the context of the Hall criteria (Hall et al., 2012), through which changes in liver weight or hepatocellular hypertrophy can be considered adverse when they are accompanied by histologic or clinical pathology indicative of liver toxicity such as necrosis, inflammation, and/or fibrosis. In this assessment, EPA listed hepatocellular hypertrophy or changes in serum liver enzymes as adverse only when they were accompanied by histologic pathology indicative of liver toxicity such as necrosis, inflammation, and/or fibrosis. The observance of liver necrosis indicates that cytotoxicity also could be a mode of action (MOA) for liver damage.

No physiologically based pharmacokinetic (PBPK) models are available that address the relationship between external exposure and internal dose for GenX chemicals; however, allometric scaling methodology is available to calculate a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans (EPA, 2011b). The use

of allometric scaling addresses some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes.

The toxicity values for this assessment include a chronic oral RfD (chronic RfD) and a subchronic oral RfD (subchronic RfD) for HFPO dimer acid and its ammonium salt. An RfD is an estimate of the concentration or dose of a substance (with uncertainty spanning perhaps an order of magnitude) to which a human population (including sensitive subgroups) can be exposed that is likely to be without an appreciable risk of deleterious effects during a lifetime. In addition to chronic RfDs, other durations of exposure can be considered, including subchronic exposures. RfDs are derived for noncarcinogenic toxicological endpoints of concern.

3.3 Methods

3.3.1 Literature Search Strategy and Results

EPA assembled and evaluated available information on toxicokinetics; acute, short-term, subchronic, and chronic toxicity; developmental and reproductive toxicity; neurotoxicity; immunotoxicity; genotoxicity; and cancer in animals. Most of the available data for HFPO dimer acid and its ammonium salt were submitted with PMNs to EPA by DuPont/Chemours, the manufacturer of GenX chemicals, under TSCA, as required pursuant to a consent order (EPA, 2009) or as required under TSCA reporting requirements (15 U.S.C. § 2607.8(e)). Submitted test data on HFPO dimer acid and its ammonium salt were available for numerous endpoints such as acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity in mice and rats with dosing durations of up to 2 years. Most of these submitted studies were conducted according to OECD TGs and/or EPA health effects TGs for pesticides and toxic substances, which:

> …are generally intended to meet testing requirements for human health impacts of chemical substances under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and TSCA (EPA, 2021b).

All available studies were considered for inclusion. Most of the studies considered for dose-response analysis in this assessment adhered to the principles of GLP, and full study reports were submitted for Agency review. As noted by OECD,[3] the OECD TGs are accepted internationally as standard methods for safety testing and:

> …are covered by the Mutual Acceptance of Data, implying that data generated in the testing of chemicals in an OECD member country, or a partner country having adhered to the Decision, in accordance with OECD Test Guidelines and Principles of GLP, be accepted in other OECD countries and partner countries having adhered to the Decision, for the purposes of assessment and other uses relating to the protection of human health and the environment.

To identify public literature available for HFPO dimer acid and its ammonium salt, literature searches were conducted of four databases (PubMed, Toxline, Web of Science (WOS), and Toxic Substances Control Act Test Submissions (TSCATS)) using CASRN, synonyms, and additional relevant search strings (see Table A-2 in appendix A for a full list). Because the results of this core search were so limited, additional databases were searched for

[3] http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm.

physicochemical property information, health effects, toxicokinetics, and mechanistic information. A list of the additional databases searched is provided in Table A-3 and Table A-4 in appendix A. The initial searches of these databases specific to HFPO dimer acid were conducted in July 2017 and specific to the HFPO dimer acid ammonium salt in January and February 2018. They returned 27 studies for HFPO dimer acid and its ammonium salt, after accounting for duplicates. Additional updates to the literature search were completed in February 2019, October 2019, and March 3, 2020 using the same search strategy as described in appendix A. These searches returned an additional 48 studies.

The submitted studies from DuPont/Chemours and the literature identified by the search of publicly available sources are available through EPA's Health & Environmental Research Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

3.3.2 Study Screening and Evaluation

In accordance with EPA's Office of Research and Development (ORD) systematic review practices, relevancy screenings were conducted on all the studies submitted from DuPont/Chemours and the publicly available, peer-reviewed literature resulting from the literature searches mentioned above (EPA, 2020). These studies were subjected to title and abstract screening to determine relevancy according to the PECO criteria statement/inclusion and exclusion criteria outlined in Table A-6 in appendix A. The title and abstract of each study were independently screened by two screeners using Distiller SR[4]. The studies that met the PECO criteria were tagged as having relevant human data, animal data in a mammalian model, or a PBPK model. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion or exclusion criteria. Studies that did not meet the inclusion criteria but provide supporting information were categorized as supplemental, relative to the type of supporting information they provided. These supplemental categories are outlined in Table A-7 in appendix A. When two screeners did not agree if a study should be included, excluded, or tagged as supplemental, a third reviewer made the final decision. The title and abstract screening resulted in 12 studies tagged as relevant (i.e., containing dose-response information). The relevancy of these studies was confirmed by a full-text review.

The twelve studies providing dose-response information were then evaluated for study quality using an approach consistent with the draft ORD Handbook for developing IRIS assessments (DuPont-24447, 2008; DuPont-24459, 2008; DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1037, 2010; DuPont-18405-841, 2010; DuPont-18405-1238, 2013; Rushing et al., 2017, Conley et al., 2019, 2021; Thompson et al., 2019; Blake et al., 2020; EPA, 2020). Study quality was determined by two independent reviewers who assessed risk of bias and sensitivity for the following domains: reporting quality, risk of bias (selection or performance bias, confounding/variable control, and reporting or attrition bias), and study sensitivity (exposure methods sensitivity, and outcome measures and results display) using EPA's version of HAWC[5]. A third reviewer made the final decision on the quality ratings based on the primary ratings. The results of the study quality evaluation are provided in Figure 3 and an interactive version of the heatmap can be found here:

[4] Distiller SR is a fee-based, multi-user, web-based platform that manages, tracks, and streamlines the screening of literature reviews.
[5] HAWC is a free and open-source web-based software application that enables multiple users to synthesize multiple data sources into an overall human health assessment of chemicals.

https://hawcprd.epa.gov/summary/visual/assessment/100500273/GenX-SQE-Heatmap/. All twelve studies were rated as medium or high-quality studies and were summarized in section 4 and considered for dose response in section 7.

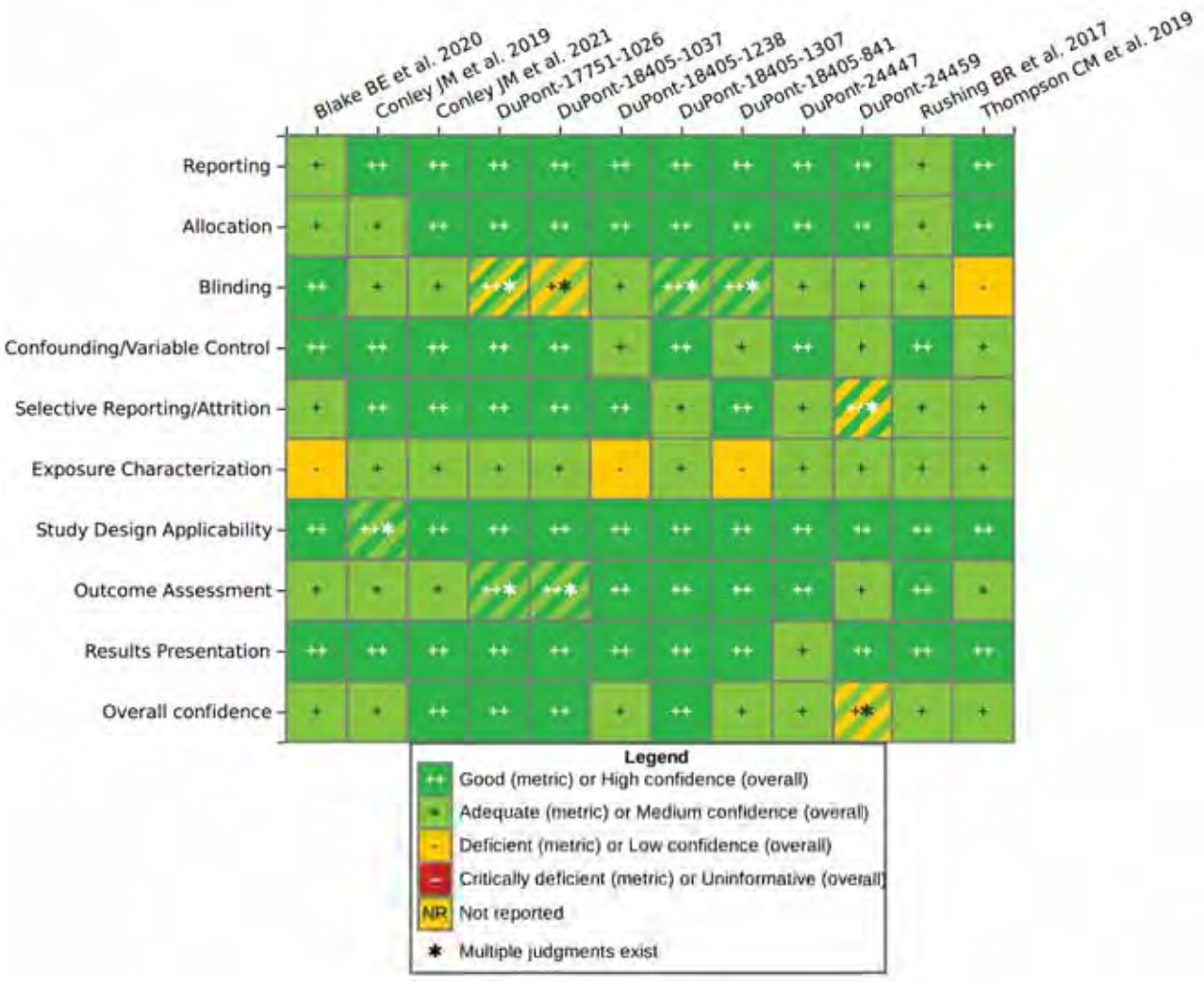


Figure 3. Evaluation Results for Animal Studies Assessing Effects of GenX Chemicals Exposure (Click to see interactive data graphic for rating rationales)

Additionally, all studies tagged as supplemental that provided toxicokinetic or mechanistic information were summarized and incorporated into the assessment in sections 2.3 and 4.6, respectively. Study summaries were also provided for all acute toxicity studies in section 4.1. Finally, two mechanistic studies were included in this assessment that were published after the final literature search (Gaballah et al., 2020; Cannon et al., 2020).

3.4 Approach to Deriving Reference Values

Development of the hazard identification and dose-response assessment for HFPO dimer acid and its ammonium salt has followed the general guidelines for risk assessment published by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a). Additional EPA guidelines and other Agency reports used in developing this assessment include the following:

- *Guidelines for Developmental Toxicity Risk Assessment* (EPA, 1991)
- *Guidelines for Reproductive Toxicity Risk Assessment* (EPA, 1996)
- *Guidelines for Neurotoxicity Risk Assessment* (EPA, 1998)
- *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002)
- *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a)
- *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005b)
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (EPA, 2006a)
- *Exposure Factors Handbook* (EPA, 2011a)
- *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b)
- *Benchmark Dose Technical Guidance Document* (EPA, 2012)
- *Child-Specific Exposure Scenarios Examples* (EPA, 2014b)
- *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (EPA, 2014c)

EPA's *A Review of the Reference Dose and Reference Concentration Processes* describes a multistep approach to dose-response assessment, including analysis in the range of observation followed by extrapolation to lower levels (EPA, 2002). EPA conducted a dose-response assessment to define a POD and extrapolated from the POD to an RfD. For HFPO dimer acid and its ammonium salt, EPA used benchmark dose (BMD) modeling to refine the critical effect POD in deriving the RfD.

The steps for deriving an RfD are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints related to exposure to GenX chemicals. This step involves determining the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study quality, and characterized for adverse outcomes, the risk assessor selects endpoints judged to be relevant and the most sensitive (typically defined by the NOAEL value). Considerations that might influence selection of endpoints include data with dose response, percent change from controls, adversity of effect, and consistency across studies.

Step 2: Conduct BMD Modeling. Using EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012), a benchmark response (BMR) is selected and BMD modeling is applied to the endpoints selected as most relevant. The BMR is a predetermined change in the response rate of an adverse effect. It serves as the basis for obtaining the benchmark dose lower limit (BMDL), which is the 95% lower bound of the BMD. A family of BMD models are fit to the

dose-response data that describe the dataset of the identified adverse effect. From the family of models, either a best fitting model with the corresponding BMD and BMDL is derived or, if no adequate models are found, the NOAEL or lowest-observed-adverse-effect level (LOAEL) identified in step 1 is used as the POD.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (either a BMDL, NOAEL, or LOAEL) is then converted to an HED following the method described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b).

Step 4: Provide rationale for selecting UFs. UFs are selected in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the critical study compared to the lifetime of the species studied, and the completeness of the toxicology database.

Step 5: Calculate the chronic and subchronic RfDs. The RfDs are calculated by dividing POD_{HED} by the selected UF.

$$RfD = \frac{POD_{HED}}{\text{Total UF}}$$

where:

- POD_{HED} = calculated from the BMDL or NOAEL/LOAEL using a $BW^{3/4}$ allometric scaling approach consistent with EPA guidance (EPA, 2011b).
- UF = Total UF established in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, duration of exposure in the critical study compared to the lifetime of the species studied, and completeness of the toxicology database.

3.5 Measures of Effect

The available dataset regarding the toxicity of these GenX chemicals includes *in vivo* and *in vitro* studies. The *in vivo* studies were considered in the dose-response assessment for HFPO dimer acid and its ammonium salt. The available data indicate that the liver, kidney, RBCs, immunological responses, and reproductive and developmental effects (BW and fetal development) are adversely impacted by exposure to GenX chemicals. Tumors were also observed following oral exposure to GenX chemicals (DuPont-18405-1238, 2013). In this analysis, all reported changes in relative organ weights were presented as relative to BW (data relative to brain weight were not included). The endpoints presented in this assessment represent potentially adverse effects that were statistically significantly different ($p < 0.05$ or 0.01) from control unless otherwise noted. Additionally, statistically significant changes from the control are presented as the percent change from control, unless otherwise noted.

The animal studies demonstrated dose-related effects on the liver in rodent species (rats and mice) following exposure to HFPO dimer acid and/or its ammonium salt for durations of 28 days to 104 weeks. The studies and endpoints reviewed as possible critical studies and effects for determination of the POD were evaluated for experimental design, data quality, and dose response identified through the range of experimental NOAELs/LOAELs. A route-to-route

extrapolation of oral toxicity data from which to derive an inhalation reference concentration was not conducted because of data limitations. For example, no toxicokinetic data are available characterizing the uptake of GenX chemicals through the lung for systemic distribution, and only one acute inhalation toxicity study is available (DuPont-17751-723, 2009). This study identifies the portal of entry effects, albeit at a high dose.

4.0 Study Summaries

4.1 Acute Toxicity Studies

There are over 10 studies available detailing the acute toxicity and irritation effects of HFPO dimer acid and its ammonium salt. This section summarizes the available acute oral, dermal, and inhalation toxicity studies as well as dermal and eye irritation studies for HFPO dimer acid and its ammonium salt. Appendix B provides additional details on each of the studies.

Oral Toxicity. Several studies have evaluated oral toxicity in rats and mice from single doses of the HFPO dimer acid ammonium salt at doses ranging from 1.5 mg/kg to 17,000 mg/kg (DuPont-22932, 2007; DuPont-24126, 2007; DuPont-25438 RV1, 2008; DuPont-2-63, 1963; DuPont-770-95, 1996). Also, male and female rats were evaluated with doses of 175–5,000 mg/kg HFPO dimer acid (DuPont-25875, 2008). The rats and mice in these studies received a single dose of the compound and were observed for clinical effects of toxicity for 14 days.

Four studies were conducted according to OECD TG 425 (OPPTS 870.1100) (OECD, 2008c) using the Up-and-Down Procedure (DuPont-22932, 2007; DuPont-25438 RV1, 2008; DuPont-25875, 2008; DuPont-24126, 2007). Two studies that estimated approximate lethal doses (ALDs) did not have identified TGs (DuPont-2-63, 1963; DuPont-770-95, 1996). For HFPO dimer acid, the oral median lethal doses (LD_{50}s) were 1,730 mg/kg and 1,750 mg/kg in male rats and female rats, respectively (DuPont-25875, 2008). For the HFPO dimer acid ammonium salt, the LD_{50} was 3,129 mg/kg for female rats (DuPont-22932, 2007); 1,030 mg/kg for female mice (DuPont-24126, 2007); and 1,750 mg/kg for male rats (DuPont-25438 RV1, 2008). The estimated ALD for male rats for the ammonium salt ranged from 5,000 mg/kg to 7,500 mg/kg (DuPont-2-63, 1963; DuPont-770-95, 1996).

The more common clinical signs observed across studies included wet fur, fur/skin stain or discoloration, altered posture, and lethargy; changes in BW were also seen (DuPont-770-95, 1996; DuPont-22932, 2007; DuPont-24126, 2007; Dupont-25438 RV1, 2008; DuPont-25875, 2008). Effects in mice were observed after exposure to HFPO dimer acid ammonium salt (86% purity) doses at 550 mg/kg and higher. Effects in rats were observed after exposure to either HFPO dimer acid (98% purity) or its ammonium salt (82.6% to 99% purity) at doses of 175 mg/kg and higher (DuPont-22932, 2007; DuPont-25875, 2008).

Gross evidence of organ or tissue damage included discoloration of lungs, stomach, skin, lymph nodes, liver, and/or esophagus (DuPont-22932, 2007; DuPont-25438 RV1, 2008; DuPont-25875, 2008). Enlarged livers and enlarged hepatocytes were observed in young male rats following single doses of 2,250, 3,400, or 5,000 mg/kg for HFPO dimer acid ammonium salt (DuPont-2-63, 1963).

Dermal Toxicity. Two studies reported acute dermal toxicity of HFPO dimer acid ammonium salt in rats or rabbits following acute dermal exposure (DuPont-24113, 2007; DuPont-839-95, 1996). In an OECD TG 402 (OPPTS 870.1200) (OECD, 2017) study, 5,000 mg/kg HFPO dimer acid ammonium salt (86% purity) was applied to shaved, intact skin of male and female rats under a semi-occlusive dressing for 24 hours. The dermal LD_{50} was more than 5,000 mg/kg (both sexes). Erythema was observed only in females, whereas hyperkeratosis and ulceration were observed in rats of both sexes. All dermal effects cleared by 13 days posttreatment (DuPont-24113, 2007). In another study (in which no guideline is cited), HFPO dimer acid ammonium salt (99% purity) was applied to shaved, intact skin of New Zealand white rabbits for 24 hours. The ALD was determined to be more than 5,000 mg/kg. In this study, erythema persisted for 13 days post application and was accompanied by scaling and sloughing of skin. One of the rabbits also exhibited necrosis for 2–6 days post application (DuPont-839-95, 1996).

Inhalation Toxicity. One study (conducted using the GLP Compliance Statement in compliance with Title 40 of the Code of Federal Regulations (CFR) part 792) evaluated acute inhalation toxicity of HFPO dimer acid ammonium salt (84% purity) in male and female rats following a single 4-hour nose-only exposure to aerosol concentrations of 0, 13, 100, and 5,200 mg/m^3. The median lethal concentration (LC_{50}) was more than 5,200 mg/m^3. Red discharge from the nose, eyes, and mouth was observed in rats at doses of 100 and 5,200 mg/m^3 for up to 2 days postexposure. No gross lesions were observed. Microscopic evaluation of respiratory tract tissue (lung, larynx/pharynx, trachea, and nose) from rats exposed to concentrations of 0, 13, and 100 mg/m^3 detected no substance-related effects (DuPont-17751-723, 2009).

Dermal Irritation. In an OECD TG 404 (OPPTS 870.2500) (OECD, 2002) dermal irritation study, very slight-to-well-defined erythema was observed in three male New Zealand white rabbits following a single application of a 0.5-mL aliquot of HFPO dimer acid ammonium salt (86% purity) in an area of shaved skin for a period of 4 hours on the day of application. Erythema cleared by 24 hours postexposure (DuPont-24030, 2007).

Eye Irritation. New Zealand white rabbits were administered a single application of a 0.1 mL aliquot of HFPO dimer acid ammonium salt (86% purity) to the lower conjunctival sac in an eye irritation study conducted according to OECD TG 405 (OPPTS 870.2400) (OECD, 2020a). At 28 hours after instillation of the compound, necrosis, corneal opacity, iritis, conjunctival chemosis (swelling), discharge, and corneal injury were observed (DuPont-24114, 2007).

4.2 Short-Term Toxicity Studies

Seven-Day Toxicity Studies. Hepatic effects were observed in 6-week-old mice and rats of both sexes in four 7-day studies (in which no TG is cited) evaluating repeated-dose oral toxicity of HFPO dimer acid and its ammonium salt (DuPont-24010, 2008; DuPont-25281, 2008; DuPont-24116, 2008; DuPont-24009, 2008). Water was used as the vehicle control in all studies. Two 7-day studies evaluated the toxicity of HFPO dimer acid ammonium salt (86.6% purity) and HFPO dimer acid (99% purity) at doses of 30 mg/kg/day in male mice and rats, respectively. In both studies, a twofold increase in liver weight relative to control, cell necrosis of hepatocytes, and hepatocellular hypertrophy were observed in all exposed animals (DuPont-24010, 2008; DuPont-25281, 2008). A third 7-day study evaluating toxicity of HFPO dimer acid (99% purity) also detected increased liver weight in male rats (at 30, 100, and 300 mg/kg/day) and in female rats (at 300 mg/kg/day). Hepatocellular hypertrophy was present in both sexes at all doses (DuPont-

24116, 2008). Hypertrophy and increased liver weight were observed in another similar 7-day gavage study evaluating effects of HFPO dimer acid ammonium salt (86.6% purity). Males appeared to be more sensitive to hepatic effects because increases in liver weight were observed at 30, 300, and 1,000 mg/kg/day, whereas increased liver weight was observed in females only at 1,000 mg/kg/day. These effects were accompanied by increases in β-oxidation and increases in cytochrome P450 enzyme activity, biomarkers for activation of PPARα nuclear receptors. Mild-to-minimal hepatocellular hypertrophy was observed in both sexes at 1,000 mg/kg/day (DuPont-24009, 2008).

Twenty-Eight-Day Toxicity Studies. Two 28-day studies evaluating systemic toxicity in rats and mice are available for HFPO dimer acid ammonium salt.

DuPont-24447 (2008)
In a study with 7-week-old Crl:CD(SD) rats (10/sex/group) conducted according to OECD TG 407 (OECD, 2008a), HFPO dimer acid ammonium salt (purity 88%) was administered on 28 consecutive days via gavage (vehicle was deionized water) (OECD, 2008a; DuPont-24447, 2008). Male rats received doses of 0, 0.3, 3, or 30 mg/kg/day while females received 0, 3, 30, or 300 mg/kg/day. In this study, there were no mortalities and clinical signs were confined to high-dose females (e.g., urogenital staining).

Hematological evaluation revealed statistically significantly decreased RBC count, hemoglobin, and hematocrit at greater than or equal to 3 mg/kg/day in males. The maximum decreases compared to control at 4 weeks were observed at the highest dose (30 mg/kg/day) and were 6%, 7%, and 8% for RBC count, hemoglobin, and hematocrit, respectively. Increases in absolute reticulocyte counts were also observed in males at all dose levels, but this increase was only statistically significant from control at the highest dose (27%) at 4 weeks. No statistically significant hematological effects were observed in the females (DuPont-24447, 2008).

Alterations in serum clinical chemistry parameters were seen in both sexes, but most of the significant effects were observed in the male rats. Decreases in total globulin and increases in the A/G ratio were observed in males and females. In males, total serum albumin increased (15% at 30 mg/kg/day) while total globulin decreased 13% and 22% compared to control at 3 mg/kg/day and 30 mg/kg/day, respectively. This resulted in an increase in the A/G ratio to 16% and 41% in the 3 mg/kg/day and 30 mg/kg/day males, respectively, most likely the result of underproduction of globulin. Females exhibited a 9% decrease in total globulin and a 20% increase in the A/G ratio compared to control at 300 mg/kg/day. Males also showed statistically significant decreases in serum cholesterol at all doses, with the largest decrease compared to control (28%) in the 30-mg/kg/day group. Triglyceride levels were decreased at all doses but were significantly decreased (22%) only at 3 mg/kg/day. Males also exhibited increases in blood urea nitrogen (BUN) (24%) and glucose (15%) at 30 mg/kg/day when compared to controls (DuPont-24447, 2008).

In males, relative kidney weight was significantly increased (15% compared to control) only at the highest dose tested. Minimal mineralization of the kidneys was also observed in 1/10 male rats in the high-dose group. There were no statistically significant changes in kidney weight in the females; however, there was minimal basophilic staining of cells in the tubules for 3/10 female mice in the 300-mg/kg/day group, while none were observed in the control group. Dose response could not be determined for basophilic tubules because no rats were examined in the 3-

mg/kg/day-dose group and only one rat was examined in the 30-mg/kg/day-dose group. No statistical analyses were completed on these microscopic observations.

Relative liver weights were statistically increased in a dose-response manner in males, 19% and 56% compared to control at 3 mg/kg/day and 30 mg/kg/day, respectively. These increases were accompanied by decreases compared to control in sorbitol dehydrogenase (SDH) at 0.3 mg/kg/day (-36%) and 30 mg/kg/day (-21%) in males. In females, the only statistically significant change in liver weight was a 12% increase compared to control at the highest dose (300 mg/kg/day). Microscopically, 4/10 and 7/10 male rats exhibited hepatocellular hypertrophy at 3-mg/kg/day and 30-mg/kg/day doses, respectively. In female rats, hepatocellular hypertrophy was observed in 4/10 rats in the high-dose group. Hepatocellular necrosis (3/10) and single-cell necrosis (1/10) were observed in males at 30 mg/kg/day. No statistical analyses were completed on these histological observations. The authors note that hepatic peroxisomal β-oxidation activity was induced in both sexes at the middle and high doses. Specifically, β-oxidation activity was determined using [14C] palmitoyl-coenzyme A (CoA) as the substrate and total cytochrome P450 content as markers of peroxisome proliferation. In the males, β-oxidation activity was significantly increased compared to control at dosages of 0.3 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day by 42%, 274%, and 772%, respectively, and total cytochrome P450 content was significantly increased by 23% at 30 mg/kg/day (DuPont-24447, 2008). In female rats dosed with 30 mg/kg/day and 300 mg/kg/day, β-oxidation activity was significantly increased compared to control by 49% and 198%, respectively, while total cytochrome P450 content remained unaltered (DuPont-24447, 2008). EPA identified the NOAEL to be 0.3 mg/kg/day and the LOAEL to be 3 mg/kg/day based on hematological (decreased hemoglobin, RBC count, and hematocrit) and immune (decreased globulin levels) findings in males (DuPont-24447, 2008). These findings were also accompanied by liver effects, including an increase in relative liver weight and hepatocellular hypertrophy; however, necrosis was observed only at the high dose (30 mg/kg/day).

DuPont-24459 (2008)

In another repeated-dose study conducted according to OECD TG 407 (OECD, 2008a), 7-week-old Crl:CD-1 mice (10/sex/group) were administered 0, 0.1, 3, or 30 mg/kg/day HFPO dimer acid ammonium salt (purity 88%) for 28 consecutive days via gavage (vehicle was deionized water) (DuPont-24459, 2008). Increases in mean BW gain were observed at 30 mg/kg/day in both males and females. In males, increases in mean cumulative BWs were reported as statistically different from the control group in the 30-mg/kg/day group during study weeks 1, 2, 3, and 4. In females, mean cumulative BW gains were significantly increased in the 30-mg/kg/day group during study weeks 2, 3, and 4.

Similar to the findings observed in the 28-day toxicity study in Crl:CD(SD) rats (DuPont-24447, 2008), decreases of 5.0% in hemoglobin and hematocrit were reported at greater than or equal to 3 mg/kg/day, and RBC count was significantly decreased by 7.6% in the Crl:CD-1 male mice at 30 mg/kg/day. In both males and females, the A/G ratio was statistically increased compared to control at greater than or equal to 3 mg/kg/day. Albumin alone was significantly increased by 31.3% compared to controls in males at 30 mg/kg/day, and globulin alone was decreased in females at greater than or equal to 3 mg/kg/day by 15.8% and 21.1% at 3 mg/kg/day and 30 mg/kg/day, respectively. Finally, in males, the serum liver enzymes aspartate aminotransferase (AST) (478%), alanine aminotransferase (ALT) (1,254%), alkaline

phosphatase (ALP) (1,222%), and SDH (1,800%) were significantly increased from control at the 30-mg/kg/day dose. Note that the hematology measures in female mice were inexplicably underpowered. Though a sample size of 9-10 mice per dose group was expected, only 2, 6, 3, and 5 female mice had hematology measurements in the 0, 0.1, 3 and 30 mg/kg/day dose groups, respectively.

In male mice, no statistically significant effect was observed on kidney weight. Female kidney weight findings were equivocal with the mean relative kidney weight showing statistically significant increases compared to control only at the low dose (8%) and high dose (17%). Minimal increases in basophilic tubular cells and tubular dilatation were observed in females at 30 mg/kg/day (3 of 10 animals for both effects) (DuPont-24459, 2008).

Macroscopic and microscopic tissue pathology evaluations were conducted for all dose groups. The inspection of male adrenal cortex at the highest dose found minimal hypertrophy in 8 of 10 tissue samples examined, while females showed mild or minimal adrenal cortex congestion at only the highest dose (DuPont-24459, 2008). No statistical analyses were completed on these microscopic observations.

Liver effects were also reported in both males and females in this study. In males, relative liver weights were significantly increased compared to control at 3 mg/kg/day and 30 mg/kg/day by 78% and 163%, respectively. In females, relative liver weights were increased at 3 mg/kg/day and 30 mg/kg/day by 32% and 103%, respectively, compared to controls. Absolute liver weights also increased at these doses in both sexes and to similar extents. Increases in liver weight correlated with microscopic liver findings (including single-cell necrosis, increased mitosis, and hepatocellular hypertrophy). Single-cell necrosis was observed in 40% (4/10) and 100% (10/10) of the male mice at 3 mg/kg/day and 30 mg/kg/day, respectively, while no liver necrosis was observed in the control mice. As noted above, serum liver enzymes were significantly increased from control at the 30 mg/kg/day dose: AST (478%), ALT (1,254%), ALP (1,222%), and SDH (1,800%). Single-cell necrosis was also detected in 40% (4/10) of female mice at 30 mg/kg/day compared to zero in the control. This was associated with an increase in serum SDH (186%) at 30 mg/kg/day. Hepatic peroxisomal β-oxidation activity was induced in both sexes. Specifically, β-oxidation activity was determined using [14C] palmitoyl-CoA as the substrate and total cytochrome P450 content as markers of peroxisome proliferation. In the male mice, β-oxidation activity significantly increased compared to control at doses of 0.1 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day HFPO dimer acid ammonium salt by 57%, 744%, and 648%, respectively, yet total cytochrome P450 content significantly decreased at 3 mg/kg/day and 30 mg/kg/day by 26% and 53%, respectively (DuPont-24459, 2008). β-oxidation activity significantly increased relative to control in female mice at 3 mg/kg/day and 30 mg/kg/day by 495% and 823%, respectively, with no alterations in total cytochrome P450 content. EPA identified the NOAEL for this study as 0.1 mg/kg/day and the LOAEL as 3 mg/kg/day based on increase in single-cell necrosis in males, which was accompanied by increased relative liver weight and hepatocellular hypertrophy, hematological, and immune effects.

4.3 Subchronic Toxicity Studies

DuPont-17751-1026 (2009)

In a repeated-dose study with rats, HFPO dimer acid ammonium salt (purity 84%) was administered to 8-week-old Crl:CD(SD) rats (10–20/sex/dose) on 90 consecutive days via oral

gavage (vehicle was deionized water) in accordance with OECD TG 408 (DuPont-17751-1026, 2009; OECD, 1998). Male rats were administered the test substance at doses of 0, 0.1, 10, or 100 mg/kg/day while females received 0, 10, 100, or 1,000 mg/kg/day. In this study, three high-dose females died before dosing was complete (two deaths considered as treatment-related; one death of undetermined cause).

Hematological evaluations revealed decreased hemoglobin, erythrocyte counts, and hematocrit in males administered greater than or equal to 10 mg/kg/day. The decreases in all three parameters for males were significantly different from control at 10 and 100 mg/kg/day and decreased in a dose-dependent manner at 90 days (study week 13). The maximum decreases from control in males were observed at the highest dose and were 11%, 13%, and 12% for RBC count, hemoglobin, and hematocrit, respectively. Likewise, female rats exhibited significant and dose-dependent decreases in RBC count (28%), hemoglobin (21%), and hematocrit (18%), but only at the 1,000 mg/kg/day dose. In males, absolute (52%) and percent (67%) reticulocytes and platelet count (17%) were significantly increased from control at the highest dose and exhibited a dose response. Additionally, both the absolute and percent of basophils (a type of white blood cell) were significantly decreased relative to control at 10 mg/kg/day (25%) and 100 mg/kg/day (50%) in males. Finally, female rats saw significant increases from control in mean corpuscular volume (15%), mean corpuscular hemoglobin (11%), mean corpuscular hemoglobin concentration (4%), platelet count (30%), and absolute (212%) and percent (392%) reticulocytes and a decrease relative to control in the percent of basophils (33%) at the high dose (1,000 mg/kg/day) (DuPont-17751-1026, 2009).

There were alterations in the clinical chemistry values in both sexes. Males exhibited a dose-dependent increase in total albumin and the A/G ratio and a decrease in total globulin compared to control. These changes were statistically significant at 10 mg/kg/day and 100 mg/kg/day. The maximum increases compared to control observed at the highest dose in total albumin, total globulin, and A/G ratio were 12%, 15%, and 35%, respectively. As in the 28-day study, females exhibited a dose-dependent decrease in globulin (33%) and an increase in A/G ratio (58%) that was significantly different from control for both effects at the highest dose only. Males and females also showed dose-dependent decreases in serum cholesterol that were statistically significantly different from control at 100 mg/kg/day (31%) in males and at both 100 mg/kg/day (20%) and 1,000 mg/kg/day (31%) in females. BUN was significantly increased relative to control in males at 100 mg/kg/day (38%). The trend for BUN was dose-related and positive in both sexes. ALP levels were significantly increased from control in a dose-dependent manner at 10 mg/kg/day (48%) and 100 mg/kg/day (106%) in the males and at 1,000 mg/kg/day (66%) in the females. Serum phosphorus levels increased dose-dependently in males and females and were significantly different from control at 10 mg/kg/day (10%) and 100 mg/kg/day (11%) in males and at 1,000 mg/kg/day (18%) in females. Total bilirubin was significantly decreased from control in a dose-dependent manner at the mid-dose (25%) and high dose (50%) only in females. Total protein and γ-glutamyl transferase decreased 10% and 69%, respectively, at the high dose in females. Finally, a slight but significant and dose-dependent decrease compared to controls in urine pH (8%) and a large increase in total urine volume (252%) were observed in female rats at 1,000 mg/kg/day (DuPont-17751-1026, 2009).

Kidney weight relative to BW was significantly and dose-dependently increased from control at 10 mg/kg/day (13%) and 100 mg/kg/day (16%) in male rats. Likewise, kidney weight relative to

BW was significantly increased at all dose levels in females and reached a maximum increase of 23% from control; however, microscopic damage of the kidney (tubular and papillary necrosis) was observed in only one of the rats at the highest dose. Additionally, one of the females that died prior to study termination exhibited tubular and papillary necrosis of the kidney. Transitional cell hyperplasia and mild acute inflammation were observed in the kidney of 1/10 male rats at the 100-mg/kg/day dose. Statistical analyses were not completed for the microscopic renal findings.

Liver weight relative to BW was significantly and dose-dependently increased from control at 10 mg/kg/day (31%) and 100 mg/kg/day (67%) in male rats. Females exhibited an 85% increase from control in liver weight at the high dose (1,000 mg/kg/day). Hepatocellular hypertrophy was observed in 3/10 and 10/10 males at the 10-mg/kg/day dose and 100-mg/kg/day dose, respectively, and in 10/10 females at the 1,000-mg/kg/day dose. Statistical analyses were not conducted for hepatocellular hypertrophy. Furthermore, it is not documented in the data tables whether other histological effects such as liver necrosis were detected in the 90-day study, although the pathology report states that the hypertrophy was not associated with microscopic changes indicative of liver injury such as necrosis (DuPont-17751-1026, 2009). EPA has determined the study NOAEL to be 0.1 mg/kg/day and the LOAEL to be 10 mg/kg/day based on blood effects (i.e., decreased RBC count, hemoglobin, and hematocrit) in males.

DuPont-18405-1307 (2010)

DuPont-18405-1307 (2010) was submitted to EPA under a TSCA Consent Order (see section 1.1 for more detail). Subsequently, in comments submitted to regulations.gov (Docket EPA-HQ-OW-2018-0614) by ToxStrategies LLC (2019a,b) a reevaluation of the study results for DuPont-18405-1307 (2010) and DuPont-18405-1037 (2010) was submitted. The reevaluation of DuPont-18405-1037 (2010) was published as Thompson et al. (2019) (discussed in section 4.5); however, the results of the reevaluation of DuPont-18405-1307 (2010) were not included in this publication. In response to these comments and the publication, EPA requested an independent review of DuPont-18405-1307 (2010) by the National Toxicology Program (NTP, 2019) Pathology Working Group (PWG) (appendix D). The results of the DuPont-18405-1307 (2010) and the NTP PWG review are described next.

In a repeated-dose, subchronic study with 7-week-old Crl:CD1(ICR) mice, the HFPO dimer acid ammonium salt (purity 84%) was administered to 10/sex/group for 95 days (males) or 96 days (females) via gavage (vehicle was deionized water) at doses of 0, 0.1, 0.5, and 5 mg/kg/day in accordance with OECD TG 408 (DuPont-18405-1307, 2010; OECD, 1998). A statistically significant increase in male BW and overall BW gain was observed at the high dose only. Mean daily food consumption was statistically increased in males between days 0 and 91 in a dose-related manner. The study authors reported that there were no treatment-related deaths. Two female mice (one at 0.5 mg/kg/day on day 6 and one at 5 mg/kg/day on day 20) died during the study. The authors reported that these animals displayed signs indicative of injury from gavage misdosing. The mice that died prematurely were included in the study results presented in the report.

A small decrease compared to control in mean corpuscular hemoglobin concentration (3%) in males and increased bilirubin (14%) in females was reported at 5 mg/kg/day. Clinical chemistry changes were more evident among male mice than female mice. Specifically, AST, ALT, and

ALP were statistically increased from control 106%, 420%, and 1,134%, respectively, at the 5-mg/kg/day dose in males. Comparatively, female mice saw significant increases relative to control in ALT (42%) and ALP (143%). SDH levels significantly increase compared to control in both males (308%) and females (32%) at 5 mg/kg/day. Albumin levels were increased relative to control in the 5-mg/kg/day-dose group in both males (14%) and females (4%), but total serum protein was significantly increased (14%) only in males at this dose (DuPont-18405-1307, 2010).

Macroscopic and microscopic tissue pathology evaluations were conducted for all dose groups. Male mice exhibited kidney tubular epithelial hypertrophy (9/10 treated mice compared to 0 in control) while females exhibited dilated kidney tubules (4/10 in treated compared to 2/10 in control) in the 5-mg/kg/day-dose group. Both effects were classified as minimal by the study authors. Female mice exhibited a decrease in relative spleen weight (10%, 21%, and 18% at 0.1 mg/kg/day, 0.5 mg/kg/day, and 5 mg/kg/day, respectively). No effects on the spleen were observed in male mice in any dose group. The study authors reported that changes in female spleen weight did not occur in a dose-related manner and were not associated with changes in absolute spleen weights or histological abnormalities in the spleen (DuPont-18405-1307, 2010).

Increased relative liver weights compared to control in both male mice (130%) and female mice (69%) were accompanied by minimal-to-mild hepatocellular hypertrophy at 5 mg/kg/day in all dosed mice. Minimal hepatocellular hypertrophy was also observed at the 0.5-mg/kg/day dose as well in males (8/10 mice). No hepatocellular hypertrophy was observed in the control group. Large and discolored livers were observed at doses greater than or equal to 0.5 mg/kg/day in males, but only in the 5-mg/kg/day-dose group in females. Key treatment-related findings considered as adverse at 5 mg/kg/day included increased enzymes indicative of liver injury (i.e., AST, ALT, ALP, and SDH) and increased total bile acids that co-occurred with histopathological findings in the liver. Histopathological findings in male mice included an increase in the incidence of single-cell necrosis (10/10 treated mice versus 0 in control), Kupffer cell pigments (10/10 treated mice versus 0 in control), and mitotic figures (9/10 treated mice versus 0 in control). Females also exhibited histopathological liver findings, but to a lesser degree. For example, 3/10 female mice exhibited focal necrosis and only 1/10 mice presented single-cell necrosis at 5 mg/kg/day (DuPont-18405-1307, 2010).

EPA concluded that the NOAEL in this study is 0.5 mg/kg/day and the LOAEL is 5 mg/kg/day based on the histological findings for the liver (i.e., necrosis and mitotic figures) accompanied by the clinical chemistry changes (i.e., AST, ALT, ALP, and SDH).

Reanalysis of DuPont 18405-1307 (2010) by National Toxicology Program Pathology Working Group (NTP, 2019)

The National Institute of Environmental Health Sciences (NIEHS), NTP in Research Triangle Park, NC convened a pathology working group (PWG) to provide an independent review of slides from the 90-day mouse study (DuPont-18405-1307, 2010) and the reproductive/developmental study (DuPont-18405-1037, 2010). All pathology slides provided by DuPont/Chemours were reviewed by the NTP PWG, including those of animals that died on study. The data and slides were reviewed per NTP standards (Sills et al., 2019).

As part of this PWG, one pathologist reviewed slides from the two studies and classified liver effects according to the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) Organ Working Group’s diagnostic criteria which describes how pathologists can

distinguish between apoptosis and single-cell necrosis in standard hematoxylin and eosin- (H&E) stained tissue sections (Elmore et al., 2016). The PWG coordinator then confirmed the classifications and selected example slides representative of the observed liver effects for review by the full, eight-member PWG. The selected slides included three examples each of normal liver, hepatocellular apoptosis, hepatocellular single-cell necrosis, and hepatocellular cytoplasmic alteration; two examples each of focal necrosis, pigment, increased mitoses, mixed-cell infiltrates, and cytoplasmic vacuolation; and one example of oval cell hyperplasia. The PWG's description of cytoplasmic alteration indicates that this endpoint includes hepatocellular hypertrophy occurrence along with eosinophilic change to the hepatocytes. There was a majority consensus for all reviewed lesions. The PWG consensus opinion for each slide, including any additional diagnoses made by the PWG panel, was recorded and presented in the final PWG report (appendix D of this revised assessment).

The PWG's classification of liver lesions included, but was not limited to, the following: apoptosis, single-cell necrosis, cytoplasmic alteration, and focal necrosis. Single-cell necrosis was observed in the high-dose group for male and female mice (DuPont-18405-1307, 2010). The PWG agreed that the observed single-cell necrosis was often accompanied with inflammation. Findings of apoptosis were also observed in the high-dose groups in both sexes.

Additionally, the PWG offered general observations about the histopathology reported in the original study. The NTP pathologists identified hepatocellular hypertrophy, including morphological changes such as eosinophilic stippling. The pathologists agreed that hypertrophy was present, but often less severe than reported in the original study. In addition, the pathologists recommended adding the diagnosis of cytoplasmic alteration to account for the eosinophilic, granular appearance of the cytoplasm of the hepatocytes. The pathologists recommended using this term to account for hypertrophy and eosinophilic changes as they are considered part of the same process. Cytoplasmic alteration was noted in the mid- and high-dose groups in males.

The PWG majority consensus opinion for each slide was recorded in review worksheets in a final report to EPA (see appendix D). Overall, the PWG review confirmed the results of the original study. Specifically, the PWG confirmed that single-cell necrosis was observed and is a treatment-related, adverse effect. The PWG concluded that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion individually, represents adversity within these studies (appendix D). EPA interpreted the NTP PWG's definition that the constellation of liver lesions is adverse applies to the dose group level instead of the individual animal level since the histopathological evaluation represents a snapshot in time of a biological process within one portion of the liver that can vary across animals. Table 10 presents a comparison of the incidence data for the 90-day mouse study (DuPont-18405-1307, 2010) and the NTP (2019) PWG reevaluation. Because the PWG analysis reflects more recent histopathological criteria for the grading of liver lesions, the incidence data as reported by NTP (see appendix D) were considered the more appropriate measure of response in the liver from the 90-day mouse study (DuPont-18405-1307, 2010). The NTP PWG reported that 10 out of 10 male mice exhibited cytoplasmic alteration, compared to 0 in control at the 0.5-mg/kg/day dose in this study. Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions considered adverse, no other liver lesions indicative of liver damage (i.e., single-cell or focal necrosis or apoptosis) were observed at the 0.5-mg/kg/day dose level in males. Consistent with the Hall criteria, EPA did not consider the cytoplasmic alteration findings

alone as an adverse effect in the 0.5 mg/kg/day dose group but considered the constellation of liver lesions observed across the male mice in the high-dose group as adverse. Additionally, the female mice in this study did not exhibit a dose response for the constellation of liver lesions. Based on EPA's interpretation of the NTP PWG results, EPA derived the study NOAEL for DuPont-18405-1307 (2010) of 0.5 mg/kg/day and the LOAEL is 5 mg/kg/day based on the histological findings for the liver (i.e., cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) in male and female mice.

Table 10. Comparison of Results from 90-Day Mouse Study (DuPont-18405-1307, 2010) and NTP PWG Reevaluation (NTP, 2019)

Reference	Results				
DuPont-18405-1307 (2010)	**Doses mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	9/10 [90]
	Hepatocellular hypertrophy [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	8/10 [80]	9/10 [90]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Mitotic figures [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	9/10 [90]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	0/10 [0]
	Pigment increased, Kupffer cells [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	2/10 [20]
NTP (2019) PWG Reevaluation of DuPont-18405-1307 (2010)	**Doses mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	0/10 [0]	9/10 [90]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	3/9[b] [33]
	Cytoplasmic alteration [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	10/10 [100]	10/10 [100]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	9/9[b] [100]
	Focal necrosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	1/10 [10]

Reference	Results				
	Female	1/10 [10]	0/9[a] [0]	2/9[b] [22]	3/9[b] [33]
	Apoptosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	3/9[b] [33]
	Combined Necrosis (single cell and focal necrosis) [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	0/10 [0]	9/10 [90]
	Female	1/10 [10]	0/9[a] [0]	2/9[b] [22]	4/9[b] [44]
	Constellation of lesions (cytoplasmic alteration, focal necrosis, single-cell necrosis, apoptosis) [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	10/10 [100]	10/10 [100]
	Female	1/10 [10]	0/9[b] [0]	2/9[b] [22]	9/9[b] [100]
	Mitotic figures increased [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	7/10 [70]
	Female	0/10 [0]	0/9[b] [0]	0/9[b] [0]	0/9[b] [0]
	Pigment increased [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/9[b] [0]	0/9[b] [0]	4/9[b] [44]

Notes:
[a] Slides for animal number 251 were not provided for analysis.
[b] EPA did not include animals that died due to gavage misdosing in the presentation of incidence data from the NTP PWG.

4.4 Chronic Toxicity and Carcinogenicity Studies

DuPont-18405-1238 (2013)

In a combined chronic toxicity/carcinogenicity study in 7-week-old Crl:CD(SD) rats, HFPO dimer acid ammonium salt (purity 84%) was administered by oral gavage (vehicle was deionized water) for up to 104 weeks (80/sex/group, of which 10/sex/group were designated for a 12-month interim necropsy in accordance with OECD TG 453) (DuPont-18405-1238, 2013; OECD, 2009, 2018b; Caverly Rae et al., 2015). Dose levels administered were 0, 0.1, 1, and 50 mg/kg/day for males and 0, 1, 50, and 500 mg/kg/day for females. Numerous animals in all dose groups (both male and female) were found dead or euthanized *in extremis* over the course of the study. Across all dosing groups in both male and female rats, 25.4% of the test animals survived to their planned terminal necropsy while 74.6% of the animals experienced unscheduled death/ moribundity prior to the scheduled study termination at 104 weeks. The authors state that mean survival in males and females was unaffected by treatment; however, all females were sacrificed before study termination at 101 weeks because of decreased survival across all groups, including the control. There were no statistically significant differences in survival across the female

dosing groups and female survival was comparable across all dosing groups. Among the animals that experienced unscheduled death/moribundity on study due to effects determined to be unrelated to treatment, DuPont stated the males most commonly died from pituitary tumors and undetermined causes while the females most commonly died from pituitary tumors and mammary tumors.

The females in the high-dose group were observed to have papillary necrosis and inflammation of the kidneys deemed by the authors to be related to treatment. BW and BW gain were unaffected in males but reduced compared to control (13% and 20%, respectively) in high-dose females at 52 weeks. The incidence of alopecia and hypotrichosis (abnormal patterns of hair growth) was statistically significantly increased in females at 500 mg/kg/day.

Statistically significant hematological effects were observed in this study, primarily in female rats. Blood samples were taken at 3, 6, and 12 months. At 3 months, RBC count, hemoglobin, and hematocrit were significantly decreased at the highest dose in males and females, although these decreases did not occur in a dose-dependent manner. Similarly, at 6 months, hemoglobin and hematocrit were significantly decreased at the highest dose in males, yet these decreases did not occur in a dose-dependent manner. There were no significant differences in any of these parameters in male rats at the 12-month time point. At 6 and 12 months, female rats exhibited a significant decrease in RBC count, hemoglobin, and hematocrit at 500 mg/kg/day and in a dose-dependent manner. The RBC count was also significantly decreased at 50 mg/kg/day in females at the 12-month time point; however, hemoglobin and hematocrit were not. The largest decreases compared to control in RBC count, hemoglobin, and hematocrit in female rats were 28%, 24%, and 20%, respectively, which were observed at 12 months. Additionally, the percent change from control of these effects increased over time (i.e., 3 months < 6 months < 12 months). At 12 months, serum albumin levels increased in males at 1 mg/kg/day and 50 mg/kg/day by 8% and 16% from control, respectively, which led to a concomitant increase in the A/G ratio by 16% and 28%, respectively.

Statistically significant changes from control were observed in the kidneys of females, but only at the highest dose (500 mg/kg/day). For example, there were increased incidences of tubular dilatation (increased by 34% compared to control), edema of the renal papilla (increased by 56% compared to control), transitional cell hyperplasia (increased by 39% compared to control), tubular and pelvic mineralization (increased by 15% and 24% compared to control, respectively), renal papillary necrosis (increased by 23% compared to control), and chronic progressive nephropathy (increased by 36% compared to control), all statistically significant from control. These microscopic indications of kidney damage were also associated with a 15% increase in relative kidney weight compared to control in females administered 500 mg/kg/day of HFPO dimer acid ammonium salt.

Liver enzyme levels also were affected by exposure to HFPO dimer acid ammonium salt at 12 months in the chronic study. In males, statistically significant increases in ALP (180%), ALT (228%), and SDH (141%) were observed at 50 mg/kg/day. These enzyme changes were correlated with microscopic findings in the liver, including focal necrosis. Relative liver weights were increased in high-dose males (16% compared to controls) and females (69% compared to controls) at the 12-month sacrifice. The change in liver weight in females corresponded to centrilobular hypertrophy in the high-dose females at the interim sacrifice. Females exposed to 500 mg/kg/day of HFPO dimer acid ammonium salt for 2 years also had significantly increased

relative liver weights (43% compared to control) at terminal sacrifice. There was no difference in organ weights in males at any dose at terminal sacrifice despite the changes observed at 12 months. Male and female rats exposed to 50 mg/kg/day and 500 mg/kg/day, respectively, had statistically significantly increased centrilobular hepatocellular hypertrophy compared to control rats (7/70 in treated males compared to 0/70 in control; 65/70 in treated females compared to 0/70 in control) and centrilobular hepatocellular necrosis (5/70 in treated males compared to 1/70 in control; 7/70 in treated females compared to 1/70 in control). Male rats also saw a decrease in incidence from control of 16% and 10% in focal and periportal vacuolization, respectively, at 50 mg/kg/day, and female rats had a 4% decrease from control in centrilobular vacuolation at 500 mg/kg/day. Finally, in females, panlobular hepatocellular hypertrophy (increase in incidence compared to control of 4%), individual cell hepatocellular necrosis (increase in incidence compared to control of 4%), and angiectasis (i.e., dilation of a blood or lymphatic vessel) (increase in incidence compared to control of 6%) were reported at the high dose.

Nonneoplastic effects also were observed in the stomach and tongue of females exposed to the high dose. Specifically, there were increased incidences of hyperplasia of the limiting ridge of the nonglandular stomach (increased by 13% compared to control; incidence was 9/70 for treated females and 0/70 in control) and of the squamous cell in the tongue (increased 16% from control; incidence was 13/70 in treated females and 2/70 in control). The tongue also exhibited an increased incidence of inflammation (increased 14% from control; incidence was 13/70 in treated and 3/70 in control). EPA concluded that the NOAEL for chronic toxicity in this study was 1 mg/kg/day and the LOAEL was 50 mg/kg/day for the liver effects in males.

Statistically significant increases in the incidence of liver tumors in females at 500 mg/kg/day and pancreatic acinar cell tumors in males at 50 mg/kg/day were reported. An increase in testicular interstitial (Leydig) cell tumors was noted at the high dose but was not statistically significant. Because of the observed early deaths in both control and treated male rats, EPA recommended that the submitter (a) reexamine their test data, (b) identify the animals that died without Leydig cell tumor within the first year, (c) exclude the animals identified in the previous step (i.e., those that died within the first year and had no tumors) from consideration for cancer data analysis, (d) recalculate tumor incidences, and (e) perform statistical analyses. Because the initial results indicated that the increased incidences of liver tumors in female rats (500 mg/kg/d) and combined pancreatic acinar tumors in male rats (50 mg/kg/d) were significantly increased from control despite the inclusion of early deaths, EPA decided to limit the reanalysis to testicular hyperplasia and tumors in male rats only. Additional discussion of tumor findings for the liver, pancreas, and testes is presented below.

Females. There were increases in the incidence of liver tumors at the high dose only (500 mg/kg/day), where degenerative and necrotic changes were also observed. The tumor incidences were 0/70 (0%), 0/70 (0%), 0/70 (0%), and 11/70 (15.7%) for hepatocellular adenomas and 0/70 (0%), 0/70 (0%), 0/70 (0%), and 4/70 (5.7%) for hepatocellular carcinomas at the doses of 0, 1, 50, and 500 mg/kg/day, respectively. The increased incidences of hepatocellular adenomas were statistically significant by the Cochran-Armitage trend test, the Peto test, and the pairwise Fisher Exact test and the increased incidences of hepatocellular carcinomas were statistically significant by the Cochran-Armitage trend test and the Peto test. The incidences of adenomas and carcinomas observed at 500 mg/kg/day also exceeded the test laboratory historical control ranges of 0%–5% and 0%–1.7%, respectively.

Males: A statistically significant increase was reported in the incidence of pancreatic acinar cell adenomas/carcinomas combined (but not adenomas or carcinomas alone) at 50 mg/kg/day. Incidences of pancreatic acinar cell adenomas were 0/70 (0%), 1/70 (1.4%), 0/70 (0%), and 3/70 (4.3%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively. The increased incidence at the high dose was not statistically significant and was within the test laboratory historical control range (0%–5%). The incidence of pancreatic acinar cell carcinomas was 0/70 (0%) in all groups other than the high-dose group, in which 2/70 (2.9%) were observed. The incidence of carcinomas at 50 mg/kg/day was not statistically significant but was slightly higher than the upper end of the laboratory's historical control range (0%–1.7%). When these two types of tumor were combined, the incidences of adenoma/carcinoma were 0/70 (0%), 1/70 (1.4%), 0/70 (0%), and 5/70 (7.1%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively, with the increased incidence at the high dose significant by the Cochran-Armitage trend test and the Peto test. For reference, the incidences of pancreatic acinar cell hyperplasia were 16/70 (22.9%), 18/70 (25.7%), 7/70 (10%), and 21/70 (30%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively, indicating a lack of dose-response relationship for this finding. Furthermore, the increased incidence of hyperplasia at the high dose was not statistically significant (compared to control).

In the testes, the incidences of interstitial cell adenomas were 4/70 (5.7%), 4/70 (5.7%), 1/70 (1.4%), and 8/70 (11.4%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively at 2 years. An interstitial cell adenoma was also present in 1/10 high-dose males at the interim sacrifice (12 months). The increased adenoma incidence at 50 mg/kg/day (11.4%) was not statistically significant but was slightly higher than the upper end of the testing laboratory's historical control range (0%–8.3%). For reference, the incidences of interstitial cell hyperplasia were 7/70 (10%), 7/70 (10%), 3/70 (4.3%), and 15/70 (21.4%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively. The increased incidence of hyperplasia at the high dose was not statistically significant (compared to control), although the incidence of hyperplasia at 50 mg/kg/day exceeded the historical control range (0%–8.3%). The observed incidences in the control and low-dose groups (both 10%) were also slightly above the upper end of historical controls. DuPont's reanalysis of these findings in the testes indicated that the number of male rats that died before 1 year was 4, 9, 8, and 3 in the 0 mg/kg/day (control), 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day groups, respectively. The causes of death were generally dosing injury or undetermined causes, and there were no testicular lesions or tumors in the testicular tissues of these animals. Excluding these early deaths, the incidences of testicular interstitial cell hyperplasia were 7/66 (10.6%), 7/61 (11.5%), 3/62 (4.8%), and 15/67 (22.4%) in the 0 mg/kg/day (control), 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day groups, respectively. The corresponding incidences of testicular interstitial cell adenomas were 4/66 (6.0%), 4/61 (6.6%), 1/62 (1.6%), and 8/67 (11.9%). Thus, there were no statistically significant differences for either hyperplasia or adenoma, consistent with results from the original report in which all early deaths were included. Although the incidence of testicular interstitial cell adenomas was not statistically significant compared to controls, the authors of the study conclude that "a relationship to treatment for these findings in the 50 mg/kg/day group cannot be ruled out" while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015).

Based upon EPA's review of the study, the increased incidence of liver tumors in females at 500 mg/kg/day and combined pancreatic acinar adenomas and carcinomas in males at

50 mg/kg/day are treatment related. The increased incidence of testicular interstitial cell adenoma was not statistically significant, and EPA accepted the results of the reanalysis that excluded the early deaths. EPA concluded that the NOAEL is 1 mg/kg/day and the LOAEL is 50 mg/kg/day based on the reported liver effects (i.e., centrilobular necrosis in both sexes; increased ALP, ALT, and SDH in males; and increased centrilobular hepatocellular hypertrophy and cystic focal degeneration in males).

4.5 Reproductive and Developmental Toxicity Studies

DuPont-18405-1037 (2010)

DuPont-18405-1037 (2010) was submitted to EPA under a TSCA Consent Order (see section 1.1 for more detail). Subsequently, Thompson et al. (2019), a contractor to Chemours (previously DuPont), performed a reevaluation of the study results for DuPont-18405-1037 (2010). In response to this publication, EPA requested an independent review of DuPont-18405-1037 (2010) by the NTP PWG (appendix D). The results of the original DuPont study, and these two reanalyses are described next.

In a combined oral gavage reproductive/developmental toxicity study in mice with HFPO dimer acid ammonium salt, the test compound (purity 84%) was administered by oral gavage (vehicle was deionized water) to Crl:CD1(ICR) mice (25/sex/group) at doses of 0, 0.1, 0.5, or 5 mg/kg/day, according to a modified OECD TG 421 (DuPont-18405-1037, 2010; OECD, 2016a). The male mice were approximately 6 weeks old and the female mice were approximately 10 weeks old. Parental (F_0) males were dosed 70 days prior to mating and throughout mating through 1 day prior to scheduled termination, for a total of 84 to 85 total doses. Parental F_0 females were dosed for 2 weeks prior to pairing and were dosed through LD20 for a total of 53 to 65 doses (exceptions include females with no evidence of mating or those that failed to deliver yet were administered a total of 37 to 50 doses). F_1 animals (offspring) were dosed daily beginning on PND21 through PND40.

In this study, increases in BWs and food consumption were observed at 5 mg/kg/day in F_0 animals. In F_0 males, increased mean BW gains were reported in the 5-mg/kg/day group during study days 0–49; differences from the control group achieved significance during study days 0–7, 14–21, and 21–28. Significantly higher mean BW gains were observed in this high-dose male group when the overall premating period (study days 0–69) and treatment period (study days 0–84) were evaluated. Mean BW gains were statistically significantly increased in females during both the premating period and throughout gestation at 0.5 and 5 mg/kg/day. Specifically, during the pre-mating period, BW gain increased by 100% and 70% in the 0.5- and 5-mg/kg/day-dose groups, respectively. Mean maternal GWG, calculated from individual differences, also significantly increased over the gestational period (0–18 days) by 18% and 22% in the 0.5- and 5-mg/kg/day-dose groups, respectively. At the high dose, mean BW gains were increased (5.1%–14.0%) compared to controls throughout lactation; the differences were significant on LD1, LD4, and LD21. BWs were unaffected at 0.1 and 0.5 mg/kg/day during lactation. Overall, final BW was significantly increased from control by 9% and 14% in males and females, respectively, administered 5 mg/kg/day.

The authors reported no treatment-related deaths in the F_0 mice. However, three males (one in each of the dose groups) and six females (one in the control, three in the low-dose group, and one each in the mid- and high-dose group) did not survive until scheduled sacrifice. The cause of

death was undetermined in all cases except the male in the mid-dose group, which appeared to have ulcerative dermatitis. Due to the lack of dose response, the study authors concluded that these deaths were not related to treatment. The study authors did not include the mice with premature deaths in the study results (e.g., histopathological incidence counts).

An increase in relative kidney weight compared to control by 6.5% was observed only in F_0 females at the 5-mg/kg/day dose. Mild increases in tubular cell hypertrophy were observed in the kidneys of males at greater than or equal to 0.5 mg/kg/day–6/24 mice or 25% and 18/24 mice or 75% of male mice at 0.5 mg/kg/day and 5 mg/kg/day, respectively, compared to 1/25 mice or 4% in the control. Chronic progressive nephropathy was also noted in males at 0.5 mg/kg/day (4/24 mice or 17%) and 5 mg/kg/day (5/24 mice or 21%). This effect was not associated with any evidence of tubular cell degeneration.

Liver effects also were reported in both males and females in this study. In males, mean absolute liver weights were increased 26% and 142% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, as compared to control values. Mean relative liver weights were increased by 26% and 121%, respectively, at the 0.5-mg/kg/day and 5-mg/kg/day doses. In females, mean absolute liver weights were increased by 26% and 101% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, as compared to control values. Mean relative (% BW) liver weights were increased by 17% and 80%, respectively. Microscopic findings observed in the liver of F_0 males and females administered 0.5–5 mg/kg/day included increases in hepatocellular hypertrophy, single-cell necrosis, mitotic figures, and lipofuscin pigment. F_0 females exhibited an increase in the incidence of gross white areas in the liver at 5 mg/kg/day, which correlated with microscopic focal and single-cell necrosis. At doses greater than or equal to 0.5 mg/kg/day, minimal-to-moderate hepatocellular hypertrophy was observed in both sexes, along with the corresponding increases in relative liver weight outlined above. Specifically, male mice exhibited a 50% and 100% increase in the incidence of hepatocellular hypertrophy compared to control at 0.5 mg/kg/day and 5 mg/kg/day, respectively, and similar increases in incidence was also observed in female mice (58% and 100% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, compared to control). At greater than or equal to 0.5 mg/kg/day, single-cell necrosis of hepatocytes was observed in males. Specifically, single-cell necrosis was observed in 5/24 mice at 0.5 mg/kg/day and 24/24 mice at 5 mg/kg/day compared to 1/25 mice in the control. Female mice exhibited an increase compared to control in both focal/multifocal necrosis and single-cell necrosis at 5mg/kg/day. Specifically, 5/24 mice had focal/multifocal necrosis compared to 1/24 in the control and 21/24 mice had single-cell necrosis compared to 1/24 mice in the control. Finally, the incidence of mitotic figures increased in males and females administered 5 mg/kg/day by 75% and 21% compared to control, respectively, while the incidence of lipofuscin pigment increased by 88% and 21% compared to control, respectively.

No treatment-related effects were identified for reproductive parameters (mating, fertility, and copulation indices; mean days between pairing and coitus), although male epididymal weight relative to final BW was statistically decreased at 5 mg/kg/day in both the left and right testes (12% decrease relative to control). No treatment-related effects were observed for mean gestation length, mean numbers of implantation sites, mean numbers of pups born, live litter size, percentage of males at birth, postnatal survival, or general condition of pups. At 5 mg/kg/day, however, F_1 male and female pups exhibited lower mean BWs at PND4, PND7, PND14, PND21, and PND28. F_1 male pups continued to exhibit lower mean BWs at PND35 and PND40.

Although values for the attainment of balanopreputial separation and vaginal patency (markers of pubertal onset) were within the range of historical control values, the pups showed statistically significant delays in these endpoints at 5 mg/kg/day (a finding that might be related to the observed effects on BW during the preweaning period). Additionally, the day for attainment of vaginal patency did not exhibit a dose response. The NOAEL (F_0) is 0.1 mg/kg/day, and the LOAEL is 0.5 mg/kg/day based on liver effects (single-cell necrosis in males). The NOAEL (F_1) is 0.5 mg/kg/day based on decreased pup BW and delays in attainment of balanopreputial separation and vaginal patency at the high dose.

Reanalysis of DuPont 18405-1037 (2010) published by Thompson et al. (2019)

In a publication presenting alternative approaches to deriving toxicity values and subsequent drinking water concentrations for GenX chemicals, Thompson et al. (2019) present a reevaluation of slides of liver sections in the reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Thompson et al. (2019) presents the reevaluation of the liver sections from the reproductive/developmental toxicity study in mice in the supplemental file, Table S3.

Thompson et al. (2019) reevaluated these slides using more current diagnostic criteria (Elmore et al., 2016) than those used in the original study (DuPont-18405-1037, 2010) to distinguish between apoptosis and single-cell necrosis in standard H&E-stained tissue sections. Cell death was classified as apoptosis and necrosis based on the proposed nomenclature from the Terminology Recommendations from the INHAND Apoptosis/Necrosis Working Group described by Elmore et al. (2016). The INHAND Nomenclature for Non-neoplastic Findings of the Rodent Liver was also consulted for final diagnostic nomenclature (Thoolen et al., 2010). The samples were specifically evaluated for the presence and type of individual hepatocyte necrosis. The veterinary pathologist who reviewed the slides concluded that apoptosis was the primary adverse effect of note at 5 mg/kg/day. Thompson et al. (2019) also reported increased mitosis at doses with apparent increased apoptosis; the study authors concluded that it is well established that peroxisome proliferator-activated receptor (PPAR) activators can increase mitosis and apoptosis *in vivo*. Therefore, the authors conclude that this effect is likely a part of PPARα signaling pathways that are specific to rodents. EPA identified the NOAEL for this study as 0.5 mg/kg/day and the LOAEL as 5 mg/kg/day based on increased apoptosis in male mice.

Reanalysis of DuPont 18405-1037 (2010) by National Toxicology Program Pathology Working Group (2019)

As described in section 4.3, slides from the 90-day mouse study (DuPont-18405-1307, 2010) and the reproductive/developmental study (DuPont-18405-1037, 2010) were reevaluated by an NTP PWG (see appendix D). The same protocol was used by the PWG in their analysis of each of these studies (see section 4.3 for protocol details). The NTP PWG consensus opinion for each slide was recorded on a review worksheet. Worksheets for the slides were provided as appendices A and B in the final PWG report to EPA (see the full report provided in appendix D of this assessment). Similar to the Thompson et al. (2019) publication, the NTP used the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al., 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al., 2016). For the reproductive/developmental study (DuPont-18405-1037, 2010), the PWG confirmed single-cell necrosis and focal necrosis in the mid- and high-dose groups of both sexes. Single-cell

necrosis alone and single-cell and focal necrosis combined exhibited a dose-response relationship in both sexes. The PWG agreed that the observed single-cell necrosis was often accompanied with inflammation in this study. Findings of apoptosis were observed but were limited to the highest dose groups in both sexes. Additionally, cytoplasmic alteration (which includes hepatocellular hypertrophy occurrence along with eosinophilic change to the hepatocytes) was noted in the mid- and high-dose groups in both males and females.

The PWG review confirmed the results of the original DuPont study and did not agree with the conclusion of the reanalysis published by Thompson et al. (2019). Specifically, the PWG concluded that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of the study. Table 11 presents a comparison the incidence data for the reproductive/developmental toxicity study (DuPont-18405-1037, 2010), Thompson et al. (2019), and the NTP PWG reevaluation (NTP, 2019) of DuPont-18405-1037 (2010). The incidence data as reported by NTP (see appendix D) were considered the more appropriate measure of response in the liver from the reproductive/developmental study (DuPont-18405-1037, 2010) because the PWG analysis reflects the more recent scientific histopathological criteria developed for the grading of liver lesions and the PWG results were the consensus of eight pathologists. The NTP PWG confirmed that the study NOAEL for DuPont-18405-1037 (2010) is 0.1 mg/kg/day and the LOAEL is 0.5 mg/kg/day based on the constellation of liver effects (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) in male and female mice.

Table 11. Comparison of Study Results from DuPont-18405-1037 (2010), Thompson et al. (2019), and NTP PWG Reevaluation of DuPont-18405-1037 (NTP, 2019)

Reference	Results				
DuPont-18405-1037 (2010)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	1/25 [4]	1/24 [4]	5/24 [21]	24/24 [100]
	Female	1/24 [4]	3/22 [14]	2/24 [8]	21/24 [88]
	Focal /multifocal necrosis [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	1/24 [4]	1/24 [4]
	Female	1/24 [4]	0/22 [0]	0/24 [0]	5/24 [21]
	Hepatocellular hypertrophy [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	12/24 [50]	24/24 [100]
	Female	0/24 [0]	0/22 [0]	14/24 [58]	24/24 [100]
	Mitotic figures [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	0/24 [0]	18/24 [75]

Reference	Results				
	Female	0/24 [0]	0/22 [0]	0/24 [0]	5/24 [21]
	Pigment increased [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	0/24 [0]	21/24
	Female	0/24 [0]	0/22 [0]	0/24 [0]	5/24
Thompson et al. (2019)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Apoptosis [incidence (%)]				
	Male	2/25 [8]	1/25 [0]	0/25 [0]	23/25 [92]
	Female	N/A	N/A	N/A	N/A
	Necrosis[a] [incidence (%)]				
	Male	2/25 [8]	0/25 [0]	1/25 [4]	1/25 [4]
	Female	N/A	N/A	N/A	N/A
	Mitosis [incidence (%)]				
	Male	0/25 [0]	0/25 [0]	0/25 [0]	15/25 [60]
	Female	N/A	N/A	N/A	N/A
NTP (2019) PWG Reevaluation of DuPont-18405-1037 (2010)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	1/25 [4]	1/24[b] [4]	2/24[b] [8]	23/24[b] [96]
	Female	0/24[b] [0]	2/22[b] [9]	3/24[b] [13]	19/24[b] [79]
	Cytoplasmic alteration [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	10/24[b] [42]	24/24[b] [100]
	Female	0/24[b] [0]	1/22[b] [5]	16/24[b] [67]	24/24[b] [100]
	Focal necrosis [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	4/24[b] [17]	3/24[b] [13]
	Female	2/24[b] 8]	1/22[b] [5]	4/24[b] [17]	5/24[b] [21]
	Apoptosis [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	21/24[b] [88]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	10/24[b] [42]
	Combined Necrosis (single cell and focal necrosis) [incidence (%)]				

Reference	Results				
	Male	1/25 [4]	1/24[b] [4]	6/24[b] [25]	24/24[b] [100]
	Female	2/24[b] [8]	3/22[b] [14]	6/24[b] [25]	20/24[b] [83]
	Constellation of lesions (cytoplasmic alteration, focal necrosis, single-cell necrosis, apoptosis) [incidence (%)]				
	Male	1/25 [4]	1/24[b] [4]	13/24[b] [54]	24/24[b] [100]
	Female	2/24[b] [8]	3/22[b] [14]	17/24[b] [71]	24/24[b] [100]
	Mitotic figures increased [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	17/24[b] [71]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	2/24[b] [8]
	Pigment increased [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	20/24[b] [83]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	2/23[b] [9]

Notes: N/A = not applicable
[a] Thompson et al. (2019) stated that "Emphasis was placed on evaluating the samples for the presence and type of individual hepatocyte necrosis. The two terms recommended for hepatocyte death were apoptosis and necrosis based on the proposed nomenclature from the Terminology Recommendations from the INHAND Apoptosis/Necrosis Working Group."
[b] EPA did not include animals that died due to gavage misdoing in the presentation of incidence data from the NTP PWG.

DuPont-18405-841 (2010)

In a prenatal and developmental toxicity study in 12-week-old female Crl:CD(SD) rats, HFPO dimer acid ammonium salt (purity 84%) was administered via oral gavage (vehicle was deionized water) once daily from GD6 through GD20 at doses of 0, 10, 100, and 1,000 mg/kg/day (22 females/group), according to OECD TG 414 (DuPont-18405-841, 2010; OECD, 2001b). The parental males and females were not dosed prior to or during mating and dosing for the dams was not initiated until GD6. Lack of dosing for males and females prior to and during mating and failure to dose the dams during the GD0 to GD6 period are limitations when evaluating this study to fully reflect the ability of the HFPO dimer acid ammonium salt to cause reproductive/developmental toxicity.

The dams' BW decreased at all doses, but significantly decreased (-22% compared to control) at 1,000 mg/kg/day. This decrease in BW also resulted in a decrease (-25%) in maternal GWG compared to control at 1,000 mg/kg/day. Moreover, gravid uterine weight was significantly decreased by 10% and 25% compared to control at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Food consumption in the dams was significantly decreased by 9% over the dosing period (GD6–GD21) at the highest dose. Early delivery on GD21 was observed in 18% and 41% of the dams at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Importantly, the authors noted that, in the available historical controls data for early deliveries in this rat strain (17 datasets), no females showed early deliveries (i.e., before GD21).

Statistically significant increases relative to control in absolute liver weight (12% and 34%) were observed at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Changes in liver weight relative to BW were not documented. This increase in liver weight was associated with hepatocellular hypertrophy at the high dose (19/22 rats, or 86%) and focal necrosis was observed in 9% and 23% of the dams dosed with 100 mg/kg/day and 1,000 mg/kg/day, respectively. Additionally, absolute kidney weight increased dose-dependently in the dams and was significantly increased compared to control (10%) at the highest dose. Changes in kidney weight relative to BW were not documented, and there were no notable microscopic changes in the kidney tissue of the dams. Of note is that a 1,000-mg/kg/day dam that died on GD20 had moderate multifocal/focal necrosis of the liver and disseminated intravascular coagulation in the kidney glomerular capillaries.

The pups experienced a 9% and 28% decrease compared to control in fetal weight at doses of 100 mg/kg/day and 1,000 mg/kg/day, respectively. The percentage of male (47%) and female (53%) pups born were significantly altered from control (55% male; 45% female) at 1,000 mg/kg/day. Additionally, a 14th rudimentary rib developed in 9% of the control fetuses, 10% of fetuses in the 10-mg/kg/day-dose group, 12% of fetuses in the 100-mg/kg/day-dose group, and 27% of the fetuses in the 1,000-mg/kg/day-dose group. Statistical analyses were not completed for the development of the 14th rudimentary rib in individual pups, but a statistically significant increase in the number of litters developing a 14th rudimentary rib was observed for those receiving the high dose.

The NOAEL for this prenatal and developmental toxicity study is 10 mg/kg/day based on an increase in early deliveries, decreases in gravid uterine weight, and decreased fetal weights for both sexes, all occurring at the LOAEL of 100 mg/kg/day.

Conley et al. (2019)

Conley et al. (2019) reported on two experiments evaluating the effects of oral gestational exposures to HFPO dimer acid ammonium salt. In the first experiment, pregnant Crl:CD(SD) rats were dosed from GD14 to GD18 with either water (control), or 1, 3, 10, 30, 62.5, 125, 250, or 500 mg/kg/day of HFPO dimer acid ammonium salt. HFPO dimer acid purity was 100% as determined by the supplier via perchloric acid titration. Dams were dosed during GD14 to GD18 because this window is identified as the critical period for masculinization of the male reproductive tract. The study authors stated that the experiment was completed in three separate "blocks" of animals (15 animals/block). There was a total of nine control animals (three control animals/block), three animals each for the 62.5-, 125-, 250-, and 500-mg/kg/day doses (first block) and six animals each for the 1-, 3-, 10-, or 30-mg/kg/day doses (second and third blocks). Across all three blocks, GWG, reproductive output (number of fetuses and absorptions), maternal sera, and maternal liver weight were measured. In the first two blocks, fetal testis gene expression and testosterone production, fetal BW, fetal and maternal liver gene expression, and maternal serum thyroid hormone and lipid concentrations were also evaluated. In the third block, fetal plasma was collected for determining HFPO dimer acid ammonium salt concentrations (see section 2.3.3 for detail).

A variety of effects were observed in the dams at doses greater than or equal to 30 mg/kg/day. Serum total triiodothyronine (T3) levels were decreased at doses greater than or equal to 30 mg/kg/day and total thyroxine (T4) levels decreased at doses greater than or equal to 125

mg/kg/day. Liver weight was increased on GD18 at doses greater than or equal to 62.5 mg/kg/day. Decreases in serum low-density lipoprotein (LDL) were observed at doses greater than or equal to 125 mg/kg/day and in serum high-density lipoprotein (HDL) and total cholesterol at doses greater than or equal to 250 mg/kg/day. Additionally, serum triglycerides were decreased at the highest dose tested. GWG was also decreased at doses greater than or equal to 250 mg/kg/day.

No significant effects from control were observed on the number of fetuses or resorptions.

In a second pilot experiment evaluating postnatal development, five Crl:CD(SD) dams were dosed from GD14 through GD18 with either water (control; *n*=2 pregnant dams) or 125 mg/kg/day of HFPO dimer acid ammonium salt (*n*=3 pregnant dams) (Conley et al., 2019). The single dose of 125 mg/kg/day was selected because it was the highest dose evaluated that did not cause a significant decrease in GWG during the study described above. Pup delivery began on GD22 and the following schedule was followed for postnatal monitoring:

- PND2: Pups were weighed and sexed and anogenital distance (AGD) was measured.
- PND13: Pups were weighed, sexed, and evaluated for retention of female-like nipples/areolae.
- PND27: Dams were euthanized, and uterine implantation sites scored. Pups were weaned to 2/sex/treatment group.
- PND31–PND37: F_1 female offspring were examined daily for vaginal opening (a marker of pubertal onset).
- PND41–PND45: F_1 male offspring were evaluated daily for balanopreputial separation (a marker of pubertal onset).
- PND128: F_1 females were weighed, euthanized, and examined for reproductive tract malformations. Tissue weights were recorded for the uterus, paired ovaries, liver, paired kidneys, and visceral adipose tissues.
- PND146: F_1 males were weighed, euthanized, and examined for reproductive track abnormalities. Tissue weights were collected for glans penis, ventral prostate, paired seminal vesicles, paired testes, paired epididymis, levator ani-bulbocavernosus, paired bulbourethral (Cowper's) glands, paired kidneys, and visceral and epididymal adipose tissues. Total sperm counts were measured in epididymal sections.

Viable pup number was not affected by treatment. The only significant effect in the treated F_1 generation was a decrease in right epididymis weight on a litter mean basis compared to control. However, multiple significant effects were observed on an individual pup basis. For example, F_1 female BW was significantly decreased compared to control on PND2, PND27, and at the time of vaginal opening (PND31–PND37). Additionally, AGD and liver weight were significantly decreased in F_1 female offspring on an individual pup basis. For F_1 males, paired testes, paired epididymides, right testis, right corpus/caput, right epididymis, left testis, and epididymal adipose tissue were significantly decreased compared to control on an individual pup basis.

Conley et al. (2019) conducted gene expression analyses to determine if HFPO dimer acid ammonium salt activates PPAR signaling pathways. Maternal and fetal livers and fetal testes were collected on GD18 for gene expression analyses. Gene expression was assessed using reverse transcriptase real-time polymerase chain reaction (PCR) of complementary

deoxyribonucleic acid (DNA). Maternal and fetal livers were assessed for 84 target genes relevant to PPARα, PPAR beta/delta (PPAR-β/δ), and -γ signaling pathways in the rat.

Maternal and fetal livers shared upregulation of 16 genes and most of these shared genes were associated with fatty acid metabolism. Enoyl-CoA, hydratase/3-hydroxyacyl-CoA dehydrogenase *(Ehhadh*) was the most highly upregulated gene in both the maternal (fifty-five-fold at 500 mg/kg/day) and fetal (321-fold at 500 mg/kg/day) livers. Other shared upregulated genes were associated with adipogenesis (e.g., *Ech1*), PPAR transcription factors (e.g., *Rxrg*), and PPAR ligand transporters (e.g., *Slc27a5*). Generally, the fetal liver tended to display a greater sensitivity to HFPO dimer acid ammonium salt exposure with respect to the number of genes upregulated and the magnitude of upregulation. For example, the fetal liver exhibited upregulation of 12 genes that were not affected in the maternal liver (e.g., *Pck1, Aqp7, Gk* (gluconeogenesis) and *Angptl4* (lipid transport)). Additionally, all but one of the upregulated genes shared by maternal and fetal livers (i.e., *Ech1*) was upregulated to a greater extent in the fetal liver. In maternal livers, the genes most sensitive to HFPO dimer acid ammonium salt exposure were *Ech1* and *Rxrg* and, in the fetal livers, *Cpt1b* (mitochondrial fatty acid metabolism), *Acox1* (fatty acid metabolism) and *Angptl4* were the most sensitive. These genes were significantly increased at 1 mg/kg/day of HFPO dimer acid ammonium salt.

Overall, Conley et al. (2019) concluded that HFPO dimer acid ammonium salt activated PPAR signaling pathways in maternal and fetal livers, but the effects observed in this study are not exclusive to PPARα or even general PPAR signaling.

Conley et al. (2019) also measured fetal testis testosterone production and gene expression to understand if HFPO dimer acid ammonium salt exposure produces effects similar to those of some phthalate ester metabolites. Fetal testes were collected from male pups on GD18, with a single testis from the first three male pups used for the *ex vivo* testosterone production assay and the remaining testes for gene expression analysis. Unlike some phthalate ester metabolites, there was no effect of HFPO dimer acid ammonium salt exposure on fetal testis testosterone production or on the expression of genes that are typically changed in the fetal testis by exposure to phthalates (e.g., steroidogenic enzymes).

HFPO dimer acid ammonium salt was also assessed for *in vitro* agonism and antagonism of transcriptional activation for estrogen (100 picomolar (pM) to 10 μM), androgen (100 pM to 100 μM), and glucocorticoid (100 pM to 100 μM) receptors (Conley et al., 2019). HFPO dimer acid ammonium salt displayed no agonism of any of the receptors. At 100 μM, the study authors classified HFPO dimer acid ammonium salt antagonism as slight for the glucocorticoid receptor (28% reduction in luciferase expression) and as moderate for the androgen receptor (AR) (42% reduction in luciferase expression). The study authors noted that the 100 μM dose was approaching the cytotoxic dose of 300 μM.

EPA concluded that the study NOAEL is 62.5 mg/kg/day and the LOAEL is 125 mg/kg/day based on the indications of reduced BW in F_1 females and tissue weights in F_1 animals, decreased maternal GWG, and decreased maternal serum total T4 levels. Although maternal serum total T3 levels were significantly decreased compared to control at 30 mg/kg/day, EPA selected the LOAEL at 125 mg/kg/day because the deiodination of free T4 results in the formation of T3 (Forhead and Fowden, 2014), and T4 is the thyroid hormone that preferentially crosses the placenta of humans and rodents during early gestation (Calvo et al., 2002).

Blake et al. (2020)

Blake et al. (2020) evaluated the effects of gestational PFOA and HFPO dimer acid exposure on maternal and embryonic endpoints in mice. Pregnant CD-1 dams were dosed from E1.5 to E11.5 or E17.5 with either deionized water (vehicle control), 1 or 5 mg/kg/day PFOA, or 2 or 10 mg/kg/day HFPO dimer acid. PFOA results, as they compare to HFPO dimer acid, are presented in section 2.3.3. These time points were selected because the placenta had not fully matured at E11.5 and this time point overlaps with critical periods of placental development, including vascularization with the uterine wall and chorioallantoic branching of vessels. The E17.5 time point was selected to capture treatment-related effects on embryo weight and because the placenta is fully mature at E17.5.

Blake et al. (2020) evaluated albumin, ALP, ALT, AST, BUN, total cholesterol, creatine, glucose, HDL, LDL, SDH, total bile acid, total protein, triglycerides, and urinary creatine in maternal serum at E11.5 and E17.5. Total cholesterol and HDL were significantly increased 66% and 56%, respectively, compared to vehicle control in the 2 mg/kg/day HFPO dimer acid-dose group at E11.5, but these effects did not reach statistical significance at E17.5. Additionally, serum triglyceride levels were significantly decreased at 2 mg/kg/day (-43%) and 10 mg/kg/day (-61%) of HFPO dimer acid at E11.5 and remained significantly decreased in the 10-mg/kg/day (-74%) dose group at E17.5. Finally, serum ALP was significantly increased (53%) compared to vehicle control at E17.5 in the 10-mg/kg/day HFPO dimer acid-dose group.

Absolute and relative maternal liver weights significantly increased compared to vehicle control at both time points and in both HFPO dimer acid dose groups. Specifically, absolute liver weight increased by 41% and 91% and relative liver weights increased 37% and 73% compared to vehicle control at 2 and 10 mg/kg/day, respectively, at E11.5. At E17.5, absolute liver weight increased by 30% and 70% and relative liver weights increased 31% and 69% compared to vehicle control at 2 and 10 mg/kg/day, respectively. A variety of hepatocellular lesions were observed to increase as compared to vehicle control, including cytoplasmic alteration, mitotic figures, cell death (included both apoptosis and single-cell necrosis), and vacuolation. At E11.5, all dosed livers presented with cytoplasmic alteration, which increased in severity at the 10 mg/kg/day HFPO dimer acid dose. Mitotic figures and cell death increased in both dose groups and vacuolation rated as minimal was observed in 100% of the 10 mg/kg/day-dose group livers. At E17.5, all dosed livers presented with more severe cytoplasmic alteration than at E11.5, and this cytoplasmic alteration was most severe in the 10-mg/kg/day HFPO dimer acid-dose group. Mitotic figures were no longer increased at E17.5 and increased cell death was only observed in the 10-mg/kg/day-dose group. Vacuolation rated as minimal and mild was observed in the 10-mg/kg/day-dose group. Additionally, a portion of E17.5 livers from all dose groups were processed for transmission electron microscopy (TEM). As compared to vehicle control, the livers from the 2- and 10-mg/kg/day HFPO dimer acid-dose groups exhibited “abnormal ultrastructure with enlarged hepatocytes containing more abundant cytoplasmic organelles consistent with mitochondria and peroxisomes and vacuolation” (Blake et al., 2020). Additionally, the livers in the 10-mg/kg/day HFPO dimer acid-dose group presented “vacuolation often with remnant membrane material as myelin figures, abundant rough endoplasmic reticulum with few ribosomes present, and unevenly dispersed glycogen appearing as clustered clumps” (Blake et al., 2020).

Absolute and relative kidney weights were unchanged at E11.5. Absolute (19%) and relative (16%) kidney weight was increased compared to vehicle control in the 10-mg/kg/day HFPO dimer acid-dose group at E17.5. No histopathologic changes in kidneys were noted in any dose groups.

GWG was significantly increased (30%) relative to vehicle control at E11.5 in the 10-mg/kg/day HFPO dimer acid-dose group. When controlling for litter size, GWG was significantly greater in the 10-mg/kg/day HFPO dimer acid-dose group than in vehicle control at both E11.5 (7.1%) and E17.5 (19.1%). Finally, GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at E17.5 using effect estimates from mixed effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day.

Implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than vehicle control in any dose group. Placental weight was significantly increased by ~15.5 milligrams (mg) and the embryo:placental weight ratio significantly decreased by 15% in the 10-mg/kg/day HFPO dimer acid-dose group relative to vehicle control at E17.5. Additionally, placentas from litters (an average of seven individual placentas per litter) per treatment group and sacrifice time point were evaluated for histopathology. There were no significant histopathological changes at E11.5 between vehicle control and the HFPO dimer acid dose groups, with nearly all the placentas evaluated within normal limits. However, 58% and 83% of placentas evaluated at E17.5 were classified as abnormal in the 2 and 10 mg/kg/day HFPO dimer acid dose groups, respectively, compared to 2% in the vehicle control group. The number of abnormal placentas in the 10 mg/kg/day HFPO dimer acid dose group was significantly different than vehicle control. The most frequent lesion detected was labyrinth atrophy, which was observed in 0/41 (0%), 15/31 (48%), and 16/35 (46%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Labyrinth congestion and early fibrin clots increased with increasing HFPO dimer acid dose. Specifically, labyrinth congestion was observed in 0/41 (0%), 1/31 (3%), and 8/35 (23%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively, and early fibrin clot was observed in 0/41 (0%), 1/31 (3%), and 4/35 (11%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Placental lesions were evaluated against the proportion of placentas within a litter within normal limits to account for litter effects, and the proportion of abnormal placentas was significantly higher at the 2- and 10-mg/kg/day HFPO dimer acid-dose groups relative to vehicle control. Finally, placental thyroid hormones (reverse triiodothyronine (rT3), T3, and T4) were quantified at E17.5 from 2-3 pooled placental tissues of same-sex embryos. Each pooled sample was considered as one biological replicate and three replicates were used for each sex and treatment group. There was no significant effect of sex or treatment on rT3, T3, T3:T4 ratio, or rT3:T4 ratio. A significant increase (60%) in T4 relative to vehicle control was reported for the 10-mg/kg/day HFPO dimer acid-dose group.

The authors noted that, in some HFPO dimer acid-exposed dams, gross anomalies were apparent, including excess abdominal fluid, edematous tissues, and clotted placentas.

EPA concluded that there is no NOAEL for this study because the study LOAEL is 2 mg/kg/day, which is the lowest dose tested. The LOAEL is based on increased incidence of placental lesions within a litter and increased GWG using effect estimates from mixed-effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day in maternal mice.

Conley et al. (2021)

In a follow-up to their 2019 study, Conley et al. (2021) reported on two experiments evaluating the effects of oral gestational exposures to HFPO dimer acid ammonium salt. In one experiment, pregnant Crl:CD(SD) rats were dosed once daily by gavage from GD16 to GD20 with either water (control) or 1, 3, 10, 30, 62.5, or 125 mg/kg/day of HFPO dimer acid ammonium salt. HFPO dimer acid purity was 100% as determined by the supplier via perchloric acid titration. The study authors stated that the experiment was completed in two separate "blocks" of animals (15 animals/block). There were three control animals/block (total of six control animals) and two animals/treatment group/block (total of four treated animals/group). In both blocks, dams and fetuses were euthanized on GD20 and maternal and fetal sera were collected for determining HFPO dimer acid concentrations (see section 2.3.3 for detail). Maternal serum was also analyzed for thyroid hormone concentrations (total T3 and total T4) and clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)). Maternal weight gain, reproductive output (number of fetuses and resorptions), and maternal liver weight were measured. Maternal liver samples were collected for determining HFPO dimer acid concentrations and gene expression analyses. In the first block, two male and two female fetuses were randomly selected from each litter for measurements of body and liver weight and HFPO dimer acid concentration in liver samples. The individual body weights of the remaining fetuses were recorded irrespective of sex. Because there was no indication of an effect of sex on fetal body weight in the first block, body weights in the second block were recorded for three randomly selected fetuses per litter (irrespective of sex); out of those fetuses, one was randomly selected per litter to determine liver weight and HFPO dimer acid concentration in the liver and for gene expression analyses.

There were no significant differences observed for fetal body weight or liver weight (broken out by sex or combined), maternal body weight gain, or maternal terminal body weight in any dose groups compared with controls. Maternal liver weight was increased at 62.5 and 125 mg/kg/day. Maternal serum T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day. Albumin was decreased at 3, 62.5, and 125 mg/kg/day. Triglycerides were decreased at doses ≥10 mg/kg/day and cholesterol was decreased at doses ≥30 mg/kg/day. There were no significant effects on the numbers of viable fetuses or resorptions in any dose groups compared with controls.

In another experiment reported in Conley et al. (2021), pregnant Crl:CD(SD) rats (five per group) were dosed once daily by gavage from GD8 to PND2 with either water (control) or 10, 30, 62.5, 125, or 250 mg/kg/day of HFPO dimer acid ammonium salt. Dams gave birth naturally and were checked for parturition beginning on GD22. Once delivery was complete, pups were counted and the litter weight was recorded. All pups were returned to the nest except for two randomly selected pups per litter that were sacrificed. Trunk blood was collected, and serum was analyzed for HFPO dimer acid concentration and clinical chemistry parameters. Livers were collected for histopathological examination and gene expression analyses. The carcasses of three deceased newborn pups (one each from 30, 125, and 250 mg/kg/day dose groups) were sent for histopathological examination. On PND2, dams received their final dose in the morning and were weighed and euthanized 2–5.5 hours later. Maternal trunk blood was collected, and serum was analyzed for thyroid hormone concentrations (total T3 and total T4), clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)), and HFPO dimer acid concentration. Maternal liver weight was recorded, and liver samples were collected for gene expression analyses and HFPO dimer acid determination. Uterine implantation

sites were scored. The pups were sexed and weighed, anogenital distance was measured, trunk blood was collected, liver weight was recorded (one male and one female per litter), and liver samples were analyzed for HFPO dimer acid concentration. PND2 pup serum was analyzed for clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)) and HFPO dimer acid concentration.

All pups were alive at birth with no remarkable gross external malformations. Dams displayed typical nesting behaviors; however, shortly after delivery, pups in the higher dose groups began displaying lethargy, morbidity, or were found dead. Pups continued to die or require euthanasia throughout PND0 and PND1. Pup survival was reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day. Many of the pups that died had visible milk bands indicating they had nursed. Pup survival scores on PND2 were 100 ± 0, 96 ± 2, 97 ± 2, 87 ± 5, 38 ± 13, and 5 ± 5% in the 0, 10, 30, 62.5, 125, 250 mg/kg/day groups, respectively. Pup survival scores were significantly decreased at doses ≥62.5 mg/kg/day Pup body weight gain (birth to PND2) and PND2 body weight in the surviving pups were both reduced at doses ≥30 mg/kg/day. Anogenital distance was not affected in male or female pups, but relative liver weight was increased in all dose groups. No remarkable histopathological lesions were observed in pup livers, but glycogen accumulation scores in the liver were significantly lower in all dose groups compared with control pups. Significant changes were observed in some pup serum clinical chemistry parameters. Glucose was decreased at doses ≥62.5 mg/kg/day in newborn pups and at doses ≥125 mg/kg/day in PND2 pups. Albumin was decreased at 62.5 and 250 mg/kg/day only in newborn pups. Cholesterol was increased at doses ≥125 mg/kg/day in newborn pups and at doses ≥62.5 mg/kg/day in PND2 pups. Triglycerides were increased at doses ≥125 mg/kg/day in newborn pups, and AST was increased at doses ≥30 mg/kg/day in PND2 pups.

Purple discoloration of the entire right hind limb was observed in one pup each from the 30, 125, and 250 mg/kg/day dose groups beginning on PND1 and those pups were examined for histopathology. All three had milk protein in the stomach lumen, vascular thrombi in various vessels, and small dense basophilic cells throughout liver lobes. The two from the higher dose groups also had moderate subcutaneous hemorrhage in the area of the umbilical artery and vein. Subcutaneous edema or vascular congestion of the lower limb was observed in the pups from the 30 and 250 mg/kg/day dose groups.

A variety of significant adverse effects were observed in the dams. Maternal body weight (on GD22 and PND2) and gestational weight gain were reduced at doses ≥125 mg/kg/day. At necropsy on PND2, maternal absolute liver weight was increased at doses ≥30 mg/kg/day, and relative liver weight was increased at all dose levels. There was no significant effect on the number of uterine implants. Maternal serum total T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day (with the exception of T3 at 250 mg/kg/day). Albumin was decreased at 250 mg/kg/day, and triglycerides were increased at 125 and 250 mg/kg/day. Serum AST was increased at all dose levels. The study authors noted that, even though maternal serum and levels of HFPO dimer acid ammonium salt did not increase when dosing was extended from 4 days in the fetal study to 16 days in the postnatal study (see section 2.3.3), maternal liver weight was more affected in the postnatal study and at lower dose levels.

Conley et al. (2021) also conducted gene expression analyses using liver samples from both experiments (GD16–20 and GD8–PND2) to determine if HFPO dimer acid ammonium salt activates PPAR signaling pathways or alters genes related to glucose and glycogen metabolism.

Gene expression was assessed using reverse transcriptase real-time polymerase chain reaction (PCR) of complementary deoxyribonucleic acid (DNA) synthesized from ribonucleic acid (RNA) extracted from sample homogenates. Maternal (GD20), fetal (GD20), and neonatal (PND0) livers were assessed for 84 target genes relevant to PPARα, PPARβ/δ, and PPAR-γ signaling pathways in the rat. Fetal and neonatal livers were also assessed for 84 genes involved in the regulation and enzymatic pathways of glucose and glycogen metabolism.

Expression of five genes related to glucose metabolism were affected in the GD20 fetal livers. Four genes (*Pck1, Pdk4, G6pc, Pdp2*) were significantly upregulated compared with controls and one (*Ugp2*, critical to glycogen synthesis) was significantly downregulated. All genes were significantly different at doses ≥10 mg/kg/day, except for *G6pc* (critical to gluconeogenesis) which was significantly different at doses ≥3 mg/kg/day. *Pck1* (critical to gluconeogenesis) was the most highly upregulated gene (37.5-fold compared with control at the highest dose). No genes were significantly affected at 1 mg/kg/day.

Conley et al. (2021) compared the gene alterations observed in PPAR signaling pathways for fetal livers exposed GD16 to 20 (this study) with those exposed GD14 to 18 (reported in Conley et al., 2019). All 28 genes involved in PPAR signaling that were significantly upregulated on GD18 were also upregulated on GD20, and 16 of these genes had a highly significant interval effect with greater upregulation on GD20 than on GD18. The remaining upregulated genes did not differ significantly between GD18 and GD20. There were no significantly downregulated PPAR signaling genes. Overall, Conley et al. (2021) concluded that greater gene expression effects were observed later in gestation on genes that code for proteins critical to mitochondrial (*Acaa2, Acadm, Cpt1a*) or peroxisomal (*Acox1, Ech1, Ehhadh*) fatty acid β-oxidation or both (*Mlycd*), gluconeogenesis (*Pck1*), glycerol metabolism (*Gk*), fatty acyl-CoA conversion (*Acsl1, Acsl3*), mediation of triglyceride clearance (*Angptl4*), triglyceride biosynthesis (*Dgat1*), fatty acid biosynthesis (*Fads2, Scd1*), and PPAR coactivation (*Rxrg*).

Analysis of maternal livers showed that the 19 PPAR signaling genes that were upregulated on GD18 (Conley et al., 2019) were also upregulated on GD20, and seven of those showed greater upregulation on GD20. The upregulated genes code for proteins critical to mitochondrial and peroxisomal fatty acid β-oxidation, ketogenesis, fatty acid transport, fatty acyl-CoA conversion, triglyceride turnover, carnitine transport, mitochondrial protein import, accumulation of reactive oxygen species, and transcriptional coactivation. Conley et al. (2021) concluded that the data from this study provide evidence for PPARα activation in the maternal, fetal, and neonatal livers following exposure to HFPO dimer acid ammonium salt.

Gene expression analyses of newborn pup livers showed that 13 glucose metabolism genes were upregulated and 15 were downregulated, 11 of which were significantly different from controls in all dose groups. *Pdk4* was upregulated and *Ugp2* was downregulated, similar to fetal livers, but *Pck1* and *G6pc* were unaffected. The most highly affected upregulated genes were *Fbp2* (gluconeogenesis) and *Ldha* (anaerobic glycolysis); the most highly affected downregulated genes included *Aldob* (glycolysis), *Agl* (glycogen degradation), *Ugp2* (glycogen synthesis), and *Gsk3a* (glycogen synthesis). There were 21 upregulated and 8 downregulated PPAR signaling pathway genes, 21 of which were significantly different from controls in all dose groups. Several gene expression changes were unique to PND0 livers including *Fabp2* (downregulated, a lipid sensor and high affinity long-chain fatty acid binding protein), *Slc27a5* (downregulated, activates very long-chain fatty acids and bile acids), *Apoc3* (downregulated, associated with metabolism of

triglyceride-rich lipoproteins), *Ppara* (downregulated, codes for the PPAR alpha nuclear receptor), and *Cd36* (upregulated, has pleiotropic effects associated with angiogenesis, inflammation, and fatty acid metabolism). Overall, Conley et al. (2021) concluded that many genes associated with carbohydrate and lipid metabolism were affected at multiple stages of development by HFPO dimer acid ammonium salt exposure.

Conley et al. (2021) observed multiple significant adverse effects when dams were dosed with HFPO dimer acid ammonium salt from GD8 to PND2. Significant pup mortality was observed at doses ≥62.5 mg/kg/day and growth rates were significantly lower at doses ≥30 mg/kg/day. Newborns displayed hypoglycemia at doses ≥62.5 mg/kg/day, elevated serum lipid levels at doses ≥125 mg/kg/day, and significantly lower glycogen accumulation scores in the PND2 livers of all dose groups. Maternal body weights were decreased at doses ≥125 mg/kg/day, and maternal relative liver weight was increased in all dose groups. Maternal serum total T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day (with the exception of T3 at 250 mg/kg/day). The authors noted that disruption of carbohydrate and lipid metabolism across the maternal-placental-fetal unit (beginning in the 1 mg/kg/day dosing group) were likely key events in the observed adverse effects, including decreases in pup body weight and survival. EPA concluded that the study NOAEL is 10 mg/kg/day and the LOAEL is 30 mg/kg/day based on reduced BW in F_1 pups at PND0 and PND2.

4.6 Other Studies

4.6.1 Immunotoxicity Studies

Rushing et al. (2017)
Male and female C57BL/6 mice (6–12/sex/group) were administered HFPO dimer acid by gavage at doses of 0, 1, 10, or 100 mg/kg/day for 28 days (Rushing et al., 2017). The animals were immunized with sheep RBC antigen on day 24 and, 5 days later, were evaluated for TDARs and splenic lymphocyte subpopulations. Organs were collected 1 day after the final gavage exposure.

T lymphocyte numbers were significantly increased (the average increase of $CD8^+$, $CD4^+/CD8^+$, and $CD4^-/CD8^-$ T cells was 74%) in males at 100 mg/kg/day, yet suppression of TDAR was observed in female mice only at 100 mg/kg/day. TDAR suppression was measured through immunoglobulin M (IgM) antibody production, which decreased by 7.3% in females at the high dose. Liver weight relative to BW significantly increased (40%–160%) in both sexes at 10 mg/kg/day in a dose-dependent manner. Relative spleen weights significantly decreased by 11% in females treated with 100 mg/kg/day, and there were no significant changes in thymus weight.

Peroxisomal fatty acid oxidation was measured using hepatic acyl-CoA oxidase activity as a readout. In male mice, hepatic acyl-CoA oxidase activity increased 122% and 222% at 10 mg/kg/day and 100 mg/kg/day, respectively. Female mice had a 100% increase in acyl-CoA oxidase activity at the highest dose tested. The NOAEL for immune effects that include TDAR suppression in females and increased T cells in males is 10 mg/kg/day.

4.6.2 Mechanistic Studies

The studies in this section provide mechanistic insight into the effects of HFPO dimer acid and/or its ammonium salt. Available studies address biological mechanisms applicable to liver

effects, serum lipids and lipoproteins, thyroid hormones, and developmental effects. Of note, many of the studies outlined here report using dimethyl sulfoxide (DMSO) to prepare HFPO dimer acid or its ammonium salt. This is important because a 2020 publication (Gaballah et al., 2020) demonstrates that HFPO dimer acid is unstable in DMSO but is stable in deionized water. Where reported, EPA has listed the vehicle that the study authors used to dissolve these chemicals and the vehicle control.

Wang et al. (2017)

In one study investigating changes in gene expression, male ICR mice (*n*=12/group) were administered either (control) or 1 mg/kg/day HFPO dimer acid ammonium salt prepared in 0.5% Tween-20 via oral gavage for 28 days (Wang et al., 2017). Although the authors state that HFPO dimer acid was tested and its chemical structure is presented, the CASRN is listed as 62037-80-3, which is the HFPO dimer acid ammonium salt. Nevertheless, whether the chemical evaluated was the acid or the ammonium salt does not impact the form dissolved in serum or plasma. In both cases, the HFPO dimer anion is present in solution.

At the end of 28 days, blood samples were collected and analyzed. After sacrifice the liver was recovered for measurement of organ weight and histological examination. High-throughput ribonucleic acid (RNA)-sequencing was conducted to gain mechanistic insights into the observed liver effects. Liver tissue samples from three controls and three treated animals were frozen for RNA isolation, library preparation, and sequencing.

Statistically significant treatment-related findings reported include increased absolute liver weight (31%) and relative liver weight (28%), ALP (51%), LDL cholesterol (50%), decreased total bilirubin (-37%), and decreased direct bilirubin (-45%) when compared to control. Qualitative hepatic histopathological findings documented abnormalities from the treated animals, including lipid droplet accumulation, hepatocellular hypertrophy, mild steatosis, and karyolysis.

High-throughput RNA-sequencing of liver tissues resulted in the identification of 146 transcripts (101 upregulated and 45 downregulated) with altered differential gene expression due to treatment with the HFPO dimer. Pathway analyses (using the National Center for Biotechnology Information, Ensemble, gene ontology, and Kyoto Encyclopedia of Genes and Genomes databases) revealed four enriched pathways from these altered hepatic transcripts: the PPAR signaling pathway, arachidonic acid (an essential polyunsaturated fatty acid) metabolism, retinol metabolism, and fatty acid degradation. All four of these pathways are associated with lipid metabolism. Gene ontology analyses of the 146 altered transcripts identified several other enriched processes, cellular components, and molecular functions related to immune system function, lipid metabolism, membrane parameters, and others that were altered by HFPO dimer acid treatment.

Behr et al. (2018)

H295R, MDA-kb2, HEK293T, LNCaP, and MCF-7 cell lines were cultured and incubated with various individual PFAS in a variety of experiments to investigate effects on cytotoxicity, estrogen and AR activity, and steroidogenesis (Behr et al., 2018). The study authors do not report how the HFPO dimer acid ammonium salt was prepared but report 99% purity.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to assess cell viability in HEK293T, H295R, MCF-7, and LNCaP cell lines following exposure to various concentrations of each PFAS. HEK293T and LNCaP cells were exposed for 24 hours, H295R cells for 48 hours, and MCF-7 cells for 6 days. The WST-1 assay was used to determine viability of MDA-kb2 cells following 24 hours of exposure to each PFAS. Although the study authors do not report how the HFPO dimer acid ammonium salt was prepared, 0.1% DMSO was used as the vehicle control in this assy. HFPO dimer acid ammonium salt (referred to as "PMOH" (ammonium perfluoro(2-methyl-3-oxahexanoate)) in this study) was not cytotoxic in HEK293T, MDA-kb2, H295R, or LNCaP cell lines up to concentrations of 500 μM and caused cytotoxicity in the MCF-7 cell line at 500 μM.

HEK293T cells were assayed for agonistic and antagonistic estrogen receptor (ER) alpha and beta (ERα and ERβ) transactivation. None of the tested PFAS were found to affect either ERα or ERβ at the highest tested concentrations (500 μM for HFPO dimer acid ammonium salt). Estrogen co-exposure with 500 μM HFPO dimer acid ammonium salt was found to co-stimulate ERβ activity. Additionally, HFPO dimer acid ammonium salt was found to enhance estrogen-mediated ERβ activation.

The agonistic and antagonistic AR reporter gene assay was performed in MDA-kb2 cells. HFPO dimer acid ammonium salt was found to be negative for AR transactivation and inhibition up to concentrations of 100 μM. HFPO dimer acid ammonium salt enhanced dihydrotestosterone-stimulated AR activity in a dose-responsive fashion at concentrations above 50 μM.

A steroidogenesis assay was performed in which H295R cells were exposed for 48 hours and enzyme-linked immunosorbent assay (ELISA) kits were used to quantify estradiol (E2), estrone, testosterone, and progesterone levels. HFPO dimer acid ammonium salt significantly decreased testosterone at 100 μM.

An E-screen assay was used to evaluate proliferation of MCF-7 cells following 6 days of exposure to various PFAS. HFPO dimer acid ammonium salt did not significantly affect cell proliferation compared to estrogen. Exposure to high concentrations of HFPO dimer acid ammonium salt (100 μM) in combination with estrogen slightly diminished cell proliferation, but the effect was not statistically significant.

MCF-7 and LNCaP cells were cultured with HFPO dimer acid ammonium salt for 24 hours, and H295R cells were cultured for 48 hours prior to RNA extraction followed by quantitative reverse transcription PCR. HFPO dimer acid ammonium salt did not stimulate estrogenic responsive gene expression of *TFF1, GREB1, PGR, ESR1, ESR2,* or *CTSD* in MCF-7 cells, or *AR, PSA, NKX3-1, TMPRSS2,* or *CDKN1A* in LNCaP cells at concentrations up to 100 μM. Additionally, HFPO dimer acid ammonium salt did not affect expression of *CYP19A1, CYP17A1, CYP21A2, CYP11A1, STAR,* or *HSD3B* at concentrations up to 100 μM.

Sheng et al. (2018)

Sheng et al. (2018) used *in vitro* experiments to investigate perfluoroalkyl cytotoxicity and binding to proteins for HFPO dimer acid ammonium salt (referred to as "HFPO-DA" in this study), HFPO dimer acid trimer, HFPO dimer acid tetramer, PFOA, and perfluorooctane sulfonate (PFOS) in a human liver HL-7702 cell line. The study authors assessed cell viability to determine the cytotoxicity of the various perfluoroalkyl substances and used flow cytometry to

investigate effects on cell proliferation. The authors noted, however, that no effects of HFPO dimer acid ammonium salt on cytotoxicity and cell proliferation could be determined through these assays because of the chemical's low boiling point and high volatility. The study authors do not report how the HFPO dimer acid ammonium salt was prepared.

Data quantifying the HFPO dimer acid anion's ability to bind to human liver fatty acid-binding protein (hL-FABP) was also generated. Binding affinity was explored because other PFAS compounds have exhibited effective binding to hL-FABP and such binding might explain how PFAS can enter into hepatocytes, a potential target cell for HFPO dimer acid and/or its ammonium salt (Luebker et al., 2002; Sheng et al., 2016; Zhang et al., 2013). Binding affinity was measured in a fluorescence competitive binding assay and found that HFPO dimer acid anion exhibited a weaker binding affinity than PFOA or PFOS. However, the study found that the HFPO dimer acid anion fit well in the hL-FABP binding pocket with a docking energy in between PFOS and PFOA. This indicates direct interaction between the HFPO dimer acid anion and hL-FABP. Additionally, the HFPO dimer acid anion bound differently to hL-FABP than PFOA and PFOS (Sheng et al., 2018). These results were replicated using a predictive model of binding affinity to hL-FABP (Cheng and Ng, 2018).

Li et al. (2019)

Li et al. (2019) investigated the binding affinity of HFPO dimer acid (referred to as "HFPO-DA" in the paper), HFPO trimer acid (HFPO-TA), and PFOA to human and mouse PPAR gamma (PPARγ) ligand binding domains. PPARγ is a second member of the PPAR family of nuclear receptors. It functions as a regulator of cell proliferation and differentiation in addition to impacting lipid metabolism. The study authors report that HFPO dimer acid was dissolved in DMSO to make stock solutions and was reported as 97% pure. Binding affinity was measured in a fluorescence competitive binding assay. The study authors observed a higher affinity for the human PPARγ ligand binding domain for HFPO-TA and PFOA, while HFPO dimer acid bound with greater affinity for the mouse PPARγ ligand binding domain. Among the three PFAS tested, a binding potency order of HFPO-TA> PFOA>HFPO dimer acid was identified for both human and mouse ligand binding. Li et al. (2019) also assessed the activity of HFPO dimer acid, HFPO-TA, and PFOA using HEK293 cells transfected with a luciferin-tagged PPARγ vector. After exposure to HFPO dimer acid, HFPO-TA, and PFOA, the luciferase activity of the cells was quantified as an indicator of the PFAS's ability to impact PPARγ transcription. The authors conclude that HFPO dimer acid, HFPO-TA, and PFOA acted as transcriptional agonists, resulting in enhanced PPARγ transcriptional activity in a dose-dependent manner.

Because PPARγ activation is involved in the modulation of adipogenesis (Tontonoz et al., 1994), Li et al. (2019) also exposed cultured human (HPA-s) and mouse (3T3-L1) preadipocytes to the three compounds for ten days during a period of cellular differentiation into adipocytes. To quantify adipogenic activity, an Oil Red O staining assay was performed to quantify lipid accumulation using the dosed human and mouse adipocytes. HFPO dimer acid significantly increased lipid accumulation at 6 μM and 25 μM for the human HPA-s cells and mouse 3T3-L1 cells, respectively. HFPO dimer acid showed comparable or weaker adipogenesis activity than PFOA and HFPO-TA. Relative PPARγ messenger RNA (mRNA) levels were statistically significantly increased in human HPA-s cells exposed to 25 μM HFPO dimer acid and at 50 μM HFPO dimer acid in mouse 3T3-L1 cells.

Sun et al. (2019)

Three dimensional (3D) spheroids were used to evaluate the cytotoxicity of PFOA, HFPO dimer acid (referred to as "HFPO-DA" in this study) and PFO4DA (3,5,7,9-tetraoxadecanoic perfluoro acid) (Sun et al., 2019). HFPO dimer acid was diluted with serum-free DMEM/F-12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) medium and was reported as 95% pure. 3D spheroids were exposed to 100 μM of each of these three substances for 28 days. No changes in adenosine triphosphate (ATP) content or albumin secretion were observed. Lactic dehydrogenase and reactive oxygen species levels were significantly increased (compared with controls) after PFOA, HFPO dimer acid, or PFO4DA exposure. A 1.5- to twofold increase in *Scd1* expression (compared with control) was observed in PFOA, HFPO dimer acid, and PFO4DA exposure groups. PFOA exposure, but not HFPO dimer acid exposure, significantly increased PPARα expression compared to the control. For the apoptosis-related genes, PFOA exposure significantly increased expression of *caspase3*, *p53*, and *p21* compared to control, whereas HFPO dimer acid exposure produced no changes. For the oxidative stress genes, only PFOA significantly increased *Nqo1* expression, and PFOA, HFPO-dimer acid, and PFO4DA all significantly induced expression of *Gsta2* and *Ho-1*.

Xin et al. (2019)

Estrogenic effects of PFOA and HFPO dimer acid (referred to as "HFPO-DA" in this study) were evaluated in a series of *in vitro* assays (Xin et al., 2019). All tests were also performed on additional HFPO homologs (HFPO-TA and hexafluoropropylene oxide tetramer acid (HFPO-TeA)). HFPO dimer acid was prepared in DMSO at a concentration of 20 millimolar (mM) and was reported as 97% pure.

HFPO dimer acid binding affinity to the human ERα and ERβ ligand binding domains were compared to that of PFOA using a fluorescence polarization-based competitive fluorescence binding assay. HFPO dimer acid did not bind either ERα or ERβ (not detected; no IC_{50} (concentration at which 50% inhibition is observed) could be derived). PFOA displaced estrogen in a concentration-dependent manner, with IC_{50} values of 469.5 μM for ERα and 384.4 μM for ERβ.

The cytotoxic effects of HFPO dimer acid at concentrations ranging from 0.8 to 1,600 μM were determined in MVLN cells using the WST-1 assay. HFPO dimer acid produced no cytotoxicity, whereas PFOA inhibited cell viability at concentrations above 800 μM. MVLN cells were also exposed to PFOA or HFPO dimer acid at concentrations ranging from 1.6 to 800 μM, with or without E2 for 12 or 24 hours, and estrogenic/anti-estrogenic activity was assessed. Exposure to HFPO dimer acid did not result in any effects on ERs. PFOA exposure resulted in concentration-dependent antagonism of ERs and PFOA was also found to compete with E2 to activate ERs.

ELISA kits were used to measure E2, testosterone, and vitellogenin (VTG) in wildtype zebrafish larvae exposed to 0.4 or 1.6 μM PFOA or HFPO dimer acid for 168 hours post-fertilization. HFPO dimer acid and PFOA significantly increased E2, testosterone, and VTG compared to controls in all dose groups, except for VTG levels at 0.4 μM HFPO dimer acid.

Molecular docking and molecular dynamics simulations were performed using AutoDock 4.2 to compare binding interactions of HFPO dimer acid and PFOA with ERα and ERβ. The simulations illustrated that both HFPO dimer acid and PFOA fit into the binding cavity of ERα

and ERβ. The calculated energies from the simulations indicated that the order of the binding affinity for these compounds is HFPO-TeA > HFPO-TA > PFOA > HFPO dimer acid.

Behr et al. (2020)

The cytotoxicity, human nuclear receptor activation, and gene expression changes induced by HFPO dimer acid ammonium salt (referred to as "PMOH" (ammonium perfluoro(2-methyl-3-oxahexanoate)) in this study) was investigated *in vitro* using HEK293T and HepG2 cells. Seven other PFAS (PFOA, PFOS, PFHxA, perfluorobutanesulfonic acid (PFBS), PFBA, PFHxS, and 3H-perfluoro-3-((3-methoxypropoxy) propanoic acid (PMPP) were also analyzed in this study (Behr et al., 2020). The study authors do not report how the HFPO dimer acid ammonium salt was prepared.

The cytotoxicity of HFPO dimer acid ammonium salt was assessed in HepG2 cells. The cells were exposed to 50–500 μM of HFPO dimer acid ammonium salt for 24 hours, and cellular viability was determined using the MTT assay. Cell viability was not significantly decreased at any concentration.

Luciferase-based reporter gene assays were used to determine the ability of HFPO dimer acid ammonium salt to activate various human nuclear receptors that function in the regulation of lipid or xenobiotic metabolism. HEK293T cells were transfected with expression plasmids for hCAR, hFXR, hLXRα, hPPARα, hPPARδ, hPPARγ, hPXR, hRARα, or hRXRα. The cells were co-transfected with a luciferase reporter plasmid and the *Renilla*-luciferase construct pcDNA3-Rluc for normalization. Positive controls were included. Receptor activity was measured after 24 hours of exposure to 25, 50, or 100 μM of each chemical. Values were normalized to *Renilla reniformis* luciferase activities and compared to untreated cells. HFPO dimer acid ammonium salt significantly induced PPARα activation (sevenfold) at 25 μM and higher. HFPO dimer acid ammonium salt also significantly induced activation of PPARγ (2.4-fold) at 100 μM. The other human nuclear receptors were not significantly affected. Reporter gene assays for PPARα were repeated for PFOA and PMOH using concentrations up to 250 μM. Concentration-response curves were calculated and EC_{10} values were determined relative to a positive control (GW7647). HFPO dimer acid ammonium salt activated PPARα to a level of 10% at 5 μM, and a comparable activation was induced by PFOA at 50 μM.

HepG2 cells were exposed to concentrations up to 250 μM HFPO dimer acid ammonium salt for 24 hours, and the RNA was extracted for analysis of PPARα-dependent target gene expression. Untreated cells served as control. At 250 μM, PMOH significantly induced expression of *CPT1A* (1.7-fold), *HMGCS2* (2.8-fold), and *PLIN2* (1.4-fold). Compared to PFOA, the effects of PMOH were not as substantial. PFOA produced similar effects on the target genes as PPARα agonists GW7647 and WY14,643.

Although HFPO dimer acid ammonium salt was a more potent PPARα agonist than PFOA under the conditions of this study, it produced weaker effects on PPARα-dependent target gene expression.

Wen et al. (2020)

The epigenetic toxicities of HFPO dimer acid (referred to as "GenX" in this study) and PFOA were explored and compared *in vitro* using a liver hepatocellular carcinoma cell line (Wen et al., 2020). HepG2 cells were exposed to concentration gradients of the ammonium salt form of

PFOA (20–600 μM) or HFPO dimer acid (20–1,000 μM) for 48 hours. The test chemicals were first dissolved in DMSO (< 0.4% volume/volume), and vehicle controls were included. HFPO dimer acid was reported as 97% pure.

The MTT and neutral red assays were used to assess cell metabolism rates and viability. Following HFPO dimer acid exposure, cell metabolic activity was only slightly increased at all concentrations compared to control. Cell viability was increased from 20 to 200 μM, and then decreased linearly from 200 to 1,000 μM.

Following PFOA exposure, cell metabolic activity increased in a concentration-dependent fashion from 0 to 100 μM, peaked at 100 μM, and decreased in a concentration-dependent fashion from 100 to 400 μM. Cell viability decreased with increasing PFOA concentration (decreased by 87% at 600 μM).

Gene expression analysis was performed for 22 genes related to cell cycle, proliferation, apoptosis, and lipid metabolism. HepG2 cells were cultured in flasks and treated with 100–600 μM HFPO dimer acid for 48 hours, and the RNA was extracted for gene expression analysis. Overall, HFPO dimer acid did not have a strong impact on the genes examined; expression of most lipid metabolism and transport-related genes was either decreased or not significantly affected by HFPO dimer acid. In contrast, expression of lipid synthesis-related genes was mostly elevated, and expression of lipid transport genes was mostly decreased by PFOA.

Global methylation assays were performed using genomic DNA from HepG2 cells extracted immediately after the treatment period. Expression profiles of 10–11 translocation methylcytosine dioxygenases (*TET*s) and DNA methyltransferases (*DNMT*s) were also evaluated. In HFPO dimer acid-treated cells, global methylation (5mc) levels significantly decreased from 100 to 400 μM, and then increased from 600 to 800 μM. GenX caused decreased expression of *DNMT*s but had no clear effect on *TET*s. PFOA caused a significant, concentration-dependent decrease in global methylation (5-mC) levels from 20 to 400 μM, and significant concentration-dependent changes in *TET*s (*TET1* decreased whereas *TET2* and *TET3* increased with increasing PFOA concentration), but no significant trends in the expression of *DNMT*s.

Cannon et al. (2020)

The effects of HFPO dimer acid ammonium salt (referred to as “GenX” in this paper) on expression and activity of three ATP binding cassette (ABC) transporters at the blood-brain barrier were studied using rat brain capillaries exposed *ex vivo* to low nanomolar (nM) concentrations (Cannon et al., 2020). Rats were also exposed to 97% pure HFPO dimer acid ammonium salt *in vivo* followed by *ex vivo* measurement of transport activity. ATPase levels were measured *in vitro*, and protein levels were measured with Western blotting. The cytotoxicity of HFPO dimer acid ammonium salt was assessed using two human cell lines. HFPO dimer acid ammonium salt was prepared in fresh DMSO (0.1% volume/volume) prior to each experiment.

The brains from 4–6 male or female Hsd:Sprague Dawley rats (age 12–15 weeks) were harvested and capillaries were isolated from cortical gray matter. Capillaries were exposed to varying concentrations of HFPO dimer acid ammonium salt (0.01–1,000 nM) for 3 hours and P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-

associated protein 2 (MRP2) transporter activities were measured with a confocal microscopy-based method. Hourly changes in transport activities were also measured during a 4-hour exposure to 100 nM HFPO dimer acid ammonium salt. In addition, reversibility assays were performed using 100 nM HFPO dimer acid ammonium salt exposure for 1 hour for P-gp and 2 hours for BCRP. After transport activities were measured, HFPO dimer acid ammonium salt was removed from the assay media and transport activities were measured at 0.5, 1, 2, and 3 hours after removal. In the main assays, P-gp transport activity was significantly lower in male capillaries exposed to 0.1–100 nM HFPO dimer acid ammonium salt and in female capillaries exposed to 1.0–100 nM HFPO dimer acid ammonium salt compared with controls. P-gp transport activity was significantly decreased beginning at 15 or 30 minutes of treatment with 100 nM and persisted to the 4-hour mark. BCRP transport activity was significantly lower in male capillaries exposed to 1.0–1,000 nM HFPO dimer acid ammonium salt and in female capillaries exposed to 0.1–1,000 nM, and a significant decrease in activity during a 4-hour exposure to 100 nM was observed beginning at the 1-hour time point for both sexes. MRP2 transport activity was not significantly affected by HFPO dimer acid ammonium salt. In the reversibility assays, P-gp transport activity in capillaries from both sexes was restored to control levels within 1 hour of HFPO dimer acid ammonium salt removal, but BCRP transport activity remained lowered for 2 hours after removal for both sexes.

A reconstituted transport assay system containing vesicle membranes and transport proteins was used to determine the effects of HFPO dimer acid ammonium salt on transport-associated ATPase activity *in vitro*. Purified P-gp and BRCP transport proteins were exposed to 0.001–1.0 μM HFPO dimer acid ammonium salt for 20 minutes and the enzymatic hydrolysis of ATP to inorganic phosphate was measured. The substrate was stimulated with paclitaxel for P-gp and sulfasalazine for BCRP. HFPO dimer acid ammonium salt did not alter ATPase activity associated with P-gp or BCRP transport either when the substrate was stimulated or when no substrate was added, indicating that HFPO dimer acid ammonium salt was not a substrate for either transporter using this particular *in vitro* reconstituted transport assay system.

Isolated brain capillaries pooled from male or female rats (n=6 rats/sex) were exposed to 100 nM HFPO dimer acid ammonium salt for 4 hours, and P-gp and BCRP protein levels were measured by Western blotting. No significant differences in P-gp or BCRP protein levels were identified for treated capillaries compared with control (vehicle-treated) capillaries for either sex.

Isolated brain capillaries from male or female rats (n=6 rats/sex) were also treated with HFPO dimer acid ammonium salt (1.0 or 100 nM) for 4 hours with or without the PPARγ inhibitor GW9662. HFPO dimer acid ammonium salt decreased P-gp transport activity at both concentrations for both sexes compared with controls. The addition of GW9662 blocked the reduction in activity in male capillaries at both concentrations, but only at 1.0 nM HFPO dimer acid ammonium salt in female capillaries. BCRP transport activity was lowered by treatment at both concentrations in both sexes, and co-treatment with GW9662 had no effect on the reduced BCRP transport activities for either sex.

Male and female Hsd:Sprague Dawley rats (5/sex/group) were administered a single oral gavage dose of 0, 10, 100, or 1,000 ng/kg (30 picomole (pmol)/kg, 300 pmol/kg, or 3 nanomole/kg) of HFPO dimer acid ammonium salt and sacrificed 5 hours later. Brains from each dose group were pooled, and capillaries were isolated for measurement of P-gp and BCRP transport activities. All

dose levels produced significant decreases in P-gp and BCRP transport activities in both sexes compared with controls.

Cell survival following HFPO dimer acid ammonium salt exposure was determined using the human ovarian cell line NCI/ADR-RES (with high P-gp expression) and human mammary epithelial cell line MX-MCF-7 (with high BCRP expression). The cells were first exposed to HFPO dimer acid ammonium salt alone at increasing concentrations of 10^{-9}–10^{-4} M for 72 hours, and the remaining cells were counted. No significant differences in survival were observed. Next, to determine if HFPO dimer acid ammonium salt affected the toxicity of known cytotoxic substrates for P-gp or BCRP, each cell line was exposed to 100 nM HFPO dimer acid ammonium salt for 72 hours in the presence of Adriamycin (for NCI/ADR-RES cells) or mitoxantrone (for MX-MCF-7 cells). Co-treatment of 100 nM HFPO dimer acid ammonium salt and 10 μM Adriamycin significantly reduced NCI/ADR-RES cell survival from 85% to 45%, and co-treatment of 100 nM HFPO dimer acid ammonium salt and 100 μM mitoxantrone significantly reduced MX-MCF-7 cell survival from 63% to 37%.

The results of these assays show that both *ex vivo* and *in vivo* exposure to low nM levels of HFPO dimer acid ammonium salt can inhibit P-gp and BCRP transport in rat brain capillaries. The effect of HFPO dimer acid ammonium salt on P-gp transport was shown to involve PPARγ.

4.6.3 Genotoxicity Studies

HFPO dimer acid ammonium salt was not observed to induce genetic mutations both with and without metabolic activation of the test substance by rat liver S9 fraction in two species of prokaryotes: *Escherichia coli* (strain WP2uvrA) and *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA 1537) (DuPont-19713 RV1, 2008; DuPont-22734 RV1, 2008). An *in vitro* gene mutation test of the HFPO dimer acid ammonium salt in mouse lymphoma cells (strain L5178Y/TK+/-) was negative in the presence and absence of rat liver S9 fraction (DuPont-26129, 2008). HFPO dimer acid ammonium salt was observed to induce chromosomal aberrations in Chinese hamster ovary cells *in vitro* in the presence and absence of S9 activation (DuPont-19714 RV1, 2008; DuPont-22620 RV1, 2009). In *in vivo* mammalian studies, exposure to HFPO dimer acid ammonium salt by the oral route did not induce chromosomal mutations in the form of structural aberrations, numerical aberrations, or micronuclei nor DNA effects in the form of unscheduled DNA synthesis (DuPont-23219, 2007; DuPont-23220, 2007). A table summarizing the findings of the available genotoxicity studies is provided in appendix C.

5.0 Summary of Hazard

The available studies indicate adverse effects including liver, developmental, hematological, and immune effects occur following exposures in the range of 0.5–1,000 mg/kg/day GenX chemicals. Table 12 presents the available studies and their NOAELs and LOAELs. Discussion of the weight of evidence for hazard is presented following the table.

Table 12. Summary of Study NOAELs/LOAELs

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
28 Day Oral (Gavage) Toxicity Study in Rats (OECD, 2008a) DuPont-24447 (2008)	Medium	Males: 0, 0.3, 3, and 30 Females: 0, 3, 30, and 300	NOAEL = 0.3 LOAEL = 3	Hematological effects (↓ RBC count, hemoglobin, and hematocrit in males) Immune effects (↓ globulin, and ↑ A/G ratio in males)
28 Day Oral (Gavage) Toxicity Study in Mice (OECD, 2008a) DuPont-24459 (2008)	Medium/Low	Males and Females: 0, 0.1, 3, and 30	NOAEL = 0.1 LOAEL = 3	Liver effects (single-cell necrosis in males, ↑ relative liver weight in in males, and ↑ hepatocellular hypertrophy in males) Hematological effects (↓ hemoglobin and hematocrit in males) Immune effects (↓ globulin in females, and ↑ A/G ratio in both sexes)
28 Day Oral (Gavage) Immunotoxicity Study in Mice Rushing et al. (2017)	Medium	Males and Females: 0, 1, 10, and 100 Note: HFPO dimer acid	NOAEL = 10 LOAEL = 100	Immune effects (TDAR suppression in females, and ↑ lymphocytes in males)
90 Day Oral (Gavage) Toxicity Study in Rats (OECD, 1998) DuPont-17751-1026 (2009)	High	Males: 0, 0.1, 10, and 100 Females: 0, 10, 100, and 1,000	NOAEL = 0.1 LOAEL = 10	Hematological effects (↓ RBC count, hemoglobin, and hematocrit in males)

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
90 Day Oral (Gavage) Toxicity Study in Mice (OECD, 1998) DuPont-18405-1307 (2010); Reevaluation by NTP PWG Pathology (NTP, 2019)	High	Males and Females: 0, 0.1, 0.5, and 5	NOAEL = 0.5 LOAEL = 5	Liver effects (↑AST, ALT, and ALP in males; ↑ relative liver weight in males and females; and ↑ in constellation of liver lesions: cytoplasmic alteration, single-cell necrosis, focal necrosis, and hepatocellular apoptosis in males and females)
Combined Chronic Toxicity/ Oncogenicity Study in Rats (OECD, 2009) DuPont-18405-1238 (2013)	Medium	Males: 0, 0.1, 1, and 50 Females: 0, 1, 50, and 500	NOAEL = 1 LOAEL = 50	Liver effects (centrilobular necrosis in both sexes; ↑ ALP, ALT, and SDH in males; and ↑ centrilobular hepatocellular hypertrophy and cystic focal degeneration in males)
Oral (Gavage) Reproduction/ Developmental Toxicity Study in Mice (OECD, 2016a; modified according to the Consent Order) DuPont-18405-1037 (2010); Reevaluation by NTP PWG Pathology (NTP, 2019)	High	Males and Females: 0, 0.1, 0.5, and 5	NOAEL (F_0) = 0.1 LOAEL (F_0) = 0.5 NOAEL (F_1) = 0.5 LOAEL (F_1) = 5	Liver effects ((single-cell necrosis, focal necrosis, and cytoplasmic alteration), and ↑ relative liver weight in males and females); reproductive/developmental effects (↑ maternal GWG from GD0 through GD18) Developmental effects (↓ pup weights, and delays in the attainment of balanopreputial separation and vaginal patency)

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
Prenatal and Developmental Toxicity Study in Rats (OECD, 2001b) DuPont-18405-841 (2010)	Medium	Females: 0, 10, 100, and 1,000	NOAEL (F_0 and F_1) = 10 LOAEL (F_0 and F_1) = 100	Developmental effects (↑ early deliveries, ↓ fetal weights in both sexes, ↓ gravid uterine weight, and focal liver necrosis)
Reproductive and Developmental Toxicity in Rats Conley et al. (2019)	Medium	Females: 0, 1, 3, 10, 30, 62.5, 125, 250, and 500	NOAEL (F_0 and F_1) = 62.5 LOAEL (F_0 and F_1) = 125	Reproduction/developmental effects (↓ maternal GWG, , and indications of reduced body (females) and reproductive and non-reproductive organ weights in F_1 animals) Thyroid effects (↓maternal serum total T3 and T4 levels)
Reproductive and Developmental Toxicity in Mice Blake et al. (2020)	Medium	Females: 0, 2, and 10	NOAEL = NA LOAEL = 2	Reproductive/developmental effects (↑ mean abnormal placental lesions (including labyrinth atrophy, labyrinth congestion, labyrinth necrosis, early fibrin clot, and placental nodule), and ↑ maternal GWG)
Reproductive and Developmental Toxicity in Rats Conley et al. (2021)	High	Females: 0, 10, 30, 62.5, 125, or 250	NOAEL (F_0) = 30 LOAEL (F_0) = 62.5 NOAEL (F_1) = 10 LOAEL (F_1) = 30	Thyroid effects (↓maternal serum total T3 and T4 levels) Reproductive/developmental effects (↓ BW in F1 pups at PND0 and PND2)

5.1 Hepatic

The liver is a target organ for toxicity from oral exposure to HFPO dimer acid and its ammonium salt. Liver effects are observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals. Liver effects are also the endpoints that are observed at the lowest doses for these chemicals. Hepatocellular hypertrophy and an increased liver-to-BW ratio are common findings in rodents but are considered nonadverse and less relevant to humans when there is evidence that PPARα activation is the only MOA. The increased relative liver weight and hepatocellular hypertrophy were only considered adverse when accompanied by effects such as necrosis, fibrosis, inflammation, and significantly increased serum levels for enzymes indicative of liver tissue damage (Hall et al., 2012).

Significant increases in liver weight relative to BW were observed in male and female Crl:CD(SD) rats and several strains of male and female mice treated with 0.5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 28–90 days (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017). These increases were observed in doses as low as 0.5 mg/kg/day in male Crl:CD-1 mice (26% increase) over 84–85 days (DuPont-18405-1037, 2010), and the greatest increases were observed when male (163%) and female (102.7%) Crl:CD-1 mice were administered 30 mg/kg/day for 28 days. Likewise, male Crl:CD(SD) rats exhibited increased relative liver weights of 19%–61% compared to control when administered 3 mg/kg/day–100 mg/kg/day for 28–90 days, while female rats' relative liver weights compared to control did not increase until much higher doses (12% at 300 mg/kg/day for 28 days and 85% at 1,000 mg/kg/day for 90 days) were administered. Comparatively, the one available chronic study in rats indicates that liver weight may increase and return to control levels after a time. For example, relative liver weights in male rats increased only 15% when administered 50 mg/kg/day for 1 year and did not exhibit a significant increase from control at 2 years. Likewise, female rat relative liver weights increased 67% and 42% after administration of 500 mg/kg/day for 1 and 2 years, respectively (DuPont-18405-1238, 2013).

Indications of liver damage were also reflected through increases in serum liver enzymes of Crl:CD-1 mice, particularly males, and Crl:CD(SD) rats administered HFPO dimer acid ammonium salt. For example, significant increases in ALT (420%–1,254%), AST (106%–478%), ALP (1,134%–1,221%), and SDH (1,134%–1,221%) were observed in male mice administered the ammonium salt at 5–30 mg/kg/day for 28–90 days. Female mice saw smaller increases in ALP (140%–143%) and SDH (32%–186%) compared to male mice administered the same dose. Overall, rats exhibited far fewer and smaller increases in serum liver enzyme levels following subchronic exposure than the mouse, with increases in AST (106%) and ALP (52%) at 100 mg/kg/day in male rats and AST (66%) in female rats at 1,000 mg/kg/day. In the chronic study, however, ALT (228%), ALP (180%), and SDH (140%) significantly increased in male rats only when administered 50 mg/kg/day for 1 year (DuPont-18405-1238, 2013).

Liver damage was confirmed microscopically in male and female mice and rats in several less-than-chronic studies (15–90 days) and one chronic study (DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Wang et al., 2017; Thompson et al., 2019; NTP, 2019). The most prevalent liver effects following both subchronic and chronic exposure to

HFPO dimer acid and/or its ammonium salt were hepatocellular hypertrophy (also referenced here as cytoplasmic alteration per NTP PWG's review) and single-cell and/or focal necrosis.

In both sexes of mice exposed either short term (28 days) or subchronically (30–90 days), hepatocellular hypertrophy was observed at 0.5 mg/kg/day, while male and female rats showed this effect at 3 mg/kg/day and 30 mg/kg/day, respectively. Interestingly, in the chronic study, male rats did not show any significant increases in hepatocellular hypertrophy when administered 0.1–50 mg/kg/day of HFPO dimer acid ammonium salt for 1 year, and only 10% of the rats exhibited minimal hypertrophy with 50 mg/kg/day administered for 2 years (DuPont-18405-1238, 2013). Conversely, female rats had significant hepatocellular hypertrophy at 500 mg/kg/day after 1 year (100%) and 2 years (93%).

Single-cell and focal necrosis were detected in all the available studies. The reanalysis of the liver pathology slides from DuPont 18405-1037 (2010) by Thompson et al. (2019) did not report necrosis in mice. This interpretation conflicts with the results from the original pathology conducted in DuPont 18405-1037 (2010) and the 2019 NTP PWG reanalysis (NTP, 2019) of DuPont 18405-1037 (2010). The incidence data as reported by NTP (see appendix D) were considered the appropriate measure of response in the liver from the reproductive/developmental study (DuPont-18405-1037, 2010) because the PWG analysis reflects more recent histopathological criteria for the grading of liver lesions and the PWG results were the consensus of eight pathologists.

In the subchronic toxicity studies in mice, males and females presented with single-cell and focal necrosis in doses as low as 0.5 mg/kg/day, which significantly increased at 5 mg/kg/day. Specifically, the incidence rates for single-cell and focal necrosis at 5 mg/kg/day were 100% and 83% in males and females, respectively, in DuPont 18405-1037 (2010) and 90% and 44% in males and females, respectively, in DuPont 18405-1307 (2010) (NTP, 2019). Apoptosis was observed in the 5 mg/kg/day-dose groups in these studies as well, but not in the 0.5 mg/kg/day-dose group (NTP, 2019). As noted in section 4.0 and appendix D in this assessment, the NTP PWG agreed that the dose response and constellation of liver lesions (i.e., hepatocellular hypertrophy, single-cell and focal necrosis and apoptosis) observed in DuPont 18405-1037 (2010) and DuPont 18405-1307 (2010) should be considered as adverse (NTP, 2019). Male and female rats exhibited hepatocellular necrosis at much higher doses in the available short-term study, with males exhibiting what was classified as general necrosis (30%) at 30 mg/kg/day and females presenting focal liver necrosis at 100 mg/kg/day (9%) and 1,000 mg/kg/day (23%). Interestingly, no liver necrosis was reported for either sex in the subchronic rat study (DuPont-17751-1026, 2009). It is possible that apoptosis could have been present in the other DuPont studies, but these studies might not have separated apoptotic lesions from other liver lesions reported (i.e., single-cell necrosis) since they were conducted prior to the histopathological guidance on separating apoptosis from single-cell necrosis (i.e., Elmore et al., 2016) and were not reanalyzed by the 2019 NTP PWG.

These findings suggest that mice are more sensitive to liver necrosis than rats in short-term and subchronic exposure scenarios. In the 2-year chronic rat study, centrilobular necrosis increased at 50 mg/kg/day and 500 mg/kg/day for males (7%) and females (4%), respectively, while single-cell necrosis was observed only in females (4%) at 500 mg/kg/day. Taken together, the male rat liver necrosis data appear to be inconsistent. Specifically, 30% of male rats have necrotic liver cells after 28 days of dosing with 30 mg/kg/day of HFPO dimer acid ammonium salt, yet no

necrosis is reported in male rats after 90 days of dosing with 0.1–100 mg/kg/day. However, necrosis returns in 50% of male rats after 1 year of dosing with 50 mg/kg/day to then be reduced to 7–13% incidence after 2 years of dosing.

Similarly, these data suggest that the pregnant rodent might be more susceptible than nonpregnant rodents to liver effects following exposures to GenX chemicals. Liver effects were reported in the pregnant dams in the available reproductive/developmental studies dosing during gestation (DuPont-18405-841, 2010; Conley et al., 2019; DuPont 18405-1037, 2010; Blake et al., 2020). All the studies reported increases in liver weight ranging from 12% to 34% in rats and 26% to 101% in mice over the gestational period. Conley et al. (2019) did not conduct liver histopathology, but both DuPont-18405-841 (2010) and Blake et al. (2020) reported hepatocellular hypertrophy and increased cell death as compared to control with increasing HFPO dimer acid ammonium salt concentration. Specifically, focal necrosis was observed in 2/22 (9%) and 5/22 (23%) pregnant rats after 15 days (GD6–GD20) of 10 mg/kg/day or 100 mg/kg/day of HFPO dimer acid ammonium salt, respectively, compared to 0 in the control group. Comparatively, nonpregnant female rats dosed from 28 to 90 days did not exhibit necrosis when treated with doses up to 1,000 mg/kg/day of HFPO dimer acid ammonium salt. Necrosis was observed in female rats only after 2 years of dosing with 500 mg/kg/day of HFPO dimer acid ammonium salt. Increased cell death (including both apoptosis and single-cell necrosis) or focal necrosis was observed in pregnant mice after 11 and 17 days (GD1.5–GD11.5 or 17.5) of 2 mg/kg/day or 10 mg/kg/day of HFPO dimer acid ammonium salt. Similarly, and as noted above, female mice dosed 14 days prior to mating and throughout gestation/lactation exhibited cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis after 53–64 days of dosing (NTP, 2019 reanalysis of DuPont 18405-1037, 2010). The incidence of single-cell and focal necrosis in the F_0 females was 6/24 (25%) and 20/24 (83%) in the 0.5- and 5-mg/kg/day-dose groups, respectively (NTP, 2019).

5.2 Hematological

The hematologic system could be a target of HFPO dimer acid ammonium salt toxicity as effects have been observed across studies of varying durations of oral exposure to the chemical. The primary effects observed are decreases in RBC number, hemoglobin, and percentage of RBCs in the blood, indicating that oral exposure to HFPO dimer acid ammonium salt might promote anemic conditions. In male mice and rats, the percent change in these effects from the controls was relatively small. For example, male Crl:CD-1 mice and Crl:CD(SD) rats treated with 3 mg/kg/day–100 mg/kg/day of HFPO dimer acid ammonium salt for 28–180 days had maximum decreases of 12%, 11%, and 12% in hemoglobin, erythrocyte count, and hematocrit, respectively (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008). Interestingly, in the available chronic study, no hematological effects were observed at the 12-month time point in male rats (DuPont-18405-1238, 2013). Female Crl:CD-1 mice and Crl:CD(SD) rats presented hematological effects at greater than 90 days and typically at higher doses than males, with one exception. Hemoglobin significantly decreased by 4% when female Crl:CD(SD) rats were administered 1 mg/kg/day of HFPO dimer acid ammonium salt for 90 days (DuPont-18405-1238, 2013). Otherwise, hematological effects occurred at doses greater than or equal to 50 mg/kg/day and the maximum decreases from control were 24%, 28%, and 20% for hemoglobin, erythrocyte count, and hematocrit, respectively (DuPont-18405-1238, 2013; DuPont-24447, 2008).

5.3 Renal

The kidney could also be a target organ for toxicity from oral exposure to HFPO dimer acid and/or ammonium salt; however, kidney effects typically presented at higher doses than the liver effects.

Significant increases in kidney weight relative to BW were observed in several less-than-chronic studies in Crl:CD-1 mice and Crl:CD(SD) rats treated with 0.1 mg/kg/day–1,000 mg/kg/day (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-24459, 2008; DuPont-24447, 2008). The maximum increase in kidney weight for male rodents was an increase of 16% compared to control in male rats treated with 100 mg/kg/day of HFPO dimer acid ammonium salt over 90 days. Likewise, the maximum kidney weight relative to BW increase in female rodents was 23% in female rats administered 1,000 mg/kg/day over 90 days (DuPont-17751-1026, 2009). Interestingly, increases in relative kidney weights were not observed in the same type of male rat when administered HFPO dimer acid ammonium salt for 1 or 2 years (DuPont-18405-1238, 2013). Relative kidney weight did increase in female Crl:CD(SD) rats by 25% and 14% when administered 500 mg/kg/day of HFPO dimer acid ammonium salt for 1 and 2 years, respectively (DuPont-18405-1238, 2013).

These increases in kidney weight were often associated with increases in BUN, which can be used as an indicator of renal damage. In several studies, urea nitrogen levels were significantly increased (16%–38%) in male mice and rats administered doses greater than or equal to 30 mg/kg/day of HFPO dimer acid ammonium salt for 28–180 days (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-24447, 2008; DuPont-24459, 2008). Female rats exhibited an increase in urea nitrogen levels (35%) only when administered 500 mg/kg/day of HFPO dimer acid ammonium salt for 1 year (DuPont-18405-1238, 2013). Kidney damage was equivocal microscopically in the less-than-chronic studies (28–90 days), and typically presented as increases in basophilic tubular cells and tubular epithelial hypertrophy or dilation without tubular degeneration and/or necrosis (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-24459, 2008; DuPont-24447, 2008).

In the chronic study, the increases in BUN and relative kidney weight noted above for female rats were associated with multiple microscopic observations of kidney damage when female rats were treated with HFPO dimer acid ammonium salt for 2 years. For example, at 50 mg/kg/day–500 mg/kg/day, female rats exhibited transitional cell hyperplasia, tubular dilation, pelvic and tubular mineralization, and papillary edema, which ultimately resulted in papillary necrosis at 500 mg/kg/day (DuPont-18405-1238, 2013).

To summarize, significant and dose-dependent increases in relative kidney weight occurred in rats at lower doses (e.g., 10 mg/kg/day) in a subchronic study (DuPont-18405-1307, 2010). Kidney hypertrophy, however, was not associated with microscopic damage of the kidney such as necrosis in this study. Additionally, there are instances in which kidney hypertrophy occurred at low doses in female mice (e.g., 0.1 mg/kg/day (DuPont-24459, 2008) or 5 mg/kg/day (DuPont-18405-1037, 2010)), but there was not a dose response in these datasets, and microscopic damage to the kidney tissues was not reported. Of the available studies, kidney hypertrophy was associated with significant microscopic damage only in female rats treated with 500 mg/kg/day of HFPO dimer acid ammonium salt for 2 years (DuPont-18405-1238, 2013). Thus, the observed kidney effects are potentially of concern. The biological significance,

however, of the observed hypertrophy and increases in BUN without microscopic evidence of kidney damage is not clear.

5.4 Reproductive/Developmental

Evidence in animals suggests HFPO dimer acid and/or ammonium salt could target the reproductive system and the developing fetus.

In a reproduction/developmental toxicity mouse study, there were no effects on mating, fertility, or copulation indices; mean days between pairing and coitus; mean gestation length; mean numbers of implantation sites; mean numbers of pups born; live litter size; percentage of males at birth; postnatal survival; or the general condition of pups (DuPont-18405-1037, 2010). Similarly, implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than control when pregnant mice were dosed with 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E17.5 (Blake et al., 2020). In the rat developmental toxicity study, however, early delivery on GD21 was observed in 18% and 41% of the dams at 100 mg/kg/day and 1,000 mg/kg/day, respectively, and the percentage of male (47%) and female (53%) pups born was significantly altered from control at 1,000 mg/kg/day (DuPont-18405-841, 2010). Conley et al. (2019) reported no significant effects from control on the number of fetuses or resorptions in pregnant rats dosed with 1–500 mg/kg/day HFPO dimer acid from GD14 through GD18. Conley et al. (2021) also reported no significant effects on the numbers of viable fetuses or resorptions in pregnant rats dosed with 1–125 mg/kg/day HFPO dimer acid from GD16 through GD20. However, pup survival was significantly reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day in pups born to dams dosed from GD8 to PND2. Specifically, pup survival percentages on PND2 were 100 ± 0, 96 ± 2, 97 ± 2, 87 ± 5, 38 ± 13, and 5 ± 5% in the 0–250 mg/kg/day groups, respectively.

Changes in maternal GWG were a consistently observed effect. In pregnant rats dosed during gestation and through PND2, maternal GWG significantly decreased 25%–70% compared to control at doses greater than or equal to 125 mg/kg/day of HFPO dimer acid ammonium salt (DuPont-18405-841, 2010; Conley et al., 2019, 2021). Conversely, pregnant mice dosed during gestation saw increases in maternal GWG ranging from 7% to 22% at doses as low as 0.5 mg/kg/day (DuPont-18405-1037, 2010; Blake et al, 2020). It is unclear why this response is different for mice and rats, but in Blake et al. (2020), the study authors hypothesize that it could be the result of differences in the exposure window or interspecies toxicokinetic differences in elimination rates. Specifically, the available rat studies dosed from GD6 through GD20 (DuPont-18405-841, 2010), GD14–GD18 (Conley et al., 2019), GD16-GD20 (Conley et al., 2021), or GD8-PND2 (Conley et al., 2021), while the mice were dosed earlier in gestation (GD1.5–GD17.5) in Blake et al. (2020) and 14 days prior to mating through LD21 in DuPont-18405-1037. Additionally, the elimination $T_{1/2}$ in urine (see section 2.3.5) for female mice (18 hours) is much longer than for female rats (8 hours) and there are also differences in the alpha and beta phase $T_{1/2}$ for female rats and mice (see Table 8).

Blake et al. (2020) presented data indicating that the placenta might be a target of GenX chemical exposure. Placental lesions were detected in 58% and 83% of mouse placentas evaluated after dosing with 2 and 10 mg/kg/day of HFPO dimer acid from E1.5 to E17.5, respectively, compared to 2% in the control group (Blake et al., 2020). The most frequent lesion detected was labyrinth atrophy, which was observed in 0/41 (0%), 15/31 (48%), and 16/35 (46%)

placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Labyrinth congestion and early fibrin clots increased with increasing HFPO dimer acid doses. These placental lesions are indicative of a placental insufficiency phenotype (Blake et al., 2020). Additionally, placental weights increased in the 10-mg/kg/day-dose group and large placentas are associated with adverse health outcomes in neonates and adult offspring (Hutcheon et al., 2012; Risnes et al., 2009). It is unclear how these effects might impact reproductive and developmental outcomes.

In some of the available developmental studies, there was also a decrease in rodent pup weight that ranged from 9% to 24% when the pups were exposed to 5 mg/kg/day–1,000 mg/kg/day in utero (DuPont-18405-841, 2010; DuPont-18405-1037, 2010; Conley et al., 2019, 2021). The mouse pups showed delays in attaining balanopreputial separation and vaginal patency at 5 mg/kg/day of 2.6 days and 3.4 days, respectively, which could be related to the observed effects on BW during the preweaning period (DuPont-18405-1037, 2010). Additionally, the attainment of vaginal patency did not exhibit a dose-response relationship. The decrease in pup weight was associated with a decrease in gravid uterine weight by 10% and 25% at 100 mg/kg/day and 1,000 mg/kg/day, respectively, in the rat prenatal developmental toxicity study (DuPont-18405-841, 2010). Moreover, in a rat prenatal developmental study, a 14th rudimentary rib developed in 9% of the control fetuses, 10% of fetuses in the 10-mg/kg/day dose, 12% of fetuses in the 100-mg/kg/day dose, and 27% of the fetuses in the 1,000-mg/kg/day dose (DuPont-18405-841, 2010). Statistical analyses were not completed on the development of the 14th rudimentary rib in individual fetuses, but a statistically significant increase in the number of litters developing a 14th rudimentary rib was observed at the high dose. Conley et al. (2019) reported significant effects for the F_1 generation in their postnatal pilot study where F_0 pregnant rats were dosed with 125 mg/kg/day of HFPO dimer acid ammonium salt from GD14 through GD18. F_1 male pups had a decrease in right epididymis weight on a litter mean basis compared to control. Multiple significant effects were observed on an individual pup basis, including AGD and liver weight decreases in female F_1 offspring and paired testes, paired epididymides, right testis, right corpus/caput, right epididymis, left testis, and epididymal adipose tissue decreases in F_1 male mice. Similarly, F_1 male mice in the 5 mg/kg/day-dose group exhibited a decrease of 12% in the relative epididymis weight in a reproduction/developmental toxicity mouse study (DuPont-18405-1037, 2010).

Changes in thyroid hormones, which are important for neurodevelopment, were reported in Conley et al. (2019), Conley et al. (2021) and Blake et al. (2020). In pregnant rats (*n*=3) dosed with 0–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 through GD18 (Conley et al., 2019), maternal serum total T3 levels were decreased at greater than or equal to 30 mg/kg/day and total T4 levels at greater than or equal to 125 mg/kg/day. The decreases in maternal serum total T4 levels compared to control were -50%, -63%, and -76% in the 125-, 250-, and 500-mg/kg/day-dose groups, respectively. The decreases in maternal serum total T3 levels compared to control were -27%, -39%, and -48% in the 30-, 62.5-, and 125-mg/kg/day-dose groups, respectively. Maternal total T3 levels in the 250 and 500 mg/kg/day-dose groups were below the detection limit. Similar findings were reported in Conley et al. (2021) for pregnant rats (*n*=4-5) dosed with 0–250 mg/kg/day of HFPO dimer acid ammonium salt from GD16 through GD20 and GD8 through PND2. Notably, significant decreases in maternal total T4 (-35% for GD16-GD20 and -51% for GD8-PND2) were also observed in the 62.5 mg/kg/day dose groups in Conley et al. (2021). In Blake et al. (2020), placental thyroid hormones (rT3, T3, and T4) were quantified at GD17.5 from 2–3 pooled placental tissues of same-sex embryos. A

significant increase (60%) in T4 relative to control was reported for the 10-mg/kg/day HFPO dimer acid-dose group.

5.5 Immune System

In the one available study specifically addressing immunotoxicity, suppression of TDARs was measured through IgM antibody production in mice (Rushing et al., 2017). IgM antibody production was decreased by 7.3% in female C57BL/6 mice treated with 100 mg/kg/day of HFPO dimer acid. In male mice treated with the same dose of HFPO dimer acid, significant increases in the number of T lymphocytes were observed, but no suppression of TDARs.

In two studies of less-than-chronic duration (28–90 days), decreases in spleen weight relative to BW were observed in female mice and rats (DuPont-18405-1307, 2010; Rushing et al., 2017). For example, in C57BL/6 mice, relative spleen weights significantly decreased by 11% in females treated with 100 mg/kg/day of HFPO dimer acid for 28 days (Rushing et al., 2017).

Changes in early markers of potential immunotoxic effects were observed in multiple studies examining the oral toxicity of HFPO dimer acid and/or ammonium salt. The most prevalent indications were statistically significant decreases from control in serum globulin levels (6%–22%), which resulted in an increase in the serum A/G ratio (7%–58%) from the controls when both sexes of Crl:CD-1 mice and Crl:CD(SD) rats were treated with 1 mg/kg/day–500 mg/kg/day of HFPO dimer acid ammonium salt for 12 months or less (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008). Alterations in the serum levels of globulin can be associated with decreases in antibody production (FDA, 2002). To determine the biological significance of the apparent decrease in globulin production, however, immune function tests (such as TDAR) need to be conducted. Finally, female Crl:CD-1 mice exhibited a 21% and 18% decrease in spleen weight relative to BW when administered 0.5 mg/kg/day and 5 mg/kg/day of HFPO dimer acid ammonium salt for 90 days, respectively (DuPont-18405-1307, 2010). For HFPO dimer acid and/or ammonium salt, there were also two local lymph node assays (LLNAs) conducted in mice that showed equivocal results (DuPont-19897, 2006; DuPont-22616 RV1, 2007).

In summation, the results of the Rushing et al. (2017) TDAR assay in combination with the supportive findings of decreased globulin levels and spleen weight provide evidence that GenX chemicals can induce immune suppression in female mice.

5.6 Cancer

The single cancer bioassay for HFPO dimer acid ammonium salt showed increased incidence of liver tumors (females) and combined pancreatic acinar adenomas and carcinomas (males) in rats at the high doses only. Additionally, a statistically insignificant increase in the incidence of testicular interstitial cell adenoma was noted at the high dose. Although that result was not statistically significant compared to controls, the authors of the study conclude that “a relationship to treatment for these findings in the 50 mg/kg/day group cannot be ruled out,” while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015). Given these uncertainties and the large number of early deaths in the study (see section 4.4), the existing evidence from this single chronic study is considered inadequate to justify a quantitative assessment. Further, the available data for HFPO dimer acid ammonium salt suggest that mice might be more sensitive to exposure to GenX chemicals than

rats. The available study (DuPont-18405-1238, 2013) only evaluated rats; there are no studies measuring cancer endpoints in mice. Given the evidence that the liver is the target organ for toxicity and primary organ for tumor development, the lack of data evaluating cancer in mice is a database deficiency. Thus, under EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a), there is *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans, based on the female hepatocellular adenomas and hepatocellular carcinomas and male combined pancreatic acinar adenomas and carcinomas. No data are available to evaluate cancer risk via dermal or inhalation exposure.

6.0 Mode of Action

The available data indicate that multiple MOAs could be involved in the liver effects observed after GenX chemical exposure. The available studies provide support for a role for PPARα, cytotoxicity, mitochondrial dysfunction, and PPARγ. The potential MOA(s) for the observed reproductive and developmental effects (e.g., changes in GWG and placental lesions) are unknown. Additionally, no data support identification of a potential carcinogenic MOA for tumors in the pancreas and testes as being related to any of the proposed MOAs for the tumor development in either organ.

For some PFAS (e.g., PFOA), PPARα activation has been proposed as a potential MOA for some of the effects in the liver (i.e., liver tumors) (Klaunig et al., 2003, 2012; Maloney and Waxman, 1999). PPARα is primarily expressed in the liver, but also is present in the kidney, intestines, heart, and brown adipose tissue (Hall et al., 2012). Klaunig et al. (2003) describes the causal key events of the PPARα MOA for liver tumors as activation of PPARα, perturbation of cell proliferation and apoptosis, and selective clonal expansion. There are multiple effects associated with the PPARα MOA such as hepatocellular hypertrophy, peroxisome proliferation, expression of peroxisomal genes, Kupffer cell-mediated events, and increased liver weight. However, these associative effects might not be specific to the PPARα MOA (e.g., hepatocellular hypertrophy) or might not be causal to the development of liver tumors (e.g., peroxisome proliferation) (Klaunig et al., 2003). According to Klaunig et al. (2003), demonstration of PPARα agonism combined with microscopic evidence for peroxisome proliferation or increases in liver weight and one or more of the specific *in vivo* markers of peroxisome proliferation (e.g., induction of acyl-CoA oxidase or cytochrome P450 4A) are sufficient to establish a PPARα MOA.

For HFPO dimer acid and/or ammonium salt, there are data that demonstrate peroxisome proliferation in the liver. Activation of PPARα was measured in multiple 28-day studies in rodents (DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017). Using acyl-CoA oxidase activity as a measure, Rushing et al. (2017) showed increased activity compared to control in male C57BL/6 mice administered 10 mg/kg/day and 100 mg/kg/day of HFPO dimer acid (122% and 222%, respectively) and a 100% increase compared to control in C57BL/6 female mice at 100 mg/kg/day. Notably, there were no significant increases in acyl-CoA oxidase activity at 1 mg/kg/day, indicating that it might be a high dose effect.

The DuPont studies used β-oxidation activity and total cytochrome P450 content as markers of peroxisome proliferation in the livers of rats and mice (DuPont-24447, 2008; DuPont-24459, 2008). In Crl:CD-1 male mice, β-oxidation activity significantly increased compared to control at

doses of 0.1 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day of HFPO dimer acid ammonium salt by 57%, 744%, and 648%, respectively, and total cytochrome P450 content significantly decreased at 3 mg/kg/day and 30 mg/kg/day by 26% and 53%, respectively (DuPont-24459, 2008). β-oxidation activity significantly increased compared to control in female Crl:CD-1 mice at 3 mg/kg/day and 30 mg/kg/day by 495% and 823%, respectively, with no alterations in total cytochrome P450 content (DuPont-24459, 2008). In male Crl:CD(SD) rats, β-oxidation activity was significantly increased relative to control at dosages of 0.3 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day by 42%, 274%, and 772%, respectively, and total cytochrome P450 content was significantly increased by 23% at 30 mg/kg/day (DuPont-24447, 2008). In female rats dosed with 30 mg/kg/day and 300 mg/kg/day, β-oxidation activity was significantly increased compared to control to 49% and 198%, respectively, while total cytochrome P450 content remained unaltered (DuPont-24447, 2008).

Induction of genes associated with peroxisome proliferation in the liver was also demonstrated (Wang et al., 2017; Conley et al., 2019). Wang et al. (2017) demonstrates significant increases in hepatic mRNA levels of many PPAR targets (e.g., CD36 antigen, acyl-CoA oxidase 1, and cytochrome P450 family members) after administration of 1 mg/kg/day of HFPO dimer acid ammonium salt for 28 days. Relatedly, Conley et al. (2019) found upregulation of gene expression associated with PPARα signaling in maternal and fetal livers following *in vivo* exposure during GD14–GD18.

Additionally, significant increases in liver weight relative to BW were observed in male and female Crl:CD(SD) rats and several strains of male and female mice treated with 0.5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 28–90 days (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017; NTP, 2019). Increases in liver weight were also reported in the pregnant dams in the available reproductive/developmental studies dosing during gestation (Blake et al., 2020; Conley et al., 2019; DuPont-18405-841, 2010; DuPont 18405-1037, 2010). Additionally, hepatocellular hypertrophy was observed at 0.5 mg/kg/day in both sexes of mice, while male and female rats showed these effects at 3 mg/kg/day and 30 mg/kg/day, respectively, in subchronic studies. Interestingly, in the chronic study, male rats showed only a 10% incidence of hepatocellular hypertrophy with dosing at 50 mg/kg/day for 2 years (DuPont-18405-1238, 2013). Conversely, female rats had significant hepatocellular hypertrophy at 500 mg/kg/day after 1 year (100%) and 2 years (93%).

There is evidence of perturbations to cell proliferation and apoptosis in the liver following short-term and subchronic exposure to HFPO dimer acid ammonium salt, particularly in the high-dose groups. In the 28-day mouse study, increased mitosis was observed in male (9/10) and female (5/10) mice in the high-dose groups only (30 mg/kg/day) and apoptosis was not reported (DuPont-24459, 2008). In the 90-day mouse study, increases in mitotic figures and apoptosis were reported in 7/10 and 10/10 male mice in the high-dose (5 mg/kg/day) group, respectively (NTP, 2019). No mitotic figures were detected in female mice, but an increase in apoptosis was observed in 3/9 mice (NTP, 2019). In the reproductive/developmental mouse study, mitotic figures were observed in 17/24 males and 2/24 females in the 5-mg/kg/day-dose group, but in no other dose groups (NTP, 2019). Similarly, apoptosis was reported in 21/24 males and 10/24 females in the 5-mg/kg/day high-dose group (NTP, 2019). Notably, decreases in the rates of apoptosis are typically observed with PPARα agonists, with Klaunig et al. (2003) describing

decreased rates of apoptosis as a "hallmark of liver growth seen in the early stages of treatment with PPARα agonists." Interestingly, increases in mitoses/mitotic figures and apoptosis are consistently restricted to the high-dose group in all available mouse studies; however, necrosis is observed in both the mid- and high-dose groups. These data suggest that PPARα's role in the observed liver effects may be dose dependent. In the 28-day rat study, mitosis/mitotic figures, hyperplasia, and apoptosis were not reported (DuPont-24447, 2008). In the 90-day rat study, mitosis/mitotic figures, hyperplasia, and apoptosis were not reported (DuPont-17751-1026, 2009). In the chronic rat study, mitotic figures and apoptosis were not reported, and hyperplasia was no different than control in the male and female rats in any dose group (DuPont-18405-1238, 2013). It is possible that the rat studies might not have separated apoptotic lesions from other liver lesions reported (i.e., single-cell necrosis) since these studies were conducted prior to the guidelines outlined in Elmore et al. (2016) and were not reanalyzed by the NTP PWG.

Although there is evidence for a PPARα MOA in the liver, particularly in the high-dose groups in the available studies, data indicate that liver toxicity extends beyond a single PPARα-based MOA. For example, liver necrosis was consistently observed in rodent toxicity studies with HFPO dimer acid ammonium salt and was reaffirmed by the NTP PWG's review of the 90-day subchronic study in mice and the reproductive and developmental toxicity study in mice (appendix D), which suggests that cytotoxicity is also a possible MOA. Felter et al. (2018) identified the following key events for establishing a cytotoxicity MOA:

1.) The chemical is not DNA reactive.
2.) Clear evidence of cytotoxicity by histopathology such as the presence of necrosis and/or increased apoptosis.
3.) Evidence of toxicity by increased serum enzymes that are relevant to humans.
4.) Presence of increased cell proliferation as evidenced by increased labeling index and/or increased number of hepatocytes.
5.) Demonstration of a parallel dose response for cytotoxicity and formation of tumors.
6.) Reversibility (ideally).

The available data for HFPO dimer acid support cytotoxicity as a potential MOA. For example, HFPO dimer acid does not appear to be DNA reactive *in vivo* (see section 4.6.3 and appendix C). It did not induce chromosomal mutations in the form of structural aberrations, numerical aberrations, or micronuclei or DNA effects in the form of unscheduled DNA synthesis (DuPont-23219, 2007; DuPont-23220, 2007). Secondly, clear evidence of cytotoxicity in the form of increased liver necrosis and apoptosis was confirmed microscopically in male and female mice and rats in several less-than-chronic studies (15–90 day) and one 2-year chronic study (DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Wang et al., 2017; NTP, 2019). There is also evidence of increased serum liver enzymes. Hall et al. (2012) identifies significant increases in ALT/AST, ALP, and bilirubin/bile acids as potentially clinically relevant. Additionally, other enzymes such as SDH might reflect alterations in liver function (Hall et al., 2012). For HFPO dimer acid, significant increases in ALT (420%–1,254%), AST (106%–478%), ALP (1,134%–1,221%), and SDH (1,134%–1,221%) were observed in male mice administered the ammonium salt at 5–30 mg/kg/day for 28–90 days. Female mice had smaller increases in ALP (140%–143%) and SDH (32%–186%) as compared to male mice administered the same dose over the same duration. Overall, rats exhibited far fewer and smaller increases in serum liver enzyme levels following subchronic exposure compared to the mouse,

with increases in AST (106%) and ALP (52%) at 100 mg/kg/day in male rats and AST (66%) in female rats at 1,000 mg/kg/day. In the chronic study, however, ALT (228%), ALP (180%), and SDH (140%) significantly increased in male rats only when administered 50 mg/kg/day for 1 year. Typically, an increase in bilirubin, when accompanied with increased bile acids, is a reliable index of liver toxicity (Hall et al., 2012). For HFPO dimer acid, however, a decrease in serum bilirubin is a consistent effect observed across multiple studies, especially in female rodents (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; Wang et al., 2017).

Data gaps exist for the other key events related to a cytotoxic MOA. Studies investigating if exposure to HFPO dimer acid result in increased labeling index and/or increased number of hepatocytes are unavailable. A 2-year chronic study in rats reported centrilobular and single cell necrosis in females in the 500-mg/kg/day high-dose group only (DuPont-18405-1238, 2013). Additionally, treatment-related liver tumors were also observed in the 500-mg/kg/day dose group (0/70 in control versus 11/70 in 500 mg/kg/day), which suggests a parallel response for cytotoxicity and formation of tumors. However, these effects were observed only in the high-dose group and dose selection in this study resulted in a large gap between the mid-dose (50 mg/kg/day) and high-dose (500 mg/kg/day). Therefore, the potential for a parallel dose response is unclear. Additionally, while liver necrosis exhibits a dose response in the 84/85 day modified reproductive developmental study (DuPont-18405-1037, 2010; NTP, 2019), there are no chronic studies in the mouse to determine if liver tumors form. The available data indicate that the mouse is the more sensitive to the liver effects resulting from HFPO dimer acid exposure.

Additionally, Blake et al. (2020) reports an increase in subcellular organelles consistent with peroxisomes and mitochondria in pregnant dam livers exposed to 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E11.5 or E17.5 using TEM. This increase in mitochondria is not typical of PPARα activation and suggests an alternate MOA such as mitochondrial alteration could also be operative for the liver effects resulting from exposure to HFPO dimer acid and/or ammonium salt. Further supporting this alternate MOA, a number of genes upregulated in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18 are specific to mitochondrial beta oxidation (*Cpt1a*, *Cpt1b*, *Cpt2*, *Acaa2*, *Acadl*, *Acadm*), mitochondrial ketogenesis (*Hmgcs2*), and mitochondrial electron transfer (*Etfdh*) (Conley et al., 2019).

Finally, a study of HFPO dimer acid in HEK293 embryonal kidney cells found activation of genes associated with the PPARγ signaling pathway (Li et al., 2019). Further supporting a role for the PPARγ signaling pathway, Conley et al. (2019) reports upregulation of genes in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18, which are associated with PPARγ signaling, including *Pck1*, *Aqp7*, and *Gk.* Additionally, Rosen et al. (2017) concluded that 11%–24% of the PFAS-induced increase in transcriptional activity is PPARα independent, depending on the PFAS. This study identified 67 genes that were similarly upregulated in wild type (129S1/Sv1mJ) and PPARα-null (129S4/SvJae-*Ppara*^{tm1Gonz}/J) mouse livers exposed to either 3 or 10 mg/kg/day of PFHxS, 1 or 3 mg/kg/day of perfluorononanoic acid, 3 mg/kg/day of PFOA, or 10 mg/kg/day PFOS for 7 days, indicative of PPARα independence. The authors note that genes typically associated with the activation of PPARα such as *Acox1* were similarly upregulated in wild type and PPARα-null mice livers, suggesting that these genes might not be specific indicators of PPARα activation. Interestingly,

Conley et al. (2019) found that five of the 67 genes identified as PPARα independent in the Rosen et al. (2017) study are also significantly upregulated in the liver of pregnant rats and their fetuses exposed to HFPO dimer acid at doses greater than or equal to 1 mg/kg/day (i.e., *Ehhadh*, *Slc22a5*, *Ech1*, *Cpt2*, and *Acox1*). *Slc22a5* and *Cpt2* are associated with mitochondrial fatty acid oxidation.

Taken together, the available data indicate that a PPARα MOA is plausible in the liver in response to GenX chemical exposure, especially at doses greater than 0.5 mg/kg/day; however, there are not yet enough data to conclude that PPARα activation is the sole mechanism underlying the liver effects associated with exposure to GenX chemicals. For example, there are no studies investigating GenX chemical exposure in PPARα-null mice. It is worth noting that exposure to PFOA has been demonstrated to induce liver effects in PPARα-null mice, including hepatocellular hypertrophy (Minata et al., 2010). Additionally, available studies indicate that other MOAs (e.g., PPARγ, mitochondrial dysfunction, and cytotoxicity) are also plausible. The data are not adequate to conclude that any of the MOAs described here are the sole toxicologic MOA for HFPO dimer acid and/or ammonium salt in the liver and especially in other organ systems. For example, the potential MOA(s) for the observed reproductive and developmental effects (e.g., changes in GWG, placental lesions, reduced pup body weight, and reduced pup survival) are unknown, though Conley et al. (2021) provides mechanistic evidence that dysregulation of carbohydrate and lipid metabolism in the mother and developing offspring may be contributing to some of these effects. Of note, glycogen accumulation scores in pup livers were significantly lower compared to control in pups exposed to doses as low as 10 mg/kg/day of HFPO dimer acid ammonium salt from GD8-PND2. Additionally, no data support identification of a potential carcinogenic MOA for tumors in the pancreas or testes as being related to PPARα or any of the proposed alternative MOAs for the tumor development in either organ.

7.0 Dose-Response Assessment

7.1 Identification of Studies and Effects for Dose-Response Analysis

Several studies were evaluated further for identification of specific endpoints to carry forward for dose-response (BMD) modeling. EPA evaluated studies based on identification of adverse effects, duration of exposure, use of a control and two or more doses, and provision of NOAEL and/or LOAEL values. Data from available studies indicate that the liver is the most sensitive target of toxicity from exposure to GenX chemicals. Liver effects were observed in both male and female mice and rats at varying durations of exposures and doses. These effects occurred at the lowest doses and shortest durations of exposure to GenX chemicals.

Because liver effects such as increases in liver weight and hepatocellular hypertrophy (also referenced here as cytoplasmic alteration per NTP PWG's review) can be associated with activation of cellular PPARα receptors, EPA evaluated observed liver effects resulting from HFPO dimer acid ammonium salt exposure against the Hall criteria (Hall et al., 2012). These criteria indicate that increased liver weight and hepatocellular hypertrophy must be accompanied by histologic or clinical pathology indicative of liver toxicity to be considered adverse. Histologic or clinical pathology indicative of liver toxicity can include changes in liver enzyme concentrations in the serum, necrosis, inflammation, and degeneration. With these criteria in mind, EPA concluded that some of the observed liver effects such as single-cell and focal

necrosis, increased apoptosis, and increases in serum liver enzymes indicate toxicity of relevance to humans as opposed to PPARα-induced cell proliferation unique to rodents.

For the GenX chemicals database, many studies identified the mouse as the most sensitive species and the liver as a target organ for toxicity. Liver effects at low doses (e.g., less than or equal to 5 mg/kg/day) were identified in the 28 day oral (gavage) toxicity study in mice (DuPont-24459, 2008), the 90 day oral (gavage) toxicity study in mice (DuPont-18405-1307, 2010), and the oral (gavage) reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010). In these studies, increases in relative liver weight were accompanied by increases in hepatocellular hypertrophy, single-cell/focal necrosis and apoptosis.

EPA requested that NIEHS, NTP convene a PWG to provide independent, expert review of the liver tissues from the oral (gavage) reproduction/ developmental toxicity study in mice (DuPont-18405-1037, 2010) and the 90 day oral (gavage) toxicity study in mice (DuPont-18405-1307, 2010). Given the availability of longer duration studies demonstrating effects at low doses, the 28-day study in mice was not included in this review. The NTP PWG classified cell death according to the INHAND Organ Working Group's diagnostic criteria that describes how pathologists can distinguish between apoptosis and single-cell necrosis in standard H&E-stained tissue sections (Elmore et al., 2016). These criteria were unavailable at the time the DuPont studies were conducted and submitted to EPA.

The liver effects noted in the 28 day oral (gavage) toxicity study in mice (DuPont-24459, 2008) were not considered as a potential POD in support of the derivation of the RfD. The 28 day study did not use a dose range optimized for the identification of low-dose effects compared to the 90 day and reproduction/developmental toxicity studies (0, 0.1, 3, and 30 mg/kg/day-dose groups in the 28 day study versus 0, 0.1, 0.5 and 5 mg/kg/day in the 90 day and reproduction/developmental studies). For example, in DuPont-18405-1037 (2010), the LOAEL (i.e., the lowest dose at which an adverse effect is observed) of 0.5 mg/kg/day falls between the low and mid-doses of the dosing design used in DuPont-24459 (2008). Additionally, as described above, this short-term study was not reviewed by the NTP PWG because there were two longer duration studies in the most sensitive species.

The liver effects noted in the 90 day and reproduction/developmental toxicity studies (DuPont-18405-1307, 2010 and DuPont-18405-1037, 2010) were considered for determination of PODs in support of the derivation of RfDs. The NTP PWG concluded, that the dose response and constellation of lesions (i.e., cytoplasmic alteration (including hepatocellular hypertrophy), single-cell necrosis, focal necrosis, and apoptosis), rather than each lesion individually, represent adversity in these studies (appendix D). EPA interpreted the NTP PWG's definition that the constellation of liver lesions is adverse to apply to the dose group level, as opposed to individual animal level, given that the histopathology assessment represents a snapshot in time of a biological process within one portion of the liver that can vary across animals. Therefore, if multiple liver lesion types and progression of adverse liver effects (e.g., necrosis or apoptosis) were observed within a dose group, all animals in that dose group were included in the dose-response modeling. The constellation of liver lesions in the reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010) was selected for BMD modeling based on the incidence data as reported by the NTP PWG. Multiple liver lesions, including cytoplasmic alteration, single-cell, and focal necrosis, exhibited a dose response in both male and female mice in this study. These effects were observed at doses as low as 0.5 mg/kg/day. A constellation

of liver lesions observed in the 90-day toxicity study in mice (DuPont-18405-1307, 2010) were observed at higher doses (5 mg/kg/day) than in the reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010). The NTP PWG reported that 10 out of 10 male mice exhibited cytoplasmic alteration, compared to 0 in control at the 0.5-mg/kg/day dose in the 90-day toxicity study in mice (DuPont-18405-1307, 2010). Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions considered adverse, no other liver lesions indicative of liver damage (i.e., single-cell or focal necrosis or apoptosis) were observed at the 0.5-mg/kg/day dose group in males. Consistent with the Hall criteria, EPA did not consider the cytoplasmic alteration findings alone as an adverse effect in the 0.5 mg/kg/day dose group but considered the constellation of liver lesions observed across the male mice in the high-dose group as adverse. Additionally, the female mice in this study did not exhibit a dose response for the constellation of liver lesions. For these reasons, the constellation of liver lesions observed in the 90-day toxicity study in mice were not selected for BMD modeling.

Additionally, the chronic rat 2-year cancer bioassay (DuPont-18405-1238, 2013) was not selected for the derivation of candidate RfDs for several reasons. Across all dosing groups in both male and female rats, just 25.4% of the test animals survived to their planned terminal necropsy with most of the animals experiencing unscheduled death/moribundity prior to the scheduled study termination at 104 weeks. Effects observed at low doses in this study include changes in serum albumin levels and the A/G ratio in male rats. For males, an increase in A/G ratio at 1 mg/kg/day at the 3-month time point and increases in both albumin and A/G ratio at the 12-month time point were observed, but these changes were not seen at 6 months. These changes, while indicative of an immune system effect, were deemed of unclear biological significance especially given these temporal inconsistencies. For these reasons, the changes in albumin and A/G ratio observed in DuPont-18405-1238 (2013) were not considered for determination of PODs in support of the derivation of the RfD. Liver effects were also observed in this study but did not occur at comparable doses to the oral reproductive/developmental toxicity study in mice. Also, the available chronic study evaluated only rats, and the data indicate that mice appear to be more sensitive. For example, mice presented with single-cell necrosis in doses as low as 0.5 mg/kg/day, with a large increase in response at 5 mg/kg/day in the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010; NTP, 2019). Female mice also had a large increase in incidence compared to control at 5 mg/kg/day for both focal/multifocal and single-cell necrosis (DuPont-18405-1037, 2010; NTP, 2019). Conversely, the study authors did not report subchronic hepatocellular necrosis in the 90-day study of male and female rats. (DuPont-17751-1026, 2009). Hepatocellular necrosis is observed in the 2-year chronic rat study, but at higher doses (50 mg/kg/day for male rats and 500 mg/kg/day for female rats) as compared to the developmental/reproductive mouse study (0.5 mg/kg/day for male and female mice) (DuPont-18405-1238, 2013; NTP, 2019 reread of DuPont-18405-1037, 2010). While a chronic study is typically the preferred duration for development of lifetime RfD, in this case, the oral reproductive/developmental toxicity study indicates that adverse effects in the liver are observed in the parental mice at lower doses than those reported in the chronic study in rats. For these reasons, the adverse liver effects observed in DuPont-18405-1238 (2013) were not selected for determination of PODs in support of the derivation of the RfD.

Adverse health outcomes resulting from exposure from HFPO dimer acid or its ammonium salt are not limited to the liver. Studies in both rats and mice indicate that exposure to GenX chemicals during pregnancy and gestation results in adverse effects at low doses. Specifically,

Blake et al. (2020) determined that 58% and 83% of placentas evaluated at E17.5 were classified as abnormal in the 2- and 10-mg/kg/day HFPO dimer acid dose groups, respectively, with the number of abnormal placentas in the 10-mg/kg/day HFPO dimer acid dose group reaching statistical significance. Different placental lesions were recorded in the study, including labyrinth atrophy, labyrinth congestion, labyrinth necrosis, early fibrin clot, and the presence of placental nodules. Placental lesions were also evaluated against the proportion of placentas within a litter that were within normal limits to account for litter effects. The proportion of abnormal placentas was significantly higher at the 2- and 10-mg/kg/day dose groups relative to vehicle control. The placental lesions observed in Blake et al. (2020) exhibited a dose response; however, only two dose groups were used in this study, and the study LOAEL (2 mg/kg/day) is much higher than the LOAELs observed for liver effects (0.5 mg/kg/day). It is possible that the placental lesions occur at lower doses, especially given that 58% of placentas were classified as abnormal at the lowest dose tested, but these data are lacking. While the placental lesions observed are considered adverse, additional research is needed to understand if they would be seen at lower doses. Additionally, further research should evaluate the impact of GenX chemicals-induced placental lesions on development after gestation, including latent health outcomes. Blake et al. (2020) reported that these lesions did not impact some measured reproductive and developmental outcomes such as implantation sites, viable embryos, nonviable embryos, and resorptions. Because, however, a full two-generation reproductive toxicity study is not available for mice, the impact of placental lesions on development after gestation, including latent health outcomes, is unclear.

An increase in maternal GWG ranging from 13 to 22% was reported by DuPont-18405-1037 (2010) at doses as low as 0.5 mg/kg/day. Similarly, an increase in maternal GWG in mice at E17.5 at doses greater than or equal to 2 mg/kg/day (i.e., the lowest tested dose) was also reported by Blake et al. (2020) using a mixed-effect modeling approach that adjusts for repeated measures of relative maternal GWG, litter size, and embryonic day. Furthermore, Conley et al. (2019) evaluated maternal GWG in rats and observed a *decrease* in GWG following exposure to dosing greater or less than 250 mg/kg/day of HFPO dimer acid. A decrease in maternal GWG in rats was also reported in DuPont-18405-841 (2010), which suggests that the shift in maternal GWG might be species specific. Given the lack of mechanistic clarity for the maternal GWG endpoints in two similar species, the endpoint was not considered for determination of PODs in support of the RfD derivation. According to Blake et al. (2020), the inconsistency in maternal GWG response between rats (Conley et al., 2019; DuPont-18405-841, 2010) and mice (Blake et al., 2020; DuPont-18405-1037, 2010) might be due to differing statistical methods, interspecies elimination rates, and/or developmental exposure windows. All other reproductive and developmental effects reported as a result of gestational exposure to GenX chemicals (see Table 12 for a summary) were observed at higher doses than the placental lesions and changes in GWG and were not selected for determination of PODs in support of the RfD derivation.

Immune and hematological effects were also observed at low doses; however, these endpoints are not as consistently observed as the liver effects. Additionally, there is some uncertainty regarding the biological significance of both the hematological and immune endpoints. For example, the observed changes in albumin and A/G ratio at dosing of 3 mg/kg/day (DuPont-24447, 2008; DuPont-24459, 2008) are considered early markers of potential immunotoxic effects. Evaluation of additional immune function assays, histopathology, and immune endpoints such as antibody levels, however, are not available. Currently little or no data exist on the

potential for GenX chemicals to impact aspects of immune function beyond the immunosuppression (e.g., allergic responses and autoimmunity). Furthermore, while considered adverse, the hematological effects were inconsistently observed, especially as study duration increased. For example, the hematological effects observed in the 28-day mouse study at 3 mg/kg/day were not observed in the 90-day subchronic study in mice, except for a 3% decrease in hemoglobin concentration at 5 mg/kg/day. No hematological changes were observed at the 0.1- or 0.5-mg/kg/day dose in the subchronic mouse study (DuPont-18405-1307, 2010). Likewise, the hematological effects observed in the subchronic rat study at low doses are not observed in the chronic rat study (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013). Specifically, decreases in hemoglobin, hematocrit, and RBC count that are observed at 10 mg/kg/day in the subchronic study are not observed after 12 months of dosing, which adds additional uncertainty to the significance of these effects (DuPont-18405-1238, 2013). For these reasons, hematological and immune endpoints from these studies were not considered further for determination of PODs in support of the derivation of the RfD.

7.2 Methods of Analysis

7.2.1 BMD Modeling

There are no biologically based dose-response (BBDR) models available for HFPO dimer acid and its ammonium salt. Thus, using the most current version of its Benchmark Dose Software at the time data were modeled, EPA evaluated a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of observed data (appendix E).

Consistent with EPA's *Benchmark Dose Technical Guidance* (EPA, 2012), the BMD and the BMDL were estimated using a BMR of 10% extra risk for dichotomous data, in the absence of information regarding the level of change considered biologically important, and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. Using the pathology analysis from the NTP PWG, candidate PODs were estimated from all three doses (plus control) for DuPont-18405-1037 (2010) (Table 13).

Further details, including the BMD modeling output and graphical results for the selected models, are provided in appendix E of this assessment.

7.2.2 Dosimetric Adjustment of the Experimental Animal-Based POD to POD_{HED}

EPA guidance was followed to calculate a candidate POD_{HED} from the animal-based POD using a $BW^{3/4}$ allometric scaling approach (EPA, 2011b), which is derived from the relationship between body surface area and basal metabolic rate in adults. With infants and children, surface area and basal metabolic rates are very different than for adults with a slower metabolic rate. While this $BW^{3/4}$ allometric scaling is not appropriate for infants and children because of the limited toxicokinetic data available, the critical effect of liver single-cell necrosis observed in adult mice is not a developmental endpoint nor is it specific to early life stages. However, the exposure for the parental females in DuPont-18405-1037 (2010) took place during pregnancy. EPA indicates that:

> …exposure and internal dosimetry of pregnant, nursing, and growing animals may vary compared to adult animals, so use of the administered dose for toxicity studies involving

these periods is associated with relatively greater uncertainty, absent life stage-specific information (EPA, 2011b).

In this case, however, $BW^{3/4}$ allometric scaling relied on life stage-specific BW data from the pregnant or lactating dams as appropriate. The HFPO dimer acid ammonium salt POD_{HED}s from the experimental animal studies (DuPont-18405-1037, 2010) were adjusted via the dosimetric adjustment factor (DAF) equation below:

$$DAF = (BW_a^{1/4}/BW_h^{1/4}),$$

where:

- BW_a = animal BW
- BW_h = human BW

For the chronic reproductive/developmental toxicity study (DuPont-18405-1037, 2010), a BW_a value of 0.0372 kg was identified as the mean BW of the F_0 male mouse controls on study day 84 (the final day of animal dosing). The mean BW_a for the F_0 females in this study was 0.0349 kg taken from the controls upon sacrifice on LD21.

A BW_h of 80 kg for humans was selected based on National Health and Nutrition Examination Survey (NHANES) sampling data (EPA, 2011a). For adults more than 21 years of age, EPA updated the default BW assumption from 70 kg to 80 kg based on NHANES data from 1999 to 2006 as reported in Table 8.1 of EPA's *Exposure Factors Handbook* (EPA, 2011a). The updated BW represents the mean weight for adults ages 21 and older. The resulting DAF for the allometric scaling of doses from male mice to humans is 0.15 for DuPont-18405-1037 (2010). For the female mice, the DAF is 0.14 for DuPont-18405-1037 (2010). Applying the DAF to the identified PODs identified for liver effects in adult mice yields a POD_{HED} as follows:

$$POD_{HED} = \text{POD animal dose (mg/kg/day)} \times DAF$$

Table 13. Summary of Determination of POD_{HED}

Endpoint and reference	Species/ Sex	Model	BMR	BMD_{10} (mg/kg/day)	POD (mg/kg/day)	POD Type	DAF	POD_{HED}[a] (mg/kg/day)
HEPATIC								
Constellation of liver lesions in parental males (DuPont-18405-1037, 2010)[b]	Crl:CD1(ICR) mice F_0 parental male	Benchmark dose (ver. 3.1.2) Probit	10%	0.19	0.14	$BMDL_{10}$	0.15	0.02
Constellation of liver lesions in parental females (DuPont-18405-1037, 2010)[b]	Crl:CD1(ICR) mice F_0 parental female	Benchmark dose (ver. 3.1.2) Probit	10%	0.12	0.09	$BMDL_{10}$	0.14	0.01

Notes: N/A = not applicable.
[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).
[b]Calculations for DuPont 18405-1037 (2010) rely on pathology conclusions of the NTP PWG (Appendix D)

7.3 Derivation of Candidate RfD Values

To calculate the candidate RfD values, EPA applied UFs to the POD_{HED}s from the oral reproduction/developmental toxicity study in mice as described in this section. UFs were applied according to guidance in EPA's *Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002).

An interspecies uncertainty factor (UF_A) of 3 ($10^{1/2}$ = 3.16, rounded to 3) was applied to account for uncertainty in extrapolating from laboratory animals to humans. The UF_A is generally presumed to include both toxicokinetic (i.e., absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. A POD_{HED} was derived from the BMDL using EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b). This guidance describes approaches for deriving POD_{HED}s from data from laboratory animals, with the preferred approach being PBPK modeling. For HFPO dimer acid and ammonium salt, no PBPK models have been developed or published. Other approaches described by the guidance include the use of chemical-specific data to inform the derivation of human equivalent oral exposures. In the absence of either PBPK models or chemical-specific information, a BW scaling to the ¾ power approach is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans. Although this scaling addresses most aspects of cross-species extrapolation of toxicokinetic processes, there is some residual uncertainty for toxicokinetics and uncertainty around toxicodynamic processes (EPA, 2011b). Thus, in the absence of chemical-specific data to quantify this uncertainty, a UF of 3 was applied.

An intraspecies uncertainty factor (UF_H) of 10 is applied to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (lifestyle) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation uncertainty factor (UF_L) of 1 is applied because a BMDL is used as the basis for the POD_{HED} derivation. When the POD type is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, the BMR of a 10% change for the modeled liver endpoints was selected under the assumption that it represents a minimal, but biologically significant, change for these effects.

A UF for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 10 was applied for the derivation of the chronic RfD, but not of the subchronic RfD. The reproduction/ developmental study (DuPont-18405-1037, 2010) considered for dose-response analysis is shorter than the duration of a chronic study. Chronic studies typically employ repeated dosing for longer than 90 days or for more than 10% of the human life span (EPA, 2002). In DuPont-18405-1037 (2010), F_0 females that delivered were dosed daily starting 14 days prior to pairing and were dosed through LD20 for a total of 53 to 64 days of exposure, depending on delivery date. By contrast, F_0 males in this study were dosed 70 days prior to mating and throughout mating through 1 day prior to scheduled termination, for a total of 84 to 85 days of exposure.

Because a 2-year chronic mouse study is unavailable and since female mice were dosed well below the 90-day exposure window typically employed in a subchronic study, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because duration of exposure appears to play a role in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, necrosis in female rats was not reported in the 28- or 90-day rat studies or the interim 1-year time point in the 2-year chronic rat study, which dosed the rats from 3 to 1,000 mg/kg/day. However, at the completion of the 2-year chronic rat study, centrilobular and single-cell necrosis are reported in the 500-mg/kg/day-dose group. Moreover, treatment-related liver tumors were observed in the 500-mg/kg/day rat dose group (0/70 in control versus 11/70 in the 500-mg/kg/day group). These data demonstrate progression of liver effects over the 2-year dosing period. Additionally, Blake et al. (2020) did not find clear evidence of changes in maternal liver serum enzymes (i.e., ALP, ALT or AST) or increases in liver necrosis as compared to control after 10-16 days of dosing at 2 mg/kg/day. Similarly, DuPont-24459 (2008) did not report single cell necrosis in female mice treated with 0.1 or 3 mg/kg/day after 28 days of dosing, though 4/10 mice displayed single cell necrosis in the 30 mg/kg/day dose group. However, DuPont-18405-1037 (2010) found liver necrosis in mice after 53-85 days of dosing at 0.5 mg/kg/day, indicating progression of liver effects as the duration of dosing increases. Because the mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat and because the mode of action for these liver effects is uncertain (see section 6), it is critical to have a 2-year chronic study in the mouse to understand the progression of these liver effects. Specifically, a longer duration study would likely result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies (DuPont-24459, 2008; DuPont-18405-1307, 2010; DuPont-18405-1037, 2010). For these reasons, EPA applied a UF of 10 to account for duration of exposure for the chronic RfD. For the subchronic RfD, a UF was not applied to account for duration as the study is of subchronic duration.

A database uncertainty factor (UF_D) of 10 was applied to account for database deficiencies. The database uncertainty factor is applied to account for a potentially lower reference value as a result of an incomplete characterization of a chemical's toxicity (EPA, 2002). The GenX chemicals database contains a number of toxicological studies including acute studies in both mice and rats, subchronic studies in mice and rats, a chronic study in rats, a one generation reproductive and developmental study in mice and gestational reproductive and developmental toxicity studies in mice and rats, as well as a single immunotoxicity study; however, when evaluating the available endpoints and studies to ensure comprehensive characterization of the potential toxicity, there are important deficiencies that need to be considered, particularly for understanding developmental toxicity. If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment (EPA, 2002). For GenX chemicals, there are reproductive or developmental effects of concern in mice occurring at similar dose levels to the liver effects (changes in maternal GWG and placental lesions indicative of placental insufficiency) or ongoing research related to these and other endpoints or effects that have not been studied yet

(skeletal ossification, mammary gland development, altered metabolism in offspring, changes in thyroid hormones in the mouse).

For example, increases in maternal gestational weight gain and placental lesions were observed in mice at doses similar to the observed liver effects. In DuPont-18405-1037 (2010) mean maternal GWG, calculated from individual differences, significantly increased over the gestational period (0–18 days) by 18% and 22% in the 0.5- and 5-mg/kg/day-dose groups, respectively. The NOAEL for this effect would be 0.1 mg/kg/day which is the same as the liver effects. Additionally, Blake et al., 2020 found that maternal GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at gestational day 17.5 using effect estimates from mixed effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day. This is a consistent effect observed in two studies conducted by two different groups. Conley et al. (2019, 2021) also evaluated GWG in rats and observed a decrease following exposure to dosing greater than 125 mg/kg/day of HFPO dimer acid. A decrease in GWG in rats was also reported in DuPont-18405-841 (2010), which suggests that the shift in GWG might be species specific. Blake et al. (2020) also suggested that differing statistical methods, interspecies elimination rates, and exposure windows could explain these disparate results. In humans, altered GWG has been shown to adversely impact both mothers and infants. Effects including pregnancy-induced hypertension, gestational diabetes, postpartum weight retention, difficulty breast feeding, increased risk of stillbirth and infant mortality, and preterm birth have been associated with increased GWG (Rasmussen and Yaktine, 2009).

Secondly, Blake et al. (2020) reports a statistically significant increase (58%) in placental lesions over control at 2 mg/kg/day, the lowest dose used in this study. The placenta is critical to the transfer of nutrients, oxygen, and waste between mother and baby. Because of its role in maintaining pregnancy and programming latent health outcomes it is a relevant endpoint to evaluate maternal and embryo health. Placental insufficiency, as evidenced by effects such as those observed by Blake can result in reduced transfer of vital oxygen and nutrients. Additionally, deficiencies in placental development or function can result in hypertensive disorders of pregnancy which increases the risk of post-pregnancy hypertension, heart disease, and stroke in affected women, as well as increased risk for adverse cardiometabolic outcomes in adult offspring (Pinheiro et al., 2016). EPA notes that it is unclear how the placental lesions might impact reproductive and developmental outcomes. For example, implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than control in Blake et al. (2020). Because, however, a full two-generation reproductive toxicity study is not available for mice, the impact of placental lesions on development after gestation or latent effects resulting from a placental insufficiency phenotype are unclear. Notably, Blake et al. (2020) also reported placental lesions for PFOA and, studies in humans have shown associations between PFOA exposure and health outcomes resulting from placental insufficiency such as pregnancy-induced hypertension or preeclampsia (EPA, 2016a).

As mentioned above, other database deficiencies include the absence of a full two-generation reproductive and developmental toxicity study to understand if latent effects occur as a result of exposure to GenX chemicals during development (e.g., adverse cardiometabolic outcomes in adult offspring associated with placental insufficiency). Additionally, Conley et al. (2021) reported that survival of pups born to dams dosed from GD8-PND2 was significantly reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day. Pup body weight gain (birth to PND2) and PND2

body weight in the surviving pups were both reduced at doses ≥30 mg/kg/day. These effects were attributed to the hypoglycemia and elevated serum lipid levels newborns displayed, consistent with impaired fetal glycogen storage. Metabolic disturbance during fetal development is likely to lead to long-term negative metabolic outcomes in the offspring. These effects are among the most sensitive effects observed in the rat resulting from exposure to GenX chemicals and highlight the importance of having a full two-generation reproductive and developmental toxicity study.

Additionally, the evaluation of particular developmental endpoints during early organogenesis (i.e., GD0 to GD6) such as delayed skeletal ossification and mammary gland development in the mouse that have been observed following exposure to other PFAS like PFOA (EPA, 2016a,b) are lacking. For PFOA, the LOAEL for mammary gland developmental effects was 0.01 mg/kg/day, with no study NOAEL. There are no published studies looking at mammary gland development for GenX chemicals at this time. Similarly, studies that evaluate skeletal ossification in the more sensitive species, mice, do not exist for GenX chemicals. The LOAEL for reduced skeletal ossification was 1 mg/kg/day for PFOA (no study NOAEL) and studies looking at lower dose ranges were not available. These studies are especially important considering that Blake et al. (2020) demonstrated accumulation of HFPO dimer acid in whole mouse embryos from E1.5 to E11.5 to E1.5 to E17.5. The lack of studies evaluating these endpoints at or below doses included in the critical study identifies this as a significant gap in the understanding of the developmental toxicity of GenX chemicals.

In addition to the gaps in the database concerning reproductive and developmental toxicity, other database gaps are noted for GenX chemicals with respect to potential immune, hematological and neurological effects, which are outlined below. Additionally, there are no human toxicity data from epidemiological studies in the general population or worker cohorts evaluating the health effects of exposure to these GenX chemicals.

The immunotoxicity of GenX chemicals has not yet been fully elucidated. PFAS chemicals, including PFOS and PFOA, interact with the immune system in studies of both humans and animals (NTP, 2016; EPA, 2016a,b). The GenX chemical immunotoxicity database is less robust than PFOA and PFOS, but does include two LLNAs (DuPont-19897, 2006; DuPont-22616 RV1, 2007) and a 28-day immunotoxicity study (Rushing et al., 2017). Rushing et al. (2017) identified suppression of TDAR by a reduction in antigen-specific IgM antibody production in females and increased T cell numbers in males at the high dose only (100 mg/kg/day). The LLNA is typically used to identify potential skin-sensitizing chemicals through their ability to induce allergic immune response (OECD, 2010b). The LLNAs were conducted with HFPO dimer acid ammonium salt preparations of varied purity and yielded equivocal results (one positive (DuPont-19897, 2006) and one negative (DuPont-22616 RV1, 2007). Evaluations of additional immune function assays, histopathology, and immune endpoints such as antibody levels are not available. The combined GenX chemicals immunotoxicity dataset was found to be incomplete as it did not include sufficient measures of immunopathology, humoral immunity, cell-mediated immunity, nonspecific immunity, or host resistance, but the available studies are suggestive of a potential immune hazard. Data on the potential for these GenX chemicals to impact aspects of immune function beyond immunosuppression are lacking. Additional studies, therefore, would be useful to support a more conclusive determination of immunotoxic potential.

Finally, additional research is needed to help determine if the inconsistent hematological effects observed in many of the studies are adverse and to investigate potential neural effects of GenX chemical exposure. As mentioned above, the hematological effects observed in the 28-day mouse study at 3 mg/kg/day were not observed in the 90-day subchronic study in mice, except for a 3% decrease in hemoglobin concentration at 5 mg/kg/day. No hematological changes were observed at the 0.1- or 0.5-mg/kg/day dose in the subchronic mouse study (DuPont-18405-1307, 2010). Likewise, the hematological effects observed in the subchronic rat study at low doses are not observed in the chronic rat study (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013). Cannon et al. (2020) demonstrated that HFPO dimer acid can modify the activity of transporters at the blood-brain barrier. Specifically, HFPO dimer acid inhibited P-gp and BCRP transport in rat brain capillaries. The potential neural effects that might result from inhibition of transport activity are unknown and require additional investigation.

Furthermore, Conley et al. (2019), Conley et al. (2021) and Blake et al. (2020) observed alterations in thyroid hormones in the pregnant dam after gestational exposure to GenX chemicals. Specifically, Conley et al. (2019, 2021) demonstrated significant decreases in maternal serum total T3 and T4 levels in the pregnant rat (e.g., a 51% decrease in total T4 in pregnant dams dosed with 62.5 mg/kg/day from GD8-PND2) while Blake et al. (2020) reported a significant increase in mouse placental total T4 levels relative to control. In the Blake et al (2020) study, there was a trend towards a significant effect of higher T4 in placentas exposed to 2 mg/kg/day GenX (38% increase) though not statistically significant. Maternal serum thyroid hormones could not be measured due to volume constraints in the study. The potential neurodevelopmental effects that might result from the disruption of these thyroid hormones are unknown and require additional investigation at lower doses.

Given the residual concerns for potentially more sensitive effects outlined above, a database uncertainty factor is considered necessary to account for the possibility that the currently available database for GenX chemicals may result in an under-protective point of departure. Specifically, a value of 10 was selected for the UF_D to account for the uncertainty surrounding reproductive or developmental effects of concern occurring at similar dose levels to the liver effects (maternal GWG, placental lesions indicative of placental insufficiency, changes in thyroid hormones) or effects that observed to occur with exposure to other PFAS (e.g., PFOA) but have not been studied or do not have published studies currently for GenX chemicals (skeletal ossification, changes in thyroid hormones, mammary gland development, and altered metabolism in the mouse).

The UFs described above were applied to the POD_{HED}s from section 7.2.2 to derive a candidate RfDs applicable to both subchronic and chronic exposures. Table 14 summarizes the results of this quantification for the subchronic scenario. The subchronic candidate RfDs range from 0.00003 mg/kg/day to 0.00007 mg/kg/day. Likewise, Table 15 summarizes the results of this quantification for the chronic scenario. The chronic candidate RfDs range from 0.000003 mg/kg/day to 0.000007 mg/kg/day. Each POD_{HED} is impacted by the doses used in the subject study, the endpoints monitored, and the animal species/gender studied. Thus, the array of outcomes, combined with knowledge of the individual study characteristics, helps to inform selection of a subchronic and chronic RfDs that will be protective for humans.

Table 14. Candidate Subchronic RfD Values

Endpoint and reference	POD_{HED}[a] (mg/kg/day)	POD Type	UF_L	UF_S	UF_A	UF_H	UF_D	UF_{TOT}	Candidate RfD value (mg/kg/day)
HEPATIC									
Liver constellation of lesions in parental male mice (DuPont-18405-1037, 2010)	0.02	$BMDL_{10}$	1	1	3	10	10	300	7×10^{-5}
Liver constellation of lesions in parental female mice (DuPont-18405-1037, 2010)	0.01	$BMDL_{10}$	1	1	3	10	10	300	3×10^{-5}

Note:
[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).

Table 15. Candidate Chronic RfD Values

Endpoint and reference	POD_{HED}[a] (mg/kg/day)	POD Type	UF_L	UF_S	UF_A	UF_H	UF_D	UF_{TOT}	Candidate RfD value (mg/kg/day)
HEPATIC									
Liver constellation of lesions in parental male mice (DuPont-18405-1037, 2010)	0.02	$BMDL_{10}$	1	10	3	10	10	3000	7×10^{-6}
Liver constellation of lesions in parental female mice (DuPont-18405-1037, 2010)	0.01	$BMDL_{10}$	1	10	3	10	10	3000	3×10^{-6}

Note:
[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).

7.4 Selection of Overall RfD

The oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and its pathologic demonstration of liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the critical study and effect, respectively, for deriving the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt. The RfD based on this grouping of effects occurred at the lowest dose and therefore provides the most health-protective RfD among the modeled endpoints based on the available data. The selection of the constellation of lesions is supported by the NTP PWG conclusion that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of the study. Because there is a negligible difference between the molecular weight of the HFPO dimer acid ammonium salt (347.08 grams per mol (g/mol)) and the free HFPO dimer acid (330.06 g/mol), the subchronic and chronic RfDs presented here are applicable for both chemicals.

Several of the other studies provide support for the selection of the DuPont-18405-1037 (2010) study as the critical analysis and the constellation of liver lesions as the critical effect (DuPont-24447, 2008; DuPont-24459, 2008; DuPont-18405-841, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013) on which to base the subchronic and chronic RfDs. The liver is the primary target organ for toxicity from oral exposure to HFPO dimer acid and its ammonium salt. Liver effects are observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals. Specifically, changes in liver enzyme levels, histopathological lesions, and tumors are observed in both male and female mice and rats at varying durations of exposures (15 days to 2 years) and doses of these GenX chemicals (0.5–1,000 mg/kg/day).

7.4.1 Subchronic RfD

This section provides the calculation for the subchronic RfD. The values and rationale describing the input parameters for the RfD calculation can be found in sections 7.2 and 7.3, and appendix E.

$$\text{Subchronic RfD} = \frac{POD_{HED}}{Total\ UF}$$

$$= \frac{0.01 \frac{mg}{kg}/day}{300}$$

$$= \mathbf{3 \times 10^{-5}\ mg/kg/day\ or\ 0.03\ \mu g/kg/day}$$

where:

- POD_{HED} = 0.01 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPO dimer acid ammonium salt by gavage for 53–64 days (DuPont-18405-1037, 2010).
- Total UF = 300, including 10 for UF_H, 3 for UF_A, and 10 for UF_D.

7.4.2 Chronic RfD

This section provides the calculation for the chronic RfD. The values and rationale describing the input parameters for the RfD calculation can be found in sections 7.2 and 7.3, and appendix E.

$$\text{Chronic RfD} = \frac{POD_{HED}}{Total\ UF}$$

$$= \frac{0.01\frac{mg}{kg}/day}{3000}$$

$$= \mathbf{3 \times 10^{-6}\ mg/kg/day\ or\ 0.003\ \mu g/kg/day}$$

where:

- POD_{HED} = 0.01 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPO dimer acid ammonium salt by gavage for 53–64 days (DuPont-18405-1037, 2010).
- Total UF = 3000, including 10 for UF_H, 3 for UF_A, 10 for UF_S, and 10 for UF_D.

8.0 Effects Characterization

8.1 Uncertainty and Variability

The uncertainty and variability in an RfD are a function of both intrinsic and extrinsic factors. EPA has identified multiple short-term subchronic and chronic studies that provide dose-response information and were considered during the quantitative assessment of risk. The range of external dose NOAELs among these studies is 0.1 mg/kg/day–10 mg/kg/day. The LOAELs range from 0.5 mg/kg/day to 100 mg/kg/day.

The intrinsic uncertainties in the assessment reflect the fact that the NOAELs and LOAELs are derived using central tendency estimates for variables such as BW, food and drinking water intakes, and dose. The central tendency estimates are derived from small numbers of relatively genetically similar animals representing one or more strains of rats or mice living in controlled environments. The animals lack the heterogeneous genetic complexity, behavioral diversity, and complex habitats experienced by humans. These differences, to some extent, have been minimized using the modeled outcomes and use of allometric scaling to help inform the application of the UF.

While EPA has routinely used BW to allometrically scale toxicity data from animal test species to HEDs during the development of human health risk assessments, the applied methodology is not without limitation (EPA, 2011b). Allometric scaling using BW scaled to the ¾ power primarily addresses uncertainty associated with toxicokinetics, although the exact amount of uncertainty addressed by this method for any specific chemical is often not quantifiable. In following the recommended method to apply $BW^{3/4}$ scaling, it remains possible that the toxicokinetic uncertainty associated with GenX chemicals might be more or less than what is accounted for using this scaling methodology.

For all selected candidate studies, $BW^{3/4}$ scaling was found to be appropriate because GenX chemicals are not metabolized and have relatively short clearance times, especially compared to other longer chain PFAS chemicals such as PFOA (DuPont-18405-1017 RV1, 2011; EPA, 2011b; Gannon et al., 2016). The $BW^{3/4}$ scaling methodology is not appropriate, however, when using children's BWs. This limitation exists due to the absence of quantitative information describing the toxicokinetic and toxicodynamic differences between test animals and early life-stage humans (EPA, 2011b). Because the liver effects observed following exposure to GenX chemicals were in adult animals, the allometric scaling methodology was scaled to the average adult human BW.

Variability in the study outcomes is extrinsically a function of study design and the endpoints monitored. Studies of systemic toxicity monitor an array of endpoints that are not evaluated in studies of reproductive, developmental, neurological, and immunological toxicity. The reverse is true for the other types of toxicity studies compared to standard short-term and long-term systemic studies. Studies of systemic toxicity do not often examine neurological or immunological endpoints. Increases in liver weight were seen in many of the studies with dose-response information, and the histological evaluation of the liver supported a determination that the increase in liver weight when it is accompanied by necrosis can be considered as adverse rather than adaptive, according to the Hall et al. (2012) criteria. Increases in relative liver weight with confirmed liver necrosis were observed in DuPont-24447 (2008), DuPont-24459 (2008), DuPont-18405-1037 (2010), DuPont-18405-1307 (2010), and DuPont-18405-1238 (2013).

The subchronic and chronic RfDs are based on the POD_{HED} derived from the parental females from the oral reproductive/developmental toxicity study in mice with application of UFs to account for variability in the human population, database uncertainties, and possible differences in the ways in which humans and rodents respond to HFPO dimer acid and/or its ammonium salt that reaches their tissues (DuPont-18405-1037, 2010). Uncertainty associated with relying on a less-than-chronic study to derive a chronic RfD is addressed with a UF applied only for the chronic RfD calculation. The selected RfDs are based on the adverse liver effects observed in the parental female animals. Selection of this endpoint is expected to provide protection to both the sensitive life stages and the general population. The RfDs are supported by the outcomes from other studies based on different endpoints, including hematological, immune, and developmental effects (DuPont-24459, 2008; DuPont-17751-1026, 2009; DuPont-18405-1037, 2010). These supporting data from the HFPO dimer acid and its ammonium salt database increase confidence in the RfD.

8.2 Composition of Test Substance

Most of the available data for HFPO dimer acid and its ammonium salt with PMNs were submitted to EPA by DuPont, the manufacturer of GenX chemicals, under TSCA, as required pursuant to a consent order for these chemicals (EPA, 2009) or as required under TSCA reporting requirements (e.g., section 8(e) 15 U.S.C. § 2607.8(e)). In these submissions, DuPont provided information on the purity of the test substance used in each of the studies. Purity ranged from 84% to 88% across the toxicity studies considered in this assessment. DuPont provided a certificate of these analyses and noted that they were conducted under EPA GLP standards (40 CFR part 792). The major impurity identified is water (12.7%–13.3%). Trace amounts of PFOA were also identified in the test substance (3.4–150 parts per million). DuPont noted that test results were adjusted for purity based on the reported test article formulations. Based on the

information provided, administered doses of PFOA present as a contaminant in the formulations used by DuPont are low. For example, in the critical study chosen for the derivation of the RfDs, the dose of administered PFOA is 0.000075 mg/kg/day at the GenX chemicals NOAEL (0.1 mg/kg/day) (DuPont-18405-1037, 2010). For PFOA, NOAELs ranging from 0.01 mg/kg/day to 30 mg/kg/day have been identified for effects including developmental, liver, and immune endpoints (EPA, 2016a). Despite trace amounts of PFOA that might be present as an impurity, EPA recognizes the potential for this impurity to contribute to the observed toxicity at very high doses of GenX chemicals. At present, however, discerning the contribution of this low level of PFOA to observed toxicity is not possible. Thus, EPA concluded that the presence of PFOA at these low levels is not the primary driver of toxicity observed in the studies. Of note is that the same test substance (Lot H-28548) was used in the 90-day mouse and rat studies, the chronic rat study, and the oral reproductive and developmental toxicity and prenatal developmental toxicity studies (DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013). Additionally, the same test substance (Lot H-28397) was used in both the mouse and the rat 28-day studies (DuPont-24447, 2008; DuPont-24459, 2008). Despite differences in test substance purity, adverse effects were observed consistently across the DuPont studies. Many of the peer-reviewed studies did not report purity in their methods or formulations of HFPO dimer acid and ammonium salt (Behr et al., 2020; Blake et al., 2020; Rushing et al., 2017; Sheng et al., 2018; Wang et al., 2017).

Given the database for GenX chemicals, the quality of these studies—including adequacy of reporting of methods and results—and the weight of evidence for effects on the liver, hematological and immune systems, and reproductive and developmental endpoints, EPA concluded that the DuPont studies demonstrated adverse effects as a result of exposure to the HFPO dimer acid ammonium salt formulations and were appropriate for derivation of toxicity values for these chemicals.

8.3 Use of Data-Derived Extrapolation Factors

For HFPO dimer acid and/or ammonium salt, there are limited human half-life data (see section 8.4) and no BBDR or PBPK models available to evaluate toxicokinetic and toxicodynamic differences between humans and animals. Additionally, only a few repeat-dose studies are available on rats and mice that evaluate toxicokinetics. These studies indicate that there is little-to-no metabolism and that clearance is relatively rapid compared to other longer chain PFAS. MOA (both *in vivo* and *in vitro*) data are also inadequate. EPA considered the 2014 *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* in determining UF_A and UF_H (EPA, 2014c). Using the decision process described in Figure 2, EPA concluded that data are not adequate to support derivation of data-derived extrapolation factors. Specifically, given the lack of available models and data to address external dose and clearance in humans, default approaches to the application of UF_A and UF_H were employed, including BW scaling for oral exposure (EPA, 2011b). These approaches are described further in section 7.3.

8.4 Use of Data-Derived Dosimetric Adjustment Factor

EPA guidance recommends a hierarchical approach to deriving human equivalent oral exposures from animal studies, with the preferred approach being physiologically based toxicokinetic modeling. There are no such toxicokinetic models available for GenX chemicals. The next

preferred approach is to use chemical-specific information to derive a data-informed dosimetric adjustment factor (DAF). For GenX chemicals there are limited human data (outlined below) and a few repeat-dose studies available on rats and mice that evaluate toxicokinetics (see section 2.3.6).

In the one available human half-life study, twenty-five workers from a Chemours facility in the Netherlands volunteered blood samples before an off-work weekend and twenty-two workers provided a second sample at the start of the next shift (72-96 hours between sample collections) (Clark, 2021). Samples were sent to two independent laboratories. HFPO dimer acid concentrations ranged from below the level of detection (less than 0.5 µg/L) to 25 µg/L (Arbo Unie, 2020). Samples containing measurable amounts of HFPO dimer acid at both time points were used to calculate an average approximate half-life of 81 ± 55 hours, assuming an exponential rate of decay (Clark, 2021). The range was 42 to 333 hours with a median of 66 hours. Serum from eighteen of the twenty-two workers contained HFPO dimer acid at detectable levels (i.e., at or above the limit of detection) at both time points.

A letter summarizing the data and briefly outlining the methods used to calculate the human half-life was provided to TSCA in 2021 by Chemours (similar information can also be found on ECHA). However, EPA has not received the full study report and these data have not been peer reviewed. The dataset used by Chemours to calculate the half-life is limited to only 18 individuals. Chemours also provided EPA with an unpublished report containing the raw data (Arbo Unie, 2020); however, this report did not stratify the data based on sex or provide any additional details on the test subjects (including sex). Sex-stratification of the human worker data is potentially important because the critical effect in mice is more severe in females (DuPont-18405-1037, 2010). Because the information provided are insufficient, EPA did not use the human half-life data to estimate a data-informed DAF. Instead, EPA employed the default procedure of body weight scaling to the ¾ power (i.e., $BW^{3/4}$) to derive human equivalent oral exposures from animal studies in concordance with EPA guidance (EPA, 2011b; outlined in section 7.2.2).

Although the Chemours human half-life data are insufficient for use in the allometric scaling of animal to human dose for toxicity and risk assessment purposes, EPA conducted an exploratory analysis to determine the magnitude of the impact on the resulting POD_{HED} if this information was used to calculate a POD_{HED} in place of the default $BW^{3/4}$ DAF (which, as outlined above, is the agency's standard approach where acceptable data are not available) (Table 16).

Table 16. Comparison of POD_{HED} using different allometric scaling methods

Endpoint and reference	POD_{HED} (mg/kg/day) calculated using…	
	$BW^{3/4}$ DAF	Data Derived Human DAF
Liver constellation of lesions in parental males (DuPont-18405-1037, 2010)	0.02	0.06
Liver constellation of lesions in parental females (DuPont-18405-1037, 2010)	0.01	0.03

The method used to calculate the data-derived DAF is outlined in Section 6.1.1.2 of the Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3) (EPA, 2021a). Briefly, the ratio of elimination half-life in animals from which the POD is obtained ($t_{1/2A}$) to that in humans ($t_{1/2H}$) can be used to calculate the DAF, and the human equivalent dose (HED) can be calculated as follows:

$$POD_{HED} = POD \times \frac{t_{1/2A}}{t_{1/2H}}$$

For the comparison exercise in Table 16, the $t_{1/2A}$ used for GenX chemicals were the male and female mouse data from the beta elimination phase outlined in Table 8 and the $t_{1/2H}$ was the 81 hours calculated from the data outlined above (Arbo Unie, 2020; Clark, 2021). Although the Chemours human half-life data were found to be insufficient for this purpose (Chemours, 2021) describes the dataset as "limited"), this comparison demonstrates that the POD_{HED} calculated using either the $BW^{3/4}$ DAF or the Data Derived Human DAF are similar. This comparison exercise illustrates a degree of consistency between the $BW^{3/4}$ approach and the use of the only available human half-life dataset for deriving human equivalent oral doses for GenX chemicals.

8.5 Limited Data on Carcinogenicity

One study is available on evaluating carcinogenicity of HFPO dimer acid and its ammonium salt in rats (DuPont-18405-1238, 2013). In this study, liver and pancreatic tumors were noted at the highest doses tested. Although the incidence of testicular interstitial cell adenomas was not statistically significant compared to controls, the authors of the study conclude that "a relationship to treatment for these findings in the 50 mg/kg/day group cannot be ruled out" while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015).The available data for HFPO dimer acid ammonium salt suggest that mice might be more sensitive than rats to exposure to these GenX chemicals. Given the evidence that the liver is the target organ for toxicity and the primary organ for tumor development, additional research is needed using chronic duration exposures in mice. This uncertainty was not considered in the application of the UF_D because a noncancer toxicity value was developed for this assessment.

8.6 Internal Dosimetry Data for GenX Chemicals

EPA recognizes that there are similarities in the health effects observed across various PFAS. Specifically, GenX chemicals are linked to adverse effects on the liver, kidney, immune system, development, and cancer and these health effects have also been associated with PFOA exposure (EPA, 2016a,b). There are data available that demonstrate that the toxicokinetic profile for GenX chemicals is different than PFOA in that GenX chemicals are more rapidly excreted than PFOA and appear not to bioaccumulate like PFOA. These data lead one to question whether administering the same dose of these chemicals could result in a much lower internal dose for GenX chemicals than PFOA or PFOS and thus differences in potency between the two chemicals.

There are currently two studies evaluating the internal dose of the HFPO dimer acid and comparing it to the internal dose of either PFOA (Blake et al., 2020) or PFOS (Conley et al.,

2021). Specifically, Blake et al. (2020) evaluated internal dose of both chemicals in pregnant mice and their embryos. Concentrations of PFOA and HFPO dimer acid were measured in the maternal serum, maternal liver, amniotic fluid, and whole embryo dosed with 0, 1, or 5 mg/kg/day of PFOA or 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E11.5 or E17.5. Although concentrations in the maternal serum were relatively similar, the total concentration of HFPO dimer acid is an order of magnitude less than PFOA in the maternal liver. Additionally, PFOA appears to accumulate in the liver from E11.5 to E17.5 in mice exposed to 1 mg/kg/day PFOA (48.3 ± 12.5 ug/mL to 181.1 ± 46.0 ug/mL); however, the concentration of HFPO dimer acid at 2 mg/kg/day is similar at both time points (5.45 ± 3.43 ug/mL to 4.56 ± 2.80 ug/mL). These differences are noteworthy because PFOA and HFPO dimer acid affected the maternal liver similarly in this study (e.g., increased liver weight and increased incidence of liver lesions) despite the concentration of HFPO dimer acid being an order of magnitude lower than PFOA and displaying no apparent accumulation between E11.5 and E17.5. The concentrations of PFOA and HFPO dimer acid are similar in the amniotic fluid and whole embryo in the 1- and 2-mg/kg/day-dose groups, respectively. These data suggest that a lower internal dose of HFPO dimer acid elicits the same effects on the liver as a higher internal dose of PFOA in the pregnant mouse. Additional research is needed to further elicit whether internal dosimetry is in fact different between these chemicals and to determine if these results are specific to the pregnant mouse.

Conley et al. (2021) compared the maternal serum levels of HFPO dimer acid and PFOS with respect to neonatal mortality. Conley et al. (2021) concluded that based on maternal serum concentrations, HFPO dimer acid (EC_{50} = 35.4 ug/mL) was ~2-fold more potent than PFOS (EC_{50} = 74.5 ug/mL). However, given that the molecular weight of PFOS (500 g/mol) is 34% greater than HFPO-DA (330 g/mol), the potency of PFOS (EC_{50} =148.9 uM) and HFPO dimer acid (EC_{50} = 107.1 uM) are very similar when correcting for molecular weight differences.

8.7 Effects on Bilirubin

A decrease in serum bilirubin is a consistent effect observed across multiple studies, especially in female rodents (DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013; Wang et al., 2017). This finding was surprising given that increased rather than decreased levels of serum bilirubin are typically indicative of liver damage, and multiple studies outlined above have confirmed microscopic liver damage (DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013; Tietze, 2012). In female mice and rats, however, serum bilirubin levels were significantly decreased by 14%–50% relative to controls when the females were administered 5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 3–12 months (DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013). Additionally, male ICR mice treated with 1 mg/kg/day of HFPO dimer acid ammonium salt exhibited a significant 37% and 45% decrease in total and direct bilirubin, respectively, when compared to controls (Wang et al., 2017); this finding was not replicated in the other 28-day studies (DuPont-24447, 2008; DuPont-24459, 2008). The biological or mechanistic significance of this effect is unknown, yet its consistency across multiple studies is noteworthy.

8.8 Susceptible Populations and Life Stages

Data for the elucidation of differential susceptibility dependent on life stage (e.g., developing embryo/fetus, women of reproductive age, or pregnant women) are not available. Children are

frequently more vulnerable to pollutants than the average adult because of the differences in their behaviors and biology. These differences can result in greater exposure and/or unique windows of developmental susceptibility during the prenatal and postnatal periods for both the pregnant mother and the developing fetus. No human toxicity or epidemiological studies are available in the literature that address early developmental or reproductive life stages. Peer-reviewed literature and DuPont submitted data examining reproductive and developmental endpoints in both mice and rats (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-18405-841, 2010; DuPont-18405-1037, 2010) and summaries of these studies can be found in section 5.4 (Reproductive/Developmental). HFPO dimer acid ammonium salt can be transferred from a pregnant animal to the fetus during gestation and lactation (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-18405-1037, 2010; Dupont-18405-849 RV1, 2011). When present, developmental and reproductive effects were found at doses similar to and higher than those associated with the selected critical effect: liver effects in females (constellation of lesions as defined by the NTP PWG to include cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis). The UF_H of 10 accounts for variability in the responses within human populations because of both intrinsic (including life stage) and extrinsic (lifestyle) factors that can influence the response to dose. No information to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics is available. Thus, the RfDs provided in sections 7.4.1 and 7.4.2 (Subchronic RfD and Chronic RfD) are applicable to all life stages. When reviewing data pertinent to the hazard potential of GenX chemicals, EPA adhered to the requirements of its 2013 reaffirmation of the Policy on Evaluating Health Risks to Children (EPA, 2013).

There is some sex-specific variation in the toxicokinetics of these two GenX chemicals in rodents. Toxicokinetic data from DuPont calculate clearance times from the urine and plasma, which is defined by DuPont as the time when 98.4% of the anion from the HFPO dimer acid ammonium salt was cleared from the urine or plasma. These data show the HFPO dimer acid and its ammonium salt clearance time in the plasma to be considerably faster for female rodents than for male rodents (see the summary in section 2.3.6 (Clearance and Half-Life Data). For example, Dupont-25300 (2008) identified 143 hours as the clearance time for HFPO dimer acid ammonium salt in male mice at 10 mg/kg and 139 hours for 30 mg/kg. In female mice, the clearance values were 57 and 62 hours for the low dose and the high dose, respectively. However, this difference was not as pronounced in mice in the $T_{1/2}$ estimates. Specifically, the alpha (distribution) phase $T_{1/2}$s were 5.8 and 4.6 hours for male and female mice, respectively, and the beta (elimination) phase $T_{1/2}$s were 36.9 hours and 24.2 hours for male and female mice, respectively. It is unknown if or how these observed sex-specific toxicokinetic differences in rodents contribute to the toxic response.

The available data suggest that the pregnant rodent might be more susceptible to liver effects following exposure to GenX chemicals during gestation. Liver effects were reported in the pregnant dams in the available reproductive/developmental studies dosed during gestation (Blake et al., 2020; Conley et al., 2019; DuPont-18405-841, 2010; DuPont 18405-1037, 2010). All the studies reported increases in liver weight ranging from 12% to 34% in rats and 26% to 101% in mice over the gestational period. Conley et al. (2019) did not conduct liver histopathology, but both DuPont-18405-841 (2010) and Blake et al. (2020) reported hepatocellular hypertrophy and increased cell death as compared to controls with increasing HFPO dimer acid ammonium salt concentration. Specifically, focal necrosis was observed in 2/22 (9%) and 5/22 (23%) pregnant

rats after just 15 days (GD6–GD20) of 10 mg/kg/day or 100 mg/kg/day of HFPO dimer acid ammonium salt, respectively, compared to 0 in the control group. Comparatively, nonpregnant female rats dosed from 28 to 90 days did not exhibit necrosis when treated with doses up to 1,000 mg/kg/day of HFPO dimer acid ammonium salt. Necrosis was observed in nonpregnant female rats only after 2 years of dosing with 500 mg/kg/day of HFPO dimer acid ammonium salt. Increased cell death (including both apoptosis and single-cell or focal necrosis) was observed in pregnant mice after 11 and 17 days (GD1.5–GD11.5 or GD17.5) of 2 mg/kg/day or 10 mg/kg/day of HFPO dimer acid ammonium salt. Similarly, and as noted above, female mice dosed 14 days prior to mating and throughout gestation and lactation exhibited cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis after 53–64 days of dosing (NTP, 2019 reread of DuPont 18405-1037, 2010). The incidence of single-cell and focal necrosis in the F_0 females was 6/24 (25%) and 20/24 (83%) in the 0.5- and 5-mg/kg/day-dose groups, respectively (NTP, 2019). A chronic study in mice is not available to compare to the gestational exposures in female pregnant mice, and comparisons to the 90-day subchronic study in mice is potentially limited by sample size (n = 9) in the 0.1 and 0.5 mg/kg/day-dose groups.

Susceptible populations include groups who have relatively high exposure to GenX chemicals. While data are currently unavailable, there is the potential for highly exposed populations. For example, formula fed infants, who have high daily water ingestion relative to body weight, have the potential for relatively high exposure to GenX chemicals when GenX chemicals are present in tap water and this tap water is used to reconstitute formula. As a second example, workers and their families who work at and/or live near facilities that use the GenX processing aid technology have the potential for greater exposure levels and duration of exposure. Finally, communities living in close proximity to facilities using the GenX processing aid technology have the potential for increased exposure as evidenced by the detection of GenX chemicals in drinking water, surface water, soil and rainwater samples collected close to the facility (see section 1.3).

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Appendix A: Literature Search Strategy

This appendix presents the details of the literature search strategy U.S. Environmental Protection Agency (EPA) used to identify primary, peer-reviewed literature pertaining to hexafluoropropylene oxide (HFPO) dimer acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6) and its ammonium salt (CASRN 62037-80-3). The literature searches were conducted using the databases listed in Table A-1.

The initial literature searches for these GenX chemicals were conducted in July 2017 (acid) and January/February 2018 (ammonium salt). Subsequent literature searches were conducted from 2018 to March 2020. The searches were conducted using CASRN, synonyms, and additional relevant search strings (see Table A-2). Because the results of this core search were so limited, additional databases were identified and searched for physiochemical property information, health effects, toxicokinetics, and mechanistic information (see Table A-3 and Table A-4). Combined, these initial literature searches returned 27 studies for HFPO dimer acid and HFPO dimer acid ammonium salt after duplicates across the two chemicals were deleted. The literature searches conducted after publication of the public comment draft in November 2018 resulted in 48 additional studies for HFPO dimer acid and HFPO dimer acid ammonium salt after duplicates were deleted.

As previously stated, the available data for GenX chemicals come primarily from studies submitted under the Toxic Substances Control Act (TSCA). Those studies were combined with the results of the search of the publicly available peer-reviewed literature for evaluation for relevance to the assessment. The submitted studies and literature identified by the search of publicly available sources are available through EPA's Health & Environmental Research Online (HERO) website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627. Potential relevance was based primarily on a title and abstract screen. Table A-5 presents the inclusion/exclusion criteria applied to conducting the literature searches. An additional 48 studies from peer-reviewed literature were identified during the updated literature searches conducted in February 2019, October 2019, and March 3, 2020. These studies were subjected to title and abstract screening to determine relevancy according to the inclusion/exclusion criteria outlined in Table A-6. Relevancy was confirmed by review of the full text of studies included in the title abstract screen. Studies that did not meet the inclusion criteria but provide supporting information were categorized as supplemental, relative to the type of supporting information they provided. These supplemental categories are outlined in Table A-7.

Table A-1. Summary of Core Database Search Results

Search date	PubMed	WOS	Toxline	TSCATS via Toxline/NLM	Other sources	Combined dataset after duplicate removal
HFPO dimer acid (CASRN 13252-13-6)						
7/24/17	3	12	0	0	3	16
7/17–2/19	6	11	0	0	0	11
2/19–10/19	9	8	0	0	9	16
10/19–3/20	7	4	N/A[a]	0	1	9
HFPO dimer acid ammonium salt (CASRN 62037-80-3)						
1/18 and 2/18	9	12	0	0	3	18
2/18–2/19	8	13	0	0	1	15
2/19–10/19	15	11	0	0	2	20
10/19–3/20	7	3	N/A[a]	0	1	8

Note: N/A = not applicable; NLM = National Library of Medicine; TSCATS = Toxic Substances Control Act Test Submissions; WOS = Web of Science.

[a] Toxline was no longer available in March 2020.

Table A-2. Database Search Strings

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
PubMed	13252-13-6[rn] OR "2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid"[tw] OR "Perfluoro(2-methyl-3-oxahexanoate) "[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)- "[tw] OR "Perfluorinated aliphatic carboxylic acid"[tw] OR "Perfluoro(2-methyl-3-oxahexanoic) acid"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid"[tw] OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid"[tw] OR "perfluoro-2-(propyloxy)propionic acid"[tw] OR "perfluoro-2-methyl-3-oxahexanoic acid"[tw] OR "perfluoro-2-propoxypropanoic acid"[tw] OR "perfluoro-2-propoxypropionic acid"[tw] OR "perfluoro-α-propoxypropionic acid"[tw] OR "propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-"[tw] OR "propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-"[tw] OR (GenX AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw])) OR (("2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic"[tw] "Perfluorinated aliphatic carboxylic"[tw] OR "Perfluoro(2-methyl-3-oxahexanoic)"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic"[tw] "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic"[tw] OR "perfluoro-2-(propyloxy)propionic"[tw] OR "perfluoro-2-methyl-3-oxahexanoic"[tw] OR "perfluoro-2-propoxypropanoic"[tw] OR "perfluoro-2-propoxypropionic"[tw] OR "perfluoro-α-propoxypropionic"[tw]) AND (acid[tw] OR acids[tw]))	(62037-80-3[rn] OR "62037-80-3"[tw] OR "Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate"[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt"[tw] OR "Perfluorinated aliphatic carboxylic acid, ammonium salt"[tw] OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt"[tw] OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate"[tw] OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)"[tw] OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid"[tw] OR "Ammonium perfluoro-2-methyl-3-oxahexanoate"[tw] OR "FRD-902"[tw] OR "GenX-H3N"[tw] OR "HFPO-DA"[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt"[tw] OR "Undecafluoro-2-methyl-3-oxahexanoic acid"[tw] OR ((GenX[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw])) OR (("Undecafluoro-2-methyl-3-oxahexanoic"[tw] OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)"[tw] OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)"[tw] OR "Perfluorinated aliphatic carboxylic"[tw]) AND (salt[tw] OR salts[tw] OR acid[tw] OR acids[tw])))) OR (((Undecafluoro AND oxahexanoic) OR (Ammonium AND perfluoro AND oxahexanoic) OR (Tetrafluoro AND heptafluoropropoxy) OR "Perfluorinated aliphatic carboxylic"[tw] OR "Perfluorinated aliphatic carboxylic"[tw]) AND (salt[tw] OR salts[tw] OR acid[tw] OR acids[tw]))

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
WOS	TS="2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid" OR TS="Perfluoro(2-methyl-3-oxahexanoate)" OR TS="Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-" OR TS="Perfluorinated aliphatic carboxylic acid" OR TS="Perfluoro(2-methyl-3-oxahexanoic) acid" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid" OR TS="2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid" OR TS="perfluoro-2-(propyloxy)propionic acid" OR TS="perfluoro-2-methyl-3-oxahexanoic acid" OR TS="perfluoro-2-propoxypropanoic acid" OR TS="perfluoro-2-propoxypropionic acid" OR TS="perfluoro-α-propoxypropionic acid" OR TS="propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR TS="propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR (TS="GenX" AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) OR ((TS="2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic" OR TS="Perfluorinated aliphatic carboxylic" OR TS="Perfluoro(2-methyl-3-oxahexanoic)" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic" OR TS="2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic" OR TS="perfluoro-2-(propyloxy)propionic" OR TS="perfluoro-2-methyl-3-oxahexanoic" OR TS="perfluoro-2-propoxypropanoic" OR TS="perfluoro-2-propoxypropionic" OR TS="perfluoro-α-propoxypropionic") AND TS=(acid OR acids))	TS=("Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt" OR "Perfluorinated aliphatic carboxylic acid, ammonium salt" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt" OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate" OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid" OR "Ammonium perfluoro-2-methyl-3-oxahexanoate" OR "FRD-902" OR "GenX-H3N" OR "HFPO-DA" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt" OR "Undecafluoro-2-methyl-3-oxahexanoic acid") OR ((TS=GenX AND (TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated)))) OR ((TS=("Undecafluoro-2-methyl-3-oxahexanoic" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)" OR "Perfluorinated aliphatic carboxylic" OR "Perfluorinated aliphatic carboxylic")) AND (TS=(salt OR salts OR acid OR acids))) **Timespan:** All years. **Indexes:** SCI-EXPANDED, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC.

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
Toxline	(13252-13-6[rn] OR "2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid" OR "Perfluoro(2-methyl-3-oxahexanoate) " OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)- " OR "Perfluorinated aliphatic carboxylic acid" OR "Perfluoro(2-methyl-3-oxahexanoic) acid" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid" OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid" OR "perfluoro-2-(propyloxy)propionic acid" OR "perfluoro-2-methyl-3-oxahexanoic acid" OR "perfluoro-2-propoxypropanoic acid" OR "perfluoro-2-propoxypropionic acid" OR "perfluoro-α-propoxypropionic acid" OR "propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR "propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR (GenX AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) OR (("2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic" OR "Perfluorinated aliphatic carboxylic" OR "Perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic" OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic" OR "perfluoro-2-(propyloxy)propionic" OR "perfluoro-2-methyl-3-oxahexanoic" OR "perfluoro-2-propoxypropanoic" OR "perfluoro-2-propoxypropionic" OR "perfluoro-α-propoxypropionic") AND (acid OR acids))) AND ((aneupl [org] OR biosis [org] OR cis [org] OR dart [org] OR pubdart [org] OR emic [org] OR epidem [org] OR fedrip [org] OR heep [org] OR hmtc [org] OR ipa [org] OR riskline [org] OR mtgabs [org] OR niosh [org] OR ntis [org] OR pestab [org] OR ppbib [org]) AND NOT pubmed [org] AND NOT pubdart [org])	(62037-80-3[rn] OR "Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt" OR "Perfluorinated aliphatic carboxylic acid, ammonium salt" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt" OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate" OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid" OR "Ammonium perfluoro-2-methyl-3-oxahexanoate" OR "FRD-902" OR "GenX-H3N" OR "HFPO-DA" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt" OR "Undecafluoro-2-methyl-3-oxahexanoic acid" OR "GenX" OR (("Undecafluoro-2-methyl-3-oxahexanoic" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)" OR "Perfluorinated aliphatic carboxylic" OR "Perfluorinated aliphatic carboxylic") AND (salt OR salts OR acid OR acids))) AND ((aneupl [org] OR biosis [org] OR cis [org] OR dart [org] OR pubdart [org] OR emic [org] OR epidem [org] OR fedrip [org] OR heep [org] OR hmtc [org] OR ipa [org] OR riskline [org] OR mtgabs [org] OR niosh [org] OR ntis [org] OR pestab [org] OR ppbib [org]) AND NOT pubmed [org] AND NOT pubdart [org])
TSCATS1	13252-13-6[rn] AND (TSCATS [org])	62037-80-3[rn] AND (TSCATS [org])

Notes: PFAS = per- and polyfluoroalkyl substances; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; TSCATS = Toxic Substances Control Act Test Submissions; WOS = Web of Science.

Table A-3. Processes Used to Augment the Search of Core Databases for HFPO Dimer Acid (CASRN 13252-13-6)

System used	Selected key reference(s) or sources
TSCATS[a]	TSCA Test Submissions 2.0; website now retired (https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm)
	Chemical Data Access Tool (CDAT); website now retired (https://java.epa.gov/oppt_chemical_search/)
	ChemView (https://java.epa.gov/chemview)
Resources searched for physiochemical property information	Agency for Toxic Substances and Disease Registry (ATSDR) (https://www.atsdr.cdc.gov/) Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (https://www.nicnas.gov.au/chemical-information) CAMEO Chemicals (https://cameochemicals.noaa.gov/) Canada DSL List (http://webnet.oecd.org/CCRWEB/Search.aspx) Chemical Risk Information Platform (CHRIP) (http://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop) ChemIDplus (https://chem.nlm.nih.gov/chemidplus/) ChemSpider (http://www.chemspider.com/) CRC Handbook of Chemistry and Physics (http://hbcponline.com/faces/contents/ContentsSearch.xhtml;jsessionid=9408875156F724E0E945D3A6D0454891) ECHA Information on Chemicals (https://echa.europa.eu/) eChemPortal (https://www.echemportal.org/echemportal/index.action) Hazardous Substances Data Bank (HSDB) https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) HSNO Chemical Classification and Information Database (CCID) updated link[b] (https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/) IARC Monographs (http://www.inchem.org/pages/iarc.html) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris) J-Check (http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en) Kirk-Othmer Encyclopedia of Chemical Technology updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/0471238961) NIEHS (https://www.niehs.nih.gov/) OSHA Occupational Chemical Database (https://www.osha.gov/chemicaldata/) PubChem (https://pubchem.ncbi.nlm.nih.gov/search/index.html) SRC Fate Pointers (http://esc.syrres.com/fatepointer/search.asp) Ullmann's Encyclopedia updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/14356007) EPA ACToR (https://actor.epa.gov/actor/home.xhtml) EPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) EPA ChemView (https://java.epa.gov/chemview) EPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents

System used	Selected key reference(s) or sources
Resources searched for health effects, toxicokinetics, and mechanistic information	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA OEHHA (http://www.oehha.ca.gov/risk.html) CPSC (http://www.cpsc.gov) ECHA (http://echa.europa.eu/information-on-chemicals) eChemPortal[c] (http://www.echemportal.org/echemportal/) EFSA Europe (http://www.efsa.europa.eu/) Environment Canada (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) European Union Risk Assessment Reports (https://ec.europa.eu/jrc/en/publications-list) Federal Docket (http://www.regulations.gov) Health Canada (https://www.canada.ca/en/health-canada.html) IARC (http://monographs.iarc.fr/ENG/Classification/index.php) ITER (http://www.tera.org/iter/) Japan Existing Chemical Data Base (http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp) NICNAS (http://www.nicnas.gov.au/chemical-information) NIEHS (http://www.niehs.nih.gov/) NTP (http://ntpsearch.niehs.nih.gov/home) OEHHA Toxicity Criteria Database (http://www.oehha.ca.gov/tcdb/index.asp) EPA NSCEP (https://www.epa.gov/nscep) FDA (http://www.fda.gov/) WHO (http://www.who.int/ipcs/assessment/en/)

Notes: TSCATS = Toxic Substances Control Act Test Submissions

[a] Only relevant TSCATS studies from these interfaces were added to the HERO project page.

[b] The URL has been updated (as listed here) since the literature search; during the search, a previous URL was used.

[c] eChemPortal includes the following databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, EPA-IRIS, EPA-SRS, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, and UK CCRMP Outputs.

Table A-4. Processes Used to Augment the Search of Core Databases for HFPO Dimer Acid Ammonium Salt (CASRN 62037-80-3)

System used	Selected key reference(s) or sources
TSCATS[a]	Chemical Data Access Tool (CDAT); website now retired (https://java.epa.gov/oppt_chemical_search/)
	ChemView (https://java.epa.gov/chemview)
Resources searched for physiochemical property information	ATSDR (https://www.atsdr.cdc.gov/) CAMEO Chemicals (https://cameochemicals.noaa.gov/) Canada DSL List (http://webnet.oecd.org/CCRWEB/Search.aspx) ChemIDplus (https://chem.nlm.nih.gov/chemidplus/) CRC Handbook of Chemistry and Physics (http://hbcponline.com/faces/contents/ContentsSearch.xhtml;jsessionid=9408875156F724E0E945D3A6D0454891) ECHA Information on Chemicals (https://echa.europa.eu/) eChemPortal (https://www.echemportal.org/echemportal/index.action) Hazardous Substances Data Bank (HSDB) (https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) HSNO Chemical Classification and Information Database (CCID) updated link[b] (https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/) IARC Monographs (http://www.inchem.org/pages/iarc.html) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris) J-Check (http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en) Kirk-Othmer Encyclopedia of Chemical Technology updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/0471238961 NICNAS (https://www.nicnas.gov.au/chemical-information) NIEHS (https://www.niehs.nih.gov/) OSHA Occupational Chemical Database (https://www.osha.gov/chemicaldata/) PubChem (https://pubchem.ncbi.nlm.nih.gov/search/index.html) SRC Fate Pointers (http://esc.syrres.com/fatepointer/search.asp) Ullmann's Encyclopedia updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/14356007) EPA ACToR (https://actor.epa.gov/actor/home.xhtml) EPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) EPA ChemView (https://java.epa.gov/chemview) EPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents

System used	Selected key reference(s) or sources
Resources searched for health effects, toxicokinetics, and mechanistic information	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA - OEHHA (http://www.oehha.ca.gov/risk.html; http://www.oehha.ca.gov/tcdb/index.asp) CPSC (http://www.cpsc.gov) ECHA (http://echa.europa.eu/information-on-chemicals) eChemPortal[c] (http://www.echemportal.org/echemportal/) EFSA Europe (http://www.efsa.europa.eu/) Environment Canada (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) EPA-NSCEP (https://www.epa.gov/nscep) European Union Risk Assessment Reports (https://ec.europa.eu/jrc/en/publications-list) Federal Docket (http://www.regulations.gov) Google (Quick search only www.google.com) Health Canada (https://www.canada.ca/en/health-canada.html) IARC (http://monographs.iarc.fr/ENG/Classification/index.php) ITER (TERA database) (http://www.tera.org/iter/) Japan Existing Chemical Data Base (JECDB) (http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp) NICNAS (http://www.nicnas.gov.au/chemical-information) NIEHS (http://www.niehs.nih.gov/) NTP (http://ntpsearch.niehs.nih.gov/home) FDA (http://www.fda.gov) WHO (http://www.who.int/ipcs/assessment/en/)

Notes: TSCATS = Toxic Substances Control Act Test Submissions

[a] Only relevant TSCATS studies from these interfaces were added to the HERO project page.

[b] The URL has been updated (as listed here) since the literature search; during the search, a previous URL was used.

[c] eChemPortal includes the following databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, EPA-IRIS, EPA-SRS, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, and UK CCRMP Outputs.

Table A-5. Inclusion-Exclusion Criteria for HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt Studies

PECO Parameter	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog • Alternative animal models in standard laboratory conditions (e.g., *Xenopus*, zebrafish, minipig) • Human or animal cells, tissues, or organs (not whole animals); bacteria; nonmammalian eukaryotes; other nonmammalian laboratory species	• Ecological species
Exposure	• Exposure is to HFPO dimer acid and/or its ammonium salt • Exposure via oral, inhalation, dermal, intraperitoneal, or intravenous injection routes • Exposure is measured in air, dust, drinking water, diet, gavage, or injection vehicle, or via a biomarker of exposure (PFAS levels in whole blood, serum, plasma, or breast milk) • Exposure is via cells in culture or subcellular matrices	• Study population is not exposed to HFPO dimer acid and/or its ammonium salt • Exposure is to a mixture only without evaluating HFPO dimer acid and/or its ammonium salt individually
Outcome	• Studies that include a measure of one or more health effect endpoints, including effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer • *In vivo* and/or *in vitro* studies related to toxicity mechanisms or physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action • Qualitative or quantitative description of absorption, distribution, metabolism, elimination, and toxicokinetic and/or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD) • Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations	

PECO Parameter	Inclusion criteria	Exclusion criteria
Other	• Structure and physiochemical properties • Reviews and regulatory documents	Not on topic, including:[a] • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant • Bioremediation, biodegradation, or chemical or physical treatment of HFPO dimer acid and/or its ammonium salt, including evaluation of wastewater treatment technologies and methods for remediation or contaminated water and soil • Ecosystem effects, studies in ecological species that are not relevant to health effects in humans • Studies of environmental fate and transport of HFPO dimer acid and/or its ammonium salt compounds in environmental media • Analytical methods for detecting/measuring HFPO dimer acid and/or its ammonium salt compounds in environmental media and use in sample preparations and assays • Studies describing the manufacture and use of HFPO dimer acid and/or its ammonium salt compounds • Not chemical-specific (studies that do not involve testing of HFPO dimer acid and/or its ammonium salt compounds) • Studies that describe measures of exposure to HFPO dimer acid and/or its ammonium salt compounds without data on associated health effects

Notes: PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; PBTK/TD = physiologically based toxicokinetic and toxicodynamic; PFAS = pre- and polyfluoroalkyl substances.

[a] Although these criteria were used for the peer-reviewed literature, the current document describes environmental fate data submitted by DuPont (now the Chemours Company). A subsequent targeted search for bioconcentration and bioaccumulation data was also conducted. In addition, a summary of occurrence data is also provided in the current document to give context to the toxicity values.

Table A-6. Inclusion-Exclusion Criteria for HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt Studies after the Public Comment Draft

PECO Parameter	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog	• Ecological species (*supplemental tag —non-PECO model*) • Alternative animal models in standard laboratory conditions (e.g., *Xenopus*, zebrafish, minipig) (*supplemental tag—non-PECO model*) • Human or animal cells, tissues, or organs (not whole animals); bacteria; nonmammalian eukaryotes; other nonmammalian laboratory species (*supplemental tag—mechanistic*)
Exposure	• Exposure is to HFPO dimer acid and/or its ammonium salt • Must include 2 or more levels of exposure to HFPO dimer acid and/or its ammonium salt (if not stated, include at title/abstract screening) • **Humans**: Exposure is measured in air, dust, drinking water, diet, or gavage or injection vehicle, or via a biomarker of exposure (PFAS levels in whole blood, serum, plasma, or breast milk) • Any exposure length is acceptable • **Animals**: Exposure via oral route only • Any exposure length for an animal study is acceptable for reproductive or developmental exposures • Exposure duration for all other animal study designs require an exposure duration of 28 days or more (if not stated, include at title/abstract screening)	• Study population is not exposed to HFPO dimer acid and/or its ammonium salt • There is only 1 exposure group (*supplemental tag—single-dose group in study*) • Exposure is to a mixture only without evaluating HFPO dimer acid and/or its ammonium salt individually (*supplemental tag—mixture study*) • Exposure via inhalation, dermal, intraperitoneal, or intravenous injection routes (*supplemental tag—non-oral route of administration*) • Exposure is via cells in culture or subcellular matrices (*supplemental tag—mechanistic*) • Acute exposures (< 28 days) in animal studies (*supplemental tag—acute/short-term duration exposures*)
Comparator	• A concurrent control group exposed to vehicle-only treatment or an untreated control • A comparison or referent population exposed to HFPO dimer acid and/or its ammonium salt at lower levels (or no exposure/exposure below detection limits) or for shorter periods of time • Biological monitoring (e.g., whole blood, serum, plasma, or breast milk) that can be used to establish a range of exposure	• Case reports and case series (*supplemental tag—case report or case series*)

PECO Parameter	Inclusion criteria	Exclusion criteria
Outcome	• Studies that include a measure of one or more health effect endpoints, including effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer • Qualitative or quantitative description of absorption, distribution, metabolism, elimination, and toxicokinetic and/or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD)	• *In vivo* and/or *in vitro* studies related to toxicity mechanisms, physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action (*supplemental tag—mechanistic*) • Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations (*supplemental tag—susceptible population*)
Other Exclusion Criteria		• Not on topic, including: • Structure and physiochemical properties (*supplemental tag—structure and physiochemical properties*) • Reviews and regulatory documents (*supplemental tag—other assessments or records with no original data*) • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant (*supplemental tag—conference abstract*) • Ecosystem effects, studies in ecological species that are not relevant to health effects in humans (*supplemental tag—non-PECO model*) • Bioaccumulation of the target chemical in fish (*supplemental tag—bioaccumulation data in fish*) • Studies of environmental fate and transport of HFPO dimer acid and/or its ammonium salt compounds in environmental media or food (*supplemental tag—environmental fate or occurrence*) • Studies that describe measures of exposure to HFPO dimer acid and/or its ammonium salt compounds without data on associated health effects (*supplemental tag—exposure characteristics*) • Bioremediation, biodegradation, or chemical or physical treatment of HFPO dimer acid and/or its ammonium salt, including evaluation of wastewater treatment technologies and methods for remediation or contaminated water and soil • Analytical methods for detecting/measuring HFPO dimer acid and/or its ammonium salt compounds in environmental media and use in sample preparations and assays • Studies describing the manufacture and use of HFPO dimer acid and/or its ammonium salt compounds • Not chemical specific (studies that do not involve testing of HFPO dimer acid and/or its ammonium salt compounds)

Notes: **PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; PBTK/TD = physiologically based toxicokinetic and toxicodynamic; PFAS = per- and polyfluoroalkyl substances.**

Table A-7. Supplemental Tags for the GenX Chemicals Literature Search

Category	Evidence
Mechanistic studies	Studies reporting measurements related to a health outcome that inform the biological or chemical events associated with phenotypic effects, in both mammalian and non-mammalian model systems, including *in vitro*, *in vivo* (by various routes of exposure), *ex vivo*, and *in silico* studies. When possible, mechanistic studies will be sub-tagged as pertinent to cancer, non-cancer, or unclear/unknown.
Non-mammalian model systems	Studies in non-mammalian model systems (e.g., fish, birds, *C. elegans*).
ADME and toxicokinetic	Studies designed to capture information regarding absorption, distribution, metabolism, and excretion, including toxicokinetic studies. Such information might be helpful in updating or revising the parameters used in existing PBPK models.
Acute/short-term duration exposures	Animal studies of less than 28 days.
Single-dose group	Studies that used only a single-dose group were tagged as supplemental due to the GenX chemicals database having several multi-dose group studies.
Exposure characteristics	Studies that include data unrelated to toxicological endpoints, but which provide information on exposure sources or measurement properties of the environmental agent (e.g., demonstrate a biomarker of exposure).
Susceptible populations	Studies that identify potentially susceptible subgroups (e.g., studies that focus on a specific demographic, life stage, or genotype).
Mixture studies	Studies not considered PECO-relevant because they do not contain an exposure or treatment group assessing only the chemical of interest.
Non-oral routes of exposure	Studies not addressing routes of exposure that fall outside the PECO scope, and include inhalation and dermal exposure routes
Case studies or case series	Case reports and case series will be tracked as potentially relevant supplemental information.
Records with no original data	Records that do not contain original data such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Conference abstracts	Records that contain insufficient documentation to support study evaluation and data extraction.

Category	
Bioaccumulation in fish	BAFs were mentioned in the public comment draft assessment.

Notes: ADME = absorption, distribution, metabolism, and excretion; BAFs = bioaccumulation factors; PBPK = physiologically based pharmacokinetic; and PECO = population, exposure, comparator, and outcome.

Appendix B: Acute and 7-Day Study Summaries

This appendix summarizes studies evaluating acute exposure to hexafluoropropylene oxide (HFPO) dimer acid or HFPO dimer acid ammonium salt by the oral, dermal, and inhalation routes of exposure and investigating dermal and eye irritation.

Oral Toxicity. In a study of the HFPO dimer acid ammonium salt (no Test Guideline (TG) cited), a single dose of 1.5, 12, 130, 1,000, 2,250, 3,400, 5,000, 7,500, 11,000, 12,963, or 17,000 milligrams per kilogram (mg/kg) of HFPO dimer acid ammonium salt was administered by stomach tube to young male rats. The approximate lethal dose (ALD) was determined to be 7,500 mg/kg. Discomfort, gasping, and tonic convulsions were observed before death at lethal doses (7,500 mg/kg and higher). Discomfort, increased water intake, inactivity, polyuria, and initial weight loss were observed in rats at the three highest sublethal doses (2,250 mg/kg, 3,400 mg/kg, and 5,000 mg/kg). Slightly enlarged livers with enlarged hepatocytes and pronounced cell membranes were also observed in rats at the three highest sublethal doses. Slight-to-moderate degenerative changes in the pancreas were also observed in doses at 2,250 mg/kg and higher. No effects were observed at doses of less than or equal to 1,000 mg/kg (DuPont-2-63, 1963).

In another study evaluating toxicity of HFPO dimer acid ammonium salt by the oral route of exposure (no TG identified), a single dose of 670, 2,300, 3,400, 5,000, 7,500, or 11,000 mg/kg of HFPO dimer acid ammonium salt (purity > 99%) was administered to 7-week-old male rats (1/dose group). Rats were evaluated for clinical signs of toxicity over a 14-day observation period. No clinical signs of toxicity were observed in the rat dosed at 670 mg/kg. Rats dosed at 2,300 and 3,400 mg/kg exhibited weight loss (17% and 14%, respectively); ruffled fur; and a wet, yellow-stained perineum at 1 day postexposure. The rats dosed at 2,300 and 3,400 mg/kg no longer exhibited these effects at 2 days and 4 days postexposure, respectively. Rats dosed with greater than or equal to 5,000 mg/kg died by 1 day after dosing. The rat dosed with 11,000 mg/kg exhibited lethargy, low carriage, and low posture before its death. The ALD was determined to be 5,000 mg/kg (DuPont-770-95, 1996).

A single dose of HFPO dimer acid ammonium salt (82.6% purity) was administered by oral gavage to 10- to 11-week-old female rats at a dose of 175, 550, 1750, or 5,000 mg/kg (1–3 rats/group) in a study conducted according to Organization of Economic Cooperation and Development (OECD) TG 425 (Up-and-Down Procedure) (OECD, 2008c). Rats were then evaluated for clinical signs of toxicity over a 14-day observation period. All rats exhibited clinical signs of toxicity such as hair loss, lethargy, high posture, stained fur/skin, clear ocular discharge, prostrate posture, partially closed eyes, or salivation. With the exception of hair loss, clinical signs disappeared by 2 days postexposure. All three rats dosed at 5,000 mg/kg died within 2 days after dosing. Grossly observable evidence of organ or tissue damage in these rats included discoloration of lungs (rat #1651), discoloration of lungs and mandibular lymph nodes (rat #1746), and discoloration of lungs and liver (rat #1975). No visible lesions were observed in females dosed at 175 mg/kg, 550 mg/kg, or 1,750 mg/kg. With the exception of rats dosed at 5,000 mg/kg, increases in body weight (BW) were observed in all rats over the course of the study. The oral median lethal dose (LD_{50}) was estimated to be 3,129 mg/kg for female rats (DuPont-22932, 2007).

Oral toxicity of HFPO dimer acid ammonium salt was also evaluated in male rats in a study conducted according to OECD TG 425 (OECD, 2008c). A single dose of HFPO dimer acid ammonium salt (86% purity) was administered by oral gavage to 9- to 11-week-old male rats at a dose of 175, 550, 1,750, or 5,000 mg/kg (three rats). Rats were then evaluated for clinical signs of toxicity over a 14-day observation period. All rats exhibited clinical signs of toxicity such as lethargy, wet fur, stained fur/skin, decreased muscle tone, low posture, or lung noise. One rat dosed at 1,750 mg/kg and all three rats dosed at 5,000 mg/kg died either the day dosed or by the day after dosing. Grossly observable evidence of organ or tissue damage in rats dosed at 5,000 mg/kg included expanded lungs and discolored stomach, discoloration and cloudiness of eyes, and stained skin. With the exception of rats dosed at 5,000 mg/kg, increases in BW were observed in all rats over the course of the study. The oral LD_{50} was determined to be 1,750 mg/kg for male rats (DuPont-25438 RV1, 2008).

Another study evaluated oral toxicity of HFPO dimer acid in both male and female rats in a study conducted according to OECD TG 425 (OECD, 2008c). A single dose of HFPO dimer acid (98% purity) was administered to 9- to 11-week-old rats. Males were dosed at 175, 550, 1,750, or 5,000 mg/kg (2–6 rats/group). Female rats were also dosed at 175, 550, 1,750, or 5,000 mg/kg (1–4 rats/group). Clinical signs were not observed in rats dosed at 175 mg/kg or in one male rat dosed at 550 mg/kg. The rest of the rats in this study exhibited clinical signs of toxicity. Clinical signs of toxicity in male rats observed up to 5 days after dosing included lung noise, absent feces, lethargy, not eating, stained fur/skin, wet fur, labored breathing, decreased muscle tone, prostrate posture, tremors, clear oral discharge, diarrhea, ataxia, and/or high posture. Clinical signs in female rats were observed for up to 3 days after dosing and included wet fur, stained fur/skin, ataxia, labored breathing, cold to touch, clear ocular or oral discharge, lethargy, lung noise, absent feces, not eating, and/or rubbing face on the bottom of the cage (DuPont-25875, 2008).

All rats dosed at 5,000 mg/kg died by the day after dosing. Among rats dosed at 1,750 mg/kg, two males and three females died by the day after dosing. One male rat dosed at 550 mg/kg (rat #274) was sacrificed in extremis on the fourth day after dosing following a 23% reduction in BW. Gross findings were detected in three male rats dosed at 5,000 mg/kg, in four rats dosed at 1,750 mg/kg, and in one rat dosed at 550 mg/kg. Small testes and epididymis were observed in rat #274. A discolored, glandular stomach was observed in two of the male rats dosed at 1,750 mg/kg. Gross findings for male rats dosed at 5,000 mg/kg included a glandular stomach; a glandular, discolored stomach (rats #640, #796, and #821); and discolored skin (rat #796). Gross findings for female rats dosed at 1,750 mg/kg included a glandular, discolored stomach (rats #478, #527, and #626); discolored lymph nodes (rat #527); and discolored skin (#527). The female rat dosed at 5,000 mg/kg exhibited wet skin; a discolored esophagus with foamy fluid; and a thick, discolored stomach. Increases in BW were observed in animals that survived until the end of the study. The oral LD_{50} was estimated to be 1,730 mg/kg for male rats and 1,750 mg/kg for female rats (DuPont-25875, 2008).

Another study conducted according to OECD TG 425 (OECD, 2008c) evaluated toxicity of HFPO dimer acid by the oral route of exposure in female mice. A single dose of HFPO dimer acid ammonium salt (86% purity) was administered to 8- to 9-week-old female mice at a dose of 175, 550, or 1,750 mg/kg (1–3 mice). No clinical signs of toxicity were observed in mice dosed at 175 mg/kg or in two mice dosed at 550 mg/kg. One mouse dosed at 550 mg/kg, however,

exhibited wet fur on the day of dosing. All three mice dosed at 1,750 mg/kg died on the day of dosing. Discoloration of lungs and an ovarian cyst were observed in a mouse dosed at 550 mg/kg. Skin stain was also observed in two mice dosed at 1,750 mg/kg. These observations were considered by study authors to be nonspecific and not indicative of test substance related. With the exception of mice dosed at 1,750 mg/kg, increases in BW were observed in all mice over the course of the study. The oral LD_{50} was estimated to be 1,030 mg/kg for female mice (DuPont-24126, 2007).

Dermal Toxicity. In a study evaluating toxicity through dermal absorption (no TG identified), 5,000 mg/kg of HFPO dimer acid ammonium salt (purity > 99%) was applied directly onto the shaved, intact skin of two young adult male New Zealand white rabbits for a period of 24 hours. One rabbit exhibited necrosis from days 2–6 post-application in a small area of treated skin. The necrotic area sloughed off by day 7, and alopecia was then observed in this area until the study was completed. Moderate erythema was observed in both rabbits at 1 day post-application and was still observed up to 3 days post-application. Erythema persisted until 13 days post-application, with the degree of severity decreasing over time. Both rabbits exhibited scaling and sloughing of skin 6–13 days after application. Increases in BW were observed for both rabbits at the conclusion (day 14) of the study. The ALD was determined to be higher than 5,000 mg/kg (DuPont-839-95, 1996).

The dermal toxicity of HFPO dimer acid ammonium salt (86% purity) was also evaluated in rats in a study conducted according to OECD TG 402 (OPPTS 870.1200) (OECD, 2017). A single dose of 5,000 mg/kg (five males and five females) was applied directly onto the shaved, intact skin for 24 hours. Rats were then observed daily for 14 days posttreatment. All female rats exhibited mild erythema on the test site 1 day post-application. Erythema was no longer detectable by the second day after application. Erythema was not observed in male rats. Hyperkeratosis was observed in four male and four female rats. Ulceration was observed in one male and two female rats. All dermal effects cleared up by 13 days posttreatment. Increases in BW were observed for male and female rats by the conclusion (day 14) of the study. The LD_{50} of the compound was determined to be higher than 5,000 mg/kg (DuPont-24113, 2007).

Inhalation Toxicity. The toxicity of HFPO dimer acid ammonium salt by the inhalation route of exposure was evaluated in 8-week-old male and female rats (no TG identified) (DuPont-17751-723, 2009). One group of five male and five female rats were exposed to an aerosol atmosphere containing 5,200 milligrams per cubic meter (mg/m^3) of HFPO dimer acid ammonium salt (84% purity) to determine the inhalation median lethal concentration (LC_{50}). Two other groups of three male and three female rats were exposed to HFPO dimer acid ammonium salt at concentrations of 0, 13, and 100 mg/m^3 in air to evaluate respiratory tract pathology. All rats were exposed nose-only for a single 4-hour period. Rats exposed to 0, 13, and 100 mg/m^3 of HFPO dimer acid ammonium salt in air were evaluated for clinical signs of toxicity for 2 days following exposure and rats exposed to 5,200 mg/m^3 were evaluated for a period of 14 days following exposure. Respiratory tract tissues (lung, larynx/pharynx, trachea, and nose) of the 0-, 13-, and 100-mg/m^3 exposure groups were also evaluated microscopically. According to study authors, no clinical signs of toxicity were observed for any animals at any exposure in this study. However, following the 100 mg/m^3 exposure, all rats displayed a red nasal discharge immediately after exposure. Rats exposed to 5,200 mg/m^3 exhibited red discharge from eyes, nose, and mouth as well as red stains on skin/fur immediately after exposure. Red discharge and staining were absent

within 1 or 2 days after exposure. Rats in the 5,200-mg/m^3 exposure group lost 2.5% to 6.8% of their original BW for 1 or 2 days after exposure but exhibited normal weight gain for the remainder of the experiment. The LC_{50} was determined to be greater than 5,200 mg/m^3 (DuPont-17751-723, 2009).

Dermal Irritation. The dermal irritation of HFPO dimer acid ammonium salt (86% purity) was evaluated in three male New Zealand white rabbits in a study conducted according to OECD TG 404 (OPPTS 870.2500) (OECD, 2002). A 0.5-mL aliquot of the compound was applied to an area of shaved skin for a period of 4 hours. Very slight erythema was observed in one rabbit following removal of the compound. At 60 minutes post-application, very slight erythema was observed in one rabbit and well-defined erythema was observed in the other two rabbits. Erythema had cleared by 24 hours postexposure (DuPont-24030, 2007).

Eye Irritation. In an OECD TG 405 (OPPTS 870.2400) (OECD, 2020a) study evaluating eye irritation of HFPO dimer acid ammonium salt (86% purity), a 0.1-mL aliquot of compound was administered to one eye of a young adult male New Zealand white rabbit. Necrosis, characterized by brown and white discoloration of the conjunctival membrane of the treated eye, was observed at 1, 24, and 28 hours after application. Corneal opacity, iritis, conjunctival chemosis, and discharge were also observed. Fluorescein stain examination of the treated eye indicated corneal injury (DuPont-24114, 2007).

Seven-Day Toxicity Studies. Four 7-day studies are available for HFPO dimer acid or ammonium salt in rats or mice. The toxicity of HFPO dimer acid ammonium salt (86.6% purity) by the oral route of exposure was evaluated in 6-week-old male and female rats (DuPont-24009, 2008). Five rats of each sex were exposed to 0, 30, 300, or 1,000 mg/kg HFPO by oral gavage for 7 days. No clinical signs of toxicity were observed in either sex at any dose level tested. A significant decrease in BW was observed on test day 7 in males exposed to 1,000 mg/kg versus control. Significant decreases in red blood cells (RBCs), hemoglobin, and hematocrit were observed in male rats at 300 milligrams per kilogram per day (mg/kg/day) and in both male and female rats at 1,000 mg/kg/day. A significant increase in red cell distribution width, reticulocytes, and neutrophils was also observed in female rats exposed to 1,000 mg/kg/day. Decreases in serum lipids and globulins were observed in males at all dosage groups as well as in females at 300 and 1,000 mg/kg/day. Increased alanine aminotransferase, urea nitrogen, and glucose as well as decreased sorbitol dehydrogenase, creatinine, and calcium were observed at doses of 300 and/or 1,000 mg/kg/day. Increases in liver weight were observed in males at all doses and in females at 1,000 mg/kg/day and corresponded with increases in B-oxidation and/or increases in P450 enzyme activity. Mild-to-minimal hepatocellular hypertrophy was also observed in both sexes at 1,000 mg/kg/day. Decreases in heart weight were observed in males at 1,000 mg/kg and increases in kidney weight were observed in females at 1,000 mg/kg/day: No microscopic changes were observed in these organs.

In another study evaluating toxicity of HFPO dimer acid (99% purity) by the oral route of exposure, 6-week-old male and female rats (5/sex) were exposed to 0, 30, 100, and 300 mg/kg of HFPO dimer acid by gavage over a period of 7 days (DuPont-24116, 2008). No clinical signs of toxicity were observed. Significant decrease in RBC count and a significant increase in red cell distribution width were observed in females at 300 mg/kg/day. Significant decreases in hemoglobin and hematocrit were observed in male rats at 300 mg/kg/day. A significant increase in mean corpuscular cell volume was observed in males at 30 mg/kg/day. Decreases in serum

lipids were detected in all dosed male groups versus control. Increased alkaline phosphatase and urea nitrogen and decreased bilirubin, creatinine, total protein, globulin, and calcium were observed at 30 and/or 300 mg/kg/day. Increased liver weight was observed in males at all doses and in females at 300 mg/kg/day. Microscopic examination of livers detected hepatocellular hypertrophy in all treated males and females. Lesions observed in males and females were mild and minimal, respectively. A statistically significant increase in β-oxidation was detected in females exposed to 300 mg/kg/day versus control.

A 7-day study was conducted in 6-week-old male mice to evaluate toxicity of HFPO dimer acid ammonium salt (86.6% purity) by the oral route of exposure (DuPont-24010, 2008). Doses of 0 or 30 mg/kg/day were administered over a period of 7 days. By test day 7, BWs were significantly higher in exposed males versus controls. A twofold increase in liver weight relative to control was detected in exposed males. No grossly observable lesions in the liver were observed. Microscopic changes in the liver observed at 30 mg/kg/day included minimal single-cell necrosis of hepatocytes, moderate hepatocellular hypertrophy, and moderate increases in mitotic figures. Minimal vacuolation of hepatocytes was also observed in one treated mouse.

Another 7-day gavage study was conducted in 6-week-old male mice to evaluate toxicity of HFPO dimer acid (99% purity) by the oral route of exposure (DuPont-25281, 2008). Doses of 0 or 30 mg/kg/day were administered over a period of 7 days. By test day 7, BWs were significantly higher in exposed males versus controls. A twofold increase in liver weight was detected in exposed males versus control. Microscopic changes to the liver of exposed animals included minimal single-cell necrosis of hepatocytes, moderate hepatocellular hypertrophy, and moderate increases in mitotic figures. Minimal vacuolization was also observed in 2/5 treated mice.

Appendix C: Genotoxicity Study Summary

Table C-1 provides a summary of the available genotoxicity data for hexafluoropropylene oxide (HFPO) dimer acid and/or its ammonium salt.

Table C-1. Genotoxicity Study Summary

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-19713 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD TG 471) (OECD, 2020b)	*Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (strain WP2*uvr*A)	HFPO dimer acid ammonium salt (85% purity) 33.3, 66.7, 100, 333, 667, 1,000, 3,333, and 5,000 µg/plate for preliminary toxicity test 333, 667, 1,000, 3,333, and 5,000 µg/plate for toxicity-mutation test Negative control (sterile water) and positive control (benzo[a]pyrene, 2-nitrofluorine, 2-aminoanthracene, sodium azide, acridine mutagen Institute of Cancer Research (ICR)-191, or 4-nistroquinoline-N-oxide) also included in study	With S9	Negative.
				Without S9	Negative.
DuPont-22620 RV1 (2009)	*In vitro* Mammalian Chromosome Aberration Test (OECD TG 473) (OECD, 1997a)	Chinese hamster ovary cells (CHO-K_1 line)	HFPO dimer acid ammonium salt (83% purity) 49, 98, 244, 489, 977, 1954, and 3391 µg/mL for preliminary toxicity test* 977, 1954, and 3391 µg/mL for the 4-hour nonactivated and activated test conditions* 489, 977, and 1954 µg/mL for the 20-hour nonactivated test condition* Negative control (sterile water) and positive control (mitomycin C or cyclophosphamide) also included in study * Doses have been corrected to account for 83% HFPO dimer acid ammonium salt purity.	With S9	Positive at 3,391 µg/mL* in 4-hour activated test conditions.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-23219 (2007)	*In vivo* Unscheduled DNA Synthesis Test in Mammalian Cells (OECD TG 486) (OECD, 1997b)	Primary hepatocytes harvested from male rats (5/dose group)	HFPO dimer acid ammonium salt (83% purity) 1, 10, 100, 1,000, and 2,000 mg/kg for preliminary toxicity test 500, 1,000, and 2,000 mg/kg/day for Unscheduled DNA Synthesis Test Negative control (distilled water) and positive control (dimethylnitrosamine) also included in study	Negative–No significant increase in the mean number of net nuclear grain counts in hepatocytes at 2–4 or 12–16 hours after dosing.	
Dupont-26129 (2008)	*In vitro* Mammalian Cell Gene Mutation Test (OECD TG 476) (OECD, 1997c)	L5178Y/TK$^{+/-}$ Mouse lymphoma cells	HFPO dimer acid ammonium salt (87% purity) 0.5, 1.5, 5, 15, 50, 150, 500, 1,500, and 3,500 µg/mL for both non-activated and S9-activated cultures at both 4-hour and 24-hour exposures for preliminary toxicity assay 500, 750, 1,000, 1,500, and 2,000 µg/mL for nonactivated cultures with a 4-hour exposure 150, 250, 500, 600, and 750 µg/mL for S9-activated cultures with a 4-hour exposure 250, 500, 600, 750, and 1,000 µg/mL for nonactivated cultures with a 24-hour exposure Negative control (sterile, distilled water) and positive control (methyl methanesulfonate or 7,12-dimethylbenz(a)anthracene) also included in study	With S9	Negative.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
Dupont-19714 RV1 (2008)	*In vitro* Mammalian Chromosome Aberration Test (OECD TG 473) (OECD, 1997a)	Chinese hamster ovary cells (CHO-K_1 line)	HFPO dimer acid ammonium salt (85% purity) 0.3, 1, 3, 10, 30, 100, 300, 1,000, and 3,471 µg/mL for preliminary toxicity test 100, 500, 1,000, 2500, and 3,471 µg/mL for the chromosome aberration assay for the 4-hour nonactivated, 4-hour S9-activated, and 20-hour nonactivated test conditions Cytogenetic evaluations were conducted at 1,000, 2,500, and 3,471 µg/mL for the 4-hour nonactivated and 4-hour S9-activated test conditions and at 100, 500, and 1,000 µg/mL for the 20-hour nonactivated test condition	With S9	The percentage of cells with structural aberrations in the test substance-treated groups was not increased above that of the vehicle control at any concentration. The percentage of cells with numerical chromosome aberrations at 2,500 and 3,471 µg/mL in the 4-hour S9-activated test conditions was increased in a dose-dependent manner above that of the vehicle control. The change was outside the historical control range and considered biologically relevant.

Study	Assay	Strain/Species	Dosing	Activation	Results
			Negative control (sterile water) and positive control (mitomycin-C or cyclophosphamide) also included in study	Without S9	In the 20-hour nonactivated test condition, substantial toxicity was observed at 3,471 µg/mL and a substantial reduction in mitotic index relative to vehicle control was observed in the mitotic index relative to vehicle control. The percentage of cells with structural aberrations in the test substance-treated groups was not increased above that of the vehicle control at any concentration. An increase in the percentage of cells with numerical chromosome aberrations was observed at 3,471 µg/mL in the 4-hour nonactivated condition relative to vehicle control.
DuPont-22734 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD TG 471) (OECD, 2020b)	*Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (strain WP2*uvr*A)	HFPO dimer acid ammonium salt (82.6% purity) 32.5, 65.2, 97.7, 325, 652, 977, 3,256, and 4,885 µg/plate for the toxicity-mutation assay* 325, 652, 977, 3256, and 4885 µg/plate for the mutagenicity test* * Doses have been correct to account for 82.6% HFPO dimer acid ammonium salt purity.	With S9	Negative.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-23220 (2007)	*In vivo* Micronucleus and Chromosome Aberration Assay (OECD TGs 474 and 475) (OECD, 2014, 2016b)	Primary bone marrow cells harvested from male and female ICR mice (2 males or 5 of each sex/dose for preliminary toxicity study) (5 of each sex/dose for toxicity study) (5 of each sex/dose for Micronucleus and Chromosome Aberration Assay)	HFPO dimer acid ammonium salt (82.6% purity) 1, 10, 98, 975, and 1,950 mg/kg by oral gavage for preliminary toxicity study* 1170, 1365, 1560, and 1,755 mg/kg by oral gavage for toxicity study* 317, 634, and 1,268 mg/kg by oral gavage for Micronucleus and Chromosome Aberration Assay* Positive control (colchicine) and negative control (sterile water) also included in the study * Doses have been corrected to account for 82.6% HFPO dimer acid ammonium salt purity.	Negative–No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes or structural or numerical chromosomal aberrations in bone marrow of male and female ICR mice at doses up to and including the maximum tolerated dose (1,268 mg/kg).	

Notes: DNA = deoxyribonucleic acid; µg/mL = micrograms per milliliter; µg/plate = micrograms per plate; mg/kg = milligrams per kilogram; mg/kg/day = milligrams per kilogram per day; OECD = Organization for Economic Cooperation and Development; TG = test guideline.

Appendix D: NTP PWG Final Report on the Pathology Peer Review of Liver Findings

FINAL REPORT

December 4, 2019

PATHOLOGY PEER REVIEW
OF LIVER FINDINGS

H-28548: SUBCHRONIC TOXICITY 90 DAY GAVAGE STUDY IN MICE
(PROJECT ID: DUPONT-18405-1307)
&
AN ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING STUDY OF H-28548 IN MICE
(STUDY NUMBER WIL-189225)
(STUDY SPONSOR NUMBER: DUPONT-18405-1037)

Prepared by:

Susan A. Elmore, MS, DVM, DACVP, DABT, FIATP
Amy Brix, DVM, PhD, DACVP

INTRODUCTION

The study report, summary tables and individual animal findings, along with hematoxylin and eosin stained microscope slides used in the Subchronic Toxicity 90 Day Gavage Study In Mice (Project ID: DuPont-18405-1307) and An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 In Mice (Study Number WIL-180225) (Project ID: DuPont-18405-1037) were received by the NTP reviewing pathologist. The slides for review each contained two liver lobes presumed to be the left and median lobes. The data and slides of the liver were reviewed per NTP standards (Sills et al, 2017), and the results are summarized in this report. The experimental design for this study is as follows:

DUPONT-18405-1307 **SUBCHRONIC TOXICITY STUDY**		
DOSAGE (mg/kg/day)	**MICE**	
	MALE	**FEMALE**
0	10	10
0.1	10	10
0.5	10	10
5	10	10

DUPONT-18405-1037 **REPRODUCTIVE/DEVELOPMENTAL TOXICITY STUDY**		
DOSAGE (mg/kg/day)	**F_0 MICE**	
	MALE	**FEMALE**
0	25	25
0.1	25	25
0.5	25	25
5	25	25

SUMMARY OF ORIGINAL STUDY RESULTS

DUPONT-18405-1307 (Subchronic Toxicity 90 Day Gavage Study)

The following information is excerpted from the Final Report entitled "H-28548: Subchronic Toxicity 90 Day Gavage Study in Mice," dated February 19, 2010:

> In 5 mg/kg/day male and female dose groups, increases were observed in the incidence of single cell necrosis, mitotic figures, and/or pigment. The liver effects at 5 mg/kg/day correlated with clinical chemistry effects and were considered test substance related and adverse. Other test substance-related effects were observed in the livers of 0.5 and 5 mg/kg/day males and 5 mg/kg/day females, including increases in absolute and/or relative liver weight, enlarged and/or discolored livers, and centrilobular hepatocellular hypertrophy. The liver effects observed in 0.5 mg/kg/day males were considered to be non-adverse adaptive responses as they were not correlated with clinical or microscopic pathology evidence of liver toxicity.

DUPONT-18405-1037 (Reproduction/Developmental Toxicity Screening Study)

The following information is excerpted from the Final Report entitled "An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice," dated December 29, 2010:

> In male and female mice given 5 mg/kg/day, mild to moderate hepatocellular hypertrophy was observed microscopically. The hepatocellular hypertrophy was characterized by cytoplasmic eosinophilic stippling that is consistent with peroxisome proliferation and was associated with correlative increases in liver weights. Other microscopic changes in the liver at 5 mg/kg/day included increases in single cell necrosis, mitotic figures, pigment, and focal necrosis (females only). In male and female mice given 0.5 mg/kg/day, the incidence and severity of hepatocellular hypertrophy, as well as the correlative liver weight changes was reduced. Other lesions at the 0.5 mg/kg/day dose level were limited to minimal single cell necrosis in 5 of 24 males.

SLIDE REVIEW WORK SHEETS (SRWS)

The Slide Review Work Sheets are presented in appendix A (Dupont-18405-1307 Subchronic Toxicity Study) and appendix B (Dupont-18405-1037 Reproduction/ developmental Toxicity Screening Study). These work sheets list, in animal ID number order, the original study pathologist's findings, along with the reviewing pathologist's comments. Entries other than "Agree" under the reviewing pathologist's comments indicate a disagreement with the study pathologist's (SP's) diagnosis. In each instance, space is provided to record remarks made during the Slide Review.

FINDINGS OF THE SLIDE REVIEW

DUPONT-18405-1307 (Subchronic Toxicity 90 Day Gavage Study)

The slides reviewed during this quality assessment were of adequate quality and had no artifacts that interfered with making diagnoses. The liver was reviewed from all animals for all lesions. It was requested by the NTP pathologist that the reviewing pathologist use the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al, 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al, 2016). The study pathologist diagnosed hepatocellular hypertrophy which included the morphologic change of eosinophilic stippling commonly observed with peroxisome proliferators. The reviewing pathologist agreed that there was hypertrophy of the hepatocytes, but often regarded the severity to be less than recorded by the study pathologist. In addition, the reviewing pathologist recommended adding the diagnosis cytoplasmic alteration to account for the brightly eosinophilic, frequently granular, appearance of the cytoplasm of hepatocytes. After reviewing these lesions with the NTP pathologist, the NTP pathologist recommended using the term cytoplasmic alteration to encompass both hypertrophy and eosinophilic change to the hepatocytes, as she considered them part of the same process. The reviewing pathologist agreed with most occurrences and severities of single cell necrosis. However, the reviewing pathologist also observed apoptosis, and recommended adding the diagnosis of "apoptosis, hepatocellular" when present. Descriptions of individual lesions recorded during this review are listed below. The summary incidences are found in Table 1.

DUPONT-18405-1037 (Reproduction/Developmental Toxicity Screening Study)

The slides reviewed during this quality assessment were of adequate quality and had no artifacts that interfered with making diagnoses. The liver was reviewed from all animals for all lesions. For the most part, the reviewing pathologist agreed with the study pathologist's diagnoses and severities. It was requested by the NTP pathologist that the reviewing pathologist use the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al, 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al, 2016). The study pathologist diagnosed hepatocellular hypertrophy which included the morphologic change of eosinophilic stippling commonly observed with peroxisome proliferators. The reviewing pathologist agreed that there was hypertrophy of the hepatocytes, but used the terminology "cytoplasmic alteration" at the request of the NTP pathologist, based upon review of the slides from the 18405-1307 subchronic study. The reviewing pathologist agreed with most occurrences and severities of single cell necrosis. However, the reviewing pathologist also observed apoptosis, and recommended adding the diagnosis of "apoptosis, hepatocellular" when present. The reviewing pathologist recorded additional occurrences of mixed cell infiltrates in most groups of animals. Descriptions of individual lesions recorded during this review are listed below. The summary incidences are found in Table 2.

DESCRIPTIONS OF LESIONS

Single cell necrosis (Figures 1 & 2) consisted of individual hepatocytes that had pale, granular, vacuolated or eosinophilic cytoplasm; nuclei were either swollen or pyknotic and karyorrhectic.

The cells frequently appeared fragmented and were often surrounded by degenerative inflammatory cells. Inflammatory cells were not documented separately as they were considered a response to the necrosis. All of the lesions were considered minimal in severity, which was recorded when 1-10 cells were observed in ten 20X fields. Single cell necrosis was not recorded unless at least two affected cells were observed in the entirety of the liver sections examined; if 2 or more necrotic cells were observed, counting of ten 20X fields was done to determine severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

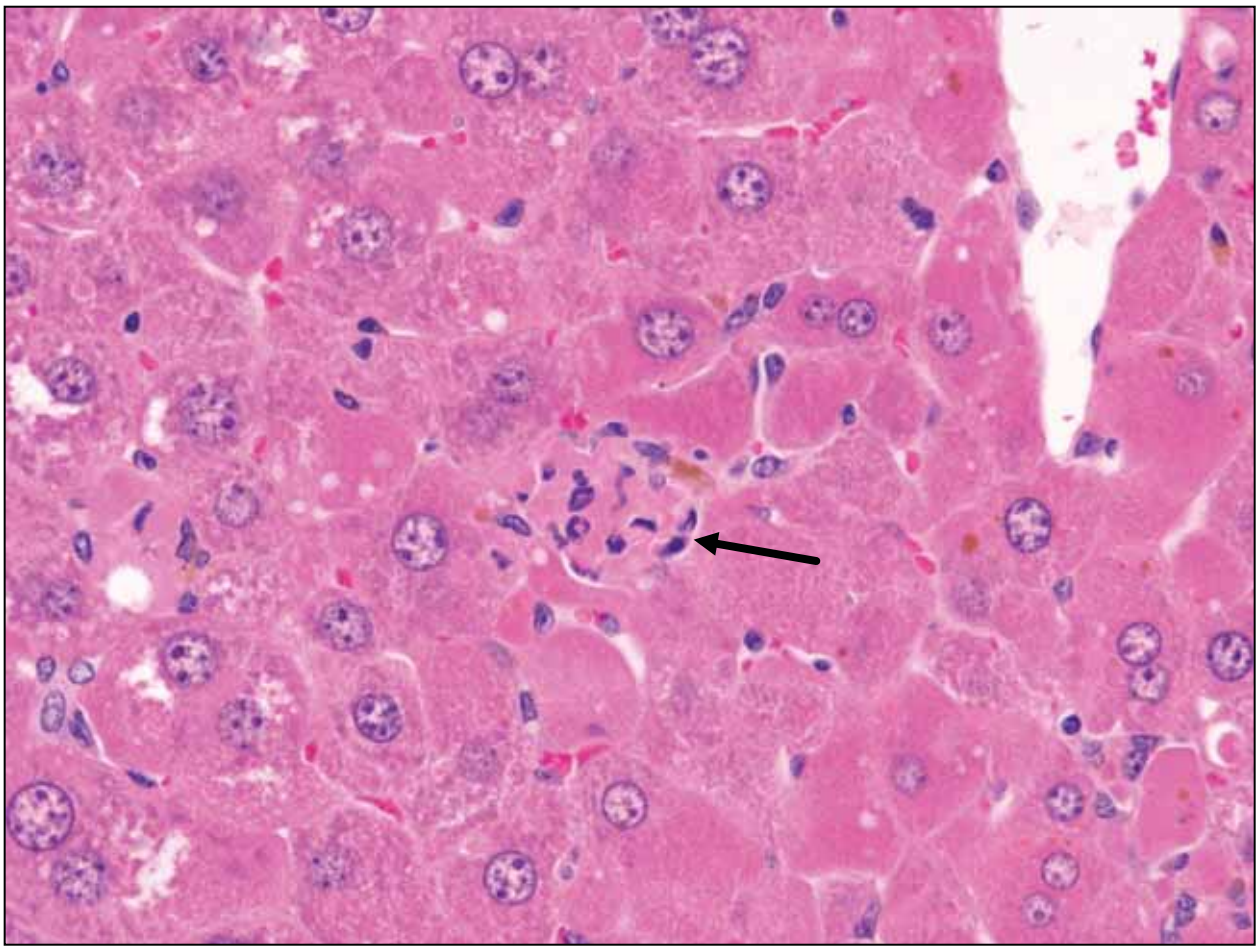

Figure 1. Single cell necrosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The necrotic cell (arrow) is fragmented and surrounded by inflammatory cells.

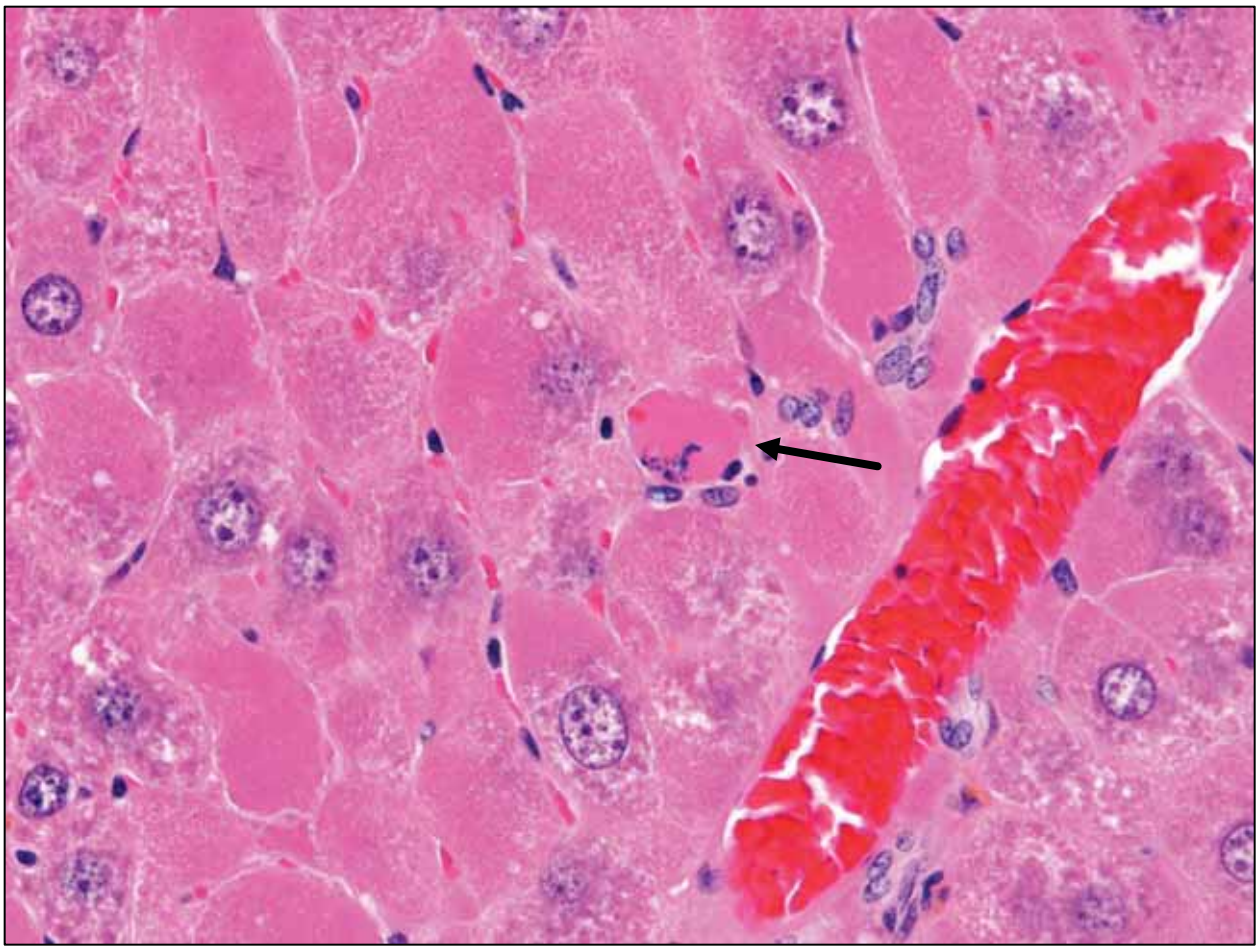

Figure 2. Single cell necrosis in the liver of a Group 4 male mouse (animal 405) from the 18405-1307 subchronic study. The necrotic cell (arrow) is swollen, and has brightly eosinophilic cytoplasm and a karyorrhectic nucleus.

Apoptosis; hepatocellular was recorded when individual hepatocytes were observed that had characteristics, as described in the article by Elmore (Elmore et al, 2016), of apoptosis. Briefly, affected cells were typically shrunken, with hyper-eosinophilic cytoplasm and condensed, pyknotic or karyorrhectic nuclei. The cells were round and often small; occasionally they were phagocytosed by surrounding cells. There was a lack of associated inflammatory cells with apoptotic hepatocytes. Grading was done based upon the Thompson article (Thompson et al, 2018) to be consistent with the reviewing pathologist's grading criteria. All of the lesions were considered minimal to mild in severity, which was recorded when 1-10 cells, or 11-40 cells, respectively, were observed in ten 20X fields. Apoptosis was not recorded unless at least two affected cells were observed in the entirety of the liver sections examined; if 2 or more apoptotic cells were observed, counting of ten 20X fields was done to determine severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

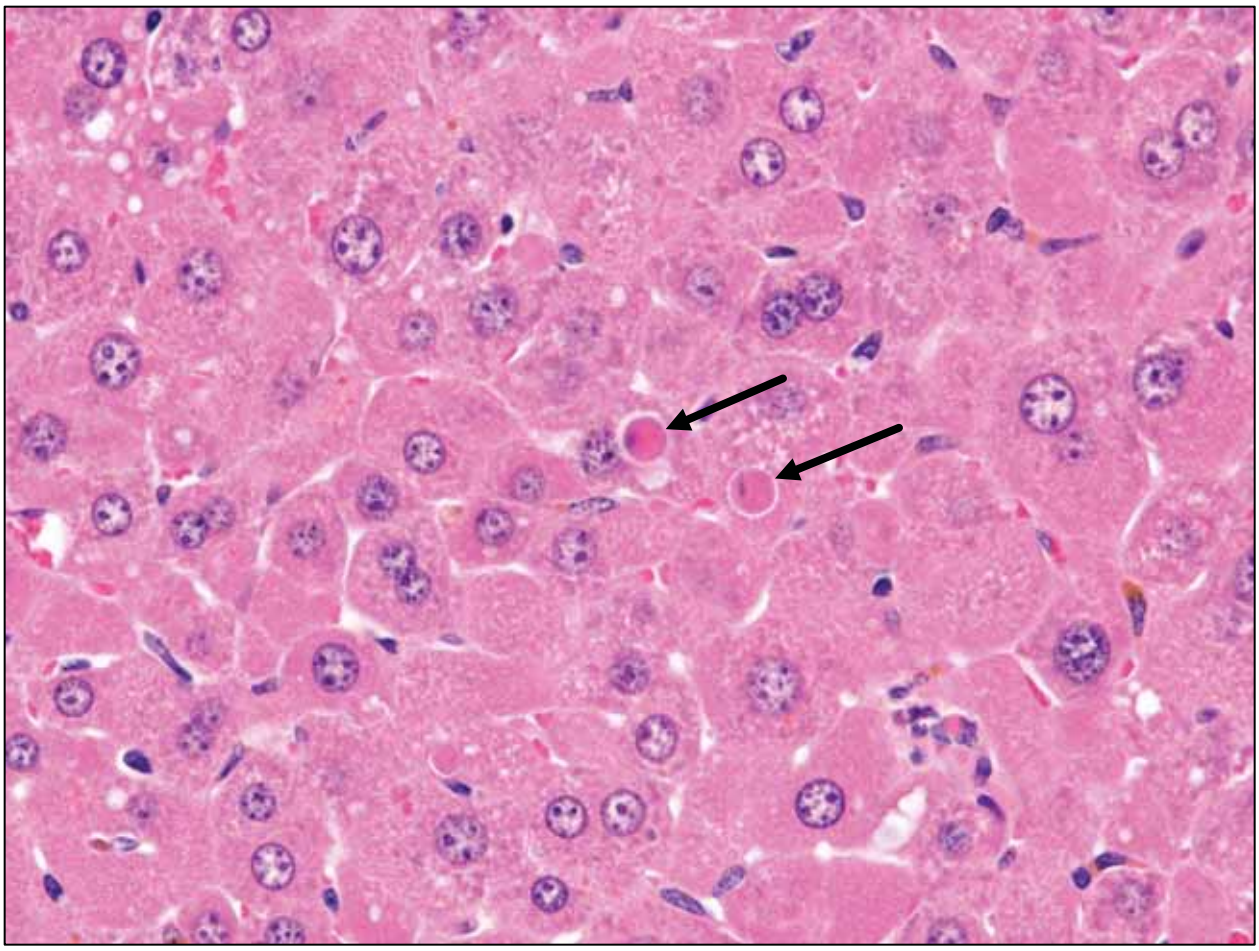

Figure 3. Apoptosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The apoptotic hepatocytes are small, rounded, and brightly eosinophilic (arrows).

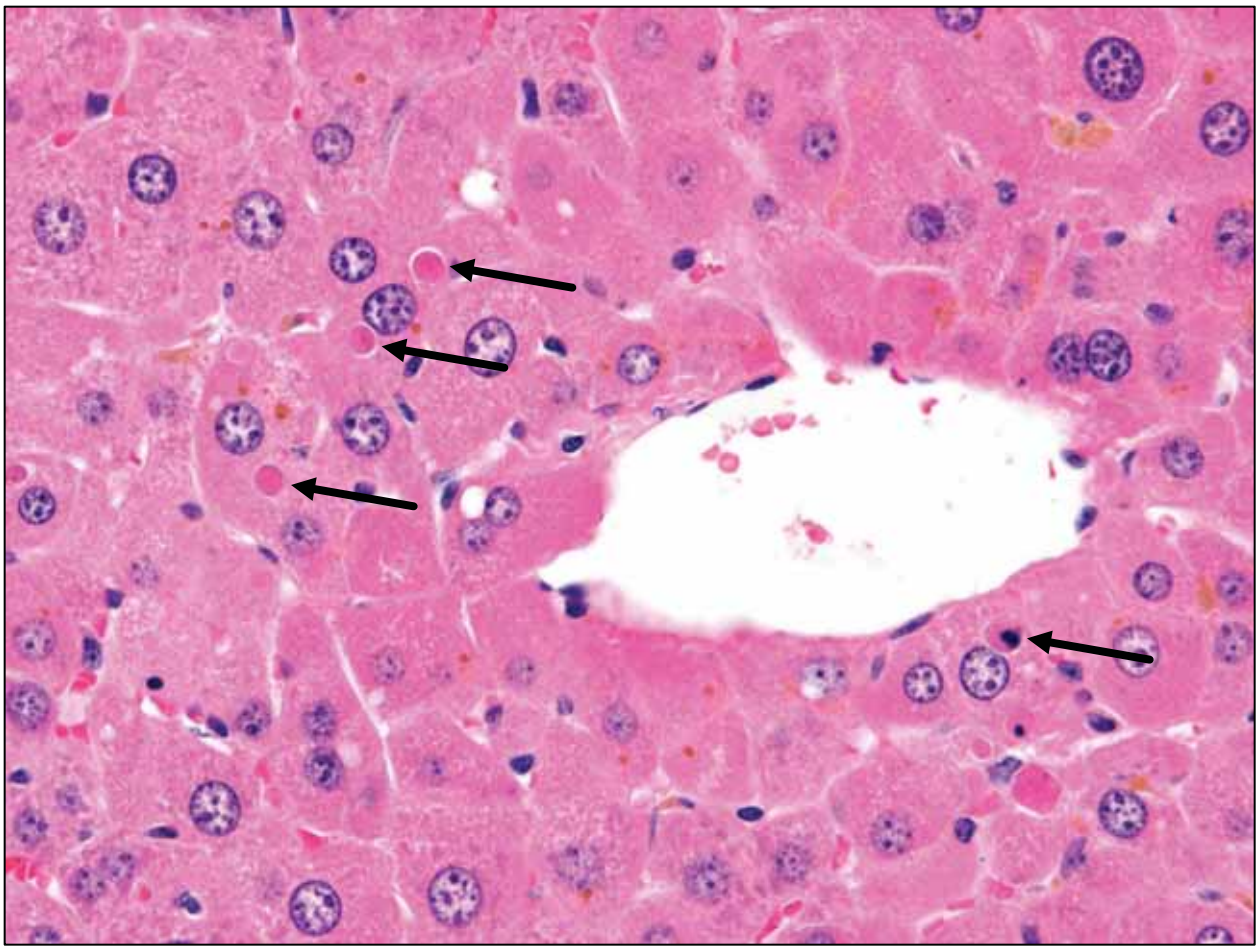

Figure 4. Apoptosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. Evidence of apoptosis is provided by small round eosinophilic remnants of hepatocytes (arrows).

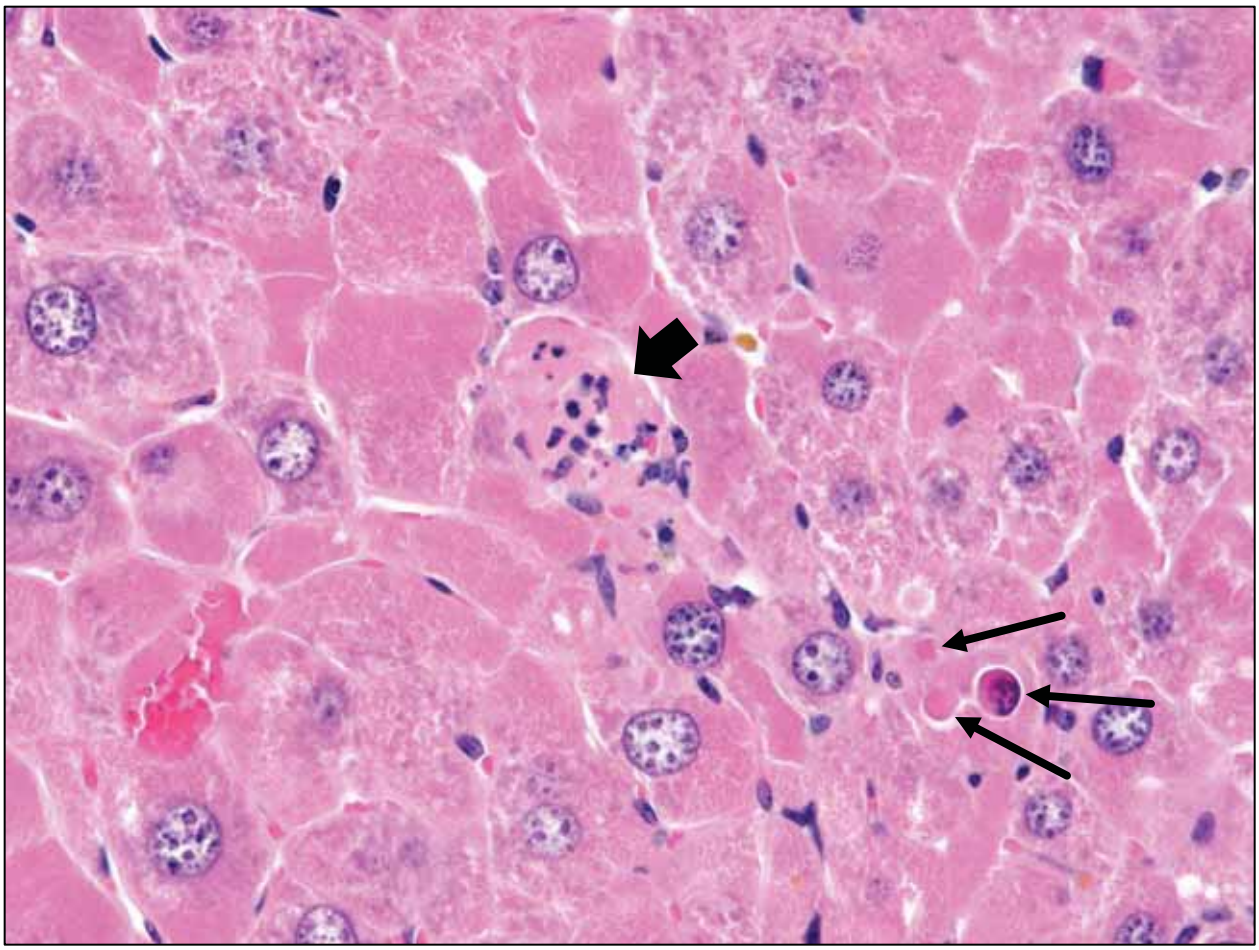

Figure 5. Apoptosis (long arrows) and single cell necrosis (short arrow) in the liver of a Group 4 male mouse (animal 405) from the 18405-1307 subchronic study.

Focal necrosis consisted of a localized area of coagulative necrosis. Generally, there was a loss of cellular detail of the affected hepatocytes; rarely there was a small amount of mineral (dystrophic) associated with the areas of necrosis. Inflammatory cell infiltrates typically ringed the region of necrotic hepatocytes. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

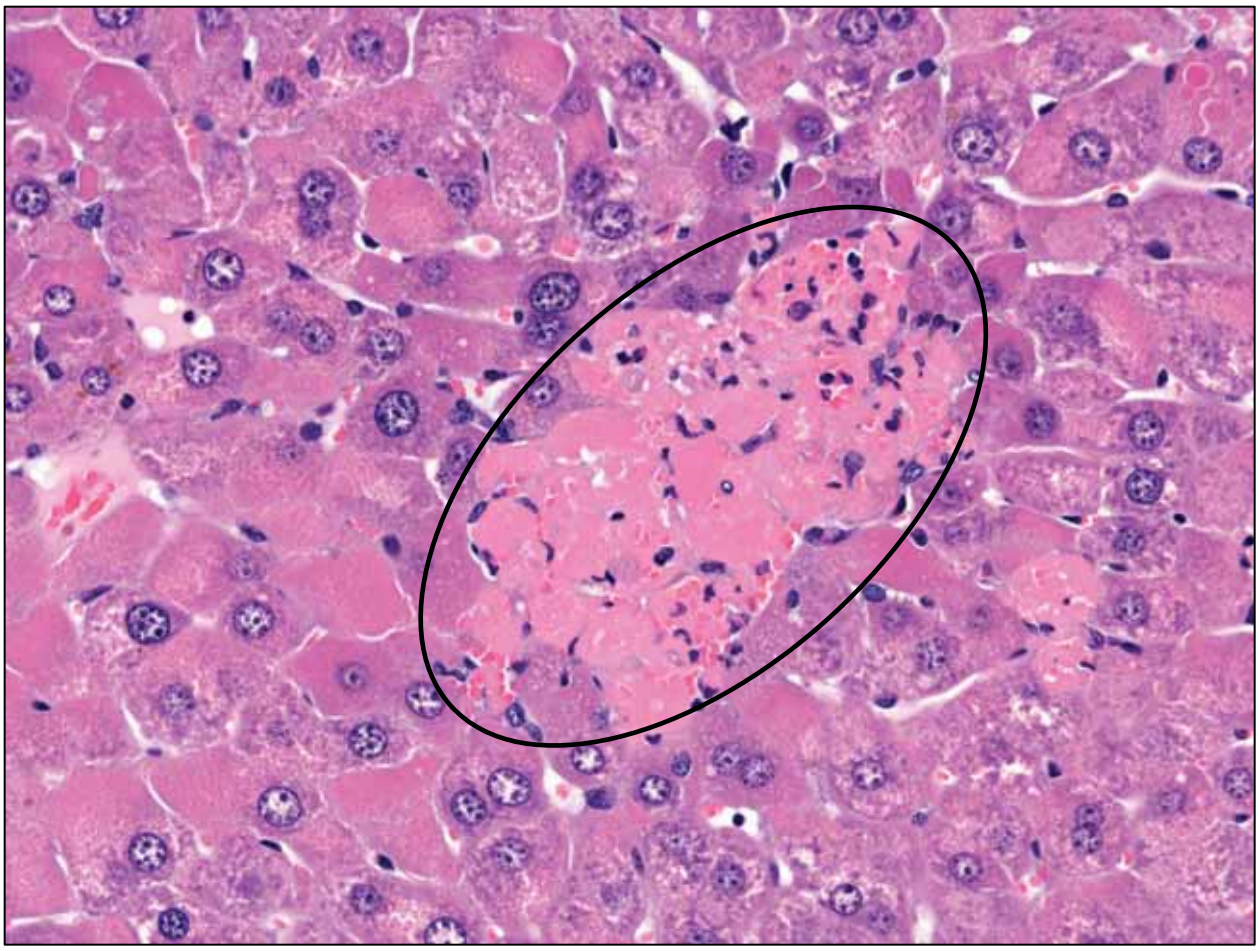

Figure 6. Focal necrosis (outlined) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. There is a small area of contiguous hepatocytes that are necrotic. Many of the cell borders are indistinct.

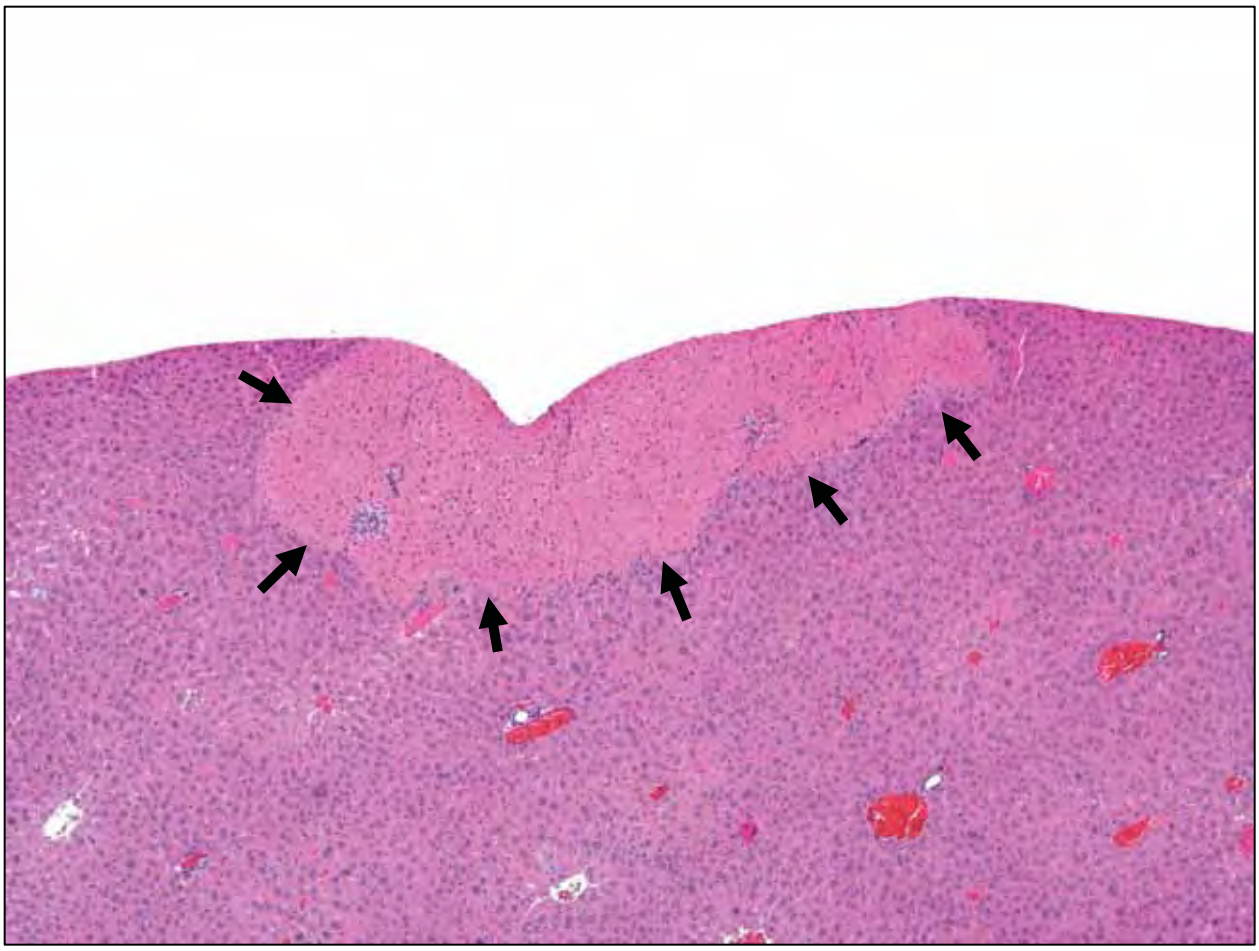

Figure 7. Focal necrosis (area delineated by arrows) in the liver of a Group 4 male mouse (animal 456) from the 18405-1307 subchronic study. This example of focal necrosis is more extensive than that shown in figure 6.

Cytoplasmic alteration was characterized by a bright eosinophilia to the cytoplasm of hepatocytes, usually accompanied by a slight increase in cell, and sometimes nuclear, size. The cytoplasm usually had a granular appearance to it, although with greater severities, the cytoplasm lost its granular appearance, and was just filled with smooth, homogeneous, brightly eosinophilic material. Severity grading was subjectively based on the number of hepatocytes involved and the amount of material within the affected hepatocytes. Minimal (+1) cytoplasmic alteration was recorded when there was an eosinophilic granular appearance to the hepatocytes in the centrilobular region of most hepatic lobules. With mild (+2) cytoplasmic alteration, more of each hepatic lobule was involved, so that many of the hepatic lobules appeared to be completely affected, rather than having alteration limited to the centrilobular area. All the hepatocytes appeared to be affected with moderate (+3) cytoplasmic alteration and those in the centrilobular area usually had lost the granular appearance to the cytoplasm and instead had a more solid, brightly eosinophilic appearance to it. Many of the hepatocytes with moderate cytoplasmic alteration were also larger than normal, and some also had larger than normal nuclei. These latter changes were not recorded separately, but were considered part of the cytoplasmic alteration. Marked (+4) cytoplasmic alteration was a diffuse change, with most of the hepatocytes distended by increased amounts of brightly eosinophilic cytoplasm that lacked granularity or definition,

similar to what was seen with moderate cytoplasmic alteration, but affected the entire hepatic lobule rather than just the centrilobular part. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

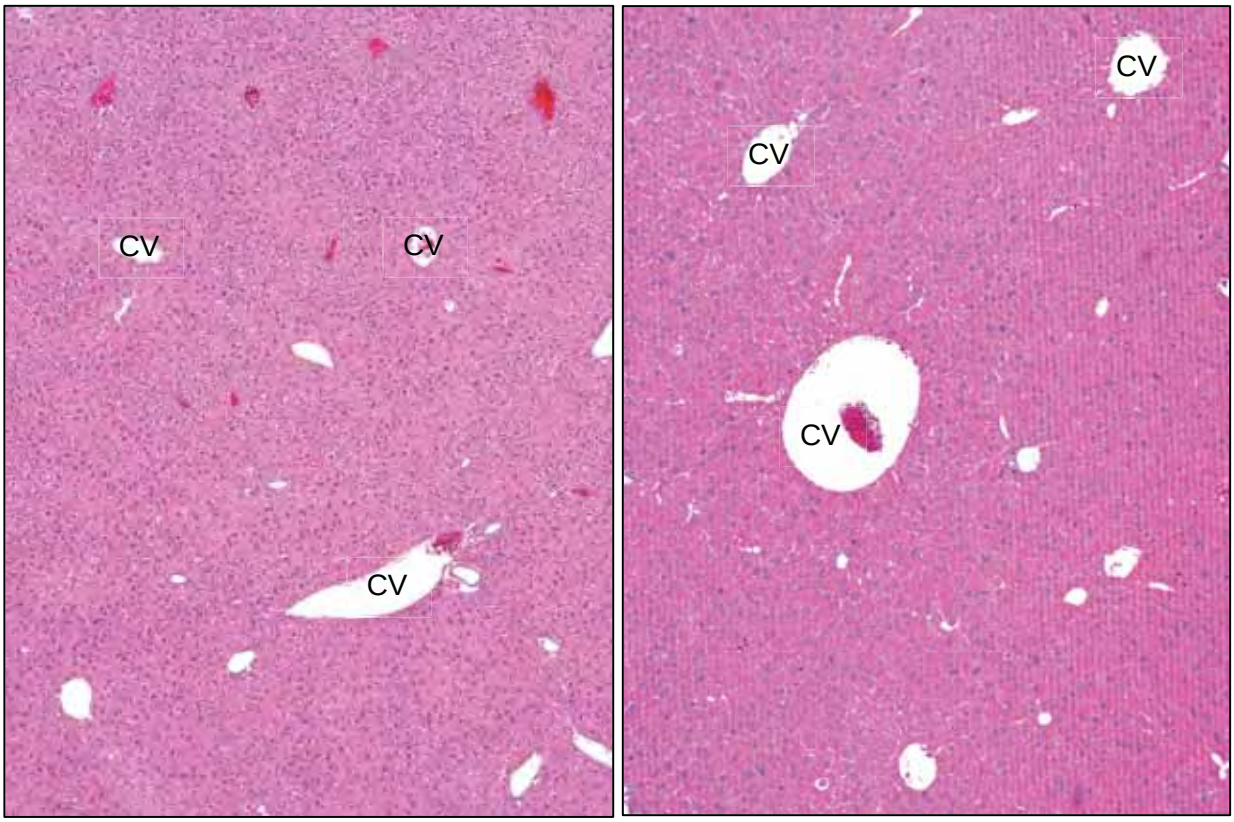

Figure 8. Control liver from a Group 1 mouse (animal 101) on the left; cytoplasmic alteration in the liver of a Group 4 male mouse (animal 401) on the right; from the 18405-1307 subchronic study. At this low magnification, the Group 4 mouse liver appears brightly eosinophilic when compared to the Group 1 (control) liver. The nuclei are also spaced further apart from each other, consistent with hypertrophied cells. CV=central vein.

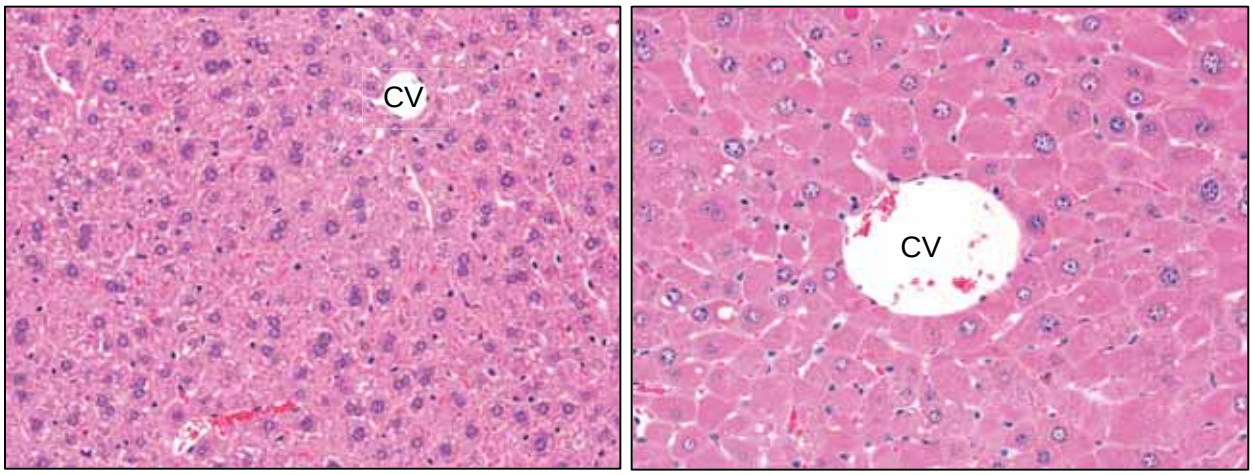

Figure 9. Control liver from a Group 1 mouse (animal 105) on the left; cytoplasmic alteration in the liver of a Group 4 male mouse (animal 401) on the right; from the 18405-1307 subchronic study. This higher magnification photo reveals the eosinophilic granular nature of the cytoplasm in the Group 4 mouse. CV=central vein.

Mixed cell infiltrate was characterized by the presence clusters of inflammatory cells within the hepatic parenchyma. They were often found randomly scattered throughout the liver, and less commonly in the periportal or centrilobular areas. The infiltrates were composed primarily of lymphocytes with fewer macrophages and plasma cells. Neutrophils were a small component of many of the foci, and were a major component of a few of them. Occasionally, a necrotic hepatocyte could be found within the focus of inflammatory cells. Mixed cell infiltrate was used as a diagnostic term as it is the term preferred in INHAND (Thoolen et al, 2010). This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies. Mixed cell infiltrates are common background lesions in mice, although they may be exacerbated with treatment.

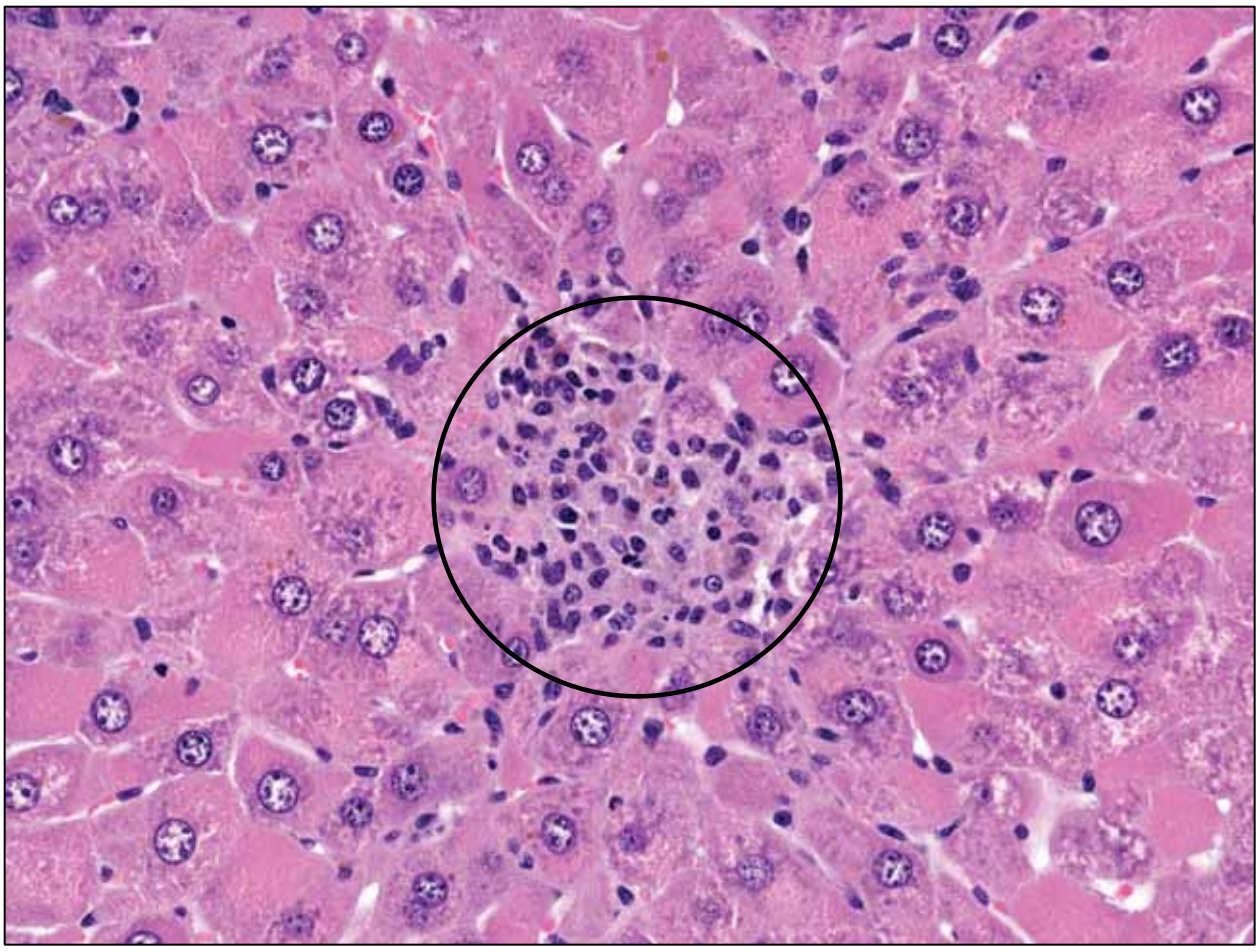

Figure 10. Mixed cell infiltrates (circled) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. Different types of inflammatory cells, including macrophages (some containing pigment consistent with cell breakdown product), lymphocytes, plasma cells, and neutrophils are present in a focal area. Some of these areas also contained an occasional necrotic hepatocyte.

Mitotic figures were considered to be present when there were an increased number of mitotic figures observed in the sections of liver examined. Typically, if 3 or more mitotic figures were observed, ten 20X fields were counted for the number of mitotic figures, and severity scores were based upon how many mitotic figures were counted: Minimal if 1-10 cells were observed in ten 20X fields; mild if 11-40 cells were observed in ten 20X fields. All the occurrences of mitotic figures were considered of minimal or mild severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

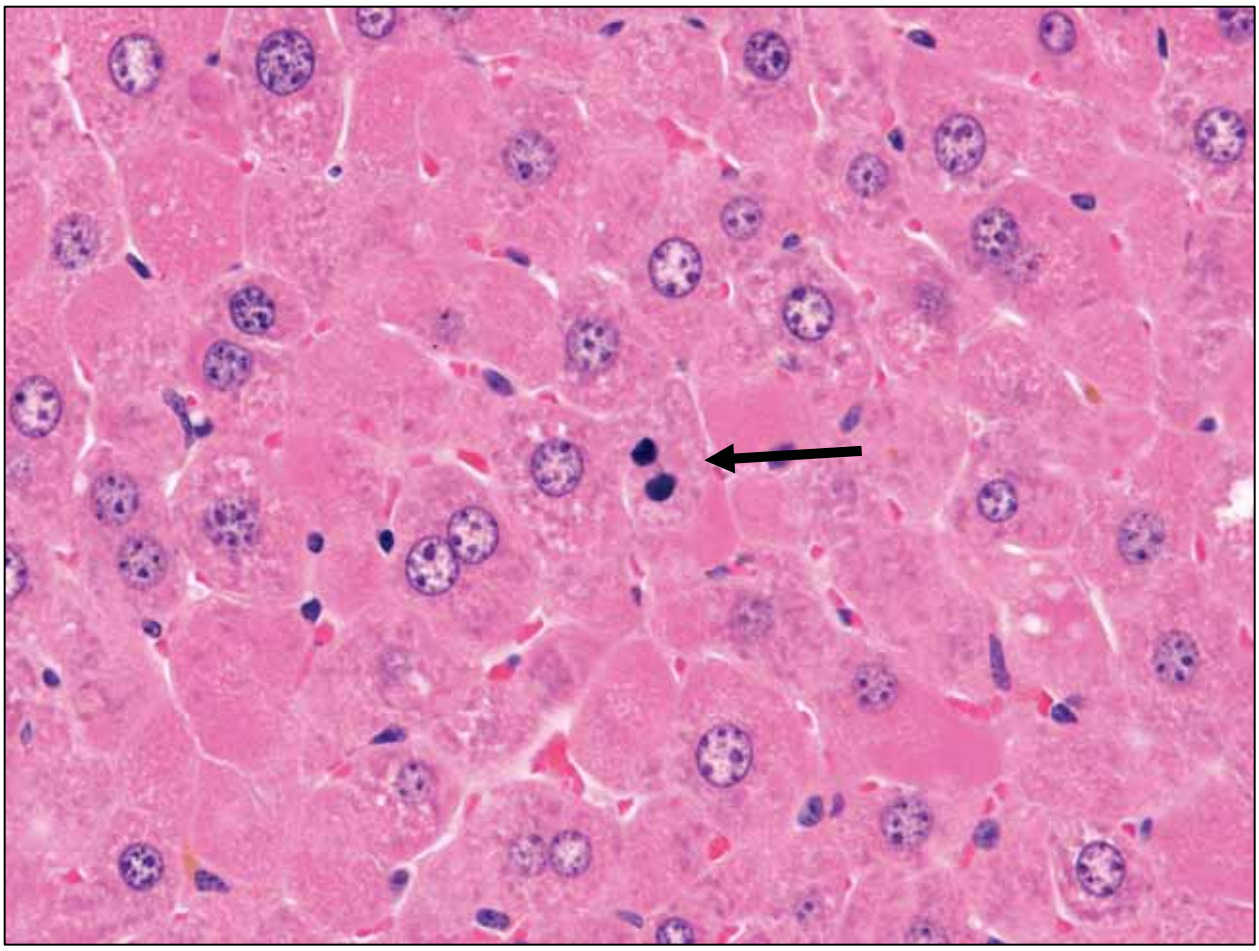

Figure 11. A mitotic figure (arrow) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. Although mitotic figures can be found in the livers of normal mice, there were increased numbers observed in the livers of some animals in this study.

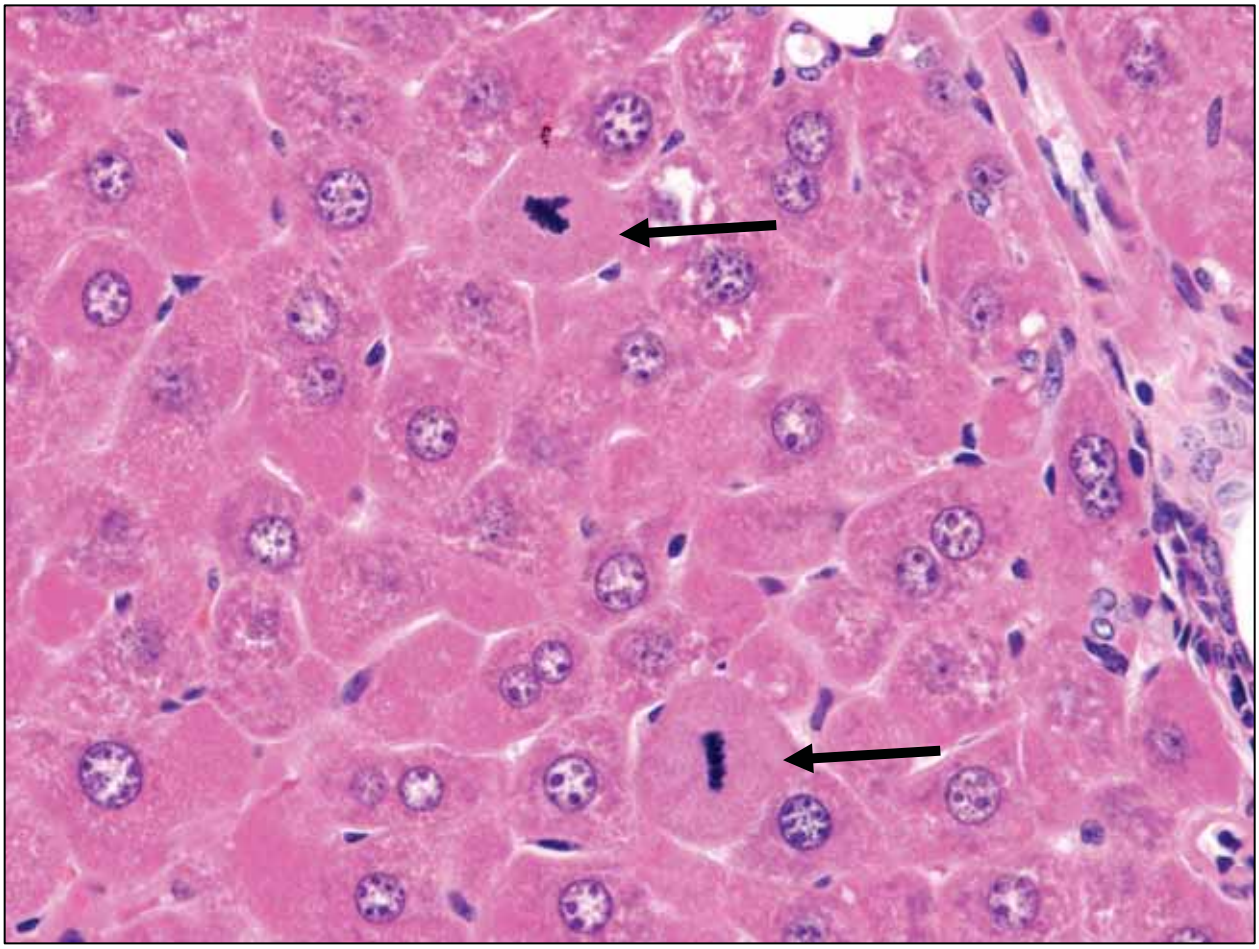

Figure 12. Mitotic figures (arrows) in the liver of a Group 4 male mouse (animal 409) from the 18405-1307 subchronic study. Increased mitotic figures were observed in both the 18405-1307 subchronic and the 18405-1037 reproduction/developmental studies.

Pigment increased; was characterized by golden brown pigment that was found primarily in bile canaliculi, Kupffer cells, but occasionally in hepatocytes as well. All the occurrences were of minimal severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

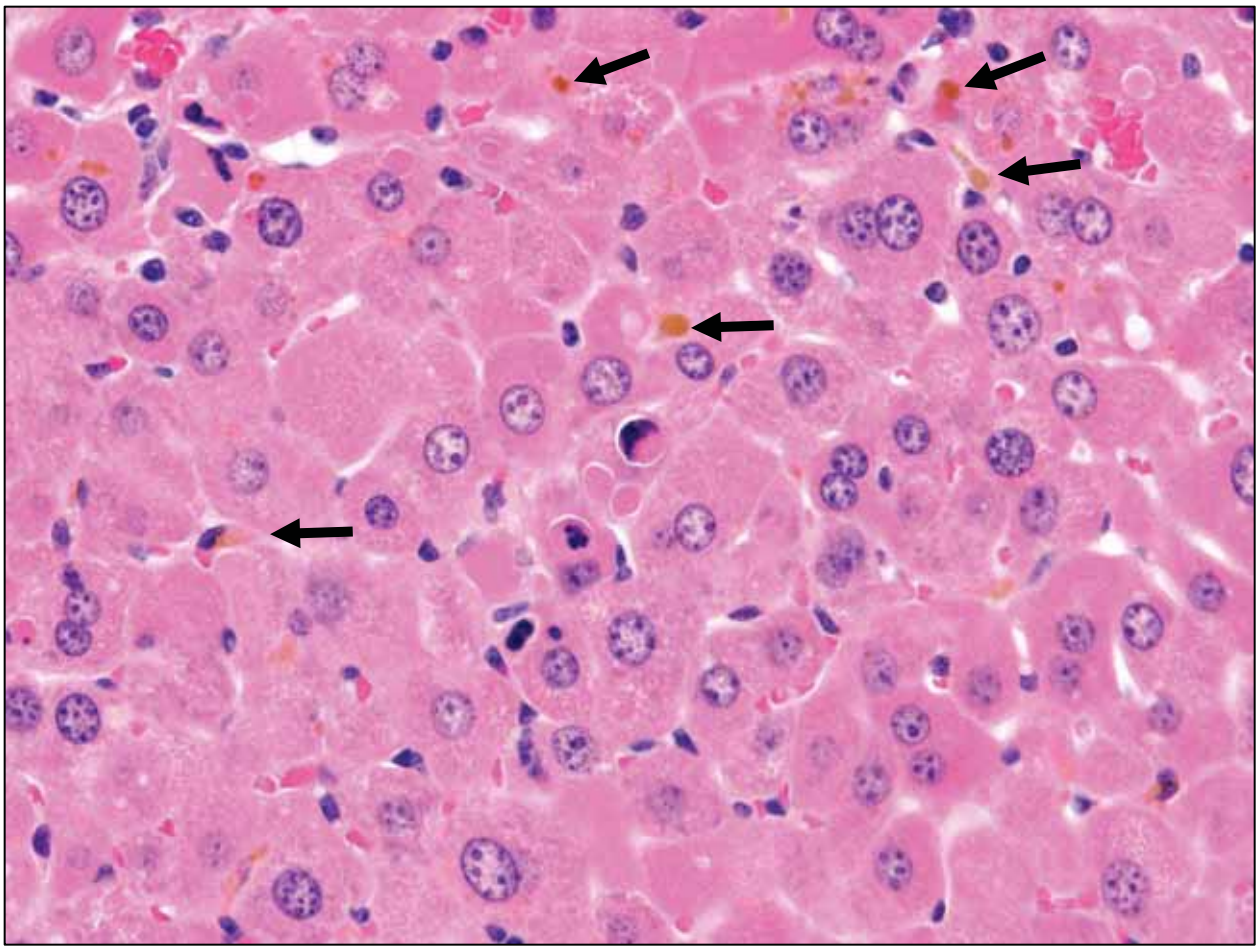

Figure 13. Pigment (arrows) in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. Special stains were not performed to identify the pigment, but the appearance and location were consistent with either inspissated bile in canaliculi (arrows) or byproducts from cell breakdown in Kupffer cells (see figure 14).

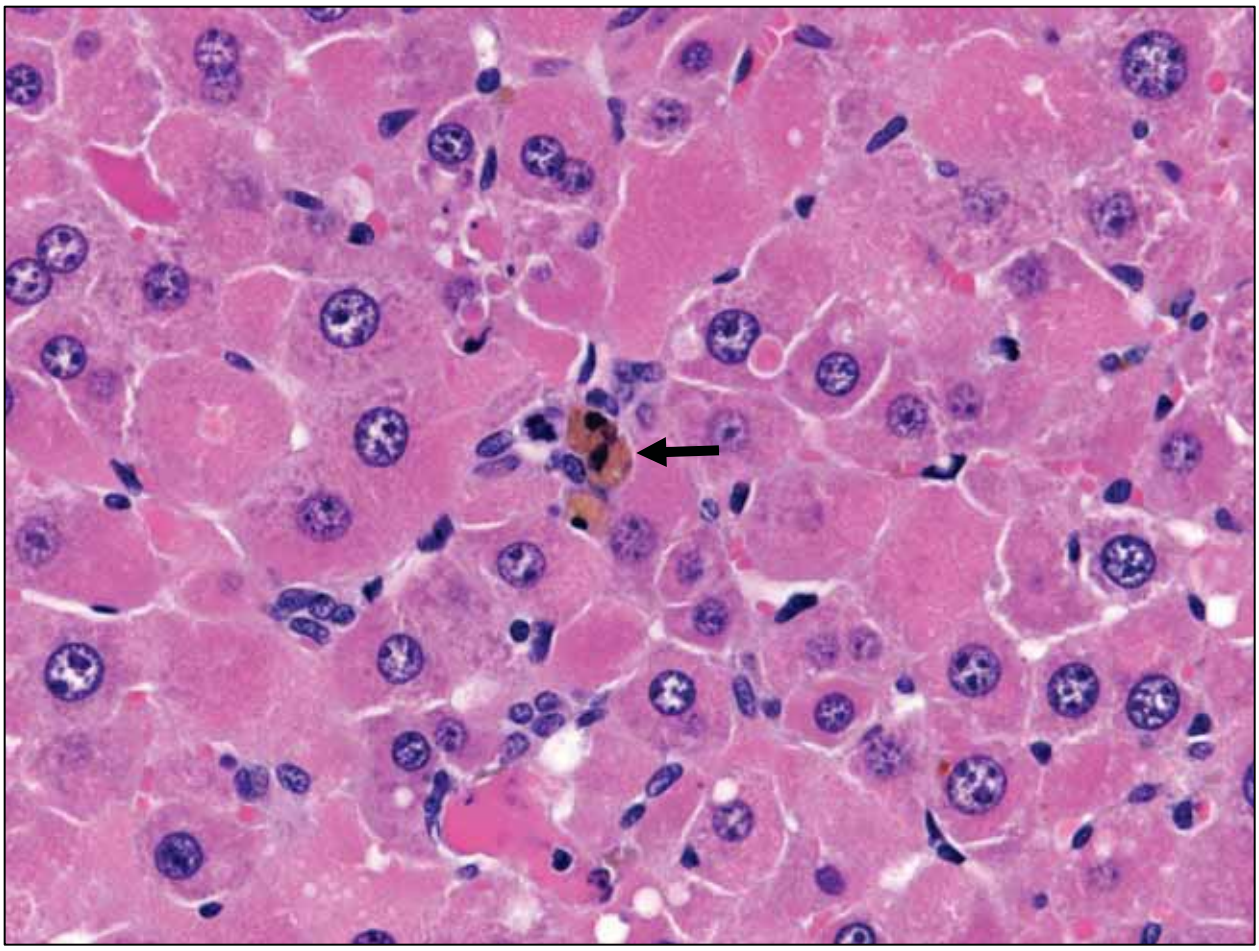

Figure 14. Pigment in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The pigment was found primarily in bile canaliculi or macrophages (Kupffer cells) but also in hepatocytes on occasion and was consistent with either inspissated bile in canaliculi or byproducts from cell breakdown (e.g. hemosiderin) (arrow).

Extramedullary hematopoiesis was recorded in one animal (a Group 4 male) in the 18405-1307 subchronic, and in 4 animals (a Group 3 male; two Group 4 males; & a Group 4 female) in the 18405-1037 reproduction/developmental study. It consisted of tight clusters of hematopoietic cells in varying degrees of maturity; most of the cells appeared to be myeloid cells.

Cytoplasmic vacuolation was recorded in several male mice in Group 3 and several female mice in Group 4 in the 18405-1037 reproduction/developmental study. It was characterized by very small vacuoles within the cytoplasm of hepatocytes, consistent with microvesicular fatty change. In most of the animals, it primarily involved the centrilobular hepatocytes, although in some animals the change was also present in midzonal hepatocytes.

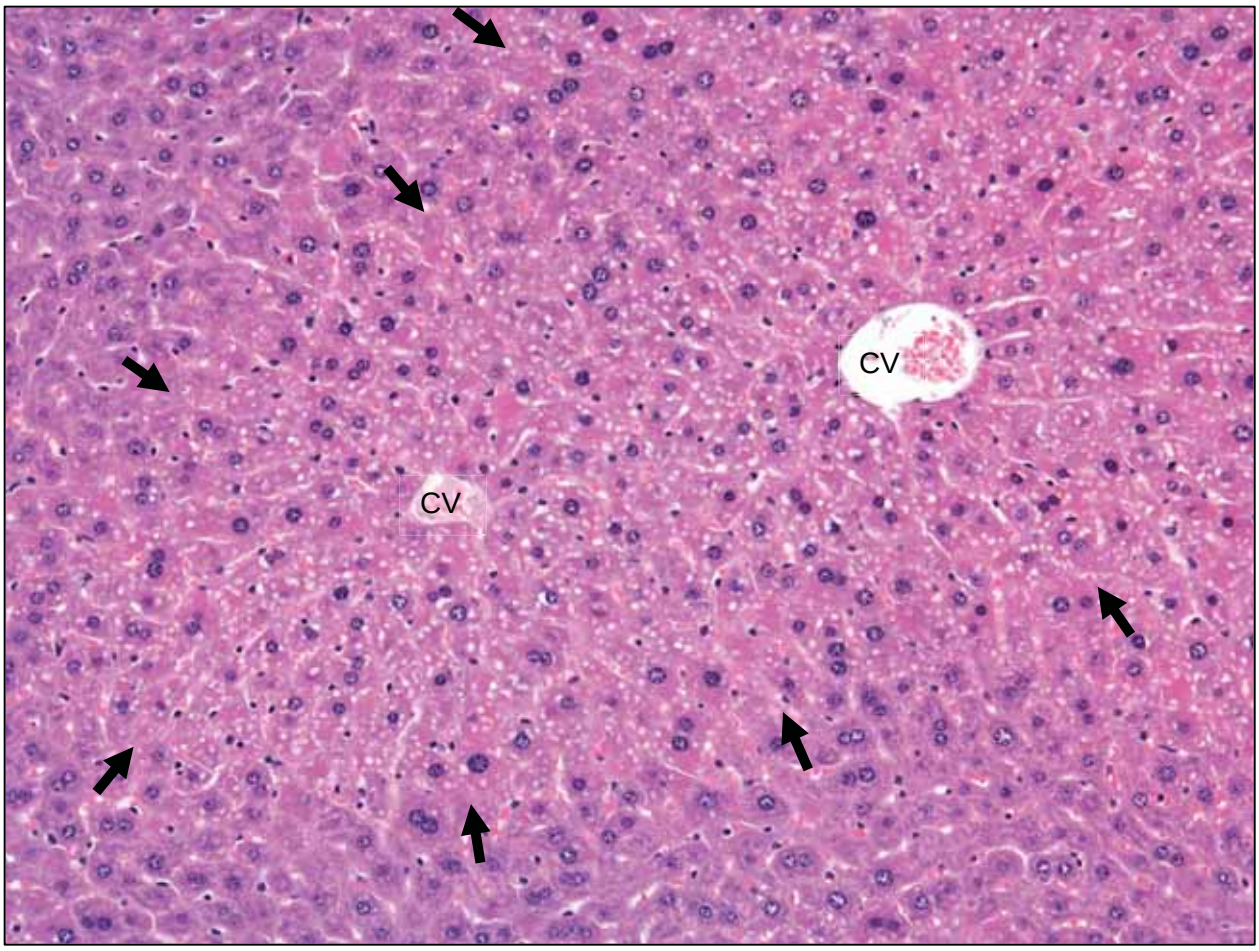

Figure 15. Cytoplasmic vacuolation in the liver of a Group 4 female mouse (animal 5073) from the 18405-1037 reproduction/developmental study. The vacuoles were small and slightly less regular than those observed in macrovesicular fatty change, and found primarily in the centrilobular region (area delineated by arrows), around the central vein. CV=central vein.

Oval cell hyperplasia was recorded in six Group 4 male mice in the 18405-1037 reproduction/developmental study. Only minimal oval cell hyperplasia was recorded, and it was characterized by an increase in oval cells in several periportal regions, with some oval cells present in the surrounding hepatic parenchyma.

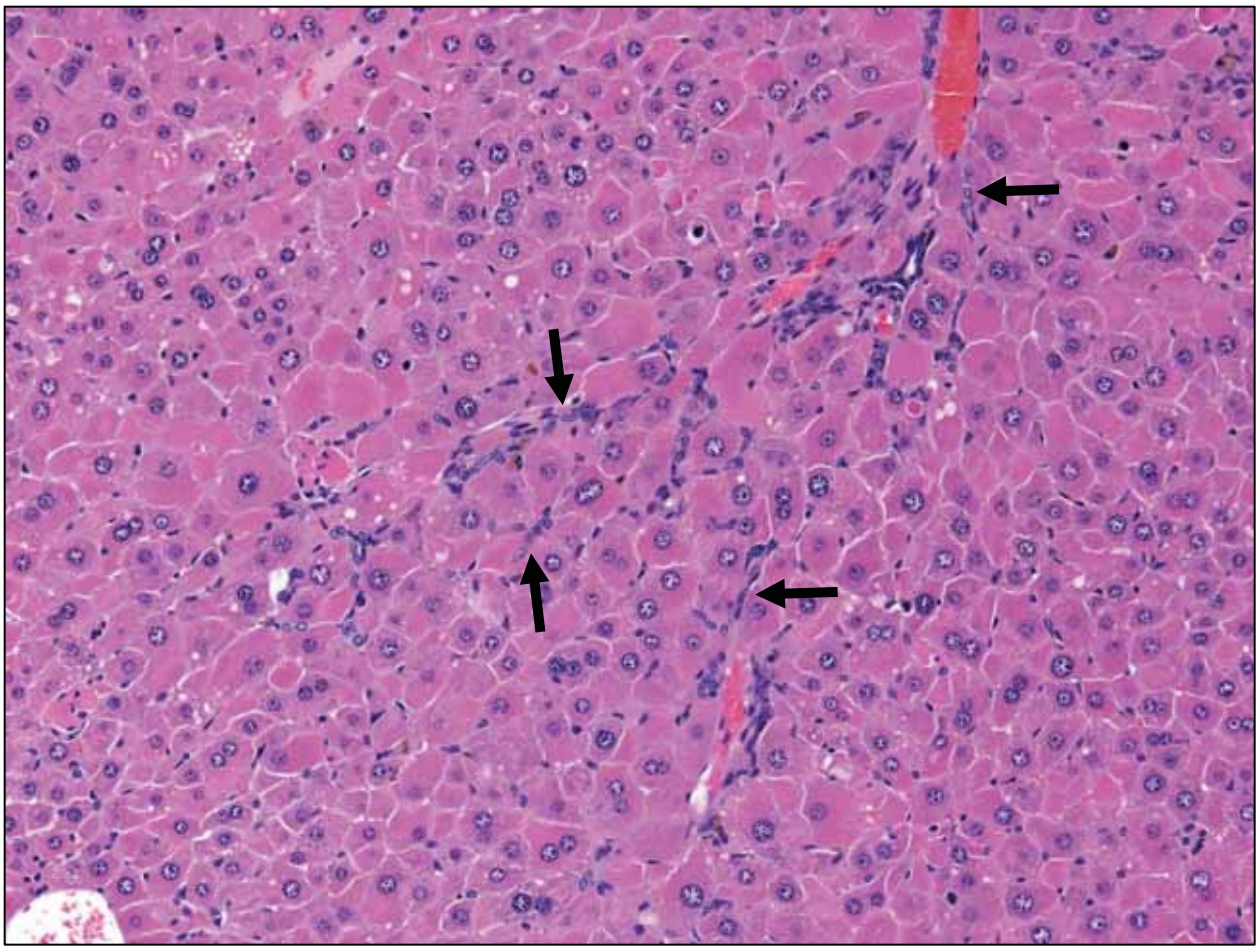

Figure 16. Oval cell hyperplasia in the liver of a Group 4 male mouse (animal 7730) from the 18405-1037 reproduction/developmental study. Oval cells (arrows), small cells with round to oval nuclei, appear to originate in the portal region and branch out from there.

Bile duct hyperplasia was recorded in one Group 4 male mouse in the 18405-1307 subchronic study, and was characterized by increased profiles of bile ducts in the periportal region; only minimal bile duct hyperplasia was recorded.

Table 1. Study 18405-1307 Subchronic Toxicity 90-Day Gavage Study
Summary incidences of lesions observed in the liver during slide review

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Within normal limits	4	4	0	0	4	6	7	0
Mixed cell infiltrate	6	6	4	6	5	3	3	7
Single cell necrosis; hepatocellular	0	1	0	9	0	0	0	3

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Cytoplasmic alteration	0	0	10	10	0	0	0	10
Focal necrosis	0	0	0	1	1	0	2	4
Cytoplasmic vacuolation	0	0	0	0	0	0	0	0
Extramedullary hematopoiesis,	0	0	0	1	0	0	0	0
Pigment, increased	0	0	0	10	0	0	0	4
Apoptosis; hepatocellular	0	0	0	10	0	0	0	3
Mitotic figures increased	0	0	0	7	0	0	0	0
Bile duct hyperplasia	0	0	0	1	0	0	0	0

Table 2. Study 18405-1037 Reproduction/developmental Toxicity Screening Study Summary incidences of lesions observed in the liver during slide review

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Within normal limits	18	21	5	0	11	14	3	0
Mixed cell infiltrate	6	3	11	8	12	7	17	15
Single cell necrosis; hepatocellular	1	1	2	24	0	2	3	19
Cytoplasmic alteration	0	0	10	25	0	1	16	25
Focal necrosis	0	0	4	3	2	2	4	5
Cytoplasmic vacuolation	0	0	3	0	0	0	0	1
Extramedullary hematopoiesis	0	0	1	2	0	0	0	1
Pigment, increased	0	0	0	21	0	0	0	3

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Apoptosis; hepatocellular	0	0	0	22	0	0	0	10
Mitotic figures increased	0	0	0	17	0	0	0	2
Oval cell hyperplasia	0	0	0	4	0	0	0	0
Inflammation, granulomatous	0	0	0	0	0	0	1	0
Polyarteritis nodosa	0	0	0	0	0	0	0	1

PATHOLOGY WORKING GROUP

A PWG was convened on October 15, 2019 at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park (RTP), NC to histologically evaluate selected tissues from this study. The participants were Drs. Susan A. Elmore, MS, DVM, DACVP, DABT (NTP/NIEHS - PWG Coordinator), Amy Brix, DVM, PhD, DACVP (EPL - Reviewing Pathologist), David Malarkey, DVM, PhD, DACVP (NTP/NIEHS), Arun Pandiri, BVSc&AH, PhD, DACVP, DABT (NTP/NIEHS), Robert Sills, DVM, PhD, DACVP (NTP/NIEHS), Brian Berridge, DVM, PhD, DACVP (NTP/NIEHS), Robert Maronpot, DVM, MS, MPH (Maronpot Consulting, LLC) and Michael Elwell, DVM, PhD (Apex ToxPath, LLC).

The PWG Coordinator selected slides for review by the PWG that included 3 examples each of normal liver, hepatocellular apoptosis, hepatocellular single cell necrosis and hepatocellular cytoplasmic alteration, as well as 2 examples each of focal necrosis, pigment, increased mitoses, mixed cell infiltrates, cytoplasmic vacuolation and 1 example of oval cell hyperplasia. There was a majority consensus for all reviewed lesions. The PWG consensus opinion for each slide, including any additional diagnoses made by the PWG panel, was recorded on the slide review worksheet attached to the end of this report.

After review of all lesions, there was discussion about potential adversity. Adversity is a term indicating “harm” to the test animal within the constraints of a given study design (dose, duration, etc.). Assessment of adversity should represent empirical measurements (i.e. objective data) integrated with well-informed subjective judgements to determine whether or not a response is considered harmful to an organism (Kerlin et al. 2016). After discussion, the PWG members agreed that the dose response and constellation of lesions (i.e. cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of this study.

SUMMARY

This review generally supported the study pathologist's findings. When appropriate, the diagnosis of apoptosis, hepatocellular was added to distinguish cells with morphological characteristics of apoptosis from those with morphologic characteristics of single cell necrosis. The diagnostic term of "cytoplasmic alteration" was used to indicate hepatocyte hypertrophy, frequently coupled with a brightly eosinophilic, often granular appearance of the cytoplasm of hepatocytes. Other changes were recommended based upon using terminology preferred by the NTP. The dose response and constellation of lesions were together considered to be indicators of adversity within the confines of this study.

CONFLICT OF INTEREST STATEMENT

This statement is to certify that the reviewing pathologist, Dr. Brix, an on-site NTP Pathologist employed by Experimental Pathology Laboratories, Inc. (EPL®), participated in the pathology peer review of the liver of the Subchronic Toxicity 90 Day Gavage Study In Mice (Project ID: DuPont-18405-1307) and An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 In Mice (Study Number WIL-180225) (Project ID: DuPont-18405-1037). She has not been involved in any aspect of the study for any organization other than NTP which conducted the study nor the generation and/or evaluation of materials or data which were reviewed prior to the receipt of materials from the study lab. Hence, her participation in the review poses no apparent or actual conflict of interest.

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PWG SLIDE REVIEW WORKSHEET

Flat/#	Animal #	Study	Lesion in question	Other/ Comments
1-1	101	1307	Within normal limits	7 agreed, 0 disagreed
1-2	7722	1037	Within normal limits	7 agreed, 0 disagreed
1-3	7718	1037	Within normal limits	7 agreed, 0 disagreed
1-4	410	1307	Apoptosis	7 agreed, 0 disagreed
1-5	401	1307	Apoptosis	7 agreed, 0 disagreed
1-6	7770	1037	Apoptosis	7 agreed, 0 disagreed
1-7	405	1307	Single cell necrosis	7 agreed, 0 disagreed
1-8	7730	1037	Single cell necrosis	7 agreed, 0 disagreed
1-9	7804	1037	Single cell necrosis	7 agreed, 0 disagreed
1-10	406	1307	Cytoplasmic alteration	7 agreed, 0 disagreed
1-11	404	1307	Cytoplasmic alteration	7 agreed, 0 disagreed
1-12	7759	1037	Cytoplasmic alteration	7 agreed, 0 disagreed
1-13	7744	1037	Focal necrosis	7 agreed, 0 disagreed
1-14	456	1307	Focal necrosis	7 agreed, 0 disagreed
1-15	403	1307	Pigment	7 agreed, 0 disagreed
1-16	7780	1037	Pigment	7 agreed, 0 disagreed
1-17	408	1307	Increased mitoses	7 agreed, 0 disagreed
1-18	409	1307	Increased mitoses	7 agreed, 0 disagreed
1-19	407	1307	Mixed cell infiltrates	5 agreed, 2 voted for “inflammation”
1-20	7723	1037	Mixed cell infiltrates	5 agreed, 2 voted for “inflammation”
2-1	5073	1037	Cytoplasmic Vacuolation	7 agreed, 0 disagreed
2-2	7799	1037	Cytoplasmic Vacuolation	7 agreed, 0 disagreed
2-3	7778	1037	Oval cell hyperplasia	6 agreed, 1 voted for biliary hyperplasia

Appendix A. Slide Review Worksheets

Project 18405-1307 Males

Animal #	Organ	SP Diagnosis	NTP Diagnosis
101	Liver	Within normal limits	Agree with SP
102	Liver	Within normal limits	Agree with SP
103	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
104	Liver	Within normal limits	Mixed cell infiltrate; minimal
105	Liver	Within normal limits	Agree with SP
106	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
107	Liver	Within normal limits	Agree with SP
108	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
109	Liver	Within normal limits	Mixed cell infiltrate; minimal
110	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
201	Liver	Within normal limits	Agree with SP
202	Liver	Within normal limits	Agree with SP
203	Liver	Within normal limits	Agree with SP
204	Liver	Within normal limits	Agree with SP
205	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal
206	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
207	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
208	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
209	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
210	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
301	Liver	Within normal limits	Cytoplasmic alteration; mild
302	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild
303	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
304	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
305	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
306	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
307	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
308	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
309	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
310	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
401	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Not present in section Apoptosis; hepatocellular; minimal
402	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hyperplasia; bile duct; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal	Agree with SP Pigment, increased; minimal Agree with SP Cytoplasmic alteration; marked Agree with SP Apoptosis; hepatocellular; minimal
403	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
404	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
405	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Apoptosis; hepatocellular; minimal
406	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Not present in section Pigment, increased; minimal Cytoplasmic alteration; marked Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
407	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Not present in section Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
408	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Mitotic figures; mild Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
409	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Not present in section Apoptosis; hepatocellular; minimal Extramedullary hematopoiesis; minimal
410	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; mild

Project 18405-1307 Females

Animal #	Organ	SP Diagnosis	NTP Diagnosis
151	Liver	Within normal limits	Mixed cell infiltrate; minimal
152	Liver	Within normal limits	Mixed cell infiltrate; minimal
153	Liver	Within normal limits	Agree with SP
154	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
155	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
156	Liver	Within normal limits	Agree with SP
157	Liver	Focal necrosis, moderate: diffuse and restricted to one lobe (likely due to lobular torsion)	Agree with SP
158	Liver	Within normal limits	Agree with SP
159	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
160	Liver	Within normal limits	Agree with SP
251	Liver	Within normal limits	Slide missing
252	Liver	Within normal limits	Agree with SP
253	Liver	Within normal limits	Agree with SP
254	Liver	Within normal limits	Agree with SP
255	Liver	Within normal limits	Agree with SP
256	Liver	Within normal limits	Mixed cell infiltrate; minimal
257	Liver	Within normal limits	Agree with SP
258	Liver	Within normal limits	Agree with SP
259	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
260	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
351	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
352	Liver	Within normal limits	Agree with SP
353	Liver	Within normal limits	Agree with SP
354	Liver	Within normal limits	Agree with SP
355	Liver	Within normal limits	Agree with SP
356	Liver	Within normal limits	Agree with SP

Animal #	Organ	SP Diagnosis	NTP Diagnosis
357	Liver	Focal necrosis; minimal Mononuclear cell infiltrate; minimal	Agree with SP Mixed cell infiltrate; minimal
358	Liver	Focal necrosis; minimal Mononuclear cell infiltrate; minimal	Agree with SP Mixed cell infiltrate; minimal
359	Liver	Within normal limits	Agree with SP
360	Liver	Within normal limits	Agree with SP
451	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild
452	Liver	Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; minimal	Pigment, increased; minimal Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal
453	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
454	Liver	Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal Focal necrosis; minimal Singe cell necrosis; minimal Pigment, increased; minimal Apoptosis; hepatocellular; minimal
455	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal Pigment, increased; minimal
456	Liver	Focal necrosis; mild: sub-capsular Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Focal necrosis; mild Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
457	Liver	Hepatocellular hypertrophy; mild	Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal
458	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
459	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Focal necrosis; mild Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; mild	Agree with SP Pigment, increased; minimal Focal necrosis; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; mild Apoptosis; hepatocellular; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
460	Liver	Focal necrosis; mild Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Focal necrosis; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal

Appendix B. Slide Review Worksheets

Project 18405-1037 Males

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7714	Liver	Within normal limits	Agree with SP
7717	Liver	Within normal limits	Agree with SP
7718	Liver	Within normal limits	Agree with SP
7722	Liver	Within normal limits	Agree with SP
7723	Liver	Hematopoiesis, extramedullary; minimal	Mixed cell infiltrate; mild
7732	Liver	Within normal limits	Agree with SP
7734	Liver	Within normal limits	Agree with SP
7742	Liver	Within normal limits	Agree with SP
7750	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
7752	Liver	Within normal limits	Mixed cell infiltrate; minimal
7758	Liver	Within normal limits	Agree with SP
7763	Liver	Within normal limits	Agree with SP
7765	Liver	Within normal limits	Agree with SP
7769	Liver	Within normal limits	Agree with SP
7772	Liver	Within normal limits	Agree with SP
7775	Liver	Within normal limits	Agree with SP
7788	Liver	Within normal limits	Agree with SP
7792	Liver	Within normal limits	Agree with SP
7798	Liver	Within normal limits	Mixed cell infiltrate; minimal
7800	Liver	Within normal limits	Mixed cell infiltrate; minimal
7803	Liver	Within normal limits	Mixed cell infiltrate; minimal
7810	Liver	Within normal limits	Agree with SP
7813	Liver	Within normal limits	Mixed cell infiltrate; minimal
7823	Liver	Within normal limits	Agree with SP
7825	Liver	Within normal limits	Agree with SP
7710	Liver	Within normal limits	Agree with SP

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7728	Liver	Within normal limits	Agree with SP
7731	Liver	Within normal limits	Mixed cell infiltrate; minimal
7737	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
7743	Liver	Within normal limits	Agree with SP
7748	Liver	Within normal limits	Agree with SP
7749	Liver	Within normal limits	Agree with SP
7754	Liver	Within normal limits	Agree with SP
7768	Liver	Within normal limits	Agree with SP
7776	Liver	Within normal limits	Mixed cell infiltrate; minimal
7777	Liver	Within normal limits	Agree with SP
7779	Liver	Within normal limits	Agree with SP
7783	Liver	Within normal limits	Agree with SP
7784	Liver	Within normal limits	Agree with SP
7786	Liver	Within normal limits	Agree with SP
7787	Liver	Within normal limits	Mixed cell infiltrate; minimal
7794	Liver	Within normal limits	Agree with SP
7797	Liver	Within normal limits	Agree with SP
7805	Liver	Within normal limits	Agree with SP
7807	Liver	Within normal limits	Agree with SP
7808	Liver	Within normal limits	Agree with SP
7809	Liver	Within normal limits	Agree with SP
7811	Liver	Within normal limits	Agree with SP
7817	Liver	Within normal limits	Agree with SP
7826	Liver	Within normal limits	Agree with SP
7711	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
7720	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7721	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
7729	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal
7740	Liver	Within normal limits	Agree with SP
7741	Liver	Within normal limits	Agree with SP
7745	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal	No remarkable lesion Focal necrosis; minimal Hepatocyte; cytoplasmic vacuolation; minimal
7746	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7756	Liver	Within normal limits	Mixed cell infiltrate; minimal Hepatocyte; cytoplasmic vacuolation; minimal
7760	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hematopoiesis, extramedullary; mild	Mixed cell infiltrate; minimal Agree with SP
7761	Liver	Within normal limits	Agree with SP
7762	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
7767	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
7774	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
7789	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; mild
7790	Liver	Necrosis, single cell; minimal	Agree with SP
7793	Liver	Within normal limits	Mixed cell infiltrate; minimal
7796	Liver	Within normal limits	Agree with SP
7799	Liver	Fatty change, centrilobular; minimal	Hepatocyte; cytoplasmic vacuolation; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7802	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; minimal
7814	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Not present in section (within normal limits)
7820	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal	Mixed cell infiltrate; minimal Focal necrosis; minimal
7822	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section
7827	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7828	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7709	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7712	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal No remarkable lesions Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
7715	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7716	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
7724	Liver	Pigment, increased; minimal Hematopoiesis, extramedullary; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
7726	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7730	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal Oval cell hyperplasia; minimal
7735	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Extramedullary hematopoiesis; minimal
7736	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7738	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7739	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7744	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal Focal necrosis; minimal
7747	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7751	Liver	Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
7759	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Oval cell hyperplasia; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7764	Liver	Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
7770	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; moderate
7778	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Oval cell hyperplasia; minimal
7780	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Oval cell hyperplasia; minimal
7781	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7782	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7785	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7801	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7804	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Focal necrosis; minimal
7815	Liver	Pigment, increased; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Not present in section Focal necrosis; minimal Apoptosis; hepatocellular; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal

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Animal #	Organ	SP Diagnosis	NTP Diagnosis
4956	Liver	Within normal limits	Mixed cell infiltrate; minimal
4958	Liver	Within normal limits	Mixed cell infiltrate; minimal
4962	Liver	Within normal limits	Agree with SP
4966	Liver	Within normal limits	Mixed cell infiltrate; minimal
4967	Liver	Within normal limits	Mixed cell infiltrate; minimal
4968	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4978	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4985	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4986	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4987	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4991	Liver	Within normal limits	Agree with SP
4999	Liver	Within normal limits	Agree with SP
5001	Liver	Within normal limits	Agree with SP
5003	Liver	Within normal limits	Agree with SP
5013	Liver	Within normal limits	Agree with SP
5018	Liver	Within normal limits	Agree with SP
5021	Liver	Within normal limits	Agree with SP
5030	Liver	Necrosis, single cell; minimal	Focal necrosis; minimal
5045	Liver	Within normal limits	Agree with SP
5058	Liver	Necrosis, focal/multifocal; minimal	Focal necrosis; minimal
5059	Liver	Within normal limits	Agree with SP
5060	Liver	Within normal limits	Agree with SP
5064	Liver	Within normal limits	Mixed cell infiltrate; minimal
5066	Liver	Within normal limits	Mixed cell infiltrate; minimal
5071	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4954	Liver	Within normal limits	Agree with SP
4957	Liver	Within normal limits	Agree with SP
4961	Liver	Within normal limits	Agree with SP
4973	Liver	Within normal limits	Agree with SP
4979	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
4981	Liver	Within normal limits	Agree with SP
4988	Liver	Within normal limits	Focal necrosis; minimal
4989	Liver	Infiltrate, neutrophil, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4990	Liver	Within normal limits	Mixed cell infiltrate; minimal
4997	Liver	Within normal limits	Mixed cell infiltrate; minimal
5000	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
5004	Liver	Within normal limits	Agree with SP
5005	Liver	Within normal limits	Agree with SP
5010	Liver	Within normal limits	Agree with SP
5015	Liver	Within normal limits	Agree with SP
5025	Liver	Within normal limits	Agree with SP
5036	Liver	Within normal limits	Mixed cell infiltrate; minimal
5040	Liver	Within normal limits	Agree with SP
5041	Liver	Within normal limits	Agree with SP
5046	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
5047	Liver	Within normal limits	Mixed cell infiltrate; minimal
5049	Liver	Within normal limits	Agree with SP
5061	Liver	Within normal limits	Agree with SP
5063	Liver	Within normal limits	Mixed cell infiltrate; minimal
5072	Liver	Necrosis, single cell; minimal	Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4960	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4963	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; minimal
4969	Liver	Within normal limits	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
4974	Liver	Within normal limits	Single cell necrosis; hepatocellular; minimal
4975	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal
4976	Liver	Within normal limits	Mixed cell infiltrate; minimal
4977	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4980	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
4993	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
5007	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5011	Liver	Within normal limits	Agree with SP
5014	Liver	Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal	Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
5022	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5023	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5031	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5034	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Within normal limits
5037	Liver	Within normal limits	Mixed cell infiltrate; minimal
5043	Liver	Within normal limits	Mixed cell infiltrate; minimal
5048	Liver	Within normal limits	Agree with SP
5050	Liver	Necrosis, focal/multifocal; minimal	Focal necrosis; minimal Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5052	Liver	Within normal limits	Mixed cell infiltrate; minimal
5056	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5057	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Focal necrosis; minimal Cytoplasmic alteration; minimal Inflammation; granulomatous; focal; minimal
5065	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5070	Liver	Within normal limits	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4955	Liver	Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Not present in section Apoptosis; hepatocellular; minimal Cytoplasmic alteration; mild Polyarteritis nodosa; moderate
4959	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4972	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
4982	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
4984	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
4998	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal Apoptosis; hepatocellular; minimal
5002	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
5006	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
5008	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5009	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
5017	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hematopoiesis, extramedullary; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Agree with SP Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
5020	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
5027	Liver	Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
5028	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal
5029	Liver	Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
5033	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
5035	Liver	Hypertrophy, hepatocellular, diffuse; mild	Cytoplasmic alteration; mild
5051	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Not present in section Cytoplasmic alteration; minimal Mitotic figures increased; minimal Mixed cell infiltrate; minimal Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5062	Liver	Pigment, increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
5068	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
5069	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
5073	Liver	Fatty change, centrilobular; mild Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Hepatocyte; cytoplasmic vacuolation; mild Cytoplasmic alteration; mild
5074	Liver	Pigment, increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
5075	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Not present in section Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
5077	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Focal necrosis; minimal Mixed cell infiltrate; minimal

Appendix E: Benchmark Dose Modeling

E.1 Oral Reproduction/Developmental Toxicity Study in Mice (DuPont-18405-1037 2010)

U.S. Environmental Protection Agency (EPA) Center for Public Health and Environmental Assessment conducted dose response modeling of this study using the Benchmark Dose Software (BMDS) 3.1.2. program. This work used data from the reevaluation of the DuPont oral reproductive/ developmental toxicity study slides by the National Toxicology Program (NTP) Pathology Working Group (see section 4.5 for a description) and addresses the constellation of liver lesions the NTP defined as adverse (i.e., cytoplasmic alteration, single-cell and focal necrosis, and apoptosis) in parental male and parental female mice.

E.1.1 Constellation of Lesions (Cytoplasmic Alteration, Apoptosis, Single-Cell Necrosis, and Focal Necrosis) in the Liver, Parental Males

Increased incidence of a constellation of lesions in the liver was observed in the parental males. Dichotomous models were used to fit dose-response data (DuPont-18405-1037, 2010). A benchmark response (BMR) of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (EPA, 2012). The doses and response data used for the modeling are listed in Table E-1.

Table E-1. Constellation of Lesions in the Male Liver Selected for Dose-Response Modeling

Dose (mg/kg/day)	Number of mice (males)	Constellation of Liver Lesions
0	25	1
0.1	24	1
0.5	24	13
5	24	24

Note: mg/kg/day = milligrams per kilogram per day.

The benchmark dose (BMD) modeling results for the constellation of lesions are summarized in Table E-2 and Figure E-1. The best fitting model was the Probit model based on adequate *p*-values (greater than 0.1), the benchmark dose lower limits (BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the Probit model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level ($BMDL_{10}$) from the selected Probit model is 0.14 milligram per kilogram per day (mg/kg/day).

Table E-2. Summary of BMD Modeling Results for Constellation of Lesions in Male Mice

Model[a]	Goodness of fit		Scaled residual for:		BMD_{10Pct} (mg/kg/day)	$BMDL_{10Pct}$ (mg/kg/day)	Basis for model selection
	p-value	AIC	Dose group near BMD	Dose group near BMDL			
Dichotomous Hill	N/A[b]	57.818	0.007	-0.007	0.29	0.11	EPA ORD selected the Weibull model. All models, except Dichotomous Hill, had adequate fit (*p*-values > 0.1), the BMDLs were sufficiently close (<3-fold difference), and the Probit model had the lowest AIC.
Gamma	0.994	55.815	-0.005	0.005	0.26	0.09	
Log-Logistic	0.977	55.816	0.000	-0.020	0.34	0.11	
Multistage Degree 3	0.997	53.820	-0.053	0.047	0.26	0.08	
Multistage Degree 2	0.905	54.026	-0.368	0.248	0.19	0.08	
Multistage Degree 1	0.279	57.026	-1.402	0.452	0.08	0.05	
Weibull	0.937	53.951	-0.290	0.205	0.20	0.08	
Logistic	0.888	54.048	-0.327	0.359	0.22	0.15	
Log-Probit	0.990	55.816	0.001	-0.001	0.24	0.10	
Probit	**0.907**	**52.444**	**-0.635**	**0.093**	**0.19**	**0.14**	

Notes: ORD = Office of Research and Development.
[a] Selected model in bold.
[b] degrees of freedom=0, saturated model (Goodness of fit test cannot be calculated).

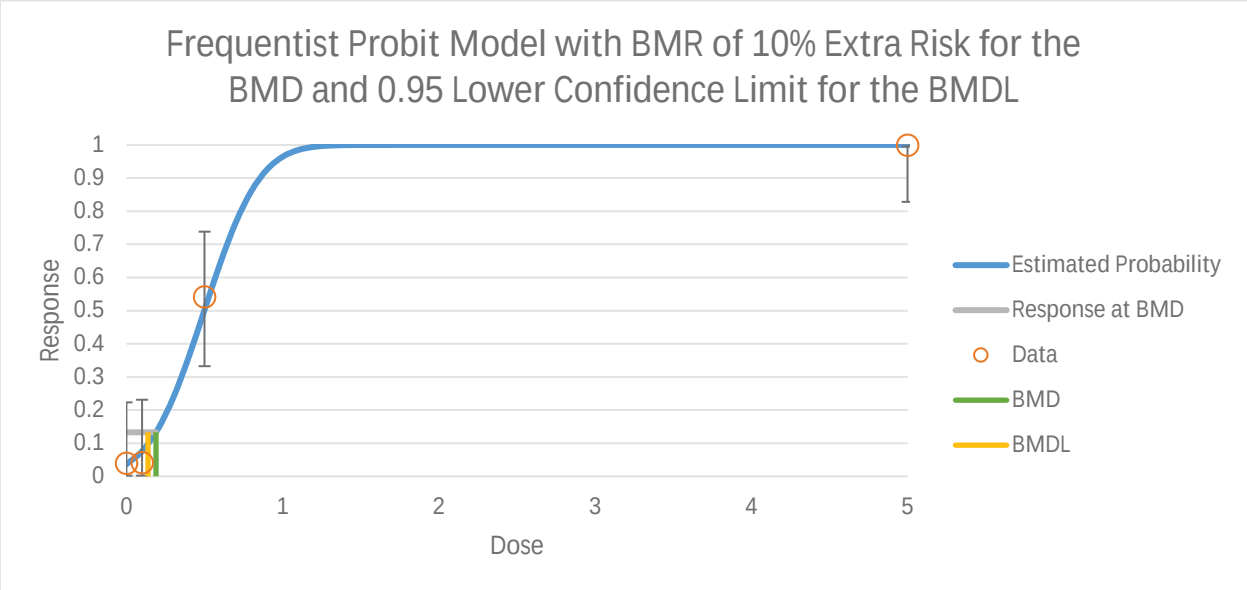

Figure E-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Constellation of Lesions in Male Mice (dose shown in mg/kg/day)

E.1.2 Constellation of Lesions (Cytoplasmic Alteration, Apoptosis, Single-Cell Necrosis, and Focal Necrosis) in the Liver, Parental Females

Increased incidence of the constellation of lesions in the liver was observed in the parental females. Dichotomous models were used to fit dose-response data (DuPont-18405-1037, 2010). A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (EPA, 2012). The doses and response data used for the modeling are listed in Table E-3.

Table E-3. Constellation of Lesions in the Female Liver Selected for Dose-Response Modeling

Dose (mg/kg/day)	Number of mice (females)	Constellation of Liver Lesions
0	24	2
0.1	22	3
0.5	24	17
5	24	24

The BMD modeling results for constellation of lesions are summarized in Table E-4 and Figure E-2. The best fitting model was the Probit model based on adequate *p*-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Probit model had the lowest AIC. The $BMDL_{10}$ from the selected Probit model is 0.09 mg/kg/day.

Table E-4. Summary of BMD Modeling Results for Constellation of Lesions in Female Mice

Model[a]	Goodness of fit		Scaled residual for:		BMD_{10Pct} (mg/kg/day)	$BMDL_{10Pct}$ (mg/kg/day)	Basis for model selection
	p-value	AIC	Dose group near BMD	Dose group near BMDL			
Dichotomous Hill	N/A[b]	68.371	0.108	-0.077	0.14	0.05	EPA ORD selected the Probit model. All models, except Dichotomous Hill and Multistage Degree 3, had adequate fit (*p*-values > 0.1), the BMDLs were sufficiently close (<3-fold difference), and the Probit model had the lowest AIC.
Gamma	1.000	66.268	0.000	0.000	0.13	0.04	
Log-Logistic	0.804	66.371	0.108	-0.077	0.14	0.05	
Multistage Degree 3	N/A[b]	68.268	0.003	-0.002	0.15	0.04	
Multistage Degree 2	0.998	66.268	-0.002	0.001	0.14	0.04	
Multistage Degree 1	0.448	66.021	-1.087	0.393	0.05	0.04	
Weibull	1.000	66.268	0.000	0.000	0.14	0.04	
Logistic	0.993	64.283	-0.086	0.085	0.13	0.09	
Log-Probit	0.932	66.282	0.024	-0.015	0.13	0.05	
Probit	**0.971**	**62.514**	**-0.328**	**-0.101**	**0.12**	**0.09**	

Notes: ORD = Office of Research and Development.
[a] Selected model in bold.
[b] degrees of freedom=0, saturated model (Goodness of fit test cannot be calculated).

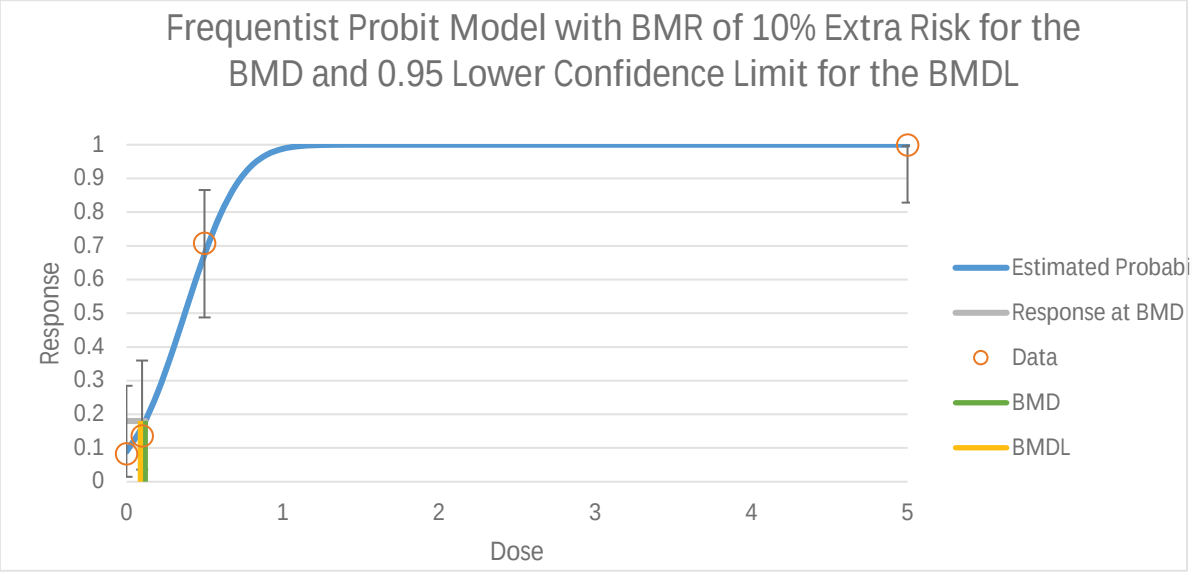

Figure E-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Constellation of Lesions in Female Mice; (dose shown in mg/kg/day)

Attachment

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EPA/630/P-02/002F
December 2002
Final Report

A REVIEW OF THE REFERENCE DOSE AND REFERENCE CONCENTRATION PROCESSES

Prepared for the
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

ACE II	Angiotensin converting enzyme II
ADI	Acceptable Daily Intake
AEGL	Acute exposure guideline level
ARE	Acute reference exposure
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	Area under the curve
BMC	Benchmark concentration
BMCL	Benchmark concentration lower confidence limit
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence level
BMR	Benchmark response
CatReg	Categorical Regression (software)
CFSAN	Center for Food Safety and Nutrition
CNS	Central nervous system
CSAF	Chemical-specific adjustment factor
DAF	Dosimetric adjustment factor
DNT	Developmental neurotoxicity
ECE-1	Endothelin-converting enzyme-1
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
FQPA	Food Quality Protection Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GD	Gestational day
GLP	Good Laboratory Practices
HA	Health Advisory
HEC	Human equivalent concentration
HED	Human equivalent dose
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
LOAEL	Lowest-observed-adverse-effect-level
MF	Modifying factor
MOE	Margin of exposure
MRL	Minimal risk level
NAAQS	National Ambient Air Quality Standards
NCEA	National Center for Environmental Assessment
NK	Natural Killer
NOAEL	No-observed-adverse-effect-level
OAR	Office of Air and Radiation
OECD	Organisation for Economic Cooperation and Development
OPP	Office of Pesticide Programs

LIST OF ABBREVIATIONS AND ACRONYMS (continued)

OPPTS	Office of Prevention, Pesticides, and Toxic Substances
OSWER	Office of Solid Waste and Emergency Response
OW	Office of Water
P	Parental
PAD	Population adjusted dose
PBPK	Physiologically-based pharmacokinetic model
PFC	Plaque-forming cell
PND	Postnatal day
POD	Point of departure
PRA	Plasma renin activity
RDDR	Regional deposited dose ratio
RGDR	Regional gas dose ratio
RfC	Reference concentration
RfD	Reference dose
SAB	Science Advisory Board
SPF	Specific pathogen free
SRBC	Sheep Red Blood Cells
TSCA	Toxic Substance Control Act
TWA	Time-weighted average
UF	Uncertainty factor

PREFACE

The U.S. Environmental Protection Agency (EPA) Risk Assessment Forum was established to promote scientific consensus on risk assessment issues and to ensure that this consensus is incorporated into appropriate risk assessment guidance. To accomplish this, the Forum assembles experts throughout EPA in a formal process to study and report on these issues from an Agency-wide perspective. For major risk assessment activities, the Forum has established Technical Panels to conduct scientific reviews and analyses. Members are chosen to ensure that necessary technical expertise is available.

The RfD/RfC Technical Panel (hereafter the Technical Panel) was established by the Risk Assessment Forum in early 1999 in response to a request from the Agency's 10X Task Force[1] to the Science Policy Council and the Forum. In the process of developing a strategy for implementing the Food Quality Protection Act (FQPA) relative to protecting children's health and application of the 10X safety factor, the 10X Task Force produced two draft reports, one on toxicology (U.S. EPA, 1999b) and one on exposure data requirements (U.S. EPA, 1999c) that were used by the Office of Pesticide Programs to develop a policy document for implementation of the FQPA safety factor (U.S. EPA, 2002b).

The draft 10X toxicology report (U.S. EPA, 1999b) raised a number of issues that relate to the derivation of the oral reference dose (RfD) and inhalation reference concentration (RfC). Examples of these issues include the following. (1) Appropriate application of a database uncertainty factor (UF) or modifying factor for studies that are considered necessary but are absent or judged inadequate that may show children to be significantly more sensitive or susceptible than adults. Addressing this issue also implicates aspects of other UFs that relate to children's health, including the factor for inter-individual variability in humans (e.g., response of the aged vs. response of the younger adult or child), and the interspecies UF (e.g., young animals vs. young humans). (2) How to account for degree of concern for potential toxicity to children in the RfD/RfC process. Degree of concern, as used in the 10X toxicology report, refers to the characterization of the database as to the likelihood that the agent under review would have effects in humans within the context of dose, route, duration, and timing of exposure. (3) The

[1]The 10X Task Force was created by the EPA Administrator to explore the adequacy of current testing approaches for pesticides for protecting children's health and to recommend approaches for implementing the additional 10X safety factor mandated by the 1996 Food Quality Protection Act.

use of developmental toxicity data as the basis for reference values[2] of chronic duration (RfDs or RfCs) and the appropriate setting of acute, short-term, and longer-term reference values, including the application of developmental toxicity data for these shorter-duration reference values. (4) The appropriateness and/or rationale for adjusting the no-observed-adverse-effect level (NOAEL) or the benchmark dose from developmental toxicity data with inhalation exposures using a concentration-times-time (C x t) adjustment, as is done for other study types.

The Technical Panel also was asked to consider the need for additional toxicity test protocols related to children's health as recommended by the 10X Task Force, when such protocols should be required, and how the data should be interpreted for risk assessment purposes. These additional protocols include (1) collection of toxicokinetic data, both in adults and at different developmental stages; (2) direct dosing of neonates, especially when early exposure is of concern; (3) perinatal carcinogenesis studies and appropriate triggers for when they should be required; (4) developmental immunotoxicity testing and appropriate triggers; (5) advanced developmental neurotoxicity testing, in particular, cognitive testing that is more similar to that used in humans; and (6) exposure assessments that are more compatible with the dose-response assessment. (See Appendix A for more a detailed discussion of the issues raised by the 10X Task Force.)

The Science Policy Council and the Risk Assessment Forum agreed that these issues should be examined—with input from various program offices within the Agency and from the outside scientific/policy community—on a broader scale than just for pesticides. This charge was expanded by the Forum to include a more in-depth review of a number of issues related to the RfD/RfC process, in part because of several other Forum activities that were underway. These activities included development of *Framework for the Harmonization of Cancer and Noncancer Risk Assessment*, revision of *Benchmark Dose Guidance Document*, and revision of *Guidelines for Carcinogen Risk Assessment*. In addition, the RfD/RfC derivation process (Barnes and Dourson, 1998; U.S. EPA, 1994, 2002c) had not been evaluated in detail for a number of years, and several scientific issues concerning children's health, for example, neurotoxicity and immunotoxicity, have become increasingly important in risk assessment. These various but related activities have prompted the need to re-examine the RfD/RfC process and to coordinate these efforts with other related activities. In particular, it was important that efforts continue to focus on moving toward the goal of harmonization of risk assessment

[2]The term reference value is used generically here to refer to values such as the RfD, RfC, acute reference exposure (ARE), Health Advisory (HA), acute exposure guideline level (AEGL), minimal risk level (MRL), or other similar values.

approaches for all health endpoints. This document represents the review and deliberations of the RfD and RfC processes by the Risk Assessment Forum Technical Panel.

EXECUTIVE SUMMARY

This document summarizes the review and deliberations of the Risk Assessment Forum's RfD/RfC Technical Panel and its recommendations for improvements in oral reference dose/inhalation reference concentration (RfD/RfC) process as well as additional efforts that are needed. It discusses revisions to the framework for the derivation of reference values. The document is a review, not guidance, but it does make recommendations that should be considered in the implementation of changes in the current process and/or development of needed guidance.

The Technical Panel reviewed most of the issues relating to hazard characterization for developing reference values and the need for developing reference values for different durations of exposure as well as the process of deriving reference values, but it did not go into detail on the quantitative aspects of the dose-response process, which is being covered in other Forum activities. The Technical Panel views the RfD/RfC process as one that should be continually evolving as new information becomes available and new scientific and risk assessment approaches are developed. This does not mean that current RfDs or RfCs are invalid, but these new scientific issues should be included in the process of re-evaluating of current reference values.

This document reviews and discusses a number of issues and provides conclusions and recommendations that are intended to improve the RfD/RfC process. The Technical Panel has provided specific recommendations for the development of guidance in some cases and more general conclusions and recommendations in others. In the latter cases, the Technical Panel felt that development of specific recommendations was beyond the scope of its efforts or that policies needed to be further developed before specific guidance could be written to implement the recommendations. The document is divided into five chapters:

Chapter 1 provides an introduction, background, purpose, and scope for the project.

Chapter 2 reviews current approaches to developing acute, short-term, and longer-term reference values as well as the chronic reference values, the RfD and the RfC. This chapter incorporates the presentations and discussions on developing less-than-lifetime values from briefings to the Technical Panel and a colloquium held August 2, 2000, and includes discussions of the proposed Acute Reference Exposure (ARE) methodology for acute inhalation exposures, the Acute Exposure Guideline Level (AEGL) Program, the Office of Pesticide Programs' procedures for setting acute and longer-term duration RfDs, the Office of Water's Health Advisories, and the Agency for Toxic Substances and Disease Registry's Minimal Risk Levels.

On the basis of its review of the various approaches to setting acute, short-term, and longer-term reference values, the Technical Panel concurred with the recommendation of the 10X Task Force that such values should be set, where possible, and that they should be incorporated into the Integrated Risk Information System (IRIS) database. In addition, the Technical Panel recommended that this process be done in a consistent manner using standardized definitions for acute, short-term, longer-term, and chronic durations that are consistent with current practice. These values can then be used by various program offices, where applicable. A framework for deriving these additional values is presented in Chapter 4.

Chapter 3 reviews the current Office of Prevention, Pesticides and Toxic Substances' harmonized health effects testing guidelines for the purpose of determining the data available for setting various duration reference values. The intent of this review is not to suggest that additional testing be conducted for each and every chemical in order to fill in the information gaps identified for those organ systems evaluated. Nor is it suggested that alternative testing protocols that are discussed in this chapter should be conducted for every chemical or become part of current toxicology testing requirements or that these alternative protocols are the only options available. Rather, it is the goal of this document to provide a basis for the development of innovative alternative testing approaches and the use of such data in risk assessment, and to then illustrate some aspects of this concept with a few examples. In reviewing the current testing protocols, target organs/systems that are evaluated were reviewed as was the thoroughness of testing with respect to life stage assessment, endpoint assessment, route, timing and duration of exposure, and latency to response. These issues were all considered important in evaluating potentially susceptible subpopulations, including life stages. The testing guideline protocols were reviewed overall for these issues; in addition, four biological systems were evaluated in depth, two that are fairly thoroughly evaluated (the reproductive and nervous systems) and two that are evaluated to a more limited extent (the immune and cardiovascular systems). In each case, an overview of the tests for the particular system is given, as well as a more specific discussion of gaps in life stage of assessment, gaps in assessment endpoints, and gaps in duration and latency assessment.

The Technical Panel has made a number of recommendations concerning toxicity testing, including development of a strategy for approaches to toxicity testing, with guidance on how and when to use existing and newly recommended guidelines; development of guidelines or guideline study protocols that will provide more systematic information on toxicokinetics and toxicodynamics (i.e., mechanism or mode of action), including at different life stages; development of protocols for acute and short-term studies that provide more comprehensive data

for setting reference values; modification of existing guideline study protocols to provide more comprehensive coverage of life stages for both exposure and outcomes; collection of more information from less-than-lifetime exposure to evaluate latency to effect and reversibility of effect; development of guidelines or guideline study protocols to assess immunotoxicity, carcinogenicity, and cardiovascular toxicity at different life stages; and exploration of the feasibility of setting dermal reference values for direct toxicity at the portal of entry, including sensitization.

A primary goal of this review was to provide the basis for recommendations for the development of a strategy for approaches to toxicity testing and for innovative alternative testing approaches to provide data for risk assessment. The Technical Panel is suggesting that alternative strategies and guidance for testing approaches be developed that incorporate information on toxicokinetics and mode of action early in the process, thus allowing a more targeted testing approach. In addition, alternative protocols are discussed that are aimed at more efficient use of animals and resources in combined studies that would provide more extensive data on life stages, endpoints, and other factors not well characterized in current testing approaches. Recommendations are also made about research areas that should be encouraged to aid in better study design and interpretation of data for risk assessment.

Finally, an example of an alternative testing protocol for acute exposure and evaluation that incorporates the types of endpoints and evaluations optimal for setting acute reference values is discussed. Two sample alternative protocols are presented for chronic exposures and options are discussed for combining studies and evaluations to include a wider array of life stage and endpoint assessments.

Chapter 4 discusses a number of modifications to the existing framework for use in deriving reference values, both for the current chronic reference values (RfD and RfC) as well as for acute, short-term, and longer-term reference values. The approach to reference values discussed here is intended for risk assessments of any type of health effect known or assumed to be produced through a nonlinear and/or threshold mode of action (which may include U-shaped or other nonmonotonic dose-response curves as well as thresholds). Thus, the Technical Panel recommends moving away from the dichotomy between “cancer” and “noncancer.” The term “noncancer” has been removed from the reference value definition, denoting the move toward defining approaches for low-dose estimation or extrapolation based on mode of action. Two case studies that illustrate many of the concepts discussed in this chapter are presented in more detail in Appendix B. The Technical Panel recommends including the acute, short-term, longer-term, and chronic reference values derived on the basis of the recommendations in this report in

IRIS after appropriate internal, external, and consensus review. Standard exposure durations are proposed, as is a definition for the reference value, including a designation for route and duration of exposure.

The Technical Panel is aware that there will be data limitations for an individual chemical that may preclude development of all four reference values, and it is aware that time and resources need to be considered when implementing these recommendations. The IRIS program has begun to implement a pilot program to test whether development of the expanded array of reference values is practical and can be accomplished without unduly delaying the completion of an IRIS file. As a part of the pilot, the IRIS program will need to identify the methods to be used in deriving these additional values.

The Technical Panel recommends that endpoint-specific reference values should not be developed, including the reference dose for developmental toxicity, RfD_{DT}. Rather, a sample reference value should be calculated for each relevant and appropriate endpoint and these should be considered in the derivation of various duration reference values. The reference values should be derived to be protective of all types of effects for a given duration of exposure.

An expanded approach to the evaluation of studies and characterization of the extent of the database as a whole is recommended; in particular, several factors are discussed that should be considered in a weight-of-evidence approach for characterizing hazard for the population as a whole as well as for potentially susceptible subpopulations. Those considerations for assessing level of concern raised by the Toxicology Working Group of the 10X Task Force have been incorporated into this approach.

In the context of this framework, the Technical Panel recommends a somewhat different approach to characterizing the extent of the database for reference values. Instead of specifying particular studies, this approach emphasizes the types of data needed (both in terms of human and animal data) for deriving reference values, and it recommends the use of a narrative description of the extent of the database rather than a single confidence ranking of high, medium, or low. To characterize the database, the Technical Panel has developed a description of a "minimal" database and a "robust" database as a way of describing the range of data that can be used for deriving a reference value, and the Panel urges the use of a great deal of scientific judgement in the process of summarizing the extent of the database, including its strengths and limitations.

The narrative approach is intended to emphasize the types of data available (both human and animal) as well as the data gaps that could improve the derivation of reference values. This approach should encourage the use of a wider range of information in deriving reference values,

taking into consideration the issues of duration, timing, and route of exposure; the types and extent of endpoint assessments (i.e., structure and function); the life stages evaluated; and the potential for latent effects and/or reversibility of effects.

Dosimetric adjustment of values for deriving a human equivalent concentration (HEC) for inhalation exposure is discussed, as is the derivation of a human equivalent dose (HED) for oral or dermal exposure. The Technical Panel recommends that duration adjustment procedures to continuous exposures based on concentration times time (C x t) be used as a default procedure for inhalation developmental toxicity studies as for other health effects from inhalation exposures. In addition, further evaluation of current dosimetric adjustments for deriving HECs should be pursued to confirm or assess the relevance for population subgroups (particularly for children).

Because of the recommendation for deriving several duration reference values, the Technical Panel recommends that the data for the point of departure (POD) be evaluated on the basis of a comparison of all relevant endpoints carried through the derivation of sample reference values, with selection of the limiting value(s) as the final step rather than on the basis of selection of a single "critical study" and "critical effect." To aid in this evaluation, the use of an exposure-response array is recommended as a visual display of all relevant and appropriate endpoints and durations of exposure in order to determine the range of numerical values for each reference value.

The Technical Panel makes a number of recommendations concerning the application of uncertainty factors (UFs) for reference value derivation. In particular, it is imperative that the IRIS documentation contain a justification for the individual factors selected for each chemical or assessment because rigid application of UFs could lead to an illogical set of reference values. Although default factors of 10 are recommended, with 3 used in place of half-power values (i.e., $10^{0.5}$) when occurring singly, the exact value of the UF chosen should depend on the quality of the studies available, the extent of the database, and scientific judgment. Sound scientific judgment should be used in the application of UFs to derive reference values that are applied to the value chosen for the POD derived from the available database (BMDL, NOAEL, or LOAEL).

The Technical Panel recommends that if there is uncertainty in more than four areas of extrapolation, it is unlikely that the database is sufficient to derive a reference value. Even when there is uncertainty in four areas, the database should be carefully evaluated to determine whether the derivation of a reference value is appropriate. In addition, the Technical Panel recommends limiting the total UF applied to a chronic reference value for any particular

chemical to 3000. This maximum of 3000 applies only to the UFs and does not include the various adjustment factors discussed in Chapter 4.

The intraspecies UF is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed. Because the RfD/RfC is defined to be applicable to "susceptible subgroups," this UF was established to account for uncertainty in that regard. In general, the Technical Panel reaffirms the importance of this UF, recommending that reduction of the intraspecies UF from a default of 10 be considered only if data are sufficiently representative of the exposure/dose-response data for the most susceptible subpopulation(s). At the other extreme, a 10-fold factor may sometimes be too small because of factors that can influence large differences in susceptibility, such as genetic polymorphisms. The Technical Panel urges the development of data to support the selection of the appropriate size of this factor, but recognizes that often there are insufficient data to support a factor other than the default.

The Technical Panel urges continued research and evaluation of the similarities and differences between the general population and susceptible subpopulations, particularly children and the elderly, in their responses to particular agents. From such evaluations, the protectiveness of the 10-fold default factor should continue to be assessed. The Technical Panel urges the development of data to support the selection of the appropriate size of this factor, but it recognizes that often there are insufficient data to support a factor other than the default. The database UF is intended to account for the potential for deriving an underprotective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity. In addition to the identification of toxicity information that is lacking, review of existing data may also suggest that a lower reference value might result if additional data were available. Consequently, in deciding to apply this factor to account for deficiencies in the available data set, and in identifying its magnitude, the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages. The Panel considers the purpose of the modifying factor (MF) to be sufficiently subsumed in the general database UF, and recommends that use of the MF be discontinued.

Given that there are several UFs that can be used to deal with data deficiencies as part of the current reference value process, and given that these are assumed to overlap to some extent, the Technical Panel agrees with the 10X Task Force Toxicology Working Group that the current interspecies, intraspecies, and database deficiency UFs, if appropriately applied using the

approaches recommended in this review, will be adequate in most cases to cover concerns and uncertainties regarding the potential for pre- and postnatal toxicity and the completeness of the toxicology database. In other words, an additional uncertainty factor is not needed in the RfC/RfD methodology because the currently available factors are considered sufficient to account for uncertainties in the database from which the reference values are derived (and does not exclude the possibility that these UFs may be decreased *or* increased from the default value of 10). The approach to using chemical-specific data for toxicokinetic and toxicodynamic components of the interspecies UF is part of the current RfC methodology. The Technical Panel encourages the Agency to develop its own guidance for chemical-specific adjustment factors (CSAFs) on the basis of some of the available methodologies (e.g., the International Programme on Chemical Safety [IPCS]).

Several other issues discussed by the Technical Panel were considered more appropriate for deliberation by other panels/committees, for example, further consideration of the use of BMD modeling approaches for deriving reference values; harmonization of the approaches for HEC and HED derivation for all types of health effects; further evaluation of approaches such as probabilistic analysis for characterizing variability and uncertainty in toxicity reference values; further evaluation of appropriate adjustment of doses for duration of exposure for acute toxicity data; and further evaluation of duration adjustment for short-term and longer-term reference values analogous to the subchronic-to-chronic duration UF for chronic reference values.

Chapter 5 summarizes the recommendations of the Technical Panel.

1. INTRODUCTION, PURPOSE, AND SCOPE

The RfD/RfC Technical Panel (hereafter the Technical Panel) was established by the U.S. Environmental Protection Agency's (EPA's, or the Agency's) Risk Assessment Forum in early 1999 to review the current oral reference dose (RfD) and inhalation reference concentration (RfC) processes, in particular with respect to how well children and other potentially susceptible subpopulations are protected; to consider new scientific issues that have become more important and of greater concern in risk assessment; and to raise issues that should be explored or developed further for application in the RfD/RfC process. This document summarizes the review and deliberations of the Technical Panel and its recommendations for improvements in the process as well as additional efforts that are needed. It discusses revisions to the framework for the derivation of RfDs and RfCs. The document is a review, not guidance, but it does make recommendations that should be considered in the implementation of changes in the current process and/or development of needed guidance.

Many of the recommendations made in this report are consistent with the Agency's commitment to harmonization of health risk assessment procedures, including the harmonization of approaches for noncancer and cancer endpoints, and to making efficient use of animal testing to achieve this goal. As noted in several places in the document, all such topics have not been discussed and resolved by the Agency. For instance, the differences in scaling factors used for cancer and noncancer derivations from oral exposure data are raised as an issue that has not been resolved; thus, there will likely be a need for revised or further guidance on this issue.

Although mixtures or multiple chemical exposures are not specifically discussed in this review, most of the recommendations are applicable to the approach to risk assessment of mixtures. The Agency's mixtures risk assessment guidelines should be consulted for issues specific to the evaluation of mixtures (U.S. EPA, 1986, 2000a). In addition, the Agency has recently issued the draft *Framework for Cumulative Risk Assessment* (U.S. EPA, 2002a), which deals with the issue of multiple stressors and their overall impacts on exposure-effect relationships. The risk assessment approaches discussed within this framework are likely to be the subject of further guidance as well.

The Technical Panel attempted to review most of the issues relating to hazard characterization for developing reference values, to the need for developing reference values for different durations of exposure, and to the process of deriving reference values. The Technical Panel did not go into detail on the quantitative aspects of the dose-response process, as this is being covered in other Forum activities (e.g., the benchmark dose [BMD] guidance document

and the quantitative dose-response aspects of the cancer guidelines revision process). The Technical Panel approached its review from the point of view that the RfD/RfC process has been and should be a continually evolving process. Thus, as new information becomes available and new scientific and risk assessment approaches are developed, they are incorporated into new RfDs and RfCs as these values are developed or as current RfDs and RfCs are reevaluated. This process of incorporating new science does not invalidate current RfDs or RfCs, because consideration of these new scientific issues is included in the reevaluation of current values; higher or lower values or, in some cases, no change in the current value may result.

This report provides conclusions and recommendations that are intended to improve the RfD/RfC process. The audience for this review is primarily the Integrated Risk Information System (IRIS) program, IRIS chemical managers, and other scientists within the Agency who are involved in developing the RfDs and RfCs, as well as IRIS users and the program offices within EPA that develop RfDs and RfCs or similar values (see Chapter 2), particularly resource managers who may be impacted by the potential for additional workload due to several of the recommendations. The Technical Panel has provided specific recommendations for guidance in some cases and more general conclusions and recommendations in others. In the latter cases, the Technical Panel felt that development of specific recommendations was beyond the scope of its efforts or that policies needed to be further developed before specific guidance could be written to implement the recommendations.

The methodology recommended in the RfD document is considered generally applicable to both cancer and noncancer endpoints where dose-response relationships are thought to be either nonlinear or consistent with a threshold. Although the emphasis in this document is on the calculation of RfDs and RfCs, the same processes and considerations are applicable to the margin of exposure (MOE), as discussed in the draft cancer risk assessment guidelines (U.S. EPA, 1999a).

The Technical Panel discussed a number of issues concerning a revised framework for the RfD/RfC process, with particular emphasis on the extent to which children and other potentially susceptible subpopulations are considered. The next three chapters summarize these issues, and several recommendations are made. Chapter 2 reviews current approaches to developing acute, short-term, and longer-term reference values as well as the chronic reference values, the RfD and the RfC. Chapter 3 reviews the current testing guidelines with respect to life stage assessment and discusses the gaps in life stage assessment, endpoint assessment, and assessment of duration and latency. Alternative testing protocols and strategies as options for combining studies and evaluations are discussed.

Chapter 4 provides constructive commentary on the current framework used in deriving reference values and on the need and possibilities for calculating reference values for different durations and routes of exposure. In addition, an expanded approach to evaluating studies and characterizing the extent of the database as a whole is presented and discussed, including dosimetric adjustment, the application of uncertainty factors (UFs), and derivation of sample reference values for each appropriate and relevant endpoint to aid in selecting the point of departure (POD) for deriving reference values.

The final chapter (Chapter 5) summarizes all of the recommendations of the Technical Panel. Two case studies that illustrate several of the recommended changes are also included as Appendix B.

2. REVIEW OF THE CURRENT USE OF ACUTE, SHORT-TERM, AND LONGER-TERM REFERENCE VALUES

The Technical Panel considered the recommendation of the 10X Task Force that acute, short-term, and longer-term reference values as well as chronic reference values should be set for environmental agents (see Appendix A). It is likely that the endpoints critical for setting acute, short-term, and longer-term reference values may differ from those for setting chronic RfDs and RfCs, although studies that use acute and short-term exposure conditions from which the appropriate data for many types of effects could be derived are not often available. Data on acute and short-term health effects must often be derived from observations after the first exposure in a repeated-exposure testing protocol.

Several acute and short-term values currently are set for various chemical types and media. For example, acute and chronic oral RfDs are set for pesticides, with some intermediate values set for occupational and residential pesticide exposures. Health advisories (HAs) of several durations have been developed for drinking water. In addition, the Office of Solid Waste and Emergency Response, the Office of Prevention, Pesticides, and Toxic Substances (OPPTS), and other program offices and regional offices use values derived through the interagency acute exposure guidelines (AEGL) process for emergency response planning. The National Center for Environmental Assessment (NCEA) is currently developing the acute reference exposure (ARE) methodology for acute inhalation exposures. These developments are reviewed in more detail below.

2.1. REVIEW OF CURRENT LESS-THAN-LIFETIME REFERENCE VALUES

The Technical Panel was briefed by representatives of several Agency offices on the methods currently used to set various less-than-lifetime reference values. Subsequently, on August 2, 2000, a Risk Assessment Forum colloquium was held on this topic (CDM Group, Inc., 2000). Each of the methods was presented and discussed. In addition, a recommendation by the Technical Panel to begin deriving acute, short-term, and longer-term reference values as well as chronic values and to standardize the definitions for each duration was presented and discussed. Each method presented is summarized below.

2.1.1. Acute Reference Exposure (ARE) Methodology

The ARE methodology is being developed at the request of the EPA's Office of Air and Radiation. It is intended for development of reference values for acute inhalation exposures of

24 hours or less. The criteria air pollutants are not included, because they are assessed within the National Ambient Air Quality Standards (NAAQS) setting process.[1] The ARE is defined as an inhalation exposure of 24 hours or less that is not likely to cause noncancer adverse effects. The ARE can be applied to intermittent exposures or to a continuous exposure. AREs are being developed in order to address the acute risk aspects of risk-related provisions of the hazardous air pollutant sections of the 1990 Clean Air Act Amendments. The ARE methodology is described in a 1998 EPA external review draft document (U.S. EPA, 1998a). The method builds on the procedures of the RfC methodology.

The ARE methodology includes three approaches in order to accommodate the varying types of data available for acute exposure. The first two approaches, the no-observed-adverse-effect level (NOAEL) and the benchmark concentration (BMC) are familiar. The third approach, categorical regression (CatReg), is newer. The NOAEL approach is useful for chemicals that have limited available data and for which no or limited dose-response relationships have been established. The BMC approach is suitable for analysis of studies that establish dose-response relationships. The CatReg approach requires multiple studies that report not only dose and response, but also duration; it is most applicable for data-rich chemicals. A feature of the CatReg approach is that effects data are grouped into severity categories (e.g., mild or severe to lethal) to which sophisticated regression procedures are then applied.

Adjustments for deriving ARE values of different durations (e.g., 15 minutes or 8 hours) are made differently for the CatReg approach than for the NOAEL and BMC approaches. For any approach, the preferred adjustment procedure is to use a pharmacokinetic model, if available. When the NOAEL or BMD approach is used, the default procedure is to use the multiple of concentration times time (C x t) (C^n x t = k; ten Berge et al., 1986) to extrapolate from short to long duration and to use the same concentration as obtained for long duration to extrapolate from long to short duration. When more than one duration is available, interpolation is performed. When the CatReg approach is used, the procedure involves reading the values directly from the concentration duration curve that is generated by the CatReg software. These approaches are explained more fully and illustrated in Chapter 4.

A minimal data set has not been defined for the ARE. Also, extrapolation from the oral to the inhalation route of exposure is not addressed in the ARE approach. UFs in the ARE approach include a lowest-observed-adverse-effect level- (LOAEL-) to-NOAEL UF of 10 and a

[1]Criteria air pollutants are those air pollutants for which NAAQS have been established under the Clean Air Act; at present, the six criteria air pollutants are particulate matter, ozone, carbon monoxide, nitrogen oxides, sulfur dioxide, and lead.

default value of 10 for interspecies and for intraspecies extrapolation. No factor is assigned for database inadequacies and study quality.

In 1998, the EPA Science Advisory Board (SAB) reviewed the ARE methodology document and made a number of comments that addressed, among other things, issues about the NOAEL and BMC approaches, the need for addressing protection of children, the dosimetry adjustment and duration extrapolation, and the CatReg approach. The SAB discussed the fact that the CatReg model, as currently set up, forces parallelism of the concentration-duration curves for the various severity categories. In addition, there were concerns about judging severity categories across various target organs and species, and there was discussion about the reliability of the confidence limits around the maximum likelihood estimate and about the appropriateness of the approach used to accommodate group versus individual data. This methodology has since (March 2001) undergone an Agency review by the Risk Assessment Forum. The principal comments from this review concerned reevaluation of whether CatReg should remain as an approach in the ARE methodology and further evaluation of the procedures for cross-species dosimetry adjustment. Revision of the ARE methodology is currently underway. In addition to revising the ARE methodology and CatReg software documents, NCEA-Research Triangle Park will develop a framework for adding AREs to the IRIS database.

2.1.2. Acute Exposure Guidelines (AEGL) Program

The primary purpose of the AEGL program is to develop guideline levels for once-in-a-lifetime short-term exposures to airborne concentrations of acutely toxic chemicals (NRC, 2000). AEGLs are needed for a wide variety of emergency planning, response, and prevention applications. AEGLs represent threshold exposure limits for the general public and are applicable to emergency exposure periods ranging from 10 minutes to 8 hours. Specific values are set for 10 minutes, 30 minutes, 1 hour, 4 hours, and 8 hours. It is believed that the recommended exposure levels are applicable to the general population, including infants and children and other individuals (e.g., asthmatics) who may be sensitive or susceptible. It is recognized that certain individuals who may be subject to unique or idiosyncratic responses could experience the effects described at concentrations below the corresponding AEGL level.

The AEGL-1, AEGL-2, and AEGL-3 levels are distinguished by varying degrees of severity of toxic effects. With increasing airborne concentrations above each AEGL level there is a progressive increase in the likelihood of occurrence and the severity of effects described for each level.

AEGL-1 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience notable discomfort, irritation, or certain asymptomatic, nonsensory effects. However, the effects would not be disabling and would be transient and reversible upon cessation of exposure.

AEGL-2 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals, could experience irreversible or other serious, long-lasting adverse health effects or an impaired ability to escape.

AEGL-3 is the airborne concentration of a substance above which it is predicted that the general population, including susceptible individuals could experience life-threatening health effects or death.

Airborne concentrations below AEGL-1 represent exposure levels that could produce mild and progressively increasing odor, taste, and sensory irritation or certain asymptomatic, nonsensory effects.

UFs are used for extrapolations. If there are no appropriate human data, an interspecies UF of 1, 3, or 10 is used. The factors considered when deciding on a specific value include (1) the species tested (type, appropriateness, and range), (2) the toxicological endpoint observed and the likely mechanism of action, (3) the range of response in the species tested, (4) the variability of response among the species tested, and (5) pharmacokinetic differences among the species tested. An intraspecies UF of 1, 3, or 10 is also used. The factors considered when assigning a specific value include (1) the toxicological endpoint observed and the likely mechanism of action, (2) the range of response among humans and subpopulations, and (3) pharmacokinetic differences among individuals. Individual factors of 3 are often used to ensure that the final values are not overly conservative.

Adjustment for duration is conducted using the equation $C^n \times t = k$. If data are available for the endpoint of concern, the value of n is derived from regression analysis. If data are not available for the endpoint of concern, the value of n is usually derived from lethality data by regression analysis and used for the other endpoints. If the study duration is greater than 1 hour, the 10-minute value is usually assigned equal to the 30-minute value. If no data are available to derive a value of n, a value of 3 is used to extrapolate to shorter durations, and a value of 1 is used to extrapolate to longer durations. As mentioned above, this procedure is further explained and illustrated in Chapter 4.

2.1.3. Office of Pesticide Programs (OPP) Procedures for Setting Acute and Intermediate RfDs

OPP developed methodologies for acute dietary as well as occupational and residential risk assessments during the process of re-registration following the 1988 revision to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). In 1998, a guidance document, *Toxicology Endpoint Selection Process* (U.S. EPA, 1998b), was presented to the FIFRA Scientific Advisory Panel for review and comment. This document, which provided the basis for procedures that are still in place, describes toxicology endpoint selection for less-than-lifetime dietary and occupational/residential risk assessments for pesticides. It includes guidance on evaluating toxicity studies that are relevant for use, selecting appropriate endpoints for hazard identification, the process of hazard identification, the influence of dermal absorption in hazard identification, the criteria for the use of the NOAEL and the LOAEL, and the use of MOEs in risk assessments. Since this guidance was first issued, some changes have evolved, such as the replacement of the acute MOE with the acute RfD and the addition of standard consideration of short- and intermediate-term incidental nondietary ingestion exposures for toddlers.

Toxicology Endpoint Selection Process (U.S. EPA, 1998b) describes the types of studies that are most likely to provide appropriate endpoints for the various exposure durations and risk assessments that will be conducted for each pesticide. OPP can rely on the availability of a wide variety of standard guideline toxicity studies from which to select endpoints because such studies are required by regulation for any pesticide registration (40 CFR Part 158). Additionally, OPP considers other sources of toxicology data, such as studies published in the open literature, as appropriate.

For the establishment of the acute RfD, OPP uses a weight-of-evidence approach in evaluating all the available data. Three guideline studies have been found to be particularly useful by OPP: the acute neurotoxicity study, the prenatal developmental toxicity study, and the developmental neurotoxicity (DNT) study.

Acute effects from subchronic and chronic dietary studies are also used in the establishment of the acute dietary RfD. Careful scrutiny of toxicological data from early in the first week of treatment can sometimes identify effects that can be described as acute. However, for a number of reasons, this option has not often been used. These reasons include the absence of detailed toxicological observations other than morbidity and mortality checks in subchronic and chronic studies before the end of the first week of treatment (i.e., after 7 days of treatment), the nature of the dietary exposure (i.e., each daily exposure results from an extended period of nightly feeding rather than from a discrete acute dose), and the possibility that apparent adverse

effects during the first week of treatment may be related to palatability issues as the animals adjust to treated feed.

OPP does not calculate short- or intermediate-term references doses. However, risk assessments are conducted for incidental nondietary ingestion exposures to toddlers—a very specific population subgroup—that result from the use of a pesticide in and around the home or other nonoccupational sources such as schools, parks, and golf courses. The post-application risk assessment considers or accounts primarily for incidental ingestion of (1) the dry pesticide materials (granules or pellets) used to treat outdoor residential areas, (2) pesticide residues in soil that are ingested by toddlers who play in treated areas (e.g., yards, gardens, playgrounds) as a result of normal mouthing activities, and (3) pesticide residues that are transferred to the skin of toddlers playing in treated areas and are subsequently ingested as a result of hand-to-mouth transfer. These risk assessments consider short-term (1 day to 1 month) and intermediate-term (1–6 months) exposure durations. Risks are expressed as MOEs. The MOE approach is used because these exposures are considered to be nondietary in source and are based on high-end values or (when adequate site- or chemical-specific field data are unavailable) on assumptions.

OPP also conducts short-term, intermediate, and long-term (longer than 6 months) dermal and inhalation risk assessments for occupational and residential exposures. The MOE approach is also used to calculate the risk for these nondietary exposure scenarios. A difficulty that OPP often faces when conducting these risk assessments is that dermal absorption and inhalation toxicity data are often not available for food-use pesticides; in that case, appropriate assumptions are applied, and the available oral toxicity data are converted for use in dermal and inhalation risk assessment.

Toxicology Endpoint Selection Process (U.S. EPA, 1998b) does not address the use of UFs in acute dietary risk assessment. In practice, however, the same 10-fold inter- and intraspecies UFs are used in calculating the acute dietary RfD as are used for the chronic RfD. Other standard UFs may be used when appropriate (e.g., the LOAEL-to-NOAEL threefold factor). Others are not appropriate for an acute risk assessment, for example, the threefold subchronic-to-chronic factor. However, no standard set of “core” studies has been defined for acute dietary risk assessment; therefore, a database UF is not used. If appropriate endpoints and doses cannot be selected for acute dietary risk assessment from the studies in the database, then an acute RfD is not calculated.

2.1.4. Office of Water (OW) Health Advisories (HAs)

The OW HA program was initiated in 1978 to provide guidance on unregulated contaminants found in drinking water. Since then, HAs have also been developed for regulated

contaminants. HAs are derived for contaminants that are known to or are likely to occur in drinking water and that may cause adverse, noncarcinogenic health effects (Orme and Ohanian, 1991). The approach for developing HAs is based on recommendations from the National Academy of Sciences (NAS, 1977). HAs are developed for specific exposure durations (1 day, 10 days, longer-term, and lifetime) that reflect different emergency contamination situations. HAs are not legally enforceable, but they do serve as technical guidance to assist in emergency spills or contamination situations or for determining unreasonable risks to health under sections 1415 and 1416 of the Safe Drinking Water Act. They also are issued at the request of State or local governments or to fill a need for criteria, guidelines, or standards. HAs undergo scientific peer review and can function as a preliminary risk assessment, if necessary.

The following assumptions are used in setting the various HAs. The 1-day HA represents a concentration of the contaminant in drinking water that is considered protective of adverse noncancer health effects in a 10 kg child. The 10 kg child serves as the protected individual for the less-than-lifetime HAs because a child of this size is likely to receive a greater dose on a mg/kg basis. This 1-day HA can serve as a guideline for each day for up to 5 consecutive days of exposure. The 1-day HA is usually derived from experimental studies of 7 days duration or less.

The 10-day HA is considered protective of these effects in a 10 kg child for each day for up to 14 days of continuous exposure and may be based on experimental studies of 30-day duration or less.

The longer-term HA, which is based on subchronic exposure studies covering 10% of an animal's lifetime, is considered protective of an exposure period in humans of up to 7 years (i.e., 10% of an individual's lifetime). The longer-term HA is developed to protect both a 10 kg child and a 70 kg adult.

The lifetime HA is considered protective of lifetime exposures and is usually based on chronic or subchronic or other more relevant experimental data. The Lifetime HA is based on the chronic oral RfD, adjusted for a 70 kg adult drinking 2 L water per day; the value is apportioned by a relative source contribution, for example, 20% of the toxicant represented by intake of water.

HA levels are generally based on available, well-conducted studies that involve humans or animals. Data from drinking water studies are preferred; however, data from dietary or gavage studies can also be used. In the absence of oral data, studies by other routes of exposure, such as inhalation or injection, are considered. Following identification of an appropriate study to develop a HA, the NOAEL or the LOAEL is adjusted for water consumption by the protected

individual. For a child, the assumed water consumption level is 1 L/day; for an adult, 2 L/day is used.

When data are absent for setting a 1-day or a 10-day HA, OW uses scientific judgment on how to handle any given situation on the basis of the overall weight of evidence. In the absence of short-term toxicity studies, a subchronic or chronic study may be used to develop a less-than-lifetime HA. Given the pressure under which HAs need to be calculated, many assessments are based on whatever toxicological data are available and on scientific judgment. Although this may be an overly conservative approach, OW considers the error to be protective of public health.

OW applies the same factors for minimum data as those outlined in the Agency's RfD methodology. For example, in emergency situations, missing data are accounted for by applying another factor of 3 or 10. Or, for instance, where inhalation data might be applied to estimate a HA based on water consumption, a factor may be applied to account for differences in absorption. Judgments based on toxicokinetic and toxicodynamic considerations are reached through intensive consultation.

Calculation of HAs is straightforward and familiar, and in most cases the NOAEL/UF approach is used. For each of the less-than-lifetime HA values, it is assumed that all of an individual's exposure to a contaminant comes from a drinking water source. The calculation of the lifetime HA differs from that of the less-than-lifetime values in that a relative source contribution factor is included. This factor adjusts the exposure to reflect the portion that is likely to be contributed from drinking water. Unless actual exposure data are available, a default factor of 20% is used to reflect the assumed contribution to exposure from drinking water. Also, in cases where there is limited evidence suggesting a carcinogenic potential of a contaminant, an additional "policy" factor of 10 is applied in calculating the lifetime HA.

The methodology for developing HAs was reviewed by the SAB and the FIFRA Scientific Advisory Panel in 1986. Each HA that is developed undergoes external peer review and Agency review before it is released to the public. The availability of the HAs is announced in the *Federal Register* and distributed through the Safe Drinking Water Hotline and the Water Docket and by the Office of Science and Technology in OW. In addition, HAs have been published in a collection of books and are available in English, Japanese, and Italian.

2.1.5. Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels (MRLs)

The ATSDR is tasked with establishing MRLs, which are defined as

> " *... an estimate of daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.*"

MRLs are considered by ATSDR to be substance-specific estimates intended to be screening levels in identifying contaminants and potential health effects that may be of concern; they do not define clean-up or action levels. The derivation procedures for MRLs have many similarities and parallels to the derivation of RfDs and RfCs; MRLs are based on careful scientific consideration of noncancer health effects only, not on consideration of cancer effects. A list of various procedural specifics employed in the derivation of MRLs, including specific effects and the level of severity, is codified in a *Federal Register* notice (ATSDR, 1996). The definition of an MRL differs expressly from EPA's definition of an RfD or an RfC in that both route and duration are included. The current routes of concern for MRL derivation are oral and inhalation (but not dermal).

The EPA procedures and methodologies discussed above address the issue of duration through a variety of extrapolation procedures. For MRLs, however, duration is addressed by providing for the designation of MRLs in three different duration categories: acute = $\leq$14 days, intermediate = 15–364 days, and chronic = $\geq$365 days. These duration categories are absolute and apply to all species, regardless of relative life span. Thus, it is possible for a contaminant to have a total of six different MRL values: two routes by three different durations.

The use of UFs is a parallel practice in RfD/RfC and MRL derivation. The UFs used by ATSDR are intraspecies 1, 3, 10; interspecies 1, 3, 10; and LOAEL/NOAEL 3, 10. The modifying factor (MF) can include database considerations, that is, deficiencies in the data or overestimates from bioaccumulative chemicals.

2.2. SUMMARY OF CURRENT METHODS FOR SETTING ACUTE, SHORT-TERM, AND LONGER-TERM REFERENCE VALUES

In summary, several methods are used by various EPA programs for setting acute, short-term, and longer-term reference values. The definitions for each of the durations used for the methods reviewed are included in Table 2-1. Because there are some differences in these

Table 2-1. Duration definitions used for various reference values

Reference value duration	Definition
Acute	
ARE	Inhalation single continuous exposure values for durations ≤ 24 hrs (to be protective of intermittent exposures)
AEGL	10 and 30 min; 1, 4, and 8 hrs
OPP acute RfD	Maximum 1-day dietary exposure
OW 1-day HA	1 day (5 successive daily doses)
ATSDR acute MRL	≤14 days
Standardized definition[a]	24 hrs or less
Short-term	
ARE	NA
AEGL	NA
OPP short-term RfD	1 day–1 month
OW 10-day HA	10 days (7–14 successive daily doses)
ATSDR MRL	NA
Standardized definition[a]	>24 hrs up to 30 days
Longer-term	
ARE	NA
AEGL	NA
OPP intermediate RfD	1–6 months
OW longer-term HA	Approximately 10% of life span in humans (90 days to 1 year in test species)
ATSDR intermediate MRL	15–364 days
Standardized definition[a]	>30 days up to approximately 10% of the life span in humans (>30–90 days in typically used laboratory species)

[a] See Chapter 4 for further discussion of these definitions.

definitions, standardized definitions were discussed at the Risk Assessment Forum Colloquium (CDM Group, 2000), and these are shown in Table 2-1. Definitions for durations are further discussed in Chapter 4.

A comparison of the UFs applied for various reference values is shown in Table 2-2. Although there is some variation in the UFs applied, those for animal-to-human extrapolation (U_A), for within-human variability (U_H), and for LOAEL-to-NOAEL (U_L) are fairly consistent. Less consistent is the way in which database deficiencies (U_D) are taken into consideration, particularly for pesticides where the Food Quality Protection Act (FQPA) safety factor is used to account for deficiencies in the database related to children's health risks.

Duration extrapolation for each of these values was also reviewed. Some type of duration adjustment of the NOAEL or the BMD is done for the ARE and the AEGL methods, and there appears to be consistency in the use of $C^n \times t$ for extrapolating from shorter to longer exposures but in using the same value (i.e., no duration adjustment) when extrapolating from longer to shorter exposures. Duration extrapolation is not done for the OPP RfDs, the OW HAs, or the ATSDR MRLs.

2.3. RECOMMENDATION

On the basis of its review of the various approaches to setting acute, short-term, and longer-term reference values, the Technical Panel concurred with the recommendation of the 10X Task Force that acute, short-term, and longer-term reference values should be set, where possible, and that they be incorporated into the IRIS database. In addition, the Technical Panel recommended that these values be set in a consistent manner, using standardized definitions for acute, short-term, longer-term, and chronic durations that are consistent with current practice. These values can then be used by various program offices, where applicable. A scheme for deriving these additional values is presented in Chapter 4.

Table 2-2. Uncertainty/safety factors for various reference values

Reference value	UF[a]				FQPA[b]
	U_A	U_H	U_L	U_D	
ARE	1, 3, 10	1, 3, 10	1, 3, 10	ND	NA
AEGL	1, 3, 10	1, 3, 10	3[c]	ND[d]	NA
OPP acute and intermediate RfDs	10	10	3, 10	ND[e]	10+
OW HAs	1, 3, 10	1, 3, 10	1, 3, 10	case-specific	NA
ATSDR MRLs	1, 3, 10	1, 3, 10	1, 3, 10	ND[d]	NA

[a] Uncertainty factors: U_A = animal-to-human; U_H = within-human variability; U_L = LOAEL-to-NOAEL; U_D = database deficiency.
[b] Additional safety factor required under FQPA.
[c] Endpoint = lethality, not really a LOAEL-to-NOAEL adjustment in this case.
[d] Database deficiencies considered, and a factor may be included for intermediate RfDs if, for example, there is no reproduction and fertility study.
[e] Overlaps with the FQPA safety factor (see U.S. EPA, 2002b)

ND = not done
NA = not applicable

3. REVIEW OF TESTING GUIDELINES WITH RESPECT TO LIFE STAGE ASSESSMENT

As a first step in determining the data necessary for setting various duration reference values for protecting potentially susceptible subpopulations, the Technical Panel reviewed the current OPPTS Series 870 health effects testing guidelines[2] to determine what information is gathered in these studies. The intent of this review is not to suggest that additional testing be conducted for each and every chemical in order to fill in the information gaps identified for those organ systems evaluated. Nor is it suggested that the alternative testing protocols that are discussed in this chapter should be conducted for every chemical or become part of current toxicology testing requirements or that these alternative protocols are the only options available. Rather, it is the goal of this document to provide a basis for the development of innovative alternative testing approaches and the use of such data in risk assessment and to then illustrate some aspects of this concept with a few examples.

Development of a toxicology testing paradigm that is based not on rigid conformance to a list of required guideline screening studies but rather on the application of knowledge about the chemical is encouraged. Under such a paradigm, both the selection of studies that would be required as well as the design of the tests themselves could be influenced by other substantive and reliable information about the chemical. For example, the incorporation of toxicokinetic and mode-of-action data early in the development of the testing strategy for a chemical would provide particularly valuable direction for development of research protocols.

Other input could include toxicity and dose-response data from other guideline or nonguideline studies, in vitro screening assays, structure-activity relationships, studies that examine age-related sensitivity or susceptibility to chemical exposure, and information on potential or actual exposure to humans. These data could be used to inform a more targeted approach in the design of individual studies or of an overall testing strategy and might in some cases result in a reduction in the number of animals used in testing or support a position that a traditionally required toxicology test should be waived.

The purpose of the review of the current OPPTS guidelines was to understand which target organ systems are evaluated in current testing protocols and how thorough the testing protocols are with respect to life stage assessment; endpoint assessment; route, timing, and

[2]The guidelines are available on the OPPTS web page (http://www.epa.gov/docs/OPPTS_Harmonized/870_Health_Effects_Test _Guidelines/Series/).

duration of exposure; reversibility; and latency to response. These issues were all considered of importance in evaluating potentially susceptible subpopulations, including children. The following sections give an overview of the current testing protocols evaluated in this way and, for certain organ/functional systems, provide a more in-depth analysis as to whether and how current protocols address these issues. The organs/functional systems that were examined in greater detail included the reproductive and the nervous systems, which were selected to represent systems that are thought to be rather well-evaluated. The immune and the cardiovascular systems were selected for review because the current evaluation of these systems is limited. It should be noted that testing guidelines were not originally designed with a focus on evaluations of different life stages or different durations of exposure. Therefore, a number of gaps in life stage assessment, endpoint assessment, timing and duration of exposure, reversibility, and latency to response were noted for each organ system that is reviewed in depth.

The last section provides recommendations for alternative testing approaches that are designed to make more efficient use of animals and resources in combined studies that would provide more extensive data on life stages, endpoints, and other factors not well characterized in current testing approaches.

3.1. EVALUATION OF CURRENT GUIDELINE TESTING PROTOCOLS

The following tables and figures summarize the exposures and endpoints covered in current testing guidelines, what is covered for each organ system/endpoint measured, and the relative depth of evaluation for each system/endpoint. In addition, the life stages covered by exposures and outcomes are illustrated. The discussions that correspond to the figures give an overview of the tests that are currently available and the gaps in assessment of life stages, endpoints, timing and duration of exposure, and latency to response. Together, these analyses provide a clear picture of the testing guidelines currently available, the systems/endpoints measured, the life stages during which exposures and outcomes are measured, the timing and duration of exposures included, and the degree of detail covered for both structural and functional outcomes.

In order to make comparisons among laboratory animal species and humans in terms of life stages covered, the approximate ages that correspond to specific events or life stages (e.g., birth, weaning, puberty, etc.) in different species are shown in Table 3-1, and these events/life stages are indicated in the figures. In a few cases, no data could be found on appropriate ages corresponding to particular life stages. In particular, the ages for mature adults and older adults often were not available, and there is some controversy about what constitutes old age in today’s

Table 3-1. Approximate age at equivalent life stages in several species

Rat		Mouse		Rabbit		Beagle dog		Human	
Life stage	**Age**	**Life stage**	**Age**	**Life stage**	**Age**	**Life stage**	**Age**	**Life stage**	**Age**
Embryonic	GD 0–16	Embryonic	GD 0–15	Embryonic	GD 0–19	Embryonic	GD 0–30?	Embryonic	GD 0–58
Fetal[a]	GD 16–22 (22–23 days)	Fetal	GD 15–20 (18–22 days)	Fetal	GD 19–32 (30–32 days)	Fetal	GD 30–63 (53–71 days)	Fetal	GD 58–267
Neonate[b]	PND 0–14	Neonate	PND 0–14	Neonate	PND 0–21?	Neonate	PND 0–21	Neonate	PND 0–30
Weaning[c]	PND 21	Weaning	PND 21 (19–28)	Weaning	PND 42 (42–56)	Weaning	PND 42	Infancy	PND 30–1 yr
								Toddler	2–3 yrs
Young	PND 22–35	Young	PND 21–35	Young	PND 42–?	Young	1.5–5 mos	Preschool	3–6 yrs
								Elementary school age	6–12 yrs
Puberty	PND 35–60	Puberty	PND 35–?	Puberty	3–8 mos	Puberty	5–7 mos	Adolescence	12–21 yrs
Sexual maturity	2.5–3 mos	Breeding age	1.5–2 mos	Breeding age	6–9 mos	Breeding age	12 mos	Young adult	21–40 yrs
Mature adult	5–18 mos	Mature adult		Mature adult		Mature adult		Mature adult	40–65 yrs?
Old adult	18 mos–2 yrs+	Old adult		Old adult		Old adult	~15 yrs	Old adult	>65 yrs?

[a] Range of gestation length in parentheses.
[b] Some neonatal events in rodents occur in utero in humans.
[c] Range of weaning ages in parentheses.

GD = gestation day
PND = postnatal day

population. A background paper on aging commissioned as part of this review discusses this issue to some extent (Versar Inc., 2001a). In animal studies, the use of dietary restriction has been shown to affect aging and life span to a significant extent, so the issue of what constitutes an older animal is also somewhat controversial.

3.1.1. Exposures and Endpoints Related to General Toxicity Testing

Table 3-2 provides an overview of the biological systems and other endpoints that are evaluated by routine toxicity test designs. The table includes all of the routine test designs that are available in Agency testing guidelines for evaluating toxicity and most of the test designs that focus on specific biological functions. The acute and subchronic studies are intended to give general information on the potential toxicity of an agent by screening the major organ systems, in particular, the liver, the kidney, and the gastrointestinal tract. This information can be used to determine where to look in more detail at specific organ system structure and function. The chronic studies, which are usually done in combination with a carcinogenicity study, evaluate general toxicity in all major organ systems. Several testing guidelines have been developed with the idea that certain systems should be evaluated frequently in more detail (e.g., the nervous system) or that the general toxicity studies do not provide any indication of a potential for effects (e.g., reproductive and developmental toxicity studies). More detailed information about specific aspects of guideline test designs for certain systems (e.g., life stages covered, exposure periods, outcomes measured, etc.) is included in the figures.

Table 3-2 is shaded and marked to indicate the extent of the evaluation of a particular system/endpoint within a particular test design. XXX indicates that the system/endpoint is a primary focus of the particular test design and that detailed assessment of the dose-response relationship of an exposure is carried out within some defined life stage and exposure period for major elements of the system/endpoint. XX indicates those systems/endpoints for which some histopathology or clinical measure of system function is carried out. X indicates those systems/endpoints that are assessed in some observational or gross manner. “0” indicates that the system/endpoint cannot be included, generally because of the design of the test. Blank cells indicate that the system/endpoint is not presently included but could be if the test design were altered appropriately.

It is obvious from the table that few systems/endpoints are examined in any significant detail. The systems/endpoints under the acute test designs are for the most part observational in nature. The acute inhalation toxicity with histopathology guideline (40 CFR 799.9135) was developed under the Toxic Substances Control Act for characterizing the exposure-response

Table 3-2. Systems/endpoints evaluated by routine toxicity guideline testing protocols[a]

Guideline[b]	Systems															Other endpoints		
	Lung-respira-tory	Cardio-vascular	Hema-tologic	Musculo-skeletal	Skin	Eye	Gastro-intest-inal	Kidney-urinary	Liver	Immuno-logical	Repro-ductive	Neuro-logical	Endocrino-logical	Pharmaco-kinetic-metabolic	Mutagenic	Cancer	Immediate death	Short life span
Acute, oral	X	X	X				X	X	X		X	X				0	XX	0
Acute, inhalation	XX	X	X			X	X	X	X		X	X				0	XX	0
Acute, dermal	X	X	X		XX		X	X	X		X	X				0	XX	0
Subchronic, oral	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX			0	XX	X
Subchronic, inhalation	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX			0	XX	X
Subchronic, dermal	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX			0	XX	X
21-day, dermal	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX			0	XX	0
Chronic, oral	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX			XXX	XX	XX
Chronic, inhalation	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX			XXX	XX	XX
Chronic, dermal	XX	XX	XX	XX	XXX	XX	XX	XX	XX	XX	XX	XX	XX			XXX	XX	XX
Prenatal developmental toxicity	X	XX		XX	X	XX	X	XX	X		XX	XX				0	X	0
Reproduction and fertility effects	X	X		X	X		X	XX	XX	X	XXX	X	XX				X	X
Neurotoxicity, acute												XXX					X	
Neurotoxicity, subchronic												XXX					X	
Neurotoxicity, acute-delayed												XXX					X	
Neurotoxicity, subchronic-delayed												XXX					X	
Neurotoxicity, chronic												XXX					X	X
Developmental neurotoxicity											X	XXX	X				X	
Operant behavior												XXX					X	
Peripheral nerve function												XXX					X	
Sensory evoked potential												XXX					X	
Eye irritation, primary						XX								0	0	0	X	0
Dermal irritation, primary					XX									0	0	0	X	0
Dermal, sensitization					X					X				0	0	0	X	0
Dermal, penetration					X									XX	0	0	X	0
Metabolism/pharmacokinetics														XXX	0	0	X	0
Genetic toxicity														0	XXX	0	X	0
Immunotoxicity										XXX				0	0	0	X	0

[a] X indicates that some observational or gross endpoints are included; XX indicates level X plus histopathology or some clinical measure of system function. The prenatal developmental toxicity study includes a more in-depth structural evaluation. XXX indicates the major focus of the evaluation. 0 indicates that this endpoint cannot be included as a major aspect in this protocol. A blank indicates that an aspect is not routinely included but could be.

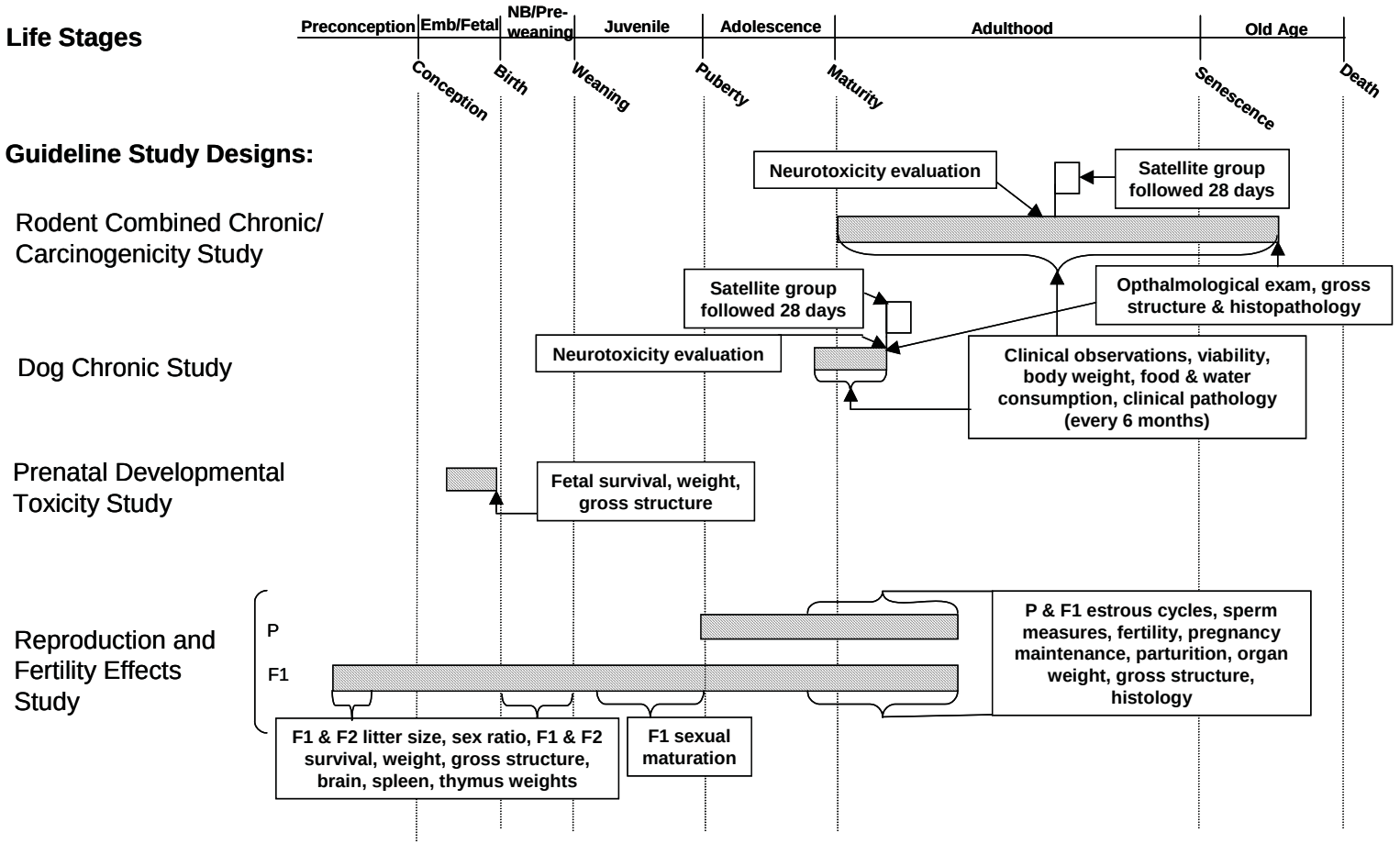
[b] A Series 870 guideline(s) exists for conducting each of the above tests.

relationship for sensitive endpoints following acute inhalation exposure and the toxicologic response following acute high exposures (see further discussion in section 3.1.1.1). Acute toxicity information is useful in establishing reference values for short-duration exposures and for establishing dose-ranges for subchronic and chronic studies. The subchronic and chronic test designs evaluate most endpoints with somewhat greater detail than do the acute test designs. Although the histopathology and/or clinical measures of system function are screening in nature, there is greater confidence that with this level of examination the dose-response relationship will be more clearly defined. Nevertheless, it should be recognized that most systems/endpoints are evaluated at a screening level, and detailed analyses of pathology and function are generally not carried out. Even in those test designs that do incorporate detailed analyses, these analyses are limited in regard to the life stages, exposure periods, and measures that are assessed.

Figure 3-1 shows the study designs that are used for general toxicity testing superimposed on a time line that indicates the life stages during which exposure occurs (hatched bars) and endpoints are measured (indicated in the boxes). The guideline studies shown represent the minimum requirement for derivation of a chronic oral RfD. Similar studies are required for the chronic inhalation RfC, with appropriate endpoints for inhalation exposure and toxicity included. In some cases, only a 90-day subchronic study is available instead of the chronic studies shown. Because the relative length of time between life stages varies among species, the placement of exposures and endpoints on the figures is not necessarily to scale. The following sections discuss the studies that address acute and short-term toxicity as well as chronic toxicity. Similar figures related to specific organ system toxicity testing are shown in subsequent sections.

3.1.1.1. *Acute and Short-Term Toxicity Studies*

3.1.1.1.1. ***Overview of tests.*** The primary purpose of the guideline acute toxicity tests (870.1100 acute oral; 870.1200 acute dermal; and 870.1300 acute inhalation) and other short-term studies (e.g., 14–28-day studies, no OPPTS guidelines available) is to identify hazards (focusing on route-specific lethality) from short-term exposure studies, provide a basis for classification and



Emb = Embryonic NB = Newborn P = Parental F1 and F2 = 1st and 2nd filial generations

Figure 3-1. Exposures and endpoints related to general toxicity evaluations. Endpoints shown are for oral exposures; endpoints specific to inhalation and dermal exposure are included for studies by those routes of exposure.

labeling, and enable the selection of exposure ranges for longer-term studies.[3] Acute guideline studies are conducted in young adult animals, with a 14-day post-exposure observation period. Other than mortality, the endpoints include cage-side observations, body weight at the end of the observation period, gross pathology changes at necropsy, and histopathological examination of organs showing evidence of gross pathology in animals surviving 24 hours or more. Two other available guideline studies cover acute exposures followed by extensive assessment of a specific organ system. The first is the acute inhalation toxicity study with histopathology (40 CFR 799.9135), which was developed for hazardous air pollutants. This study includes assessments of liver, kidney, and broncho alveolar lavage samples for several indicators of cellular damage (e.g., total protein, cell count, percent leukocytes) and a phagocytosis assay to determine macrophage activity. For the respiratory tract histopathology, detailed specifications are provided.

The second expanded study that includes observations following an acute exposure is the acute neurotoxicity study (870.6200), which was developed for the evaluation of neurotoxic chemicals and includes assessments of functional behavior and motor activity at the time of peak effect and again at 14-days post-treatment and histopathology of the central and peripheral nervous systems at 14-days post-treatment. The prenatal developmental toxicity study (870.3700) in two species (typically rats and rabbits) and the DNT study (870.6300) can also provide relevant data for acute risk assessment because maternal observations are often recorded daily and there is a presumption that effects during development may result from a single exposure.

3.1.1.1.2. ***Gaps in life stage of assessment.*** Acute/short-term testing is done only in prenatally exposed animals and in young adults. No direct information is available from any of these studies on acute or short-term exposure in postweaning young animals or aged animals.

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[3]Alternative test protocols have been adopted by the Organization for Economic Cooperation and Development for acute toxicity testing for oral, dermal, and inhalation exposure, including the fixed-dose procedure, the acute toxic class method, and the up-and-down procedure. All are designed to minimize animal usage and provide minimal hazard and dose-response information for classification, labeling, and dose selection. In the future, EPA plans to put primary reliance on the up-and-down procedure for testing of technical grade pesticides, although the other tests may be acceptable in some circumstances, e.g., testing of pesticidal products. These studies are not designed to provide information for use in less-than-lifetime risk assessments.

3.1.1.1.3. ***Gaps in assessment endpoints.*** Data on only a limited number of toxicological endpoints are available from guideline acute toxicity (lethality) studies except in the case of the acute inhalation toxicity guideline study with histopathology and the acute neurotoxicity study. Consequently, these studies often are not suitable for use in deriving reference values unless additional data, such as those from subchronic studies (e.g., hematological, clinical, histology of more organs), are collected. Some data from animals examined at early times might be available in guideline subchronic or chronic studies. These data could augment the results from guideline acute studies.

3.1.1.1.4. ***Gaps in duration of exposure/latency to response assessment.*** There is no guideline study for short-term toxicity testing, although the prenatal developmental toxicity studies in rats and rabbits and the DNT study include repeated dosing of maternal animals for periods of less than 25 days. Because of the post-exposure observation period in acute guideline studies and in the DNT study, some information on latency to effect and reversibility of effect may be available.

3.1.1.2. ***Subchronic and Chronic Toxicity Studies***

The subchronic exposure studies (870.3100, 870.3150, 870.3200, 870.3250, 870.3465) are used for setting chronic RfDs and RfCs when a chronic study is not available. The guideline studies for chronic exposures (870.4100, 870.4200, 870.4300) (1 year in rodents, although the typical study is a combined chronic and carcinogenicity study with a 2-year exposure) provide an in-depth look at a number of organ systems, and in some cases they evaluate both structure and function (see Figure 3-1). The chronic study in nonrodents, usually dogs, involves a 12-month exposure with similar endpoints assessed as in rodents. The prenatal developmental toxicity study (870.3700) in two species (typically rats and rabbits), the DNT study (870.6300), and the reproduction and fertility effects study (870.3800), typically in rats, are also considered in setting chronic RfDs or RfCs.

3.1.1.2.1. ***Gaps in life stage of assessment.*** The subchronic and chronic studies are conducted in young adult animals, with exposure in the chronic/carcinogenicity study continuing into old age. No information is available from chronic studies in pre- or postnatal animals. Exposures in subchronic study protocols do not include pre- or postnatal development, although the reproduction and fertility effects study does provide data on subchronic exposures in animals that are exposed before birth, through prenatal and postnatal development up to mating of the F1

males and females and through pregnancy (F1 young adult females). No subchronic toxicity evaluations are conducted in aged animals. No chronic studies are conducted in pre- or postnatal animals, although aged animals are exposed and evaluated as part of the chronic study protocol.

3.1.1.2.2. ***Gaps in assessment endpoints.*** The greatest gaps appear to be the lack of routine testing for subchronic neurotoxicity in adults, immunotoxicity testing in adults, and more thorough toxicokinetics in animals at various life stages. Gaps in assessment endpoints during prenatal and postnatal development are discussed in the next section. Assessment endpoints for routine toxicity testing in old age are completely lacking, as is background information on endpoints related to the aging process itself.

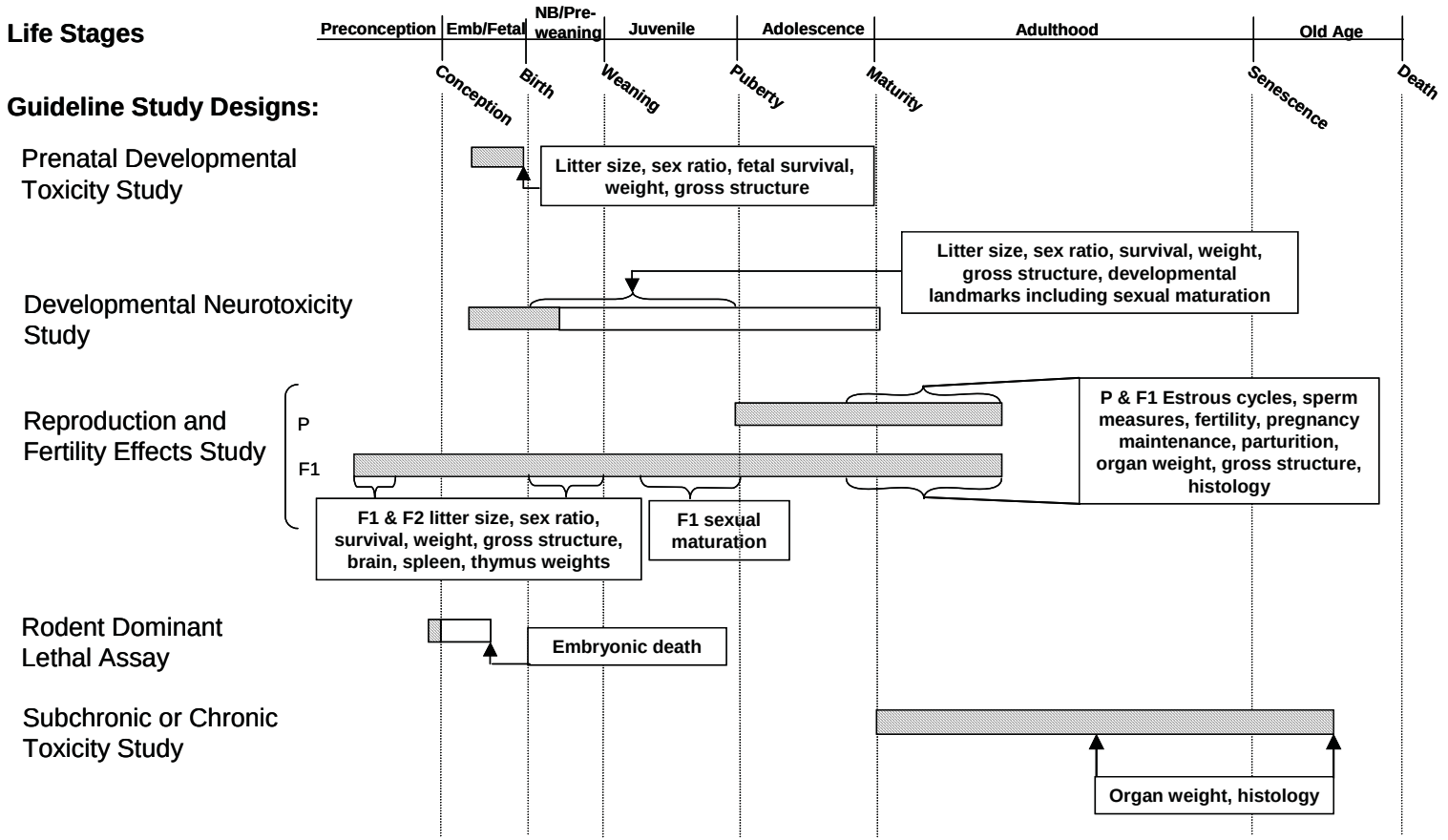
3.1.1.2.3. ***Gaps in duration/latency assessment.*** Chronic studies that include prenatal and postnatal exposure into old age are lacking. The so-called chronic study in dogs is actually a short-term study, as it does not cover at least 10% of the life span. Chronic studies that include a satellite group in which exposure is stopped after 12 months in rodents do assess latency to response for a brief period of time (28 days or more).

3.1.2. Exposures and Endpoints Related to Evaluation of Reproductive Toxicity

3.1.2.1. *Overview of Tests*

The reproductive organs are examined structurally in a number of general guideline screening studies, including the 90-day subchronic study (OPPTS 870.3100, 870.3150, 870.3250, 870.3465), chronic/carcinogenicity studies (OPPTS 870.4100, 870.4200, 870.4300), the prenatal developmental toxicity study (OPPTS 870.3700), and the reproduction and fertility effects study (OPPTS 870.3800), which is a two-generation reproduction study. In addition, extensive assessment of numerous functional aspects of the reproductive system is conducted in the reproduction and fertility effects study. Specific functional effects on the reproductive system of male animals can also be assessed in the rodent dominant lethal assay (OPPTS 870.5450). As illustrated in Figure 3-2, these studies include a variety of both structural and functional assessments of the reproductive system over a wide sampling of life stages.

In guideline subchronic and chronic/carcinogenicity studies, gross structural evaluation and general qualitative histopathology are conducted on reproductive organs and tissues. The animals in these studies are adults, but at the time of organ assessment they may be young (e.g., rats 45 days to 5 months of age from a subchronic study), mature (e.g., rats 5–18 months of age from a reproduction study), or old animals (e.g., rats 18 months to 2 years of age from a chronic study), depending on the protocol.



Emb = Embryonic NB = Newborn P = Parental F1 and F2 = 1st and 2nd filial generations

Figure 3-2. Exposures and endpoints related to reproductive evaluations.

Standard guideline prenatal developmental toxicity studies are designed to evaluate the potential effects of the test substance on the developing fetus. Observations on the reproductive capacity of the maternal animals in this study generally consist only of clinical observations (including any abnormalities of pregnancy maintenance) and gross necropsy data (including uterine). Selected fetuses are examined for gross structural changes to the internal reproductive organs. In studies that employ methods of serial sectioning in the process of soft tissue examination, a limited macroscopic evaluation of the internal structure and integrity of the reproductive organs is performed; however, the fetal tissues are not examined microscopically. Additionally, there are no assessments of organ function in this study design.

In the guideline reproduction and fertility effects study, rats are exposed to the test substance over the duration of two generations, beginning when the first generation animals are young adults of approximately 6–9 weeks of age. Daily exposure continues during all phases of development and reproductive function. Adult animals of both generations are killed as mature adults, generally prior to reaching reproductive senescence (that is, the cessation of normal reproductive function) or an age that would be considered geriatric in that species. Assessments of reproductive capability and function are conducted at least once in each generation. These assessments include direct evaluation of the age of sexual maturation, estrous cyclicity (immediately prior to mating), sperm measures (at termination), mating success, fertility and fecundity, implantation, pregnancy maintenance, gestation duration, parturition, and success of lactation (e.g., maternal nurturing and nesting behavior).

Indirect assessments of some reproductive functions are also evaluated. These observations are based on evidence of normality in a structure, function, or process that is dependent on normal functioning of the component parts, including, for example, hormonal homeostasis, ejaculation, accessory gland function, placental function, milk production, pup nursing behavior or ability, and, to some extent, reproductive senescence (although the adult animals are terminated at the end of each generation, when they are only around 6 months of age; therefore, there are no assessments conducted in older rats). Gross structural assessments of the whole animal are conducted on adult and immature animals throughout the course of the study; gross internal (organ) structural assessments are conducted on offspring that are killed at litter standardization (postnatal day [PND] 4), weaning (PND 21), and termination of each generation (mature adults). Histopathological evaluation of the reproductive organs (gonads and accessory structures) is conducted only in the mature parental adult animals that are killed at the termination of each generation. The guideline specifies a very focused pathological examination of the reproductive organs in this study.

The dominant lethal assay is not conducted for every chemical, but it may be conducted in response to a concern raised by other developmental or reproductive toxicity findings in the database. In this study, sexually mature adult males are treated with the test substance to determine whether there is an effect in the germinal tissue that does not cause dysfunction in the gamete but is lethal to the fertilized egg or developing embryo. Exposed males are mated with untreated females, and uterine contents are evaluated. Evidence of pre- and/or postimplantation loss is generally thought to be indicative of treatment-related chromosomal damage in germinal tissue.

3.1.2.2. *Gaps in Life Stage of Assessment*

Determination of gaps in the assessment of potential effects of any chemical across all life stages requires consideration of both the exposure period and the time of assessment. In the prenatal developmental toxicity study, animals are exposed from implantation through gestation. The reproductive organs are examined for gross structural changes, but no microscopic examination is conducted. There is no follow-up of the animals to determine the functional consequences of prenatal exposure. In the reproduction and fertility effects study, the F1 animals are exposed from preconception throughout prenatal and postnatal development until after mating. The reproductive organs are examined macroscopically at weaning and adulthood. The maturation of the reproductive system is assessed, as is its function. Thus, the study provides a fairly thorough assessment of structure and function following exposure during many critical periods of development. In the parental generation, the animals are exposed as young adults, and the structure and function of the reproductive organs are assessed.

The dominant lethal study, when conducted, assesses a single aspect of the function of the reproductive system for one sex, although a detailed structural assessment is not conducted. In the subchronic and chronic studies, the animals are exposed beginning as young adults, and the structure—but not the function—of the reproductive organs is assessed. Therefore, the major gaps include (1) the lack of functional assessment (particularly the age of onset of reproductive senescence) in older adult animals following adult-only exposures, and (2) the lack of structural and functional assessments in older adult animals following developmental exposures.

The onset of reproductive senescence can be marked by findings such as altered hormonal homeostasis, disruption of estrous cyclicity, diminished sperm measures (number, motility, or morphology), or gonadal atrophy. Studies in rodents have demonstrated the adverse effects of a number of agents (e.g., ionizing radiation, chemotherapeutic agents, polycyclic aromatic hydrocarbons, and agents that form epoxides, such as 1, 3-butadiene and 4-vinylcyclohexene) on reproductive senescence (reviewed by Hoyer and Sipes, 1996).

In humans, premature reproductive senescence has been associated with cigarette smoking (Jick et al., 1977). In addition to potentially diminishing fertility in individuals who are only slightly past prime reproductive age, early reproductive senescence can adversely affect the general health of the aged human. For example, hormonal alterations that are associated with early senescence have been linked to abnormalities of cardiovascular function, osteoporosis, and even a predisposition to early mortality.

3.1.2.3. *Gaps in Assessment Endpoints*

As described above, there are identifiable gaps in the endpoints that are used to assess reproductive toxicity in guideline studies. Currently, there is no assessment of functional endpoints in older animals following adult exposures, and there are no structural or functional endpoints assessed in older animals following developmental exposures, including reproductive senescence. In addition, concerns have recently been raised about the ability to detect rare malformations of the reproductive organs and abnormalities in the maturation of the reproductive system in the two-generation reproductive toxicity study. This concern relates particularly to endocrine-active chemicals. In the current guideline, three pups/sex/litter are examined macroscopically at weaning.

Questions have been raised about whether these weanlings should be retained until day 45 (females) or day 60 (males) to ensure that any later-appearing gross or functional changes are detected. This issue is currently being examined within the endocrine validation/standardization program.

3.1.2.4. *Gaps in Duration/Latency Assessment*

There are no studies that include acute or chronic exposures that can be used to assess the development of the reproductive system. As indicated above, it has been suggested that animals be retained until older ages in the two-generation study in order to assess later-appearing structural or functional changes in reproductive organs. In addition, there is no consideration of latent responses for reproductive toxicity, such as early onset of reproductive senescence, as a result of an exposure earlier in life in any of the studies that can be used to evaluate reproductive toxicity, except for a few endpoints in the DNT study.

3.1.3. Exposures and Endpoints Related to Evaluation of Neurotoxicity

3.1.3.1. *Overview of Tests*

Observation of the animals for signs of overt toxicity and routine gross pathological assessment of the nervous system is required under OPPTS acute, subchronic, and chronic study protocols (870.100–870.400 series). In rat studies, age at initiation of testing is to be 8–12 weeks under acute and subchronic testing protocols. In acute studies, cage-side observation and gross neuropathology are the only endpoints required under 870.100 (oral, dermal, or inhalation exposure). Motor activity, grip strength, and sensory reactivity and neuropathology are measured in the rodent oral study, the dermal 21–28- and 90-day subchronic studies, and the 90-day inhalation study. In rodent subchronic studies, specific assessment for neurotoxicity is performed at or near the end of the study, although observations of the animals, including those for detection of overt neurotoxicity, are made routinely throughout the study. No specific functional tests for neurotoxicity are required for nonrodent subchronic studies, although observation and neuropathology are required.

Chronic toxicity studies (oral, dermal, inhalation) are to be performed in two species (one rodent) over a 12-month period, regardless of the life span of the species. Exposure in rodents is to begin no later than 8 weeks of age. Motor activity, grip strength, and sensory reactivity are to be assessed at or near the end of the study, but no earlier than the 11th month. Clinical observation is performed weekly throughout the study and would presumably detect gross neurological abnormality. In current practice, the chronic study is often combined with the carcinogenicity test, in which dosing extends for 24 months in rats and 18 months in mice (OPPTS 870.4300). Motor activity would be performed at 11–12 months only, as in the chronic study, and not again until near the end of exposure.

The neurotoxicity screening battery (870.6200) is designed to be included in acute, subchronic, or chronic toxicity studies (Figure 3-3). The endpoints examined extend those required in the 870.100 series, although there is no guidance as to when these extended batteries would be required. The functional observation battery includes a ranking system for general reactivity, activity, and gait abnormalities, as well as forelimb and hindlimb grip strength, landing foot splay, sensorimotor reactivity to sensory stimuli, and pain reception. Motor activity and a more detailed neuropathological observation are also required in this battery. For acute studies, assessments are made before initiation of dosing, at the estimated peak of activity within 8 hours of dosing, and at 7 and 14 days post-dosing. For subchronic studies, assessments are performed pre-exposure and at 7, 8, and 13 weeks of exposure. For chronic studies, assessment is at pre-exposure and every 3 months post-exposure. There is no specific guidance regarding

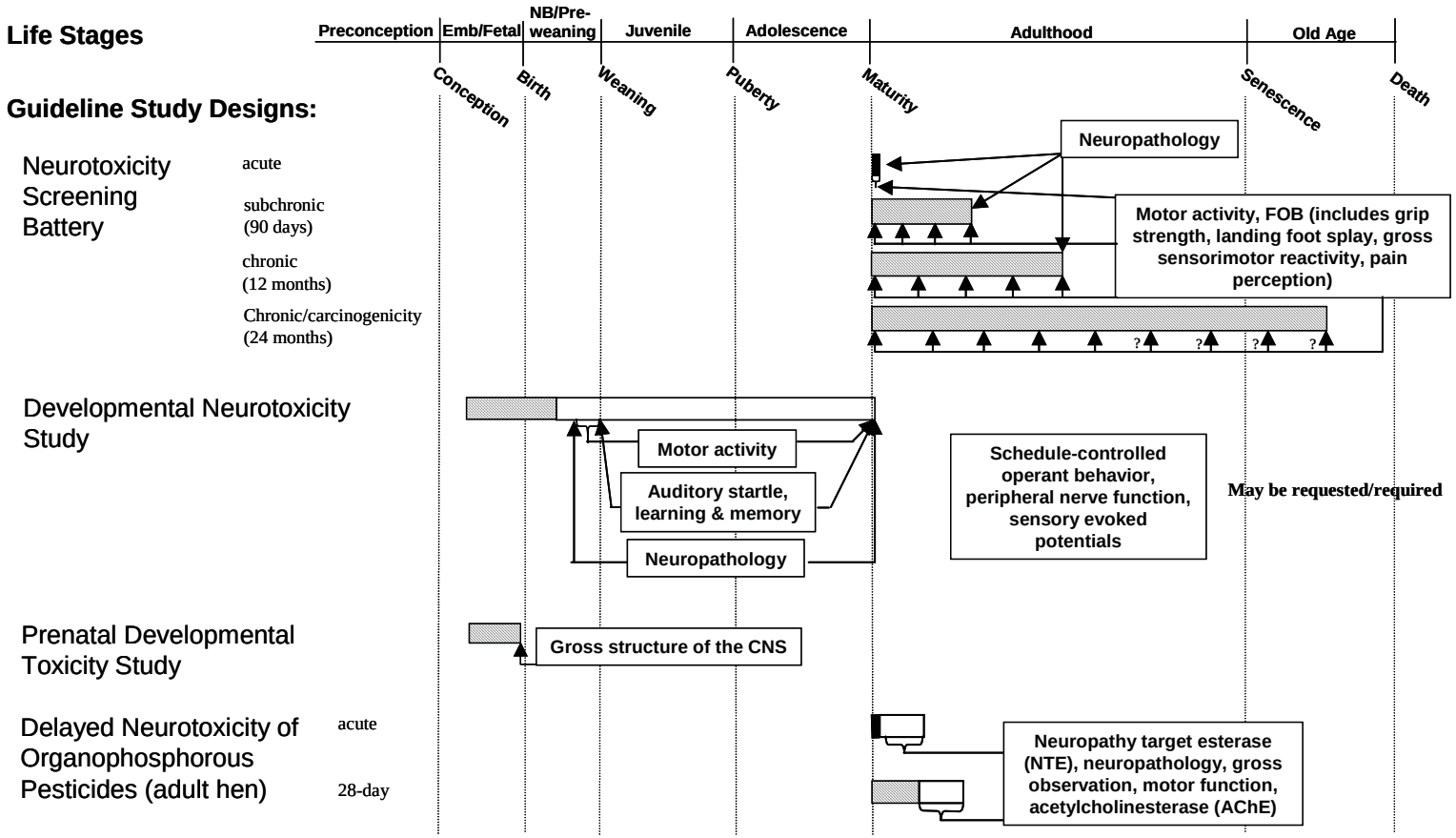


Figure 3-3. Exposures and endpoints for neurotoxicity evaluations.

the assessment schedule for the combined chronic/carcinogenicity study, but presumably the schedule required for the chronic study would be maintained.

The DNT study protocol (870.6300) currently requires dosing of the dams from gestational day (GD) 6 through PND 10, although the requirement may soon be extended to PND 21 (i.e., until weaning). Motor activity is measured at PNDs 13, 17, 21, and 60. Auditory startle is measured around weaning and at PND 60, as is a test of learning and memory, which may be the same test or different tests at the two time points. Cage-side observation of both dams and pups is required, and neuropathology in the pups is required at PND 11 and at the termination of the study (usually PND 60). The prenatal developmental toxicity study (870.3700) requires dosing of the dams on GDs 6–20 in rats and 6–29 in rabbits. Gross structural evaluation of the nervous system is evaluated as part of the fetal examinations conducted in this study.

3.1.3.2. *Gaps in Life Stage of Assessment*

One of the most significant gaps revealed by Figure 3-3 is the lack of exposure or assessment under any protocol during old age. For example, following acute exposure, assessment is for 14 days in juvenile or young adult animals. The chronic exposure protocol extends exposure into adulthood and the combined chronic/carcinogenicity protocol extends exposure up to approximately the aged period in the rat, but neurotoxicology assessments are not performed in aged animals. Thus, none of the protocols assess potential effects of chemicals on aging as a function of exposure during development. This may be important, because studies in animals have shown that developmental exposure to agents that cause neurotoxicity, such as trimethyl tin, can accelerate the onset of cognitive deficits measured later in life. Other studies with methyl mercury have documented early-onset sensory dysfunction in monkeys exposed during development. Furthermore, current testing protocols do not provide information collected at different life stages—that is, comparison of effects of exposure during infancy, adulthood, or old age. This is important, because life stage-dependent differences in pharmacokinetic, and possibly toxicodynamic, parameters could result in quantitatively or qualitatively different effects at different life stages.

Under the DNT protocol, there currently is no requirement to perform kinetic studies to ascertain either in utero or postnatal exposure. There is no mechanism to guarantee exposure postnatally (i.e., direct dosing of pups) because the compound may not be excreted into breast milk or it may be excreted only at very low concentrations. This is of particular importance, because the early postnatal period in the rodent is equivalent to a prenatal life stage in humans.

There is no long-term follow-up assessment to detect delayed neurotoxic effects, a situation that is arguably more worrisome for developmental exposure than for exposure later in life.

3.1.3.3. *Gaps in Assessment Endpoints*

The nervous system is one of the most fully assessed organ systems in the EPA/OPPTS 870 guidelines. Nonetheless, most of the endpoint assessments are designed to be screening procedures rather than sensitive assessments of nervous system function. In addition, the assessments required are different in the neurotoxicity screening battery than in the DNT study. The adult neurotoxicity screening battery does not require assessment of learning and memory or auditory startle. The lack of assessment of cognitive function in the neurotoxicity screening battery constitutes a significant omission that should be addressed.

It may also be pointed out that even in the developmental protocol, the tests that are used to assess learning and memory may be very simple, potentially revealing only relatively gross deficits. In addition, although potentially more sensitive cognitive, sensory, and motor tests are available (Figure 3-3), there is no guidance as to what would trigger a requirement for these assessments. Except for the protocol for delayed neurotoxicity for organophosphorous pesticides in the hen, there is no assessment of neurochemical endpoints. Additionally, the required neuropathological assessments may also be considered screening.

Minimal morphometric analysis, consisting of the thickness of “representative” layers in the neocortex, hippocampus, and cerebellum, is required in the DNT study. No morphometric analyses are required in the adult neurotoxicity testing protocols. Although more sophisticated tests would presumably not be performed on all agents, more sophisticated measures could be triggered by results from screening tests. It also may be advisable to require more sensitive tests in instances of particular concern, for example, adding more extensive morphometric analysis to the DNT protocol.

In summary, although the nervous system is one of the most thoroughly assessed systems in the 870 test guideline studies, it must be kept well in mind when interpreting the results that these are screening tests. Positive findings must be viewed as indicative of relatively overt toxicity, not so-called subtle effects.

3.1.3.4. *Gaps in Duration/Latency Assessment*

One of the principles in the neurotoxicity risk assessment guidelines (U.S. EPA, 1998c) is that neurotoxicity could occur after one or a few exposures, such as in the case of an organophosphate insecticide that produces a delayed neuropathy, or only after a series of repeated exposures, as in the case of acrylamide. For DNT, it is assumed that a single exposure

to a chemical during a critical period of development could result in an adverse effect on the developing nervous system. There are, however, few data that compare the effects of a single exposure to a chemical with the effects of the same chemical given multiple times during development.

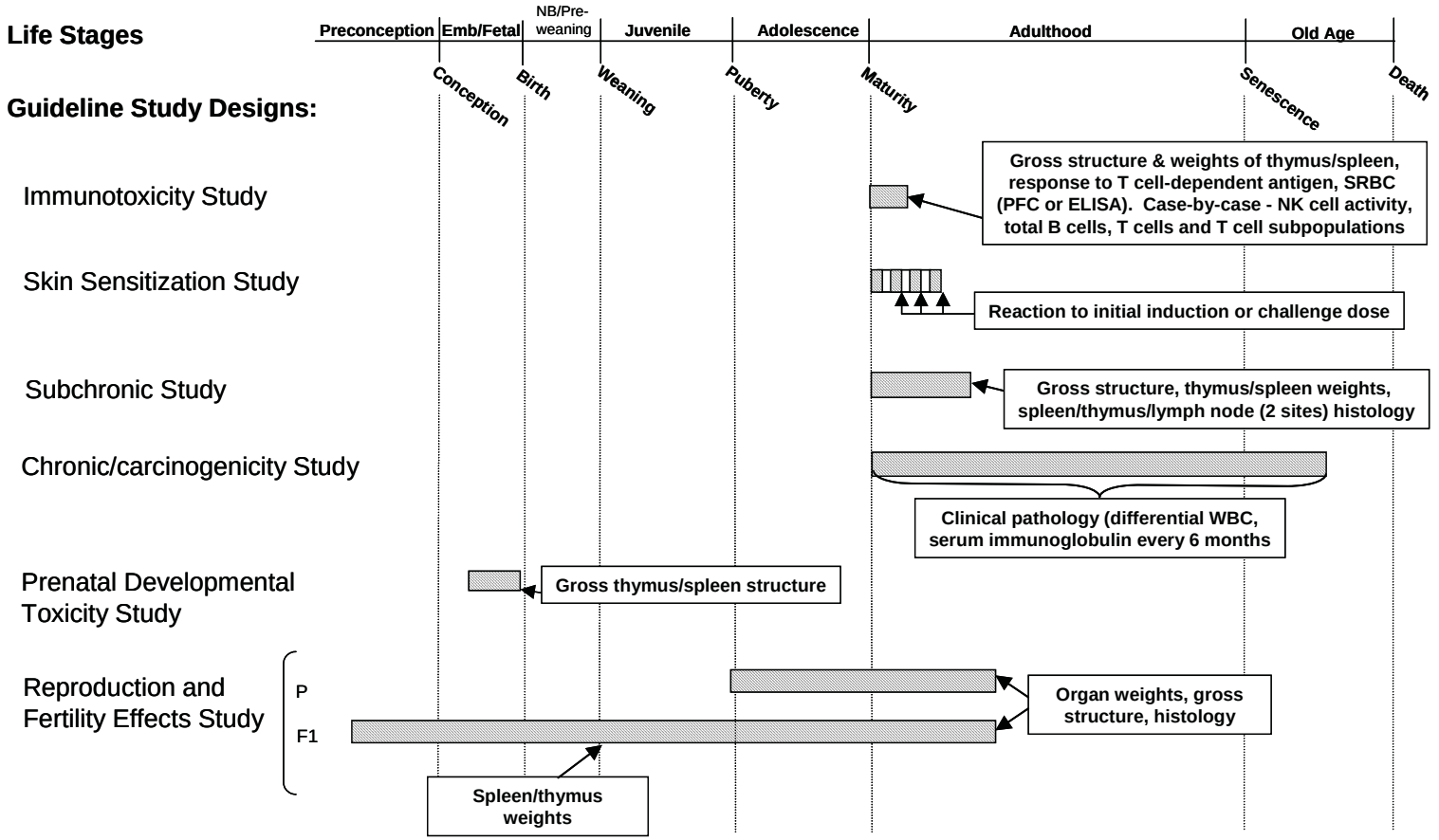
3.1.4. Exposures and Endpoints Related to Evaluation of Immunotoxicity

3.1.4.1. *Overview of Tests*

Examination of the macro- and/or microscopic structural anatomy of immune system organs and tissues is performed in a number of general guideline screening studies, including the acute inhalation toxicity with histopathology guideline (40 CFR 799.9135), the 90-day subchronic study (OPPTS 870.3100, 870.3150, 870.3250, 870.3465), the chronic/carcinogenicity studies (OPPTS 870.4100, 870.4200, 870.4300), the prenatal developmental toxicity study (OPPTS 870.3700), and the two-generation reproduction study (OPPTS 870.3800). In addition, functional assessments of the immune system are evaluated in the skin sensitization study (OPPTS 870.2600) and the immunotoxicity testing guideline (OPPTS 870.7800) (see Figure 3-4).

In the guideline immunotoxicity study, young adult rats (6–8 weeks of age) are exposed to the test substance for 28 days, at which time they are terminated. The spleen and thymus are examined macroscopically, and organ weights are recorded; a histopathological evaluation is not performed. Assessments of immune system function include an evaluation of the response to the T cell-dependent antigen, sheep red blood cells (SRBC). The SRBC antigen response assays can be conducted either by an antibody plaque-forming cell (PFC) assay or an immunoglobulin quantification by enzyme-linked immunosorbent assay (ELISA). In addition, an assessment of natural killer (NK) cell activity and/or enumeration of splenic or peripheral blood total B cells, total T cells, and T cell subpopulations may be required on a case-by-case basis.

The skin sensitization study has been generally conducted in guinea pigs as a Guinea Pig Maximization Test (GPMT) or a Buehler test. In a recent review by the FIFRA Science Advisory Panel (U.S. EPA, 2001a), it was recommended that in the future, skin sensitization methods should preferentially include the local lymph node assay (LLNA), which uses young adult mice. The skin sensitization test involves an initial intradermal (GPMT) and/or epidermal (Buehler, LLNA) exposure of the test animal to a substance, followed by a challenge exposure approximately 1 week later. In the guinea pig tests, sensitization is determined by examining the reaction to the challenge exposure and comparing this reaction with that of the initial induction



Emb = Embryonic NB = Newborn P = Parental F1= 1st filial generation SRBC = Sheep red blood cell
PFC = Placque-forming cells ELISA = Enzyme-linked immunosorbent assay NK = Natural killer
WBC = White blood cells

Figure 3-4. Exposures and endpoints for immunotoxicity evaluations.

exposure. In the LLNA, proliferation of lymphocytes is measured (as a function of in vivo radioisotope incorporation into cellular DNA) in draining lymph nodes proximal to the application site. Hence, although the GPMT and Buehler tests result in a qualitative assessment of hypersensitivity, the LLNA provides a quantitative dose-response evaluation. Histopathological evaluation of the skin is not required with any of these methods, but it may be conducted. No other immune system endpoints or organs are evaluated in this study.

In guideline subchronic and chronic/carcinogenicity studies, an evaluation of macroscopic structure and general qualitative histopathology are conducted on only a few immune system tissues. In studies that include young adult animals (e.g., rats 45 days to 5 months of age from a subchronic study), the spleen, thymus, and lymph nodes from two locations (one near to and the other distant from the site of administration) are examined; the spleen and thymus are weighed. In chronic and carcinogenicity study guidelines, there is no requirement that the thymus be examined and/or weighed. For rodents (e.g., rats or mice 18 months to 2 years of age), it is reasonable to assume that the thymus would have undergone normal age-related atrophy by study termination. However, the thymus might be present at early interim sacrifices of rodents (e.g., at 6 months or 12 months of study) during a long-term study, and it would certainly be present at study termination in a canine chronic study (at which point the dogs are young adults of only approximately 1.5 years of age).

Differential white cell counts in the circulating blood are examined at study termination in the subchronic study and at approximately 6-month intervals in long-term studies. Serum immunoglobulin levels may be measured at the same intervals. Perturbations may indicate increased immune system response to some unspecified initiator, but this information does not address the adequacy of immune system function. In the same manner, histopathological evaluation of other organ systems in the subchronic and chronic/carcinogenicity studies may identify cellular alterations that are nonspecific indicators of an effect on immune response, for example, the presence of increased numbers of macrophages in lung tissue or an increased incidence of inflammatory dermal lesions.

In the reproduction and fertility effects study in rats, a macroscopic evaluation of all organ systems is conducted in a sample of offspring at weaning and in the mature adult parental animals at the termination of each generation. Additionally, the spleen and thymus are weighed for those pups that are necropsied at weaning; these measurements are intended to provide information on the need for further evaluation of the immunotoxic potential of a chemical to the immature animal.

In the prenatal developmental toxicity study, an evaluation of the macroscopic structure of the thymus and spleen is conducted in at least half of the fetuses from each litter.

3.1.4.2. *Gaps in Life Stage of Assessment*

In the available guideline studies, assessments of organs with immune system function are conducted in fetuses following prenatal exposure, in weanling animals following pre- and postnatal exposure, and in young and/or mature adult animals at a variety of time points. With prenatal exposures and evaluation at early life stages, these assessments consist entirely of the evaluation of macroscopic changes, with no microscopic examination. Toxicokinetic data that characterize the exposure in the young (i.e., exposure of the fetus to the chemical or its metabolites via the placenta or of the neonate via breast milk) are not routinely required and are seldom available.

Some detailed structural assessment (histopathology) is conducted in mature or older adult animals. Indirect assessment of immune system function is conducted in adult animals of various ages via the evaluation of peripheral blood cells and chemistry. Direct functional assessments of the immune system are conducted only in young adult animals; generally this age group is selected for assessment because of the anticipated robustness of the immune response.

There is no guideline that examines potential perturbation of immune system function following early pre- and/or postnatal exposure (often referred to as a developmental immunotoxicity study). Comparisons of immune effects following exposure at various life stages (i.e., during in utero or postnatal development, adulthood, or old age), including data that analyze whether these effects are more severe in one age group or whether the effects are persistent, are not required. To achieve even a minimal assessment of immune system structure and function, a broad variety of studies would need to be conducted and assessed; yet there could still be relatively low confidence in the ability of the results of these combined studies to predict the outcome of age-specific insults to the immune system.

3.1.4.3. *Gaps in Assessment Endpoints*

There are identifiable gaps in the endpoints that are used to assess immunotoxicity in guideline studies. For example, for fetuses, immature animals, and old animals (rodents), assessments are composed entirely of the evaluation of macroscopic structural changes, with no histopathological or functional evaluations. In mature adult animals, thorough macroscopic and microscopic structural assessments, as well as routine hematological testing (e.g., blood cell counts), are performed; however, those assessments are generally very limited in young animals, and guideline requirements do not consider species differences. The only assessments of functional integrity of the immune system are provided by the guideline sensitization study and the 28-day immunotoxicity study. These studies are conducted only in young adult animals, and

they include only a few examples of potential immune system response (e.g., hypersensitivity, humoral immunity, or nonspecific cell-mediated immunity). In very young and very old animals, there is no direct assessment of immunological function. No assessment of autoimmune effects is conducted in any of the current guideline protocols.

3.1.4.4. *Gaps in Duration/Latency Assessment*

Latent effects on immune function that result from early lifetime exposure are not assessed; these can include effects in aged animals that result from in utero, neonatal, or young-adult exposure. Exacerbation of effects in relation to aging and response to subsequent immunological challenge are not routinely or systematically assessed to any extent. The two-generation reproduction study offers an opportunity to evaluate immunotoxic response in adulthood that resulted from prenatal or early postnatal exposure. In the chronic toxicity studies in rodents, aged animals are available for evaluation. However, in both cases there is little focus on the evaluation of the immune system. Only indirect evidence of perturbation of the immune system may be observed through macroscopic and microscopic evaluation of various organs; corollary functional assessment is not performed. Response to an immunological challenge is examined only in the hypersensitization study, and even when the results from this study are positive, no further specific assessment of the immune system is pursued.

3.1.5. Exposures and Endpoints Related to Evaluation of Cardiovascular Toxicity

3.1.5.1. *Overview of Tests*

Gross observation of the heart and major vessels augmented by conditional standard pathology is mentioned in most applicable OPPTS Series 870 health effect guidelines (Figure 3-5).

3.1.5.2. *Gaps in Life Stage of Assessment*

The period from birth to maturity is essentially without toxicological monitoring of cardiovascular endpoints for both repeated chronic and single acute-exposure regimes.

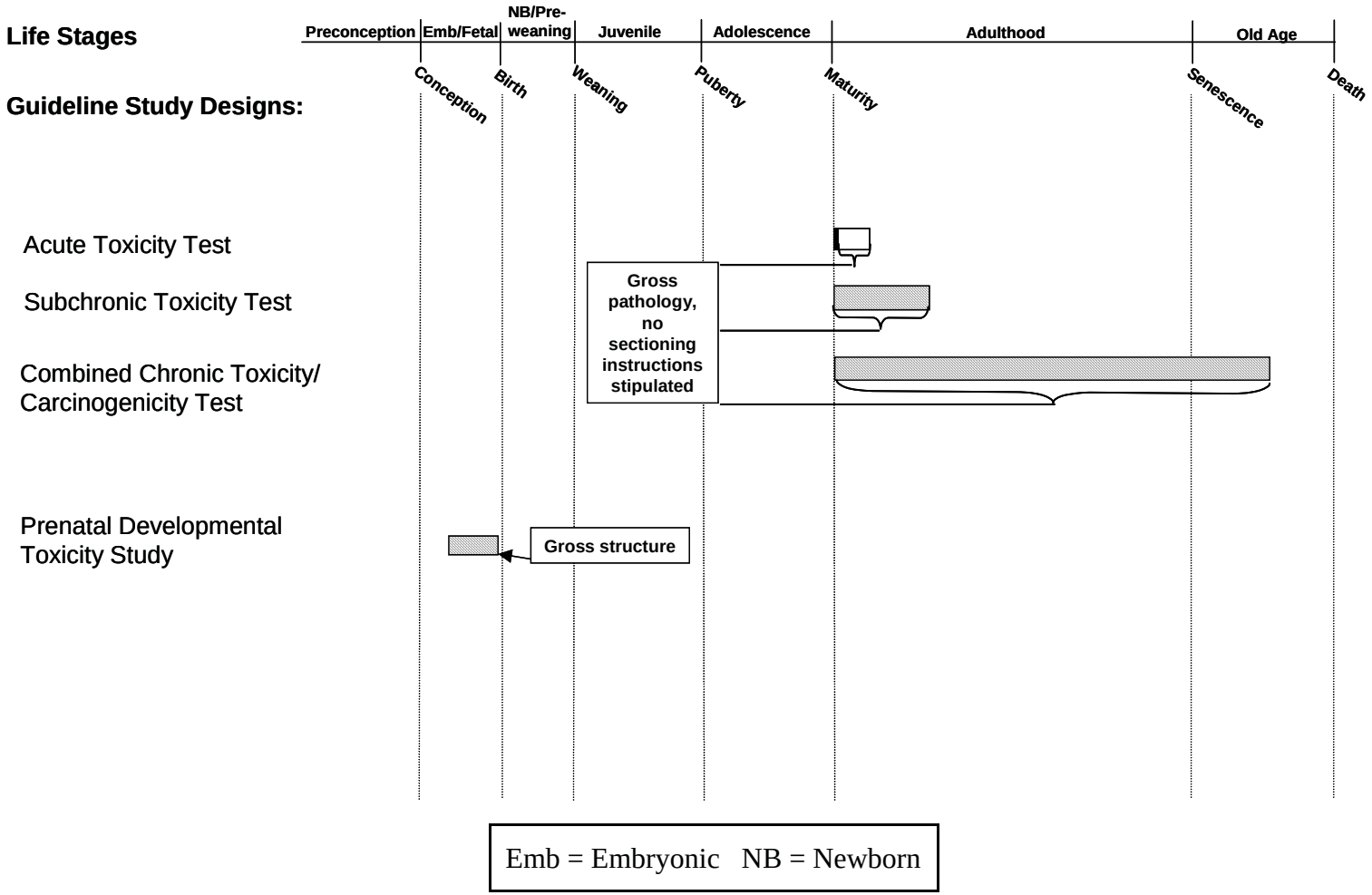


Figure 3-5. Exposures and endpoints related to cardiovascular evaluations.

3.1.5.3. ***Gaps in Assessment Endpoints***

Gross observation only of the heart is provided for in most OPPTS guidelines. Functional clinical or histopathological cardiac examination is not currently part of any testing guideline. Even gross pathology could be improved and brought into line with current cardiovascular evaluation by separating, weighing, and constructing right and left ventricle-to-body weight ratios to give an evaluation of cardiac hypertrophy. Also, guidelines regarding sectioning procedures for the heart, either number or plane, could be provided.

No simple cardiac functional evaluation is currently available, including even systolic or diastolic blood pressures. It should be noted that telemetric in-dwelling echocardiograms (ultrasound examinations of the heart) can be used to detect occlusions and atherosclerosis and to detect alterations in cardiac output. Combination echocardiograms and electrocardiogram analysis can detect cardiac wave forms as well as heart rate variability in high- and low-frequency power ranges (i.e., beat-to-beat changes in heart rate ascribed to varying control by the autonomic nervous system). Heart rate variability may be critical in explaining toxicity, as was shown in recent work associating exposures to fine particulate matter with decreases in heart rate variability in elderly humans (Creason et al., 2001). Both echocardiograms and electrocardiograms can be done on rats down to 100 g, well within the size range of juvenile and adolescent rats.

Chemicals can produce degenerative and/or inflammatory changes in the peripheral blood vessels as a consequence of an excessive pharmacologic effect or by an interaction with a vascular structural or functional macromolecule. As a result of sustained arterial vasoconstriction, peripheral arterial lesions consisting of intimal proliferation and medial degenerative changes could result in gangrene. Also, chemicals can induce or enhance atheroma formation, which is characterized by endothelial damage with increased permeability, monocyte adhesion, and endothelial proliferation.

Selected representative techniques to study the peripheral vascular system consist of flow measurement techniques (Smith et al., 1994), such as electromagnetic flowmetry, pulsed Doppler flowmetry, transit time flowmetry, laser Doppler fluxmetry, and laser scanner methods. These techniques allow investigation of blood flow in vessels as large as the aorta and as small as the capillary, determination of the level of perfusion in tissues, and calculation of the derived hemodynamic variable of resistance.

The two major noninvasive techniques for determining microvascular velocity are the flying spot technique and the dual-slit technique. External ultrasound may be used to examine internal vascular dimensions. A noninvasive assessment of arterial flow in rodents and monkeys can be performed using Doppler spectrum analysis (duplex ultrasound technology) (Leopold et

al., 1997). This test detects arterial compromise in extremities, functional severity, and the hemodynamic significance of vascular lesions. In most cases, the locations in the arteries involved can be designated. Information regarding the extent and effectiveness of collateral circulation can also be gained. This testing is a valuable tool for monitoring early flow compromise secondary to chronic reoccurrence of anastomotic or distal disease.

Several blood/plasma tests for clinical assessment are in active use in cardiovascular research. In general, these are tests that may be used to document a cardiovascular accident (within 48–96 hours). Their utility for risk assessment has yet to be evaluated. Specific enzymes currently being used by the research community for these purposes include LDH-I, creatinine kinase-II, and troponin. Other enzymes useful as prognostic indicators of risk of a cardiovascular accident include angiotensin converting enzyme II, plasma renin activity, endothelin-converting enzyme-1, and catecholamines (epinephrine and norepinephrine).

3.2. CONCLUSIONS AND RECOMMENDATIONS

A review of current testing guidelines was conducted to determine the types of data available for setting reference values. The approach used was to evaluate testing guidelines from the point of view of (1) life stages covered, (2) endpoints assessed generally and for specific organ systems, (3) timing and duration of exposure, and (4) evaluation of reversibility and latency to response.

The relevance of these issues to the health evaluation of children and other potentially susceptible subpopulations should be apparent from the gaps identified in each of the above sections regarding life stage assessment, endpoints assessed, timing and duration of exposures included in guideline studies, reversibility, and latency to response. Although a number of areas of toxicity testing have been discussed, this review should not be considered exhaustive, and other health effects may be as or more important for particular chemicals than those reviewed in detail here.

Issues of particular concern for children’s health that have not been discussed in great detail here are effects related to asthma and other respiratory tract toxicity. For both children and the elderly, renal and liver function can be a major factor in the disposition, metabolism, and excretion of chemicals and, therefore, their toxicity. Thus, the evaluation of toxicity and the interpretation of data in terms of its completeness will always require scientific judgment about whether or not adequate data have been collected on effects of importance at the appropriate life stages and timing and duration of exposure, for example, for a given agent.

Effects seen at the termination of a chronic study may be due to cumulative damage from a continued repeated chemical insult, but they could also be a latent response from an earlier

single or short-term multiple exposure. Thus, latent effects might be revealed in chronic studies, but it would not be clear whether they were the result of acute/short-term exposure or the chronic exposure. Specific information on the latency of a response would follow only from a clearer understanding of the mechanism of the effect and from actual "stop exposure" protocols (e.g., the satellite studies depicted in Figure 3-1) or from shorter-term exposures with follow-up over a much longer period of time. It thus follows that any chemical database that does not have exposure-response studies of lifetime duration or any specific exposure-latency protocols would not cover the possibility of latent effects.

Effects that persist throughout a designated post-exposure period may be considered irreversible; those that do not are reversible. For chronic lifetime exposures, designation of an effect as irreversible or reversible is academic, as exposure is presumed to be lifetime (i.e., there is no post-exposure period). For shorter-term values (e.g., acute, short-term) where an appreciable period of time post-exposure is anticipated, designation of an effect as reversible or irreversible becomes more relevant. Derivation of a reference value based on shorter-term exposure guideline protocols would have to fully consider the aspect of reversibility in interpretation of the data. It is important to understand the difference between an endpoint that is truly reversible and one that is related to or is a precursor of other adverse effects. For example, low birth weight may be "reversible" through catch-up growth postnatally, but it also may be related to developmental delays or other health outcomes that result from prenatal growth reduction/retardation.

3.2.1. Conclusions

From this review, the Technical Panel reached the following major conclusions:

1. There are a number of gaps in life stages covered in current guideline testing protocols, particularly in terms of the exposure periods included. In particular, there is minimal evaluation of aged animals, especially after exposures that include early development.

2. There are a number of gaps in the evaluation of endpoints included for certain systems; for example, the evaluations of the cardiovascular and immune systems in various guideline studies were reviewed as examples of systems that are minimally covered. Other systems, for example, the reproductive and nervous systems, are evaluated in more detail, but even in these systems there are gaps that need to be

considered; notably, functional evaluations are not always included or integrated with structural evaluations of particular systems.

3. Acute and short-term exposure studies are either not available or include only gross effects, so that the data needed to derive acute and short-term reference values are often not available.

4. Latency to response and reversibility are only rarely evaluated directly. These types of effects could have a major impact on hazard characterization, especially in designing acute and short-term test guideline protocols and ultimately on the risk management options that can be used for intervention or prevention.

5. Although not more specifically discussed, it is clear that there is a lack of information on toxicokinetics. The available data are generally limited to studies that are conducted in young adult animals, but there are no guideline protocols for toxicokinetic evaluations during development or in older age related to exposures and outcomes.

6. The underlying assumption that the internal dose of the active form of an agent to the target site is the relevant measure of dose clearly underscores toxicokinetics as an essential tool that must be used in both hazard identification and dose-response evaluations. This should not only continue to be a central and critical area of exploration, it should be an area of direct application to assessment activities to address various issues, including but not limited to (a) design of studies, (b) delivery to the fetus/neonate, (c) dose scaling, (d) toxicokinetic and toxicodynamic considerations, and (e) route extrapolation.

A white paper on pharmacokinetics commissioned by the Technical Panel (Versar Inc., 2001b) is meant to serve as a technical resource for the application of toxicokinetics to these and other issues addressed throughout this document. Another white paper on aging (Versar Inc., 2001a) also addresses issues of changing pharmacokinetics during this life stage.

7. Portal-of-entry effects (i.e., respiratory, gastrointestinal, dermal) are acknowledged as being important in the effects of chemicals, and they may preclude systemic toxicity as being sentinel. Chronic oral RfDs and inhalation RfCs have been developed for a

number of agents, but rarely have dermal RfDs been derived. In some cases, oral RfDs and oral cancer potency factors have been used to assess systemic toxicity from dermal exposures. However, the dermal route of exposure can result in different patterns of distribution, metabolism, and excretion than those that occur from the oral route. Dermal contact with a chemical may also result in direct dermal toxicity, such as allergic contact dermatitis, urticaria reactions, chemical irritation, and skin cancer.

The dose-response relationship for the portal-of-entry effects in skin is likely to be independent of any associated systemic toxicity exhibited by a particular chemical. Therefore, there is a long-term need for the development of dermal RfDs that consider both the systemic toxicity effects and the portal-of-entry effects of individual chemicals. In addition, there is a need for data on the dermal uptake of chemicals from soil, water, and air, including information about specific chemical forms and bioavailability from different soil types that contribute to variations in uptake. Different exposure duration RfDs, such as acute chemical injury to the skin, need to be developed.

3.2.2. Recommendations

On the basis of the review of the guideline toxicity studies, the Technical Panel makes the following recommendations (in no particular order) regarding the development of testing procedures and guidance for their use. In identifying the need for development of specific protocols, the Technical Panel is not recommending that these tests be used for every chemical or in all circumstances, as pointed out at the beginning of the chapter.

- *Develop a strategy for alternative approaches to toxicity testing, with guidance on how and when to use existing and newly recommended guidelines.* Information on all aspects of a chemical should be considered in the strategy for testing, including chemical-physical characteristics, intended use, and toxicokinetic and toxicodynamic (mode of action) data, to allow a more efficient and targeted testing approach. In addition, the strategy should consider life stages in evaluating exposures and outcomes, as well as other sensitive subpopulations.

- *Develop guidelines or guideline study protocols that will provide more systematic information on toxicokinetics and toxicodynamics (i.e., mechanism or mode of action), including at different life stages.* Such studies could provide information that would be relevant to susceptible subpopulations, including life stages (i.e., inform the

selection of the intraspecies UF). Such studies also could provide information on species differences (i.e., inform the selection of the interspecies UF). Finally, such studies can provide information to conduct route-to-route extrapolations and reduce the number of route-specific tests required to derive a reference value.

- *Develop protocols for acute and short-term studies that provide more comprehensive data for setting reference values (see Section 3.3).* The existing protocols for acute studies (except for the acute inhalation protocol with histopathologic evaluation) generally collect data only on what could be called frank effects, which may not be protective of more subtle effects.

- *Modify existing guideline study protocols to provide more comprehensive coverage of life stages for both exposure and outcomes (see Section 3.3).* Existing guideline studies do not include, for example, the evaluation of toxic effects that may occur in old age from prenatal or early postnatal exposure (including carcinogenesis) or premature aging from exposure earlier in life.

- *Collect more information from less-than-lifetime exposure to evaluate latency to effect and to evaluate reversibility of effect.* Existing guideline studies, with the exception of the acute tests and some developmental toxicity studies, expose animals up to the time of testing. Some form of "stop exposure" studies would provide useful information that could increase or decrease the level of concern for an observed toxic event.

- *Develop guidelines or guideline study protocols to assess immunotoxicity, carcinogenicity, and cardiovascular toxicity at different life stages.* Immunotoxicity and cardiovascular toxicity are presently looked at only in a cursory manner. Carcinogenicity is currently evaluated only after chronic exposure to adult animals. There is a need to integrate functional measurements into evaluations of these and other systems.
- *Explore the feasibility of setting dermal reference values for direct toxicity at the portal of entry, including sensitization.* Reference values have been derived for lesions in the gastrointestinal and respiratory tracts from direct exposure. The lack of procedures for dealing with similar effects on the skin is a glaring omission.

3.3. OPTIONS FOR ALTERNATIVE TESTING APPROACHES

The Technical Panel explored alternative testing protocols for acute toxicity testing as well as alternative protocols for subchronic/chronic toxicity testing. These are offered here as alternatives that may be used, depending on the agent being tested or the type of reference values needed.

3.3.1. Alternative Acute Toxicity Testing Protocol

The current EPA test guidelines for acute toxicity focus on the determination of an LD50 in adult test species. A gross necropsy is conducted on the animals, and histologic evaluation of target organs may or may not be conducted. Therefore, very limited information is obtained from the current protocol that would be useful for determining an acute reference value. However, a number of alternative study designs are available that would provide information for consideration in establishing the acute reference value (Gad and Chengelis, 1998).

One basic study design is shown in Figure 3-6. In this protocol, a control group and a minimum of three dose groups with 10 animals/sex/group are used. The animals are dosed once on day 1 and followed for 2 weeks. Clinical signs of toxicity are recorded daily, food consumption and body weights are recorded on days 1–4, 8, and 14. There is an interim sacrifice of 5 animals/sex/group at 3 days after dosing and a final sacrifice of the remaining animals at 2 weeks after dosing. At both sacrifices, hematological and clinical chemistry analyses are conducted, as is a urinalysis. The animals are necropsied, organ weights are recorded, and the organs are examined histologically.

Because the purpose of this study design is to provide hazard and dose-response information rather than determination of an LD50, the dose levels should be chosen accordingly. This study would initially be conducted on adult animals. As information is obtained from other toxicology and/or toxicokinetic studies, it may be necessary to conduct the study with animals at different life stages and to include other endpoints.

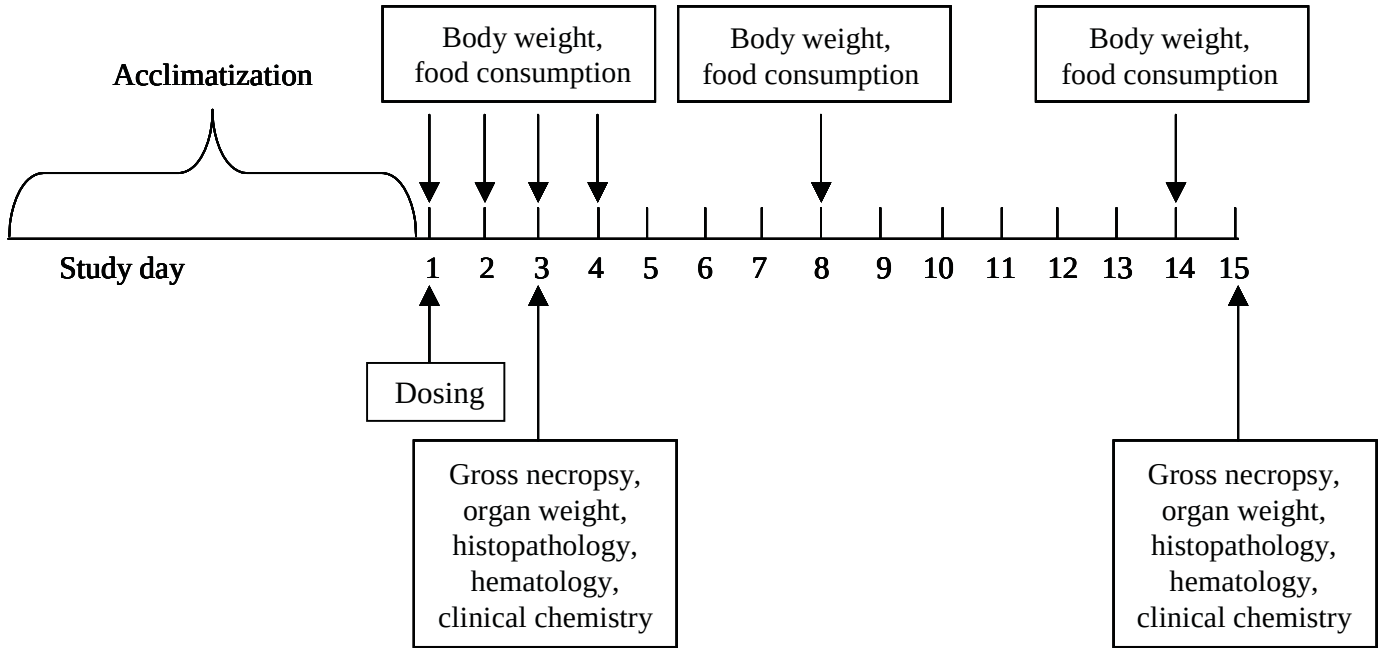


Figure 3-6. Alternative acute toxicity protocol.

3.3.2. Alternative Chronic Toxicity Testing Protocols

As stated, a review of currently available EPA guideline toxicology studies (OPPTS 870 Series) demonstrates that there is no single protocol that addresses continuous exposure through all life stages of any test species. To address this issue, two possible alternative study designs were considered: the "expanded chronic/carcinogenicity study" and the "unified screening study." These are described in some detail below and are illustrated in accompanying figures. The intent of this discussion is to demonstrate the advantages (and disadvantages) of exploring nontraditional testing paradigms; however, such discussion does not constitute a recommendation for implementation. For many chemicals, the existence of adequate (by Agency standards) stand-alone studies would preclude the need for further testing, with or without expanded or combined protocols such as those described below. In any case, any proposal to use alternative study designs in a regulatory setting should be thoroughly discussed by Agency and registrant scientists prior to study initiation.

3.3.2.1. *The Expanded Chronic/Carcinogenicity Study*

An example of a study design that would incorporate lifetime (in utero through old age) exposure is the expanded chronic/carcinogenicity study (shown in Figure 3-7), which could serve as a replacement for a standard guideline chronic/carcinogenicity study in rats. In this expanded study, female rats are assigned to treatment groups, mated, and treated with test substance throughout gestation and lactation. When pups are weaned on PND 21, they are assigned individual animal numbers and maintained within their established treatment group. Prenatal and early postnatal exposure to the test substance in this study is similar to that required for the in utero carcinogenicity study that is used to evaluate food additive chemicals for regulation by the Food and Drug Administration's Center for Food Safety and Nutrition.

The difference here is that the study duration is extended to a period of 3 years (vs. a typical chronic duration of 2 years for rats), with interim sacrifices scheduled at yearly intervals. The total number of animals used in this expanded study is greater than for a standard guideline chronic/carcinogenicity study because of the additional interim sacrifice; for each annual segment, the sacrifice of 25 rats/sex/group is required. To reduce this number, the study could be conducted with fewer animals per segment (e.g., 20/sex/group), or only two sacrifices could be scheduled (e.g., at 1.5 and 3 years). Of course, such actions will either reduce the power of the evaluation for tumor data or will eliminate examination of an important life phase.

Parameters typical of a guideline chronic/carcinogenicity study are examined in this expanded study (e.g., mortality, clinical observations, body weight, food consumption, clinical

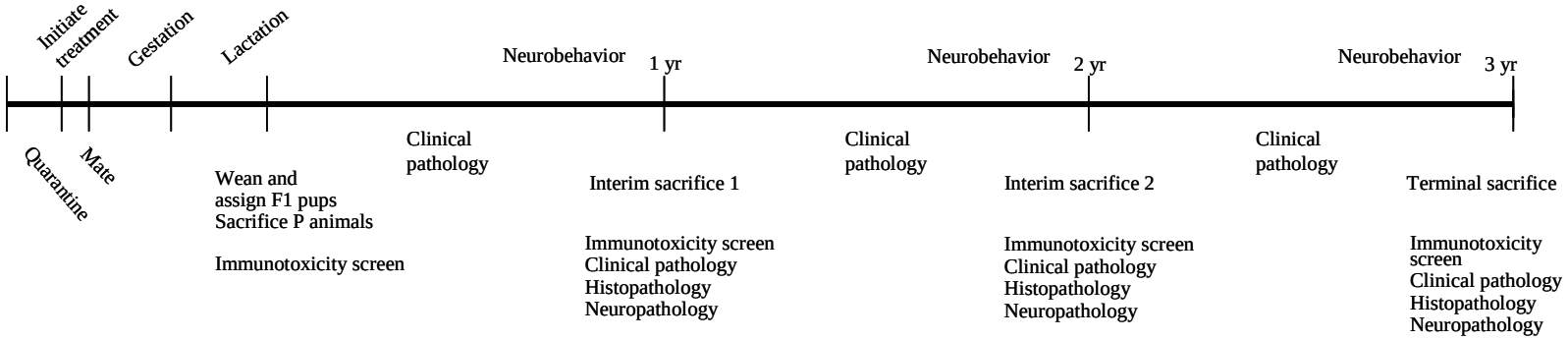


Figure 3-7. Expanded chronic/carcinogenicity study.

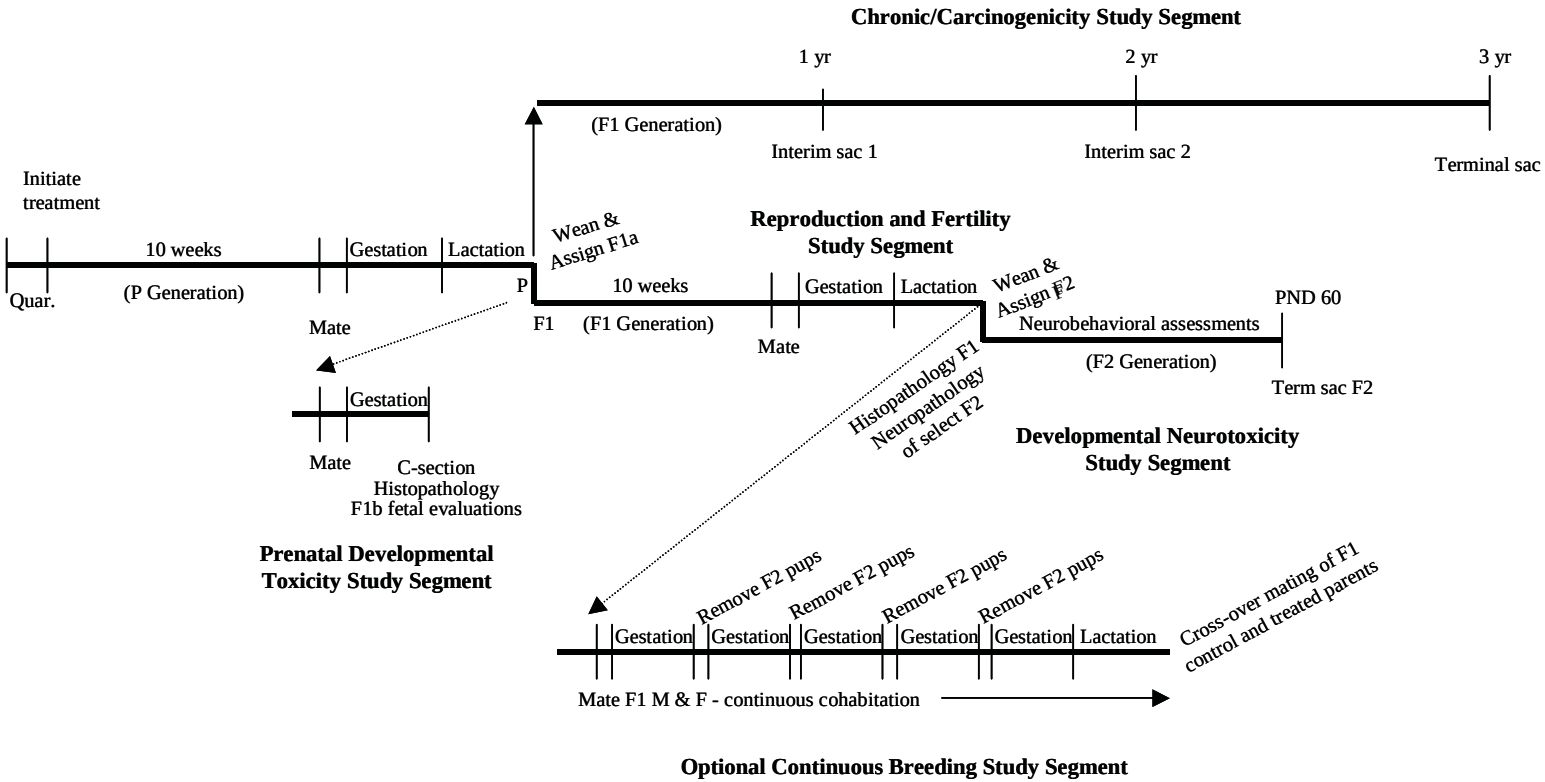
chemistry and hematology, ophthalmology, gross pathology, and histopathology). In addition, neurological and immunological evaluations are performed in the adult animals at multiple intervals into old age, which, along with the fact that the animals are exposed to the chemical during all life stages, contributes to the superiority of this study design.

Although the temporal linear nature of this study protocol makes it less complicated to conduct in the laboratory, this attribute also results in the inability to easily assess some other important endpoints, such as prenatal development, reproduction and endocrine function, and DNT. Additionally, by 3 years of age, when this study would be terminated, survival in laboratory rats may be compromised; therefore, it may be necessary to consider using feed restriction to maximize the number of animals available for in vivo and post mortem assessment of aged animals. In addition, housing from birth in specific-pathogen-free facilities may be necessary to maintain sufficient viable animals for such an extended period of time (see the background white paper on aging, Versar Inc., 2001a).

3.3.2.2. *The Unified Screening Study*

An alternative study design, the unified screening study, is illustrated in Figure 3-8. This study is composed of at least four segments: a two-generation reproduction and fertility study, an expanded chronic/carcinogenicity study, a developmental toxicity study, and a DNT study. Each of these is currently conducted as a separate study. An optional continuous-breeding study segment could be added to the design. When conducted in the rat, the unified screening study assesses all life stages of the animals and provides a means to evaluate prenatal developmental toxicity, DNT, reproduction, and endocrine function, all within animals that are derived from the same gene pool and are evaluated within two generations of the progenitor rodents that are initially placed on study.

The unified screening study begins as a typical two-generation reproduction and fertility study, with 10 weeks of treatment, mating, gestation, and lactation phases conducted according to OPPTS 870.3800. The F1 weanlings are selected for either the second generation of the reproduction and fertility study or the expanded chronic/carcinogenicity study. (As a point of clarification, at any point that animals are selected and/or assigned to a different study segment, it is assumed that the treatment group remains constant for each animal.) The parental (P) animals from the first generation are not immediately terminated; rather, they are transferred to a prenatal developmental study segment. After a short rest, they are mated. The P males can be terminated at any time point; the P females are continued through to caesarian section on approximately GD 20. The resulting F1b fetuses are processed and examined for external, soft tissue, and skeletal



Quar. = Quarantine P = Parental F1 and F2 = 1st and 2nd filial generations

Figure 3-8. Unified screening study. Study lines are not drawn to scale.

abnormalities, as is typical in an OPPTS 870.3700 study. At necropsy, however, the P-generation animals receive an extended postmortem examination, according to the procedures for the two-generation reproduction and fertility study, that includes sperm measures for the males and extensive histopathology of the reproductive and other organ systems for both sexes.

The expanded chronic/carcinogenicity study segment, using F1 animals, would continue as described above concurrently with all other segments of the unified screening study but continuing well past the time that the others have been terminated. The other F1 pups that are selected as second-generation parental animals in the reproduction and fertility study segment are treated for 10 weeks and then undergo the standard reproductive functional assessments, as specified in the OPPTS 870.3800 guideline. Because a number of F2 pups from this generation will continue on into the DNT study segment, some additional observations are required during the lactation segment of the second generation. Specifically, F2 pups are selected and assigned for neurobehavioral assessments on PND 4 (at the time of litter standardization). Preweaning observations include weekly age-appropriate clinical/functional behavioral observations conducted outside of the home cage and motor activity assessments on PNDs 13 and 17. Additional assessments of physical, reflex, and sensory development may also be conducted during this period.

At the time of weaning of the F2 pups on PND 21, those preselected for neurobehavioral assessment continue into the DNT segment and other weanlings are sacrificed for postmortem evaluations that address the considerations of both the reproduction protocol (including organ weight data) and the DNT protocol (requiring in situ perfusion fixation of tissues and neuropathology, including morphometric analysis). The DNT-segment F2 animals are evaluated as per OPPTS 870.6300, which includes multiple assessments of clinical and functional observations, motor activity, auditory startle habituation, and learning and memory. They are maintained until termination (with postmortem evaluations, including neuropathology following perfusion fixation) at approximately PND 60.

Also at the time of weaning of the F2 pups, a decision could be made to either sacrifice the F1 parental animals immediately (with the usual sperm measures and postmortem evaluations) or to maintain them through a continuous-breeding reproduction study segment, sequentially mating the F1 adults for the production of five litters (the pups from these litters are terminated in early lactation). This continuous-breeding study segment, which would extend the reproduction study for about 100 additional days, uses a standardized assessment protocol that has been well characterized in the peer-reviewed literature (Lamb, 1985; Lamb et al., 1985; Morrissey et al., 1989) but does not have a corresponding OPPTS guideline.

As previously stated, in this unified study protocol, the animals are both exposed and assessed during all life stages, and the evaluation of both structural and functional endpoints for multiple organ systems are maximized in the overall design, for example, by the inclusion of immunotoxicity and neurotoxicity endpoints. There is one notable exception to this statement in that reproductive senescence is not standardly examined. Nevertheless, if the two-generation reproduction study segment identifies problems with fertility or cyclicity, this could be pursued more rigorously by the addition of testing during the second or third year of the expanded chronic/carcinogenicity study, for example, evaluating cyclicity in aged female rats and/or evaluating ovarian follicular counts and atrophy at sacrifice.

Another benefit of using the unified screening study design is that it results in the purchase and use of many fewer naive animals for study initiation and it increases the efficient utilization of animals, particularly of the F2 offspring from the reproduction study.

Although there are obvious benefits in using a unified screening study, there are also a number of concerns or potential problems involved with its conduct. Although it is assumed that treatment levels and route of administration will remain constant across all study segments, this approach to dose-setting and route selection may not always be optimal for every phase. Generally, a temporal nonlinear design of this nature is more difficult to manage in the laboratory. The strain of rat generally used in toxicity studies is the Sprague-Dawley, whereas the Fischer 344 rat is often used in the standard chronic/carcinogenicity study. Fischer 344 rats have not typically been used in reproductive and developmental toxicity studies. The use of either strain for the unified study could compromise the use of historical data for comparison, for example, for the chronic/carcinogenicity study if the Sprague-Dawley is used and for the reproductive and developmental toxicity studies if the F344 is used.

As study complexity increases, so does the opportunity for error. In some cases, a serious technical error in one study segment could compromise subsequent study segments and result in an extensive waste of animals and resources. As with the expanded chronic/carcinogenicity study discussed above (section 3.3.2.1.), survival during the chronic/carcinogenicity study segment in this design may need to be enhanced via feed restriction. Also, if the test substance interferes with reproduction or results in increased mortality, the number of offspring that are available for assignment to subsequent study segments (e.g., the selection of F2 animals for the DNT phase) may be critically reduced. An additional but similar problem could arise when selecting F1 animals for the expanded chronic/carcinogenicity study segment at the same time as for the second generation of the reproduction and fertility study segment, because a large number of offspring needs to be available all at one time. Additionally, the offspring that are assigned to the

chronic/carcinogenicity segment should be genetically diverse within each dose group and should originate from as many litters as possible (i.e., not be siblings).

A number of possible solutions that could be used alone or in combination to increase the number of F1 pups available for selection in other study phases include the following:

1. Reducing the number of animals needed for the expanded chronic/carcinogenicity segment by examining fewer animals at each serial sacrifice or by abandoning the final year of evaluation, as described above.

2. Reducing the number of animals assigned to the second generation of the reproduction and fertility study segment; however, this could compromise the number of F1 offspring that would be available for the DNT study segment.

3. Standardizing litters to 10 rather than 8 pups per sex and assuming that no litter has less than 10 pups and that no pups die during lactation. Because some small litters and neonatal pup deaths almost always occur, even in controls, it is wiser to design the study more conservatively in order to avoid discovering that there are not enough F1 pups to assign to the later segment(s).

4. Assigning additional females to the two-generation reproduction and fertility study segment in order to produce extra F1 pups for selection. Although even a modest increase in the number in each group would increase the probability of producing a sufficient number of F1 pups, a larger number of litters would generally be required in order to ensure genetic diversity among the weanlings that are assigned to the chronic/carcinogenicity study segment. This could be accomplished by placing additional P-generation females or breeding pairs on study, perhaps combined with 2:1 mating procedures, or by mating the males with the reproduction and fertility study-segment females first and then with an extra set of females. One adverse consequence of placing additional females on study so that their litters can be used for selection of genetically diverse offspring for the chronic/carcinogenicity study segment is that this method results in a larger number of excess F1 weanling pups that would not be used for evaluations in this protocol. However, these pups could be used for other evaluations, such as immunotoxicity, specialized neurotoxicity tests, or adult onset disease or diseases of aging.

Some of the above options appear to be more advantageous and preferable than others; however, no recommendation is proffered because the list is presented only to illustrate some of the many possibilities that could be used in a customized study design. It should be noted that simply combining the reproduction and fertility study and the DNT study when a two-generation reproduction and fertility study has not already been conducted greatly reduces the total number of animals that would be required to conduct the two studies individually. No additional animals are required over the reproduction and fertility study alone, and there is greater efficiency in the use of the F2 offspring when the DNT study is conducted in that group.

4. FRAMEWORK FOR SETTING ACUTE, SHORT-TERM, LONGER-TERM, AND CHRONIC REFERENCE VALUES

As noted in Chapter 2, the Technical Panel is recommending that EPA begin deriving acute, short-term, and longer-term reference values in addition to chronic reference values. The approach to reference values discussed here is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear and/or threshold mode of action. Although there has been a dichotomy between cancer and noncancer risk assessment in terms of the underlying assumption about the linearity or nonlinearity of the dose-response curve, there is a move toward harmonization among approaches for all health effects (Butterworth and Bogdanffy, 1999; Bogdanffy et al., 2001). This includes recognition of the possibility that some carcinogenic agents may work through nonlinear mechanisms (U.S. EPA, 1999a), whereas some agents that produce effects other than cancer may work through linear mechanisms (see discussion in U.S. EPA, 1998d). Thus, the decision to use a linear extrapolation approach or a reference value approach should take into consideration the underlying mode of action and presumed dose-response relationship.

The approach described here is the default approach to be used when the assumption is a nonlinear and/or threshold mode of action, except for cases where other methods have been developed (e.g., in support of the NAAQS). This approach can and should be improved upon or replaced when more specific data on toxicokinetics and mode of action are available to allow the development of a chemical-specific or a biologically based dose-response model for prediction of risks to humans and to susceptible individuals within the population. The acute, short-term, longer-term, and chronic reference values derived on the basis of the recommendations in this report should be included in IRIS after appropriate internal, external, and consensus review. These values would then be available for use by program offices, where appropriate.

In this chapter, we discuss the definitions of the exposure durations and the proposed changes in the definition of the corresponding reference values. In addition, several issues are discussed regarding the adequacy of studies and characterization of the extent of the database with regard to sufficiency of data for deriving reference values. The derivation of reference values also is discussed with regard to dosimetric adjustment and application of UFs. A number of recommendations are made with regard to this process. In particular, the Technical Panel recommends incorporating the concept of life stage and expanding the endpoints evaluated as well as consideration of duration and timing of exposure and latency to response in characterizing the extent of the database used for setting reference values. The Technical Panel

strongly encourages the use of a narrative description of the database, including strengths and limitations, rather than a single confidence statement for support of a reference value.

The adjustments required for derivation of the human equivalent dose (HED) for oral and dermal exposure and the human equivalent concentration (HEC) for inhalation exposure are described and discussed. This is followed by recommendations about the evaluation and comparison of data for the POD, based on an analysis of each potentially limiting endpoint carried through the reference value derivation process, followed by selection of the appropriate health-protective reference value.

Finally, the Technical Panel emphasizes that considerable use of scientific judgment is advisable and necessary in practically all phases of the process, especially in the application of UFs. This review and its recommendations build on the principles in the Agency's handbook on risk characterization (U.S. EPA, 2000b), which calls for transparency, clarity, consistency, and reasonableness in the risk assessment process.

4.1. DEFINITIONS OF EXPOSURE DURATIONS FOR USE IN SETTING REFERENCE VALUES

The Technical Panel proposes the following definitions of exposure duration as a first step in the development of consistent approaches for the Agency. These definitions are based on exposure durations for humans; analogous exposure durations for rodents are indicated for the longer-term and chronic durations. The definitions are not intended to be rigid specifications, but simply general descriptions of the relevant exposure time period. Their application is meant to be flexible, so that, for example, if a 4-month animal study is available, it may be used as the basis for both a longer-term and a chronic reference value.

The definitions were developed on the basis of the review of values currently set by various program offices (see Chapter 2), and they have been standardized to be compatible with those definitions currently used by various program offices within the Agency. The definitions for various durations, as follow, were discussed at an EPA Risk Assessment Forum Colloquium (CDM Group Inc, 2000).

Acute: Exposure by the oral, dermal, or inhalation route for 24 hours or less.

Short-term: Repeated exposure[4] by the oral, dermal, or inhalation route for more than 24 hours, up to 30 days.

Longer-term: Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the life span in humans[5] (more than 30 days up to approximately 90 days in typically used laboratory animal species[6]).

Chronic: Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species).

The Technical Panel believes there is an advantage in having a central source of consensus reference values of various exposure durations available to risk assessors throughout EPA. EPA Program Offices could use the values for risk assessments in which the known or assumed exposure duration approximated the exposure duration in the appropriate reference value definition. The Panel recognizes that Program Offices may make further adjustments to the reference values depending on circumstances that are unique in their assessments.

The Technical Panel recommends that the principles of sound science be used when the expanded array of reference values are developed. The Panel cautions that the exposure-response relationships for all durations of exposure and issues of latency need to be carefully considered to ensure that there are no obvious conflicts in the series of recommended reference values for any specific chemical. This analysis can become complex in a case where the toxicological endpoint may differ for the different durations of exposure.

The Technical Panel is aware that there will be data limitations for an individual chemical that may preclude development of all four reference values. For example, currently, a chronic RfD or RfC would not ordinarily be considered for inclusion in the IRIS database unless

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[4]A repeated exposure may be either continuous, periodic, or intermittent. A continuous exposure is a daily exposure for the total duration of interest. A periodic exposure is one occurring at regular intervals, e.g., inhalation exposure 6 hrs/day, 5 days/wk or oral exposure 5 days/wk. An intermittent exposure is one in which there is no effect of one exposure on the effect of the next; this definition implies sufficient time for the chemical and its metabolites to clear the biological system before the subsequent exposure, that is, noncumulative pharmacokinetics. A periodic exposure may or may not be intermittent.

[5]The lifespan value used depends on the situation under consideration. For example, an average of 70 years has been the typical default used for chronic exposures, but the average life span based on U.S. census data is 75.5 years (U.S. EPA, 1997a).

[6]Typically used laboratory animal species refers to rats, mice, and rabbits, for example.

a subchronic or chronic study were available. Similarly, where data of the type needed for deriving acute, short-term, or longer-term reference values are not available, theses values would not ordinarily be considered for inclusion in the IRIS database. In situations where an acute, short-term, or longer-term reference value is needed but appropriate data fitting the definition for duration are not available, then the Program Office may wish to consider several options. One option would be to not develop a reference value for that particular duration of exposure. Another option would be to use the reference value for the next longer duration of exposure as a conservative estimate of a reference value that would be protective for a short-term exposure duration. For example, the Office of Water (see Chapter 2) will use a longer-term health advisory for a child as a conservative estimate for a 10-day exposure in the absence of data to derive a 10-day health advisory. Other program-specific options might also be considered.

The Technical Panel is aware that time and resources need to be considered when implementing its recommendations. The IRIS program has begun to implement a pilot program to test whether development of the expanded array of reference values is practical and can be accomplished without unduly delaying the completion of an IRIS file. As a part of the pilot, the IRIS program will need to identify the methods to be used in deriving these additional values.

4.2. PROPOSED CHANGES IN THE REFERENCE VALUE DEFINITIONS

In the process of considering definitions for different duration reference values, the Technical Panel discussed several issues that have been raised about the current definitions of the chronic RfD and RfC (Box 4-1). The following items describe the issues and the recommended changes.

1. The parenthetical statement in the current RfD and RfC definitions—"with uncertainty spanning perhaps an order of magnitude"—has been variously used by risk assessors and risk managers to mean that the estimate is at the upper end, the lower end, or the middle of the range of an

Box 4-1. Current definitions for the chronic oral RfD and inhalation RfC

RfD: an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or BMD, with UFs generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments.

RfC: an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or BMD, with UFs generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments.

order of magnitude. This statement has been removed from the proposed revision of the definition for reference value (Box 4-2), and it is recommended that issues of uncertainty/variability be discussed qualitatively as part of the weight of evidence and characterization of the database. Attempts to adapt such a qualitative derivation process to formal quantitative procedures for prediction of accuracy presents major difficulties. A particularly obvious difficulty is that the same definition and phrase were applied to different reference values that may have varied markedly in their underlying data and, thus, their potential for accuracy. For example, the same "order-of-magnitude" range applied equally to a robust reference value with known exposures plus observable and quantifiable dose-response data derived from a segment of the human population and to a marginal reference value based only on animal data with minimal supporting information.

Box 4-2. Proposed revisions in the reference value definitions

Reference Value: an estimate of an exposure, designated by duration[a] and route, to the human population (including susceptible subgroups[b]) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a BMDL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors[c] applied to reflect limitations of the data used.

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[a] The generalized durations are similar to those given in Section 4.1. for acute (≤24 hours), short-term (up to 30 days), longer-term (up to 10% of average lifespan), and chronic (up to a lifetime), all considered to be continuous exposures throughout the duration specified.

[b] Susceptible subgroups may refer to life stages, e.g., children or the elderly, or to other segments of the population, e.g., asthmatics or the immune-compromised, but they are likely to be somewhat chemical specific and they may not be consistently defined in all cases. See below (Section 4.3.2.3) for further discussion.

[c] See discussion later in this chapter (Section 4.4.5) on application of uncertainty/variability factors.

The Technical Panel notes a lack of support from the external reviewers of this document for any such prediction and recommends that the database characterizations for reference values be approached in a comprehensive way, as discussed in Section 4.3 to ensure that they are authoritative and as complete as possible in order to yield qualitative information about the range that could be predicted around the individual estimates rather than attempting quantitative evaluations of accuracy and ranges.

2. The term “deleterious” is considered ambiguous by some, so it has been replaced with the term “adverse,” because the latter is more commonly understood in the context of data evaluation and selection of endpoints for setting reference values.

3. In the spirit of harmonization of risk assessment approaches for human health effects, it has been recommended that health effects no longer be categorized as “cancer” or “noncancer” for the purposes of hazard characterization and dose-response analysis (U.S. EPA, 1997b, 1998d; Bogdanffy et al., 2001). As indicated earlier, the approach to reference values discussed here is intended for risk assessments for any type of health effect known or assumed to be produced through a nonlinear and/or threshold mode of action (which may include U-shaped or other nonmonotonic dose-response curves as well as thresholds). In light of this recommendation, the term “noncancer” has been removed from the definition, denoting the move toward defining approaches for low-dose estimation or extrapolation based on mode of action. It is recommended that this issue be considered further in the deliberations by the Risk Assessment Forum’s Technical Panel on a framework for harmonization of approaches for human health risk assessment.

To fulfill the need for consistency in the designation of various duration reference values, the Panel recommends that the terminology for reference values be standardized. Rather than continuing to use RfD and RfC only to denote chronic oral and inhalation reference values, respectively, standardized terminology should be developed that denotes both duration and route of exposure. Although Technical Panel members did not come to agreement on the best way to do this (and we welcome alternative suggestions), the terminology shown below is offered as an example of the way in which consistent labels could be developed and used. Either new standard terminology, (e.g., reference value) could be used, or RfD and RfC could continue to be used, but they would always need to be accompanied by the qualifying duration of exposure and, in the case of the RfD, by the route of exposure. Thus, the following alternatives for terminology are offered:

Acute (Oral, Dermal) Reference Value or Dose, Acute (Inhalation) Reference Value or Concentration: RfV_{AO}, RfV_{AD}, RfV_{AI}; RfD_{AO}, RfD_{AD}, RfC_{AI} or RfC_{A}

Short-term (Oral, Dermal) Reference Value or Dose; Short-term (Inhalation) Reference Value or Concentration: RfV_{SO}, RfV_{SD}, RfV_{SI}; RfD_{SO}, RfD_{SD}, RfC_{SI} or RfC_{S}

Longer-term (Oral, Dermal) Reference Value or Dose; Longer-term (Inhalation) Reference Value or Concentration: RfV_{LO}, RfV_{LD}, RfV_{LI}; RfD_{LO}, RfD_{LD}, RfC_{LI} or RfC_{L}

Chronic (Oral, Dermal) Reference Value or Dose; Chronic (Inhalation) Reference Value or Concentration: RfV_{CO}, RfV_{CD}, RfV_{CI}; RfD_{CO}, RfD_{CD}, RfC_{CI} or RfC_{C}

The Panel recommends that endpoint- or life stage-specific reference values such as the RfD_{DT} (reference dose for developmental toxicity), which were originally proposed in *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), not be derived. Rather, a sample reference value should be calculated for each relevant and appropriate endpoint and these should then be considered in the derivation of various duration reference values. Reference values should be derived to be protective of all types of effects for a given duration of exposure and are intended to protect the population as a whole, including potentially susceptible subgroups. Thus, the RfD_{DT} concept of a critical window of exposure for some health effects is addressed in the adoption of the less-than-chronic reference values. This recommendation does not preclude, however, using specific common endpoints in the assessment of cumulative risk for mixtures or chemicals that have a common mode of action or for risk management purposes.

4.3. CHARACTERIZATION OF THE EXTENT OF THE HEALTH-RELATED DATABASE FOR SETTING REFERENCE VALUES

A necessary first step in hazard characterization is the critical evaluation of all pertinent and relevant human and animal data that are available in the open literature as well as data submitted to the Agency in response to various regulatory standards, data call-ins, or other requirements and agreements.

4.3.1. Review of Studies

Data will be available from a wide variety of sources, including studies conducted according to EPA guidelines, studies conducted by industry using Organization for Economic Cooperation and Development or other protocols, experimental studies conducted by academic researchers, epidemiology studies, case reports or series, and controlled clinical studies in

volunteers.[7] These studies will be of widely differing quality; EPA must evaluate each study to determine whether it is of acceptable quality.

4.3.1.1. *Adequacy of Studies*

The following list of questions could be helpful in the process of evaluating data from animal and human studies.

All types of studies:

- What was the purpose of the study and is there a clearly delineated hypothesis?
- Is there sufficient description of the protocol, statistical analyses, and results to make an evaluation?
- Were the appropriate endpoints assessed in the study?[8] Were the techniques used for the assessment scientifically sound?
- Were appropriate statistical techniques applied for each endpoint? Was the power of the study adequate to detect effects?
- Did the study establish dose-response relationships? Was a BMD lower confidence level (BMDL), LOAEL or NOAEL established?
- Is the shape of the dose-response curve consistent with the known toxicokinetics of the test compound?

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[7]Currently, OPP is reviewing its policy concerning use of human data from studies in which there is intentional pesticide exposure, and it has asked the National Academy of Sciences for input on the acceptability of such studies and ethical criteria for their use under the Protection of Human Subjects Rule (the "Common Rule") (EPA, 2001c).

[8]A chemical may cause a variety of toxic effects depending on the amount, duration, timing, and pattern of exposure (i.e., continuous, periodic, or intermittent). These effects may range from severe—such as death—to more subtle biochemical, physiological, or pathological changes in one or more organ systems. In addition, the effects will vary depending on their latency following exposure and when the observations are made. Primary attention is given in risk assessment to those effects in the lower exposure range and/or the effects most biologically appropriate for a human health risk assessment.

- Do effects fit with what is known about mode of action?

- Is the dose-response curve for precursor events consistent with the dose-response curve for clinical effects?

- Are the results of the study biologically plausible?

- What uncertainties exist? Do the results of the study indicate the need for follow-up studies to reduce uncertainties?

- Are the study conclusions supported by the data?

Human studies:

- What were the data sources for exposure, health status, and risk factors (e.g., questionnaires, biological measurements, exposure/work history record reviews, or exposure/disease registries) and what were their strengths and limitations?

- What methods were used to control, measure, or reduce various forms of error (e.g., misclassification or interviewer bias, confounding factors and potential effect modifiers) and their potential impact on the findings? What is the validity (accuracy) and reliability (reproducibility) of the methods used to determine exposure and outcome? What were the response rates?

- What major demographic and other personal factors were examined (e.g., age, sex, ethnic group, socioeconomic status, smoking status, and occupational exposure)? What other climate or life stage factors were important for the endpoints and exposures assessed?

- Were the findings examined for biologic plausibility, internal and external consistency of the findings, and the influence of limitations of the design, data sources, and analytic methods?

Animal studies:

- Was the study sufficiently documented (e.g., conducted in accordance with good laboratory practices)?

- Were appropriate analytical techniques used to measure the stability, homogeneity, and actual level of the test substance in the study (in the water, feed, air, etc.)?

- Was an appropriate animal species used?[9] Was an appropriate number of animals used? Were sex and age considered?

- Were the dose levels appropriate? What was the basis for choosing the dose levels?

- Was an appropriate method used to assign the animals to dose groups?

- Was an appropriate route and matrix of exposure employed?[10]

- Was the duration of exposure adequate for the particular study design?

- Were possible alterations in metabolism considered at the higher exposure levels?

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[9]The laboratory animals used most often are the rat, mouse, rabbit, guinea pig, hamster, dog, or monkey. When reviewing these studies, the risk assessor makes judgments about the ability of the study to predict the potential for toxicity in humans and tries to select data from the species that is most relevant to humans using the most defensible biological rationale. When available, comparative toxicokinetics can be used to support this decision. Absent a clearly most-relevant species, the most sensitive mammalian species is used, that is, the species that shows toxicity at the lowest exposure level.

[10]The most appropriate route of exposure is the route for which an evaluation is to be made. The toxicity of the chemical may differ with route of exposure because of differences in mechanism of action or toxicokinetics (absorption, distribution, metabolism, and excretion). Development of data to establish dosimetry for the purpose of route-to-route extrapolation is encouraged; however, route-to-route extrapolation is inappropriate when based exclusively upon default assumptions regarding exposure and toxicokinetics. Even within the same route of exposure, responses may differ due to alterations in toxicokinetics, for example, dietary or water exposure versus oral gavage.

Professional judgment is required to decide, on the basis of a thorough review of all available data and studies, whether any observed effect is adverse and how the results fit with what is known about the underlying mode of action. These judgments require the input of experts trained in toxicology, statistics, and epidemiology and, often, of specialists in the structure and function of the target organ systems. Both the biological and the statistical significance of the effects are considered when making these judgments. Biological significance is the determination that the observed effect (a biochemical change, a functional impairment, or a pathological lesion) is likely to impair the performance or reduce the ability of an individual to function or to respond to additional challenge from the agent. Biological significance is also attributed to effects that are consistent with steps in a known mode of action. Statistical significance quantifies the likelihood that the observed effect is not due to chance alone. Precedence is given to biological significance, and a statistically significant change that lacks biological significance is not considered an adverse response.

For many discrete or quantal endpoints (e.g., birth defects, tumors, or some discrete pathological changes), this judgment is more straightforward because criteria have been established for deciding what type and incidence of effects are to be considered to be adverse, and an increase above the background rate can be judged using statistical tools. In the case of continuous measures (e.g., body weight, enzyme changes, physiological measures), this tends to be more difficult, because the amount of change to be considered adverse has not been defined by toxicologists or health scientists. Consequently, the endpoint is often decided in the context of the endpoint itself, the study, and the relationship of changes in that endpoint to other effects of the agent.

Decisions about the amount of change to consider adverse must always be made using professional judgment and must be viewed in light of all the data available on the endpoint of concern. All toxicological data on a chemical must be reviewed before deciding whether an effect is biologically significant and adverse. Using a default cutoff value to define adversity for continuous measures may result in an inappropriate interpretation of data and less than optimum evaluation of a chemical's effects.

4.3.2. Issues to be Considered in Characterizing the Database for Risk Assessment

4.3.2.1. *The Weight-of-Evidence Approach*

A weight-of-evidence approach such as that provided in EPA's RfC Methodology (U.S. EPA, 1994) or in EPA's proposed guidelines for carcinogen risk assessment (U.S. EPA, 1999a) should be used in assessing the database for an agent. This approach requires a critical

evaluation of the entire body of available data for consistency and biological plausibility. Potentially relevant studies should be judged for quality and studies of high quality given much more weight than those of lower quality. When both epidemiological and experimental data are available, similarity of effects between humans and animals is given more weight. If the mechanism or mode of action is well characterized, this information is used in the interpretation of observed effects in either human or animal studies. Weight of evidence is not to be interpreted as simply tallying the number of positive and negative studies, nor does it imply an averaging of the doses or exposures identified in individual studies that may be suitable as PODs for risk assessment. The study or studies used for the POD are identified by an informed and expert evaluation of all the available evidence.

4.3.2.2. *Use of Human and Animal Data in Risk Assessment*

Adequate human data are the most relevant for assessing risks to humans. When sufficient human data are available to describe the exposure-response relationship for an adverse outcome(s) that is judged to be the most sensitive effect(s), reference values should be based on human data. Much more data on a wide range of endpoints typically are required to establish confidence that there are no effects of exposure. If sufficient human data are not available to provide the basis for reference values, data from animal studies must be employed. It is advantageous if some human data are available to compare with effects observed in animals, even if the human data are not adequate for quantitative analysis. Availability of data on effects in humans at least allows qualitative comparison with effects observed in animals for determining whether toxicity occurs in the same organ systems and whether the nature of the effects is similar or different. If no human data are available, reliance must be exclusively on animal data. In that case, attention should be paid to whether data are available in more than one species and, if so, whether the same or similar effects occur in different species and possible sources of any observed differences.

One of the major default assumptions in EPA's risk assessment guidelines is that animal data are relevant for humans (e.g., U.S. EPA, 1991, 1996, 1998c). Such defaults are intended to be used in the absence of experimental data that can provide direct information on the relevance of animal data.

Several types of information should be considered when determining the relevance or nonrelevance of effects observed in animal models for humans. This information is used in a variety of ways, from determining the role of metabolism in toxicity (Is the parent chemical or a metabolite responsible for toxicity?), to assessing whether homologous activity would be

expected across species (Do humans share the sensitivity of the animal model, or is the response due to some species-specific idiosyncratic reaction?), to determining whether or not a threshold is likely to exist for the response (Are repair mechanisms capable of maintaining a homeostatic process?). All of this information must be weighed in light of the known heterogeneity of the human population versus the relatively inbred status of laboratory animals used in toxicity testing studies and housed under carefully controlled environmental conditions.

Table 4-1 presents several factors to consider when evaluating the weight of evidence about the likelihood of the occurrence of effects in humans that is based on animal data (in conjunction with human data, if available). The table is not necessarily intended to delineate all factors that may need to be considered, but rather to provide a framework for evaluation and interpretation. It is important to evaluate the database in a holistic manner, determining strengths and weaknesses that are relevant to the overall assessment. Each chemical and database presents a unique set of issues that must be evaluated critically and thoughtfully.

The dose-response nature of the data is an important characteristic of the database or individual study. When data are **dose related**, that is, when the incidence and/or intensity of response changes in an orderly manner as a function of dose, the effect should be considered to be of greater importance than when there is no apparent association between exposure and toxicity. Note, however, that the dose-response relationship need not be monotonic. U-shaped (or inverted U-shaped) dose-response functions are not uncommon in toxicology. For example, a chemical may induce an enzyme at low doses and inhibit it at high doses. Similarly, many solvent-like chemicals (including alcohol) produce increased motor activity at lower doses and depressed activity at high doses.

Similarly, comparative **toxicokinetic/metabolism** data that suggest qualitative and quantitative comparability to that in humans would support the relevancy of animal data. Evidence suggesting a difference in toxicokinetics/metabolism would require additional exploration regarding whether the difference(s) results in a major qualitative or quantitative difference in internal dose in humans.

The **similarity of effects** between species is also an important aspect in characterizing the database. Similar effects in more than one species indicate that the effect provides increased weight of evidence for the risk assessment process, even if such data are not available in humans. In contrast, response data that show inconsistency of effects among studies and/or species that cannot be explained by differences in toxicokinetics/metabolism or timing and/or magnitude of exposure, may suggest that less emphasis be placed on the effect. “Similarity” does not necessarily require identical effects between species. For example, changes in motor activity in

Table 4-1. Factors for evaluation of the weight of evidence regarding the likelihood of effects in humans

Factor	Increased weight	Decreased weight
Dose-response relationship	Orderly change in effect as a function of exposure (need not be monotonic)	No identified relationship between exposure and magnitude of effect
Toxicokinetics/ metabolism	Qualitative and quantitative comparability between humans and animals	Qualitative and quantitative differences between humans and animals
Similarity of effects	Similar effects in more than one animal species or in animals and humans	Inconsistency of effects among studies and/or species that cannot be explained by differences in timing and/or magnitude of exposure or toxicokinetics/metabolism
Mode of action	Demonstration of homologous mode of action in animal model and humans	Evidence suggesting that the mode of action is species specific and irrelevant to humans
Temporal relationship	Consistent temporal relationship between exposure and effect	Lack of temporality between exposure and effect

animals evaluated in the neurotoxicity screening test and cognitive effects in humans would generally be considered similar, because both are indicative of changes in nervous system function.

Mode of action information is also important in understanding whether a particular effect may be important for humans. For example, a transient reduction in anogenital distance in the postnatal animal following perinatal exposure to an anti-androgen has increased weight if the chemical is also known to act as an anti-androgen in humans. Likewise, the interpretation of increased skeletal variants observed following exposure to many chemicals would be enhanced by data indicating that the mechanistic pathways for these agents and the overall biological significance defined were also a possibility in humans. Mode of action data are also important in determining whether various chemicals work by common modes or mechanisms of action, which would then be considered in a cumulative risk assessment.

Another criterion that is important in evaluating data is the **temporal relationship** between exposure and effect. The exposure should precede the effect at an interval that is consistent with what is known about the toxicokinetics and mode of action of the agent. It may be the case, however, that higher doses produce a shorter latency to effect than do lower doses.

4.3.2.3. *Characterization of Effects in Potentially Susceptible Subpopulations*

A dose-response analysis for potentially susceptible subpopulations should be done as part of the overall dose-response analysis for health effects in general. "Susceptible" in this context means a differential (greater) response at the same internal dose in a particular segment of the population due to intrinsic (possibly unknown) factors. "Susceptible subpopulations" is used here to refer both to life stages and to other factors that may predispose individuals to greater response to an exposure. Life stages may include the developing individual before and after birth up to maturity (e.g., embryo, fetus, young child, adolescent), adults, or aging individuals. Other susceptible subpopulations may include people with specific genetic polymorphisms that render them more vulnerable to a specific agent or people with specific diseases or pre-existing conditions (e.g., asthmatics). The term may also refer to gender differences, lifestyle choices, or nutritional state.

It is important to recognize that little basis currently exists for a priori identification of susceptible subpopulations for many chemicals. Without other data to raise suspicions, only the evaluation of effects in various segments of the population such as those mentioned above can identify susceptible subpopulations for a particular chemical and a particular set of exposure conditions. In some situations, differential exposure rather than differential susceptibility per se may be the critical issue (e.g., hand-to-mouth activity in toddlers). Economic differences may also result in differential exposure and susceptibility.

A great deal of attention has been given in recent years to the issue of children as a susceptible subpopulation. Several approaches have been proposed for characterizing the database concerning the potential pre- and postnatal toxicity of a particular chemical and providing some guidance as to the weight of evidence or degree of concern for children's health. However, each approach has been developed for a slightly different purpose and, as such, is generally complementary to, but not the same as, the other approaches.

EPA's developmental toxicity (U.S. EPA, 1991) and reproductive toxicity (U.S. EPA, 1996) risk assessment guidelines describe an approach that characterizes the database as sufficient or insufficient to judge whether a chemical does or does not pose a hazard within the context of dose, route, duration, and timing of exposure. The International Programme on Chemical Safety (IPCS) (IPCS, 1995) proposed an approach based on the quality of information gathered in developmental and reproductive toxicity studies and the types of data that were not available from these studies. EPA's draft 10X toxicology report (U.S. EPA, 1999b) further extended the recommendations for characterizing risks to children's health within the context of the FQPA by discussing issues that would increase or decrease the level of concern.

The present report endorses and extends the recommendations of the 10X Toxicology Working Group's report by incorporating the issues dealing with level of concern into a framework for evaluating the evidence regarding the identification and characterization of susceptible subpopulations (see below). A workshop was held recently to discuss aspects of a framework for children's health risk assessment and to emphasize a broader perspective on the issues that should be considered in hazard characterization, dose-response assessment, exposure assessment, and risk characterization for children as a susceptible subpopulation (ILSI RSI, 2001).

In contrast with the attention paid to children and asthmatics as potentially susceptible subpopulations in recent years, little attention has been focused on risk assessment for other potentially susceptible subgroups. As outlined in Chapter 3, there currently are no requirements in EPA animal study protocols for exposure during old age or for outcome evaluations near the end of the life span following earlier life stage exposures. Similarly, healthy animals that are more genetically homogeneous than humans are used in standard toxicity testing protocols, and information on pre-existing conditions or genetic polymorphisms is largely unavailable from animal studies.

Human studies also usually employ healthy nonelderly individuals, although some studies in more susceptible populations have been conducted, such as studies of the effects of air pollutants in asthmatics. Individuals who have identified risk factors that are not the focus of a study are usually excluded from the study sample. It is important to consider such characteristics of the database if human data are used as the basis for the risk assessment.

As can be seen in Table 4-2, several issues must be considered in assessing the potential for some subpopulations, including different life stages, to have greater susceptibility than others to a chemical. These include the **timing (life stage)-response relationship,** indicating greater susceptibility to exposure at some life stages than at others; whether effects are of a **different type** in identifiable subgroups of the population; and the **dose-response relationship**, that is, whether effects are observed at different levels of exposure in different subpopulations.

Another important consideration is whether effects are observed at the same dose but with a shorter **latency** in different subpopulations. Additionally, differences among groups in terms of the **seriousness** and **reversibility of effects** must be considered. For example, an agent may produce relatively mild and reversible neurological effects in adults but produce permanent behavioral impairment following in utero exposure. It is also important to keep in mind that effects that may initially appear to be reversible may re-appear later or be predictive of later adverse outcomes. This is probably best exemplified by certain outcomes following a

Table 4-2. Factors for evaluating evidence regarding identification and characterization of susceptible subpopulations[a]

Factor	Increased weight	Decreased weight
Timing (life stage) - response relationship	Effects occur at greater magnitude at one or more life stage(s)	No difference in effects at different life stage(s)
Type of effect	Different types of effects in specific subpopulations	Same effect(s) across all potential subpopulations
Dose-response relationship	Effect occurs at lower exposures in one or more subpopulation(s)	No evidence for differential dose-response across different subpopulations
Latency of effect	Latency to observed effect different in specific subpopulations	No difference between subpopulations in latency to effect
Seriousness/ reversibility of effects	Effects different in seriousness or degree of reversibility in specific subpopulations and/or differences in later consequence of an initially reversible effect	No differences between subpopulations in seriousness and/or reversibility of effects, or in later consequences of an initially reversible effect

[a] Subpopulations may be defined by gender, individuals at different life stages (fetus, child, adult, elderly), differences in genetic polymorphisms, and/or pre-existing diseases or conditions that may result in differential sensitivity to adverse effects from exposure to a specific toxic agent.

developmental exposure; for example, an initial depression in birth weight or weight gain or subtle developmental retardation may be indicators of more serious abnormalities later in life.

4.3.3. Characterization of the Extent of the Database

The derivation of an RfD or an RfC is a multifaceted process that involves the coordination of data gathering and evaluation, analysis and judgment in varying proportions, and integration of all the information available. A vital part of the chronic RfD and RfC derivation process that relies heavily on judgment, for example, is the current approach to characterizing the database. For example, the minimum dataset for low-confidence and high-confidence RfDs and RfCs has been specifically defined as follows (U.S. EPA, 1994, 2002c): *minimum dataset for a low confidence chronic RfD or RfC* is a single subchronic study. The *minimum dataset for*

a high confidence chronic RfD or RfC is a chronic study in two species, a single two-generation reproductive toxicity study, and a developmental toxicity study in two species by the appropriate route of exposure.

The Technical Panel is recommending a somewhat different approach. Instead of specifying particular studies, this approach emphasizes the types of data needed (in terms of both human and animal data) for deriving reference values and recommends the use of a narrative description of the extent of the database rather than a single confidence statement. The Technical Panel believes that this approach encourages the use of a wider range of information in deriving reference values that take into consideration the issues of duration and route of exposure, the timing of exposures, the types and extent of endpoint assessment (i.e., structural and function), the susceptible subpopulations evaluated, and the potential for latent effects and/or reversibility of effects. In addition, this approach encourages the identification of data that would be needed or useful for improving the risk assessment for a particular chemical or group of chemicals.

To characterize the database, the Technical Panel has developed a description of a "minimal" database and a "robust" database as a way of describing the range of data that can be used for deriving a reference value (Box 4-3). A great deal of scientific judgment is necessary when evaluating the extent of the database for a particular chemical. Defining the extent of the database requires an overall evaluation and judgment as to where in the minimal–robust continuum the available database should be characterized. The Technical Panel purposely did not define additional categories between minimal and robust (moderate), and the Panel has serious concerns about developing such categories because of the tendency to try to characterize a database with single word descriptors. Instead, we strongly support a narrative description of the extent of the database, with emphasis on the strengths and limitations of the data. It should also be noted that a database that is less than minimal should not be used to derive a reference value.

Rather than presenting separate "minimal" and "robust" database descriptions for each type of reference value that might be derived, the descriptions in Box 4-3 are intended to apply generally across the various reference value types (e.g., acute, short-term, longer-term , or chronic durations for oral, dermal, or inhalation routes of exposure). Additionally, it is expected that the different types of reference values for a particular chemical will be developed within the same assessment. In this manner, the entire database for a chemical may be relied upon in the development of each of the different values (e.g., important and relevant insights may be gleaned

from toxicity studies for exposure durations other than those directly corresponding to the type of reference value being developed).

A minimal database as defined above can be used to set reference values, but the limitations of such a database should be clearly recognized and discussed in the narrative description. For example, a minimal database may provide data on only one duration or route of exposure or it may be specific to only one endpoint or organ system. Thus, the uncertainties related to such a database will be great and should be reflected in the size of the UFs applied for reference value derivation (see further discussion below).

Box 4-3. Description of minimal and robust databases

Minimal Database: no human data available, route-specific toxicity data are limited to dose-response data applicable to the duration in question with assessment of endpoints other than mortality. A study showing only effect levels for mortality or other extremely severe toxicity would not be sufficient to set a reference value.

Robust Database: includes extensive human and/or animal toxicology data that cover route-specific information on many health endpoints, durations of exposure, timing of exposure, life stages and susceptible subpopulations. In the absence of complete human data, mechanistic and other data show the relevance of the animal data for predicting human response. Specifically, the dose-response data for the reference value in question includes endpoint-specific data (e.g., developmental toxicity, neurotoxicity) coupled with toxicokinetic information as needed for route-to-route extrapolation. The toxicity studies include the evaluation of a variety of endpoints (e.g., hematological, clinical, histology of target organs) and endpoints specific to any known hazard characterization. The database for a reference value of less-than-chronic duration has also addressed the issue of reversibility of effects and latency to response, taking into consideration the possibility that less-than-chronic exposure may lead to effects at some period of time after exposure. Biological and chemical characteristics of the exposure and outcomes, as well as known limits on reserve capacities and repair of damage, form the basis for determining the appropriate length of follow-up.

On the other hand, a robust database would address issues of potential toxicity in humans and animals and include data on several durations and routes of exposure as well as a thorough assessment of a variety of health endpoints. It would also include sufficient data on toxicokinetics and mode action to provide extensive information for extrapolation of effects to humans, including potentially susceptible subpopulations. A complete database on a single health endpoint that does not contain information on other endpoints of possible relevancy would not necessarily constitute a robust database, nor would a database that provides complete information on one route and/or duration of exposure be considered robust.

It is clear that a robust database represents a “gold standard” that will rarely, if ever, be available. However, a lack of robustness does not mean that the database is deficient to the extent that a reference value could not be derived or that large UFs would need to be applied. Sound scientific judgement will be required to determine which UFs are appropriate in each case.

A critical assessment of the extent and quality of the database will inform the selection of the endpoints to be used to derive the reference values and the appropriate UFs. A reference value based on a single study would likely have a high degree of uncertainty. As more information from additional toxicology studies, toxicokinetic studies, structure-activity relationships, and human data becomes available, EPA can have greater assurance that the appropriate species, route of exposure, and target organ system(s) are known for each duration reference value needed for a human health risk assessment. As this additional information becomes available, the use of UFs will likely decrease. The ultimate objective is to account for all human health endpoints resulting from exposures over all life stages from before conception to the elderly adult.

The optimum assessment considers subtle effects that impact an individual's quality of life as well as so-called "frank" effects (death and major disease). The evaluation should encompass immediate health outcomes as well delayed responses to an exposure (i.e., latent responses), although most current testing guidelines do not explicitly evaluate latency to response.

4.3.3.1. *Extent of the Database*

The following series of questions regarding the extent of the database can help guide the assessment process:

- Have adequate studies been conducted to establish the target organs/endpoints?
- Have the effects been characterized for both sexes and all life stages?
- Are data pertaining to potentially susceptible subpopulations available?
- Are the responses consistent across species? Are the results of the studies biologically plausible?
- Is the route and matrix of exposure relevant to the specific reference value being derived?
- Is the duration of exposure appropriate for the specific reference value being derived?

- Is the animal species and strain appropriate for extrapolation to humans?

- To what degree may the biological endpoints be extrapolated (qualitatively and quantitatively) to humans?

- Are toxicokinetic data available? Are they available for both sexes, for relevant life stages, for other susceptible subpopulations?

- Is the shape of the dose-response curve consistent with the known toxicokinetics of the test compound?

- Are the metabolism and toxicokinetics in the animal species similar to those of humans?

- Has the dose-response curve been replicated by or is it consistent with data from other laboratories and other test species?

- Have the data for all relevant endpoints been adequately modeled by the BMD or other appropriate quantitative analysis to determine the most sensitive endpoint(s)?

- How well is the toxicity characterized? Do the results of all the studies indicate the possibility of effects on particular systems that have not yet been explored sufficiently or do they indicate that additional studies may reveal effects not yet characterized?

4.4. DERIVATION OF REFERENCE VALUES

After the database has been thoroughly evaluated for quality and extent, as outlined above, several decisions must be made and procedures applied before the final derivation of a reference value. This section summarizes the current procedures and points out assumptions made and areas for improvement and clarification. A variety of factors related to the derivation of reference values is discussed, including the selection of relevant endpoints for the POD for various duration reference values (Section 4.4.1). Adjustment of the study dose/exposure for duration is described in Section 4.4.2, and derivation of a HED or HEC is discussed in Section 4.4.3.

Other issues are discussed briefly in Section 4.4.4, such as varying levels of response at the BMDL, BMCL (lower confidence limit on the BMC), or NOAEL due to varying study designs and test sensitivity and considerations of adversity and severity (i.e., nature of the response) for choosing the benchmark response (BMR) level. The nature and application of uncertainty/variability factors and MFs are discussed and critiqued in Section 4.4.5, and future directions are briefly discussed in Section 4.4.6. Section 4.4.7 summarizes key points from two case studies that are presented in detail in Appendix B.

4.4.1. Sample Reference Values and Selection of Endpoints to Use as the POD for Reference Values

Currently, the "critical effect" is used as the basis for the POD, and various UFs are applied to the dose at the critical effect to derive the RfD or the RfC. The critical effect is defined as "the first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases" (U.S. EPA, 2002c). The underlying assumption is that if the RfD or the RfC is derived to prevent the critical effect from occurring, then no other effects of concern will occur; in addition, this approach assumes that the relationship of various health effects for a particular chemical is maintained across species.

The Technical Panel is concerned that presenting only a single critical effect and the critical study from which it was derived in the IRIS summary table that appears at the beginning of each RfD or RfC file may not provide enough information to the reader who is unfamiliar with risk assessment and thus could be misleading. Presenting a single endpoint as a POD for a systemic effect, for example, cannot capture the nature of the dose-response curve for that particular endpoint, nor does it convey the possibility that other more serious endpoints may have a dose-response character markedly different from the less serious endpoint. For example, an agent may have a clear progression of responses with increasing dose that is seen as one type of effect at the lowest exposure level (e.g., proteinuria in the case of cadmium), but at a higher level it produces additional effects (proteinuria PLUS GFR decrements) and at the highest level even more types of effects (proteinuria PLUS GFR decrements PLUS osteomalacia). Each of these effects could have a markedly different dose-response character.

Focusing on a single critical effect also does not reflect the situation in which other types of effects may be found at similar levels of exposure or the variety of health outcomes that may result when an exposure significantly exceeds the RfD or the RfC. Most importantly, in light of the Technical Panel's recommendations for deriving an expanded number of reference values for different durations and routes of exposure, the limitations of focusing only on the critical effect

become apparent because the most sensitive endpoint may be different for different durations or routes of exposure.

Layered upon this complex consideration of dose-response is the further complication that all of the exposure levels producing these effects are or should be adjusted to a human equivalent exposure at the time of their comparison. These adjustments may profoundly affect what is considered the most sensitive organ or system. Effects that occur at the same external inhaled concentration but in different organs in the same exposed animals (e.g., effects in the liver and the nasal cavity) may have quite different HECs, based on the current RfC methodology (U.S. EPA, 1994), because the underlying basis for the adjustment used for systemic effects is markedly different from that used for portal-of-entry effects between animals and humans. This adjustment procedure is discussed further below but is noted here because of its interrelationship with identifying what is to be considered a critical effect.

These aspects all support the case that a more comprehensive approach to setting reference values requires a more extensive and systematic analysis of endpoints than has typically been conducted in the past. In the approach proposed here, the selection of the POD would be similar to the current critical effect approach (e.g., U.S. EPA, 1994) and would include the use of sound scientific judgment in evaluating the strength and validity of studies and the extent of the database, as described in Section 4.3. In this approach, however, the selection of the POD would be based on consideration of all relevant and appropriate endpoints carried through the derivation of sample reference values, with selection of the limiting value(s) protective of all endpoints as the final step (the same approach would be used for deriving a POD for low-dose modeling, as discussed in the proposed cancer risk assessment guidelines [U.S. EPA, 1999a]).

For example, the dose-response curves would be modeled for several adverse endpoints and the corresponding BMDs and BMCs and their lower 95% confidence limits (BMDLs/BMCLs) calculated (U.S. EPA, 2000c) or NOAELs determined if dose-response modeling is not possible. Next, duration adjustment to the continuous exposure scenario would be performed for each endpoint, with further adjustment to the corresponding HECs using the RfC methodology (U.S. EPA, 1994) or adjusted BMDLs or NOAELs for oral or dermal exposures (see Section 4.4.3 for further discussion). These adjusted values would represent the POD for each relevant endpoint. Then, uncertainty/variability factors that take into account a variety of issues, including chemical-specific data, such as known toxicokinetic differences between the laboratory animal species tested and humans, and mode of action information would be applied to the adjusted values for each relevant endpoint. The sample reference values would

then be compared across endpoints and organ systems to determine which are the most relevant for use in deriving the final reference value for each exposure duration that will be protective of the human population (including susceptible subgroups).

The Technical Panel recommends the use of a more visual and graphic exposure-response array to depict the PODs for all relevant endpoints for various routes and durations of exposure, somewhat like those shown in the ATSDR toxicology profiles but with appropriate changes for the purpose of deriving reference values. The exposure-response array of the PODs would facilitate the evaluation and comparison of relevant endpoints and values. (See examples of the proposed approach discussed in Section 4.4.7 and in two case studies in Appendix B.)

4.4.2. Dose Adjustment for Duration of Exposure

Available studies from which reference values are derived seldom if ever match the intent of the reference value regarding species or duration. For example, chronic RfD and RfC values are intended by definition to be for "a continuous exposure to the... human population." Doses or exposures from studies in which animals are exposed for less than a lifetime or in which worker populations are exposed only during working hours require adjustment to continuous exposure in order to be concordant with the intended duration of the reference value (see Rozman and Doull, 2000; Rozman et al., 2001, for further discussion). This section describes various procedures that are currently used by the Agency to adjust a LOAEL, a NOAEL, or a BMDL with regard to duration. The basis for these adjustments is discussed, as is the applicability of these procedures to various routes of exposure.

The Agency has invested considerable time and effort into exploring these aspects for the inhalation route. A major point that will become apparent in this discussion is that methodologies for duration adjustment via the inhalation route are currently in place as part of the existing methodology for the chronic RfC and as proposed for ARE derivations, whereas no comparable documents yet exist for the oral or dermal routes of exposure.

4.4.2.1. *Duration Adjustment Procedures for Inhalation Exposures to Continuous-Exposure Scenarios*

Adjustment of duration to a continuous exposure scenario is regularly applied as a default procedure to studies with repeated exposures but not to single-exposure inhalation toxicity studies in animals and humans (U.S. EPA, 1994). Operationally, this is accomplished by

applying a C^n x t product[11] for both the number of hours in a daily exposure period and the number of days per week that the exposures are performed. In an inhalation study in which animals are exposed to 100 mg/m^3 for 6 hours, 5 days per week, the adjustment to a continuous exposure concentration would consider both hours per day and days per week:

$$100 \text{ mg/m}^3 \times 6/24 \text{ hrs x } 5/7 \text{ days/wk} = 17.9 \text{ mg/m}^3,$$

with 17.9 mg/m^3 being the concentration adjusted for continuous exposure. Study designs that include exposures 7 days/wk, for example, prenatal developmental toxicity studies and DNT studies, do not require the 5/7 days/wk adjustment.

Exposures from human occupational studies are most often reported as 8-hr time-weighted averages (TWAs) and are therefore also discontinuous. Adjustment of these exposures to derive a HEC is explained below in Section 4.4.3.

These adjustment procedures imply that the C × t product and not C is associated with the endpoints observed; this may be restated as implying that the area under the curve (AUC), C × t, rather than the peak concentration, C, is the dosimeter associated with toxicity. Although neither of these dosimeters may be demonstrable experimentally to be the appropriate measure of dose, the Agency uses adjustment to a continuous inhalation exposure based on the C × t relationship as a matter of policy.

When applied to a discontinuous inhalation exposure regimen from an experimental study, adjustment to a continuous exposure will always result in a lower value of C and maintain a measure of total exposure, that is, C × t. Thus, application of this procedure provides an automatic margin of protectiveness for chemicals for which C alone may be appropriate, and it reflects the maximum dose for agents for which total or cumulative dose is the appropriate measure. When considered in this way, this policy can be regarded as being protective of public health. However, assessors are encouraged to look for data on specific chemicals that support the use of C x t or that offer alternative models for adjustment of exposure duration.

4.4.2.2. *Duration Adjustment for Inhalation Developmental Toxicity Studies—A Current Exception*

A notable exception to duration adjustment of inhalation exposures is for inhalation developmental toxicity studies in which this practice historically has not been done. The current

[11]Where $C^n = C^1$, as described in Section 4.4.2.3.

guidelines for developmental toxicity risk assessment (U.S. EPA, 1991) recommend against duration adjustment (i.e., from a discontinuous to a continuous exposure) as a default procedure unless toxicokinetic data are available to indicate an accumulation with continuous exposure. This is contrary to the default approach used for other types of studies in which duration adjustment is done without a requirement for toxicokinetic information. In fact, for other types of studies, toxicokinetic information is often used as the basis for moving away from the default adjustment.

Furthermore, although the effects of some agents that cause developmental toxicity have been shown to be more a function of peak concentration (Nau, 1991), the effects of other agents have been shown to be related to either AUC or C, depending on the timing of exposure and the developmental timing of the organ system affected (Terry et al., 1994). In addition, recent studies have shown that the developmental effects of certain agents that have a short half-life, such as all-trans-retinoic acid (Tzimas et al., 1997) and ethylene oxide (Weller et al.,1999), or a very discrete exposure period, for example, hyperthermia (Kimmel et al., 2002), are a function of AUC.

On the basis of this information and the rationale used for duration adjustment for other health effects (i.e., that exposure adjustment based on $C \times t$ tends to be more health protective), the Technical Panel recommends that duration adjustment procedures to continuous exposures based on $C \times t$ be used as a default procedure for inhalation developmental toxicity studies as it is for other health effects from inhalation exposure. The Technical Panel also urges continued development of data, modeling, and improved procedures for dose-duration adjustments related to developmental toxicity.

4.4.2.3. *Duration Adjustment for Acute Reference Values—Discontinuous Scenarios of 24 Hours or Less*

As discussed above, the magnitude of response to a toxic chemical exposure usually depends on both the concentration and the duration of the exposure, such that the combination of these components, $C \times t$, determines the response and, by logical extension, the internal dose of a chemical at the target tissue. In deriving acute, short-term, or longer-term reference values, there may be a need to specifically adjust or present these values under alternative $C \times t$ combinations. For example, an acute reference value may be required for both a 1-hour duration and an 8-hour duration, but the available data are from a 4-hour exposure. The current guidance on this issue is contained in the draft methodology for development of AREs (U.S. EPA, 1998a). This section presents the adjustment procedures recommended in the draft ARE methodology.

Because of the recognized limitations of the C x t model, a modification has been developed such that $C^n \times T = k$, with n being empirically derived. The consequences of varying the values of the "n" exponent are shown in Figure 4-1. This figure, which was derived from the

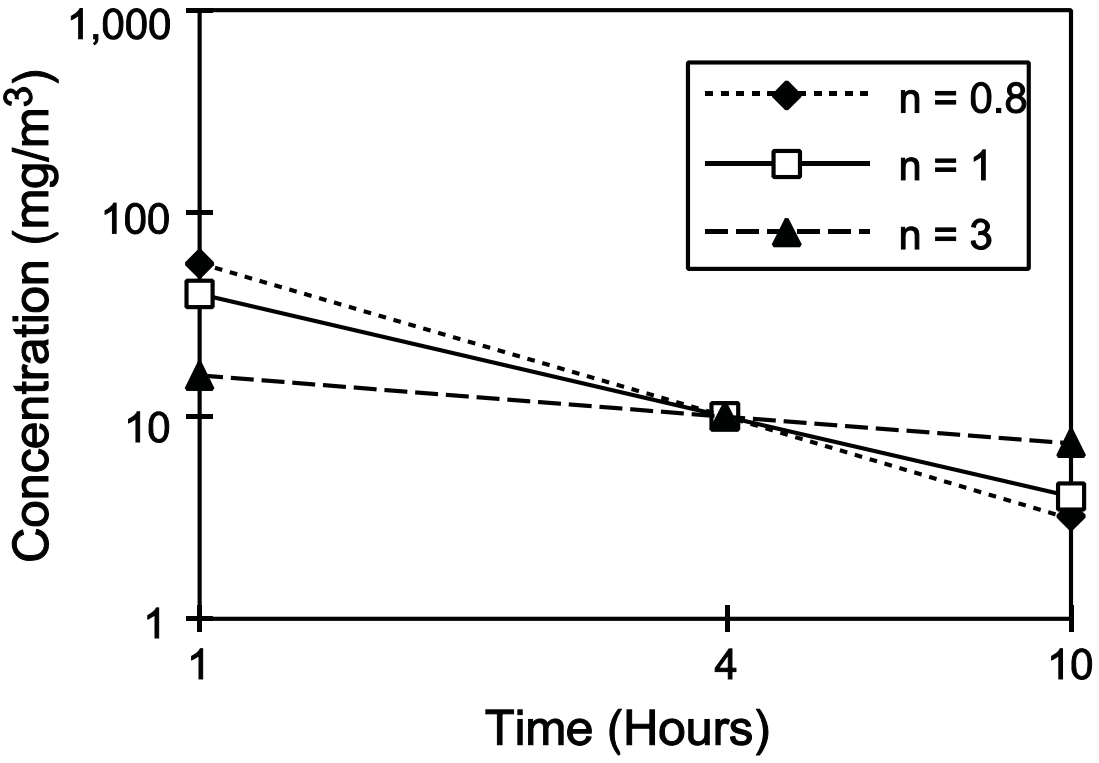


Figure 4-1. Concentration-by-duration plot showing the effect of the exponent in the C^n x T = k on extrapolation across time.

Source: Adapted from ten Berge et al., 1986.

current version of the Agency's ARE methodology, is based on the data of ten Berge et al. (1986). These investigators were able to empirically derive values of "n" that ranged from 0.8 to 3.5 for a number of chemicals on the basis of acute lethality. A value of 1 for the exponent "n" would indicate that the relationship described by Haber's law holds and that the response is related to total dose.

Note that for any degree of downward slope with increasing duration (lines marked with n = 1 or n = 0.8), an extrapolation from a longer to a shorter duration (i.e., from right to left) would result in a higher value for C. Extrapolating from a shorter to a longer duration (i.e., from left to right), however, would have a different consequence in that with any degree of downward slope, C would always be lower for the longer duration. Several possible approaches for extrapolation in this situation could be envisioned. One approach would be to assume a value of 1 for "n," such that $C^n \times T = k$ and lower values of C would always result; this approach is likely to be the actual case, because the value of "n" for most chemicals so far examined has shown an appreciable downward slope (e.g., $0.8 < n < 3.5$ [ten Berge et al., 1986]).

The optimal approach for extrapolating from one dose-duration response situation to another is the use of a physiologically based pharmacokinetic model (PBPK) model. The principle of using PBPK models as the basis for describing the correlations between level and duration of exposure, internal dose, and biological effect has been stated clearly by Andersen et al. (1987). Integration of information using PBPK models requires a chemical database that is rich in toxicity data; therefore, this approach is not applicable to most chemicals for which toxicokinetic data are scarce or nonexistent.

In the absence of such a database to support the development of a PBPK model, the approach recommended by the draft ARE methodology is the use of chemical-specific data on duration dependence from other adequate but longer-duration data, if they exist (e.g., in extrapolating to 28 days using 7-day data, the 90-day repeated-dose data should also be considered). This is considered a conservative approach, because the duration adjustment approach (i.e., averaging to continuous exposure), when applied to multiple exposure studies always results in decreased values for C (i.e., extrapolation would be from shorter to longer durations on the curves in Figure 4-1).

In the absence of chemical-specific data to inform duration adjustment, the response has most often been related to the simple $C \times t$ product. This is also the default in the draft ARE methodology for adjustment to longer durations. For adjustment to shorter durations, the ARE methodology conservatively recommends that there be no change in concentration.

Further investigation would increase confidence in the basic assumptions made for the latter two methods of duration adjustment, including the applicability of the C x t relationship over spans of exposure from months to years and assessing the "conservativeness" of these approaches in relation to public health. Further investigation of C x t relationships relative to life stage is also recognized as a research need.

4.4.3. Derivation of a HEC or a HED

Animal data often form the basis for dose-response assessment. By definition, the IRIS risk values are for humans, thereby making animal-to-human extrapolation requisite. The specific point of this extrapolation is to estimate from animal exposure information the human exposure scenario that would result in the same response. The simplest manner in which this may be done is application of an animal-to-human UF (discussed further below), typically with a value of 10; in application this means that humans are assumed to be more sensitive to effects than are animals by a factor of 10.

Much of the RfC methodology (U.S. EPA, 1994) focused on improving the science underlying the animal-to-human UF, segregating it into toxicokinetic and toxicodynamic components and providing generalized procedures to derive dosimetric adjustment factors (DAF). Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the HEC. Application of a DAF in the calculation of a HEC is considered to address the toxicokinetic aspects of the animal-to-human UF (i.e., to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue).

Current Agency practice is to accommodate uncertainty about the remaining toxicodynamic component through application of a partial animal-to-human UF ($10^{0.5}$, which is typically rounded to 3). The theoretical basis for deriving DAFs used in calculating HECs, along with recommendations for improvement of this process, is discussed in this section.

Exposures from human occupational studies are most often reported as 8-hr TWAs for exposures during work days (5 days/wk). As with discontinuous exposures of animal studies (e.g., 6 hrs/day, 5 days/wk), exposures from occupational studies are also adjusted to derive continuous HECs relevant to the human population (U.S. EPA, 1994). As described below for animal data, the optimal approach is to use a biologically motivated mathematical, or PBPK, model. An occupational exposure can be extrapolated in the same fashion as intermittent exposure regimens from experimental laboratory animals, using particle deposition or PBPK models with human exertion (work) ventilation rates and exposure durations appropriate to the occupational setting.

In the event that a PBPK model or required physicochemical and physiological parameters are not available, the default approach for human exposure scenarios is to adjust by the default occupational ventilation rate and for the intermittent work week schedule. The ventilation rate adjustment is based on the assumed amount of air used by a worker during the work period, that is, half of the daily ventilatory capacity of an adult male human is assigned (10

m^3 of 20 m^3 total) to the 8-hour occupational exposure (i.e., instead of 1/3 or 8/24 hrs) (ICRP, 1994). By basing this adjustment on a functioning physiological parameter, that is, a fractional ventilatory capacity based on the assumption that activity levels are higher in this setting than in others, such as at rest or asleep, this adjustment may be considered to have a toxicokinetic basis. The 8-hour TWA concentrations are multiplied by this factor, 10/20 m^3, and the product is considered to be an average continuous airborne concentration.

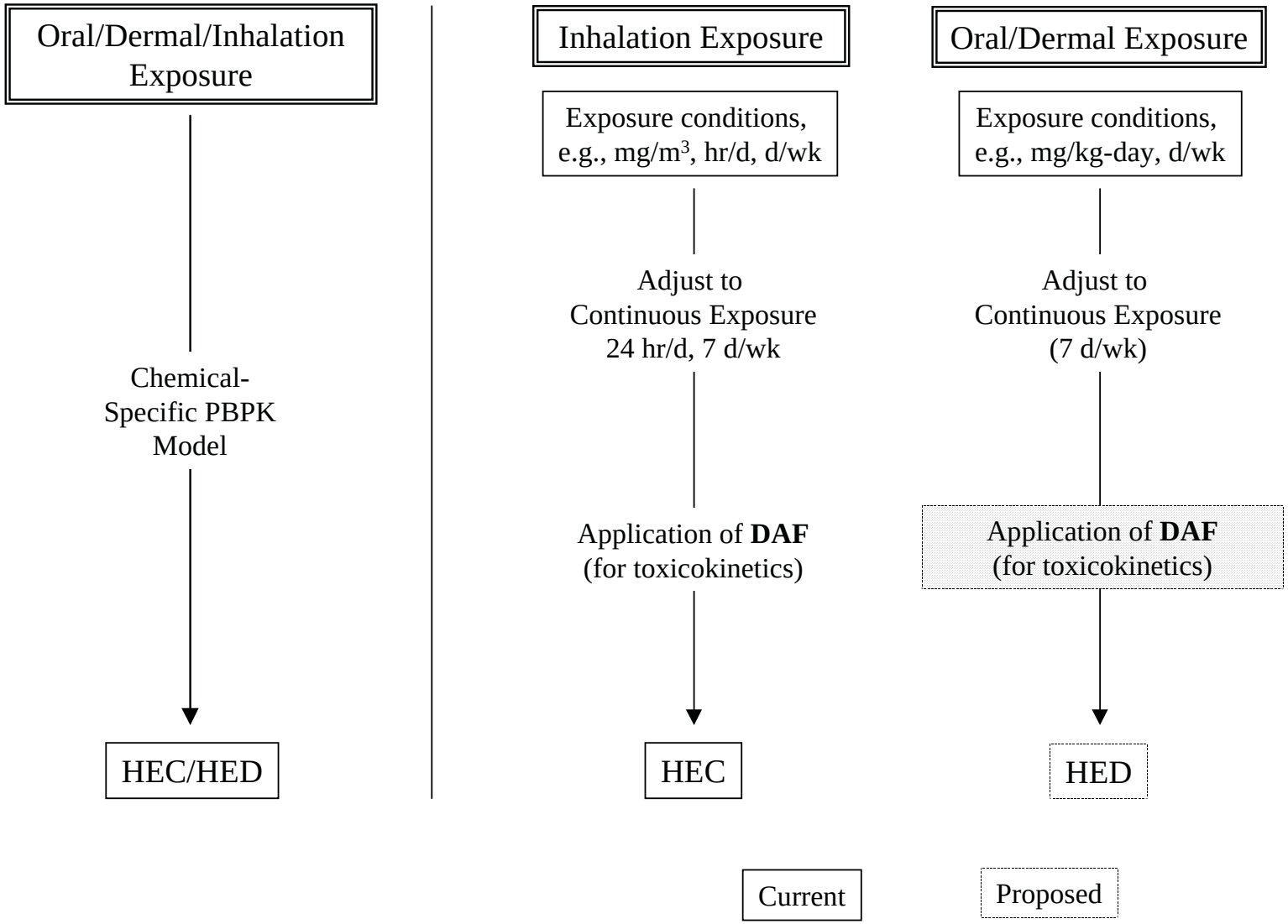
In parallel with the animal studies, an adjustment for days per week (usually 5/7 days/wk) is also made, if applicable. This adjusted airborne concentration is considered to be a HEC. This default calculation, as with those described below for extrapolation from animal data, was developed for the general human population. It may be appropriate to further evaluate this approach or to develop an alternate default approach to ensure adequate consideration of intrahuman variation.

Currently, no procedures parallel to the inhalation RfC methodology exist for deriving either oral or dermal human equivalents from animal data. Default factors (usually of 10) are routinely applied to address the issue of animal-to-human extrapolation. Thus, no parallel to the HEC, that is, a HED, is derived nor are other adjustments applied to the animal oral or dermal dose.

This section recommends that dose adjustments similar to those by which HECs are estimated be explored in deriving HEDs for oral and dermal exposures. This would be accomplished in a manner parallel to the HEC derivation, by instituting and applying a DAF to animal oral or dermal exposures. Specific recommendations are also presented and discussed concerning the basis for deriving DAFs for HED calculation. These recommendations, along with current procedures for estimating human equivalent values, are illustrated in Figure 4-2. This figure also demonstrates how calculation of the HEC through application of a DAF is considered to address the toxicokinetic but not the toxicodynamic component of the animal-to-human extrapolation. Procedures outlined in this figure for deriving a HEC may be applied to any animal inhalation exposure, regardless of whether it is a BMDL, a NOAEL, a LOAEL, or another effect level.

4.4.3.1. *PBPK Models and Derivation of HEDs and HECs: Estimating Internal Dose*

The preferred option for calculating a HED or a HEC is to use a chemical-specific PBPK model parameterized for the species and regions (e.g., respiratory tract) involved in the toxicity, as shown on the left-hand side in Figure 4-2. When sufficiently parameterized, a PBPK model is capable of calculating internal doses to a target organ from any exposure scenario in an animal



DAF = Dosimetric Adjustment Factor

Figure 4-2. Current and proposed generalized procedures for deriving HECs or HEDs from animal exposures.

and then estimating what human exposure would result in this same internal dose, that is, the HED or the HEC. A formal DAF is not calculated in this process; rather, the model itself serves as a DAF in estimating HECs or HEDs. However, constructing a PBPK model is an information-intensive process that requires much chemical-specific data, including route-specific data. Such sophisticated data and models are available usually for only a subset of chemicals that have extensive databases.

It should be noted that even these sophisticated models are often parameterized on the basis of adult members of the species. Many of the parameters critical to PBPK model solutions are sensitive to life stages, such as lung function/development in humans (Pinkerton and Joad, 2000), for which no or few data are available. Thus, these models are available but often cannot specifically address species differences at life stages other than mature adults (and then usually males). The Technical Panel encourages research and data gathering to support the construction of PBPK models, it endorses attempts to produce PBPK models that are sensitive to life stages, and it supports fully attempts to produce template models for suites of related chemicals, as has recently been done by Barton et al. (2000).

4.4.3.2. *Default Procedures and Derivation of HECs from the RfC Methodology: Derivation and Application of DAFs*

The next lower level of complexity in deriving HECs is less data intensive than the PBPK approach. As shown in Figure 4-2, this procedure involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (e.g., particle or gas) and categorized with regard to elicitation of response either locally (i.e., within the respiratory tract) or remotely. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (e.g., to a weekly average). The generalized DAF procedures may also employ chemical-specific parameters, such as mass transport coefficients, when available. In lieu of such data, however, default procedures that yield generalized adjustments are recommended. Although these generalized procedures were developed from the existing scientific understanding of the relevant processes, they have not been comprehensively evaluated (e.g., using data from humans and animals). They are explained fully in the RfC methodology (U.S. EPA, 1994).

For example, the manner in which a HEC is calculated for a reactive gas that elicits an effect in the extrathoracic region of the respiratory tract (i.e., the nasal tract) of a rat is by creating a surface area/ventilation ratio for both humans and rats and applying it to the external exposure concentration for rats. The current default values used for both the human and the rat

extrathoracic surface area are single estimates from the literature and are apparently estimated from adult specimens. The ventilation measure for humans is set at a default value of 20 m^3, and the ventilation measure for rats is based on an algorithm of body weight (from U.S. EPA, 1988).

A major assumption made in this particular adjustment is that the distribution of a gas in the region of interest is uniform, although it is known to be highly nonuniform (Kimbell et al., 1993, 1997). Data are not available to address this simplified assumption directly. Use of the method, for example on effects in the extrathoracic region, results in a DAF of about 0.2, such that the resultant HECs are about 20% of the animal-duration-adjusted concentration. Although information is not yet available to address this assumption, indications are that resolution with actual data may produce DAFs that are much closer to unity, that is, that are near the animal-adjusted concentration.

In comparison to the procedure for gases that elicit respiratory effects, calculation of a HEC for a category 3 gas (i.e., a gas that is relatively water-insoluble and unreactive in the respiratory tract and for which the site of toxicity is generally remote to the site of absorption in the pulmonary region) is usually accomplished by creating a ratio of the blood:gas partition coefficient for the laboratory animal species to the human value. The ratio is used as the DAF and applied to the experimental exposure concentration. In lieu of data on the values for blood:gas partition coefficients for the chemical or when the data indicate the ratio to be >1, the default assumption is that the ratio of animal coefficient to human coefficient is 1, and therefore the DAF would be 1. However, available data on partition coefficients for a number of compounds indicate that the animal/human ratio is usually >1 (Gargas et al., 1989; Jepson et al., 1994) such that the DAF would also be >1. In the context of substituting data-derived values for UFs, the Technical Panel recommends further investigation into using data-derived values in constructing the animal/human ratios—even when much greater than 1—in place of the default.

The default dosimetric adjustment procedure for particulate substances is an empirical model that estimates regional deposition only, although it is recognized that with the development of the relevant data, clearance and the retained dose may be used as a DAF (U.S. EPA, 1994). The DAF for particles is more specifically termed the regional deposited dose ratio and is derived from a normalizing factor (surface area being the recommended factor for all three regions of the respiratory tract), the ratio of animal-to-human minute volumes (where the human default value is the traditional adult value of 13.8 L vs. the adult value for the relevant animal), and the ratio of animal-to-human regional fractional deposition. Physiological parameters used in estimating the regional deposition include body weight, minute volume, and surface area for the three areas of the respiratory tract. Defaults for the human values are based on adult data

(e.g., 70 kg body weight, 13.8 L minute ventilatory volume, etc); the animal values are also traditionally based on adult data. To evaluate protectiveness of these default calculations for different life stages, it may be appropriate to perform ratio calculations using data for other life stages.

As a general recommendation, the Technical Panel encourages further consideration of the existing animal-to-human extrapolation procedures described in current methodologies (e.g., the chronic RfC methodology [U.S. EPA, 1994]) and the development of procedures for inhalation adjustment to incorporate the most current scientific thought and data to address, as needed, issues of variability due to life stage and other intrinsic factors. This consideration would include examining the extent to which calculating a HEC (or any recommended HED) addresses cross-species toxicokinetics as well as identification and parallel investigation into issues of toxicodynamics.

4.4.3.3. *HECs and Children—A Special Case?*

Children are often characterized as constituting a potentially susceptible subgroup because they could be at greater risk than adults for inhaled toxic agents (including both gases and particulates) for reasons relating to either toxicokinetics or toxicodynamics. It is clear for any of a variety of reasons related to toxicokinetics that an adult and a child breathing the same concentration of an agent such as a reactive gas may receive different doses to the body or to the lungs. A generalized theoretical approach to judging whether children would receive greater doses than would adults when both breathe the same concentration of a reactive gas, for example, would be to compare the amount of gas breathed in (which would be directly proportional to the ventilatory volume) with the overall surface area in the respiratory tract on which the gas may impinge. The current Agency default assumption used in deriving HECs for particles and reactive gases that elicit respiratory effects is that the surface area of the total respiratory tract of an adult male, estimated at 54.3 m^2, is exposed to a total daily air intake of 20 m^3, a volume for an adult male derived from a combination 24-hour activity pattern in ICRP (1994) of sitting awake for 8 hours, exercising lightly for 8 hours, and sleeping for 8 hours.

It has been well established that the human respiratory system passes through several distinct stages of maturation and growth that involve branching morphogenesis and cellular differentiation during the first several years of life and into adolescence (Pinkerton and Joad, 2000). The proportion of surface area to ventilation volume may be markedly different during these developmental stages. The significance of these disproportions with regard to toxicant exposure overall or to the sites of active cellular differentiation have yet to be elucidated.

The Technical Panel recommends that issues involving dose to the young from inhalation exposures be pursued both theoretically and experimentally in order to establish the basis on which children should be considered as a susceptible subpopulation for inhalation exposures. It should also be reiterated that this is an estimate of the toxicokinetic aspect of dose only, and toxicodynamic differences between the lungs of young children and adults are not addressed.

4.4.3.4. *Deriving a HED for Oral and Dermal Exposure—Use of $BW^{3/4}$ as a Cross-Species DAF*

As indicated above, the Agency currently does not provide a procedure for calculating a HED for oral or dermal exposure scenarios that would parallel calculation of the inhalation HEC. Instead, assumptions are made regarding the comparability of ingested or applied dose, based on a mg/kg body-weight basis, and there is no adjustment for portal-of-entry alterations to internal dose or on portal-of-entry versus systemic effects. The Technical Panel recognizes the work of an interagency workgroup to develop and propose dosimetric adjustment procedures for both dermal and oral routes of exposure in order to address those aspects of cross-species dosimetric adjustment that are missing in Figure 4-2. Some of these proposals have already appeared in abstract form (Jarabek, 2000; Hanna and Jarabek, 2000; Hubal et al., 2000; Rigas et al., 2000).

Figure 4-2 demonstrates that dosimetric adjustment procedures for estimating human equivalents from animal values are not consistent for different exposure routes. Other procedures, both from within and external to the Agency, could be explored for the purposes of deriving a DAF and employing it to estimate a HED. For example, in the absence of more sophisticated physiologically based models, the Agency has endorsed scaling of doses for carcinogens between species according to body mass raised to the 3/4 power ($BW^{3/4}$) (U.S. EPA, 1992). This procedure presumes that equal doses in these units (i.e., in $mg/kg^{3/4}/day$) when administered daily over a lifetime, will result in equal lifetime cancer risks across mammalian species. This same relationship (i.e., $BW^{3/4}$) has been affirmed to apply across entire phyla, including plants (Gillooly et al., 2001), for general metabolic rates.

The basis for the less-than-full-power relationship for general metabolic processes (i.e., $< BW^{1}$) is thought to be related to species differences in exchange surfaces and distribution networks that constrain concentration and flux of metabolic reactants (West et al., 1997; Enquist et al., 1998). Thus, when this procedure is applied to animal data, the resulting scaled human dose may be viewed as a valid cross-species relationship not only of cancer potency but also for general metabolic processes and, by extension, for other phenomena involving the fundamental determinants of concentration and flux, the same ones that drive basic toxicokinetics.

This brief analysis of the $BW^{3/4}$ cross-species relationship and toxicokinetic processes and the Agency's endorsement of this procedure for carcinogenic agents makes this process a possible candidate for estimating cross-species toxicokinetic relationships in the absence of adequate toxicokinetic information. That is, $BW^{3/4}$ factors could be applied as DAFs for deriving a HED. This procedure would parallel the one used for deriving the HEC. As with the HEC, however, this process applies only to toxicokinetic aspects of cross-species extrapolation and does not address toxicodynamic differences that may exist between species. As with the HEC, consideration of toxicodynamics is proposed to be through application of a portion of the animal-to-human extrapolation ($10^{0.5}$, which is typically rounded to 3). Table 4-3 shows the general magnitude of the DAFs that would be applied to various species to obtain the HED along with the default UF of 3 to cover toxicodynamic differences.

Table 4-3. DAFs based on $BW^{3/4}$ for various species

Species	Weight (kg)	DAF[a]
Mouse	0.03	7
Rat	0.25	4
Guinea pig	0.5	3
Rabbit	2.5	2
Human	70	1

[a] Derived on the basis of $BW^{3/4}$ relationship. All variables in $BW^{3/4}$ relationship containing time will scale $BW^{-1/4}$, such that animal $BW^{-1/4}$/human $BW^{-1/4}$ = DAF.

The Technical Panel encourages consideration of cross-species extrapolation procedures for oral and dermal reference values, including evaluation of the most current scientific thought and data to address, as needed, issues of variability due to life stage and other intrinsic factors. This consideration would include examination of the extent to which calculation of a HED addresses cross-species toxicokinetics and identification and parallel investigation into issues of toxicodynamics.

4.4.4. Other Issues

The Technical Panel considered several other issues related to the application of a factor (data-derived or default) to the BMDL, the BMCL, the NOAEL, or the LOAEL selected as the POD from data considered adequate for risk assessment. In particular, there was controversy about the application of such a factor on the basis of the level of response at the BMD, the BMC, the NOAEL, or the LOAEL. For example, the use of a quantitative dose-response modeling approach results in the calculation of a BMD or a BMC, which is based on a particular level of response, that is, the BMR. The BMR is usually selected to be at the low end of the observable range of the data, which is dependent on the power of the study to detect changes from control values. The limit of sensitivity for most long-term bioassays is in the range of 10%, as determined from both the typical number of animals used in bioassays (~50/group) and a low spontaneous background rate (e.g., 0.1%) for a given effect (Haseman, 1984; Haseman et al., 1989).

For other types of studies, however, the limit of sensitivity may be lower or higher than 10%. For example, in an analysis of a large number of standard prenatal developmental toxicity studies with an average sample size of 15–20 litters, the limit of sensitivity averaged 5% for the proportion of pups affected per litter, whereas when the quantal endpoint (i.e., the number of litters affected) was analyzed in dams from the same studies, the limit of sensitivity averaged 30% (Allen et al., 1994). For data from some human studies (e.g., high-quality, large epidemiology studies), the limit of sensitivity may be in the range of 1 to 5%.

In the BMD guidance document (U.S. EPA, 2000c), the BMDL or BMCL is recommended for the POD in order to ensure that a majority of the population is below the selected BMR. However, a concern has been raised that a BMD or BMC based on a response rate of $\geq$10% may not be appropriate to use in deriving an exposure to the human population (including sensitive or susceptible subgroups) *that is likely to be without appreciable risk of deleterious or adverse effects* (from current and proposed reference value definitions [Boxes 4-1 and 4-2]) without application of a factor to extrapolate to a lower dose/exposure level considered to reflect a more appropriate level of risk (e.g., <10%).

Similarly, the NOAEL is not necessarily a no-effect level, and it depends on the study design, including sample size, background rate, and response variability, which can be used to determine the limit of detection for a particular study. Thus, a NOAEL may be equivalent to no response or it may actually represent a substantial response rate. Previously, there has been no attempt to apply a factor to the NOAEL on the basis of power calculations, sample size, or response variability for deriving a POD, although professional judgment is recommended in deciding whether the study is acceptable for use in deriving a POD.

Adjustment for the steepness of the dose-response curve has been noted as another critical aspect of the dose-response character that is not currently considered in the choice of a response level using either a BMD/BMC or a NOAEL approach.

The Technical Panel was unable to fully evaluate these issues or to reach agreement about any recommendation for change to the current methodology, and it recommends that they be considered further by the Agency. The Technical Panel also recommends that factors such as the response rates at the BMD or the NOAEL, the power of the study, and the slope of the dose-response curve be included in the description of the database, where possible, as part of risk characterization.

Box 4-4. Variability and Uncertainty

Variability refers to true heterogeneity or diversity. For example, among a population that drinks water from the same source and with the same contaminant concentration, the risks from consuming the water may vary. This may be due to differences in exposure (i.e., different people drinking different amounts of water and having different body weights, different exposure frequencies, and different exposure durations) as well as differences in response (e.g., genetic differences in resistance to a chemical dose). Those inherent differences are referred to as variability. Differences among individuals in a population are referred to as inter-individual variability, while differences for one individual over time is referred to as intra-individual variability.

Uncertainty occurs because of a lack of knowledge. It is not the same as variability. For example, a risk assessor may be very certain that different people drink different amounts of water but may be uncertain about how much variability there is in water intakes within the population. Uncertainty can often be reduced by collecting more and better data, while variability is an inherent property of the population being evaluated. Variability can be better characterized with more data, but it cannot be reduced or eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization.

Source: U.S. EPA, 1997b.

4.4.5. Application of Uncertainty/Variability Factors

Reference values are derived in a way that attempts to account for both the uncertainty and the variability in the data available (see Box 4-4). The existing definition of UF in the IRIS glossary mixes the above concepts. The present definition for UF is as follows.

> **Uncertainty Factor**: One of several, generally 10-fold, factors used in operationally deriving the RfD and RfC from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population (i.e., interhuman or intraspecies variability); (2) the uncertainty in extrapolating animal data to humans (i.e., interspecies variability); (3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) the uncertainty associated with extrapolation from animal data when the database is incomplete.

Following the logic above, the LOAEL-to-NOAEL extrapolation, the subchronic-to-chronic extrapolation, and the database deficiency factors are UFs. The variation in susceptibility among members of the human population is a variability factor. When a default factor is used for intrahuman variability, however, this factor also contains some degree of uncertainty, because the range of uncertainty is not really known, although it is presumed to be no more than 10-fold. Rather than adding a new definition of variability factor, we propose to modify the wording of the UF definition as follows.

> **Uncertainty/Variability Factor:** One of several, generally 10-fold, default factors used in operationally deriving the RfD and the RfC from experimental data. The factors are intended to account for (1) the variation in sensitivity among the members of the human population (i.e., inter-individual variability); (2) the uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty); (3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) the uncertainty associated with extrapolation when the database is incomplete.

In setting pesticide tolerances, the FQPA directs EPA to use an additional 10-fold margin of safety to protect infants and children, taking into account the potential for pre- and postnatal toxicity and the completeness of the toxicology and exposure databases. The statute authorizes EPA to replace this additional 10X factor with a factor of a different value (higher or lower, including 1) only if, on the basis of reliable data, the resulting level of exposure would be safe for infants and children. The Agency use of this FQPA safety factor has been discussed in several documents (U.S. EPA, 1999b, c, 2002b).

The Agency has concluded that in many cases, concerns regarding pre- and postnatal toxicity can be addressed by calculating an RfD or an MOE using pre- or postnatal developmental endpoints and applying traditional UFs to account for deficiencies in the toxicity data (U.S. EPA, 2002b). These traditional UFs include extrapolation from the LOAEL when a NOAEL is not available, extrapolation from a subchronic study to a chronic-exposure scenario when no chronic study data are available, and application of a database UF when there are gaps in the data considered essential for setting a reference value, including lack of data on children.

In addition to considering these FQPA-relevant areas of uncertainty, which are addressed in the development of an RfD/RfC, OPP assessments of pesticide risk to children also consider applying part or all of the FQPA factor in certain situations to account for areas of residual

uncertainty that the traditional UFs do not address or for which they are believed to be insufficient. These areas of residual uncertainty include exposure uncertainties and high concern for an observed susceptibility. This risk management approach is consistent with procedures used in the past for managing potential risks, although the FQPA has brought a significant new focus on improving the process of risk assessment relative to children's health risks from environmental exposures.

In considering the robustness of the RfC/RfD methodology and its adequacy for assessing hazards to infants and children, the Technical Panel also recognized the overlap of areas covered by the FQPA factor and those addressed by the traditional UFs. For example, the database UF may be invoked where data are unavailable or are insufficient to explicitly consider the potential sensitivity of the developing organism. The Technical Panel agrees with the 10X Task Force draft Toxicology Working Group report (U.S. EPA, 1999b) that the current interspecies, intraspecies, LOAEL-to-NOAEL, subchronic-to-chronic, and database-deficiency UFs, if appropriately applied using the approaches recommended in this review, will be adequate in most cases to cover concerns and uncertainties regarding the potential for pre- and postnatal toxicity and the completeness of the toxicology database. In other words, an additional UF is not needed in the RfC/RfD methodology because the currently available factors are considered sufficient to account for uncertainties in the database from which the reference values are derived (and it does not exclude the possibility that these UFs may be decreased *or* increased from the default value of 10).

Guidance is needed on the use of developmental toxicity data in all reference values, including the appropriate application of UFs, because of the assumption that a single exposure during development may produce an effect (U.S. EPA, 1991) and the concomitant recognition that multiple exposures may result in effects at lower doses in many cases or cause tolerance in other cases. These issues are chemical specific, and scientific judgement about when and how to apply UFs must include consideration of toxicokinetics/metabolism as well as the mode of action for each agent.

4.4.5.1. *Recommendations for Application of UFs*

The exact value of the UFs chosen should depend on the quality of the studies available, the extent of the database, and scientific judgment. It is imperative that the IRIS documentation contain a justification for the individual UFs selected for a particular agent. The default factors typically used cover a single order of magnitude (i.e., 10^{1}). By convention, in the Agency, a value of 3 is used in place of one-half power (i.e., $10^{0.5}$) when appropriate. The Technical Panel recommends that these half-power values be factored as whole numbers when they occur singly

but as powers or logs when they occur in tandem. A composite UF of 3 and 10 would be expressed as 30 (3×10^1), whereas a composite UF of 3 and 3 would be expressed as 10 ($10^{0.5} \times 10^{0.5} = 10^1$). It should be noted, in addition, that rigid application of log or ½ log units for UFs could lead to an illogical set of reference values; therefore, the Technical Panel emphasizes that application of scientific judgment is critical to the overall process.

It is imperative that the IRIS documentation contain a justification for the individual factors selected for each chemical or assessment and for each duration reference value. Although default factors of 10 are recommended, with 3 used in place of half-power values (i.e., $10^{0.5}$) when occurring singly, the exact value of the UF chosen should depend on the quality of the studies available, the extent of the database, and scientific judgment. Sound scientific judgment should be used in the application of UFs to derive reference values that are applied to the value chosen for the POD derived from the available database (BMDL, NOAEL, or LOAEL).

The Technical Panel recognizes that there is overlap in the individual UFs and believes that the application of five UFs of 10 for the chronic reference value (yielding a total UF of 100,000) is inappropriate. In fact, in cases where maximum uncertainty exists in all five areas, it is unlikely that the database is sufficient to derive a reference value. Uncertainty in four areas may also indicate that the database is insufficient to derive a reference value. In the case of the RfC, the maximum UF would be 3000, whereas the maximum would be 10,000 for the RfD. This is because the derivation of RfCs and RfDs have evolved somewhat differently. The RfC methodology (U.S. EPA, 1994) recommends dividing the interspecies UF in half, one-half ($10^{0.5}$) each for toxicokinetic and toxicodynamic considerations, and it includes a DAF to account for toxicokinetic differences in calculating the HEC, thus reducing the interspecies UF to 3 for toxicodynamic issues. RfDs, however, do not incorporate a DAF for deriving a HED, and the interspecies UF of 10 is typically applied.

The Technical Panel recommends limiting the total UF applied for any particular chemical to no more than 3000 and avoiding the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation. This maximum of 3000 applies only to the UFs discussed in the following sections and does not include the various adjustment factors that have been discussed previously (Sections 4.4.2. and 4.4.3.). Similar concerns would need to be considered for the less-than-lifetime reference values, taking into account those UFs that are appropriate for each duration reference value.

4.4.5.2. *Interspecies UF*

The interspecies UF is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both toxicokinetic and toxicodynamic aspects. The toxicokinetic aspects of this factor were addressed in the section on deriving HEDs and HECs (Section 4.4.3). This UF is intended also to account for differences in species sensitivity (i.e., toxicodynamics) between the laboratory animal species used for testing and humans. Seldom are there data available to inform toxicodynamic differences. One-half the default 10-fold interspecies UF (i.e., $10^{0.5}$) is assumed to account for such differences, but more specific data should be used when available (see discussion of chemical-specific adjustment factors, Section 4.4.6.1 below), and the flexibility for applying a factor greater than 10 should be recognized. Unless data support the conclusion that the test species is more or equally as susceptible to the pollutant as are humans, and in the absence of any other specific toxicokinetic or toxicodynamic data, a default factor of 3 (in conjunction with HEC derivation) or 10 is applied.

4.4.5.3. *Intraspecies UF*

The intraspecies UF is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed. As the reference concentration/dose is defined to be applicable to "susceptible subgroups," this UF was established to account for uncertainty in that regard. In general, the Technical Panel reaffirms the importance of this UF, recommending that reduction of the intraspecies UF from a default of 10 be considered only if data are sufficiently representative of the exposure/dose-response data for the most susceptible subpopulation(s).

Various authors who have evaluated the intraspecies UF using data from animal or human studies (as summarized by Dourson et al. [1996]) have concluded that the 10-fold default factor appears to be protective when starting from a median response—by inference a NOAEL assumed to be from an average group of humans. Renwick and Lazarus (1998) considered data on toxicokinetics and toxicodynamics to support the idea that the 10-fold intraspecies factor can be divided into two factors to account for kinetics and dynamics. When they evaluated the composite 10-fold factor to account for variability in both kinetics and dynamics, they concluded that a 10-fold factor would cover the vast majority (>99%) of the population. These evaluations, however, did not specifically consider children as part of the range of human variability when evaluating the adequacy of the intraspecies UF.

In papers that have evaluated this factor for the general population as well as for specific subpopulations, including children (Renwick and Lazarus,1998; Renwick, 1998) and the elderly (Abdel-Mageed et al., 2001), the 10-fold intraspecies factor appears to be sufficient in most cases, and chemical-specific factors often indicate a requirement for less than a l0-fold factor. Renwick (1998) indicated that the 10-fold factor is more likely to be sufficient if developmental toxicity data are available on the specific agent. Calabrese (2001) reviewed the data available on a number of chemical classes and concluded that the young are often more susceptible than adults but that there is a not-infrequent occurrence of greater susceptibility in adults. The sometimes greater sensitivity among the elderly than among mature adults appears to be related primarily to reduced renal clearance (Abdel-Mageed et al., 2001; Skowronski and Abdel-Rahman, 2001).

The Technical Panel urges continued research and evaluation of the similarities and differences between the general population and susceptible subpopulations—particularly children and the elderly—in their responses to specific agents. From such evaluations, the protectiveness of the 10-fold default factor can continue to be assessed.

The cases on IRIS in which the intraspecies UF has been reduced from the default of 10-fold have been documented by Dourson et al. (1996). These include 2/46 RfCs and 13/346 RfDs (overall frequency 3.6%). In those cases where developmental effects were the most sensitive endpoint (0 RfCs, 6 RfDs), reduction of the intraspecies UF from 10 to 3 was based on data derived either from human data showing which age groups or time periods were most susceptible (e.g., methyl mercury exposure to the developing fetus) or from an animal study with support from strong human or other data (e.g., Aroclor 1016 in utero exposure in monkeys, strontium-induced rachitic bones in young rats). In three cases the intraspecies UF was reduced to 1, based on very specific data about the particular vulnerability of infants and children within certain age ranges to an agent (e.g., nitrate, nitrite, fluorine/soluble fluoride). However, even within these populations it is possible that some variability exists, based on genetics, lifestyle, or other factors.

In cases where the susceptible subpopulation is quite specifically defined (e.g., through knowledge of the chemical’s mode of action) so that the resultant RfC is truly applicable to the susceptible subpopulation (although not necessarily to hypersensitive individuals), reduction of the intraspecies UF is warranted. Thus, the Technical Panel supports and expands the recommendation of the Toxicology Working Group of the 10X Task Force (U.S. EPA, 1999b) that reduction of the intraspecies UF from a default of 10 be considered only if data are sufficient to support the conclusion that the data set on which the POD is based is representative of the exposure/dose-response data for the susceptible subpopulation(s). Given this, whether and how

much the intraspecies UF may be reduced must be linked to how completely the susceptible subpopulation has been identified and their sensitivity described (vs. assumed). At the other extreme, a 10-fold factor may sometimes be too small because of factors that can influence large differences in susceptibility, such as genetic polymorphisms. The Technical Panel urges the development of data to support the selection of the appropriate size of this factor, but recognizes that often there are insufficient data to support a factor other than the default.

4.4.5.4. *LOAEL-to-NOAEL UF*

A UF (default 10) is typically applied to the LOAEL when a NOAEL is not available. The size of the LOAEL-to-NOAEL UF may be altered, depending on the magnitude and nature of the response at the LOAEL. It is important to consider the slope of the dose-response curve in the range of the LOAEL in making the determination to reduce the size of the LOAEL-to-NOAEL UF. Several papers have described the magnitude of the difference between the dose at the LOAEL and at the NOAEL. For example, Lewis et al. (1990) and Faustman et al. (1994) showed that the ratio of the LOAEL-to-NOAEL in many cases was approximately threefold, but in a few cases the difference was as much as 10-fold.

In general, the ratio of the doses at the LOAEL and the NOAEL is likely to vary considerably among studies and may not be informative. This is because the lowest dose in a study is often selected to ensure that no statistically significant response above control is observed and the next higher dose is selected to ensure that some significant response is observed, rather than selecting doses that will give a maximum NOAEL and a minimum LOAEL. Data should be carefully evaluated, taking into consideration the level of response at the LOAEL and the NOAEL and the slope of the dose-response curve before reducing the size of the UF applied to the LOAEL.

4.4.5.5. *Database UF*

The database UF is intended to account for the potential for deriving an underprotective RfD/RfC as a result of an incomplete characterization of the chemical's toxicity. In addition to identifying toxicity information that is lacking, review of existing data may also suggest that a lower reference value might result if additional data were available. Consequently, in deciding to apply this factor to account for deficiencies in the available data set and in identifying its magnitude, the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages.

In many respects, the additional 10-fold factor for infants recommended by the National Research Council (NRC, 1993) and by Schilter et al. (1996) and called for in the 1996 FQPA is

similar to the database UF. If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing (Dourson et al., 1996). Dourson et al. (1992) examined the use of the database UF by analyzing ratios of NOAELs for chronic dog, rat, and mouse studies and reproductive and developmental toxicity studies in rats. They concluded that reproductive and developmental toxicity studies provide useful information for establishing the lowest NOAEL, and if one or more bioassays are missing, a factor should be used to address this scientific uncertainty in deriving a chronic RfD.

If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD for the particular duration RfD or RfC under development.

If the RfD/RfC is based on human data, a similar assessment regarding the completeness of the database is necessary. Information on life stages and organ systems may come from either animal or human studies. If data on specific life stages or organ systems are unavailable or limited data suggest that availability of more extensive data might decrease the POD, this should be taken into account in assigning a database UF. For example, depending on the database and what is known about the chemical, the lack of a two-generation animal reproductive toxicity study might be considered a deficiency even if the reference value is based on human data. In any case, the size of the database factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical and, consequently, the POD.

4.4.5.6. *Subchronic-to-Chronic-Duration UF*

As indicated earlier, a duration adjustment currently in use is the application of a UF when only a subchronic duration study is available to develop a chronic reference value such as the RfC or the RfD (U.S. EPA, 1994). A default value of 10 for this UF is applied to the NOAEL/LOAEL or BMDL/BMCL from the subchronic study on the assumption that effects from a given compound in a subchronic study occur at a 10-fold higher concentration than in a corresponding (but absent) chronic study. This factor would be applied subsequent to the adjustment of the exposures from intermittent to continuous, as above.

The specific use of a UF applied to a subchronic study in the derivation of a chronic reference value is reasonable. Some work has been published on this aspect of extrapolation (Lewis et al., 1990; Pieters et al., 1998). Guidance for replacement of the default factor of 10 by

CSAFs may be forthcoming. It would be appropriate to incorporate such data into applicable assessments. In the current practice, this factor is applied when a chronic reference value is derived from a database in which the critical study is of subchronic duration. No chronic reference value is derived if neither a subchronic nor chronic study is available. The application of a UF to less-than-subchronic studies is not part of the current practice, but further exploration of this issue may be appropriate. For short-term and longer-term reference values, the application of a UF analogous to the subchronic-to-chronic duration UF also needs to be explored, as there may be situations in which data are available and applicable but they are from studies in which the dosing period is considerably shorter than that for the reference value being derived.

4.4.5.7. *Modifying Factor (MF)*

A clear definition of intended usage for an MF is lacking. The only comments located about the MF are in the RfC methodology (U.S. EPA, 1994), and they indicate that the MF is intended to account for scientific uncertainties in the study or database that are not explicitly treated by other UFs. It is further stated that use of the factor depends principally on professional judgment and assessment. Some example applications are also given, such as accounting for small sample size or for poor exposure characterization in the principal study. The definition in the IRIS glossary gives similar examples.

The description of the database UF shows substantial similarity to that of the MF. Text on the database UF indicates that this factor attempts to recognize that without a comprehensive array of endpoints there is uncertainty as to whether all possible toxicologic endpoints at the various life stages are adequately addressed. Without this information, uncertainty remains as to whether the critical effect chosen for RfD or RfC derivation is either the most sensitive or the most appropriate. There are only seven cases in IRIS for which an MF has been applied: RfDs for chromium III, chromium VI, nitrite, 1,1-biphenyl, and manganese and RfCs for methyl ethyl ketone and acetonitrile. The rationale for these varies considerably but in all cases appeared to be for reasons that could be considered under other UFs.

Recent developments in the IRIS process include the obligation for risk characterization within the assessments. A central aspect of risk characterization includes discussing confidence and uncertainties in the quality of data used and the “clarity, transparency, consistency and reasonableness” of the assessment (U.S. EPA, 2000b). Within the risk characterization, the assessor has a pathway provided to discuss and analyze all aspects of uncertainty about the database, including the adequacy or limitations of the database, directly in the assessment.

The Panel considers the purpose of the MF to be sufficiently subsumed in the general database UF. The Panel also notes that the risk characterization section of assessments may be used to provide a full and complete characterization of all uncertainty, including any residual uncertainty that may not be addressed by the other UFs. In view of these factors, the Panel recommends that use of the MF be discontinued.

4.4.6. Future Directions

4.4.6.1. *Chemical-Specific Adjustment Factors (CSAFs)*

There is growing support for the use of CSAFs in place of DAFs (see Section 4.4.3.), and this will provide an incentive to fill existing data gaps (Murray and Andersen, 2001; Meek, 2001; Meek et al., 2001; Bogdanffy et al., 2001). Additional chemical-specific data permit the replacement of components of interspecies or inter-individual variation with data-derived values in the context of the traditional default framework as developed by Renwick (1993) and revised by IPCS (1994). The following is a brief discussion of available methodologies that promote the use of CSAFs in risk assessment.

Renwick (1993) described the use of toxicokinetic and toxicodynamic data as a means of replacing the traditional 10-fold safety factors for human sensitivity and experimental animal-to-human extrapolation in developing acceptable daily intakes. His data-derived approach assigns default values for both toxicokinetic and toxicodynamic differences within each traditional 10-fold safety factor. Specifically, Renwick proposed dividing both the interspecies and the inter-individual UFs into a factor of 2.5 for toxicodynamics and a factor of 4.0 for toxicokinetics. IPCS (1994) has adopted the data-derived approach initially proposed by Renwick (1993), with a slight modification in the UF for inter-individual variation (3.16 for toxicodynamics and 3.16 for toxicokinetics). IPCS has used this approach in several of its recent risk assessments (e.g., IPCS, 1998), and EPA is proposing a similar approach for boron (U.S. EPA, 2001b).

IPCS has developed a draft guidance document (IPCS, 2001) to assist risk assessors in the use of experimental data in deriving CSAFs for interspecies differences and human variability in dose/concentration response assessment. CSAFs have been adopted because they better describe the nature of the refinement to the usual default approach.

For several years, EPA used a more qualitative approach to modify the usual 10-fold default values (Dourson et al., 1996). Recently, it has used a data-derived approach as one of the methods to derive a UF for boron (U.S. EPA, 2001b).

EPA has not yet established guidance for the use of chemical-specific data for deriving UFs, but the division of UFs into toxicodynamic and toxicokinetic components is in the RfC methodology (U.S. EPA, 1994). EPA's assessments of data assume a division of both

interspecies and intraspecies UFs into toxicokinetic and toxicodynamic components that have assigned default values of 3.16 ($10^{0.5}$) each. The Agency will develop its own guidance for the use of CSAFs in risk assessment, based on some of the available methodologies (e.g., IPCS).

The Technical Panel would like to caution the user that for many substances there are relatively few data available to serve as an adequate basis to replace defaults for interspecies differences and human variability with more informative CSAFs. Currently, relevant data for consideration are often restricted to the component of uncertainty related to interspecies differences in toxicokinetics. Although there are fewer relevant data with which to address the other four components namely—interspecies (animal-to-human) differences in toxicodynamics, intraspecies (human) variability in toxicokinetics, intraspecies (human) variability in toxicodynamics, and adequacy of the database—it is anticipated that availability of such information will be needed to apply CSAFs. Specifically, the data-derived CSAF approach for any single substance is necessarily determined principally by the availability of relevant data. The extent of data available is, in turn, often a function of the economic importance of the substance, and this is frequently related to the extent of potential human exposure.

4.4.6.2. *Probabilistic Approaches*

Another approach to quantifying uncertainty in RfD or RfC derivation when data are not sufficient to develop a chemical-specific or biologically based dose-response model is probabilistic analysis. When the available data are sufficient to meaningfully characterize the distributions of interest, a probabilistic approach would provide results as a distribution rather than as a single measure for the dose/concentration-response. For example, distributions could be used for inputs into a toxicokinetic model to derive a distribution of internal dose metrics. Also, the approaches described in the draft IPCS guidance document (IPCS, 2001) are amenable to probabilistic analysis.

Probabilistic analysis for human health assessments generally has been confined to the exposure variables. In deriving human health toxicity reference values, inter-individual variability in toxicokinetics and toxicodynamics is usually represented with a UF because data are insufficient to support a more quantitative representation of these sources of inter-individual variability. Several studies have been published addressing the use of probabilistic data for health assessments (Baird et al., 1996; Maull et al., 1997; Slob and Pieters, 1998; Swartout et al., 1998; Brand et al., 1999; Gaylor and Kodell, 2000; Evans et al., 2001). The Technical Panel recommends that the Agency further evaluate approaches such as probabilistic analysis for characterizing variability and uncertainty in toxicity reference values.

4.4.7. Summary of Key Points from the Case Studies

Two case studies were developed to illustrate many of the recommendations in this report. The studies are for two hypothetical chemicals: Inhalate, a synthetic halogenated aliphatic alkene, and Luteinate, a new pesticide that acts via the neuroendocrine system.

The available database on Inhalate was considered adequate for deriving inhalation reference values for all four durations of exposure (acute, short-term, longer-term, and chronic). Very little is known about the mode of action for Inhalate except for the tumorigenic effects in liver, which are thought to be produced as a result of prolonged cytotoxicity caused by oxidative metabolism. Thus, a nonlinear mode of action is assumed for Inhalate carcinogenesis, and a chronic reference value is derived that takes into account these effects along with others seen after chronic exposure. Acute, short-term, longer-term, and chronic reference values were derived for Inhalate. This case study illustrates the use of a variety of types of data from toxicity testing studies in deriving a set of inhalation reference values, including carcinogenic effects assumed to have a nonlinear dose-response.

Luteinate belongs to a class of chemicals known to work through a neuroendocrine mode of action. In order to ascertain its potency and confirm a similar mode of action, a number of short-term studies were conducted, followed by testing in more traditional toxicology studies to establish its long-term effects and dose response relationships. The data were considered adequate to derive oral reference values for all four durations of exposure. This case study provides an example of the usefulness of mode-of-action information in establishing the short- and long-term effects of Luteinate on relevant target organ systems at different life stages. Such information enables the development of a targeted robust data set for use in establishing reference values for various durations of exposure.

A detailed summary of the case studies is provided in Appendix B. Several key points are described here that demonstrate the use of the proposed framework outlined in this chapter. First, the data are reviewed and characterized on the basis of the hazard and dose-response information, including consideration of the weight-of-evidence factors discussed in Section 4.3.2, above. A narrative statement is used to describe the extent of the database for each chemical as well as the gaps in information that would make the database more robust. Dosimetric adjustments were made to derive HECs in the case of Inhalate. For Luteinate, adjustments for oral exposure were made on a BW^{1} basis and do not incorporate the $BW^{3/4}$ scaling factor or other DAF, as further work is needed on the harmonization of approaches for deriving of oral and dermal HEDs.

The data are presented both in tabular form and in graphical form as an exposure response array to provide a visualization of the data applicable to each duration of exposure.

Then, the reference values are derived by considering all of the relevant data for each duration reference value, weighing the evidence in the database, developing sample values on the basis of various endpoints considered for each duration, and selecting a final reference value for each duration on the basis of an evaluation of each of the relevant endpoints rather than on a single critical study and critical effect.

The approaches illustrated by the case studies showing derivation of multiple-duration reference values are not without precedent. Several offices within EPA, as well as ATSDR and the AEGL committee, derive multiple duration values for various purposes (see review in Chapter 2). The derivation of sample reference values in selecting the final reference value also is not a new idea. For example, EPA's assessment for methylmercury included the derivation of sample RfDs from prospective longitudinal studies of the effects of in utero exposure to methylmercury (Table 4-4) in deriving a chronic RfD (U.S. EPA, 2001d).

Sample RfDs were derived from a number of neuropsychological endpoints from two studies in which an association was observed (New Zealand and the Faroe Islands) as well as an integrative analysis of those studies plus a study in the Seychelles Islands in which no association between in utero methylmercury exposure and deficits in neuropsychological function were reported. The sample RfDs converged on 0.1 μg/kg/day, providing strong support for the appropriateness of this value. This RfD is not a developmental RfD per se, and its use is not restricted to pregnancy or developmental periods. The RfD, derived from an overall evaluation of the database, is applicable to lifetime daily exposure for all populations, including sensitive subgroups.

In a recently released health assessment document on 1,3-butadiene (U.S. EPA, 2002d), sample RfCs were derived for determining the chronic RfC.

Table 4-4. BMDLs, ingested dose, and RfDs for various endpoints from the Faroe Islands, New Zealand, and the NRC integrative analysis

Test[b]	BMDL[a] (ppb mercury cord blood)	Ingested dose[b] (µg/kg/day)	RfD[c] (µg/kg/day)
BNT Faroes			
Whole cohort	58	1.081	0.1
PCB adjusted[d]	71	1.323	0.1
Lowest PCB tertile	40	0.745	0.1
CPT Faroes			
Whole cohort	46	0.857	0.1
PCB adjusted	49	0.913	0.1
Lowest PCB tertile	28	0.522	0.05
CVLT Faroes			
Whole cohort	103	1.920	0.2
PCB adjusted	78	1.454	0.1
Lowest PCB tertile	52	0.969	0.1
Finger Tap Faroes			
Whole cohort	79	1.472	0.1
PCB adjusted	66	1.230	0.1
Lowest PCB tertile	24	0.447	0.05
Geometric mean			
Whole cohort	68	1.268	0.1
PCB adjusted	65	1.212	0.1
Lowest PCB tertile	34	0.634	0.1
Median values			
Faroes	48	0.895	0.1
New Zealand	24	0.447	0.05
Smoothed values			
BNT Faroes	48	0.895	0.1
CPT Faroes	48	0.895	0.1
CVLT Faroes	60	1.118	0.1
Finger Tap Faroes	52	0.969	0.1
MCCPP New Zealand	28	0.522	0.05
MCMT New Zealand	32	0.596	0.1
Integrative			
All endpoints	32	0.596	0.1

[a] $BMDL_{05}$s from NRC (2000), Tables 7-4, 7-5, 7-6. Hair mercury was converted to blood mercury using a 250:1 ratio and an assumption of equivalent maternal and cord levels.
[b] Calculated using a one-compartment model.
[c] Calculated using an UF of 10.
[d] There was significant co-exposure to PCBs in the Faroe Islands study, with PCB cord tissue concentrations available for about half of the whole cohort. Analyses were performed adjusted for PCBs (half the cohort) as well as unadjusted for PCBs in those individuals in the lowest PCB tertile (i.e., one-sixth of the whole cohort).

BNT = Boston Naming Test; CPT = Continuous Performance Test; CVLT = California Verbal Learning Test; MCCPP = McCarthy Perceived Performance; MCMT = McCarthy Motor Test.

5. RECOMMENDATIONS

A number of recommendations have been made in other parts of this report. This chapter summarizes those recommendations, based on the Technical Panel's review of the RfD and RfC process. The Technical Panel assumes that it will be possible to implement some of the recommendations in the near future, given adequate resources and personnel, whereas others will require additional effort. In particular, testing strategies are needed that consider toxicokinetic and mode of action information early in the process, as well as when to implement new testing guidelines in the process of developing a data package on a particular chemical. OPPTS, together with scientists in other parts of the Agency, will consider the recommendations to develop additional or alternative testing guidelines as part of the Harmonized Health Effects Test Guidelines (870 Series).

As part of its deliberations, the Technical Panel considered the recommendations of the Toxicology Working Group of the 10X Task Force (U.S. EPA, 1999b, and Appendix A). The Technical Panel endorses those recommendations and extends and expands them to deal with a broader view of life stages, timing and duration of exposure, and evaluation of endpoints, both structural and functional. The recommendations are presented here in the order of the chapters in which they appear. Further discussion of the specific recommendations can be found in the earlier chapters.

Chapter 2

The Technical Panel concurred with the recommendation of the 10X Task Force that reference values should be derived, where possible, for acute, short-term, and longer-term as well as chronic exposures for oral, dermal, and inhalation routes and that they be included in the IRIS database for use by EPA programs, where applicable. The definitions for duration should be standardized but left flexible so they can be adjusted depending on the exposure situation of concern.

Chapter 3

The Technical Panel reviewed and evaluated current testing guidelines and testing approaches as a follow-up to its recommendation in Chapter 2 concerning the derivation of less-than-lifetime reference values. This review was undertaken to determine what information is currently gathered with regard to life stage assessment, endpoint assessment, route and duration of exposure, and latency to response. The intent of this review is not to suggest that additional

testing be conducted for each and every chemical in order to fill in the information gaps identified for those organ systems evaluated. Nor is it suggested that the alternative testing protocols discussed in this chapter be conducted for every chemical or become part of current toxicology testing requirements or that these alternative protocols are the only options available. Rather, it is the goal of this document to provide a basis for the development of innovative alternative testing approaches and the use of such data in risk assessment. The recommendations include:

- Develop a strategy for alternative approaches to toxicity testing, with guidance on how and when to use existing and newly recommended guidelines.

- Develop guidelines or guideline study protocols that will provide more systematic information on toxicokinetics and toxicodynamics (i.e., mechanism or mode of action), including at different life stages.

- Develop protocols for acute and short-term studies that provide more comprehensive data for setting reference values.

- Modify existing guideline study protocols to provide more comprehensive coverage of life stages for both exposure and outcomes.

- Collect more information from less-than-lifetime exposures to evaluate latency to effect and reversibility of effect.

- Develop guidelines or guideline study protocols to assess immunotoxicity, carcinogenicity, and cardiovascular toxicity at different life stages.

- Explore the feasibility of setting dermal reference values for direct toxicity at the portal of entry, including sensitization.

Chapter 4

The Technical Panel discussed a number of modifications to the existing framework for reference value derivation, both for the current chronic reference values (RfD and RfC) and for the acute, short-term, and longer-term reference values. In addition, two case studies that

illustrate many of these concepts are summarized in Chapter 4 and discussed in detail in Appendix B. The recommendations for improvement and expansion of the existing approaches are aimed at taking a broader approach to the characterization of the entire database and what impact that will have on the dose-response assessment and risk characterization of a chemical. Included are recommendations for setting several less-than-lifetime reference values, broader characterization of the database instead of using a checklist of a minimum set of studies for setting a reference value, using an exposure-response array and carrying appropriate and relevant endpoints through the derivation of sample reference values before deciding which endpoint(s) to use for the POD, and deriving reference values in a way that is protective of all relevant endpoints rather than setting reference values on particular endpoints (e.g., the RfD_{DT}) but using a process that facilitates the evaluation of risk to particular subgroups for specific program office needs, including cumulative risk assessment.

The specific recommendations follow:

1. Include the acute, short-term, longer-term, and chronic reference values derived on the basis of the recommendations in this report in IRIS after appropriate internal, external, and consensus review.

2. Use consistent definitions for the duration of exposure in deriving acute, short-term, longer-term, and chronic reference values.

3. Use the revised definition for reference values shown in Chapter 4. This definition is aimed at clarifying that the approach to reference values discussed here is intended for risk assessments for any type of health effect known or assumed to be produced through a nonlinear and/or threshold mode of action (which may include U-shaped or other nonmonotonic dose-response curves as well as thresholds). Thus, the term “noncancer” has been removed from the definition in the spirit of overall harmonization of risk assessment approaches for human health effects because it has been recommended that health effects no longer be categorized as “cancer” or “noncancer” for the purposes of hazard characterization and dose-response analysis. This change denotes the move toward defining approaches for low-dose estimation or extrapolation based on mode of action.

The term “deleterious” has been replaced with the term “adverse,” because the latter is more commonly used and understood in data evaluation and selection of endpoints for setting reference values. The parenthetical statement in the current RfD and RfC definitions, “with uncertainty spanning perhaps an order of magnitude,” has been removed from the proposed revision of the definition for reference value, and it is recommended that issues of uncertainty/variability be discussed qualitatively as part of the weight of evidence and characterization of the database.

4. For consistency in the designation of various duration reference values, the Panel recommends that the terminology for reference values be standardized; this standardized terminology should reflect both duration and route of exposure. Consistent terminology recommendations for reference values are proposed in this report, but additional suggestions are welcome.

5. The Technical Panel recommends that endpoint-specific reference values per se not be developed, including the RfD_{DT}, which was originally proposed in *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991). Rather, a sample reference value should be calculated for each relevant and appropriate endpoint and these should then be considered in the derivation of various duration reference values. The reference values should be derived to be protective of all types of effects for a given duration of exposure.

6. An expanded approach to the evaluation of studies and characterization of the extent of the database as a whole is recommended; in particular, several factors are discussed that should be considered in a weight-of-evidence approach for characterizing hazard for the population as a whole as well as for potentially sensitive subpopulations. Those considerations for assessing level of concern raised by the Toxicology Working Group of the 10X Task Force (U.S. EPA, 1999b) have been incorporated into this approach.

7. A narrative approach rather than a confidence ranking of high, medium, or low should be used in describing the extent of the database. The extremes for the extent of the database (i.e., minimal or robust) are defined in Chapter 4. The narrative approach is intended to emphasize the types of data available (both human and animal) as well as research needed to fill the data gaps that could improve the derivation of reference

values, and it should encourage the use of a wider range of information in deriving reference values, taking into consideration the life stages evaluated; the issues of timing, duration, and route of exposure; the types and extent of endpoint assessment (i.e., structure and function); and the potential for latent effects and/or reversibility of responses.

8. Duration adjustment procedures to continuous exposures for inhalation developmental toxicity studies should be done in the same way as for other health endpoints.

9. Additional consideration of the HEC and HED derivation methodology is needed to confirm or assess the relevance for all population subgroups (particularly children).

10. An exposure-response array should be used as a visual display of all relevant endpoints and durations of exposure, as shown in the case studies. This type of array can be used to evaluate the range of exposure-response data for different durations of exposure in order to determine the range of numerical values available for each route and duration reference value.

11. The POD should be selected on the basis of an evaluation of all appropriate and relevant endpoints carried through to sample reference value derivation, with selection of the limiting value(s) as the final step, rather than on a single “critical study” and “critical effect.”

12. It is imperative that the IRIS documentation contain a justification for the individual factors selected for each chemical or assessment, because rigid application of UFs could lead to an illogical set of reference values. Although default factors of 10 are recommended, with 3 used in place of half-power values (i.e., $10^{0.5}$) when occurring singly, the exact value of the UF chosen should depend on the quality of the studies available, the extent of the database, and scientific judgment. Sound scientific judgment should be used in the application of UFs to derive reference values that are applied to the value chosen for the POD derived from the available database (BMDL, NOAEL, or LOAEL).

13. The Technical Panel recommends that if there is uncertainty in more than four areas of extrapolation, it is unlikely that the database is sufficient to derive a reference value. Even when there is uncertainty in four areas, the database should be carefully evaluated to determine whether the derivation of a reference value is appropriate. In addition, the Technical Panel recommends limiting the total UF applied to a chronic reference value for any particular chemical to 3000. This maximum of 3000 applies only to the UFs and does not include the various adjustment factors discussed in Chapter 4.

14. The intraspecies UF is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed. As the reference concentration/dose is defined to be applicable to "susceptible subgroups," this UF was established to account for uncertainty in that regard. In general, the Technical Panel reaffirms the importance of this UF, recommending that reduction of the intraspecies UF from a default of 10 be considered only if data are sufficiently representative of the exposure/dose-response data for the most susceptible subpopulation(s).

At the other extreme, a 10-fold factor may sometimes be too small because of factors that can influence large differences in susceptibility, such as genetic polymorphisms. The Technical Panel urges the development of data to support the selection of the appropriate size of this factor, but it recognizes that often there are insufficient data to support a factor other than the default.

15. The Technical Panel urges continued research and evaluation of the similarities and differences between the general population and sensitive subpopulations in their responses to particular agents, particularly children and the elderly. From such evaluations, the protectiveness of the 10-fold default factor can continue to be assessed.

16. Given that several UFs can be used to deal with data deficiencies as part of the current reference value process, and given that these are assumed to overlap to some extent, the Technical Panel agrees with the 10X Task Force Toxicology Working Group (U.S. EPA, 1999b) that the current interspecies, intraspecies, and database deficiency UFs, if appropriately applied using the approaches recommended in this review, will be

adequate in most cases to cover concerns and uncertainties about children's health risks. Any residual concerns about toxicity and/or exposure can be dealt with in risk characterization/risk management (e.g., by retention of all or part of the FQPA safety factor for pesticides).

17. The Panel considers the purpose of the MF to be sufficiently subsumed in the general database UF. Therefore, the Panel recommends that use of the MF be discontinued.

18. EPA has not yet established guidance for the use of specific data to replace UFs (i.e., CSAFs), but the division of the interspecies UF into toxicodynamic and toxicokinetic components is in the RfC methodology (U.S. EPA, 1994) and may apply to the intraspecies UF as well. The Agency is encouraged to develop its own guidance, based on some of the available methodologies (e.g., IPCS).

The following issues were discussed by the Technical Panel but were considered more appropriate for discussion and recommendation by other panels/committees:

1. There have been inconsistencies in the use of BMD modeling approaches to deriving RfDs and RfCs currently in IRIS. The Technical Panel was unable to fully evaluate these issues or to reach agreement about any recommendation for change to current methodology and recommends that they be considered further by the Agency. The Technical Panel also recommends that factors such as the response rates at the BMD or NOAEL, the power of the study, and slope of the dose-response curve be included in the description of the database, where possible, as part of risk characterization.

2. The Technical Panel recommends harmonization of the approaches for HEC and HED derivation for all types of health effects. Development of the appropriate adjustment procedure is referred to the Harmonization Framework Technical Panel.

3. The Technical Panel recommends that the Agency further evaluate approaches such as probabilistic analysis for characterizing variability and uncertainty in toxicity reference values.

4. The Technical Panel recommends further evaluation of appropriate adjustment of doses for duration of exposure. The method derived from ten Berge et al. (1986) is raised as a possibility for acute exposures on the basis of its recommendation in the ARE methodology. Duration adjustment for short-term and longer-term reference values analogous to the subchronic-to-chronic duration UF for chronic reference values is raised in the case study and should be explored further.

APPENDIX A: ISSUES RAISED BY THE 10X TASK FORCE

A number of issues were raised by the 10X Task Force[1] in its discussions of the requirements for protecting children's health and application of an additional 10X safety factor, as mandated by the 1996 FQPA. The Task Force felt that these issues, which include the following, should be discussed on a broader Agency-wide basis as well as with the outside community for both pesticides and other agents.

1. *Appropriate application of the database modifying factor for additional required developmental and adult toxicity studies.* It appears from the data available that the default intraspecies 10-fold uncertainty factor may be adequate in the majority of cases for protecting children's health. However, when data specific to children's health are missing or inadequate for a particular agent, application of the database modifying factor in addition to the intraspecies variability factor may be sufficient to account for the possibility that children may be significantly more sensitive than adults. This issue needs further examination.

2. *How to account for the level of concern in the RfD/RfC process.* Criteria for assessing the level of concern for children's health were developed by the Toxicology Working Group of the 10X Task Force and include factors such as (a) human data on pre- and postnatal toxicity; (b) pre- and postnatal toxicity in animal studies, including effects of a different or similar type as those in adults; (c) dose-response nature of the experimental animal data, including the dose-related incidence of response, relative potency of response, slope of the dose-response curve when the margin of exposure is small, and how well the NOAEL or BMD is defined; and (d) relevance of the experimental animal data to humans, including toxicokinetics, similarity of the biological response, and knowledge of the mechanism of action. For each of these areas, criteria are given for estimating a level of concern for children's health as high, moderate, or low. The level of concern may be taken into account in the uncertainty and modifying factors applied to the RfD, although there is currently no formal process for doing so.

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[1]See 10X Task Force documents: *Toxicology Data Requirements for Assessing Risks of Pesticide Exposure to Children's Health* (U.S. EPA, 1999b) and *Exposure Requirements for Assessing Risks from Pesticide Exposure to Children's Health* (U.S. EPA, 1999c).

3. As indicated in the toxicology document appended to the Task Force report, *the current default recommended for using developmental toxicity data for different duration reference values is to apply most endpoints for all durations*. This is because it is assumed that most endpoints of developmental toxicity can be caused by a single exposure. If, however, developmental effects are more sensitive than those seen after longer-term exposures, then even the chronic RfD/RfC should be based on such effects to reduce the risk of potential greater sensitivity in children. Because the standard studies currently conducted for developmental toxicity involve repeated exposures, data are not often available on which endpoints may be induced by acute, subacute, subchronic, or chronic dosing regimens and, therefore, on which should be used in setting various duration reference values. Further consideration of the appropriate application of developmental toxicity endpoints to various duration reference values is recommended. As part of this recommendation, an in-depth review of the HED document on Hazard Identification—Toxicology Endpoint Selection System, should be undertaken.

4. *Appropriate setting of intermediate RfDs/RfCs for pesticides and other agents.* The focus of the RfD and the RfC has been on chronic exposure reference values. Acute RfDs are also set for pesticides, and intermediate reference values are set for residential exposures as well as for drinking water. Data on developmental toxicity will often be a greater factor in calculating the acute and intermediate reference values, and exposures to children are more often of this type as well. Consideration should be given to setting intermediate reference values for environmental agents. In addition, the question of whether or when to set RfDs/RfCs specific for children should be considered.

5. *Appropriate adjustment of the NOAEL or the BMD from inhalation exposure studies for extrapolation of developmental toxicity data using less-than-continuous exposure to a continuous-exposure scenario.* Currently, NOAELs/BMDs from inhalation exposure studies other than those for developmental toxicity using, for example, a 6-hr/day exposure regimen, are adjusted to a continuous (24 hr/day) exposure for calculating RfDs/RfCs. The developmental toxicity risk assessment guidelines (U.S. EPA, 1991) recommended against making this adjustment because it was assumed that there was a threshold above which exposure would have to occur before an effect would result. This recommendation needs to be reconsidered, along with the adjustment of NOAELS/BMDs in general.

Several improvements in testing approaches were also proposed for consideration in the 10X Task Force report as a way to improve the assessment of potential risks to children. The Technical Panel was asked to consider the need for such tests, when they should be required, and interpretation of the data for risk assessment purposes. The improvements to be considered include

- pharmacokinetics that include data from different developmental stages, perhaps done in a tiered approach as suggested in Kimmel and Francis (1990);

- direct dosing of neonates, especially when early exposure is of concern, because this is the time when differences in metabolic capability are greatest;

- perinatal carcinogenesis studies and appropriate triggers for when they should be required;

- developmental immunotoxicity testing and appropriate triggers; and

- advanced DNT testing, in particular, cognitive testing that is more similar to that used in humans.

An additional issue was how to make exposure assessments compatible with the dose-response assessment. For example, how should the appropriate durations of exposure be determined for toxic endpoints of concern? Should standard exposure durations be used?

APPENDIX B: CASE STUDIES—EVALUATING AND SELECTING HEALTH ENDPOINTS FOR DERIVING REFERENCE VALUES

Two case studies were developed by the Technical Panel to illustrate many of the points discussed in this report. The first case study is of a hypothetical volatile chemical for which limited data are available and little information is known about the mode of action except that there is support for a nonlinear mode of action for cancer.

The second case study is of a hypothetical endocrine disruptor for which the mode of action is known or assumed from other chemicals in the same class. This case study is used to illustrate in part how the information on mode of action can inform a more focused collection of data as well as the interpretation of the data and its use in risk assessment.

In both case studies, NOAELs and LOAELs rather than BMDLs or BMCLs are used to derive reference values, in large part because the data are fictitious and were not developed to the point that they could be readily modeled. However, the Technical Panel strongly encourages the use of dose-response modeling and calculation of BMDLs or BMCLs for selection of the POD to be used as the basis for deriving reference values.

CASE STUDY 1: INHALATE

Inhalate, a synthetic halogenated aliphatic alkene, is a nonflammable volatile liquid at room temperature. The chemical enters the air through its industrial and commercial use, primarily as a solvent. It is also found in surface and ground water and soil upon disposal. The most important route of human exposure is inhalation of the chemical in the ambient and indoor air, although there is a lower possibility of ingestion through contaminated drinking water. Because of its high volatility, dermal exposure to the chemical is expected to be minimal.

This case study illustrates the use of single or multiple health endpoints for deriving reference values for different durations of exposure following inhalation exposure. It also illustrates the harmonized approach for all effects (including cancer) that are known or assumed to be produced through a nonlinear or threshold mode of action. For the purpose of illustration, results of key studies are summarized in Table B-1, including dose-response data for different health endpoints relevant to different durations of exposure via inhalation exposure. Although oral data for this chemical were available, a brief description is included here only to show the consistency with which effects were seen after either inhalation or oral exposure.

SUMMARY OF HEALTH EFFECTS INFORMATION

Absorption, Distribution, Metabolism, and Elimination

There is very little information on the absorption and distribution of Inhalate in humans and laboratory animals following oral, inhalation, or dermal exposure. However, similar effects are seen by oral and inhalation exposures, suggesting that Inhalate or its metabolites reach their target sites after absorption from either exposure route. Available in vitro metabolic studies indicate that Inhalate is extensively metabolized in target tissues including the liver and kidney of rats and mice. Limited in vitro studies with human tissues show a similar pattern of metabolism. As discussed below, much of Inhalate-induced toxicity appears to be due to its metabolites. These metabolites have been detected in the urine of rats and mice following inhalation and oral exposure to the parent chemical.

Postulated Mode of Action

No information is available on mode of action except for the carcinogenicity of Inhalate. The carcinogenic effects of Inhalate in rodent liver are attributed to oxidative metabolism-mediated cytotoxicity in the target organ. The oxidative metabolism produces highly tissue-reactive metabolites that lead to tissue injury and cell death. The persistent cell proliferation

Table B-1. Summary results of major inhalation exposure studies on Inhalate

Species	Sex	Exposure duration and frequency	Concentrations (mg/m^3)	LOAEL/ NOAEL (mg/m^3)	HEC [a] (mg/m^3)	Responses
Human	M/F	2 hrs	4, 40, 400	40/4	40/4, 2-hr	Headache, dizziness, incoordination, drowsiness, anesthesia at 2000 mg/m^3
		Accidental exposure	NA	NA	-	Narcosis, proteinuria, hematuria
		6 hrs/day for 7 days (clinical exposure)[b]	10, 20, 100, 150	20/10	4/2	Headache, dizziness, incoordination, drowsiness
		Occupational (>15 yrs)	TWA of 56	20/NA	20/NA	Dizziness, forgetfulness; changes in serum liver enzymes; increased urinary levels of lysozymes, beta-glucuronidase
	F	Occupational	NA	NA	-	Menstrual disorders; spontaneous abortion; cardiac anomalies in children of workers
Rat/ mouse	M/F	4 hrs	0, 13, 24, 50	13/NA	13/NA, 4-hr	Dose-related hyperactivity, ataxia, hypoactivity, narcosis
		6 hrs/day, 5 days/wk for 2 wks	0, 50, 100, 200	50/NA	9/NA	Dose-related hyperactivity, ataxia, hypoactivity, narcosis
Rat	M/F	6 hrs/day, 5 days/wk for 13 wks	0, 30	30/NA[c]	–	Changes in fatty acid composition of the brains

Table B-1. Summary results of major inhalation exposure studies on Inhalate (continued)

Species	Sex	Exposure duration and frequency	Concentrations (mg/m^3)	LOAEL/ NOAEL (mg/m^3)	HEC [a] (mg/m^3)	Responses
Rat (cont)	M/F	6 hrs/day, 5days/wk for 13 wks	0, 22, 39, 88	39/22	7/4	Dose-related hyperactivity, ataxia; liver hypertrophy, vacuolization of hepatocytes, necrosis; cytomegaly, toxic nephrosis of tubular epithelial cells
Mouse	M/F	6 hrs/day, 5 days/wk for 13 wks	0, 28, 50, 100	50/28	9/5	Dose-related hyperactivity, ataxia; liver hypertrophy, vacuolization of hepatocytes, necrosis; cytomegaly, toxic nephrosis of tubular epithelial cells
Rat	M/F	6 hrs/day, 5 days/wk for 104 wks	0, 11, 22, 44	22/11	4/2	Clinical signs of neurotoxicity; dose-related liver hypertrophy, vacuolization of hepatocytes, necrosis, hepatocellular carcinoma; cytomegaly, toxic nephrosis of tubular epithelial cells
Mouse	M/F	6 hrs/day, 5 days/wk for 78 wks[e]	0, 11, 28, 56	28/11	5/2	
Rat	F	GDs 70–13, 6 hrs/day	0, 10, 90	90/10	22.5/2.5	Decreased motor activity in pups; ataxia in dams at high dose
	M/F	Two-generation reproductive study, 6 hrs/day, 5 days/wk	0, 28, 100	100/28	18/5	Reduced litter size, reduced survival of offspring, sedation at high dose

[a] These values are approximate HECs derived in accordance with the RfC methodology (U.S. EPA, 1994) and Chapter 4 (this report) for category 3 Gases using pharmacokinetic data for Inhalate.
[b] Data included here for comparison; data from intentional human exposures are not currently used by EPA in risk assessment.
[c] Special study for neurotoxicity, no HEC calculated.
[d] Considered to be a lifetime exposure
HEC = human equivalent conentration
NA = not available
TWA = time-weighted average
GD = gestation day

presumably would lead to higher probabilities of cell mutation and subsequent cancer. Liver tumors were produced only at dose levels that resulted in repeated or sustained cytotoxicity and regenerative cell proliferation. This postulated mode of action is further supported by the observation in specialized studies that neither the cytotoxicity nor cell proliferation occurred in the CYP2E1 null mouse or in the wild type treated with a P450 inhibitor at the same exposure. The weight of the evidence indicates that a mutagenic mode of action via DNA reactivity is not a significant component of Inhalate-induced liver tumors in rats and mice. There are no data indicating that the mode of action observed in rodents is not also likely to apply to humans.

Nervous System

Inhalate has been found to elicit dose-dependent clinical signs of CNS effects in adult humans following acute inhalation exposure and accidental ingestion. CNS symptoms have been reported in several studies of occupational exposure of workers to Inhalate. Dose-dependent clinical signs of CNS effects have been observed in adult rats and mice exposed to Inhalate by inhalation following different duration of exposures. There are limited data indicating that prenatal exposure to Inhalate adversely affects the developing nervous system in rats and mice (see Growth and Development below). There are no data on the ability of Inhalate to affect the nervous system at other life stages (e.g., during the fetal period, infancy, childhood, or old age). The mechanism of action for the CNS effects has not been clearly established, but it is believed to be related to effects of the parent compound on lipid and fatty acid composition of the membranes.

Inhalation Exposure

Several reports available in the open literature indicate dose-dependent clinical signs of CNS symptoms in adults exposed acutely and subacutely via inhalation to Inhalate. Males and females exposed acutely to high concentrations (40–400 mg/m^3 for 2 hours) showed dose-dependent effects, including headache, dizziness, incoordination, drowsiness, and anesthesia. No effect was reported following acute exposure to 4 mg/m^3. Similar effects were observed in adult human volunteers at lower concentrations (10, 20, 100, 150 mg/m^3) for 6 hours per day for up to 7 days, with a NOAEL of 10 mg/m^3. An acute accidental exposure of a small group of workers to an unknown (presumably high) concentration resulted in narcosis.

Long-term and chronic neurotoxic effects have been reported in several studies of occupational exposure of workers to Inhalate in different industries. Exposure data were not provided in these reports; however, it can be presumed that these workers were exposed to a

daily TWA exposure of 56 mg/m^3. Subjective neurological symptoms, including dizziness and forgetfulness, were consistently reported across studies. No other information on possible neurological effects was collected in these studies.

Concentration-dependent clinical signs of neurological effects, including hyperactivity, ataxia, hypoactivity, and finally loss of consciousness, have also been reported in adult rats and mice following acute (13, 24, 50 mg/m^3 for 4 hours) and short-term inhalation exposure (50,100, 200 mg/m^3 6 hrs/day for 2 weeks) to Inhalate at high concentration. Similar effects were observed in rats exposed at 39 and 88 mg/m^3 and in mice at 50 and 100 mg/m^3 for 13 weeks. The subchronic NOAELs for rats and mice were 22 and 28 mg/m^3, respectively.

Chronic exposure to Inhalate at lower concentrations resulted in less serious clinical signs of CNS effects in rats (22 or 44 mg/m^3) and mice (28 or 56 mg/m^3). The chronic NOAELs for rats and mice in these studies were both 11 mg/m^3. It should be noted that neurological endpoints examined in these animal studies are limited to clinical signs and histopathology. In a special study, changes in fatty acid composition of the brain were observed in rats exposed at 30 mg/m^3 (the only tested concentration) for 90 days.

Pregnant rats were exposed by inhalation to Inhalate at 0, 10, or 90 mg/m^3 for 6 hrs/day on days 7–13 of gestation. Decreased motor activity was observed in 21- or 60-day-old pups from dams exposed to 90 mg/m^3. A NOAEL of 10 mg/m^3 for developmental neurotoxicity was identified in this study.

Oral Exposure

Acute neurological effects in adult humans after ingestion of Inhalate are similar to those seen after inhalation. Accidental exposure to approximately 6–8 mL (or about 100 mg/kg/day) resulted in narcotic effects. Neural tube defects and eye anomalies were reported in studies of offspring of residents exposed to drinking water contaminated with Inhalate and other solvents. Exposure levels were not determined in this study.

Single oral gavage administration of Inhalate to adult rats (1000 mg/kg) caused ataxia. Ataxia was also observed in pregnant rats treated by gavage at 900 mg/kg on gestation days (GDs) 6–19. No CNS effects were reported in a chronic oral gavage study in rats and mice at 50, 100, or 300 mg/kg/day. However, neurological endpoints examined in these studies were limited to clinical signs and histopathology.

In a study that investigated the effect of Inhalate on the developing nervous system, male mouse pups were treated by gavage at 50 or 300 mg/kg/day for 7 days (postnatal days [PNDs]

10–17). Hyperactivity was reported in animals during adulthood at the high dose. No studies with exposure throughout development (prenatal and postnatal) were available.

Liver

Two reports provided suggestive evidence of liver effects in workers exposed to Inhalate in different industries. Inhalate has been shown to induce dose-dependent liver toxicity in adult rats and mice following subchronic and chronic exposure by inhalation and oral gavage. Liver tumors were also observed in chronic studies of rats and mice. Available data support the conclusion that liver tumors were produced only at dose levels that resulted in repeated or sustained cytotoxicity and regenerative cell proliferation. Inhalate carcinogenic effects in rodent liver are attributed to oxidative metabolism-mediated cytotoxicity in the target organ. The oxidative metabolism produces highly tissue-reactive metabolites that lead to tissue injury and cell death. The persistent cell proliferation presumably would lead to higher probabilities of cell mutation and subsequent cancer.

This postulated mode of action is further supported by the observation in specialized studies that neither the cytotoxicity nor cell proliferation occurred in the CYP2E1 null mouse or in the wild type treated with a P450 inhibitor at the same exposure. The weight of the evidence indicates that a mutagenic mode of action via DNA reactivity is not a significant component of Inhalate-induced liver tumors in rats and mice. No data exist indicating that the mode of action observed in rodents is not also likely to apply to humans. There are no data that provide any insights into possible differential sensitivity across life stages.

Inhalation Exposure

One study reported changes in serum levels of liver enzymes in workers exposed to the chemical at a daily TWA exposure concentration of about 56 mg/m^3 over an 8-hour work shift. These workers, however, did not exhibit any clinical symptoms of liver dysfunction.

Dose-related liver effects (liver hypertrophy, vacuolization of hepatocytes, necrosis) have been observed in mice following subchronic exposure (13 weeks) to Inhalate at 50 or 100 mg/m^3, with a NOAEL of 28 mg/m^3. Dose-related liver toxicity and hepatocellular carcinomas were also found in mice following chronic exposure at 28 and 56 mg/m^3. The NOAEL for liver toxicity in mice in this chronic study was 11 mg/m^3.

Rats showed similar liver responses but at higher exposure concentrations following subchronic exposure (39 or 88 mg/m^3), with a NOAEL of 22 mg/m^3. Liver toxicity and hepatocellular carcinomas were also observed at 22 or 44 mg/m^3 in a chronic study in rats. The

NOAEL for liver effects in rats was 11 mg/m^3. It should be noted that liver effects examined in these subchronic and chronic studies were limited to clinical chemistry, morphology, and histopathology.

Oral Exposure

Liver effects were observed in mice and rats treated subchronically (100, 300, 500 mg/kg/day) or chronically (50, 100, 300 mg/kg/day) with Inhalate via oral gavage and were similar to those seen after inhalation exposure. Mice showed more severe effects than did rats. Dose-related hepatocellular carcinomas were also found in treated mice in a chronic study.

Kidney

Available human and animal studies indicate that Inhalate also has the potential to cause renal toxicity in adults. The mechanisms for the development of kidney effects in humans and animals are not known. No data were available to evaluate the effects of Inhalate at life stages other than in adults (i.e., during development or old age).

Inhalation Exposure

Symptoms of renal dysfunction (proteinuria, hematuria) have been associated with accidental human exposure to anesthetic concentrations of Inhalate. Subtle or no renal effects were reported in workers exposed chronically. Increased urinary levels of lysozyme and beta-glucuronidase suggestive of mild renal tubular damage have been observed in workers exposed for an average of 15 years to a daily TWA concentration of 56 mg/m^3.

Dose-related renal toxicity (cytomegaly, toxic nephrosis of tubular epithelial cells in the inner renal cortex) was induced in rats (39, 88 mg/m^3) and mice (50, 100 mg/m^3) exposed to Inhalate for 13 weeks. Subchronic NOAELs for renal effects in rats and mice were 22 mg/m^3 and 28 mg/m^3, respectively. Similar renal effects were observed in a chronic study in rats (22, 44 mg/m^3) and mice (28, 56 mg/m^3). Chronic NOAELs for renal effects in rats and mice were both 11 mg/m^3.

Oral Exposure

Dose-related toxic nephropathy characterized by degenerative changes in the proximal convoluted tubules and necrosis of the tubular epithelium were found in rats and mice treated with Inhalate via oral gavage for 90 days at 100, 300, or 500 mg/kg/day and for 2 years at 50,

100, or 300 mg/kg/day. Subchronic and chronic NOAELs for renal effects in rats and mice were at 100 and 50 mg/kg/day, respectively.

Growth and Development

Available studies in humans and animals indicate that Inhalate has the potential to cause developmental effects by inhalation and oral ingestion. Limitations of human studies could not resolve whether the observed developmental effects were causally related to the chemical or were a result of chance or bias. However, the epidemiologic findings are supported by animal studies with exposure to Inhalate by inhalation and oral gavage that show that the developing nervous system is the most sensitive target in rats and mice.

Inhalation Exposure

Epidemiologic studies of women occupationally exposed to Inhalate and other related solvents have reported elevated risk of spontaneous abortion and cardiac anomalies in their offspring. Due to limitations of these studies, an exposure-response could not be established. No other health endpoints were investigated in these studies.

Pregnant rats were exposed by inhalation to Inhalate at 0, 10, or 90 mg/m^3 for 6 hrs/day on days 7–13 of gestation. Decreased motor activity was observed in 21- and 60-day-old pups from dams exposed to 90 mg/m^3. A NOAEL of 10 mg/m^3 for developmental effects was identified in this study. No studies included exposure throughout gestation and lactation to determine effects at other developmental stages. Data from a two-generation reproduction study indicated a reduction in litter size and survival of offspring in rats exposure to Inhalate at 100 mg/m^3, a concentration that also resulted in sedation and renal effects. No effects were reported at 28 mg/m^3.

Oral Exposure

Neural tube defects and eye anomalies have been reported in studies of residents exposed to drinking water contaminated with Inhalate and other solvents. Exposure levels were not determined in this study.

An increased incidence of micro/anophthalmia were observed in the offspring of rats treated with Inhalate by gavage at 900 mg/kg/day on GDs 6–19. In a study that investigated the effect of Inhalate on the developing nervous system, male mouse pups were treated by gavage with Inhalate at 50 or 300 mg/kg/day for 7 days (age 10–17 days). Hyperactivity was reported in animals during adulthood at the high dose. No effects were found at the low dose. No studies

included exposure throughout gestation and lactation to determine effects at other developmental stages.

Reproductive System

Available studies in humans and animals suggest that Inhalate may have the potential to cause reproductive effects. The underlying mechanism of action for potential reproductive effects is not known.

Inhalation Exposure

There is suggestive evidence of spontaneous abortion and menstrual disorders among women occupationally exposed to Inhalate. However, no definitive conclusions can be made because of the limitations associated with these studies.

Reduced litter size and reduced survival of offspring were reported in rats exposed to Inhalate at 100 mg/m^3 in a two-generation reproduction inhalation study; this concentration also resulted in sedation and renal effects. No effects were identified at 28 mg/m^3. The protocol used, however, was not the most recent one, in which reproductive development (e.g., timing of puberty or anogenital distance) and adult reproductive function (semen quality, estrous cyclicity) are evaluated, nor were organ weights measured. No effects on the reproductive system were noted in any other studies.

Oral Exposure

No information is available on the potential reproductive effects of Inhalate in animals via oral exposure.

SELECTION OF HEALTH ENDPOINTS AND DERIVATION OF REFERENCE VALUES

Narrative Description of the Extent of the Database

No information is available on possible modes of action except for liver carcinogenic effects in rats and mice. In this case, the mode of action is attributed to oxidative metabolism-mediated cytotoxicity and persistent cell proliferation in the liver. Persistent regenerative cell proliferation presumably would lead to higher probabilities of cell mutation and subsequent cancer. No data exist to indicate that the mode of action observed in rodents is not also likely to apply to humans. Pharmacokinetic data indicate that Inhalate is extensively metabolized and that much of its toxicity is due to the metabolites.

The database for inhalation exposure is limited but adequate for deriving reference values. Some human data on acute, short-term, and longer-term exposures are available, although the range of endpoints evaluated and the dose-response information for different durations of exposure are limited. The animal data include acute, short-term, longer-term, and chronic studies with exposures beginning in young adult animals. The acute and short-term data are limited to clinical signs of morbidity and mortality, whereas the longer-term and chronic studies include some histopathology as well.

A developmental neurotoxicity (DNT) study was conducted in rats with prenatal exposure limited to GDs 7–13 (as opposed to more extensive exposure throughout a major part of CNS development, e.g., GD 6 to PND 11 or 20 in the standard DNT study testing protocol). No other studies of prenatal or postnatal developmental toxicity were done except for evaluations of survival and growth in a two-generation reproduction study in rats. However, the protocol used was one in which reproductive development (e.g., timing of puberty or anogenital distance) and adult reproductive function (semen quality, estrous cyclicity) were not evaluated, nor were organ weights measured. No studies were conducted that considered issues related to the toxicity of the agent in old age, either from earlier exposures or from exposures in aged animals.

The database for oral exposure is much more limited than the database for inhalation exposure, with acute accidental ingestion data in humans at a single, high-dose level resulting in narcosis and chronic drinking water exposure (no dose information) associated with an increase in birth defects. The animal data are likewise very limited, with a single-dose acute toxicity study in rats in which clinical signs of morbidity and mortality were evaluated and subchronic (90-day) and chronic toxicity data in rats and mice indicating effects similar to those seen with inhalation exposure. Prenatal developmental toxicity data were available in rats following exposure on GDs 6–19, and an evaluation of adult neurotoxicity was conducted in mice following developmental exposure on PNDs 10–17. No other developmental toxicity data, and no information on reproductive toxicity or adult neurotoxicity were available. No studies were conducted that considered issues related to the toxicity of the agent in old age, either from earlier exposures or from exposures in aged animals.

Exposure-Response Array

In addition to displaying the data in tabular form (Table B-1), an exposure-response array can be a useful way of visually displaying the data (see Figures B-1 through B-4) to show what data are available for each duration of exposure. The data shown in the graphs are the human equivalent concentrations (HECs) based on the dosimetric adjustments discussed in Chapter 4 of

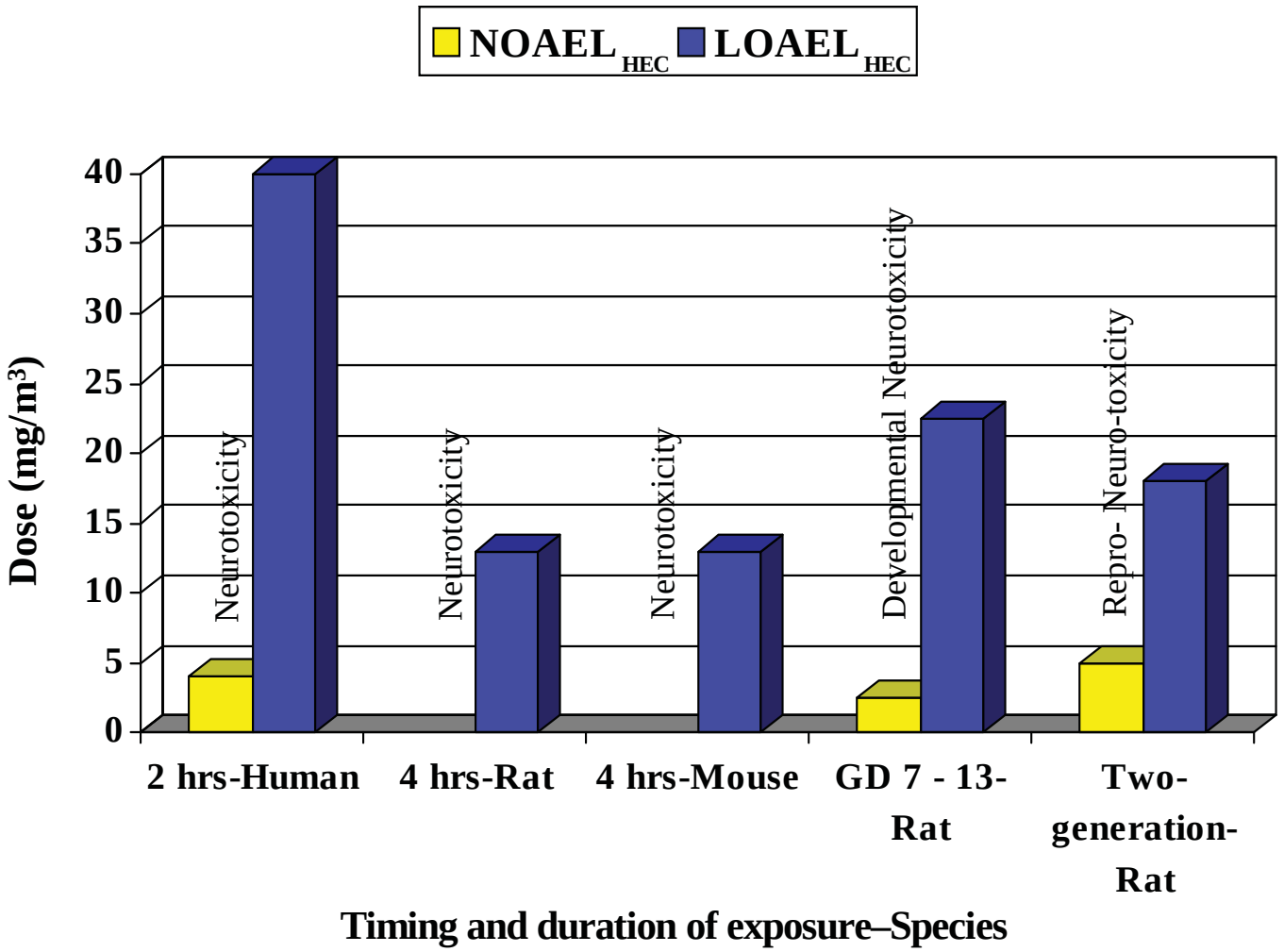

Figure B-1. Exposure-response array of data considered for the Inhalate acute reference value.

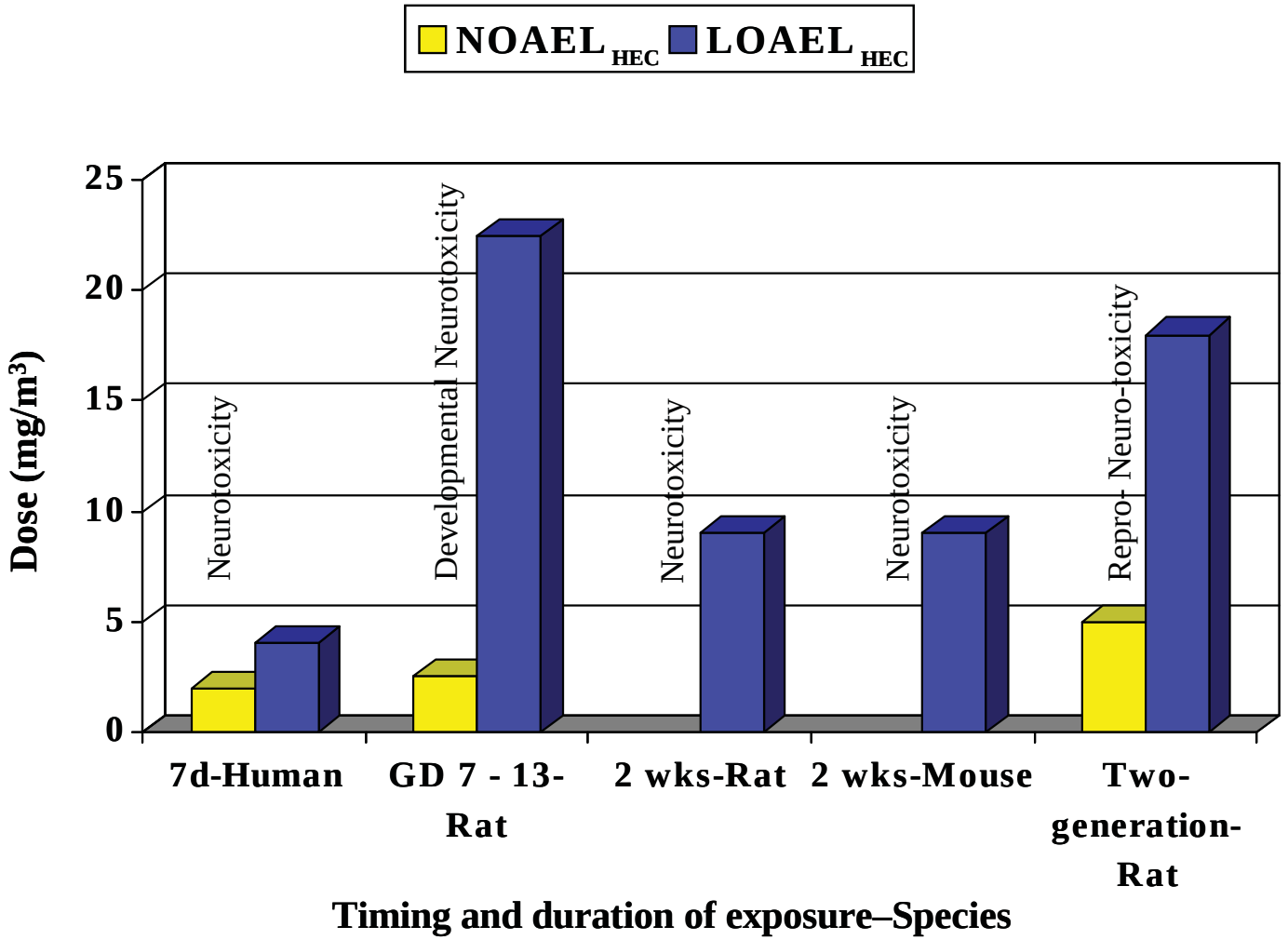

Figure B-2. Exposure-response array of data considered for the Inhalate short-term reference value.

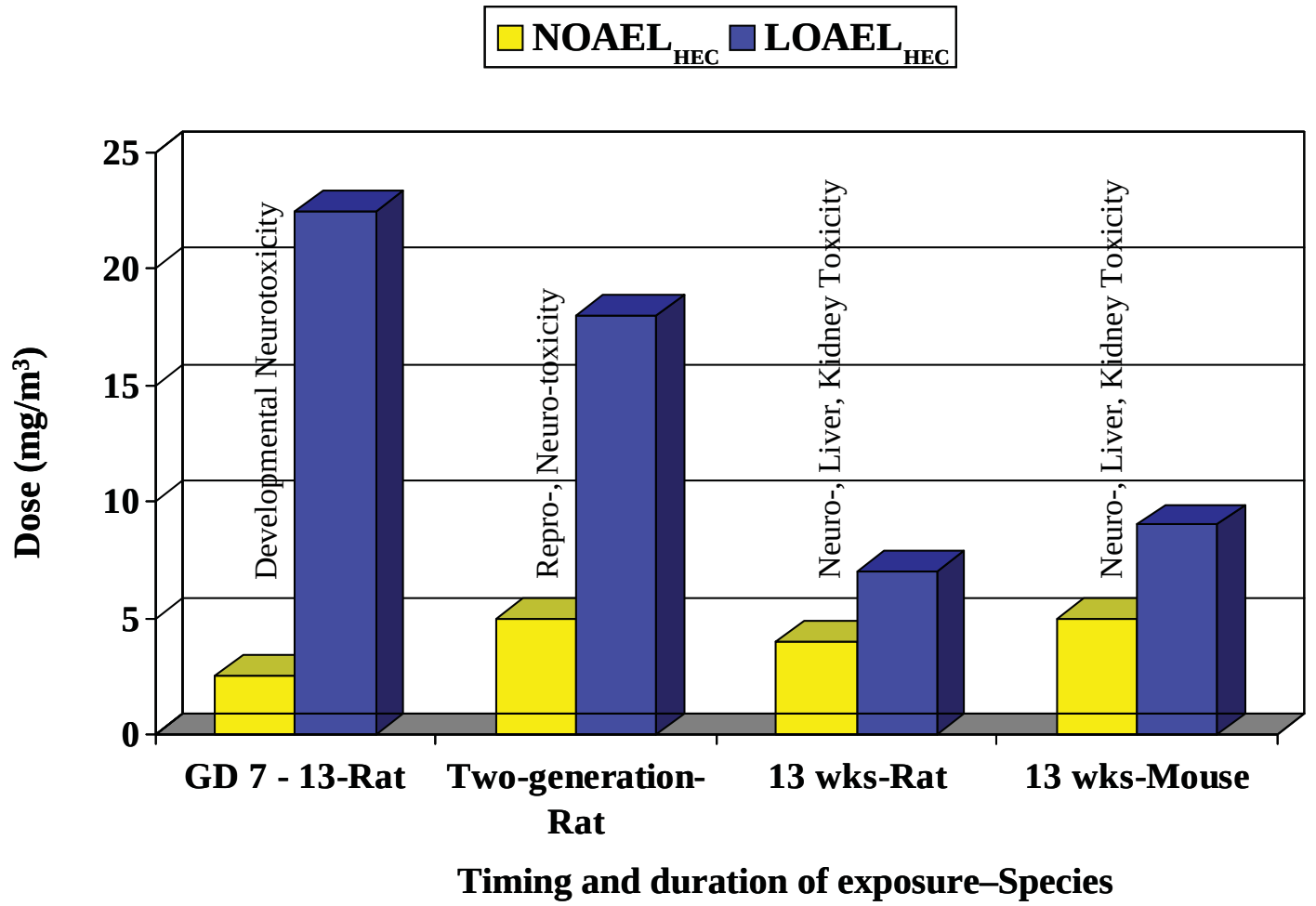

Figure B-3. Exposure-response array of data considered for the Inhalate longer-term reference value.

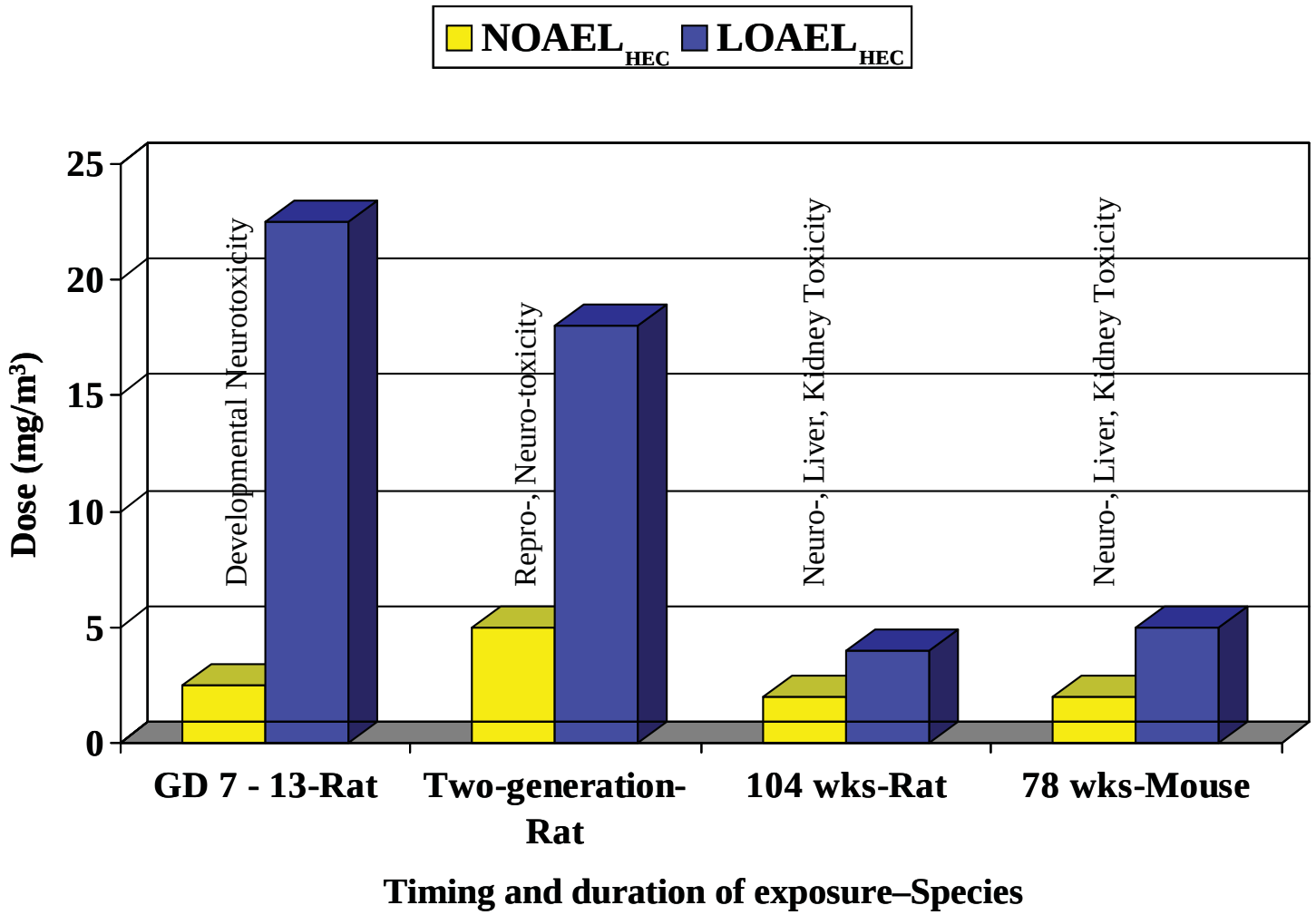

Figure B-4. Exposure-response array of data considered for the Inhalate chronic reference value.

this report, including dosimetric adjustment of the developmental toxicity data in the same manner as for other types of toxicity data, as recommended in U.S. EPA (1994) and Chapter 4.

No toxicokinetic model is available for Inhalate, so adjustments to the applied concentrations for dose adjustments and calculation of the HEC in this case study were done using the default procedures discussed in U.S. EPA (1994) and Chapter 4 of this document. As the effects observed are systemic, with no indications of portal-of-entry effects, the specific default procedures are based on the vapors of Inhalate being a category 3 gas. The principal parameter for interspecies extrapolation of category 3 gases, the blood:gas (air) partition coefficient ($H_{b/g}$), is unknown for both humans and animals and assumed to be 1. Therefore, the dosimetric adjustment factor (DAF) (see Chapter 4, Section 4.4.3) applied to the duration adjusted concentrations is 1, such that the duration adjusted values then become the HECs.

Duration adjustment was accomplished by factoring the exposure concentration (in mg/m^3) by 6/24 (for hours of exposure) and, where applicable, by 5/7 for the number of days per week exposed. As noted in Chapter 4, Section 4.4.2.1, duration adjustments were not made to acute (i.e., less than 24-hr) single exposures.

Uncertainty Factors (UFs)

An interspecies UF (A in Table B-2) of $10^{1/2}$ was applied in all cases to animal studies because the dosimetric adjustment procedures applied to Inhalate are considered to address the toxicokinetics portion of this UF, leaving the rest of the UF to cover interspecies toxicodynamics (U.S. EPA, 1994). An intraspecies UF (H in Table B-2) of 10 was applied in all cases because the data did not allow the estimation of within-human variability and the most sensitive life stage and/or susceptible subpopulation was not clearly identified in the database.

A LOAEL-to-NOAEL UF (L in Table B-2) was applied to the human data for neurotoxicity for the longer-term and chronic reference values because only a LOAEL was identified in the data for those two durations of exposure. A subchronic-to-chronic (duration) UF (S in Table B-2) was not applied, as there were data available for the appropriate duration of exposure in each case. For the longer-term and chronic reference values, a duration UF was not applied to the data from the developmental toxicity study, as this is not the usual practice when considering these data for longer-term and chronic exposures. However, as noted in the report (Chapter 4, Section 4.4.5.6), the application of a UF analogous to the subchronic-to-chronic-duration UF should be explored, as there may be situations in which data are available and applicable but they are from studies in which the dosing period is considerably shorter than that for the reference value being derived.

Table B-2. Derivation of reference values for Inhalate—inhalation exposure

Reference value duration	Exposure duration	HEC (mg/m^3)	Species	Type of effect[a]	Uncertainty factors[b]						Reference value (ppm)[c]	
					Total	A	H	L	S	D	Sample	Final
Acute	2 hrs	4	Human	NT	30	1	10	1	1	3	0.13	0.03
	GDs 7–13	2.5	Rat	DNT	100	3	10	1	1		0.03	
	4 hrs	13L[d]	Rat/ Mouse	NT	1000	3	10	10	1		0.01	
	13–16 wks	5	Rat	RT	100	3	10	1	1		0.05	
Short-term	7 days	2	Human	NT	30	1	10	1	1	3	0.07	0.03
	14 days	9L[d]	Rat/ Mouse	NT	1000	3	10	10	1		0.01	
	GDs 7–13	2.5	Rat	DNT	100	3	10	1	1		0.03	
	13–16 wks	5	Rat	RT	100	3	10	1	1		0.05	
Longer-term	>15 yrs	20L[d]	Human	NT	300	1	10	10	1	3	0.07	0.03
	13 wks	4	Rat	NT	100	3	10	1	1		0.04	
	13 wks	5	Mouse	NT	100	3	10	1	1		0.05	
	GDs 7–13	2.5	Rat	DNT	100	3	10	1	1[e]		0.03	
	13–16 wks	5	Rat	RT	100	3	10	1	1		0.05	
	13 wks	4	Rat	LT	100	3	10	1	1		0.04	
	13 wks	5	Mouse	LT	100	3	10	1	1		0.05	
	13 wks	4	Rat	KT	100	3	10	1	1		0.04	
	13 wks	5	Mouse	KT	100	3	10	1	1		0.05	
Chronic	>15 yrs	20L[d]	Human	NT	300	1	10	10	1	3	0.07	0.02
	104 wks	2	Rat	NT	100	3	10	1	1		0.02	
	78 wks	2	Mouse	NT	100	3	10	1	1		0.02	
	GDs 7–13	2.5	Rat	DNT	100	3	10	1	1[e]		0.03	
	13–16 wks	5	Rat	RT	100	3	10	1	1		0.05	
	104 wks	2	Rat	LT	100	3	10	1	1		0.02	
	78 wks	2	Mouse	LT	100	3	10	1	1		0.02	
	104 wks	2	Rat	KT	100	3	10	1	1		0.02	
	78 wks	2	Mouse	KT	100	3	10	1	1		0.02	

[a] NT = neurotoxicity; DNT = developmental neurotoxicity; RT = reproductive toxicity; LT = liver toxicity; KT = kidney toxicity

[b] A = animal-to-human (interspecies); H = inter-individual (intraspecies); L = LOAEL-to-NOAEL; S = subchronic-to-chronic duration; D = database deficiency

[c] Sample = reference value based on that particular endpoint, species, duration; Final = reference value for the entire database for a particular duration of exposure.

[d] L indicates that this value is the HEC based on the LOAEL.

[e] A duration UF was not applied to the data from the developmental neurotoxicity study for either the longer-term or chronic reference value; however, the adjustment should be considered when extrapolating from shorter to longer durations of exposure.

GD = gestation day

A database UF of $10^{1/2}$ was applied in all cases because there were data indicating that one of the main target organs for Inhalate was the nervous system, that there was neurotoxicity in adults (humans and animals), and that there were some data on developmental neurotoxicity, but with exposure limited to only a portion of the developmental period. In addition, there was no study in which pregnancy outcomes were evaluated (i.e., fetal survival, growth, and structural development) except for a possible association with spontaneous abortion and cardiac anomalies in occupationally exposed workers. These data gaps were not considered likely to reduce the NOAEL by more than a factor of 3 because there were some data on developmental exposures from a two-generation reproductive toxicity study. There were no reports of effects on fertility or reproduction per se, except for a possible association with menstrual disturbances in occupationally exposed workers.

Acute Inhalation Exposure

Results of available studies indicate that acute inhalation exposure to Inhalate can result in neurotoxic effects in human adults with a $LOAEL_{HEC}$ of 40 mg/m^3 and a $NOAEL_{HEC}$ of 4 mg/m^3 in a 2-hour exposure. Animal studies also show that Inhalate has the potential to cause neurotoxicity in adults with a $LOAEL_{HEC}$ of 13 mg/m^3 in a 4-hr exposure, and developmental neurotoxicity and other reproductive effects with $LOAEL_{HEC}$s of 22.5 mg/m^3 and 18 mg/m^3 and $NOAEL_{HEC}$s of 2.5 mg/m^3 and 5 mg/m^3, respectively.

Default UFs of $10^{1/2}$ (animal-to-human extrapolation), 10 (inter-individual differences), and $10^{1/2}$ (database deficiencies: no adequate prenatal developmental toxicity studies in two species, no adequate developmental neurotoxicity study) were applied to all the $NOAEL_{HEC}$s to derive sample reference values. In addition, a UF of 10 (LOAEL to NOAEL) was applied to the 4-hr rat and mouse $LOAEL_{HEC}$s. Human and animal studies indicate that the nervous system is vulnerable to Inhalate exposure. Although the sample reference values for the 4-hr adult rat and mouse exposures were lower than that based on the developmental neurotoxicity study, the values were within a similar range and the sample reference value for developmental neurotoxicity had less overall uncertainty. Therefore, the resultant reference value chosen for acute inhalation exposure is 0.03 mg/m^3 (Table B-2).

Short-term Inhalation Exposure

The reference value for short-term inhalation exposure is based on the human data $NOAEL_{HEC}$s of 2 mg/m^3 as well as the animal developmental neurotoxicity NOAEL of 2.5 mg/m^3 and

reproductive toxicity NOAEL of 5 mg/m^3 ($LOAEL_{HEC}$s of 4 mg/m^3, 22.5 mg/m^3, and 18 mg/m^3, respectively). In addition, the 14 day exposures of rats and mice resulted in a $LOAEL_{HEC}$ of 9 mg/m^3. For the human $NOAEL_{HEC}$ of 2 mg/m^3, applying a 10-fold default UF for intraspecies uncertainty and variability and a $10^{1/2}$-fold UF for database deficiencies results in a sample reference value for short-term inhalation exposure of 0.07 mg/m^3. Sample reference values based on animal data include default factors of $10^{1/2}$ (interspecies), 10 (intraspecies) and $10^{1/2}$ (database deficiencies) applied to the $NOAEL_{HEC}$s for developmental neurotoxicity and reproductive toxicity (2.5 and 5 mg/m^3, respectively) and result in sample reference values of 0.03 and 0.05 mg/m^3. An additional factor of 10 (LOAEL to NOAEL) applied to the adult neurotoxicity data in rats and mice results in a sample reference value of 0.01 mg/m^3. Given the close range of values and the lower overall uncertainty in the sample reference value for developmental neurotoxicity than that for adult neurotoxicity, the final reference value of 0.03 mg/m^3 is chosen (Table B-2).

Longer-term Inhalation Exposure

Subchronic and chronic inhalation exposure to Inhalate can result in multiple health effects. Available studies demonstrate neurotoxicity in adult humans. However, dose-response information is not available, and the presumed LOAEL (20 mg/m^3) for neurotoxicity in humans is somewhat higher than the HECs for other health endpoints (developmental, reproductive, liver, and renal effects) observed in animal studies, where the $LOAEL_{HEC}$s range from 7 mg/m^3 to 22.5 mg/m^3 and the $NOAEL_{HEC}$s range from 2.5 mg/m^3 to 5 mg/m^3. Dose-response data for these health endpoints in animal studies can be used as the basis for deriving a longer-term inhalation reference value for Inhalate.

UFs of $10^{1/2}$ (interspecies), 10 (intraspecies), and $10^{1/2}$ (database deficiencies) were applied to $NOAEL_{HEC}$s for the various endpoints in deriving sample reference values. If an additional factor of 3 were applied to the rat developmental toxicity data to account for the marked difference in exposure duration in the study itself (7 days of exposure: GDs 7–13) versus the duration covered by this reference value (up to 10% of the life span), a longer-term sample reference value of 0.01 mg/m^3 would result. Without this additional factor, the sample reference value from the developmental toxicity study was still the lowest value (0.03 mg/m^3), although all values from the animal studies were in a similar range (0.03–0.05).

The final reference value chosen was 0.03 mg/m^3 to be protective of the developing individual as well as adults (Table B-2). Whether an additional factor should be applied to the developmental toxicity data or to other data of much shorter duration should be explored further.

Chronic Inhalation Exposure

For the chronic inhalation reference value, the $LOAEL_{HEC}$s range from 4 mg/m^3 to18 mg/m^3 and the $NOAEL_{HEC}$s range from 2 mg/m^3 to 5 mg/m^3. UFs of $10^{1/2}$ (interspecies), 10 (intraspecies), and $10^{1/2}$ (database deficiencies) applied to the chronic exposure $NOAEL_{HEC}$s for neurotoxicity and liver, kidney, and reproductive toxicity data result in sample reference values of 0.02–0.05 mg/m^3 (Table B-2). Applying these UFs to the $NOAEL_{HEC}$ of 2.5 mg/m^3 for developmental toxicity yields a sample reference value of 0.03 mg/m^3, which falls within the range of chronic study-based values. If, contrary to current practice, an additional 3- or 10-fold UF for subchronic to chronic duration were applied to the developmental $NOAEL_{HEC}$, the sample reference value would be 0.01 or 0.003. As mentioned in Section 4.4.5.6, this issue may need further exploration. In this example, several endpoints result in a sample chronic inhalation reference value of 0.02 mg/m^3, the value chosen for the chronic inhalation reference value.

With regard to the liver effects of Inhalate, the $NOAEL_{HEC}$s and reference values for liver histopathology were the same as for the tumorigenic effects; thus, the reference value based on liver toxicity should be protective of the carcinogenic effects of Inhalate.

Overall Evaluation of Reference Values

The reference values for Inhalate were similar across all durations of exposure. This is because the same data were used as the basis for the acute, short-term, and longer-term reference value, that is, the effects on developmental neurotoxicity. Although there were human data appropriate for consideration for all four durations of exposure, the endpoints examined in these studies or reports were limited and were not indicative of effects on the developing nervous system. To be protective of developmental life stages, it was considered appropriate to base the reference values on the developmental neurotoxic effects, for which the sample reference value was slightly lower than for other endpoints. For the chronic reference value, a number of sample reference values, including that for the carcinogenic effects of Inhalate, were clustered in the same range, at 0.02 mg/m^3, slightly lower than that for developmental neurotoxicity. In this case, it was considered appropriate to use this lower reference value to be protective of all potential effects for lifetime exposures.

CASE STUDY 2: LUTEINATE

INTRODUCTION

Luteinate is a new pesticide that was developed for use as an herbicide. Environmental fate studies have shown that it will persist in soils and will therefore likely to move into ground and surface water. The general population may be exposed to Luteinate through consumption of food and drinking water.

Luteinate belongs to a class of pesticides for which the neuroendocrine mode of action is known. It was designed to be less potent than other members of the class. In order to ascertain its potency, a number of short-term studies were first conducted; they confirmed a similar mode of action and the fact that Luteinate was less potent. Following this, Luteinate was tested in more traditional toxicology studies to establish its long-term effects and dose-response relationships.

This case study provides an example of the usefulness of mode of action information in establishing the short- and long-term effects of Luteinate on relevant target organ systems at different life stages. Such information enables the development of a targeted robust data set for use in establishing reference values for various durations of exposure.

Postulated Mode of Action

Other members of this class of pesticides have been shown to act on the hypothalamic-pituitary-ovarian axis. These pesticides affect the hypothalamus, leading to a decreased secretion of hypothalamic norepinephrine (NE). Decreased NE levels result in decreased release of gonadotropin releasing hormone (GnRH) from the hypothalamus. GnRH is the hormone responsible for inducing the pituitary gland to release luteinizing hormone (LH). Thus, the decrease in GnRH leads to a suppression of the pituitary LH release. These compounds also decrease the neurotransmitter dopamine, which in turn leads to a decrease in pituitary prolactin.

Decreased LH and prolactin levels have the potential to impact several organ systems at different life stages. In humans, there are robust pulses of the LH surge in the fetus and prior to birth. The LH pulsatility continues with diminishing amplitude during the early months of postnatal life, and then LH secretion becomes barely detectable during much of the first decade of postnatal life. Around the age of 10, there is the reemergence or re-awaking of LH pulses while sleeping.

The natural progression from prepubertal to postpubertal status is dependent on the normal function of the hypothalamic-pituitary-gonadal axis. Likewise, many of the same hypothalamic mechanisms that control pituitary function and the pituitary hormones themselves

(especially LH and prolactin) play a key role in pubertal development. In the adult, ovulation is dependent on sufficient LH levels. Therefore, this class of pesticides impacts critical reproductive processes, including puberty, ovarian cyclicity, pregnancy, and lactation (milk quality/production). Given the role of NE and dopamine in the development of the CNS, this class of pesticides may also affect the developing CNS; however, relevant toxicology studies have not yet been conducted. In addition, suppression of prolactin during the early postnatal period in rodents may lead to prostate inflammation in male offspring.

This class of pesticides has also been shown to lead to mammary tumors in Sprague-Dawley (SD) rats but not in other strains of rats or mice. Mammary tumors result from the prolonged decrease in serum LH, which leads to a cessation of ovulation and eventually causes the ovarian follicles to continue to secret estradiol. In concert with prolactin, estrogen acts on the mammary gland and leads to the formation of mammary tumors. However, the induction of the tumors has been shown to be due to unique features of the reproductive aging process in Sprague-Dawley rats; because humans do not age in the same manner, this mode of action is unlikely to be operative in humans.

SUMMARY OF HEALTH EFFECTS INFORMATION

Studies with other pesticides in this chemical class have found negligible differences in response (with the exception of the induction of mammary tumors in SD rats) among various strains of rats and between rats and mice. Therefore, all studies on Luteinate were conducted on SD rats.

Luteinate exposure results in a spectrum of effects that are related to decreases in serum LH and decreases in prolactin. The toxicology studies are summarized below in the context of the specific effects associated with either decreases in LH or prolactin. In addition, the effect levels for specific toxicological endpoints are summarized in Table B-3. It is important to note that as the spectrum of effects are mechanistically related to decreases in serum LH or prolactin, there is great similarity in the doses that affect LH and prolactin levels and those that cause the related effects.

Absorption, Distribution, Metabolism, and Elimination

Studies in adult rats have shown that Luteinate is rapidly absorbed following oral exposure. It is not metabolized and is eliminated in the urine. No other information is available. The toxicokinetic profiles of other pesticides in this chemical class have not been extensively examined either.

Table B-3. Summary of endpoints and effect levels for rat studies of Luteinate

Response	Exposure period	Dose levels (mg/kg/day)	NOAEL/LOAEL (mg/kg/day)
Decreased LH	Pregnant females: GDs 1–8	 50, 100, 200	 100/200
	PNDs 22–41	25, 50, 100	50/100
	Adults: 3 days 28 days 6 months	 50, 100, 200 40, 80, 160 4, 8, 16	 100/200 40/80 4/8
	Two-generation reproduction study: 10 weeks - F0 13 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 5/10 5/10
Disruption of estrous cyclicity	PND 22-41	25, 50, 100	50/100
	Adults: 28 days 6 months	 40, 80, 160 4, 8, 16	 40/80 4/8
	Two-generation reproduction study: 10 weeks - F0 13 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 5/10 5/10
Altered pregnancy maintenance	Pregnant females: GDs 1–8 GDs 6–10 GDs 1–20	 50, 100, 200 100, 200, 400 50, 100, 200	 100/200 200/400 100/200
	Two-generation reproduction study: 11–13 weeks - F0 14–16 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 5/10 5/10
Delayed parturition	Pregnant females: GDs 6–10	 100, 200, 400	 100/200
	Two-generation reproduction study: 14 weeks - F0 17 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 5/10 5/10
Delayed vaginal opening	PNDs 22–41	25, 50, 100	50/100
	Two-generation reproduction study: 4–5 weeks - F1[a]	 5, 10, 25, 50	 25/50
Delayed preputial separation	PND 23–53	25, 50, 100	50/100
	Two-generation reproduction study: 6–7 weeks - F1[a]	 5, 10, 25, 50	 25/50

Table B-3. Summary of endpoints and effect levels for rat studies of Luteinate (continued)

Response	Exposure period	Dose levels (mg/kg/day)	NOAEL/LOAEL (mg/kg/day)
Attenuation of prolactin release	Lactating females: PNDs 1–4	 25, 50, 100	 25/50
	Adults: 3 days 28 days 6 months	 50, 100, 200 40, 80, 160 4, 8, 16	 100/200 40/80 4/8
	Two-generation reproduction study: 10 weeks - F0 13 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 5/10 5/10
Increased prostatitis in offspring	PNDs 1–4	25, 50, 100	25/50
	Adults: 28 days 6 months	 40, 80, 160 4, 8, 16	 >200/NA[b] >160/NA[b]
	Two-generation reproduction study: 16 weeks - F0 16 weeks - F1[a]	 5, 10, 25, 50 5, 10, 25, 50	 >50/NA[b] 5/10
Reduced weight of seminal vesicles and ventral prostate	PNDs 23–53	25, 50, 100	50/100
	Adults: 28 days 6 months	 40, 80, 160 4, 8, 16	 >200/NA[b] >160/NA[b]
	Two-generation reproduction study: 16 weeks - F1[a]	 5, 10, 25, 50	 5/10
Fetus: Delayed ossification Reduced fetal weight	 GDs 1–20 GDs 1–20	 50, 100, 200 50, 100, 200	 100/200 >200/NA[b]

[a] F1 exposures are indicated for the duration of postnatal exposure, but it is assumed that 3 weeks of prenatal exposure also occurred.

[b] When no effect on a particular endpoint was noted in a study, the NOAEL is indicated as > the highest dose, and the LOAEL as NA (not applicable).

Decreased LH Surge and Related Effects

Exposure to Luteinate resulted in a significant decrease in serum LH that was dependent on the dose and duration of exposure. In a 3-day gavage study of SD male and female rats exposed to levels of 0, 50, 100, and 200 mg/kg/day, there were significant decreases in serum LH at 200 mg/kg/day. Serum LH was significantly reduced at doses of 80 mg/kg/day and above in a 28-day study and at doses of 8 mg/kg/day and above in a 6-month study of SD rats. Levels in the latter study were similar to those observed in the F0 rats in a two-generation reproductive toxicity study in which significant decreases in serum LH were observed at doses of 10 mg/kg/day and higher following 10 weeks of exposure.

Serum LH was also measured in pregnant dams exposed to doses of 0, 50, 100, and 200 mg/kg/day on GDs 1–8 and was significantly reduced at 200 mg/kg/day. Exposure of weanling SD rats on PNDs 22–41 to doses of 0, 25, 50, and 100 mg/kg/day of Luteinate resulted in a significant decrease in serum LH at 100 mg/kg/day. In a two-generation reproductive toxicity study, serum LH was measured in the F1 generation prior to mating (13 weeks postnatal exposure) and was significantly reduced at doses of 10 mg/kg/day and higher.

Disruption of Estrous Cyclicity

In the adult female, ovulation is dependent on sufficient levels of LH. Because exposure to Luteinate suppresses LH, it would be anticipated to also disrupt the normal estrous cycle. Estrous cyclicity was abnormal in SD rats exposed to 80 mg/kg/day for 28 days and 8 mg/kg/day for 6 months. In the two-generation reproductive toxicity study, estrous cyclicity was abnormal in the F0 females exposed to 10 mg/kg/day for 10 weeks and in the F1 females exposed to 10 mg/kg/day for 13 weeks postnatally (as well as prenatally). In addition, exposure of weanlings to 100 mg/kg/day on PNDs 22–41 resulted in abnormal estrous cycles.

Pregnancy

Altered LH levels also impact the ability of the female to maintain pregnancy as well as the timing of parturition. Thus, in a variety of exposure scenarios, an increase in pre- and postimplantation loss was observed. Implantation was affected following exposure to 200 mg/kg/day on GDs 1–8 or 1–20 and after exposure to 400 mg/kg/day on GDs 6–10. Parturition was delayed following exposure to 200 mg/kg/day and above on GDs 6–10. In the two-generation reproductive toxicity study, there was an increase in pre- and postimplantation loss and a delay in parturition in the F0 and F1 females at doses of 10 mg/kg/day and higher.

Sexual Maturation

At the time of puberty, the CNS and pituitary respond to increased concentrations of estradiol in a positive feedback fashion culminating in the first LH surge. Thus, exposure to Luteinate would likely impact sexual maturation. Vaginal opening was delayed in female SD rats following exposure to 100 mg/kg/day on PNDs 22–41 and preputial separation was delayed in males exposed to 100 mg/kg/day on PNDs 23–53. In the two-generation reproductive toxicity study, vaginal opening was delayed in the F1 females exposed to 50 mg/kg/day and preputial separation was delayed in the F1 males exposed to 50 mg/kg/day.

Decreased Prolactin and Related Effects

Prolactin levels were significantly decreased in adult SD rats following exposure to 200 mg/kg/day of Luteinate for 3 days, to 80 mg/kg/day for 28 days, and to 8 mg/kg/day for 6 months. In a study in which lactating dams were exposed during PNDs 1–4, prolactin levels were reduced at doses of 50 mg/kg/day. In the two-generation reproductive toxicity study, prolactin levels were reduced in the F0 and F1 animals at doses of 10 mg/kg/day.

Prostatitis

As a consequence of the reduced prolactin levels, an increased incidence of prostatitis was observed in males following maternal exposure to 50 mg/kg/day during lactation days 1–4, and in the F1 males exposed to 10 mg/kg/day and higher. Prostatitis was not observed in males exposed during adulthood only (i.e., F0 males, males in 28-day and 6-month studies).

Organ Weights

In males exposed to doses of 100 mg/kg/day Luteinate during PNDs 23–53, there was a decrease in absolute and relative weights of the seminal vesicles and ventral prostate. This was also observed in the F1 males exposed to doses of 10 mg/kg/day or greater at the time of terminal sacrifice following mating. Histopathological examination revealed no lesions in either study. No organ weight changes or histopathological lesions were noted in any of the other studies.

Effects Unrelated to LH or Prolactin

A prenatal developmental toxicity study was conducted in which pregnant SD rats were exposed to doses of 0, 50, 100, or 200 mg/kg/day Luteinate on GDs 1–20. In addition to the increase in implantation loss noted above, there was an increase in delayed ossification at 200

mg/kg/day but no effect on fetal body weight. It is unlikely that this effect is related to LH levels. However, the mode of action is unknown.

SELECTION OF HEALTH ENDPOINTS AND DERIVATION OF REFERENCE VALUES

Narrative Description of the Extent of the Database

The database for oral exposure is quite robust and is adequate for deriving reference values. Because the mode of action was known for other pesticides in this chemical class, it was possible to study known targets during relevant life stages. Luteinate was shown to interfere with the pituitary-hypothalamic axis, resulting in a decrease in serum LH and prolactin. The decrease was shown to be dependent on dose and duration of exposure. Normal LH levels are known to be required for ovulation, maintenance of pregnancy, timing of parturition, and sexual maturation. Similarly, decreases in prolactin can lead to prostatitis and effects on the male reproductive organs. Each of these events were examined in several short-term studies as well as in traditional prenatal developmental toxicity studies and a two-generation reproductive toxicity study.

An acute toxicity study of Luteinate was not conducted. However, information was available on serum LH and prolactin levels from other short-term exposures that was informative for acute exposures. The database as a whole demonstrates that there is a clear dose-duration relationship for Luteinate on serum LH levels. Serum LH levels were reduced following a 3-day exposure in adult rats and in pregnant rats on GDs 1–8 at doses of 200 mg/kg/day; longer exposures to weanlings on PNDs 22–41 or to adults for 28 days required a dose of 100 or 80 mg/kg/day, respectively. The effective dose of 10 mg/kg/day was still lower following 10 to 13 weeks exposure. Therefore, it is unlikely that serum LH levels and related effects would occur at doses less than 200 mg/kg/day following an acute exposure, but higher doses may actually be necessary.

The situation is less clear for the decrease in prolactin and related effects. Prolactin levels were reduced following a 3-day exposure to adult rats at 200 mg/kg/day and following exposure to lactating dams at 50 mg/kg/day. A 28-day exposure to adult rats resulted in decreased prolactin levels at 80 mg/kg/day, whereas 10 mg/kg/day was an effective dose at 10 to 13 weeks of exposure. Thus, although there is a clear dose-duration relationship between the 4- and 10-week periods, the relationship is less clear for durations of less than 28 days. However, it is unlikely that serum prolactin levels and related effects would have NOAELs less than 25 mg/kg/day following an acute exposure.

Knowledge about the effect of Luteinate on serum LH and prolactin levels from these other studies was considered in conjunction with the prenatal developmental toxicity study for deriving the acute reference value. It is assumed that effects resulting from developmental exposures (both prenatal and postnatal) may be the result of a single exposure. Therefore, the fetal and offspring effects resulting from exposures during gestation and postnatally to the time of sexual maturation were also considered for the acute reference value. Data from the two-generation study were not considered because there were always shorter-term studies showing effects at higher doses. Taken as a whole, the lack of an acute toxicity study was not considered to be a major data gap for Luteinate.

A chronic study of Luteinate was not conducted. However, serum LH was decreased at 10 mg/kg/day in the F0 and F1 animals in the two-generation reproductive toxicity study and at 8 mg/kg/day in the 6-month study. Because there is essentially no change in the effective dose levels following 13 weeks of exposure and 6 months of exposure, it is unlikely that a longer exposure period would substantially lower the effective dose. Thus, the lack of a chronic study was not considered a major database deficiency. Although there is no knowledge of the effects of continued LH and prolactin suppression on reproductive aging, this is probably a qualitative, rather than a quantitative data gap. It is known from chronic bioassays of other pesticides in this chemical class that lifetime exposure results in mammary tumors in SD rats, but this mode of action is unlikely to be operative in humans due to differences in the aging process. Thus, this was not considered to be a data gap for Luteinate.

A developmental neurotoxicity study was not conducted. Given that Luteinate interferes with two neuroreceptors, norepinephrine and dopamine, the potential for developmental neurotoxicity exists. Thus, the lack of knowledge regarding the developing nervous system is considered to be a data gap.

Exposure-Response Array

In addition to the display in tabular form (Table B-3), the data considered useful for deriving each reference value were displayed in an exposure-response array. The arrays for acute, short-term, longer-term, and chronic exposures are shown in Figures B-5 through B-8, respectively. Although data on delayed ossification were considered in deriving the longer-term and chronic reference values, the data were not included in Figures B-7 and B-8 because the doses were substantially higher than those for all other endpoints, and inclusion would have altered the y-axis substantially. Figure B-9 is a composite of the NOAELs for each endpoint

with each exposure duration. In addition, the NOAELs for each relevant endpoint, the uncertainty factors (UFs), and the sample and final reference values are shown in Table B-4.

Uncertainty Factors

An interspecies UF (A in Table B-4) of 10 was applied in all cases because the data were insufficient to characterize toxicokinetic and toxicodynamic differences between rodents and humans. An intraspecies UF (H in Table B-4) of 10 was applied in all cases because the data did not allow the estimation of human variability. A UF for LOAEL to NOAEL (L in Table B-4) was not applied because the NOAEL was known for each exposure duration. A subchronic-to-chronic UF (S in Table B-4) was not applied because there were data available for each exposure duration. A database UF of $10^{1/2}$ was applied in all cases because there are concerns for the potential developmental neurotoxicity of Luteinate and there were no available data.

A database UF of $10^{1/2}$ (rather than a UF of 10) was applied on basis of the observation that for other pesticides in this chemical class, the doses that result in a decrease in norephinephrine and dopamine are very similar to the doses that lead to decreases in serum LH. Therefore, it is likely that any developmental neurotoxicity effects would be observed at similar doses. Because the effects of Luteinate on serum LH levels have been well characterized, additional developmental neurotoxicity information is unlikely to dramatically affect the reference values. However, a UF of $10^{1/2}$ was retained to reflect underlying uncertainties of this data gap.

Acute Exposure

For the derivation of the acute reference value, a standard prenatal developmental toxicity study provided information on the effects of Luteinate on fetal ossification in the absence of an effect on fetal weight. There was no information on serum LH or prolactin and related effects following an acute exposure. As described above in the discussion of the extent of the database, it is unlikely that the NOAEL for serum LH levels and related effects would be less than 100 mg/kg/day following an acute exposure, and it could be higher. Similarly, it is unlikely that the NOAEL for serum prolactin levels and related effects would be less than 25 mg/kg/day following an acute exposure. The effects on sexual maturation occurred at a dose of 100 mg/kg/day (NOAEL of 50 mg/kg/day) and effects on ossification occurred at a dose of 200 mg/kg/day (NOAEL of 100 mg/kg/day).

Thus, derivation of the acute reference value based on these endpoints would also be protective of effects on serum LH and related effects, but may not cover effects on prolactin and

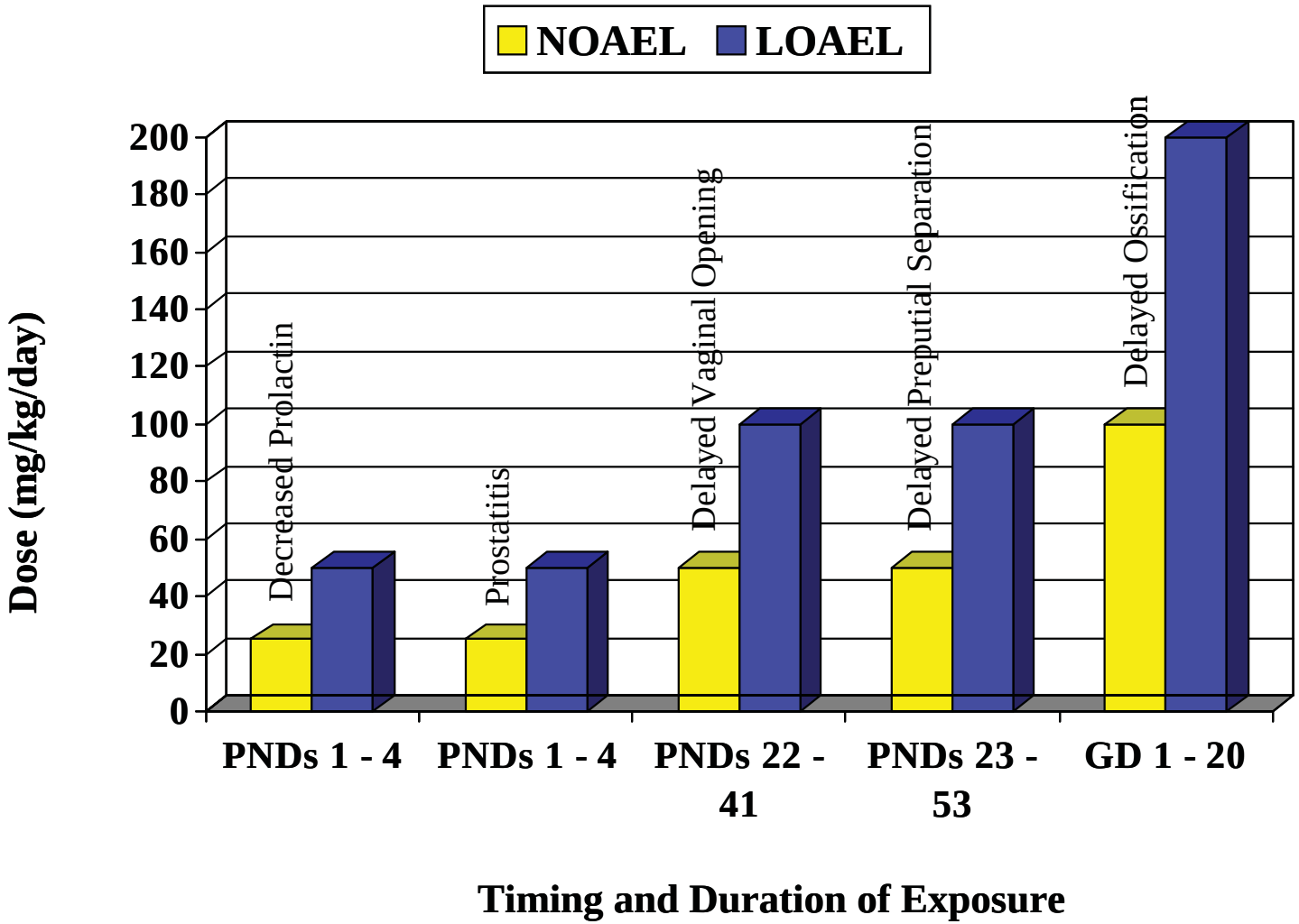

Figure B-5. Exposure-response array of data considered for the Luteinate acute reference value.

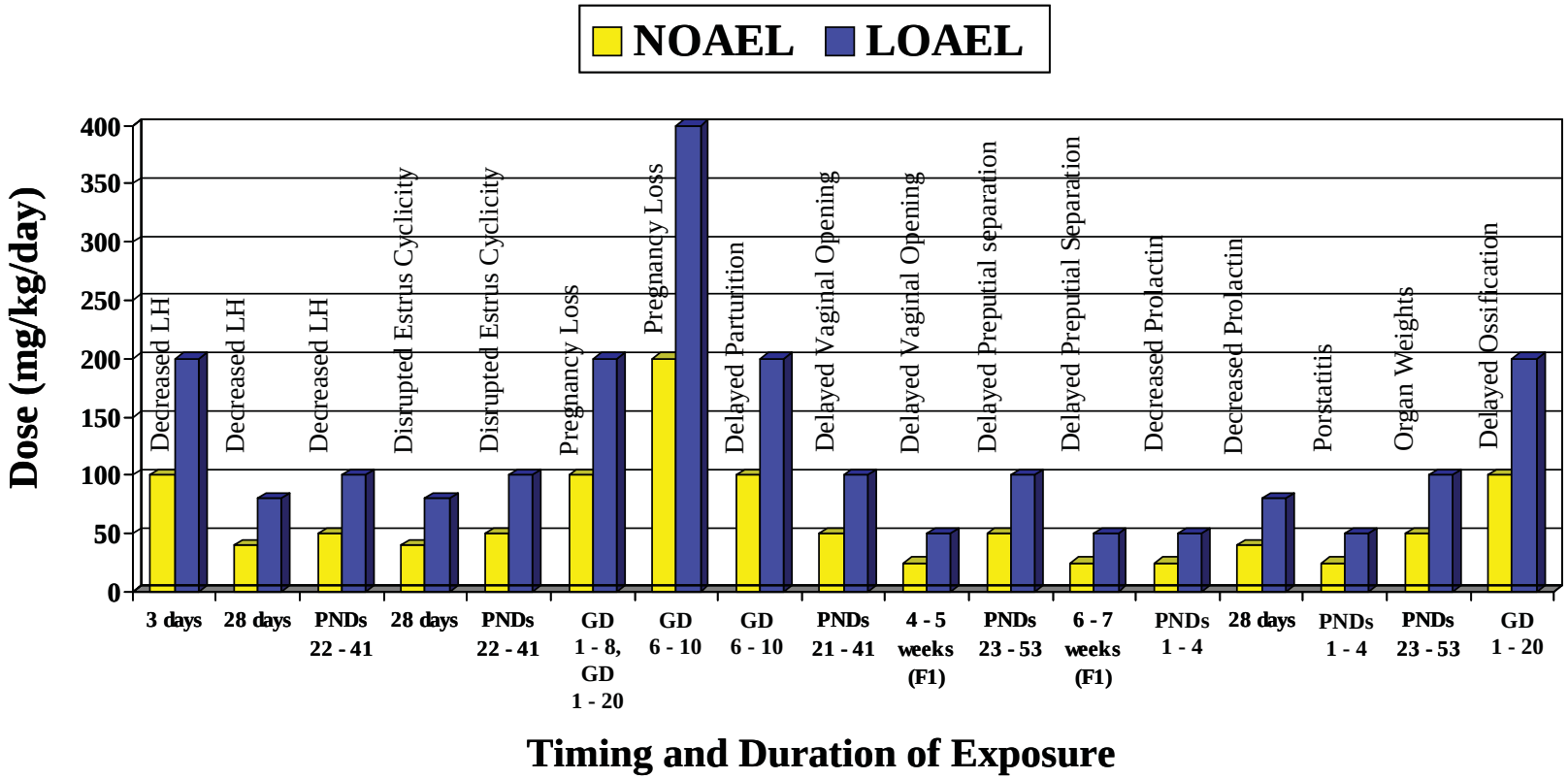

Figure B-6. Exposure-response array of data considered for the Luteinate short-term reference value.

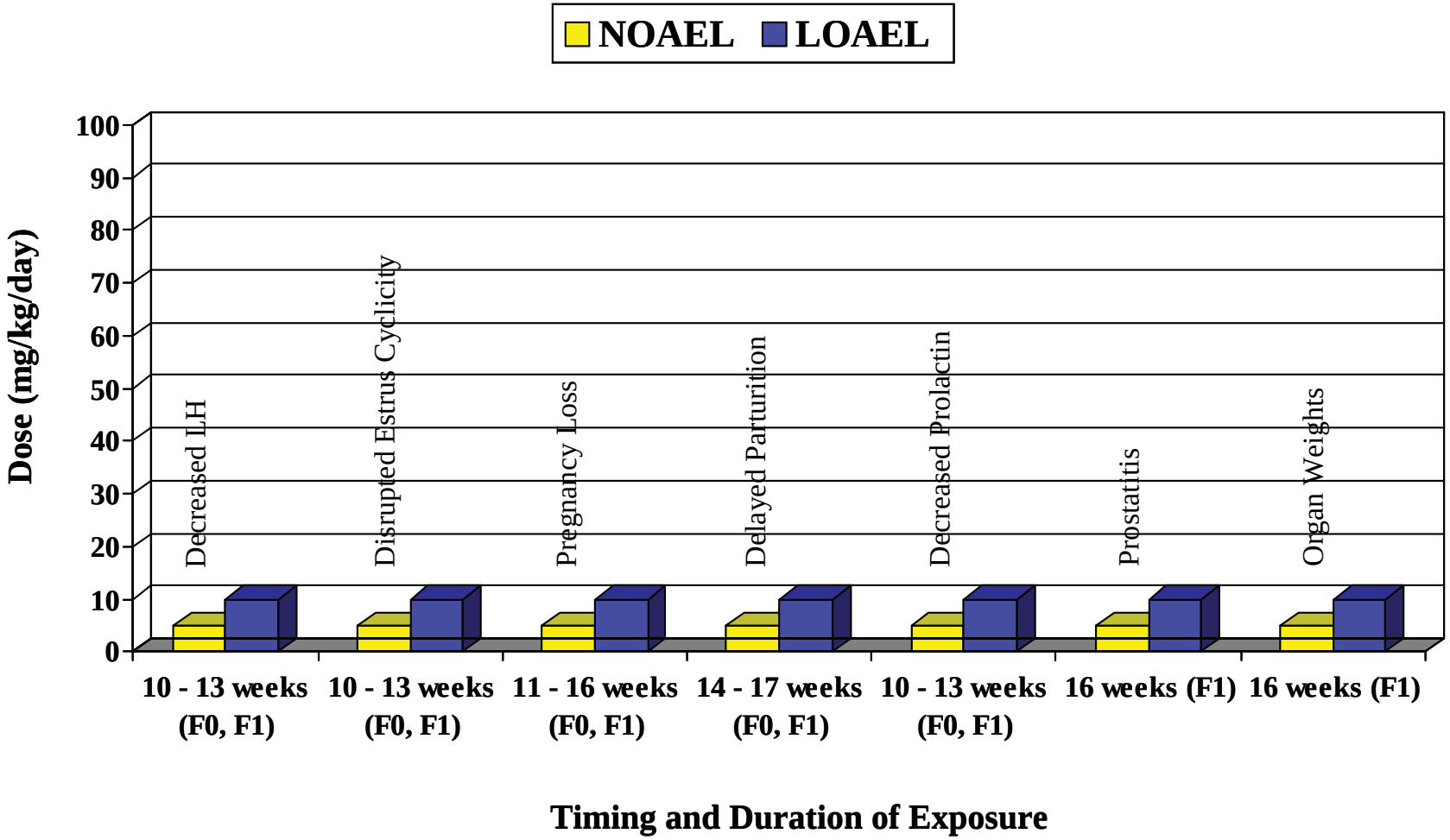

Figure B-7. Exposure-response array of data considered for the Luteinate longer-term reference value.

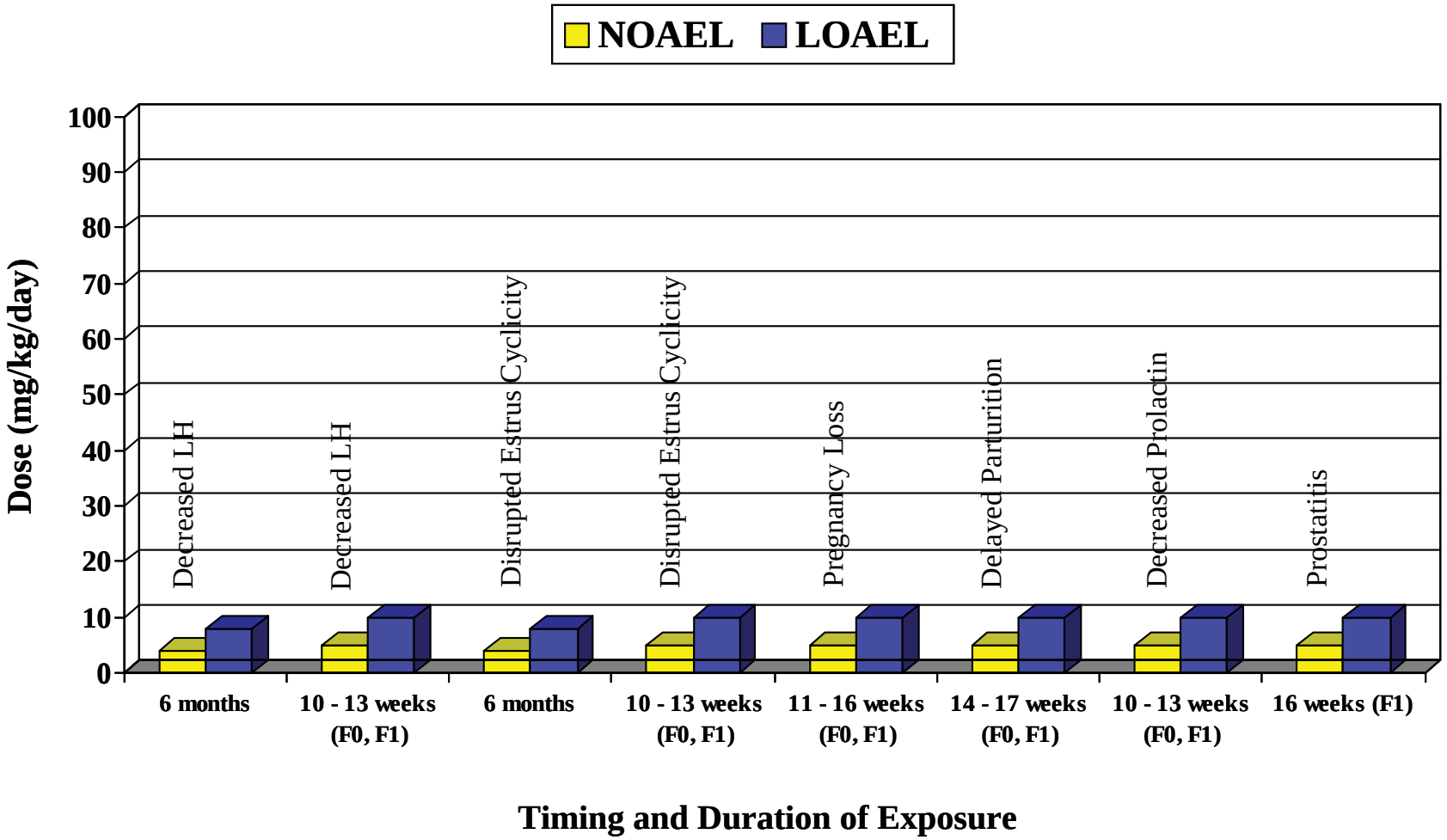

Figure B-8. Exposure-response array of data considered for the Luteinate chronic reference value.

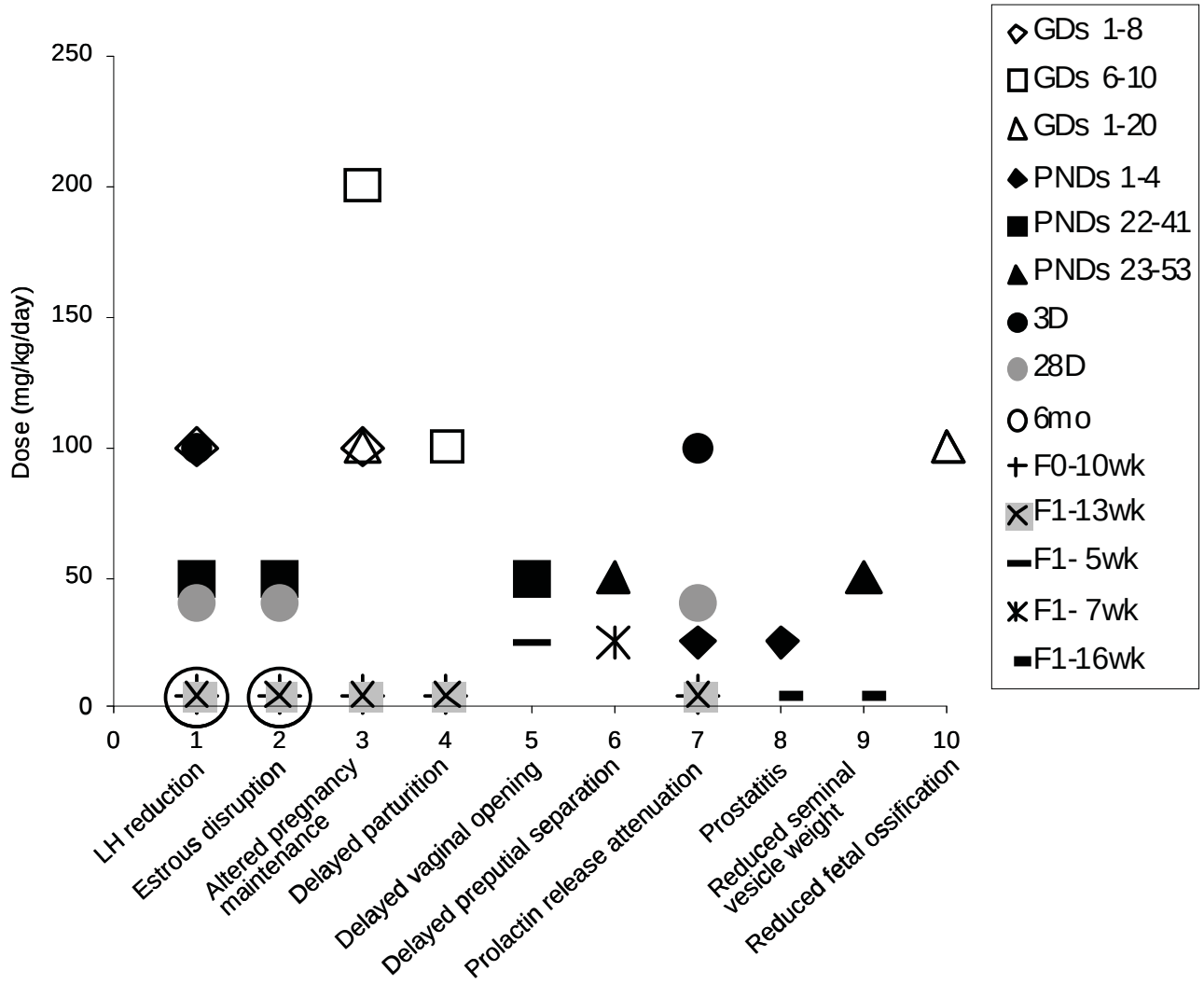

Figure B-9. NOAELs for Luteinate with timing/duration of exposure.

Table B-4. Summary of reference values for Luteinate

Reference value duration	Exposure duration	NOAEL (mg/kg/day)	Type of effect	Uncertainty factors[a]						Reference value (mg/kg/day)	
				A	H	L	S	D	Total	Sample	Final
Acute	PNDs 1–4	25	Prolactin	10	10	1	1	3	300	0.08	0.08
	PND 1–4	25	Prostatitis	10	10	1	1		300	0.08	
	PNDs 22–41	50	Sexual Maturation	10	10	1	1		300	0.17	
	PNDs 23–53	50	Sexual Maturation	10	10	1	1		300	0.17	
	GDs 1–20	100	Fetal ossification	10	10	1	1		300	0.33	
Short-term	3-day adult	100	LH	10	10	1	1	3	300	0.33	0.08
	28-day adult	40	LH	10	10	1	1		300	0.13	
	PNDs 22–41	50	LH	10	10	1	1		300	0.17	
	GDs 1–8	100	LH	10	10	1	1		300	0.33	
	28-day adult	40	Estrus	10	10	1	1		300	0.13	
	PNDs 22–41	50	Estrus	10	10	1	1		300	0.17	
	GDs 1–8	100	Pregnancy Maintenance	10	10	1	1		300	0.33	
	GDs 1–20	100	Pregnancy Maintenance	10	10	1	1		300	0.33	
	GDs 6–10	200	Pregnancy Maintenance	10	10	1	1		300	0.67	
	GDs 6–10	100	Parturition	10	10	1	1		300	0.33	
	PNDs 22–41	50	Sexual Maturation	10	10	1	1		300	0.17	
	PNDs 23–53	50	Sexual Maturation	10	10	1	1		300	0.17	

Table B-4. Summary of reference values for Luteinate (continued)

Reference value duration	Exposure duration	NOAEL (mg/kg/day)	Type of effect	Uncertainty factors[a]						Reference value (mg/kg/day)	
				A	H	L	S	D	Total	Sample	Final
Short-term	4–7 weeks	25	Sexual Maturation	10	10	1	1	3	300	0.08	0.08
	PNDs 1–4	25	Prolactin	10	10	1	1		300	0.08	
	3-day adult	100	Prolactin	10	10	1	1		300	0.33	
	28-day adult	40	Prolactin	10	10	1	1		300	0.13	
	PNDs 1–4	25	Prostatitis	10	10	1	1		300	0.08	
	PNDs 23–53	25	Organ Wt	10	10	1	1		300	0.08	
	GDs 1–20	100	Fetal ossification	10	10	1	1		300	0.33	
Longer-term	10–13 weeks	5	LH	10	10	1	1	3	300	0.02	0.02
	10–13 weeks	5	Estrus	10	10	1	1		300	0.02	
	11–16 weeks	5	Pregnancy Maintenance	10	10	1	1		300	0.02	
	14–17 weeks	5	Parturition	10	10	1	1		300	0.02	
	4–7 weeks	25	Sexual Maturation	10	10	1	1		300	0.08	
	10–13 weeks	5	Prolactin	10	10	1	1		300	0.02	
	16 weeks	5	Prostatitis	10	10	1	1		300	0.02	
	16 weeks	5	Organ Wt	10	10	1	1		300	0.02	
	GDs 1–20	100	Fetal ossification	10	10	1	1		300	0.33	

Table B-4. Summary of reference values for Luteinate (continued)

Reference value duration	Exposure duration	NOAEL (mg/kg/day)	Type of effect	Uncertainty factors[a]						Reference value (mg/kg/day)	
				A	H	L	S	D	Total	Sample	Final
Chronic	6 months	4	LH	10	10	1	1	3	300	0.01	0.01
	6 months	4	Estrus	10	10	1	1		300	0.01	
	11–16 weeks	5	Pregnancy Maintenance	10	10	1	1		300	0.02	
	14–17 weeks	5	Parturition	10	10	1	1		300	0.02	
	10–13 weeks	5	Prolactin	10	10	1	1		300	0.02	
	16 weeks	5	Prostatitis	10	10	1	1		300	0.02	
	16 weeks	5	Organ Wt	10	10	1	1		300	0.02	
	GDs 1–20	100	Fetal ossification	10	10	1	1		300	0.33	

[a] A = animal-to-human (interspecies); H = inter-individual (intraspecies); L = LOAEL-to-NOAEL; S = subchronic-to-chronic duration; D = database deficiency

related effects. For this reason, the NOAEL of 25 mg/kg/day for prolactin and related effects following a 4-day developmental (neonatal) exposure was used as the basis for the acute reference value, with the assumption that a single exposure during a critical period of development would be sufficient to produce these effects.

Although a single-exposure LOAEL, particularly for the reduction in prolactin and increase in prostatitis (which showed a dependence on exposure duration in adults), might be slightly higher than the 4-day LOAEL, lack of data on this endpoint from a single-day exposure leads us to rely on the 4-day value during what appears to be a particularly sensitive time in the early postnatal period. Effects on prolactin (and therefore dopamine) also indicate a concern for developmental neurotoxicity (Figure B-5). Because there is a strong relationship between the data gaps of the acute toxicity study and the developmental neurotoxicity study, a database UF of $10^{1/2}$ was applied. In addition, UFs of 10 for interspecies and intraspecies uncertainty/variability were applied. The resulting reference value was 0.08 mg/kg/day.

Short-term Exposure

A variety of endpoints were examined for calculating the short-term reference value. Information was available on serum LH from the 3-day and 28-day adult studies as well as the study in which pregnant dams were exposed on GDs 1–8. Information on estrous cyclicity was available from the 28-day study in adult rats and in the study in which female weanlings were exposed on PNDs 22–41. Information on pregnancy maintenance was available from studies in which pregnant dams were exposed on GDs 1–8, 6–10, and 1–20, and information on parturition was available for exposures on GDs 6–10.

Information on sexual maturation in females was available from the study in which female weanlings were exposed on PNDs 22–41, and information on sexual maturation in males was available from the study in which weanling male rats were exposed on PNDs 23–53. In addition, information on sexual maturation from the two-generation reproductive toxicity study was also considered relevant. The latter study also provided information on the effects of Luteinate on the seminal vesicles and ventral prostate. Information on prolactin was available from the 28-day study in adults and the study in which lactating dams were exposed on PNDs 1–4; information on prostatitis was also available from the latter study (Figure B-6).

Interspecies and intraspecies UFs of 10 were applied to each of these endpoints (Table B-4). For the entire database available for short-term exposure, a database UF of $10^{1/2}$ was applied in the derivation of the final reference value for the lack of a developmental neurotoxicity study. The resulting reference values ranged from 0.08 to 0.67 mg/kg/day, and the final reference value was 0.08 mg/kg/day.

Longer-term Exposure

For derivation of the longer-term reference value, the databases available for the acute and shorter-term exposures were considered. In addition, the two-generation reproductive toxicity study provided information following longer exposures for serum LH, estrous cyclicity, pregnancy maintenance, parturition, prolactin, and prostatitis (Fig. B-7). Interspecies and intraspecies UFs of 10 were applied to each of these endpoints (Table B-4). The resulting sample reference values were 0.33 mg/kg/day, based on delayed ossification and 0.08 mg/kg/day, based on sexual maturation; all the other endpoints yielded a value of 0.02 mg/kg/day. The latter was chosen as the final value for the longer-term reference value.

Chronic Exposure

Information from all of the exposure scenarios described above was considered in deriving the chronic reference value. In addition, information on serum LH and estrous cyclicity was available from the 6-month study in adult rats. For endpoints where there was a clear exposure-dose-effect relationship, only information from studies of the longest exposure period was included in Table B-4 and Figure B-8. As noted above, decreases in serum LH were observed at 8 mg/kg/day in the 6-month study and at 10 mg/kg/day in the two-generation reproductive toxicity study; the NOAELs were 4 and 5 mg/kg/day, respectively. Estrous cyclicity was affected at these same dose levels in the two studies. The NOAEL for all other endpoints in the two-generation reproductive toxicity study was also 5 mg/kg/day.

Given the similarity in effect levels in the two studies, it is unlikely that longer exposures would alter the effect level. For this reason, although there is no information on the effect of Luteinate on reproductive aging, this is considered to be a qualitative gap in hazard identification, but was not considered to be a database deficiency for the purposes of deriving a chronic reference value. UFs of 10 for interspecies and intraspecies variability and uncertainty were applied to each of these endpoints (Table B-4). The reference values ranged from 0.01 to 0.02 mg/kg/day, and 0.01 mg/kg/day was chosen as the final value.

GLOSSARY

NOTE*: The following terms are used in this document. To the extent possible, definitions were taken from other EPA sources, e.g., IRIS, the Children's Health Research Strategy, the RfC Methodology. In some cases, the definitions have been revised from the originals in IRIS for the sake of clarity or to be consistent with usage in this document. Those terms and definitions that are changed and/or newly proposed in this document to be added to IRIS are shown in italics and the definition(s) they are proposed to replace are indicated in brackets. A number of other terms are included in the IRIS glossary that are not listed here, simply because they were not used in this document.*

Acute Exposure: One dose or multiple doses of short duration spanning less than or equal to 24 hours. [Current IRIS definition.]

Acute Exposure*: Exposure by the oral, dermal, or inhalation route for 24 hours or less.* [Proposed definition to replace the current Acute Exposure definition on IRIS.]

Adverse Effect: A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge.

Benchmark Dose (BMD) or Concentration (BMC): A statistical lower confidence limit on the dose that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background. [current IRIS definition]

Benchmark Dose (BMD) or Concentration (BMC): *A dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background.* [Proposed definition to replace the current definition on IRIS.]

BMDL or BMCL: *A statistical lower confidence limit on the dose or concentration at the BMD or BMC, respectively.* [A new definition to be added to IRIS.]

Benchmark Response (BMR): An adverse effect used to define a benchmark dose from which an RfD (or RfC) can be developed. The change in response rate over background of the BMR is usually in the range of 5 to 10%, which is the limit of responses typically observed in well-conducted animal experiments.

Bioassay: An assay for determining the potency (or concentration) of a substance that causes a biological change in experimental animals.

Bioavailability: The degree to which a substance becomes available to the target tissue after administration or exposure.

Biologically Based Dose Response (BBDR) model: A predictive tool used to estimate potential human health risks by describing and quantifying the key steps in the cellular, tissue, and organismal responses as a result of chemical exposure. [Current IRIS definition.]

Biologically Based Dose Response (BBDR) Model: *A predictive model that describes biological processes at the cellular and molecular level linking the target organ dose to the adverse effect.* [Proposed definition to replace the current definition on IRIS.]

Blood-to-air Partition Coefficient: A ratio of a chemical's concentration between blood and air when at equilibrium.

Chronic Exposure: Multiple exposures occurring over an extended period of time or a significant fraction of the animal's or the individual's lifetime. [Current IRIS definition.]

Chronic Exposure*: Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species).* [Proposed definition to replace the current definition for Chronic Exposure on IRIS.]

Chronic Study: A toxicity study designed to measure the (toxic) effects of chronic exposure to a chemical.

Critical Effect: The first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases.

Critical Study: The study that contributes most significantly to the qualitative and quantitative assessment of risk; also called Principal Study.

Developmental Toxicity: Adverse effects on the developing organism that may result from exposure prior to conception (either parent), during prenatal development, or postnatally until the time of sexual maturation. The major manifestations of developmental toxicity include death of the developing organism, structural abnormality, altered growth, and functional deficiency.

Dose*: The amount of a substance available for interactions with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The* ***potential dose*** *is the amount ingested, inhaled, or applied to the skin. The* ***applied dose*** *is the amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). The* ***absorbed dose*** *is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of the skin, lung, and digestive tract) through uptake processes.* ***Internal dose*** *is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by any particular organ or cell is termed*

*the **delivered** or **biologically effective dose** for that organ or cell.* [New definition proposed to be added to IRIS.]

Dose-Response Assessment: A determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence, percent response in groups of subjects (or populations), or as the probability of occurrence within a population. [Current IRIS definition.]

Dose-Response Assessment: *A determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence or change in level of response, percent response in groups of subjects (or populations), or the probability of occurrence or change in level of response within a population.* [Proposed definition to replace the current definition on IRIS.]

Dose-Response Relationship: The relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific, biological changes (response). [Current IRIS definition.]

Dose-Response Relationship: *The relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biological changes in incidence or in degree of change (response).* [Proposed definition to replace the current definition on IRIS.]

Endpoint: An observable or measurable biological event or chemical concentration (e.g., metabolite concentration in a target tissue) used as an index of an effect of a chemical exposure.

Epidemiology: The study of disease patterns in human populations. [Current IRIS definition.]

Epidemiology: *The study of the distribution and determinants of health-related states or events in specified populations and the application of this study to the control of health problems.* [Proposed definition to replace the current definition on IRIS.]

Exposure: Contact made between a chemical, physical, or biological agent and the outer boundary of an organism. Exposure is quantified as the amount of an agent available at the exchange boundaries of the organism (e.g., skin, lungs, gut).

Exposure Assessment: An identification and evaluation of the human population exposed to a toxic agent, describing its composition and size, as well as the type, magnitude, frequency, route, and duration of exposure.

Exposure Pathway: The physical course an environmental agent takes from the source to the individual exposed.

Extrapolation, Low Dose: An estimate of the response at a point below the range of the experimental data, generally through the use of a mathematical model.

Hazard: A potential source of harm.

Hazard Assessment: The process of determining whether exposure to an agent can cause an increase in the incidence of a particular adverse health effect (e.g., cancer, birth defect) and whether the adverse health effect is likely to occur in humans.

Hazard Characterization*: A description of the potential adverse health effects attributable to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the associated dose, route, duration, and timing of exposure.* [New definition proposed to be added to IRIS]

Human Equivalent Concentration (HEC): The human concentration (for inhalation exposure) of an agent that is believed to induce the same magnitude of toxic effect as the experimental animal species concentration. This adjustment may incorporate toxicokinetic information on the particular agent, if available, or use a default procedure.

Human Equivalent Dose (HED): The human dose (for other than the inhalation routes of exposure) of an agent that is believed to induce the same magnitude of toxic effect as the experimental animal species dose. This adjustment may incorporate toxicokinetic information on the particular agent, if available, or use a default procedure, such as assuming that daily oral doses experienced for a lifetime are proportional to body weight raised to the 0.75 power.

Incidence: The number of new cases of a disease that develop within a specified population over a specified period of time.

Incidence Rate: The ratio of new cases within a population to the total population at risk given a specified period of time.

Latency Period: The time between exposure to an agent and manifestation or detection of a health effect of interest.

Linear Dose Response: A pattern of frequency or severity of biological response that varies proportionately with the amount of dose of an agent. [Current IRIS definition.]

Linear Dose Response: *A pattern of frequency or severity of biological response that varies directly with the amount of dose of an agent. This linear relationship holds only at low doses in the range of extrapolation.* [Proposed definition to replace the current definition on IRIS.]

Longer-term Exposure: *Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the life span in humans (more than 30 days up to*

approximately 90 days in typically used laboratory animal species). [Proposed new definition to be used relative to the Longer-term Reference Value. Similar to the current definition for Subchronic Exposure. Because subchronic exposure studies will continue to be used in risk assessment, the latter term should be retained as well but replaced with the definition for Longer-term Exposure.]

Lowest-Observed-Adverse-Effect Level (LOAEL): The lowest exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group. Also referred to as lowest-effect level (LEL). [Current IRIS and RfC Methodology definition.]

Lowest-Observed-Adverse-Effect Level (LOAEL): *The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group.* [Proposed to replace the current definition in IRIS and the RfC methodology, U.S. EPA, 1994]

Margin of Exposure (MOE): The LED10 or other point of departure divided by the actual or projected environmental exposure of interest.

Mechanism of Action: The complete sequence of biological events that must occur to produce the toxic effect.

Mode of Action (MOA): A less-detailed description of the mechanism of action in which some but not all of the sequence of biological events leading to a toxic effect is known.

Modifying Factor (MF): A factor used in derivation of a reference dose or reference concentration. The magnitude of the MF reflects the scientific uncertainties of the study and database not explicitly treated with standard uncertainty factors (e.g., the completeness of the overall database). A MF is greater than zero and less than or equal to 10, and the default value for the MF is1. [Current definition in IRIS; this report recommends that its use be discontinued.]

No-Observed-Adverse-Effect Level (NOAEL): The highest exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse, nor precursors to adverse effects. [Current IRIS and RfC Methodology definition.]

No-Observed-Adverse-Effect Level (NOAEL): *The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors to adverse effects.* [Proposed to replace the current definition in IRIS and the RfC methodology, U.S. EPA, 1994.]

Nonlinear Dose Response: A pattern of frequency or severity of biological response that does not vary proportionately with the amount of dose of an agent. When mode of action information indicates that responses may not follow a linear pattern below the dose range of the observed data, non-linear methods for determining risk at low dose may be justified. [Current IRIS definition.]

Nonlinear Dose Response: *A pattern of frequency or severity of biological response that does not vary directly with the amount of dose of an agent. When mode of action information indicates that responses may fall more rapidly than dose below the range of the observed data, nonlinear methods for determining risk at low dose may be justified.* [Proposed definition to replace the current definition on IRIS.]

Physiologically Based Pharmacokinetic (PBPK) Model: Physiologically based compartmental model used to characterize pharmacokinetic behavior of a chemical. Available data on blood flow rates, and metabolic and other processes which the chemical undergoes within each compartment are used to construct a mass-balance framework for the PBPK model. [Current IRIS definition.]

Physiologically Based Pharmacokinetic (PBPK) Model: *A model that estimates the dose to a target tissue or organ by taking into account the rate of absorption into the body, distribution among target organs and tissues, metabolism, and excretion.* [Proposed definition to replace the current definition on IRIS.]

Point of Departure: The dose-response point that marks the beginning of a low-dose extrapolation. This point is most often the upper bound on an observed incidence or on an estimated incidence from a dose-response model. [Current IRIS definition.]

Point of Departure: *The dose-response point that marks the beginning of a low-dose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response.* [Proposed definition to replace the current definition on IRIS.]

Ppb: A unit of measure expressed as parts per billion. Equivalent to 1×10^{-9}.

Ppm: A unit of measure expressed as parts per million. Equivalent to 1×10^{-6}.

Prevalence: The proportion of disease cases that exist within a population at a specific point in time relative to the number of individuals within that population at the same point in time.

Reference Concentration (RfC): An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a

lifetime. It can be derived from a NOAEL, LOAEL, or benchmark concentration, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Current IRIS definition.]

Reference Dose (RfD): An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used. Generally used in EPA's noncancer health assessments. [Current IRIS definition.]

Reference Value (RfV): *An estimate of an exposure for [a given duration] to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse effects over a lifetime. It is derived from a BMDL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used.* [Durations include acute, short-term, longer-term, and chronic and are defined individually in this glossary. This definition is proposed to replace those for the Reference Dose (RfD) and Reference Concentration (RfC). A subscript would be used with the RfV to denote route and duration, e.g., RfV_{AO} for the Acute Oral Reference Value.]

Regional Deposited Dose (RDD): The deposited dose of particles calculated for a respiratory tract region of interest (r) as related to an observed toxicity. For respiratory effects of particles, the deposited dose is adjusted for ventilatory volumes and the surface area of the respiratory region affected (mg/min-sq. cm). For extrarespiratory effects of particles, the deposited dose in the total respiratory system is adjusted for ventilatory volumes and body weight (mg/min-kg).

Regional Deposited Dose Ratio (RDDR): The ratio of the regional deposited dose calculated for a given exposure in the animal species of interest to the regional deposited dose of the same exposure in a human. This ratio is used to adjust the exposure-effect level for interspecies dosimetric differences to derive a human equivalent concentration for particles.

Regional Gas Dose: The gas dose calculated for the region of interest as related to the observed effect for respiratory effects. The deposited dose is adjusted for ventilatory volumes and the surface area of the respiratory region affected (mg/min-sq.cm).

Regional Gas Dose Ratio (RGDR): The ratio of the regional gas dose calculated for a given exposure in the animal species of interest to the regional gas dose of the same exposure in humans. This ratio is used to adjust the exposure effect level for interspecies dosimetric differences to derive a human equivalent concentration for gases with respiratory effects.

Risk (in the context of human health): The probability of injury, disease, or death from exposure to a chemical agent or a mixture of chemicals. In quantitative terms, risk is expressed in values ranging from zero (representing the certainty that harm will not occur) to one

(representing the certainty that harm will occur). The following are examples of how risk is expressed within IRIS: E-4 or 10-4 = a risk of 1/10,000; E-5 or 10-5 = 1/100,000; E-6 or 10-6 = 1/1,000,000. Similarly, 1.3 E-3 or 1.3 x 10-3 = a risk of 1.3/1,000=1/770; 8 E-3 or 8 x 10-3 = a risk of 1/125 and 1.2 E-5 or 1.2 x 10-5 = a risk of 1/83,000. [Current IRIS definition.]

Risk: *The probability of adverse effects resulting from exposure to an environmental agent or mixture of agents.* [Proposed definition to replace the current definition on IRIS.]

Risk Characterization*: The integration of information on hazard, exposure, and dose-response to provide an estimate of the likelihood that any of the identified adverse effects will occur in exposed people.* [New definition proposed to be added to IRIS.]

Risk Assessment (in the context of human health): The determination of potential adverse health effects from exposure to chemicals, including both quantitative and qualitative expressions of risk. The process of risk assessment involves four major steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. [Current IRIS definition.]

Risk Assessment: *The evaluation of scientific information on the hazardous properties of environmental agents (hazard characterization), the dose-response relationship (dose-response assessment), and the extent of human exposure to those agents (exposure assessment). The product of the risk assessment is a statement regarding the probability that populations or individuals so exposed will be harmed and to what degree (risk characterization).* [Proposed definition to replace the current definition on IRIS.]

Short-term Exposure: Multiple or continuous exposure to an agent for a short period of time, usually one week. [Current IRIS definition.]

Short-term Exposure: *Repeated exposure by the oral, dermal, or inhalation route for more than 24 hours, up to 30 days.* [Proposed definition to replace the current definition for Short-term Exposure on IRIS.]

Statistical Significance: The probability that a result [sic] likely to be due to chance alone. By convention, a difference between two groups is usually considered statistically significant if chance could explain it only 5% of the time or less. Study design considerations may influence the a priori choice of a different statistical significance level. [Current IRIS definition.]

Statistical Significance: *The probability that a result is not likely to be due to chance alone. By convention, a difference between two groups is usually considered statistically significant if chance could explain it only 5% of the time or less. Study design considerations may influence the a priori choice of a different level of statistical significance.* [Proposed definition to replace the current definition on IRIS.]

Subchronic Exposure: Exposure to a substance spanning approximately 10% of the lifetime of an organism. [See note for Longer-term Exposure.]

Subchronic Study: A toxicity study designed to measure effects from subchronic exposure to a chemical.

Supporting Studies: Studies that contain information useful for providing insight and support for conclusions.

Susceptible Subgroups*: May refer to life stages, for example, children or the elderly, or to other segments of the population, for example, asthmatics or the immune-compromised, but are likely to be somewhat chemical-specific and may not be consistently defined in all cases.* [New definition proposed to be added to IRIS.]

Susceptibility*: Increased likelihood of an adverse effect, often discussed in terms of relationship to a factor that can be used to describe a human subpopulation (e.g., life stage, demographic feature, or genetic characteristic).* [New definition proposed to be added to IRIS.]

Systemic Effects or Systemic Toxicity: Toxic effects as a result of absorption and distribution of a toxicant to a site distant from its entry point, at which point effects are produced. Not all chemicals that produce systemic effects cause the same degree of toxicity in all organs. [Current IRIS definition.]

Systemic Effects or Systemic Toxicity: *Toxic effects as a result of absorption and distribution of a toxicant to a site distant from its entry point.* [Proposed definition to replace the current definition on IRIS.]

Target Organ: The biological organ(s) most adversely affected by exposure to a chemical substance. [Current IRIS definition.]

Target Organ: *The biological organ(s) most adversely affected by exposure to a chemical or physical agent.* [Proposed definition to replace the current definition on IRIS.]

Threshold: The dose or exposure below which no deleterious effect is expected to occur.

Toxicity: The degree to which a chemical substance elicits a deleterious or adverse effect upon the biological system of an organism exposed to the substance over a designated time period. [Current IRIS definition.]

Toxicity: *Deleterious or adverse biological effects elicited by a chemical, physical, or biological agent.* [Proposed definition to replace the current definition on IRIS.]

Toxicodynamics*: The determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response to an environmental agent (also called pharmacodynamics).* [New definition proposed to be added to IRIS.]

Toxicokinetics*: The determination and quantification of the time course of absorption, distribution, biotransformation, and excretion of chemicals (also called pharmacokinetics).* [New definition proposed to be added to IRIS.]

Toxicology: The study of harmful interactions between chemicals and biological systems. [current IRIS definition]

Toxicology: *The study of harmful interactions between chemical, physical, or biological agents and biological systems.* [Proposed definition to replace the current definition on IRIS.]

Toxic Substance: A chemical substance or agent which may cause an adverse effect or effects to biological systems. [Current IRIS definition.]

Toxic Substance: *A chemical, physical, or biological agent that may cause an adverse effect or effects to biological systems*. [Proposed definition to replace the current definition on IRIS.]

Uncertainty*: Uncertainty occurs because of a lack of knowledge. It is not the same as variability. For example, a risk assessor may be very certain that different people drink different amounts of water but may be uncertain about how much variability there is in water intakes within the population. Uncertainty can often be reduced by collecting more and better data, whereas variability is an inherent property of the population being evaluated. Variability can be better characterized with more data but it cannot be reduced or eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization.* [New definition proposed to be added to IRIS.]

Uncertainty Factor (UF): One of several, generally 10-fold, factors used in operationally deriving the RfD and RfC from experimental data. UFs are intended to account for (1) variation in sensitivity among the members of the human population, i.e., interhuman or intraspecies variability; (2) the uncertainty in extrapolating animal data to humans, i.e., interspecies variability; (3) the uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure, i.e., extrapolating from subchronic to chronic exposure; (4) the uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) the uncertainty associated with extrapolation from animal data when the data base is incomplete. [current IRIS definition]

Uncertainty/Variability Factors (UFs): *One of several, generally 10-fold, default factors used in operationally deriving the RfD and RfC from experimental data. The factors are intended to account for (1) variation in sensitivity among the members of the human population (i.e., inter-individual variability); (2) uncertainty in extrapolating animal data to humans (i.e.,*

interspecies uncertainty); (3) uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure to lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) uncertainty in extrapolating from a LOAEL rather than from a NOAEL; and (5) uncertainty associated with extrapolation when the database is incomplete. [Proposed definition to replace the current one for Uncertainty Factor on IRIS.]

***Variability**: Variability refers to true heterogeneity or diversity. For example, among a population that drinks water from the same source and with the same contaminant concentration, the risks from consuming the water may vary. This may be due to differences in exposure (i.e., different people drinking different amounts of water and having different body weights, different exposure frequencies, and different exposure durations) as well as differences in response (e.g., genetic differences in resistance to a chemical dose). Those inherent differences are referred to as variability. Differences among individuals in a population are referred to as inter-individual variability; differences for one individual over time is referred to as intra-individual variability.* [New definition proposed to be added to IRIS.]

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Attachment

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EPA
United States
Environmental Protection
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www.epa.gov/raf

Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation

Office of the Science Advisor
Risk Assessment Forum

EPA/100/R-14/002F
September 2014

Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation

Office of the Science Advisor
Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC 20460

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ADME	absorption, distribution, metabolism, excretion
Agency	U.S. Environmental Protection Agency
AOP	adverse outcome pathway
AUC	area under the curve (optional subscripts $_{A}$ = animal AUC value; $_{gen}$ = general human population AUC value; $_{sens}$ = sensitive human population AUC value)
AUC_A	animal AUC value (AUC value produced in animals at or near the animal point of departure)
AUC_{gen}	general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population)
AUC_{sens}	sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population)
BBDR	biologically based dose-response
$BMDL_x$	lower confidence bound on benchmark dose corresponding to an *x*% increase in response
$BW^{¾}$	body weight raised to the ¾ power
CF	composite factor
Cl	clearance (optional subscripts $_{A}$ = animal Cl value; $_{H}$ = human Cl value; $_{gen}$ = general human population Cl value; $_{sens}$ = sensitive human population Cl value; $_{hep}$ = hepatic Cl value; $_{int}$ = intrinsic Cl value)
Cl_A	animal Cl value
Cl_H	human Cl value
Cl_{gen}	general human population Cl value (at a measure of central tendency in the entire or general human population)
Cl_{sens}	sensitive human population Cl value (at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)
Cl_{hep}	hepatic Cl value
Cl_{int}	intrinsic Cl value
C_{max}	maximum concentration (optional subscripts $_{gen}$ = general human population C_{max} value; $_{sens}$ = sensitive human population C_{max} value)
$C_{max\ gen}$	general human population C_{max} value (at a fixed external dose at a measure of central tendency in the entire or general human population)
$C_{max\ sens}$	sensitive human population C_{max} value (at a fixed external dose at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)
CSAF	chemical-specific adjustment factor

LIST OF ABBREVIATIONS (continued)

D_A	animal external dose (administered or external dose in the test animal species that leads to a level of a toxicologically relevant dose metric at or near the point of departure)
D_H	human external dose (administered or external dose at the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)
DDEF	data-derived extrapolation factor
DNA	deoxyribonucleic acid
EF	extrapolation factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
EF_{AD}	interspecies toxicodynamic EF
EF_{AK}	interspecies toxicokinetic EF
EF_{HD}	intraspecies toxicodynamic EF
EF_{HK}	intraspecies toxicokinetic EF
EPA	U.S. Environmental Protection Agency
HEC	human equivalent concentration
HED	human equivalent dose
IOM	Institute of Medicine
IPCS	International Programme on Chemical Safety
IRIS	Integrated Risk Information System
K_m	Michaelis constant, substrate concentration at rate of ½ V_{max}
MOA	mode of action
NRC	National Research Council
PBPK	physiologically based pharmacokinetic
POD	point of departure
RfC	reference concentration
RfD	reference dose
TD	toxicodynamic
TK	toxicokinetic
UF	uncertainty factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability)
UF_A	interspecies UF
UF_H	intraspecies UF
U.S. EPA	U.S. Environmental Protection Agency
V_{max}	theoretical maximal initial velocity
WHO	World Health Organization

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EXECUTIVE SUMMARY

The mission of the U.S. Environmental Protection Agency ("EPA" or "Agency") is to protect human health and the environment. The statutes under which EPA functions require the Agency to identify substances in the environment that may elicit a response, and determine the potential for harmful effects from exposure to those substances. Risk assessment is an analytical process routinely used by EPA to inform decisions on managing risks to human health and the environment (U.S. EPA, 2012). Because no risk assessment is definitive, uncertainties must be handled in a manner that is both consistent with EPA's policies and responsive to the needs of decision makers (U.S. EPA, 2004a). It is a common practice to resort to default assumptions (including values) to allow the assessment to proceed when data are limited. This document is consistent with the recommendations in *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) as it describes the process for developing scientifically supportable values to account for inter- and intraspecies extrapolation.

Among the default values most commonly used in human health risk assessment are those used to extrapolate toxicity data derived from animal models to humans and those that account for human variability. This document provides guidance to risk assessors who are well versed in chemical dosimetry and/or studies of tissue responses on methods used to account for the differences between the model species and the average human (interspecies variation), and for variation in the human population (intraspecies variation). Moving from the established default values for inter- and intraspecies extrapolation to empirically derived values addresses the recommendations in *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) to "…continue and expand use of the best, most current science to support and revise default assumptions." The Institute of Medicine (IOM) report *Environmental Decisions in the Face of Uncertainty* concluded "… if enough scientific information exists about the differences in the metabolism or mode of action of a chemical in animals versus in humans, then scientifically derived extrapolation factors can be used rather than the defaults." The IOM report goes on to say about data-derived values, "If those factors more accurately reflect the differences between animals and humans than default adjustment factors, the use of such data-derived extrapolation factors would decrease the uncertainty in the risk assessment" (IOM, 2013).

The goals of data-derived extrapolation factors (DDEFs) are to maximize the use of available data and improve the scientific support for a risk assessment. A DDEF approach is an accepted approach for deriving reference concentrations (RfCs), reference doses (RfDs), or counterpart values and is consistent with existing Agency guidance. This guidance presents the Agency's approach to identifying, justifying, and employing quantitatively useful data to develop nondefault values for inter- and intraspecies extrapolation. Moreover, this guidance will

aid risk assessors and researchers in identifying data gaps and developing informative experiments to yield quantitatively valuable data.

DDEF values are applicable in the derivation of RfCs and RfDs, or other relevant values or metrics (e.g., hazard index, margins of exposure). This guidance describes the process for identifying pertinent data useful for quantifying inter- and intraspecies differences to serve as the basis for empirically determined DDEFs. When using DDEFs, inter- and intraspecies extrapolation factors are divided into two components representing toxicokinetic (TK; amount of agent reaching the target tissue) variability and toxicodynamic (TD; dose at which the target tissue responds to the agent) variability. Key considerations include identifying an adverse health outcome, a measurable biological event associated with that adverse health outcome, and the concentration of the toxicant associated with the development of the biological event. Interspecies TK variability is quantified based on the external exposure that produces the same tissue concentration in animals and in humans. Intraspecies TK variability is defined as differences in tissue concentration attained from the same human external exposure (dose). TD variability is quantified on the basis of differences in the tissue or *in vitro* concentration that produce the same response between animals and humans or among humans.

1. INTRODUCTION

1.1. BACKGROUND

Risk assessment is an analytical process used by the U.S. Environmental Protection Agency ("EPA" or "Agency") to inform decisions on managing risks to human health and the environment (U.S. EPA, 2012). No risk assessment can reflect risk with absolute certainty, so it is important that uncertainties be accounted for in a predictable, scientifically defensible manner that is both consistent with EPA's policies and responsive to the needs of decision makers (U.S. EPA, 2004a). The risk assessment process involves decreasing uncertainty in estimates whenever possible, defining uncertainty and variability in estimates, and quantifying the uncertainty when feasible. In deriving reference concentrations (RfCs) and reference doses (RfDs), the Agency has historically used default uncertainty factors (UFs) to compensate for a lack of information (U.S. EPA, 2002b). As science has advanced, however, there has been a growing effort to increase reliance on available data to modify the values for these UFs (IPCS, 2005). The default UFs were developed to address data gaps in the development of RfDs and RfCs, but when appropriate data are available for an assessment, those data are given precedence over standard default values (U.S. EPA, 2004a). This guidance describes an approach for identifying and using pertinent information for developing data-derived extrapolation factors (DDEFs) for the purposes of developing RfDs, RfCs, or related metrics/approaches (e.g., hazard index, margin of exposure).

It is common to use default values and processes in risk assessments to compensate for the absence of data. EPA uses the definition of default assumption articulated by the National Research Council (NRC): "the option chosen on the basis of risk assessment policy that appears to be the best choice in the absence of data to the contrary" (NRC, 1983). In its report *Science and Judgment in Risk Assessment* (NRC, 1994), the NRC supported EPA's use of defaults as a reasonable way to consider uncertainty. The report stated that EPA should have principles for choosing default options and for judging when and how to depart from them. Specifically, the report recognized that EPA uses default assumptions (e.g., UF) and indicated that criteria for their use should be clearly articulated in situations in which "the chemical and/or site-specific data are unavailable." In the report *Science and Decisions: Advancing Risk Assessment* (NRC, 2009), the NRC recommended that "EPA should develop clear, general standards for the level of evidence needed to justify the use of alternative assumptions in place of defaults." The current document is responsive to the recommendations in *Science and Decisions: Advancing Risk Assessment* because it describes the process for developing scientifically supportable values to account for inter- and intraspecies extrapolation.

While risk assessors have generally tried to make maximum use of available data, the shift away from standard default assumptions as the starting point in risk assessment was formalized as EPA science policy with the publication of the 2005 *Guidelines for Carcinogen Risk Assessment* [or "*Cancer Guidelines*"; (U.S. EPA, 2005)]. The *Cancer Guidelines* state "these cancer guidelines view a critical analysis of all of the available information…as the starting point from which a default option may be invoked if needed to address uncertainty or the absence of critical information." Applying the available and sufficient data to avoid default UF values will improve the scientific basis of risk assessments when data are sufficient for refining UFs (IOM, 2013). In cases where data are not sufficient and default approaches are used, hazard and risk characterizations will be improved because data needs can be more clearly articulated and potentially met in the future (Bogdanffy et al., 2001; Meek, 2001; Meek et al., 2001; Murray and Andersen, 2001).

Extrapolation is most scientifically robust when data are first evaluated before using defaults. However, with a multitude of types of data, analyses, and risk assessments, as well as the diversity of needs of decision makers, it is neither possible nor desirable to specify step-by-step criteria for decisions to invoke a default option. Some risk assessments may be limited by constraints of data, time, and/or resources. Other risk assessments may require only screening-level evaluations; in these cases, the risk assessor may be more likely to resort to one or more default assumptions. On the other hand, risk assessments used to support significant risk management decisions will often benefit from a more comprehensive approach. In general, the level of effort applied in a particular assessment should be related to the needs of decision makers, as determined through planning and scoping for that assessment (U.S. EPA, 2014).

1.2. PURPOSE AND SCOPE

Efforts by the United States and international communities have improved the scientific basis for human health risk assessments by increasing the use of mechanistic and kinetic data. For example, the *Cancer Guidelines* (U.S. EPA, 2005) emphasize the use of mode-of-action (MOA) information in characterizing potential health effects of exposure to environmental agents. International efforts, including those by the International Life Sciences Institute and the World Health Organization (WHO)'s International Programme on Chemical Safety (IPCS), have developed frameworks for evaluating animal data to determine the human relevance of described MOAs (Boobis et al., 2008; Seed et al., 2005; Sonich-Mullin et al., 2001).[2] These documents

[2]Use of the term adverse outcome pathway (AOP) has become common. AOPs and MOAs are similar in that they identify an initiating event and the important biological steps associated with different levels of biological organization leading to an adverse health outcome.

guide the qualitative and quantitative evaluation of the relevance of a particular animal MOA in humans and discuss the use of *in vivo* and *in vitro* data when considering animal-to-human extrapolation. The 2005 *Cancer Guidelines* (U.S. EPA, 2005) and other documents such as IPCS's chemical-specific adjustment factors (CSAFs) guidance (IPCS, 2005), the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), and *An Examination of EPA Risk Assessment Principles and Practices: Staff Paper Prepared for the U.S. EPA by Members of the Risk Assessment Task Force* (U.S. EPA, 2004a) also encourage the use of sophisticated models like physiologically based pharmacokinetic (PBPK) and biologically based dose-response (BBDR) models in interspecies extrapolation.

This guidance deals specifically with the development and use of DDEFs in the calculation of RfDs, RfCs, and other relevant approaches (e.g., margin of exposure) to assessing risk. The goal of DDEFs is to maximize the use of available data and improve the scientific support for a risk assessment. The processes described herein have benefited from ongoing discussions in the scientific community regarding the need to refine the default 10-fold UFs historically used in deriving safety estimates (e.g., RfDs, minimal risk levels, and acceptable daily intakes). Finalized in 2005 (IPCS, 2005), the WHO CSAF guidance describes approaches for using data to refine inter- and intraspecies default UFs. *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* is based largely on analyses by Renwick (1993, 1991) and Renwick and Lazarus (1998), which describe a data-derived approach that assigns values for toxicokinetic (TK) and toxicodynamic (TD) differences as components within an established 10 × 10 framework for inter- and intraspecies extrapolation. DDEFs are similar in concept to the CSAFs in that the factors for interspecies and intraspecies extrapolation are subdivided into TK and TD components, and kinetic and mechanistic data are used to derive refined inter- or intraspecies extrapolation factors.

LINKING TOXICOKINETICS AND TOXICODYNAMICS

Interactions between the toxicologically active chemical moiety and the cellular receptor are responsible for producing an adverse response. Therefore, this guidance presents a single methodology to quantify differences in target tissue concentrations of toxicants (toxicokinetics) and differences in target tissue responses to toxicants (toxicodynamics) to avoid reliance on default values for inter- and intraspecies uncertainty factors when data are available.

The Appendix to this document contains case study examples taken from EPA's Integrated Risk Information System (IRIS) and from EPA Program Office records. These case studies present the application of principles contained in this document to data and modeling studies for actual chemicals and should serve as instructional aides.

Issues related to the derivation and use of DDEFs to avoid default UFs for intraspecies (human) variability and interspecies variability are the focus of this guidance document. Thus, concepts beyond the scope of this guidance are not discussed in detail here; they include approaches for selecting critical effects, establishing key events in an MOA analysis,[3] deriving points of departure (PODs), performing benchmark dose analysis, and developing and evaluating PBPK and BBDR models. In addition, no discussion is included on factors that have been used for other areas of uncertainty or variability (e.g., duration, database deficiencies, or lack of a no-observed-adverse-effect level).[4]

Finally, this document is written for toxicologists and risk assessors, and the methods described here should be conducted by or in conjunction with scientists with the appropriate level of expertise.

[3]MOA refers to a series of key, determinant, and necessary interactions between the toxicant and its molecular target(s) that lead to the toxic response. Refer to Section 2.3 for further information on use of MOAs in developing DDEFs.

[4]The Food Quality Protection Act mandates the use of a presumptive 10-fold factor in risk assessments performed for establishing pesticide tolerances as part of pesticide registration for the protection of infants and children in addition to inter- and intraspecies factors. This factor can only be modified based upon reliable data.

2. TECHNICAL CONCEPTS AND PRINCIPLES FOR DATA-DERIVED EXTRAPOLATION FACTORS

2.1. BACKGROUND

EPA has developed several methodologies to guide and refine the approach to estimate reference values for human exposures. This approach comprises several steps that include inter- and intraspecies extrapolation, in which UFs based on default assumptions may be required to account for inherent uncertainties and variability (U.S. EPA, 2011, 2002b; Bogdanffy and Jarabek, 1995; U.S. EPA, 1994; 1993, see Figure 1). This guidance describes an approach to performing inter- and intraspecies extrapolations based on the use of the best available science

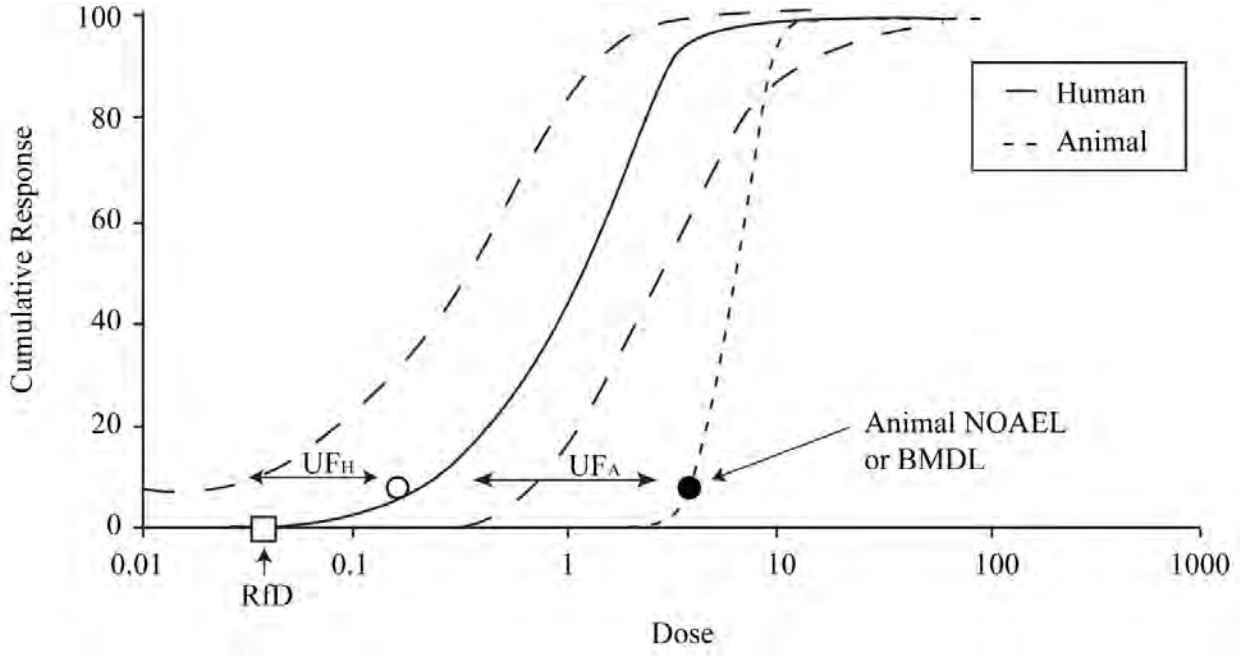

Figure 1. Derivation of reference dose/reference concentration using uncertainty factors. This figure depicts the extrapolation of the dose-response relationship between and among species. The POD (filled circle) for the animal dose-response relationship (dotted line) is extrapolated to humans (solid line) through application of the interspecies uncertainty factor (UF_A), which is "applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both TK and TD aspects." Here, per long-standing guidance, the dose at the animal POD associated with a predetermined level of response is extrapolated using UF_A to a measure of dose assumed to represent the same level of response (open circle) for a central tendency member of the general human population (solid line) (U.S. EPA, 1993). Dashed lines surrounding the solid line represent confidence bounds for human variability. Note that while the dose is extrapolated to other values, the response level remains fixed both between animals and humans, as well as within the human population.

and data. The rationale for choosing an extrapolation factor value should be presented transparently, include a full discussion of the perceived strengths and limitations of the data and describe the impact of science policy considerations include relevant science policy choices or implications

In the context of the methodologies for the derivation of RfDs, RfCs, and other relevant metrics, DDEFs are intended to address, as needed, inter- and intraspecies extrapolation of the POD from experimental data to an estimate for the sensitive human population or life stage. DDEFs are developed from data on inter- or intraspecies differences. DDEFs may consider both TK and TD properties. These factors can be derived for a single agent or chemical, for a class of chemicals with shared chemical or toxicological properties, or for a group of chemicals that share a mode or mechanism of action or TK characteristics. As described below, DDEFs can be calculated using sophisticated TD or TK models or can be calculated as ratios using key kinetic or dynamic data. With regard to interspecies extrapolation, EPA currently recognizes a hierarchy of approaches ranging from the preferred approach using PBPK modeling (U.S. EPA, 2011, 2006a, 1994) to default approaches when data do not support a more chemical-specific approach. "The intraspecies uncertainty factor (UF_H) is applied to account for variations in susceptibility within the human population (interhuman variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the groups of the human population that are most sensitive to the health hazards of the chemical being assessed" (U.S. EPA, 2002b). The default value for UF_H is 10-fold; the default value for interspecies uncertainty factor (UF_A) is apportioned into a TD component valued at one-half order of magnitude and a TK component addressed via default inhalation dosimetry methods (U.S. EPA, 1994) or body-weight scaling for orally encountered compounds (U.S. EPA, 2011). DDEFs fall within this hierarchical range of approaches.

CHEMICAL-SPECIFIC DATA

When deriving an RfD/RfC from animal data, and in the absence of information to the contrary, humans are assumed to be more sensitive to the toxic effect of chemicals than are test animal species. Humans also demonstrate population variability in response. These differences in sensitivity between species and among humans are captured in two uncertainty factors: interspecies (UF_A) and intraspecies (UF_H), respectively (U.S. EPA, 2002b). The default values for these UFs are based on our understanding and interpretation of data for a limited number of chemicals. With data relevant to the chemical of interest, DDEF may be used instead of the default values, thus increasing the confidence in the assessment.

Avoiding default assumptions with DDEFs begins with an evaluation of the strengths of the available data. Using *in vitro* data in risk assessment, as advocated in *Toxicity Testing in the 21st Century* (NRC, 2007), offers some distinct advantages over *in vivo* studies. While data derived in whole animal bioassays offer some value in deriving DDEF values, the data are accompanied by limitations. Given that humans and test animal species may differ in terms of

both dosimetry and innate sensitivity, response data from whole animal bioassays offer little opportunity to separate the TK and TD components of uncertainty. *In vitro* systems offer some advantages in that the influence of TK can be well controlled and response data can be well characterized, largely due to the avoidance of experimental constraints (e.g., less restrictive constraints on resources in areas like the number of doses/concentrations for testing). *In vitro* systems also offer some distinct advantages in studying both metabolism and response development in the human species because the ethical considerations of exposure are substantially lessened. Regardless, *in vitro* data require interpretation in the context of the intact mammalian system. For example, when *in vitro* data are derived from preparations representing only a fraction of the total biology of the cell (e.g., microsomal protein), care should be taken to ensure that the measured TD event (e.g., protein binding) or TK outcome (e.g., formation of an/the active metabolite) accurately reflects the biology of the *in vivo* effect. Regardless of the system evaluated (*in vitro* or *in vivo*), determinations regarding the strengths of the relevant data require careful consideration and characterization.

2.1.1. Uncertainty Factors Compared to Data-Derived Extrapolation Factors

DDEF values are not UFs, *per se*. UFs incorporate both extrapolation components that address variability (heterogeneity between species or within a population) and components that address uncertainty (i.e., lack of knowledge); (U.S. EPA, 2002b; Dourson et al., 1996; Dourson and Stara, 1983), whereas DDEFs focus on variability. Additionally, interspecies and intraspecies UFs are values based on general assumptions, whereas data-derived values are empirically determined based on chemical-specific data.

Thus, DDEF values are more precise and accurate than default UF values, but the values for the DDEF components may sometimes be similar to default values for UFs. Regardless of any similarity to default UF values, developing a DDEF quantifies variability and reduces uncertainty, carrying with it a change in nomenclature (IOM, 2013).

UNCERTAINTY AND VARIABILITY EXPLAINED

Variability refers to true heterogeneity or diversity. This may be due to differences in exposure as well as differences in response. Those inherent differences are referred to as variability. Differences among individuals in a population are referred to as interindividual variability, while differences for one individual over time are referred to as intraindividual variability. DDEF values quantify variability on the basis of chemical specific information.

Uncertainty occurs because of lack of knowledge. It is not the same as variability. Uncertainty can often be reduced by collecting more and better data, while variability is an inherent property of the population being evaluated. Variability can be better characterized with more data but cannot be eliminated. Efforts to clearly distinguish between variability and uncertainty are important for both risk assessment and risk characterization.

Source: U.S. EPA (2002b).

Evaluation of the sources and magnitude of uncertainty accompanying DDEF values is informative (U.S. EPA, 2005, 2001c, 1997a, b), and quantitative uncertainty analyses may be undertaken, but such analyses are not presented in this guidance. When quantitative approaches are not feasible, qualitative uncertainty analyses may be developed. Furthermore, as discussed in the 2005 *Cancer Guidelines*, "a default option may be invoked if needed to address uncertainty or the absence of critical information."

The use of human response data for the critical effect obviates the need for a $UF_{A.}$ The richness of a human data set may offer additional potential to develop DDEF values for interindividual variability. It may prove difficult to separate the contributions of TK and TD in these data sets without additional data and/or models.

RESPONSE DATA IN HUMANS

For some chemicals, the available data describing adverse effects in humans are suitable for dose-response analysis. When the POD is derived from studies with humans, the need for a UF_A is obviated, and the value for UF_A is set to 1.

The capability to develop a DDEF value depends on the availability and suitability of experimental data and/or predictions from reliable models (see Section 2.2.5). Once data sets are evaluated to justify their basis for a quantitative reliance, three primary sets of information are required to develop a DDEF value:

≠ Sufficient information on the MOA, such as understanding of the major steps leading from exposure to adverse outcome, including identification of the toxicologically active chemical species;

≠ Identification of the target tissues or organs; and

≠ Availability of information to determine whether an instantaneous (i.e., maximum concentration [C_{max}]) or a time-normalized (i.e., clearance [Cl] or area-under-the-curve, [AUC]) measure of exposure is the more appropriate basis for tissue response.

2.1.2. Sensitivity and Susceptibility in the Context of Data-Derived Extrapolation Factors

For the purpose of this DDEF guidance, the terms susceptibility and sensitivity are used interchangeably and defined as an increased response to a given exposure. [Note the term *susceptible* is also used to describe *sensitive* or *vulnerable* populations or life stages. These terms have varying definitions within EPA documents and are used interchangeably. No convention for use of the terms sensitivity, susceptibility, or vulnerably is widely accepted (U.S. EPA, 2004a). The term, vulnerability, is not used in this document.] Susceptibility in the human population may be due to life stage, health status or disease state, genetic disposition, exposure, or other factors. Therefore, with respect to intraspecies variability, it is important to consider the

factors that lead some individuals or groups to be more sensitive than others. Humans respond differently to chemical exposures based on several factors that can be exogenous and/or intrinsic. Exogenous factors relate to exposure conditions such as chemical concentration/external dose, media, pathway, or duration. Physiological, anatomical, and biochemical parameters are intrinsic factors that may also be the basis for differential susceptibility among the population and at different life stages. Intrinsic factors can mediate sensitivity by influencing the target tissue concentrations of the chemical inside the body (TK; see Section 3.3.1.1) or by modulating an increased responsiveness of the tissues to the toxicologically active chemical species (TD; see Section 4.3.1.1). For some chemicals, data may be sufficient to identify one or more sensitive populations or life stages.

Life stage is a key consideration in susceptibility. Developing organisms (e.g., fetus, infant) can be more sensitive for several reasons, some of which include a higher body mass-adjusted exposure and the potential for increased sensitivity of rapidly growing tissues. Critical windows of development, and therefore windows of sensitivity, occur at different times for various tissues, organs, and systems; therefore, considering susceptibility to more than one critical effect may require consideration of more than one life stage. The aged may also represent a sensitive life stage.

Toxicity (response) data from the sensitive life stage may be used directly to identify the POD. In other cases, TK or TD data may be used in derivation of DDEFs to extrapolate POD values, for example from the average adult to the sensitive life stage. Because every human being goes through developmental life stages, sensitive life stages are not a population *per se*, but sensitive life stages do need to be considered explicitly in the risk assessment when sufficient data are available. For purposes of this guidance, life stages are considered among the multiple potentially sensitive populations.

With respect to TK, sensitivity is the result of higher tissue concentrations being attained at a fixed dose. Elevated tissue concentrations may be the result of an increased distribution to tissues or a decreased elimination from tissues. Regarding measures of tissue concentrations, maximum concentration (C_{max}) and AUC are suitable measures, and sensitive individuals or sensitive populations will be those at or near the upper tail of the population distribution. With respect to measures of the removal of toxicant, measures of Cl are suitable, and sensitive individuals or sensitive populations will be at the lower tail of the population distribution. Sensitive individuals or sensitive populations will be those in which a predetermined level of response will be reached at lower tissue (or *in vitro*) concentrations. A quantification of DDEF values based on measures obtained from those deemed sensitive and those representing the generally responsive portion of the population are described later (TK in Section 3.3.2.2; TD in Section 4.3.2.2).

The development and evaluation of experimental data and models describing TK and TD are likely to lead to an improved understanding of population sensitivities and thus population variability, as well provide a means for quantitation. Understanding population sensitivity and characterization of population variability will improve the scientific basis for human health risk assessment.

2.2. DERIVING AND APPLYING DATA-DERIVED EXTRAPOLATION FACTORS

The foundation of DDEFs is the concept that the toxicity of a particular agent is due to a combination of both TK and TD factors, and that those factors can be quantified in animals and humans. For purposes of this guidance, TK is defined as the determination and quantification of the time course and dose dependency of absorption, distribution, metabolism, and excretion (ADME) of chemicals (sometimes referred to as pharmacokinetics) of the chemical agent, while TD is defined as the determination and quantification of the sequence of events at the cellular and molecular levels leading to a toxic response. TK and TD share a common point—each is concerned with the concentration of the toxicologically active chemical species in the target tissue. As such, it can be difficult to establish a clear separation between TK and TD because the processes leading to biological responses include aspects of both—including interactions between TK and TD processes.

SUBDIVIDING UF_A AND UF_H

The response to toxicants is based broadly on two functions: target tissue exposure (i.e., TK) and innate sensitivity to the insult that modulates the type and severity of the response (i.e., TD). Thus, both UF_A and UF_H have been divided into TK and TD components. This distinction was described in the RfC guidance for inhaled substances (U.S. EPA, 1994). The subdivision of UF_H has been applied in several assessments described in Appendix A. The subdivision of the UFs provides the framework for the quantitative inclusion of TK and TD data sets in inter- and intraspecies extrapolation.

Extrapolation from animals to humans and within the human population can be accomplished by one of several approaches ranging from the use of sophisticated BBDR models to the calculation of relatively simple ratios using TK or TD data describing critical factors in inter- or intraspecies extrapolation. The following text describes the approaches for calculating the different DDEF values. In the absence of data for performing sophisticated modeling or for deriving DDEF values, default approaches for toxicokinetics are used, but no such approaches for toxicodynamics are yet available.

Four DDEFs can be calculated given sufficient information. Two extrapolation factors are for interspecies extrapolation from animal data to humans (EF_A): (1) extrapolation factor covering interspecies toxicokinetics (EF_{AK}) is calculated to account for TK variability, while (2) extrapolation factor covering interspecies toxicodynamics (EF_{AD}) accounts for TD variability. Likewise, there are two extrapolation factors dealing with variability within the human population (EF_H): (1) extrapolation factor covering intraspecies toxicokinetics (EF_{HK}) for TK and

(2) extrapolation factor covering intraspecies toxicodynamics (EF_{HD}) for TD. Table 1 provides example equations for calculating these DDEFs. Section 3 describes how to calculate and when to use TK factors for interspecies (see Section 3.2) and intraspecies (see Section 3.3) extrapolation. Section 4 describes how to calculate and when to use TD factors for both interspecies (see Section 4.2) and intraspecies (see Section 4.3) extrapolations. Section 5 describes how to combine EF_{AK}, EF_{AD}, EF_{HK}, and EF_{HD} into the composite UF.

Table 1. Example equations used to derive data-derived extrapolation factors

Extrapolation	Toxicokinetics (Section 3)	Toxicodynamics (Section 4)
Animal to human (interspecies)	$EF_{AK} = \frac{D_A}{D_H}$	$EF_{AD} = \frac{Concentration_A}{Concentration_H}$
Within human (intraspecies)	$EF_{HK} = \frac{AUC_{sens}}{AUC_{gen}}$	$EF_{HD} = \frac{Concentration_{gen}}{Concentration_{sens}}$

EF_{AK} = interspecies TK extrapolation factor.
D_A = animal external dose (administered or external dose in the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD).
D_H = human external dose (administered or external dose at the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species).
EF_{AD} = interspecies TD extrapolation factor.
$Concentration_A$ = animal concentration (concentration of the agent in the tissue or *in vitro* in the test animal species corresponding to a level of response near the animal POD).
$Concentration_H$ = human concentration (concentration of the agent in the tissue or *in vitro* in the human corresponding to a level of response near the animal POD).
EF_{HK} = intraspecies TK extrapolation factor.
AUC_{gen} = general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population).
AUC_{sens} = sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population).
EF_{HD} = intraspecies TD extrapolation factor.
$Concentration_{gen}$ = general human population concentration (concentration producing the response corresponding to the POD at a measure of central tendency in the general human population).
$Concentration_{sens}$ = sensitive human population concentration (concentration producing the response corresponding to the POD at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population).

The benefit of DDEFs is that they maximize the use of available data and improve the overall scientific support for a risk assessment. Figure 2 provides a flowchart of the decision process for the extrapolation used in deriving DDEFs. As described in more detail in Sections 3 and 4, it is important for the human health hazard and/or risk characterizations to include

thorough and transparent discussions of methods and data used to support extrapolation approaches.

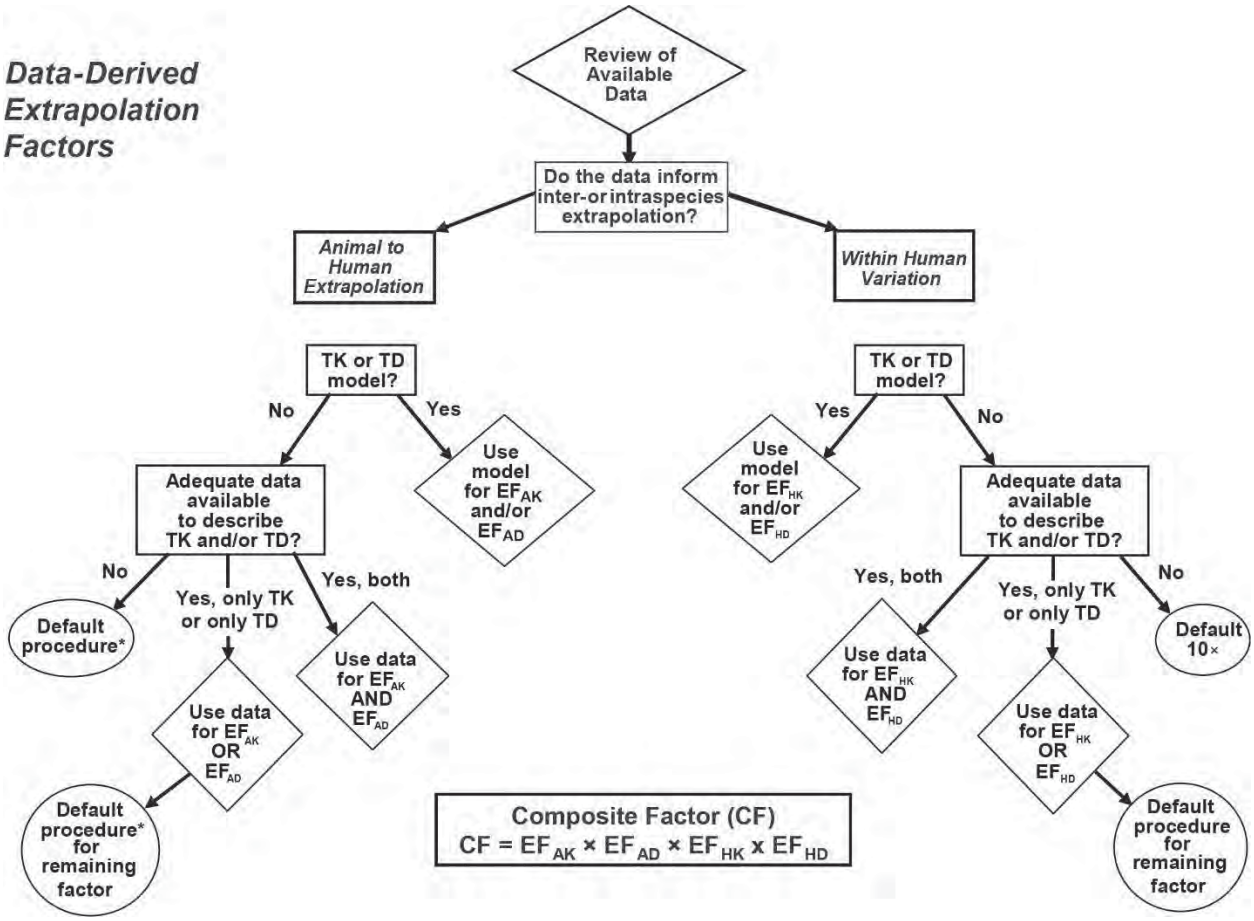

Figure 2. Decision process for data-derived extrapolation factors. The availability of an adequate TK or TD model is considered first, followed by analysis of the availability of adequate data to describe the TK and/or the TD of the chemical. With the availability of an adequate model or data, data-derived extrapolation factors for intraspecies (EF_{AK}, EF_{AD}) and interspecies extrapolation (EF_{HK}, EF_{HD}) are developed. In the absence of an adequate model or data, default factors are used.

*For interspecies extrapolation, the default procedure is ¾ body-weight scaling for RfD (U.S. EPA, 2011) and the RfC method (U.S. EPA, 1994) for inhalation to derive a human equivalent dose (HED) and human equivalent concentration (HEC), respectively. When these methods are used in deriving the RfD and RfC, the default interspecies UF is then reduced to a value of 3. The composite factor (CF) accounts for inter- and intraspecies extrapolation and can comprise default or DDEF values for the four extrapolation factor components.

2.2.1. TK and TD Models

TK and TD data and/or models represent the preferred approach to intra- and/or interspecies extrapolation. Models vary in level of complexity from classical compartmental and simple statistical response models to physiologically realistic models of TK and TD processes, up to and including BBDR models. These models provide a quantitative description of the biological processes involved in the TK and/or MOA of chemical(s). In these TK and TD models, some measure of the internal dose is related to the external dose and response, respectively. When available, BBDR models combine TK and TD modeling, using the measure of internal dose or dose metric to link the TK and TD aspects of the modeling approach (see discussion in Section 4.2.2.2).

TK modeling is the process of developing a mathematical description of ADME in a living organism. Two common types of TK models are (1) data-based noncompartmental or compartmental models and (2) PBPK models. Data-based models, also known as classical models, mathematically describe the temporal change in chemical concentration in blood, tissue, or excreta of the species for which the data were generated. The classical models often treat the body as a single homogenous or multicompartment system with elimination occurring in a specific compartment; the characteristics of the compartments (number, volume, etc.) are hypothetical in that they are chosen for the purpose of describing the data rather than based *a priori* on the physiological characteristics of the organism or the biological attributes of the response. Due to these characteristics, classical models are used for interpolation [i.e., within the range of doses, dose route, and species in which the data were generated (Renwick, 1994)].

PBPK models differ from classical compartmental models in that they are composed of compartments with realistic tissue volumes that are linked by blood flow. Other parameters used in these models account for chemical-specific characteristics that can be independently measured in both humans and laboratory animals (usually using *in vitro* techniques); these chemical-specific parameters include tissue solubility (i.e., partition coefficients), binding, and metabolism. These models are used to simulate the relationship between applied (administered) dose and internal dose at the target tissue. PBPK models require more data to develop compared to classical compartmental models, but they are advantageous because they can be used for extrapolation [i.e., across dose range, among animal species, between routes of exposure, and across exposure scenarios (U.S. EPA, 2006a; Krishnan and Andersen, 1994)].

TD models can be developed when sufficient data exist to both ascertain the MOA and to quantitatively support model parameters that represent rates and other quantities associated with key precursor events in the MOA. A BBDR model describes biological processes at the cellular and molecular levels in such a way as to link target tissue dose with adverse effect; in practice, BBDR models are often described as a combined TK/TD model. These models may be used for

extrapolation. However, with an adequate understanding of the nature of the response and sufficient empirical data describing the dose-response function in relevant species or populations, a fully developed TD model may not be required to develop a DDEF.

2.2.2. Use of Ratios to Calculate Data-Derived Extrapolation Factor

In the absence of sufficient data to develop a robust TK or TD model, the risk assessor need not necessarily use default approaches and UFs. DDEFs can be calculated as ratios using data from key studies evaluating TK or TD profiles or properties of a particular chemical. Some example equations for calculating DDEFs were provided in Table 1 and are described in more detail in Sections 3 (TK) and 4 (TD).

TARGET TISSUE CONCENTRATIONS IN DDEF CALCULATIONS

DDEF values are based on an understanding of target tissue concentrations, rather than relying solely on external concentrations or effective doses. For TK (see Section 3), interspecies differences are calculated as differences in external (administered) dose resulting in the same target tissue concentration, and intraspecies differences are calculated as differences in internal concentrations resulting from the same external dose or exposure. TD differences (see Section 4) are calculated as differences in target tissue concentrations resulting in the same response level.

In general, *interspecies* extrapolation involves calculating a ratio of animal data (for a kinetic or dynamic parameter) to human data for a kinetic or dynamic parameter. Similarly, for *intraspecies* extrapolation from the general (average) human population to the sensitive population, a ratio is calculated using data from the sensitive population and that for the central tendency of the general population. Data to derive the TK factors may come from *in vivo* or *in vitro* studies. For TD, in general, interspecies extrapolation may have its basis in data from *in vivo* studies but may often be accomplished with *in vitro* data in a relevant tissue. When adequate data on toxic effects are available in humans, these data may be considered when identifying a POD, eliminating the need for the interspecies extrapolation. Otherwise, the human information can be used to inform an interspecies factor when the POD is derived from animals, allowing a quantitation of UF_A components, rather than relying on default values.

SENSITIVITY

In the absence of data to the contrary, it is assumed that humans will exhibit response in the same tissues as in test species. When humans are more toxicodynamically sensitive than animals, humans will demonstrate the same level of response, but at lower tissue concentrations. If the same dose results in higher observed or predicted AUC or C_{max} values, or lower clearance values in humans than in animals, then humans are more (toxicokinetically) sensitive and EF_{AK} will be greater than 1.

Intraspecies susceptibility may be based on differences in TK or TD. Sensitive populations will demonstrate higher tissue concentrations at the same dose (TK), or the same type and level of response at lower tissue concentrations (TD).

For interspecies extrapolation, it is preferred that the ratio be based on data at or near the POD. When sufficient data are available, interspecies DDEF values should be calculated for a

range of doses near the POD because the shape of the dose-response curve can vary among species. Metabolism and kinetic properties can vary across doses, particularly in the higher dose ranges; thus, developing multiple estimates of DDEF values at or near the POD helps avoid potential uncertainty in the DDEF estimate that may be introduced by nonlinearity in kinetic properties. Moreover, evaluating a range of PODs takes into account the dependence of the DDEFs on the POD selected. One way to address this is to calculate interspecies DDEF values at multiple doses in the range of the POD to demonstrate the stability of the DDEF value. Likewise, when the POD is expressed with a confidence bound, some effort can be taken to include this range of POD values when developing DDEF values. The interspecies DDEF values should be derived using an estimate of central tendency, such as the mean, median, or mode, depending on the characteristics of the data. In contrast, when calculating intraspecies DDEF values, the ratio includes a measure of central tendency of the general population and percentiles of the distribution representing those potentially sensitive (see Section 2.1.2). As the needs of risk managers and decision makers vary, it is recommended that the risk assessor consult with the risk manager or decision maker to determine the risk assessment objectives (U.S. EPA, 2014). A range of percentiles may be useful and thus evaluated and their corresponding DDEFs be reported in the human health hazard and risk characterizations.

DATA FROM SENSITIVE POPULATIONS

The POD may be determined in test animals, in the general human population, or in susceptible human populations. For some well-studied chemicals (e.g., nitrate, fluoride), dose-response data from the sensitive human population may be available. When these data are sufficient to identify a POD in the sensitive population, the issue of human variability has been addressed and the need to apply an intraspecies uncertainty factor (UF_H) is obviated—UF_H is set to a value of 1.

AREA UNDER THE CURVE, CLEARANCE, AND HALF LIFE

Area under the curve (AUC = (μg/ml) × hr) is related to total dose; clearance (Cl = ml/min per kg body weight) is independent of dose and inversely related to AUC. Half-life is not an acceptable basis for DDEF calculation because it is related to neither body weight nor volume of distribution. When clearance decreases, AUC values increase; when clearance increases, AUC values decrease. When a chemical does not induce or inhibit its own metabolism or clearance, AUC or clearance values after a single dose, when extrapolated to infinity, may prove a suitable alternative basis for DDEF calculation.

TK ratios (for either interspecies or intraspecies extrapolation) are informed by the MOA and are based on the relevant dose metric, such as AUC and the C_{max}.[5] Other metrics (e.g., AUC above a threshold) may be used if supported by the data or if relevant for a particular chemical or MOA. For toxicants that bind covalently or cause irreversible damage, especially as a consequence of subchronic or chronic exposure, an integrated measure

[5]Clearance can be used to calculate this ratio when it can be assumed or demonstrated that the relevant dose metric is the AUC or concentration at steady state.

of dose over time such as AUC is preferable (O'Flaherty, 1989). In the case of effects occurring as a consequence of acute exposure or when toxicity is related to exceeding an internal concentration threshold, C_{max} may be more appropriate (Barton, 2005; Boyes et al., 2005). When data on chemical-specific AUC, C_{max}, or Cl are not available, a chemical-related physiological parameter (e.g., renal glomerular filtration rate) that is critical to the onset of toxicity or to the MOA may be used.

2.2.3. Default Methods for the Derivation of Reference Concentrations, Reference Doses, and Other Relevant Metrics

In accordance with the hierarchy of approaches, when available agent-specific data are supportive of DDEF derivation from use of models or from ratios, a data-derived approach is preferred over using the default RfC approach or ¾ body-weight scaling. When deriving reference values (or counterpart values) from an animal POD in the absence of applicable TK and/or TD data in animals and humans, a default uncertainty factor value is applied unless it can be concluded that the test species is equally or more susceptible than humans (U.S. EPA (2002b).

The default approach for the inhalation exposure route (i.e., RfC) involves applying both a categorical dosimetric adjustment factor to account for species differences in tissue exposure (i.e., TK) and a residual UF of a value of one-half order of magnitude, that is generally described as covering TD (U.S. EPA, 1994). The dosimetric adjustments are based on the following:

- ≠ Anatomical and physiological differences between species
- ≠ Physical differences between particles and gases
- ≠ Whether the toxic effect(s) are portal-of-entry or systemic in nature

For the oral exposure route, the default approach for interspecies extrapolation involves scaling the applied dose, according to body weight to the ¾ power ($BW^{3/4}$), and applying a UF of one-half order of magnitude to account for residual uncertainty (U.S. EPA, 2011).

After default adjustment between species, the residual UF associated with either route (oral or inhalation) has a default value of one-half order of magnitude, which may be modified based on available data (U.S. EPA, 2011, 1994).

2.2.4. Qualitative Considerations

Although in some cases data may be insufficient for a quantitative estimate of a DDEF, there may still be information to support a UF different from the default. For example, there may be qualitative evidence based on an MOA that humans are less sensitive than animals or that

certain groups are more sensitive than the central tendency of the general population. In these cases, where only qualitative data are available, a thorough weight-of-evidence analysis can be considered with the hazard characterization to discuss the derivation of the DDEF along with associated uncertainties in the available database.

2.2.5. Information Quality

Before conducting a DDEF analysis, it is recommended that the risk assessor perform a critical evaluation of all data that may be used to support the development of DDEFs. As an important step in the process, it is advised that data providing qualitative support for the MOA and choice of dose metric, as well as data used in the quantitative derivation of the DDEF itself, be examined. Documentation of the types of literature and data evaluated and a summary of the strengths and weaknesses of data sets should be provided. This will instill confidence in the selection of data chosen as the basis for DDEF derivation, as well as provide an increased understanding of the rationale for any dismissed data. Supporting studies can be evaluated using EPA guidance documents, including the 2005 *Cancer Guidelines*, as well as earlier guidelines specific to neurotoxic, reproductive, and developmental endpoints (U.S. EPA, 2005, 1998, 1996, 1991). In addition, general principles outlined in the EPA information quality guidelines are consulted when critically evaluating data used to support the development and application of DDEF values (U.S. EPA, 2002a).

Use of secondary data sources is one area for particular consideration. Examples of secondary data sources include compilations of pharmacokinetic parameters (e.g., Brown et al., 1997) and studies cited and summarized in toxicity profiles and review articles. In general, for principal and supporting studies used directly in the derivation of DDEF values, a review of the original literature is recommended. In the case of critical assumptions and data, contradictory results from different studies are best resolved by reviewing the original publications.

Quantitative TK and TD data used in the DDEF-derivation process require particular attention to the appropriateness of the study design, the analytical methodology used, and the statistical analysis of the data. Consideration of appropriate study design extends beyond simply verifying that the methods used were adequate for the goals of the study; it also encompasses consideration of the relevancy of the animal or *in vitro* test system used to derive the DDEF for the endpoint of concern. Relevance can be assessed in both qualitative and quantitative terms. For example, if there is a lack of concordance (i.e., a particular TK or TD process relevant to the endpoint does not occur in the test system), or if physiologically unrealistic conditions are used, or different tissue or cell types are evaluated, then the relevancy of the data may be uncertain. Particular considerations relevant to the use of *in vitro* data are discussed below. Another important factor in terms of relevancy is to consider whether the TK or TD response represents a

uniquely sensitive tissue, process, or population. This decision is a critical determinant in evaluating the use of data to describe intraspecies variability.

2.3. MODE OF ACTION

Information on MOA is important in DDEF derivation, even when a complete understanding of the mechanism is not available. DDEFs for both TK and TD are endpoint driven—that is, they are considered in the context of the toxic endpoints most relevant for purposes of the risk assessment. Understanding the MOA(s) for the agent(s) of interest ensures that the TK or TD parameter used to derive the DDEF will be causally related to the adverse outcome of interest. The key events in MOA are likely to identify important metabolite(s) and can aid in identifying potential life-stage susceptibility, sensitive population groups, and/or species differences. Moreover, data on key events may be used directly to estimate EF_{AK} or EF_{AD}.

In the 2005 *Cancer Guidelines*, EPA describes the MOA evaluation as the critical information that defines the conditions under which a toxicant causes its effect, the relevance of animal data for hazard identification, and the most appropriate approach to low-dose extrapolation. The 2005 *Cancer Guidelines* also presents a framework for evaluating data in support of an MOA determination. Major components of this framework include a description of the hypothesized MOA and a discussion of the experimental support for the hypothesized MOA based on modified Hill criteria (U.S. EPA, 2005) for demonstrating associations in human studies.

The MOA is defined as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through functional and anatomical changes, and resulting in toxicity. A key event is an empirically observable precursor step that is itself a necessary element of the MOA or is a biologically based marker for such an element. MOA is contrasted with "mechanism of action," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by MOA (U.S. EPA, 2005). As a result of the 2007 NRC report on *Toxicity Testing in the 21st Century* (NRC, 2007), the concept of the adverse outcome pathway (AOP) has been introduced (Ankley et al., 2010). An AOP links a molecular initiating event, or mechanism of action, to progressive levels of biological organization at the individual or population level. As such, this framework is conceptually similar to, but in some cases may be more comprehensive than, MOA and would be particularly useful for derivation of DDEFs.

2.4. USE OF *IN VITRO* DATA

In vitro assays play an important role in defining DDEFs; however, care must be taken to avoid taking isolated findings out of context. Consideration of interspecies differences in ADME is essential because the dose to the target tissue in any given exposure scenario is a balance among multiple and competing ADME processes. Thus, it is recommended that *in vitro* data not be used for quantitative purposes unless interpreted in the context of the intact system. Among the questions to be considered when applying *in vitro* data to DDEFs are the following:

- ≠ Was the toxicologically active form of the agent studied?
- ≠ How directly was the measured response linked to the adverse effect?
- ≠ Are the biological samples used in the assays derived from equivalent organs, tissues, cell types, age, stage of development, and sex of the animals/humans in which the target organ toxicity was identified?
- ≠ What is the range of variability (e.g., diverse human populations and life stages) that the biological materials cover?[6]
- ≠ If the effect occurs or can be measured in several tissues, is the studied tissue or tissue preparation an appropriate surrogate? Or, in situations where the effect is not localized, is the effect consistent across tissues?
- ≠ Does the design of the study allow for statistically valid comparisons based on such factors as replicate and sample size?
- ≠ Was chemical uptake considered when the chemical was applied to the samples so as to give comparable intracellular concentrations across tissues?
- ≠ Were similar tissues or samples evaluated across species?
- ≠ Do the concentrations in the *in vitro* studies allow for comparison with *in vivo* conditions?

All of these issues affect the utility of applying *in vitro* data for risk assessment. A clear discussion of these points helps clarify the appropriateness of the information used for deriving DDEFs.

[6]Quality (purity, viability, donor demographics) of the samples is of particular concern with biological materials derived from human organ donors.

2.5. MULTIPLE POTENTIAL CRITICAL EFFECTS

For some toxicants, multiple adverse effects may be identified during hazard identification; these may occur at similar doses or exposures and may be the result of a common similar, a dissimilar, or an unknown MOA. It is also possible that the uncertainty and/or variability associated with the TK and/or TD of each of the several adverse effects may differ, resulting in different DDEFs or the retention of default values for UFs, which may lead to differences in dose extrapolation and different reference values. One explanation is that risk assessors may be more certain about inter- and intraspecies differences for one effect versus another. For that reason, the results generated for the multiple responding tissues/organs can be presented for comparison (e.g., in a table that is accompanied by a discussion of the methods used), particularly if multiple MOAs are operational or unknown. It is important not to mix DDEFs derived for one tissue or one MOA with DDEFs (or default UFs) derived from a different tissue unless they can be justified on the basis of the biology of the insult. For example, DDEF values for kidney effects may not apply to liver effects due to innate differences in physiology and biochemistry of the tissues.

DDEF VALUES AND CRITICAL EFFECTS

The PODs for multiple potential critical effects should be combined with their respective default UF or DDEF values to produce an array of potential reference values.

3. DATA-DERIVED EXTRAPOLATION FACTORS BASED ON TOXICOKINETICS

3.1. GENERAL CONSIDERATIONS

TK is concerned with the ADME of chemicals, with an emphasis on the exposure of the biologically active chemical species to the target tissue of interest. Data on tissue concentrations of toxicants or clearance rates of toxicant removal serve as the basis for deriving DDEF values for TK components. This section provides a discussion of factors common to the derivation of both inter- and intraspecies values to account for TK variability. Given the UF_A and intraspecies uncertainty factor (UF_H) framework for uncertainty and extrapolation, there are three generally identifiable points bounding inter- and intraspecies extrapolation: (1) the animal model, (2) the general human population, and (3) sensitive populations or life stages. When a sensitive population(s) or life stage(s) has been identified, and when TK data in animals and the sensitive population(s) are available, these data may be employed to develop a DDEF value for TK that combines both inter- and intraspecies extrapolation. In this case, the DDEF value represents both EF_{AK} and EF_{HK}. Since this situation is not common, this section addresses inter- and intraspecies extrapolations separately.

Data on the quantitative TK differences between animals and humans are used for EF_{AK}. TK differences among the human population are used for the EF_{HK}. Thus, the factor EF_{AK} accounts for extrapolation from laboratory animals to the general human population. EF_{HK} accounts for the variation due to TK in the exposure associated with the critical effect between the human population group represented by the dose-response assessment and sensitive human individuals or populations. Developing a DDEF for TK requires knowledge about the relationship between external dose and internal (target tissue) concentrations. This information can come from studies in which tissue concentrations are measured or predicted, in which both types of data are recorded, or from adequate TK models, which expand the range of confidence from that of the empirical observations. TK models, especially PBPK models, represent an important tool through which *in vitro* observations can be interpreted in the context of the intact system. As such, they represent an advantageous means to evaluate the impact of studies (especially those using human tissues) conducted *in vitro*.

The TK portion of each factor (EF_{AK}, EF_{HK}) is combined with the corresponding TD factors to assemble the composite UF (see Section 5). When the data are not sufficient to derive a DDEF for TK, other approaches can be considered for EF_{AK} or EF_{HK}. For example, the RfC approach (U.S. EPA, 1994) describes default procedures for interspecies extrapolation for inhaled substances. Some important questions to address for TK include:

- What is/are the critical effect(s) and POD being used for this assessment?
- Has the toxicologically active chemical moiety been identified?
- What is the MOA, AOP, or mechanism for that toxicity? Have the key events been identified and quantified? Do these key events identify important metabolic steps?
- Are the processes of ADME of the chemical well characterized? If dose-response data are from an animal model, do animals and humans metabolize the chemical(s) in a similar way (qualitatively and quantitatively)?
- Are there data in human populations describing variation in important kinetic parameter values for this chemical(s)? Have sensitive populations and/or life stages been identified? Are the data for these sensitive populations adequate for quantitative analyses?

TK data may be developed empirically or through compartmental or physiologically based TK models. It is recommended that these data, models, and approaches be evaluated for their appropriateness (IPCS, 2010; U.S. EPA, 2006a). For each critical effect identified for a particular agent, separate DDEF analyses are conducted for EF_{AK} and EF_{HK}. As such, data for multiple sensitive tissues/endpoints can be evaluated, concentrating on those effects that demonstrate response levels near the POD for the critical effect.

3.1.1. Dose Metric

Dose metric is a measure of the internal dose of a chemical agent. A dose metric associated with the health outcome of interest is most useful when it describes target tissue exposure in terms of the toxic chemical moiety (parent or metabolite) and is expressed in appropriate time-normalized terms. The choice of the dose metric is an important component in TK extrapolations. This choice depends on whether toxicity is best ascribed to a transient tissue exposure or a cumulative dose to the target tissue. For a given chemical, the appropriate dose metric will also be determined by, and can vary with, the MOA, duration of exposure, and the adverse effect of concern (U.S. EPA, 2006a). Selection of an appropriate dose metric based on specific endpoints involves several elements including:

DOSE METRIC

Dose metric is a measure of the tissue concentration of the toxicologically active chemical species that reflects a time-normalized (i.e., AUC) or instantaneous (i.e., C_{max}) measure of concentration. Dose metric values may also include measures of chemical flux or clearance. In some cases, dose metrics may be expressed in direct physiological units like glomerular filtration. (See the boron and compounds case study in Appendix A to this document.)

≠ Duration of exposure and effect;

≠ Identification of the active chemical moiety;

≠ Selection of the organ or tissue group in which some measure of internal dose is desired; and

≠ Selection of the measure of exposure that best correlates with toxicity.

SELECTION OF THE APPROPRIATE DOSE METRIC

Dose metrics will differ with respect to the toxicological response of interest. While clearance values for some agents may be used to describe internal exposures, clearance may not be the dose metric most closely associated with the toxicological response of interest. Rather, the toxicological response may be mediated by the interaction of the toxicologically active chemical form with the receptors in the target tissue of interest, better represented by C_{max} or AUC values.

For example, the acute central nervous system effects of halogenated solvents may relate to C_{max} values for the parent compound in the brain, while chronically observed nephrotoxicity may best relate to averaged tissue concentrations of a metabolite or the rate at which metabolites are formed.

Whether an adverse effect is a consequence of an acute or chronic exposure impacts the choice of dose metric. For acute, reversible effects (e.g., sensory irritation, narcosis), a measure of instantaneous or peak tissue exposure such as C_{max} may be the most appropriate dose metric (Boyes et al., 2005; Alarie, 1973). For chronic effects, in the absence of MOA information to the contrary, it is generally assumed that some integrated cumulative measure of tissue exposure to the active toxicant is the most appropriate dose metric (e.g., AUC). Alternative choices, such as amount of chemical or rate of metabolite production, can be used as appropriate for a particular agent or MOA (U.S. EPA, 2006a). For example, there may be a case where a temporally large influx of active chemical to a target site in a relatively short period of time (peak exposure) is observed. In this case, a less commonly used metric, such as time above a critical concentration, may be more appropriate. It is recommended that the assessor provide the data and rationale in support of a particular dose metric.

Clearance, while not often considered a dose metric, can be used in DDEF derivation. Clearance is mathematically inversely related to AUC (i.e., AUC = dose/clearance); thus, differences in clearance values can be used in the calculation of ratios. When metabolism represents the primary or sole clearance mechanism, either of two clearance models may be applicable. The first, intrinsic clearance (Cl_{int}), has been used for interspecies scaling of administered doses in drug development (Houston and Carlile, 1997) and is applicable at doses that do not result in metabolic saturation. Cl_{int} is calculated as a ratio of the theoretical maximal initial velocity of the reaction to the Michaelis constant (V_{max}/K_m) and is in units of volume of the substrate cleared per unit time, where K_m is the substrate concentration driving the reaction rate at one-half V_{max}. The Cl_{int} can be extrapolated to the whole body with knowledge of protein binding and the recovery of the protein or cellular or subcellular fraction used in the *in vitro* investigations (Carlile et al., 1997).

INTRINSIC CLEARANCE

Cl_{int} is often calculated for therapeutics (as V_{max}/K_m). While it is a valuable measurement for purposes of comparing agents, it is not suitable for derivation of DDEFs. Measures of intrinsic clearance do not take into account the constraints of the intact system (e.g., partitioning into tissues, blood flow), which can limit metabolic clearance.

The second clearance model is hepatic clearance (Cl_{hep}), which is also based on V_{max}/K_m measurements but includes a substrate delivery term whose value is governed by hepatic blood flow. These measures of clearance differ in that Cl_{int} is not bounded by hepatic blood flow, but Cl_{hep} cannot exceed hepatic blood flow. While metabolic rate constants (V_{max} and K_m) derived from *in vitro* data can also be scaled up and incorporated into PBPK models, the use of the hepatic clearance model is a simpler approach when an appropriate PBPK model is unavailable. Classical, compartmental TK analyses and measures of clearance of the parent compound are best suited for conditions where metabolism represents a detoxication process, when substrate concentration is less than the K_m value, and when metabolism represents the major clearance mechanism.

Whether toxicity is attributable to a parent chemical, a metabolite, or some combination of metabolites is a critical consideration. The active chemical moiety can be identified through studies in which the toxicities induced by the parent chemical and metabolite(s) are compared or from the results of studies using enzyme inhibitors and/or inducers. *In vitro* studies can also be quite useful in this regard under appropriate conditions (see Sections 2.4 and 3.1.3). Quantifying differences in dosimetry can be difficult when metabolic pathways become complex (e.g., where competition among pathways may be concentration dependent). If the metabolic pathway bifurcates and the identity of the bioactive metabolite(s) is unknown or unquantifiable, determination of the appropriate dose metric can be highly uncertain.

The target organ or tissue group is the preferred site in which estimates of internal dose (tissue concentration) are generated. In practice, this information may be unavailable in the absence of an appropriate PBPK model. It may be necessary to use absorbed dose of the parent chemical as a surrogate measure of internal dose. Another surrogate dose metric is the measured concentration of the parent chemical or active metabolite in circulating blood if the relationship between target tissue concentration and blood concentration is known or can be reliably inferred from experimental data. Some data have demonstrated that blood:air partition coefficient values may vary appreciably among species but that tissue:air (e.g., liver:air) partition coefficients are similar among mammalian species (Thomas, 1975). It seems reasonable to use the cross-species similarity as the primary determinant of diffusion from blood into tissues as a justification to rely on concentrations of the toxicant in blood as a surrogate for tissue concentrations. However, when local tissue bioactivation may determine the toxic response, special care should be used when developing DDEF values on the basis of blood concentrations. Those issues

notwithstanding, measurements of internal dose in circulating blood (IPCS, 2005) may be used as the basis for DDEF derivation under either of the following conditions:

≠ When evaluating interspecies differences, the distribution from blood to target (critical) tissues is shown to be or can be assumed to be the same between animals and humans.

≠ When evaluating intraspecies differences, the distribution from blood to sensitive (critical) tissues is shown to be or can be assumed to be the same in segments of the human population representing those generally responsive and potentially sensitive human populations.

Confidence in model predictions is enhanced when predictions can be compared directly to observed data. However, few human data sets exist that describe concentrations of toxicants in solid tissues. Blood, however, is much more readily obtained, and so the ability to compare predictions of blood to observations is more readily accomplished. This situation results in a higher level of confidence in modeled blood concentrations compared to solid tissue concentrations. Since the partitioning of the active chemical from blood into systemic target tissues may be governed more by physicochemical properties than by biological processes, communication of the understanding of these processes will increase confidence in predictions of solid tissue concentrations. This difference in confidence in predictions may be considered another basis for relying on data describing the concentration and variability of the biologically active metabolite in the central compartment.[7] For example, the ratio of blood lipid to tissue lipid concentrations may be a key determinant in the diffusion of lipophilic compounds out of blood; however, differences in tissue lipid composition between species may be fairly small compared to differences in blood flow and metabolic activity.

3.1.2. Dose Selection

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (inhaled concentration or orally ingested dose) is important. In some cases, there may be nonlinearities between the external dose and the dose metric. That is, increasing or decreasing doses may not produce proportional increases or decreases in the dose metric. In this instance, the dose selected for the DDEF derivation will impact the magnitude of EF_{AK} or EF_{HK}. Using a dose at or near the POD alleviates some concerns regarding nonlinearities in

[7]The central compartment is defined as blood, plasma, or serum in the systemic circulation. All tissues except those representing the portal of entry are defined as peripheral compartments.

metabolism. This situation is especially true for interspecies extrapolation, where the basis for DDEF calculation is the dose metric. The human equivalent concentration (HEC) or human equivalent dose (HED) is defined as the human exposure producing the same level of the dose metric as attained in the animal at the POD. Alternatively, data that show a linear relationship between external dose and internal dose metrics will lessen this dependence of dose on the estimation of EF_{AK} or EF_{HK}; thus, doses that may be higher or lower than the POD for the critical effect can be used in the calculation.

3.1.3. *In Vitro* Data

Due to ethical and practical constraints, some studies with humans are not possible—especially with chemicals already known to be toxic. *In vitro* study designs offer excellent opportunities to assess the toxicity of an agent, especially when the need to isolate TK from TD is a concern. However, it is important when deriving EF_{AK} and EF_{HK} to consider interspecies differences in ADME. *In vitro* data can be used for quantitative purposes only when interpreted in the context of the intact system, as discussed in Section 2.4. Care must be taken to avoid taking isolated findings out of context.

IN VITRO CAVEATS

When investigating toxicodynamic events, *in vitro* experiments offer the opportunity to control for TK influences; concentrations of the toxicant can be well controlled. Results of *in vitro* toxicokinetic/metabolism studies should be used only when interpreted in the context of the intact system. Samples used *in vitro* should closely represent the species/population of interest, and the measured response should be one well associated with the critical effect.

3.2. INTERSPECIES TOXICOKINETIC EXTRAPOLATION

This section provides a discussion of the quantitative differences in the TK between animals and humans that are used to compute EF_{AK}. In this process, TK differences between species are characterized as the ratio of applied (administered) doses in the test species and in humans (if human data or models are available) that result in the same level of the internal dose metric (see Figure 3). Values for the dose metric may be calculated from the external doses actually used in the dose-response evaluations, or by normalizing the dose metric to account for administered dose (e.g., correcting AUC for each species by dividing the AUC by the external dose) when the relationship between the values for the dose metric and the applied dose are linear in the range of extrapolation. Predictions of dose metrics from verified TK models are often acceptable. Illustrative case studies are included in Appendix A.

3.2.1. Considerations for Interspecies Toxicokinetic Extrapolation Factor

TK differences between animals and humans are evaluated for the selected critical effect and for effects arising near the POD for the presumed critical effect. This analysis includes

consideration of MOA, identification of the active chemical agent for this particular effect, and determination of the appropriate dose metric. Selection of the dose metric is based on a weight-of-evidence approach emphasizing both qualitative and quantitative evidence. An important part of this process is evaluating concordance of metabolic processes between the animal model and humans. An additional consideration is whether the kinetic data are from a "typical" or average adult animal as opposed to an animal model system that may be unusually sensitive for a particular effect (e.g., metabolic knockout).

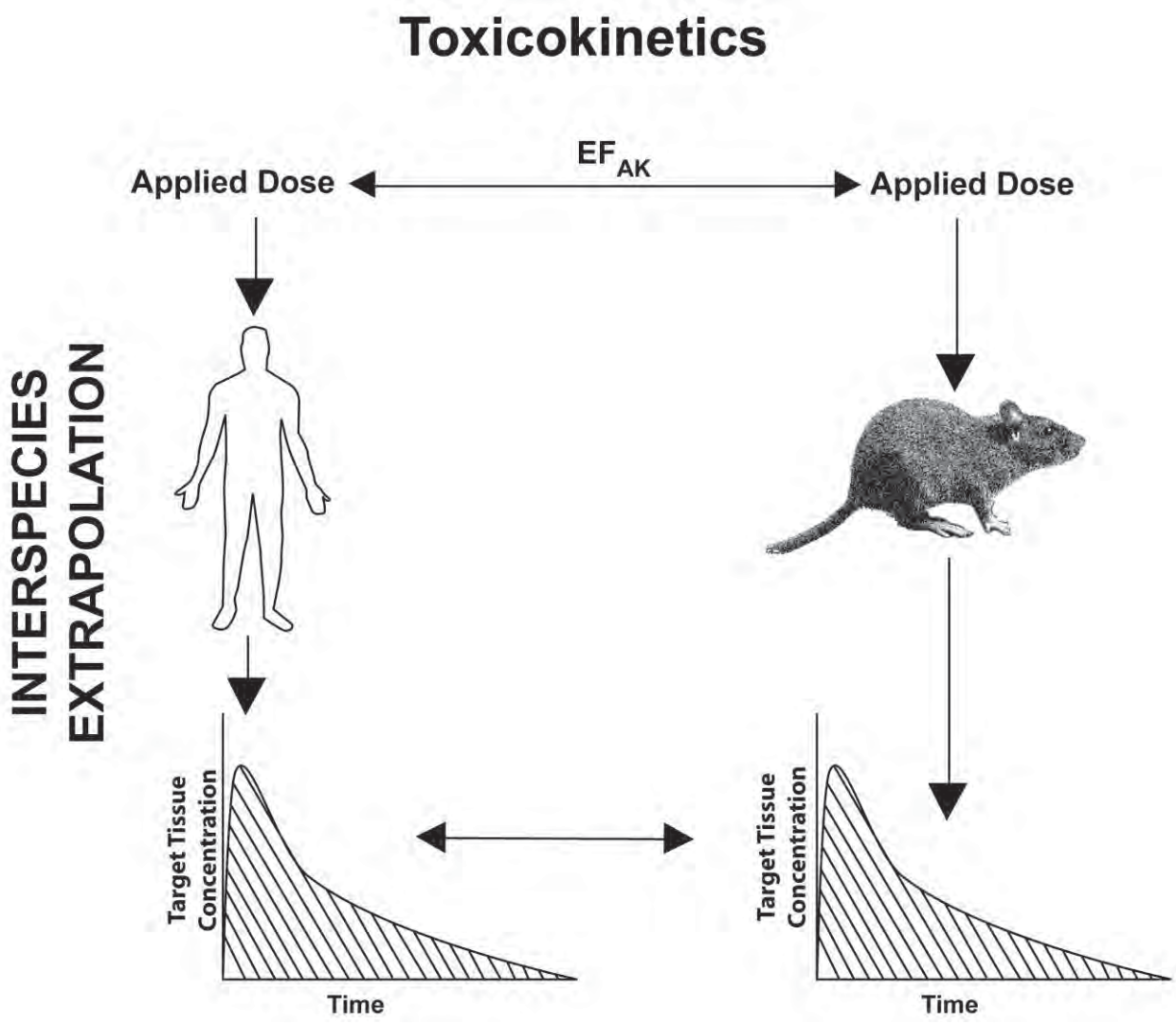

Figure 3. Interspecies toxicokinetics. In keeping with the principles established in earlier Agency guidance, which addressed inhaled toxicants (U.S. EPA, 1994), interspecies differences in TK are defined as differences in the external dose producing the same level of the dose metric in the target tissue of interest in test animals.

When animal data come from a group or from individuals expressing a condition known to be useful in identifying a sensitive human population or life stage, and when the corresponding population group in humans is determined to be the sensitive population, the extrapolation can be conducted between the sensitive animal and sensitive human. In this case, these data may cover both inter- and intraspecies extrapolation, and so represent both EF_{AK} and $EF_{HK,}$ respectively. However, it is recommended that a full weight-of-evidence evaluation be conducted. Using this example, if there are no data in the developing human, but data are available in the adult human, then the data-derived interspecies extrapolation would be from the more sensitive animal (e.g., sensitive life stage) to the general human population; intraspecies extrapolation would require default assumptions.

Furthermore, it is important to assess the relationship of externally applied dose to internal dose metric over the entire range of dose levels used in the critical study. Careful attention should be paid both to measures of central tendency and to variability, particularly in the range of concentrations or doses close to the point of inflection (where the shape or slope of the dose-response curve changes) because of potential nonlinearities in metabolism.

3.2.2. Computation

For interspecies TK extrapolation, the goal is to determine differences in dosimetry between animals and humans. For interspecies extrapolation, toxicokinetically equivalent exposures are determined by fixing the internal dose (level of the dose metric at or near the POD) and determining the ratio of external (applied) dose that results in the same level of the dose metric in animals and humans. This approach is consistent with that in the RfC guidance for inhaled toxicants (U.S. EPA, 1994).

3.2.2.1. *Use of Toxicokinetic Models*

A PBPK (or other TK) model provides the most biologically appropriate approach for evaluating interspecies TK extrapolation. The model is subjected to evaluation as previously described (IPCS, 2010; U.S. EPA, 2006a). The model can be used in different ways, depending on the model and the circumstances. In some cases, the TK model may be used directly to perform interspecies extrapolation (i.e., to derive a human equivalent concentration or dose that includes TK considerations). The use of a PBPK model would obviate the need for EF_{AK}. In other cases, the TK model may be used to derive EF_{AK}.

3.2.2.2. *Use of Ratios*

When AUC or concentration at steady state is the relevant dose metric, and if animal and human data or TK models are available, EF_{AK} is derived using a ratio of external or applied

doses producing the same AUC value. This is accomplished by identifying doses associated with the AUC value produced in animals at or near the animal POD (AUC_A) (see eq 1 and Figure 4). The human dose that produces the same AUC_A value is the toxicokinetically equivalent dose. In these cases, differences between the animal and the human dose producing the same AUC value in each species define the EF_{AK}.

$$AUC = Dose \div Clearance \quad (1)$$

$$AUC_A = D_A \div Cl_A = D_H \div Cl_H$$

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

Cl_A = animal clearance value

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

ClH = human clearance value

Using these data, EF_{AK} is calculated according to eq 2.

$$EF_{AK} = \frac{D_A}{D_H} \text{ OR } \frac{Cl_A}{Cl_H} \quad (2)$$

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

ClA = animal clearance value

Cl_H = human clearance value

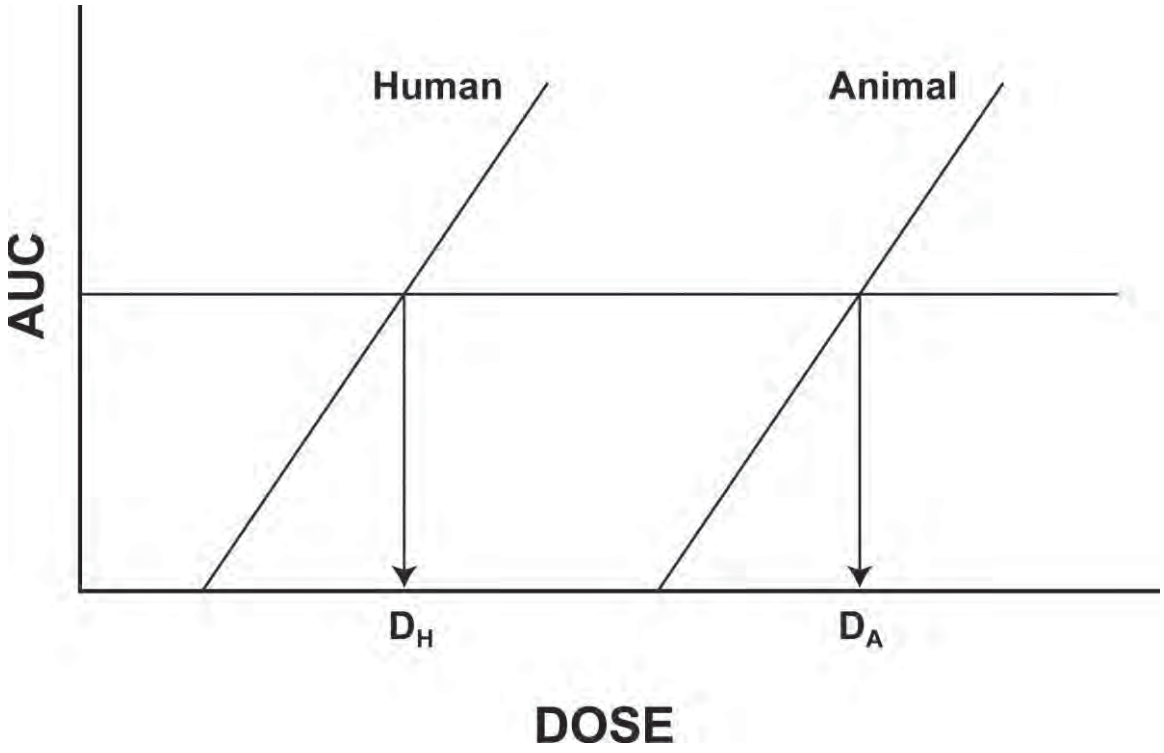

Figure 4. The conceptual relationship between dose and dose metric in animals and humans. Here, AUC is the appropriate dose metric, and the relationship between dose and AUC is determined in animals and in humans. This method can be used to develop a human equivalent dose or concentration, or in the calculation of EF_{AK}. Calculation of EF_{AK} requires knowledge of applied D_A and D_H that produce the AUC value determined in animals at the point of departure (i.e., AUC_A).

Using AUC as an example, the value for the dose metric would be AUC_A, which is the AUC value determined in animals at the POD. Thus, in this example, eq 2 can be conceptualized as

$$EF_{AK} = \frac{D_A \text{ producing } AUC_A}{D_H \text{ producing } AUC_A} \quad (3)$$

where,

D_A = animal external dose (administered or external dose to the test animal species that leads to a level of a toxicologically relevant dose metric at or near the POD)

AUC_A = area-under-the-curve value produced in animals at or near the animal POD

D_H = human external dose (administered or external dose to the central tendency in the general human population that leads to the same level of the same dose metric identified in the test animal species)

This is graphically presented in Figure 4.

Because clearance values are the mathematical reciprocal of internal dose (i.e., AUC), they may also be used to calculate a DDEF value, with the human clearance value in the denominator. Calculations using C_{max} are developed in a manner similar to that for AUC. When the dose is lower in humans than animals at the same AUC or C_{max} value, the developed DDEF will be greater than 1, demonstrating that humans are more sensitive than animals.

3.2.3. Relationship to Other EPA Guidance

The development and use of data for model predictions for tissue dosimetry to serve as the basis for quantitative, interspecies extrapolation via DDEFs is consistent with existing EPA policy (U.S. EPA, 2006a, 2002b). EPA's inhalation RfC methodology presents a continuum of approaches from rudimentary knowledge to biologically based dose-response models (U.S. EPA, 2012, 1994). The RfC methodology describes default approaches for dosimetric adjustment of animal exposure concentrations based on categorical descriptions of target tissue and target tissue concentrations in test species and humans. The first is for reactive (Category 1) gases and inhaled particles that damage portal-of-entry (respiratory tract) tissues, and the second is for gases that are absorbed and produce toxicity in tissues bathed by circulating blood (Category 3 gases). For Category 1 gases, toxicity information identifies the affected region of the respiratory tract, and species differences in the regional respiratory tract surface area and airflow (respiratory rate) serve as the basis to quantify species differences in dosimetry. For Category 3 gases, species differences in the solubility of the compound in blood (the blood:air partition coefficient) serve as the basis upon which to quantify species differences in dosimetry. The default interspecies extrapolation approach for deriving an oral RfD is dose scaling by the ratio of species' body weights raised to the ¾ power (human BW:animal BW)$^{3/4}$ (U.S. EPA, 2011). These guidance documents indicate that their approaches are default dosimetric adjustments, to be superseded when more detailed information on tissue dosimetry can be developed. The

subject of this DDEF guidance is the development and interpretation of quantitative TK data for the purpose of developing nondefault values for inter- and intraspecies uncertainty/extrapolation.

3.2.4. Conclusions for Interspecies Toxicokinetic Extrapolation Factor

Mathematically, EF_{AK} is the ratio of the external animal dose (at or near the POD) to the external human dose expected to result in the same level of the dose metric. This situation is mathematically analogous to developing the HEC or the HED.[8] If possible, EF_{AK} values should be calculated for multiple organs/effects. For a given organ or effect, the same level of the dose metric at the animal POD should be used for TK analyses conducted in test animals and humans. The quantitatively determined DDEF values for EF_{AK} will be less than 1 if the level of the dose metric at the animal POD is attained at a higher dose in humans than in animals (indicating that humans are less toxicokinetically sensitive).

EF_{AK} CAN BE LESS THAN 1

For EF_{AK}, central tendency estimates of doses or exposures producing the same measure of target tissue exposure in animals and humans are used. Lower sensitivity in humans compared to animals is demonstrated by lower AUC or C_{max} values, or higher clearance values in humans than in animals at the same exposure. In these instances, the calculated value of EF_{AK} will be less than 1.

Confidence in EF_{AK} is increased when decisions and calculations are well documented. This narrative includes descriptions of toxicity data identifying the target tissue, chemical species, MOA, and species concordance of effects. Data describing the TK, the metabolism of the compound, and the relationship between external dose and dose metric are also summarized. Data that show a linear relationship between external dose and internal dose metrics can be specifically reiterated in this description, which will indicate generalizability of the EF_{AK} value to doses that may be higher or lower than those used in DDEF calculation. Because animal-to-human differences in target tissue concentrations may not be consistent for all responding tissues or organs, a comparison of POD and DDEF values from multiple affected organs will increase confidence in the extent to which the developed DDEF value sufficiently addresses the toxic action of the assessed chemical. Results can be presented in tabular form for ease of comparison across endpoints.

3.3. INTRASPECIES TOXICOKINETIC EXTRAPOLATION FACTOR

When toxicity data defining the POD are developed in test animals, the established framework for UFs includes an initial extrapolation to the human population, then an

[8]If an HED or HEC value is developed, the residual one-half order of magnitude in the value for UF_A encompasses TD, as well as any residual uncertainty in the derived HEC or HED value.

extrapolation to account for human intraspecies or interindividual variation. The purpose of these analyses is to characterize the variation of TK among the human population, the quantitation of which will help avoid the application of default UFs. From a TK standpoint, among humans experiencing the same *external* dose, sensitivity is due to higher target tissue concentrations of the toxicant in the sensitive population or group relative to the rest of the general human population. As with interspecies extrapolation, the DDEF values may be compared to default UF values. This comparison is conducted to aid policy decisions and risk communication after the adequacy of the underlying data have been confirmed (a data quality evaluation). Characterization of the available data includes considering how completely the sensitive population has been identified and its sensitivity described (as opposed to assumed) (U.S. EPA, 2002b). The selection of a bimodal- or unimodal-based analysis will be a function of the available data. It is important to document the available information and related statistical analysis and/or assumptions that serve as the basis for selecting a unimodal or a bimodal distribution of sensitivity. The extent to which this description has been done will inform decisions regarding the application of DDEF analyses.

Sensitivity may reside in an identifiable population (e.g., distinct life stage or genetic polymorphism) or may be less distinctly distributed among humans (e.g., differences in the levels of an endogenously expressed enzyme). As described in more detail below, extrapolation among the human species is accomplished by either of two options (or both): (1) evaluating human interindividual variability among the entire human population or (2) explicit identification of the potentially sensitive population(s) for TK analysis. Differences in the scope/intent of the risk assessment and the availability of data to identify a given population or group as sensitive (e.g., the aged, those with genetic polymorphisms) may limit application of the second option. This section provides a discussion of the quantitative differences in TK among humans for intraspecies extrapolation (i.e., EF_{HK}).

Although it is important to acknowledge the complex factors that contribute to human variability, for sake of simplicity, the entire human population can be distinguished as those who are sensitive and others that make up the general population. This is an important distinction, influencing the choice of computational methods. A bimodal analysis (segregating the entire population into the general and the sensitive populations) is used when sensitive individuals can be identified on the basis of physiological, biochemical, or life-stage attributes and grouped into a distinct population. A unimodal analysis is used when sensitive individuals cannot be identified *a priori* on the basis of physiological, biochemical, or life-stage attributes. Regardless of the analysis type, it should be based on a sufficiently large and diverse population data set, including adequate sampling of potentially sensitive populations and life stages.

3.3.1. Considerations for Intraspecies Toxicokinetic Extrapolation Factor

3.3.1.1. *Sensitive Populations*

This section presents the process of considering the TK of an agent among the human population. For some chemicals, data may be sufficient to identify one or more sensitive populations but insufficient for other chemicals. Sensitivity in the human population may be due to life stage, health status or disease state, genetic disposition, and other factors (also see Section 2.1.2). Critical windows of development, and therefore "windows of susceptibility," occur at different times for various tissues, organs, and systems; therefore, considering sensitivity to more than one critical effect may require consideration of more than one life stage. As discussed in more detail below, distributional analysis of response data can be conducted to identify points for use in quantitation. In completing the analysis, it is important to describe the relationship between the dose metric and the toxicity endpoint(s) of concern (e.g., critical effect or key event). The intraspecies extrapolation step is intended to account for differences between the central tendency of the entire population and the sensitive portion of the population (unimodal analysis) or between the central tendency of the general population and some point in the distribution of the population of sensitive individuals (see bimodal analysis; Section 3.3). Considering sensitivity to more than one critical effect may require consideration of more than one potentially sensitive population.

From a TK standpoint, among humans experiencing the same *external* dose, sensitivity is due to higher target tissue concentrations of the toxicant in the sensitive population relative to the rest of the human population. Higher tissue concentrations can be demonstrated as higher AUC or C_{max} values, or by lower Cl values. Thus, when assessing sensitivity, values in the upper tail of the distribution (e.g., 95th, 97.5th, 99th percentiles) of values for AUC and C_{max} values are considered, while values in the lower tail of the distribution (e.g., 1st, 2.5th, 5th) of values for Cl are considered. The examples demonstrated in this section use AUC or C_{max} as the dose metric. Equation 4, discussed in Section 3.3.2.2, demonstrates the mathematical approach to quantitation of EF_{HK} based on AUC, C_{max}, or Cl. For AUC and C_{max} values, EF_{HK} is computed as the ratio of an internal dose metric attained in the sensitive population to that observed at or near the central tendency in the general human population exposed to the same external dose or concentration. Illustrative examples are included in Appendix A.

Sensitivity may be due to increased tissue exposure at a given dose (TK) or to increased responsiveness to a given tissue concentration (TD; see also Section 4.3.1.1). With an adequate description of the population variability of biochemical, physiological, and anatomical variability, a distributional analysis of the dose metric can confirm that TK variability influences sensitivity. If the dose metric is segregated into distinct groups and the supposed sensitive population has a higher level of the dose metric (e.g., AUC or C_{max}) than the rest of the

population (see Figure 5, left panel), these results would confirm that sensitivity may be at least partially influenced by TK. However, if the dose metric demonstrates a uniform distribution (see Figure 5, right panel) and sensitive individuals are distributed among the entire population distribution, such results would suggest that TK variability may have little influence on sensitivity. Ideally, data will be complete enough to enable more than point estimates among the populations. As discussed in more detail below, distributional analysis of response data should be conducted to identify points for use in quantitation. In completing the analysis, it is important to describe the relationship between the dose metric and the toxicity endpoint of concern (e.g., critical effect or key event). A sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.

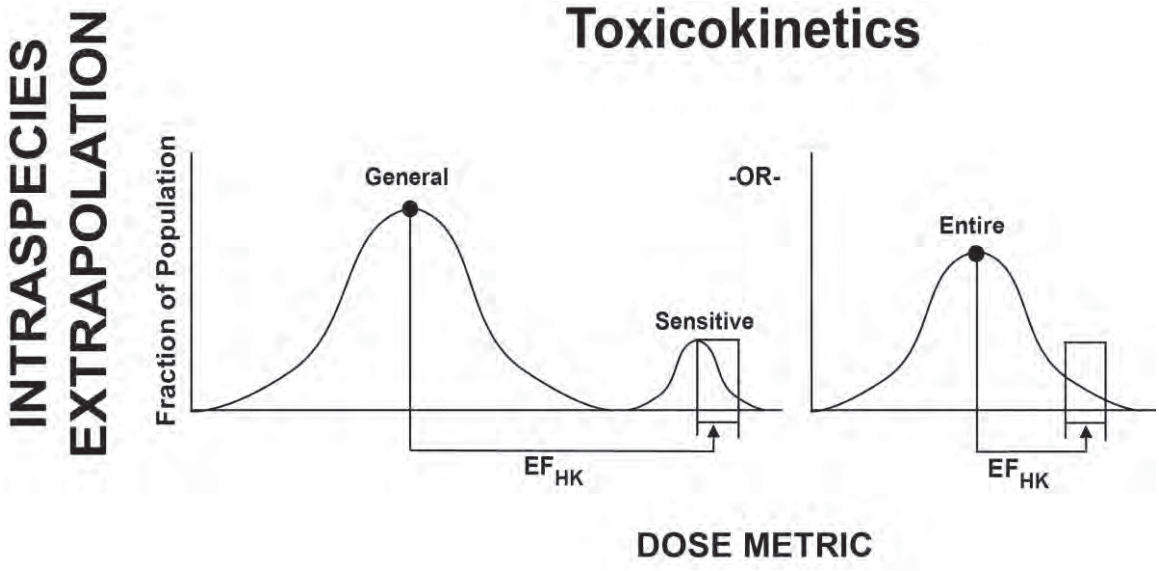

Figure 5. Intraspecies toxicokinetics. Dose metric values may be distributed among the human population in a bi- (or multi-) modal or a unimodal fashion. Even when an identifiable population is presumed or demonstrated to be sensitive on the basis of exposure, the distribution of dose metric values may be unimodal. Differences in distribution type affect quantitative methods as described in the text. EF_{HK} is computed on the basis of differences in dose metric attained at the same external dose. This figure demonstrates analysis of AUC and C_{max} data.

3.3.1.2. *Target Tissues*

When responses are observed in several organs at or near the POD for the most sensitive effect, a comparison of EF_{HK} values developed for those tissues is informative. However, the selection of a target organ for calculating human variability other than the one serving as the

basis for animal-to-human extrapolation is best accompanied with a justification. Development of candidate DDEF values should be undertaken in a manner analogous to the development of candidate RfD or RfC values per EPA (U.S. EPA, 2002b). The extrapolation approach is generally most consistent when the same tissue or organ is used for each phase of the extrapolation procedure.

3.3.1.3. *Dose-Response*

Because variability in internal dosimetry may be a function of dose, the selection of the external exposure (e.g., inhaled concentration or orally ingested dose) is important for use in estimating intraspecies differences in dosimetry. When the POD is derived in animals, several options exist for interspecies extrapolation, depending on the availability of suitable animal and human data describing TK and/or TD. In the TK part of the interspecies extrapolation step, the animal POD is extrapolated to produce a toxicokinetically equivalent human dose or concentration. In some instances, interspecies extrapolation may also include a separate, technical treatment of TD. Also, interspecies adjustment may be completed by the default value for UF_A, or the POD may be defined from dose-response studies in humans. Regardless of whether the default UF is applied or DDEF value is computed to complete *interspecies extrapolation*, it is the external dose (mg/kg-day) or concentration (mg/m^3) reflecting this adjustment that is most appropriate for application in determining human *intraspecies* TK variability.

The basis for comparison of *human variability* is at the level of the internal dose metric rather than the external dose. This choice for the level of comparison is consistent with the principle that it is the target tissue dose that drives the toxic response, not an external (applied) dose. Placing the comparison at the level of the target tissue dose (dose metric, internal dose) forces the assumption of TK linearity to maintain consistency with the established approach of applying UFs to external doses. Thus, it is important that the relationship between internal and external doses be well characterized to ensure TK linearity (the proportionality of the ratio of external dose to dose metric across some range of doses or exposures). While nonlinearities may become evident at substantially different human exposures (e.g., between the interspecies-adjusted POD and the resulting RfD or RfC), the prime point for comparison is the range of doses (or concentrations) immediately surrounding the interspecies-adjusted POD. Because EF_{HK} and preceding DDEF values will be used to extrapolate external doses, it is recommended that TK linearity be tested by documenting a consistent ratio of external doses and dose metric values in a range of exposures bounded by the value of EF_{HK}.

Nonlinearities in TK frequently arise due to metabolism, and are addressed on a case-by-case basis. Several circumstances may account for nonlinearities. When a chemical is

metabolized by a single enzyme and the concentration is below the K_m, the metabolic rate is essentially defined by V_{max}/K_m—metabolism is essentially first order. When concentrations become saturating, further increases in concentration do not result in increased metabolism. When metabolism represents a bioactivation process, no further increase in toxicity due to a bioactivated metabolite is anticipated. However, if metabolism represents a detoxication process, then a disproportionate increase in toxicity from the accumulation of the toxic parent chemical may be predicted. However, increasing concentrations of the chemical may recruit additional enzymes with a lower affinity, and these enzymes may be responsible for the production of detoxicated or bioactivated metabolites. Nonlinearities represent special cases, and when identified, are seldom evident over narrow ranges of concentrations. This DDEF guidance is concerned with the factors governing inter- and intraspecies extrapolation. When nonlinearities become evident in the range of doses pertinent to these dose extrapolation steps, they are considered on a case-by-case basis. Consideration of nonlinearities that are evident across broader ranges of concentrations (e.g., animal POD versus RfD values) are beyond the scope of this guidance.

Specific differences among humans, particularly those demonstrated *in vitro* (i.e., intrinsic clearance), are most reliably used when they are translated into differences in dosimetry based on the anatomical and physiological constraints imposed by the intact system (whole animals). This approach may include evaluations of multiple different doses.

3.3.2. Computation

For intraspecies TK extrapolation, differences in dosimetry are characterized for the human population by comparison of central tendency TK data for the sensitive population to measures of the general population or the entire population (see Section 3.3.1.1, Figure 5). The comparisons are among differences in internal dosimetry (or target site dose, dose metric) resulting from the same external exposure. To address human variability, some attention is devoted to documenting the reasons for the assumption of sensitivity among any population anticipated *a priori* to be sensitive (generating a bimodal analysis, which involves the general and sensitive populations). In the absence of a specifically identifiable population as sensitive, or when the analysis of TK among humans includes a separate population presumed to be sensitive fails to result in distinguishable distributions of the dose metric, a unimodal type analysis should be conducted. In this instance, comparisons of the dose metric between the central tendency and defined percentiles of the entire population distribution (upper percentiles for C_{max} and AUC; lower percentiles for clearance) can be made. Regardless of the distribution type, the presentation of multiple values/points in the tail of the distribution will better enable risk communication and management decisions.

3.3.2.1. *Use of Toxicokinetic Models*

A PBPK or other TK model provides the most biologically appropriate approach for evaluating intraspecies TK extrapolation. When a model is available and has been properly evaluated (IPCS, 2010; U.S. EPA, 2006a). It can be used in different ways depending on the model and the circumstances. There are several potential applications of TK models for sensitivity among humans. Some examples include the identification of the POD in a test animal species and a subsequent two-step process to perform a TK extrapolation to account for inter- and intraspecies differences. This case is the typical scenario, and in it, the initial extrapolation is from the animal POD value to a value representing the central tendency in the human population. If TK data or a reliable TK model exists that can be used to examine dosimetry in the segment of the human population deemed sensitive, then the second extrapolation step to account for human variability is undertaken to define differences in tissue concentrations between the central tendency of the general population and those in the sensitive population or in the sensitive portion of the population. For example, the DDEF value for EF_{HK} is determined as the ratio $Concentration_{sens}$:$Concentration_{gen}$.

A second circumstance is exemplified when the POD is identified in the generally responsive (e.g., 70-kg adult human male), obviating the need for interspecies extrapolation and requiring an extrapolation to account for sensitivity among humans. In this case, the data or the model used to identify the dose metric near the central tendency of the general (overall) human population and in the segment of the population representing sensitivity/susceptibility will be examined. However, when the POD is determined in the portion of the human population that is sensitive, deriving UFs or DDEF values for inter- or intraspecies extrapolation is unnecessary.

3.3.2.2. *Use of Ratios*

In addition to predictive models, EF_{HK} can be derived using a ratio (see eq 4). The value for the dose metric employed as the central-tendency measure in humans (e.g., area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population [AUC_{gen}]) would be that level of the dose metric identified from studies with animals and further extrapolated to account for interspecies TD differences, or from studies in humans. The value for the dose metric in sensitive populations or at a percentile of the entire population (e.g., area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive human population or representing sensitive individuals among the entire human population [AUC_{sens}]) should be determined from empirical data or pharmacokinetic modeling.

$$EF_{HK} = \frac{AUC_{sens}}{AUC_{gen}} \text{ OR } \frac{C_{max\ sens}}{C_{max\ gen}} \text{ OR } \frac{Cl_{gen}}{Cl_{sens}} \quad (4)$$

where,

EF_{HK} = intraspecies TK extrapolation factor

AUC_{gen} = general human population AUC value (area under the concentration-time curve at a fixed external dose at a measure of central tendency in the entire or general human population)

AUC_{sens} = sensitive human population AUC value (area under the concentration-time curve at a fixed external dose at a percentile of interest for the sensitive population or representing sensitive individuals in the entire human population)

$C_{max\ gen}$ = general human population maximum concentration value (at a fixed external dose at the central tendency in the entire or the general human population)

$C_{max\ sens}$ = sensitive human population maximum concentration value (at a fixed external dose at a percentile of interest for the sensitive population or representing sensitive individuals in the entire human population)

Cl_{gen} = general human population clearance value (at a measure of central tendency in the entire or general human population)

Cl_{sens} = sensitive human population clearance value (at a percentile of interest in the sensitive human population or representing sensitive individuals among the entire human population)

3.3.3. Conclusions for Intraspecies Toxicokinetic Extrapolation Factor

EF_{HK} is a comparison of dose metrics resulting from the same external dose across the human population(s). When using an empirical ratio, EF_{HK} is the ratio of the dose metric value at a percentile of the distribution intended to represent the sensitive population or individuals and the dose metric value at a central-tendency measure of the general or the entire population. By quantitatively, EF_{HK} cannot be less than 1.

The dose selected for quantifying human interindividual variance may have an impact on the magnitude of variability (the DDEF value). When the POD is identified in a test species, the dose adjusted from the animal POD to account for all components of UF_A is the preferred dose for quantitation of human variability. If the POD is identified in humans not deemed to represent a human population, then human variability should be characterized at that dose. DDEF values should be calculated for multiple organs/effects when multiple tissues respond near the POD for

the most sensitive tissue (the critical effect). The consistency of DDEF values should be evaluated over a range of doses surrounding the POD to increase the level of confidence. DDEF values should be developed for each of the candidate tissues or effects, combined with POD values for each candidate tissue or effect, and clearly communicated. To ensure transparency, associated documentation should describe the mathematical method employed, the type of distribution and percentile(s) of interest, the rationale for choosing percentiles of interest, the dose metrics (e.g., AUC of parent compound in kidney), and the target tissues for which EF_{HK} values are developed. Confidence in the extrapolation is improved when distribution types are justified or explained.

4. DATA-DERIVED EXTRAPOLATION FACTORS BASED ON TOXICODYNAMICS

4.1. GENERAL CONSIDERATIONS

TD describes the critical interaction of the toxicologically active chemical moiety with the target site and the ensuing sequence of events leading to toxicity. Data that describe the dose-response relationship serve as the basis for deriving extrapolation factors for TD components. This section provides a discussion of factors common to inter- and intraspecies extrapolation for TD. In contrast to TK, which focuses on *differences in internal dosimetry,* TD differences are quantified as *differences in concentration*[9] *producing the same level of response* between test animals and humans, as well as among humans. TD evaluations may include multiple response levels, critical effects, key events, or analytical methods. Developing a DDEF for TD requires knowledge about the relationship between an event measured *in vitro* or *in vivo* (e.g., receptor binding) and the end result (critical effect). Doses or concentrations producing the measured event can be obtained *in vitro* or *in vivo* in the tissue of interest or a suitable surrogate. TD models, the most complex of which may be represented by BBDR models, are an important tool through which predictions may be made to extend the range of empirical observations. Comparisons between animals and humans or to quantify human interindividual differences are made on the basis of doses or concentrations that produce the same level of the same measured response.

Quantitative differences between animals and humans are used for EF_{AD}, whereas differences in sensitivity within the human population are used for EF_{HD}. Thus, EF_{AD} is used to extrapolate findings in laboratory animals to the general human population, and EF_{HD} is used to extrapolate to sensitive human populations. The TD components (EF_{AD}, EF_{HD}) are combined with the corresponding TK components to develop the composite factor.

In rare cases, when a sensitive population or life stage has been identified, TD data in animals and the sensitive population (if available) may be employed to develop a DDEF value that combines both inter- and intraspecies TD extrapolation. In this case, the DDEF value derived represents both EF_{AD} and EF_{HD}. Because this is expected to be a rare situation, this section will address extrapolations separately.

Although a complete mechanistic understanding is not required, derivation of a DDEF for TD relies on some understanding of an MOA for the critical effect(s) identified for risk

[9]When using a biologically based dose-response model, differences may be quantified as the ratio of dose metrics in respective species or population groups producing the same level of the response. However, DDEF values for TD may also be quantified as the ratio of *in vitro* concentrations producing the same level of the response.

assessment. As for all DDEF derivations, adequate and appropriate data are essential. Important issues to address include the following:

≠ What are the critical effect(s) and POD(s) being used in this assessment? If more than one, each should be considered in this process.

≠ What is the MOA, AOP, or mechanism(s) for that toxicity? Have the key events been identified? How are they measured?

≠ Is the MOA, AOP, or mechanism(s) in the animal model relevant to humans (qualitatively and/or quantitatively)?

≠ For interspecies extrapolation, are there sufficient data on the key events amenable to modeling such that a uniform measure of response in animals and humans can be derived? If not, do the available data points include a response level that is sufficiently similar in animals and humans?

≠ For intraspecies extrapolation, are there adequate data in human populations that describe population variation in response as a function of internal dose metric? Do the available data identify sensitive population(s)? Can the degree of this sensitivity be adequately estimated?

4.1.1. Mode of Action

TD extrapolation should be endpoint driven (considered in the context of the critical effect). For clarity, the choice of critical effect is justified, when possible, based on findings of response in exposed humans (U.S. EPA, 1994). Understanding MOA for the agent(s) of interest ensures that the TD responses (the biological events) used to derive the DDEF are relevant to the adverse health outcome of interest. These responses could include receptor affinity, enzyme inhibition, and molecular changes, among others. Repair of deoxyribonucleic acid (DNA) or tissue damage, biological thresholds, residual function, and other processes that could contribute to the shape of the dose-response curve and effects are considered. Experimental systems and measured responses in each species should be the same or comparable for both inter- and intraspecies extrapolation.

4.1.2. Relating Response to Dose or Concentration

TD data may be developed from the results of *in vivo* or *in vitro* studies. In some cases, *in vivo* data may be used, but care should be taken to control for the influence of TK. If there are existing human data measuring the response, then those data can be used to derive the POD,

thereby removing the need for the UF_A. Likewise, data from the most sensitive human population, if available, could be used for the risk assessment and a UF_H may not be needed.

Care should be taken to ensure that kinetic factors do not confound the interpretation of response data. Comparisons based on an internal dose metric are preferred over external dose; that is, the internal concentration producing the level of response (rather than applied dose or concentration) is preferred so that TD response may be distinguished from TK influences. Tissue-specific metabolism could also influence the actual target tissue concentrations and must be considered. Blood levels are an acceptable measure of internal dose when it can be shown that they are proportional to concentrations of toxicologically active chemical moieties in the target tissue or biological preparation.

When using *in vitro* systems, the response measured should be representative of the toxicity; that is, the measured endpoint should be consistent with or comparable to the critical effect or key event observed *in vivo* (if known). In cases where the measured response *in vitro* is known to differ from that observed *in vivo*, it is important to describe the potential impact of these differences on the final DDEF. For the sake of transparency when using *in vitro* data, the comparability of chemical uptake between animal and human tissues or preparations is addressed. Among the factors considered when presenting *in vitro* results are data describing metabolism of the test chemical by the *in vitro* system used.

4.1.3. Range of Doses or Concentrations

The relationship between the doses, tissue concentrations, and/or *in vitro* concentrations used to derive the DDEF, and those attained in the toxicity studies from which the POD is derived, can be characterized to improve transparency of the assessment. Optimally, the concentrations used in studies of the critical effect(s) include the concentration at the POD. The need for quantitative data is important because the variability in the response may change with increasing or decreasing dose or concentration. Doses and/or tissue concentrations may also be compared to those expected from environmental exposure; this comparison is part of the consideration of overall relevance of the test system.

4.2. INTERSPECIES TOXICODYNAMIC EXTRAPOLATION FACTOR

This section provides information for calculating TD differences between species (EF_{AD}). These are characterized as the ratio of the concentrations (concentrations used *in vitro* or the level of the dose metric) in animals and humans producing the *same level of response*. Note that the level of comparison is at a fixed response level, and it is not a comparison of responses produced by the same concentration (see Figure 6). Illustrative examples are included in Appendix A.

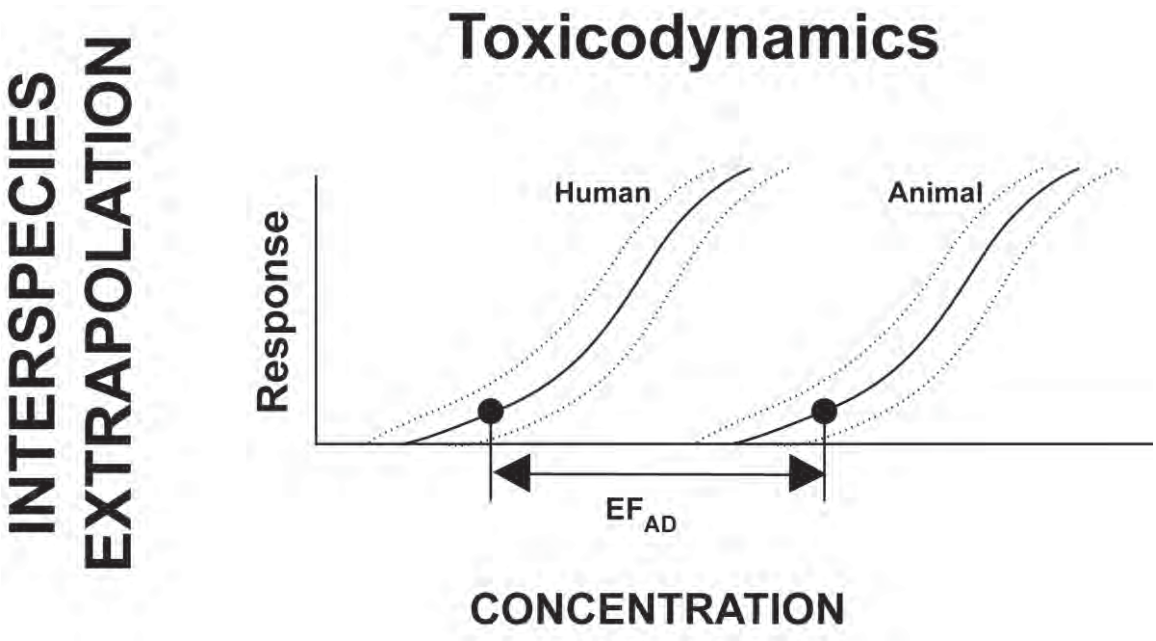

Figure 6. Interspecies toxicodynamics. The TD difference between test animals and humans is calculated from concentration-response relationships determined in test systems or animals generally representative of the respective species. The comparison is made for concentrations producing the same level of response, and comparisons are made using values representing the central tendency. Dashed lines represent variability.

4.2.1. Considerations for Interspecies Toxicodynamic Extrapolation Factor

4.2.1.1. *Mode of Action*

Endpoint(s) must be chosen for which to derive the EF_{AD}, and the endpoint(s) should be the actual critical effect or a key event in the MOA or AOP. For each endpoint evaluated, it is determined whether the data are from an animal typical of the responding species/strain/sex/life stage, as opposed to an animal model system that may be unusually sensitive for a particular effect. Characterization of the test animal might be important in transgenic animals and in animal models used to study specific human diseases (e.g., spontaneously hypertensive rats). Animal models and the MOA(s) based on them are best evaluated for human relevance (Meek et al., 2003), as has been done for forestomach tumors (Proctor et al., 2007).

4.2.1.2. *Target Tissues*

Data from the target tissues are preferred but not always available for human tissue. Where there are data from the molecular targets in both species, but the data are not from the critical target organ, the data can be used provided there is sufficient justification that one tissue

is an appropriate surrogate for another. For example, a target enzyme may be present in several tissues, including blood; however, blood is easily obtained from humans, but tissue from the target organ is not. Studies may show that the effect on this target enzyme in blood correlates very well and is entirely predictive of the effect in the target organ, and that the enzyme structure, function, and chemical affinity are the same regardless of tissue. Such information would be necessary to justify comparisons of chemical effects in the blood enzyme in both humans and laboratory animals.

4.2.1.3. *Dose-Response*

The choice of response level to use for comparison depends on several factors:

- ≠ Completeness of dose-response data,
- ≠ Shape of the dose-response curves, and
- ≠ Understanding of the effect along the toxicity pathway at that response level.

The magnitude of EF_{AD} may be a function of the response level chosen for extrapolation. Confidence is increased when the response level employed for EF_{AD} calculation approximates the response level at the POD and when the ratio of doses producing the same response level in animals and humans is similar over a range of doses. When data are available to describe the full dose-response curve, evaluating the shape of the dose curves for animals and humans can provide important information. If the shapes of the curves are different, then the magnitude of EF_{AD} will depend on the response level selected. Note that concentrations causing greater magnitudes of effect may also produce nonspecific cellular changes that could confound the comparisons.

4.2.1.4. *In vitro Data*

When using data from *in vitro* systems, the activity of the parent chemical and/or metabolites, as well as the extent of metabolism of the compound by the *in vitro* system, should be known. The *in vitro* assay should measure a response that can be linked to the toxic outcome, and the assays should employ the same (responding) tissues from the *in vivo* test animal species/strain/sex/life stage and from humans. Experimental systems should be as closely matched as possible, and the concentrations of toxicant *in vitro* should be compared to tissue concentrations (the dose metric) at the POD. See Section 2.4 for other general considerations.

4.2.2. Computation

4.2.2.1. *Use of Ratios*

When TD models are not available, EF_{AD} can be calculated as a ratio describing the relationship between the tissue concentrations producing a set response in human tissues compared to animal tissues, preferably at or near the response level at the POD. For example, "*x* response level" may be a 10% response if the effective dose producing a 10% response rate or lower confidence bound on benchmark dose corresponding to a 10% increase in response ($BMDL_{10}$ value) is used. The EF_{AD} value would be calculated as follows:

$$EF_{AD} = \frac{\text{Concentration}_{\text{A-}x\text{ response level}}}{\text{Concentration}_{\text{H-}x\text{ response level}}} \quad (5)$$

where,

EF_{AD} = factor for interspecies extrapolation covering TD

$\text{Concentration}_{\text{A}-x\text{ response level}}$ = concentration of the agent at the tissue in the animal resulting in an *x*% response

$\text{Concentration}_{\text{H}-x\text{ response level}}$ = concentration of the agent at the tissue in the human resulting in an *x*% response, where *x* is the same response value as in animals

To evaluate the extent to which the shape of the dose-response curve varies between animals and humans, and thus impacts the magnitude of EF_{AD}, a range of response levels should be evaluated (e.g., in cases where the dose-response data are extrapolated to derive levels for the POD). The rationale and implications for choosing the point for extrapolation should also be presented.

4.2.2.2. *Biologically Based Dose-Response Models*

A BBDR model often provides the most robust approach for evaluating interspecies extrapolation. These models are typically expansions of PBPK models (addressing TK) extended to include TD. By including both TK and TD components, BBDR models provide a linkage between external (applied) dose and biological response. Such models incorporate data from key events allowing direct estimation of adverse health outcome. In cases where the ultimate biological effect modeled in the BBDR model (e.g., DNA binding of the toxicant) is not the adverse health outcome of interest (e.g., tumor development), additional considerations are

needed. Perhaps the most important among these is the quantitative relationship between the modeled biological effect and the adverse health outcome. In keeping with principles established herein and elsewhere in Agency guidance, several points are important when evaluating a BBDR model for quantitative reliance. Fundamentally, the TK components of the model must be evaluated according to established principles (U.S. EPA, 2006a). TD components of the model should be evaluated according to principles established in this section. Because BBRD models translate exposure to response (without necessarily separating out TK from TD influences), they may be used to complete species extrapolation without developing distinct values for EF_{AK} and EF_{AD}. Alternatively, this human exposure may be identified as the HEC (for inhaled toxicants) or an HED (for orally encountered toxicants).

4.2.3. Conclusions for Interspecies Toxicodynamic Extrapolation Factor

Mathematically, EF_{AD} will be the ratio of the concentrations or dose metric values resulting in the same level of response in both the test species and human. DDEF values may be calculated for multiple PODs and organs/effects, but the response levels used for quantitation should be the same in animals and humans. Confidence in the value (knowing whether the DDEF value can vary depending on the response level) is improved when EF_{AD} values developed from multiple points on the concentration-response curve are comparable; the shape/slope of the curves may also influence these values. Quantitatively, EF_{AD} can be less than 1 if the data show humans are less sensitive than test species.

Preferably a summary of all conclusions and their scientific support are provided. Data describing the dose-response of the compound in animals and humans can be specifically reiterated in the summary. Finally, the summary also indicates the extent to which the EF_{AD} value can be generalized to doses that may be higher or lower than those used in its calculation.

4.3. INTRASPECIES TOXICODYNAMIC EXTRAPOLATION FACTOR

This section provides information for calculating TD variability within the human population. EF_{HD} is calculated as the ratio of concentrations or dose metric values producing the same level of the response at or near the central tendency in the general (nonsensitive) or the entire population to concentrations of the dose metric observed/predicted in the sensitive population or sensitive portion of the entire. From a TD standpoint, sensitivity is based on attaining a given level of response at a lower concentration of toxicant. For this evaluation, multiple response levels, critical effects (or key events), analytical methods, or sensitive populations may be considered.

4.3.1. Considerations for Intraspecies Toxicodynamic Extrapolation Factor

4.3.1.1. *Sensitive Populations*

Sensitivity in the human population may be due to life stage, health status or disease state, genetic disposition, or other factors as discussed in Section 2.1.2. Critical windows of development, and therefore windows of sensitivity, occur at different times for various tissues, organs, and systems. Considering sensitivity to more than one critical effect may require consideration of more than one life stage. Data to address differential sensitivities in dynamic response are becoming more available with advances in the science (IOM, 2013). For example, population variation, such as genetic polymorphisms, is an expanding area of study. The increased availability and application of newer technologies, such as omics and high through-put, will benefit the derivation of DDEFs in general, and EF_{HD} in particular. A data-derived EF_{HD} is feasible if human data are of sufficient quality, the data address aspects of the critical effect consistent with that identified from applicable human or animal studies, studies have been conducted in the segment(s) of the population deemed sensitive, and/or a sufficiently large and diverse sample set is available.

For quality purposes, data should be sufficient to enable at least a rudimentary understanding of the distribution of values. As presented in Section 3.3.1.1, sensitivity may be distributed among the entire population or segregated into an identifiable sensitive population, dictating a unimodal or bimodal distribution, respectively, with their inherent DDEF quantitative procedures. As discussed in more detail below, distributional analysis of response data may be conducted to identify points for use in DDEF quantitation. The relationship between the measured response and the toxicity endpoint of concern (e.g., critical effect or key event) should be described, whether determined *in vivo* or *in vitro*.

4.3.1.2. *Target Tissues*

For calculation of EF_{HD}, data for multiple responding tissues can be evaluated and multiple DDEFs can be derived. It is particularly important to evaluate those tissues that demonstrate response at doses or concentrations near that for the critical effect.

4.3.1.3. *In vitro Data*

Given the constraints on generation of human response data *in vivo*, *in vitro* studies offer an alternative. Samples selected for *in vitro* investigation should represent the central tendency of the human population as well as sensitive populations or life stages (see Section 2.4 for other general considerations). It is important that the *in vitro* assay measure a response that can be linked to the toxic outcome, and also that the assays employ the same (responding) tissues or

suitable surrogates for the tissues identified in the *in vivo* test animal species/strain/sex/life stage and from humans. Finally, experimental systems for each segment of the human population should be as similar as possible, and the concentrations of the toxicant *in vitro* be compared to tissue concentrations (the dose metric) at the POD.

4.3.2. Computation

For intraspecies TD extrapolation, differences in response are characterized for the human population by comparison of the TD data (doses or concentrations producing the same level of response) for the central tendency of the populations to the sensitive population or segment of the population. For quantitation, data on the critical response(s) are derived from a population that includes sensitive populations. Because the data available to define potentially sensitive populations from which to derive quantitative differences could be viewed in different ways, a statistical analysis may be helpful to determine distribution type (see Figure 7), which include:

≠ A unimodal distribution where the sensitive population represents the tail of the distribution because the sensitive individuals cannot be separated from the remainder of the human population; a sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.

≠ A bimodal (or multimodal) distribution where the sensitive populations have been identified or suggested.

4.3.2.1. *Use of TD Models*

In the absence of *in vivo* response data in the sensitive human population, a BBDR or other TD model provides the best approach for evaluating intraspecies TD extrapolation. When sufficient data are available, these TD models can be structured and used to include differences in MOA components that may be life-stage dependent or influenced by other conditions potentially conferring increased sensitivity, such as genetic polymorphisms. Specific to EF_{HD}, it is critical that the model parameter(s) conferring increased sensitivity be well documented. When an appropriate model is available, it can be used in different ways depending on the model. In some cases, the TD model may directly account for interindividual variation and/or include data from the sensitive population, thus eliminating the need for EF_{HD}. In other cases, the TD model may be used to derive EF_{HD}.

4.3.2.2. *Use of Ratios*

When TD models are not available and populations can be identified as sensitive, then the ratio approach may be used. EF_{HD} is defined as the ratio between the concentrations producing the same level of response at the central tendency of the general population and a specified percentile of the distribution in the sensitive populations and/or life stages. EF_{HD} is calculated using eq 6 and described in Figure 7.

$$EF_{HD} \cong \frac{Concentration_{gen}}{Concentration_{sens}} \quad (6)$$

where,

EF_{HD} = factor for intraspecies extrapolation covering TD

$Concentration_{gen}$ = concentration producing the response corresponding to the POD at a measure of central tendency in the entire or the general human population

$Concentration_{sens}$ = concentration producing the response corresponding to the POD at a percentile of interest for the sensitive human population

When sensitivity among the population exhibits a unimodal distribution, EF_{HD} is the ratio of the concentration that elicits a level of response at the central tendency of the entire distribution to the concentration that elicits the same level of response at a lower sensitive percentile of the distribution. It is important to define and justify the point(s) in the distribution representing sensitivity; a sufficiently large and diverse population data set must be used to ensure that it includes an adequate sampling of potentially sensitive populations and life stages.

When sensitivity among the population exhibits a bimodal (or multimodal) distribution, the DDEF is determined in a similar manner, using the concentrations that elicit the specified level of response at the central tendency of the general population and in the lower tail of the sensitive population. The values selected to describe the sensitive population are defined and presented. The selection of the response level and the percentile of the distribution used to describe the potentially sensitive populations is an important issue. A justification for selection of the response level and percentile for sensitive population should be provided.

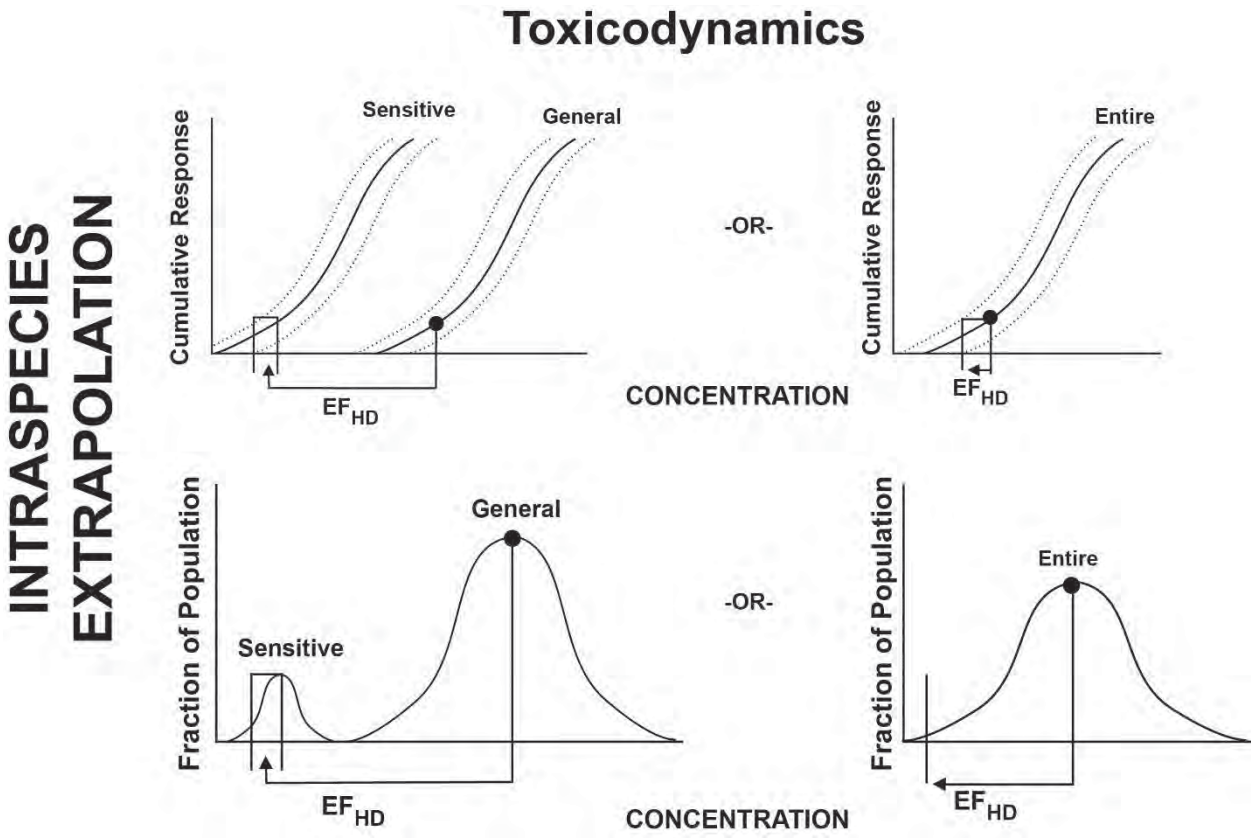

Figure 7. Intraspecies toxicodynamics. Sensitivity may be distributed among the human population in a bi- (or multi-) modal or a unimodal fashion. Even when an identifiable population may be presumed or demonstrated to be sensitive, the distribution of dose metric values may be unimodal. Differences in distribution type affect quantitative methods. TD differences are quantified on the basis of differences in concentration producing the same level of the observed response.

4.3.3. Conclusions for Intraspecies Toxicodynamic Extrapolation Factor

A BBDR or other TD model provides the most biologically appropriate approach for developing an EF_{HD} value. When using empirical ratios, EF_{HD} is the ratio of the concentration producing the specified level of response in sensitive populations to the concentration of the toxicant producing the same level of response at the central tendency of the general or the entire human population. Increased confidence in EF_{HD} is developed when the range of concentrations

INTRASPECIES EXTRAPOLATION OF RESPONSE

For the toxicodynamics component of EF_{HD}, the relationship between the response measured *in vitro* and the response observed *in vivo* should be presented, and EF_{HD} is calculated as the ratio of concentrations producing the same level of the response in the respective population groups—general or entire to sensitive population groups.

used to determine EF_{HD} is comparable to target tissue concentrations at or near the human POD (the animal POD adjusted by the value of the UF_A). Quantitatively, EF_{HD} cannot be less than 1.

The risk assessor describes all choices and rationales, including the use of multiple response levels, critical effects (or key events), analytical methods, or data from sensitive populations and/or life stage. The conclusions include a clearly worded description of the mathematical method(s) employed and a presentation of the relationship between the measured response and toxicity (i.e., critical effects or key events). This description should clearly identify and provide the justification for the selection of data and points in the distribution(s) representing sensitive groups. Attention should be paid to characterizing the distribution type employed for analysis; uncertainty in the choice of distribution type can be reduced by presenting DDEF values resulting from multiple distribution types.

5. CALCULATION OF THE DATA-DERIVED EXTRAPOLATION FACTOR

The composite factor is calculated after the appropriate DDEF values for inter- and intraspecies differences in TK and TD have been derived. The composite factor is calculated by multiplying the specific factor values (default and/or DDEFs), as shown in eq 7. This computation is entirely analogous to calculating composite UFs when using the 10× defaults for UF_A and UF_H. The composite DDEF may be less or greater than 100.

$$CF = EF_{AK} \times EF_{AD} \times EF_{HK} \times EF_{HD} \quad (7)$$

where,

CF = composite factor

EF_{AK} = interspecies TK extrapolation factor

EF_{AD} = interspecies TD extrapolation factor

EF_{HK} = intraspecies TK extrapolation factor

EF_{HD} = intraspecies TD extrapolation factor

In practice, data may only be available to develop a DDEF for one component of extrapolation or another (e.g., data for EF_{AK} but not EF_{AD}). In these situations, the remaining extrapolation is done by an appropriate default procedure. When default values are used, DDEFs and default values (i.e., UFs) are used in combination. Often this default will be one-half order of magnitude UF—as described in the Agency's RfC methodology (U.S. EPA, 1994). When data are not available to develop DDEFs for either component of inter- or intraspecies extrapolation, the default approach (e.g., $BW^{3/4}$ scaling for interspecies extrapolation for oral RfDs, RfC default for interspecies extrapolation for RfCs, or application of UFs) is employed (U.S. EPA, 2011).

Finally, the composite factor provides the total magnitude of the factor. The values derived for each of the components and the resulting extrapolations should be clearly reported and characterized. The relationship of each of these doses or concentrations to the POD should be presented.

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APPENDIX A

CASE STUDIES TO ACCOMPANY DATA-DERIVED EXTRAPOLATION FACTOR GUIDANCE

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DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

LIST OF ABBREVIATIONS

AChE	acetylcholinesterase
AML	amount of metabolite in the liver
AUC	area under the curve
BAA	2-butoxyacetic acid
BMD	benchmark dose
BMD_x	benchmark dose corresponding to an *x*% increase in response
BMDL	benchmark dose lower bound (i.e., lower confidence bound on benchmark dose)
$BMDL_x$	lower confidence bound on benchmark dose corresponding to an *x*% increase in response
BMR	benchmark response
CMG	common mechanism group
CRA	cumulative risk assessment
DAF	dosimetric adjustment factor
DDEF	data-derived extrapolation factor
DMA	dimethyl arsenic acid
EF	extrapolation factor (optional subscripts $_A$ = interspecies/animal-to-human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
EF_{AD}	interspecies toxicodynamic EF
EF_{AK}	interspecies toxicokinetic EF
EF_{HK}	intraspecies toxicokinetic EF
EGBE	ethylene glycol monobutyl ether
EPA	U.S. Environmental Protection Agency
GFR	glomerular filtration rate
HED	human equivalent dose
IRIS	Integrated Risk Information System
LC_{50}	lethal concentration for 50% of the population
LOAEL	lowest-observed-adverse-effect level
MCV	mean corpuscular volume
MeHg	methylmercury
MOA	mode of action
MOE	margin of exposure
NMC	N-methyl carbamate
NOAEL	no-observed-adverse-effect level
OPP	Office of Pesticide Programs
PBPK	physiologically based pharmacokinetic
POD	point of departure
RBC	red blood cell
RED	registration eligibility decision
RfD	reference dose
RPF	relative potency factor
SD	standard deviation(s)
TD	toxicodynamic
TK	toxicokinetic

LIST OF ABBREVIATIONS (continued)

UF	uncertainty factor (optional subscripts $_A$ = interspecies/animal to human; $_H$ = intraspecies/within human variability; $_D$ = toxicodynamic component; $_K$ = toxicokinetic component)
UF_A	interspecies UF
UF_H	intraspecies UF
UF_{AD}	interspecies UF for the TD component
UF_{HD}	intraspecies UF for the TD component
UF_{AK}	interspecies UF for the TK component
UF_{HK}	intraspecies UF for the TK component
VC	vinyl chloride

APPENDIX A.
CASE STUDIES TO ACCOMPANY
DATA-DERIVED EXTRAPOLATION FACTOR GUIDANCE

This document is an Appendix to the document *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*. It presents examples where the availability of data for given chemicals support the derivation of nondefault values for components of uncertainty factors (UFs). Each of the chemicals examined has an existing Integrated Risk Information System (IRIS) file and/or U.S. Environmental Protection Agency (EPA) Program Office risk assessment, although the derivation of data-derived extrapolation factors (DDEFs) contained in these examples may not be found in the those documents. The intent of this case study document is to present examples that instruct the calculation of DDEF values; reference values derived in these case studies should not be used in place of values found in IRIS or Program Office risk assessments.

A consistent format, shown below, has been developed and applied to the case studies. However, different components of case studies are more extensively described for some chemicals than for others. Differences are due to chemical-specific data sets, which may be more informative for some areas than others.

1. Summary
   This section communicates the current assessment(s) (e.g., IRIS, Provisional Peer Reviewed Toxicity Values, Registration Eligibility Decision (RED), premanufacture notice/existing chemical, International Programme on Chemical Safety) and reference values. It includes the individual and composite UFs, and indicates how the DDEF was applied.

2. Hazard Identification and Dose-Response
   This section lists the key studies, identifying the principal study and critical effects including supplemental studies that might help to inform the decision. Methods used to characterize the dose-response relationship and models or data describing response as a function of internal (target tissue) concentration are described. Also included is information on the mode/mechanism of action. The section is not intended to be a compendium of data; rather it should communicate the information necessary to serve as a basis for the case study.

3. Basis for Data-Derived Extrapolation Factor
   This section provides the rationale for developing a DDEF rather than relying on default values. Models or data available for evaluation, the basis for selection of DDEF method, and the connection between the measure of dose and adverse effect are described.

4. Data-Derived Extrapolation Factor Derivation
   This section presents the computation of the DDEF value so that the reader can follow the derivation. Comparisons to other possible values for the UF component are presented.

The case studies illustrate different principles described in the main document. Table A-1 provides a summary of the principles illustrated in each. Note that the Agency does not yet have experience with deriving a DDEF for intraspecies extrapolation with regard to the toxicodynamic (TD) component. As such, no case study for that component is provided here.

Table A-1. Data-derived extrapolation factor case study chemicals and issues

DDEF	Chemical	Other principles or issues
Extrapolation Factor (EF) for the Interspecies/Animal-to-Human (A) Toxicokinetic Component (K) (EF_{AK})[a]	Ethylene glycol monobutyl ether	Choice among dose metrics, physiologically based pharmacokinetic modeling, benchmark dose applied to internal, not external, doses
	Vinyl chloride	Mode-of-action analysis to identify dose metric; internal dose of reactive metabolite in liver chosen as basis for toxicokinetic equivalency between species
EF for the Interspecies/Animal-to-Human (A) Toxicodynamic Component (D) (EF_{AD})	Ethylene glycol monobutyl ether	*In vivo* toxicity evaluations to identify effects to quantify in both species *in vitro*
	Dimethyl arsenic acid	Mode of action analysis, use of *in vitro* data, use of genomics data
	N-methyl carbamate pesticides	Application of a DDEF to a common mechanism group of chemicals, use of DDEF approach to identify data gaps
EF for the Intraspecies/Within Human Variability (H) Toxicokinetic Component (K) (EF_{HK})	Boron	Clearance mechanism identified, surrogate measures of clearance employed for quantitation
	Methylmercury	Toxicokinetic model developed using human data from dietary exposures, choice of dose metric from among several surrogates

[a]The IRIS Assessments for these chemicals calculate a human equivalent dose—based on the results from physiologically based pharmacokinetic modeling. This document guides the development of a DDEF that results in the same human external dose when adjusted for interspecies differences in dosimetry. The DDEF concept applied to interspecies differences in dosimetry makes explicit the magnitude of species differences, whereas methods that calculate a human equivalent dose or human equivalent concentration do not make that calculation explicit.

A.1. INTERSPECIES EXTRAPOLATION FACTOR FOR TOXICOKINETICS

A.1.1. Ethylene Glycol Monobutyl Ether—Interspecies Extrapolation Factor for Toxicokinetics Case Study

A.1.1.1. *Summary*

Ethylene glycol monobutyl ether (EGBE; also known as 2-butoxy ethanol) has an established reference dose (RfD) of 0.1 mg/kg-day in EPA's IRIS database (U.S. EPA, 2010b). This value is based on a human equivalent dose (HED) developed from the results of a chronic inhalation study (NTP, 2000). Internal doses resulting in hemosiderin deposition in liver were extrapolated via physiologically based pharmacokinetic (PBPK) modeling to identify the HED.

As described below, the IRIS RfD is based on a PBPK modeling approach that identified an HED of 1.4 mg/kg-day. To this value, a combined UF of 10 was applied, which comprised a UF value of 10 for variation in sensitivity within the human population/intraspecies UF (UF_H), a value of 1 for the interspecies UF for the toxicokinetic (TK) component (UF_{AK}) (based on application of a PBPK model), and a value of 1 for interspecies toxicodynamic uncertainty factor (UF_{AD}) on the basis of quantified differences in red blood cell (RBC) sensitivity defined *in vitro* [as discussed in the EGBE interspecies/animal-to-human extrapolation factor (EF) for the toxicodynamic (TD) component (EF_{AD}) case study presented later in this appendix].

A.1.1.2. *Hazard Identification and Dose-Response*

No chronic-duration oral studies are currently available for EGBE; there are only two subchronic-duration, 91-day drinking water studies in rats and mice (Dieter, 1993) and a chronic inhalation study in rats and mice (NTP, 2000). Based on a comparison of no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect level (LOAELs) for hematologic and liver effects, rats are clearly more sensitive than mice. Hematologic and hepatocellular changes were noted in both sexes of rats. Hemosiderin accumulation in liver of male rats, a consequence of hemolysis, was chosen as the critical effect. Hemolysis in humans has been demonstrated to result in hemosiderin accumulation in the liver, and acute exposure to EGBE has been shown to produce hematological changes in humans (U.S. EPA, 2010b).

In the 2010 IRIS assessment, PBPK modeling was used to convert each of the inhalation exposures to levels of internal dose, and this measure of dose was combined with response levels. A benchmark dose (BMD) analysis revealed the lower confidence bound on benchmark dose (BMDL) corresponding to a 10% increase in response ($BMDL_{10}$) value, which was chosen as the point of departure (POD). The $BMDL_{10}$ value (expressed as internal dose) was 133 μmol butoxy acetic acid/liter-hour; this was used as the starting point in the development of the IRIS RfD for hemosiderin deposition in male rat liver (NTP, 2000). In the IRIS assessment, the

dose-response relationship was developed by combining PBPK modeling with BMD analysis as shown in Figure A-1.

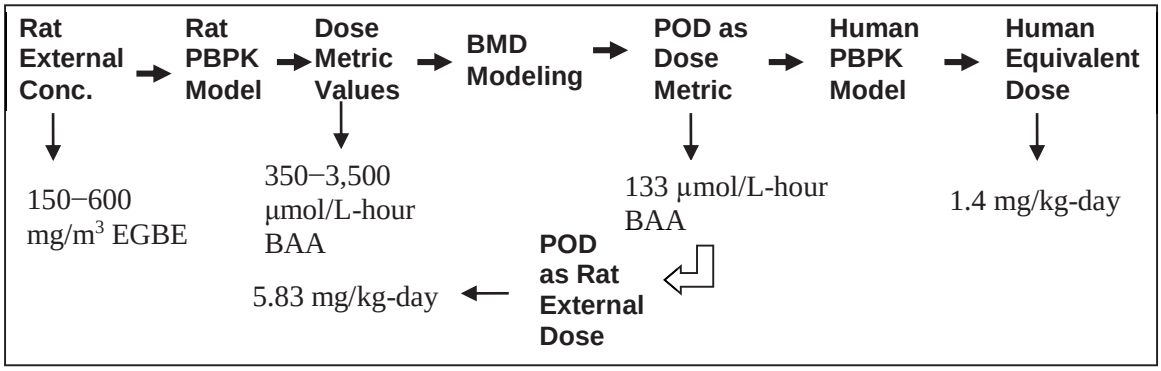

Figure A-1. Analysis approach for developing the human equivalent dose (HED) with values shown.

A.1.1.3. *Basis for Data-Derived Extrapolation Factor*

Area under the curve (AUC) values for the EGBE metabolite 2-butoxyacetic acid (BAA) in arterial blood of male rats following oral exposure were estimated using the PBPK model of Corley et al. (1994) as modified by Corley et al. (1997). BAA levels, rather than EGBE levels, were deemed appropriate measures of exposure based on the findings of Carpenter et al. (1956), who demonstrated *in vitro* that concentrations of 0.1% BAA induced hemolysis; whereas, hemolysis was not induced by EGBE until concentrations of approximately 2.5% were reached. PBPK modeling was used to translate each of the external concentrations to levels of the dose metric, AUC.

Next, BMD modeling was applied to the results of the PBPK modeling. The results of this analysis indicated that an AUC value of 133 µmol/L-hour BAA in arterial blood is the $BMDL_{10}$ for increased hemosiderin deposition in the liver in male rats.

The AUC value for BAA of 133 µmol/L-hour in arterial blood was chosen as the POD for interspecies extrapolation. Next, a human PBPK model was employed to translate this level of the dose metric to an HED of 1.4 mg/kg-day.

A.1.1.4. *Data-Derived Extrapolation Factor Derivation*

The PBPK modeling approach described above was used as the basis for the development of the DDEF. In addition to the data presented in the IRIS file, the rat PBPK model was run to identify that an external dose of 5.83 mg/kg-day in rats would produce this $BMDL_{10}$

concentration (133 μmol/L-hour) of the BAA metabolite (Dr. Richard Corley, personal communication, 2010). The human PBPK model was run to identify the external dose that produced the same level of the dose metric (133 μmol/L-hour); this value of the dose metric was produced by an external dose of 1.4 mg/kg-day in humans. The AUC dose metric was used as the basis for human equivalence.

An alternative approach for deriving the DDEF illustrated in this case study would be to use the ratio of rat-to-human external doses derived from the PBPK model to derive an interspecies toxicokinetic extrapolation factor (EF_{AK}; see eq 1, Section 3.2.2.2.). Using the data described above, a comparison of the external doses would yield an EF_{AK} value of 5.83 mg/kg-day ÷ 1.4 mg/kg-day = 4.2. The 4.2-fold DDEF would replace the default UF_{AK} threefold factor.

Applying the EF_{AK} 4.2-fold[10] to the rat external dose (5.83 mg/kg-day) yields an HED of 1.4 mg/kg-day. This HED is identical to that derived in the IRIS assessment (U.S. EPA, 2010b).

The above methods can also be compared to the default methodology that is based on body-weight scaling (U.S. EPA, 2011). Using a study-specific female rat body weight of 0.188 kg and a default human body weight of 70 kg, these values would result in a default dosimetric adjustment factor (DAF) = $(0.188 \div 70)^{1/4}$ = 0.23. The DAF is multiplied by the animal external dose (5.83 mg/kg-day) to yield an HED of 1.3 mg/kg-day. The reciprocal of the 0.23 DAF is 4.3. When using body weight to ¾ power scaling, the accompanying reduced default interspecies UF is threefold. In this case study, the body-weight scaling default approach yields a similar RfD as the data-derived approaches described above. However, the DDEF approach makes use of quantitative TK data for EGBE and uses the TK/TD framework, which preserves the option also to rely on quantitative TD information.

A.1.1.5. *References for Case Study A.1.1*

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A.1.2. Vinyl Chloride—Interspecies Extrapolation Factor for Toxicokinetics Case Study

A.1.2.1. *Summary*

Vinyl chloride (VC) has an established oral RfD of 3×10^{-3} (0.003) mg/kg-day in the current IRIS file (U.S. EPA, 2000a). The POD for liver toxicity is a NOAEL value of 0.13 mg/kg-day from a chronic feeding study in male Wistar rats. PBPK modeling was used to develop an HED of 0.09 mg/kg-day, making the UF_{AK} unnecessary. The composite UF of 30 comprises a UF of 3 to cover TD differences between species and a default value of 10 for intraspecies variability. Species differences in dosimetry were determined on the basis of external doses required to produce the same level of the toxicologically active metabolite in the critical organ (liver). This case study demonstrates the application of data to inform EF_{AK}.

A.1.2.2. *Hazard Identification and Dose-Response*

The liver was selected as the critical target organ of VC in humans and experimental animals. Strong epidemiological evidence exists for liver effects in humans. Studies involving workers in the polyvinyl chloride plastics industry from several countries have demonstrated a significant relationship between VC inhalation exposure and liver cancer. While limited evidence may suggest a risk for other, nonliver tumors (e.g., leukemia, brain, lung, pancreas, mammary), “vinylchloride is not likely to be associated strongly with cancers other than liver in humans” (U.S. EPA, 2000a). Other noncancer effects noted in epidemiologic investigations include impaired liver function and biochemical and histological evidence of liver damage and focal hepatocellular hyperplasia. Pulmonary function appeared unimpaired, and no solid evidence of teratogenicity in humans has been identified.

Limited data are available for inhalation studies in animals. Data are available from a 12-month inhalation study, which supports the liver as the critical organ. Bi et al. (1985) exposed Wistar rats to 0, 10, 100, or 3000 ppm VC for 6 hours/day, 6 days/week. Cellular alterations, degeneration, and necrosis were observed in the seminiferous tubules of the testes, with a NOAEL in the 10-ppm exposure group. This same exposure was the LOAEL for liver effects, characterized only as liver weight changes.

Like the inhalation results, studies conducted via the oral route identify the liver as the critical target organ. Til et al. (1991; 1983) reported the results of two-year rodent bioassays with VC in feed. Groups of 100 or 50 male and female Wistar rats were exposed to 0, 0.014, 0.13, or 1.3 mg/kg-day in feed for only 4 hours/day to minimize volatilization. The VC content of feed was measured before and after feeding to control for volatilization. Multiple hepatic effects were noted, including several that were deemed neoplastic or preneoplastic. The pathologists were able to delineate and determine incidences for two effects not thought to represent neoplastic or preneoplastic changes. Liver cell polymorphisms and proliferative bile duct epithelium cysts served as the basis for identifying the liver as the critical target tissue for noncancer effects in the chronic bioassay. These same changes were observed in a second study, but the doses employed in that study (Feron et al., 1981) were higher than those employed by Til and coworkers. Because of a lack of confidence in the outcome from a BMD modeling approach based on external dose, a traditional (i.e., NOAEL) approach to dose-response evaluation for events not associated with carcinogenicity was used. The POD for species extrapolation was the NOAEL of 0.13 mg/kg-day for liver cell polymorphisms and bile duct cysts.

The initial process in the mode of action (MOA) appears to be the formation of reactive and short-lived metabolites that achieve only low steady-state concentrations. These metabolites are thought to be responsible for the toxic effects of VC (Bolt, 1978). Experiments that manipulated the longevity of cytochrome P450-derived metabolites demonstrated an inverse relationship between metabolite longevity and protein and nucleotide binding (Guengerich et al., 1981). Thus, the metabolism of VC to reactive intermediates was demonstrated to be a critical determinant of toxicity. Because of the short-lived nature of the metabolite(s), a measure of their concentration in the target tissue (liver, the site of their formation) was deemed the appropriate dose metric for quantitative application. This concept and approach has also been applied to methylene chloride (Andersen et al., 1987) and chloroform (ILSI, 1997).

A.1.2.3. *Basis for Data-Derived Extrapolation Factor*

The liver was determined to be the target organ, and evidence indicated that the formation of a reactive metabolite was likely responsible for the toxicity of VC. BMD modeling of external doses failed. Because of this, the POD for extrapolation was determined as the study

NOAEL for liver effects, 0.13 mg/kg-day. Consistent with the guidance for reference concentration derivation, PBPK modeling of the formation of the active metabolite in liver was used as the basis for determining an HED. PBPK models were developed for rats and humans and used to extrapolate dosimetry between species (Clewell et al., 1995b; Clewell et al., 1995a); the models were subjected to an external peer review and deemed sufficient for quantitative reliance. The NOAEL dose (0.13 mg/kg-day) was converted into the dose metric for VC—the amount of metabolite in the liver (AML), with units of concentration (mg/L) of liver.

A.1.2.4. *Data-Derived Extrapolation Factor Derivation*

Importantly for dose extrapolation, these models demonstrated a linear relationship between applied dose and the dose metric (i.e., AML) up to doses approximating 25 mg/kg-day. This allowed linear interpolation to be used to identify levels of external doses associated with specific amounts of the internal dose, rather than specific iterations via PBPK modeling. The rat NOAEL dose of 0.13 mg/kg-day produced AML at a value of 3.0 mg/L (see Figure A-2). This level of internal exposure in the 70-kg human was determined to result from a drinking water exposure of 0.09 mg/kg-day (the HED). Thus, doses of 0.13 mg/kg-day and 0.09 mg/kg-day in the rat and human, respectively, are toxicokinetically equivalent.

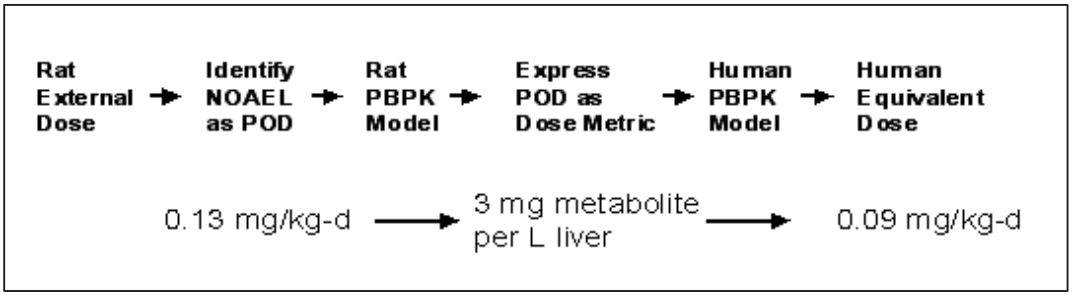

Figure A-2. Schematic for dose extrapolation for vinyl chloride. The POD was defined as the external dose in the rat, with PBPK modeling of the rat. PBPK modeling of the dose metric translated dose from units of applied dose to units of tissue concentration, representing the dose metric. PBPK modeling in the human identified the HED, the dose producing the same level of the dose metric in the rat study at the POD.

The IRIS file for VC used the HED as the POD to which UFs are to be applied. An alternative approach illustrated in this case study to deriving the DDEF for interspecies TK differences would be to use the ratio of rat and human external doses resulting in the same level of target tissue exposure at the POD.

Using the data described above, a DDEF value for EF_{AK} of 0.13 ÷ 0.09 = 1.44 is indicated. Combined with the default value of 3 for TD, a DDEF for EF_{AK} would be 3 × 1.44 = 4.32, in place of the default value of 10. By applying the DDEF calculated above (4.32-fold) and the default value of 10 for UF_H to the animal NOAEL (0.13 mg/kg-day), the RfD is 0.13 mg/kg-day ÷ 43.2 = 0.003 mg/kg-day. This is the same value that was calculated for the IRIS assessment, which was expressed as the HED/UF, or 0.09 mg/kg-day/30 (U.S. EPA, 2000a).

The above methods can also be compared to the default methodology that is based on body-weight scaling (U.S. EPA, 2011). Using a default value of 0.462 kg for adult Wistar rats (U.S. EPA, 1988) and applying the body-weight scaling approach described in *Recommended Use of Body Weight $^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011) to the NOAEL of 0.13 mg/kg-day, an HED can be calculated as:

$$0.13 \text{ mg/kg-day} \times 0.462 \text{ kg} = 0.060 \text{ mg}$$

$$0.06 \text{ mg} \times (70 \text{ kg} \div 0.462 \text{ kg})^{3/4} =$$

$$0.06 \text{ mg} \times 43.19 = 2.59 \text{ mg}$$

$$2.59 \text{ mg} \div 70 \text{ kg} = 0.037 \text{ mg/kg-day} \qquad \text{(A-1)}$$

In this case study, the body-weight scaling default approach yields a slightly lower POD value than the data-derived approaches described above. Furthermore, this approach makes use of quantitative data on VC.

A.1.2.5. *References for Case Study A.1.2*

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A.2. INTERSPECIES EXTRAPOLATION FACTOR FOR TOXICODYNAMICS

A.2.1. Ethylene Glycol Monobutyl Ether—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.1.1. *Summary*

As noted in Section A.1.1.1, EGBE has an established RfD of 0.1 mg/kg-day in EPA's IRIS database (U.S. EPA, 2010b). This value is based on the results of a subchronic-duration drinking water study in mice and rats where increases in Kupffer cell hemosiderin content secondary to hemolysis were determined to be the critical effect. Derivation of the HED of 1.4 mg/kg-day is detailed in the EGBE TK case study in Section A.1.1 and in the IRIS file for EGBE (U.S. EPA, 2010b).

The IRIS RfD is based on a PBPK modeling approach that identified an HED of 1.4 mg/kg-day. UFs were applied to this POD value to derive the RfD. A total UF of 10 was applied to this HED to derive the RfD. A default value of 10 was used to account for variation in sensitivity within the UF_H. The interspecies UF (UF_A) was reduced to 1 on the basis of dosimetry adjustments to account for TK (discussed case study A.1.1, for EGBE TK). EF_{AD} was also reduced to a value of 1, because studies indicate that humans may be significantly less sensitive than rats to the hematological effects of EGBE. *In this case study, however, issues associated with EGBE TD are described and an alternative approach to DDEF derivation based on the use of in vitro data are described.*

A.2.1.2. *Hazard Identification and Dose-Response*

Based on extensive review of the literature, hematologic effects appear to be the most sensitive of the adverse effects observed in laboratory animals exposed to EGBE. Hematologic effects (e.g., hemoglobinuria) have also been documented in worker populations exposed to technical grade EGBE (Cellosolve™) and following ingestion of cleaning products containing EGBE (U.S. EPA, 2010b).

Key events in the proposed MOA in RBCs leading to increases in hemoglobin accumulation in Kupffer cells include:

≠ Oxidative metabolism to BAA

≠ RBC swelling and lysis (probably preceded by an increase in osmotic fragility and loss of deformability)

≠ Decreased RBC count, hemoglobin, and hematocrit, and in response, increased production of immature RBCs (reticulocytes) by the bone marrow

Carpenter et al. (1956) incubated RBCs from rats, mice, rabbits, monkeys, dogs, humans, and guinea pigs with 0.1% BAA. Results demonstrating maximum time without hemolysis were 35−40, 40−45, 60−90, 103−120, 80−120, 147−268, and 360 minutes, respectively. These data indicate that guinea pigs and humans are more resistant and that rats and mice may be more sensitive to the hemolytic effects of BAA. Studies with Cellosolve and BAA demonstrated that RBCs were much more sensitive to BAA than to Cellosolve. These results led Carpenter et al. (1956) to speculate that the BAA metabolite of Cellosolve was responsible for hemolysis.

In a direct comparison of the effects of BAA on rat and human RBCs, Udden and Patton (1994) devised a study using filtration, phase contrast light microscopy, and routine hematologic methods. RBCs were obtained from healthy adults via venipuncture and from 9- to 11-week-old male Fischer 344 rats via cardiac puncture. Rat RBCs were incubated with 0.2 and 2.0 mM BAA; human RBCs were incubated with only 2.0 mM BAA. Rat RBC demonstrated 30% hemolysis after incubation with 2.0 mM BAA for 4 hours, and 4% hemolysis after incubation with 0.2 mM BAA for 6 hours or longer. The 4% measure was "mild," and the data were not shown. Incubation of human RBC with 2.0 mM BAA for 4 hours resulted in no increase in hemolysis over the background (control) level of 1%. Histologic evaluations of rat, but not human, RBC preparations postexposure demonstrated RBC "ghosts," which are cell membranes from lysed cells. These incubation conditions resulted in increases in mean corpuscular volume (MCV) in rat RBCs incubated with 0.2 mM BAA, but not human RBCs incubated with 2.0 mM BAA. The increase in MCV indicates RBC swelling, an event that leads to fragility.

Finally, these investigators examined the deformability of RBCs by pumping them through a narrow-diameter filter and monitoring an increase in inflow pressure. The results were presented graphically, as pressure versus time plots. The logic behind this study was that RBCs normally can deform to pass through tight spaces (like capillaries). However, RBCs in which swelling has been induced and in which other mechanisms may have been activated that can lead to increased membrane rigidity, will not pass through the membrane, resulting in an increase in inflow pressure. Rat RBCs demonstrated a nearly doubled pressure in rat RBCs incubated with 0.2 mM BAA for 4 hours, and a roughly tripled pressure when incubated with 2.0 mM BAA for 4 hours compared to controls. In contrast, pressure differences between human RBCs incubated in the absence of BAA and in the presence of 2.0 mM BAA for 4 hours were not distinguishable. Udden and Patton (1994) indicated that their findings that human RBCs were less sensitive *in vitro* to the hemolytic effects of BAA were consistent with multiple other findings. In a follow-up study, Udden (2002) demonstrated again that human RBCs were less susceptible to the

effects of BAA than RBCs from rats. Whereas rat RBCs demonstrated alterations including deformability and changes in MCV at 0.05 mM, human RBCs did not demonstrate changes in MCV at concentrations below 10 mM—a difference of 200-fold.

Ghanayem and Sullivan (1993) also performed a species comparison of BAA-induced RBC effects *in vitro*. An advantage of this study is that it applied a pairwise statistical design to determine the effect of treatment over control. Male Fischer 344 rats 15 weeks of age were used, and human blood was drawn from adult male donors. BAA (2.0 mM incubated up to 4 hours) produced only slight, but not statistically significant, alterations of hematological parameters: hematocrit was increased 2−4% and MCV was increased less than 4% above vehicle controls. The authors concluded that their results demonstrated that humans were "minimally sensitive" to the *in vitro* effects of BAA. In comparison, RBCs from rats were deemed "relatively susceptible" to the effects of BAA. A graphic presentation of data demonstrated that in rats, MCV and hematocrit were increased nearly 60% when exposed to 2.0 mM BAA.

Udden (2002) compared the subhemolytic and hemolytic effects of BAA in rat and human RBCs. RBC deformability, density, MCV, count, osmotic fragility, and hemolysis were measured following a 4-hour exposure. Alterations were noted in loss of deformability, but at concentrations that were 150-fold higher in rat RBCs than in human RBCs (0.05 mM vs. 7.5 mM). A larger species difference in response was noted in comparing effect levels for increases in MCV—these differences approximated 200-fold, with rat RBCs responding at 0.05 mM and human RBCs demonstrating alterations of MCV at 10 mM. Changes in osmotic fragility were similar.

A.2.1.3. *Basis for Data-Derived Extrapolation Factor*

Several data sets are available in which the responsiveness of rat and human blood at 2.0 mM can be compared. *However, comparison of effect data for TD is most appropriately accomplished by comparing the different concentrations resulting in the same response.*

Hemolytic effects were observed in rat RBCs exposed to BAA at concentrations as low as 0.05 mM. In contrast, similar hemolytic effects were observed in human blood exposed to 10 mM BAA. The IRIS file indicates that humans may be much less sensitive than rats to the hematologic effects of EGBE, and for this reason a value of 1 was selected for the UF_{AD}.

A.2.1.4. *Data-Derived Extrapolation Factor Derivation*

One potential approach would be to derive an adjustment factor of 0.005 for UF_{AD} (0.05 mM/10 mM = 0.005). This approach requires the assumption that the TD differences observed *in vitro* would be approximated *in vivo*. Uncertainty about this issue prompted EPA to

take a different approach. In the IRIS assessment (U.S. EPA, 2010b), the UF value for UF_{AD} was established at 1.

Regarding TD, *in vivo* (Carpenter et al., 1956) and *in vitro* (Udden, 2002; Udden and Patton, 1994; Udden, 1994; Ghanayem and Sullivan, 1993) studies indicate that humans may be significantly less sensitive than rats to the hematological effects of EGBE. For this reason, a value of 1 was selected for the TD portion of the UF_A.

A.2.1.5. *References for Case Study A.2.1*

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A.2.2. Dimethyl Arsenic Acid—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.2.1. *Summary*

Dimethyl arsenic acid (DMA), also known as cacodylic acid, is an herbicide used on primarily cotton and turf. DMA is also a urinary metabolite in most mammals, including humans, following direct exposure to inorganic arsenic. The Office of Pesticide Programs (OPP), in collaboration with National Health and Environmental Effects Research Laboratory scientists, developed a nonlinear MOA assessment for DMA for the development of rat bladder tumors (U.S. EPA, 2006b). The MOA and dose-response assessments were developed using the MOA (Sonich-Mullin et al., 2001) and Human Relevance (Boobis et al., 2006) Frameworks. The information provided by the MOA analysis also provides the basis for the chronic RfD and

the respective UFs in the RED for cacodylic acid. Instead of the default 10-fold factor to extrapolation from animal to human, OPP has reduced the TD component of UF_A (EF_{AD}) to a value of 1. This case study describes the determination of the one-half order of magnitude (threefold) factor, which was used to account for TD differences between animals and humans. The default value of 10 for UF_H was also applied leading to a composite factor of 30.

A.2.2.2. *Hazard Identification and Dose-Response*

In rat carcinogenicity studies, oral exposure to DMA^V leads to bladder tumors. The overall weight of the evidence provides convincing support for a nonlinear MOA for DMA^V-induced carcinogenesis in rodents. The key events include:

- ≠ Reductive metabolism of DMA^V to DMA^{III}.
- ≠ DMA^{III} causes urothelial cytotoxicity. Regenerative cell proliferation then ensues in order to replace dead urothelial cells. The amount of cell killing is a function of the severity of the cytotoxicity, which is related to the amount of DMA^{III} present. The amount of DMA^{III} is dependent on the conversion of DMA^V to DMA^{III}.
- ≠ Sustained cytotoxicity leads to regenerative cell proliferation, which in turn, ultimately leads to hyperplasia and bladder tumors.

To obtain a tumor via the proliferation/replication genetic error process, induced cell proliferation needs to be persistent. There is convincing experimental evidence to indicate that this is the case for the rat bladder. There is a clear association of DMA^V treatment and cell killing/regenerative proliferation and bladder tumors. The amount of proliferation would be a function of the amount of cell killing since the tissue will undergo regenerative proliferation in response to cell killing. As the severity of cytotoxicity increases with increasing levels of DMA^V (DMA^{III}), regenerative proliferation is the rate limiting step for tumor formation, even though the product is chromosome mutations. Thus, a tumor dose-response curve would be influenced by the induced cell proliferation curve, even though chromosomal mutations may be an output. DMA^V-induced tumors would be produced only at treatment durations and dose levels that result in significant cell killing and regenerative cell proliferation in the urothelium of the bladder. Experimental data are available to support the coincidence of key events at similar concentration levels. The levels of DMA^{III} in the urine of rats treated with 100-ppm DMA^V range from 0.5−5.0 μM. The lethal concentration for 50% of the population (LC_{50}) values for DMA^{III} in rat and human urinary epithelial cells *in vitro* are 0.5−0.8 μM. A significant increase in chromosome aberrations occurs in human lymphocytes *in vitro* at about 1.35 μM DMA^{III}. At

100 ppm, there is significant cell killing and regenerative proliferation in female rat bladders. It appears that chromosomal mutations, cytotoxicity, and cell proliferation can potentially occur concurrently at 100-ppm DMA^V, which is the tumorigenic dose in female rats via diet.

Among the several key events, all of which are necessary for tumor formation, cell proliferation has been used for deriving a POD because it is needed for increasing the likelihood of chromosome mutation formation and for the perpetuation of genetic errors, as well as for hyperplasia. A $BMDL_{10}$ value (0.43 mg/kg bw/day) is the basis for the POD in deriving an RfD or a margin of exposure (MOE). This approach is considered public health protective because a $BMDL_{10}$ of 0.43 mg/kg bw/day is approximately an order of magnitude lower than the dose (~0.7 mg/kg bw/day or 10 ppm) that resulted in a 1.5-fold statistically nonsignificant increase in cell proliferation after 10 weeks of exposure to DMA^V and about two orders of magnitude lower than the dose (~9.4 mg/kg bw/day) resulting in neoplasia in the feeding studies.

A.2.2.3. *Basis for Data-Derived Extrapolation Factor*

In the 2006 DMA risk assessment (U.S. EPA, 2006b), instead of the default 10-fold factor to extrapolate from animal to human, the OPP reduced the TD component to 1. A factor of 3 was used to account for interspecies differences in TK. UF_{AD} was reduced to 1 given that, at a similar dose at the target site (i.e., bladder urothelial), humans and rats are expected to respond pharmacodynamically similar. This case is built on a combination of information:

≠ Chemical-specific *in vitro* data from Cohen et al. (2002) which show that human and rat cells respond similarly to exposure to DMA^{III}—the LC_{50} values for cytotoxicity in human and rat epithelial cells were very similar (0.8 μM and 0.5 μM, respectively).

≠ There is microarray support (Sen et al., 2005). Qualitatively the genes that are upregulated in human urinary bladder epithelial cells (UROtsa) are similar to those upregulated in rat urinary bladder epithelial cells (MYP3) exposed to DMA^V *in vitro*. In this study, the rat cell line was quantitatively more sensitive compared to the human cell line.

≠ General information on the development and function of the bladder along with incidence of bladder tumors in human populations qualitatively supports the animal MOA in humans.

There are known pharmacokinetic differences between rats and humans. These pharmacokinetic differences include sequestration of DMA^{III} by rat hemoglobin, which results in a longer retention time in the rat compared to humans or mice, and the increased urinary output of trimethylarsine oxide in rats compared to humans. Because of uncertainties regarding

quantifying the tissue dose in humans using rat data, and in the absence of a fully developed PBPK model at the time of the risk assessment, an EF_A of 3 was applied. Pharmacokinetic analyses indicate that, for similar chronic low-level exposures, rats would take longer to achieve steady-state concentrations of DMA^V and metabolites in target tissue compared to humans, and that for a given exposure target tissue, concentrations would be elevated for a longer time after exposure ceased in the rat because rat hemoglobin acts as a slow-release storage depot. Note that the half-life in the rat for DMA^V appears to correlate with erythrocyte half-life, indicating that the binding to hemoglobin is not readily reversible. There are, however, uncertainties regarding the quantitative differences between rats and humans that prevent further reduction of the UF_A.

A.2.2.4. *Data-Derived Extrapolation Factor Derivation*

A value of 1 was developed for EF_{AD} based on the rationale above in combination with the animal MOA and implementation of the Human Relevance Framework. Together, the weight of the evidence provides a strong case for TD equivalence between rats and humans. A factor of 3 was used to account for interspecies differences in TK. The default value of 10 for UF_H was also applied, leading to a composite factor of 30.

A.2.2.5. *References for Case Study A.2.2*

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A.2.3. N-methyl Carbamate Pesticides—Interspecies Extrapolation Factor for Toxicodynamics Case Study

A.2.3.1. *Summary*

OPP released its revised cumulative risk assessment (CRA) for the N-methyl carbamates (NMCs) in 2007 (U.S. EPA, 2007). As required under the Food Quality Protection Act (FQPA) (FQPA, 1996), a CRA incorporates exposures from multiple pathways (i.e., food, drinking water, and residential/nonoccupational exposure to pesticides in air, or on soil, grass, and indoor surfaces) for those chemicals with a common mechanism of toxicity (FQPA, 1996). This CRA began with the identification of a group of chemicals, called a common mechanism group (CMG), which induces a common toxic effect by a common mechanism of toxicity. The NMCs were considered to be a CMG due to their common inhibitory actions on acetylcholinesterase (AChE), an enzyme that is normally required for ending cholinergic transmission in the nervous system.

This case study describes a sensitivity analysis conducted in the risk characterization phase of the revised CRA using a DDEF approach. TD equivalence for animal-to-human extrapolation ($EF_{AD} = 1$) was assumed for several chemicals for which human data were not available.

A.2.3.2. *Hazard Identification and Dose-Response*

The NMCs were established as a CMG by EPA in 2001 (U.S. EPA, 2001a) based on their similar structural characteristics and shared ability to inhibit AChE by carbamylation of the serine hydroxyl group located in the active site of the enzyme. When AChE is inhibited, acetylcholine accumulates and results in cholinergic toxicity, due to continuous stimulation of cholinergic receptors throughout the central and peripheral nervous systems that innervate virtually every organ in the body. An important aspect of NMC toxicity is the rapid nature of the onset and recovery of effects; following maximal inhibition of cholinesterase (typically between 15 and 45 minutes), recovery occurs rapidly (minutes to hours).

Inhibition of AChE is considered the first and critical step in the toxicity of NMCs. Human health monitoring has capitalized on the availability of blood cholinesterase measurements, and these have been widely accepted as a marker of exposure. However, since the brain may be considered more as the critical target site, data on inhibition of brain AChE are obviously only available using laboratory animals. Brain AChE data have been widely used as a POD for risk assessment.

EPA used the relative potency factor (RPF) method to determine the combined risk associated with exposure to NMCs. Briefly, the RPF approach uses an index chemical as the

point of reference for comparing the toxicity of the NMC pesticides. RPFs are calculated as the ratio of the toxic potency of a given chemical to that of the index chemical and are used to convert exposures of all chemicals in the group into exposure equivalents of the index chemical. Because of high-quality dose-response data for all routes of exposure, as well as high-quality time-to-recovery data, EPA selected oxamyl as the index chemical for standardizing the toxic potencies and calculating RPFs for each NMC pesticide.

A.2.3.3. ***Basis for a Data-Derived Extrapolation Factor***

In the single chemical assessments for most NMCs, a default value of 10 for animal-to-human/intraspecies extrapolation factor (EF_H) was used. For three NMCs (aldicarb, methomyl, and oxamyl), however, studies in human subjects were determined by EPA to be ethically and scientifically acceptable for use in risk assessment, after considering the advice of the Human Studies Review Board. These studies were used to derive the chemical-specific EF_A for these three chemicals. Table A-2 summarizes the dose-response and time-course modeling data for critical rat and human studies for these three NMCs.

Table A-2. N-methyl carbamate cumulative risk assessment: interspecies/animal-to-human extrapolation factors and corresponding rat and human BMD_{10}s and $BMDL_{10}$s

	Rat						Human			
	Brain			RBC			RBC			
Chemical	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	BMD_{10} (mg/kg)	$BMDL_{10}$ (mg/kg)	Half-life (hr)	UF_A
Aldicarb	F = 0.048 M = 0.056	F = 0.035 M = 0.035	1.5	0.031	0.020	1.1	0.016	0.013	1.7	2
Methomyl	0.486	0.331	1.0	0.204	0.112	0.8	0.040	0.028	1.6	5
Oxamyl	F = 0.145 M = 0.185	F = 0.111 M = 0.143	0.9	0.278	0.158	0.8	0.083	0.068	2.4	3

With regard to the EF_{AK}, NMCs have similar metabolic profiles across species. NMCs do not require activation; the parent compound is an active AChE inhibitor. Although some metabolites of NMCs have been shown to be active as well, none have been shown to be more potent than the parent chemical. Thus, metabolism is considered to be a detoxification process. As such, species differences in tissue dosimetry are likely correlated with differences in body weight to the ¾ power (U.S. EPA, 2011).

The mechanism of toxic action of NMCs is reproducible across a range of species, including rodents and humans. In addition, the AChE enzyme in humans and rats has similar function and structure. See reviews by Radić and Taylor (2006) and Sultatos (2006). The half-life to recovery values[11] for rats and humans provided in Table A-2 range from approximately 1 to 2 hours and demonstrate the similarity of the half-lives of the two species. Based on this information, given a similar dose or concentration at the target site, it is likely that human and rat AChE would respond similarly. This understanding can inform the interspecies DDEF (EF_{AD}).

A.2.3.4. *Data-Derived Extrapolation Factor Derivation*

For the CRA, toxic potencies for the NMCs were determined using brain AChE inhibition measured at peak inhibition following gavage exposures in rats. The Agency used an exponential dose-time-response model to develop BMD estimates at a level estimated to result in 10% brain cholinesterase inhibition (i.e., a BMD or BMD_{10}) to estimate RPF. Ratios comparing doses (administered) that produce the same magnitude of effect may then be derived. Using the data in Table A-2, and dividing the $BMD_{10\text{-rat}}$ by the $BMD_{10\text{-human}}$ for RBC cholinesterase inhibition:

$$\text{Aldicarb: } 0.031 \div 0.016 = 1.9 \approx 2 \quad \text{(A-2)}$$

$$\text{Methomyl: } 0.204 \div 0.040 = 5.1 \approx 5 \quad \text{(A-3)}$$

$$\text{Oxamyl: } 0.278 \div 0.083 = 3.3 \approx 3 \quad \text{(A-4)}$$

This analysis showed that the ratio of the BMDs for rat/human ranges from 2 to 5 for these NMCs. This range would tend to support the DDEF approach described here to reduce the standard interspecies factor value from 10 to 3. The concentration of toxicant at the active site *in vivo* is controlled by TK processes. The available data described the TD processes (enzyme regeneration) and indicated that rats and humans were very similar in this area. The remaining threefold (default) value for UF_A addresses species differences in TK.

[11]Recovery half-life differs from elimination half-life. While elimination half-life is not an acceptable basis for calculation of a DDEF for *toxicokinetic* components, enzyme regeneration measurements (the subject of the present analysis) are also expressed in half-life values. This usage refers to regeneration of enzymatic activity or *de novo* synthesis of additional enzyme (protein). In this instance, "half-life" measures are an adequate basis for derivation of DDEF values for *toxicodynamic* events.

It may be possible to use *in vitro* studies using human and rat tissues and human and rat AChE to test this hypothesis. In other words, it may be possible to use *in vitro* studies to demonstrate TD equivalence between rats and humans. If these data were available and they showed TD equivalence, the Agency could reduce the interspecies factor for those NMCs to a value of 3 without human toxicity studies. Due to the lack of these *in vitro* studies, the Agency does not believe it appropriate at this time to refine the standard for EF_A of 10 further. Instead, the Agency has used the DDEF approach as a sensitivity analysis in its risk characterization.

In this sensitivity analysis, the interspecies factor was reduced from 10 to 3 based on the assumption of TD equivalence for carbaryl, carbofuran, and formetanate HCl. These three NMCs were identified since they were shown in the CRA to contribute a large portion of the estimated human exposure to the cumulative risk to this group. The results of this sensitivity analysis for the food exposure assessment are shown below in Table A-3. The Agency has used a probabilistic approach to the food exposure assessment. The estimated exposures of the NMC as a group (i.e., oxamyl equivalents) and the MOE at the 99.9th percentile are shown here. The target MOE is 10 or higher for EF_H. The table compared the MOEs when using the standard UF to those obtained with the modified UF_A.

Table A-3. Sensitivity analyses N-methyl carbamate cumulative food assessment: Data-derived extrapolation factor approach for interspecies uncertainty factors

Age group		Estimated exposure at the 99.9th percentile (mg/kg oxamyl equivalents)	MOE at the 99.9th percentile	Percentile at which target of 10 is reached
Baseline CRA	Children 1−2	0.0229	7.9	99.848th
	Children 3−5	0.0209	8.6	99.870th
DDEF approach for interspecies UF	Children 1−2	0.0183	9.8	99.896th
	Children 3−5	0.0171	10.5	N/A

Review of this table shows that:

≠ Using the standard interspecies factor of 10 for all NMCs without human data, the (baseline) MOEs at the 99.9th percentile of exposure are less than 10 for children 1−2 and 3−5 years of age. Furthermore, MOEs reach the target of 10 at the 99.848th and 99.870th percentiles of exposures.

≠ When considering an alternative approach to the interspecies factor that assumes a value of 3 for carbaryl, carbofuran, and formetanate HCl, the MOEs at 99.9th percentile of exposure increase to 9.8 and 10.5 for children 1−2 and 3−5 years of age, respectively—a 20% increase in MOEs. The exposure for the younger age group reaches the target MOE of 10 at the 99.896th percentile of exposures.

This sensitivity analysis suggests that additional data could provide a substantial improvement in the refinement of the CRA.

A.2.3.5. *References for Case Study A.2.3*

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A.3. INTRASPECIES EXPTRAPOLATION FACTOR FOR TOXICOKINETICS

A.3.1. Boron and Compounds—Intraspecies Extrapolation Factor for Toxicokinetics Case Study

A.3.1.1. *Summary*

In the EPA IRIS summary file for Boron and Compounds (U.S. EPA, 2004b), an RfD value of 2×10^{-1} mg/kg-day has been developed based on the critical developmental effect of decreased fetal weights (U.S. EPA, 2004b). The POD for the derivation of this value is the $BMDL_{05}$ value of 10.3 mg/kg-day. The $BMDL_{05}$ is based on the combined results of two separate studies chosen for the derivation of the RfD (Price et al., 1996; Price et al., 1994; Heindel et al., 1992). Using data from rats (Vaziri et al., 2001) and humans (Pahl et al., 2001), a mathematical model was applied to the EF_{AK} to address interspecies TK. The EF_{AK} was calculated to be 3.3. An intraspecies toxicokinetic extrapolation factor (EF_{HK}) of 2.0 was estimated from three studies (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981), using glomerular filtration rate (GFR) as a surrogate for boron clearance. The remaining uncertainty in the RfD derivation was from TD. Interspecies and intraspecies TD uncertainty were each assigned the default value of one-half order of magnitude (3.16). The product of all the adjustment and subfactors served as the total adjustment factor of 66. The RfD was derived by dividing the $BMDL_{05}$ of 10.3 mg/kg-day by the adjustment factor and rounding to one digit. This case study demonstrates the use of data to develop an EF_{HK}. Specifically, it demonstrates how a value of 2.0 for EF_{HK} was identified from three studies (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981), using GFR as a surrogate for boron clearance.

A.3.1.2. *Hazard Identification and Dose-Response*

Oral animal studies have identified the testes and the developing fetus as the two most sensitive targets of boron toxicity in multiple species (U.S. EPA, 2004b). Testicular effects include reduced organ weight and organ-to-body weight ratio, atrophy, degeneration of the spermatogenic epithelium, impaired spermatogenesis, reduced fertility, and sterility. The mechanism of action for boron's effect on the testes is not known, but the available data suggest an effect on Sertoli cells. Developmental effects following oral exposure to boron have been reported in mice, rabbits, and rats, and include high prenatal mortality, reduced fetal body weight, and malformations and variations of the eyes, central nervous system, cardiovascular system, and axial skeleton. Similarities in the NOAEL values for the reproductive toxicity studies and quality control issues complicated the choosing of testicular effects as the critical effect.

Developmental effects (decreased fetal weights) are considered the critical effect and serve to identify the sensitive population—the fetus of the pregnant female. The studies by Price et al. (1996; 1994), Heindel et al. (1992), and NTP (1990) in rats were chosen as critical developmental studies because they were well-conducted studies of a sensitive endpoint that identified both a NOAEL and LOAEL. Rats were more sensitive than mice and rabbits, which were also studied for developmental toxicity.

The POD was determined by BMD modeling. BMD evaluation of multiple developmental endpoints identified decreased fetal body weight as the most suitable endpoint. Two studies (Price et al., 1996; Price et al., 1994; Heindel et al., 1992) provided data on fetal body weight, and the results were combined for BMD evaluation. The benchmark response (BMR) level for mean fetal weight was chosen to be the $BMDL_{05}$ value of 10.3 mg/kg-day.

No data are available to identify an MOA, but boron is absorbed, distributed, and eliminated unchanged in urine. It is not metabolized, so some measure of exposure to the parent compound should serve as the basis for dose (exposure) expression.

A.3.1.3. *Basis for Data-Derived Extrapolation Factor*

Following administration, boron is rapidly absorbed and distributed throughout the body. It distributes with total body water; concentrations in all tissues examined were similar. Bone and fat tissues represent outliers—boron seems to accumulate in bone and the low water content of adipose tissue reduces boron distribution to fat.

Given the relatively uniform distribution of boron to the tissues and that the majority of the compound is excreted quickly, the likelihood for sequestration of boron by a given tissue is minimal. Although there are no direct measurements of fetal boron concentrations, boron concentrations in the fetus should be the same as in the mother because boron is freely diffusible across biological membranes and will rapidly and evenly equilibrate in all body water compartments. As the boron RfD is based on developmental effects observed in rats, the most relevant kinetic data are those pertaining to pregnant rats and pregnant humans. Given the difficulty in obtaining tissue boron concentrations in the developing fetus, data on plasma boron in these species were considered; however, data were insufficient to compare plasma boron in rats and humans at the same exposure levels. Therefore, boron clearance is used as an estimator of internal dose. Again, complications of the availability of data on boron clearance in a large enough population sufficient to support reliable estimates of variability were identified.

Since boron is not metabolized, clearance from blood and tissues is via urinary elimination. Boron is a small, uncharged molecule, and data indicate a lack of protein binding. Evidence from human dialysis studies indicates clearance is via passive diffusion. These (and other) data identify passive renal mechanisms as those most governing boron clearance. Because

the molecular and physical attributes of boron were consistent with those for agents eliminated by glomerular filtration and because boron clearance correlated with measures of glomerular filtration in some studies, variability of GFR was deemed an acceptable surrogate for variability of boron clearance among pregnant women. Table A-4 lists several studies that have characterized the variability of GFR among pregnant humans. The application of these data describing variance of GFR among pregnant humans serves as the basis for estimating human intraspecies differences in internal exposure.

Table A-4. Measures of glomerular filtration rate variability among pregnant women

Study	Mean GFR (mL/min)	Standard Deviation
Dunlop	150.5[a]	17.6
Krutzen	195[b]	32
Sturgiss	138.9[c]	26.1

[a]Serially averaged observations across three time periods (16, 26, and 36 weeks) for 25 pregnant women.

[b]Third-trimester values for 13 pregnant women.

[c]Serially averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals).

A.3.1.4. *Data-Derived Extrapolation Factor Derivation*

For the assessment of intraspecies TK variability, GFR is used as a surrogate for boron clearance. Although the study of Pahl et al. (2001) provides an estimate of boron clearance variability in pregnant women, the data are judged to be inadequate for this purpose. As boron clearance is largely a function of GFR, GFR is considered to be an appropriate surrogate and there is a larger, more certain database on GFR and its variability among humans than on boron clearance. Thus, the GFR database is used to estimate boron clearance variability. Because the measured boron clearances in the rat and human kinetic studies were less than GFR, tubular reabsorption could be contributing to the variability of boron clearance. Variability in these factors, however, is judged to be minor in comparison to the variability in GFR.

GFR data have been used previously in the context of the boron RfD by Dourson et al. (1998), who proposed the ratio of the mean GFR to the GFR value two standard deviations (SDs) below the general population mean (mean ÷ [mean − 2 SD]) as the metric for the EF_{HK}. This

approach is referred to as the sigma method, which is a common term used for statistical methods using multiple SDs to establish "acceptable" lower bounds.

For the derivation of EF_{HK}, for reasons described here, the sigma method is modified by using 3 SD as the reduction factor for establishing the lower bound (i.e., mean GFR − 3 SD). The basic formula modified from Dourson et al. (1998) for EF_{HK} is:

$$GFR_{AVG}{}^{EFHK} = GFR_{AVG} - 3\ SD_{GFR} \qquad \text{(A-5)}$$

where GFR_{AVG} and SD_{GFR} are the mean and SD of the GFR (mL/minute) for the general healthy population of pregnant women. The use of 3 SD rather than 2 SD (as in Dourson et al., 1998) is based on a statistical analysis of the published GFR data, with more consideration being given to the full range of GFR values likely to be found in the population of pregnant women. In the aggregate, the data suggest that a lower bound GFR 2 SD below the mean does not provide adequate coverage of the susceptible subpopulation (those pregnant women experiencing or predisposed to preeclampsia who have lower GFR values). While no conclusive information exists from controlled-dose studies in humans, it may be possible that the variability in boron clearance might be greater than GFR variability, but this is not expected. The uncertainty surrounding this possibility is low. Therefore, EF_{HK} must also account for any residual uncertainty in using GFR as a surrogate.

The three studies listed in Table A-4 (Sturgiss et al., 1996; Krutzén et al., 1992; Dunlop, 1981) were found to address GFR variability in pregnant women. Dunlop (1981) assessed GFR for 25 women at 3 different time points during pregnancy (16, 26, and 36 weeks) and again after delivery. In this study, GFR was measured as inulin clearance and the overall average and SD was 150.5 and 17.6 mL/minute, respectively. Sturgiss et al. (1996) performed a similar assessment of GFR (also using inulin clearance) for 21 women in early (12−19 weeks) and late (30−35 weeks) pregnancy and again at 15−25 weeks postpartum and found a mean GFR of 138.9 mL/minute with an SD of 26.1 mL/minute. Krutzén et al. (1992) evaluated GFR during pregnancy for 4 different groups of women (13 normal healthy women, 16 diabetic women, 8 hypertensive women, and 12 women diagnosed with preeclampsia) by using iohexol clearance in the second and third trimester and again 6−12 months postpartum. Krutzén et al. (1992) reported the third trimester mean GFR and SD for the healthy women as 195 and 32 mL/minute, respectively. In general, the GFR values reported in this study are much higher than those reported by Sturgiss et al. (1996) and Dunlop (1981). The reason for this discrepancy is not known. The GFRs from these studies and the results of the sigma method value calculations for EF_{HK} are shown in Table A-5.

Table A-5. Sigma-method value calculation for intraspecies toxicokinetic extrapolation factor[a]

Study	Mean GFR (SD) (mL/min)	Mean GFR − (3 SD)	Sigma-Method Value
Dunlop	150.5 (17.6)[b]	97.7	1.54
Krutzen	195 (32)[c]	99	1.97
Sturgiss	138.9 (26.1)[d]	60.6	2.29
Averages	161.5	85.8	1.93

[a]Mean GFR ÷ (Mean GFR − 3 SD).

[b]Serially averaged observations across 3 time periods (16, 26, and 36 weeks) for 25 pregnant women.

[c]Third-trimester values for 13 pregnant women.

[d]Serially averaged observations across two time periods (early and late pregnancy) for 21 pregnant women (basal index plus basal control individuals).

Considering the Krutzén et al. (1992) results in the context of the sigma method, a reduction of 2 SD from the healthy population mean to establish the lower bound (which results in a GFR slightly higher than the mean of the preeclamptic GFR) would appear to be insufficient for adequate coverage of the susceptible population. Thus, the use of 3 SD below the healthy GFR mean gives coverage in the sensitive subpopulation to about 1 SD below the mean preeclamptic GFR.

As no single study is considered to be definitive for assessment of population GFR variability, EF_{HK} is determined from the average of the individual sigma-method values for each of the three studies. The mean GFR and SD values in Table A-5 are based on average GFR across the entire gestational period, except for the Krutzén et al. (1992) estimate, which was for the third trimester only. The average sigma-method value from the three studies is 1.93. Considering a small residual uncertainty in the use of GFR as a surrogate for boron clearance, the average sigma-method value of 1.93 is rounded upward to 2.0 and established as the value for EF_{HK}.

By virtue of their lower GFR, pregnant women diagnosed with preeclampsia are considered to be a sensitive subpopulation, at least toxicokinetically. TD sensitivity is presumably independent of TK sensitivity. The onset of preeclampsia generally occurs after week 20 of pregnancy and is characterized by acute hypertension, often accompanied by edema and proteinuria. Women with preeclampsia are at increased risk for premature separation of the placenta from the uterus and acute renal failure, among other adverse health effects. The fetus may become hypoxic and is at increased risk of low birth weight or perinatal death.

The approximately twofold intraspecies variability factor derived from 3 SDs below the mean of three studies for pregnancy GFR (mean = 161.5 mL/minute; mean − 3 SD = 85.8) is considered preferable for providing adequate coverage to women predisposed to adverse birth outcomes due to renal complications. Therefore, the default value of 3.16 for intraspecies UF for the TK component (UF_{HK}) was obviated by a DDEF of 2.0.

A.3.1.5. *References for Case Study A.3.1*

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A.3.2. Methylmercury—Intraspecies Extrapolation Factor for Toxicokinetics Case Study

A.3.2.1. *Summary*

This case study presents information derived from the *Water Quality Criterion for the Protection of Human Health: Methylmercury* (U.S. EPA, 2001d) and the IRIS entry for methylmercury (MeHg) (U.S. EPA, 2001b). MeHg has an RfD of 1×10^{-4} mg/kg-day in EPA's IRIS database (U.S. EPA, 2001b). No Toxicological Review is available for MeHg, but the underlying data and interpretations are published in the *Water Quality Criterion for the Protection of Human Health: Methylmercury* (U.S. EPA, 2001d).

Multiple RfDs were calculated from $BMDL_{05}$ values for various endpoints reported in three epidemiological studies measuring neurobehavioral deficits in children exposed *in utero*. Composite UFs of 10 were used in all calculations. This included a default threefold factor for human TD variability and uncertainty and a threefold factor for human TK variability and uncertainty. This latter EF_{HK} was based on published analyses of human TK data.

A.3.2.2. *Hazard Identification and Dose-Response*

MeHg can produce a variety of toxicities depending on the dose. These range from seizures and death to subtle neurobehavioral changes in humans exposed *in utero*. The choices of studies, critical effects, model, and POD were informed by an NRC advisory report and a subsequent review by an independent scientific panel (U.S. EPA, 2000b).

Mercury is methylated in soils and sediments by microorganisms and is bioaccumulated through aquatic food webs. It can reach relatively high concentrations (1 ppm or more) in predatory fish and sea mammals consumed by humans and wildlife. MeHg is absorbed readily from the human gut and is transported through the body, crossing both the blood/brain and placental barriers. Human studies from environmental exposures (fish and seafood consumption) were available and served to define the POD. Neurobehavioral effects were observed in two studies of children exposed *in utero* from maternal consumption of seafood [Faroe Islands and New Zealand; (U.S. EPA, 2001d)]. The Faroe Islands study was a longitudinal study of about 900 mother-infant pairs (Grandjean et al., 1997). The main independent variable was cord-blood mercury; maternal hair mercury was also measured as was child hair mercury. At 7 years of age, children were tested on a variety of tasks designed to assess function in specific behavioral domains. In the New Zealand study (Kjellstrom et al., 1989; Kjellstrom et al., 1986), increased maternal hair mercury was associated with decreased scores on standard intelligence quotient tests in 6-year-old children. No effects were reported in a third such study in the Seychelles Islands, but these data were also included in the modeling (NRC, 2000).

No MOA for MeHg has been established.

Test responses of children in three large studies discussed above (Faroe Islands, Seychelles, and New Zealand) were coupled with measured or calculated MeHg cord-blood concentrations for the dose-response analysis. BMD analysis was applied to the results from multiple individual neurobehavioral tests (e.g., Boston Naming Test, Continuous Performance Test, and California Verbal Learning Test). Data were modeled using a *K*-power model with $K \geq 1$; $K = 1$ generally giving the best fit. These data were continuous in exposure and effect. An abnormal response was defined as one falling into the lowest 5% of test responses ($P_0 = 0.05$). The BMR was set at 0.05, based on the NRC committee's advice that the combination of BMR and P_0 were within the observed range of responses and were, in fact, typical for these types of measurements (NRC, 2000). $BMDL_{05}$ values of 46 to 79 ppb MeHg in fetal cord blood were chosen as the points of departure for RfD calculation.

There is a correlation between maternal-blood mercury concentrations and fetal-blood mercury concentrations. A review of results from 21 studies demonstrated that the ratio of concentrations (fetal:maternal) is typically higher than 1, with overall mean values supporting a ratio close to 1.7. Based on the advice of an NRC panel, EPA (U.S. EPA, 2001b) chose not to make a numerical adjustment between cord-blood and maternal-blood mercury in calculating the RfD. The relationship between cord-blood and maternal-blood mercury was instead discussed as an area of variability and uncertainty during UF derivation.

Twenty-four RfDs were calculated using various $BMDL_{05}$ values but with the same dose conversion and a composite UF of 10. These calculations resulted in one RfD = 0.2 μg/kg bw/day, three RfD = 0.05 μg/kg bw/day, and twenty RfD = 0.1 μg/kg bw/day (or 1×10^{-4} mg/kg-day).

A.3.2.3. *Basis for Data-Derived Extrapolation Factor*

A PBPK model and a one-compartment model for pregnant women were used to examine the relationship between ingested doses of MeHg and maternal blood levels. To estimate human intraspecies variability (of MeHg concentrations in maternal blood to ingested MeHg dose), the most deterministic (sensitive) parameters of the TK model were identified and varied. Model results demonstrated that external doses required to produce maternal-blood concentrations of 1 ppm varied up to threefold. This value (3) served as a nondefault value for EF_{HK}; the TD component of the intraspecies UF (UF_{HD}) was left at a default value of 3 and the overall intraspecies UF value was 10.

A.3.2.4. *Data-Derived Extrapolation Factor Derivation*

Multiple measures of MeHg exposure are available from several human studies. For MeHg, hair and blood are considered more appropriate than urine, particularly for longer term exposure. The toxicity evaluated was induced during gestation, at a time when MeHg exposure to the developing brain of the fetus is through the placental blood supply. Thus, MeHg originated from the maternal blood circulation. Both a PBPK model and a one-compartment model were used to assess variables in fetal MeHg exposure. Independent of model type, ability to estimate maternal hair concentrations required at least two more parameters (blood-to-hair transfer and hair growth rate) than was required to estimate maternal blood concentrations. In addition, because EPA set cord-blood concentrations to equal maternal-blood concentrations, the model was able to predict fetal cord blood concentrations with less uncertainty than maternal hair concentrations. Largely for these reasons, blood MeHg concentrations were selected as the most appropriate dose metric.

EPA characterized human TK variability as differences in external (ingested) doses of MeHg that resulted in the same concentration of MeHg in maternal blood. The concentration selected for analysis was one that was relevant to the $BMDL_{05}$ for the neurobehavioral effects—namely 1 ppm. This concentration is about 12 to 20 times higher than the concentrations serving as the POD, but the choice was based in part on increasing model uncertainty when predicting concentrations lower than 1 ppm. An evaluation of the uncertainty and variability in model parameters was conducted in three studies (Swartout and Rice, 2000; Clewell et al., 1999; Stern, 1997) to identify the extent to which the external (ingested) dose might vary when compared to a fixed maternal hair or blood concentration.

Results from the Stern (1997) analysis were available in the original publication, whereas specific predictions of values at given percentiles for the Swartout and Rice (2000) and Clewell et al. (1999) studies required additional model exercises by the original authors; these analyses were published by NRC (2000). All data used in the models were from human studies. The analysis demonstrated the ratio of external (ingested) doses (in µg/kg-day) that resulted in the same blood concentration. The value of 3 was selected to represent the TK portion of the intraspecies extrapolation. This value was at or above the estimates from all three analyses for the comparison of the dose at the 50th percentile of the distribution to the dose at the 1st percentile of the distribution. The selected value of 3 thus encompassed the difference across these percentiles of the distribution. Because the dose at the 1st percentile of the distribution is lower than the dose at the 50th percentile of the distribution, the ratio has a value greater than 1.0.

Table A-6 presents the ratios developed (external dose at the 50th percentile/external dose at the 1st percentile) for each of the three studies. Considering TK variability as described

by the ratio of external doses at the specified percentiles of the distribution, values for blood and hair ranged from 1.7 to 3.3. Maximum values were 3.3 and 3.0 for hair and blood, respectively. EPA's IRIS entry for MeHg states, "Using maternal blood as the starting point, the consolidated range from the three analyses is 1.7 to 3.0." This is consistent with EPA's Water Quality Criterion document (U.S. EPA, 2001d). On this basis, a value of 3 was chosen to represent the TK portion of intraspecies variability. As no data were available to address intraspecies differences in susceptibility (TD), this portion of the UF was left at the default value of 3. Together these values for the components of UF_H combine to equal a value of 10, which was characterized as a "hybrid" value, comprising values based on default methodology and on data.

Table A-6. Comparison of results from three analyses of the intraspecies variability in the ingested dose of methylmercury corresponding to a given maternal-hair or blood mercury concentration

Study	Maternal medium	50th Percentile[a] (µg/kg-day)	50th Percentile/ 5th percentile[b]	50th Percentile/ 1st percentile[c]
Stern (1997)	Hair	0.03–0.05[d] (mean = 0.04)	1.8–2.4 (mean = 2.1)	2.3–3.3 (mean = 2.7)
	Blood	0.01	1.5–2.2 (mean = 1.8)	1.7–3.0 (mean = 2.4)
Swartout and Rice (2000)	Hair	0.08	2.2	Data not reported
	Blood[e]	0.02	2.1	2.8
Clewell et al. (1999)	Hair	0.08	1.5	1.8
	Blood[f]	0.07	1.4	1.7

[a]Predicted 50th percentile of the ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood.
[b]Ratio of 50th percentile of ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 5th percentile.
[c]Ratio of 50th percentile of ingested dose of MeHg that corresponds to 1 ppm Hg in hair or 1 ppb in blood to the 1st percentile.
[d]Range reflects minimum and maximum values among eight alternative analyses.
[e]Data from J. Swartout, U.S. EPA, personal communication; June 9, 2000.
[f]Data from H.J. Clewell, ICF Consulting, personal communication; April 19, 2000 as cited in NRC (2000).

SOURCE: This is Table 3-1 from NRC (2000).

Acknowledgment: Some passages in this document were taken from EPA's IRIS entry for Methylmercury (U.S. EPA, 2001b); some were taken from EPA's Water Quality Criterion document for Methylmercury (U.S. EPA, 2001d).

A.3.2.5. *References for Case Study A.3.2*

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EPA/630/P-03/001F
March 2005

Guidelines for Carcinogen Risk Assessment

Risk Assessment Forum
U.S. Environmental Protection Agency
Washington, DC

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1. INTRODUCTION

1.1. PURPOSE AND SCOPE OF THE GUIDELINES

These guidelines revise and replace the U.S. Environmental Protection Agency's (EPA's, or the Agency's) *Guidelines for Carcinogen Risk Assessment*, published in 51 FR 33992, September 24, 1986 (U.S. EPA, 1986a) and the 1999 interim final guidelines (U.S. EPA, 1999a; see U.S. EPA 2001b). They provide EPA staff with guidance for developing and using risk assessments. They also provide basic information to the public about the Agency's risk assessment methods.

These cancer guidelines are used with other risk assessment guidelines, such as the *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b) and the *Guidelines for Exposure Assessment* (U.S. EPA, 1992a). Consideration of other Agency guidance documents is also important in assessing cancer risks where procedures for evaluating specific target organ effects have been developed (e.g., assessment of thyroid follicular cell tumors, U.S. EPA, 1998a). All of EPA's guidelines should be consulted when conducting a risk assessment in order to ensure that information from studies on carcinogenesis and other health effects are considered together in the overall characterization of risk. This is particularly true in the case in which a precursor effect for a tumor is also a precursor or endpoint of other health effects or when there is a concern for a particular susceptible life-stage for which the Agency has developed guidance, for example, *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a). The developmental guidelines discuss hazards to children that may result from exposures during preconception and prenatal or postnatal development to sexual maturity. Similar guidelines exist for reproductive toxicant risk assessments (U.S. EPA, 1996a) and for neurotoxicity risk assessment (U.S. EPA, 1998b). The overall characterization of risk is conducted within the context of broader policies and guidance such as Executive Order 13045, "Protection of Children From Environmental Health Risks and Safety Risks"(Executive Order 13045, 1997) which is the primary directive to federal agencies and departments to identify and assess environmental health risks and safety risks that may disproportionately affect children.

The cancer guidelines encourage both consistency in the procedures that support scientific components of Agency decision making and flexibility to allow incorporation of innovations and contemporaneous scientific concepts. In balancing these goals, the Agency relies on established scientific peer review processes (U.S. EPA, 2000a; OMB 2004). The cancer guidelines incorporate basic principles and science policies based on evaluation of the currently available information. The Agency intends to revise these cancer guidelines when substantial changes are necessary. As more information about carcinogenesis develops, the need may arise to make appropriate changes in risk assessment guidance. In the interim, the Agency intends to issue special reports, after appropriate peer review, to supplement and update guidance on single topics (e.g., U.S. EPA, 1991b). One such guidance document, *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* ("Supplemental Guidance"), was developed in conjunction with these cancer guidelines (U.S. EPA., 2005). Because both the methodology and the data in the Supplemental Guidance (see Section 1.3.6) are expected to evolve more rapidly than the issues addressed in these cancer guidelines, the two were developed as separate documents. The Supplemental Guidance, however, as well as any other relevant (including subsequent) guidance documents, should be considered along with these cancer guidelines as risk assessments for carcinogens are generated. The use of supplemental guidance, such as the Supplemental Guidance for Assessing Cancer Susceptibility from Early-life Exposure to Carcinogens, has the advantage of allowing the Supplemental Guidance to be modified as more data become available. Thus, the consideration of new, peer-reviewed scientific understanding and data in an assessment can always be consistent with the purposes of these cancer guidelines.

These cancer guidelines are intended as guidance only. They do not establish any substantive "rules" under the Administrative Procedure Act or any other law and have no binding effect on EPA or any regulated entity, but instead represent a non-binding statement of policy. EPA believes that the cancer guidelines represent a sound and up-to-date approach to cancer risk assessment, and the cancer guidelines enhance the application of the best available science in EPA's risk assessments. However, EPA cancer risk assessments may be conducted differently than envisioned in the cancer guidelines for many reasons, including (but not limited to) new

information, new scientific understanding, or new science policy judgment. The science of risk assessment continues to develop rapidly, and specific components of the cancer guidelines may become outdated or may otherwise require modification in individual settings. Use of the cancer guidelines in future risk assessments will be based on decisions by EPA that the approaches are suitable and appropriate in the context of those particular risk assessments. These judgments will be tested through peer review, and risk assessments will be modified to use different approaches if appropriate.

1.2. ORGANIZATION AND APPLICATION OF THE CANCER GUIDELINES

1.2.1. Organization

Publications by the Office of Science and Technology (OSTP, 1985) and the National Research Council (NRC) (NRC, 1983, 1994) provide information and general principles about risk assessment. Risk assessment uses available scientific information on the properties of an agent[1] and its effects in biological systems to provide an evaluation of the potential for harm as a consequence of environmental exposure. The 1983 and 1994 NRC documents organize risk assessment information into four areas: hazard identification, dose-response assessment, exposure assessment, and risk characterization. This structure appears in these cancer guidelines, with additional emphasis placed on characterization of evidence and conclusions in each area of the assessment. In particular, the cancer guidelines adopt the approach of the NRC's 1994 report in adding a dimension of characterization to the hazard identification step: an evaluation of the conditions under which its expression is anticipated. Risk assessment questions addressed in these cancer guidelines are as follows.

- For hazard—Can the identified agent present a carcinogenic hazard to humans and, if so, under what circumstances?

- For dose response—At what levels of exposure might effects occur?

[1] The term “agent” refers generally to any chemical substance, mixture, or physical or biological entity being assessed, unless otherwise noted (See Section 1.2.2 for a note on radiation.).

- For exposure—What are the conditions of human exposure?

- For risk—What is the character of the risk? How well do data support conclusions about the nature and extent of the risk from various exposures?

The risk characterization process first summarizes findings on hazard, dose response, and exposure characterizations and then develops an integrative analysis of the whole risk case. It ends in the writing of a technical risk characterization. Other documents, such as summaries for the risk managers and the public, reflecting the key points of the risk characterization are usually written. A summary for managers is a presentation for those who may or may not be familiar with the scientific details of cancer assessment. It also provides information for other interested readers. The initial steps in the risk characterization process are to make building blocks in the form of characterizations of the assessments of hazard, dose response, and exposure. The individual assessments and characterizations are then integrated to arrive at risk estimates for exposure scenarios of interest. As part of the characterization process, explicit evaluations are made of the hazard and risk potential for susceptible lifestages, including children (U.S. EPA, 1995, 2000b).

The 1994 NRC document also explicitly called attention to the role of the risk assessment process in identifying scientific uncertainties that, if addressed, could serve to reduce their uncertainty in future iterations of the risk assessment. NRC recommended that when the Agency "reports estimates of risk to decisions-makers and the public, it should present not only point estimates of risk, but also the sources and magnitudes of uncertainty associated with these estimates" (p. 15). Thus, the identified uncertainties serve as a feedback loop to the research community and decisionmakers, specifying areas and types of information that would be particularly useful.

There are several reasons for individually characterizing the hazard, dose response, and exposure assessments. One is that they are often done by different people than those who do the integrative analyses. The second is that there is very often a lapse of time between the conduct of hazard and dose-response analyses and the conduct of exposure assessment and integrative

analysis. Thus, it is important to capture characterizations of assessments as the assessments are done to avoid the need to go back and reconstruct them. Finally, frequently a single hazard assessment is used by several programs for several different exposure scenarios. There may be one or several documents involved. "Integrative analysis" is a generic term; and many documents that have other titles may contain integrative analyses. In the following sections, the elements of these characterizations are discussed.

1.2.2. Application

The cancer guidelines apply within the framework of policies provided by applicable EPA statutes and do not alter such policies.

- The cancer guidelines cover the assessment of available data. They do not imply that one kind of data or another is prerequisite for regulatory action concerning any agent. It is important that, when evaluating and considering the use of any data, EPA analysts incorporate the basic standards of quality, as defined by the EPA Information Quality Guidelines (U.S. EPA, 2002a see Appendix B) and other Agency guidance on data quality such as the EPA Quality Manual for Environmental Programs (U.S. EPA, 2000e), as well as *OMB Guidelines for Ensuring and Maximizing the Quality, Utility, and Integrity of Information Disseminated by Federal Agencies* (OMB, 2002). It is very important that all analyses consider the basic standards of quality, including objectivity, utility, and integrity. A summary of the factors and considerations generally used by the Agency when evaluating and considering the use of scientific and technical information is contained in EPA's *A Summary of General Assessment Factors for Evaluating the Quality of Scientific and Technical Information* (U.S. EPA, 2003).

- Risk management applies directives in statutes, which may require consideration of potential risk or solely hazard or exposure potential, along with social, economic, technical, and other factors in decision making. Risk assessments may be used to support

decisions, but in order to maintain their integrity as decision-making tools, they are not influenced by consideration of the social or economic consequences of regulatory action.

The assessment of risk from radiation sources is informed by the continuing examination of human data by the National Academy of Sciences/NRC in its series of numbered reports: "Biological Effects of Ionizing Radiation." Although some of the general principles of these cancer guidelines may also apply to radiation risk assessments, some of the details of their risk assessment procedures may not, as they are most focused on other kinds of agents. Therefore, these cancer guidelines are not intended to provide the primary source of, or guidance for, the Agency's evaluation of the carcinogenic risks of radiation.

Not every EPA assessment has the same scope or depth, a factor recognized by the National Academy of Sciences (NRC, 1996). For example, EPA's Information Quality Guidelines (U.S. EPA, 2002a, see Appendix B) discuss influential information that "will have or does have a clear and substantial impact ... on important public policies or private sector decisions ... that should adhere to a rigorous standard of quality." It is often difficult to know *a priori* how the results of a risk assessment are likely to be used by the Agency. Some risk assessments may be used by Agency economists and policy analysts, and the necessary information for such analyses, as discussed in detail later in this document, should be included when practicable (U.S. EPA, 2002a). On the other hand, Agency staff often conduct screening-level assessments for priority setting or separate assessments of hazard or exposure for ranking purposes or to decide whether to invest resources in collecting data for a full assessment. Moreover, a given assessment of hazard and dose response may be used with more than one exposure assessment that may be conducted separately and at different times as the need arises in studying environmental problems related to various exposure media. The cancer guidelines apply to these various situations in appropriate detail, given the scope and depth of the particular assessment. For example, a screening assessment may be based almost entirely on structure-activity relationships (SARs) and default options, when other data are not readily available. When more data and resources are readily available, assessments can use a critical analysis of all of the available data as the starting point of the risk assessment. Under these conditions, default

options would only be used to address uncertainties or the absence of critical data. Default options are inferences based on general scientific knowledge of the phenomena in question and are also matters of policy concerning the appropriate way to bridge uncertainties that concern potential risk to human health.

These cancer guidelines do not suggest that all of the kinds of data covered here will need to be available or used for either assessment or decision making. The level of detail of an assessment is a matter of Agency management discretion regarding applicable decision-making needs. The Agency generally presumes that key cancer information (e.g., assessments contained in the Agency's Integrated risk Information System) is "influential information" as defined by the EPA Information Quality Guidelines and "highly influential" as defined by OMB's Information Quality Bulletin for Peer Review (OMB 2004).

1.3. KEY FEATURES OF THE CANCER GUIDELINES

1.3.1. Critical Analysis of Available Information as the Starting Point for Evaluation

As an increasing understanding of carcinogenesis is becoming available, these cancer guidelines adopt a view of default options that is consistent with EPA's mission to protect human health while adhering to the tenets of sound science. Rather than viewing default options as the starting point from which departures may be justified by new scientific information, these cancer guidelines view a critical analysis of all of the available information that is relevant to assessing the carcinogenic risk as the starting point from which a default option may be invoked if needed to address uncertainty or the absence of critical information. Preference is given to using information that has been peer reviewed, e.g., reported in peer-reviewed scientific journals. The primary goal of EPA actions is protection of human health; accordingly, as an Agency policy, risk assessment procedures, including default options that are used in the absence of scientific data to the contrary, should be health protective (U.S. EPA, 1999b).

Use of health protective risk assessment procedures as described in these cancer guidelines means that estimates, while uncertain, are more likely to overstate than understate hazard and/or risk. NRC (1994) reaffirmed the use of default options as "a reasonable way to cope with uncertainty about the choice of appropriate models or theory" (p. 104). NRC saw the

need to treat uncertainty in a predictable way that is "scientifically defensible, consistent with the agency's statutory mission, and responsive to the needs of decision-makers" (p. 86). The extent of health protection provided to the public ultimately depends upon what risk managers decide is the appropriate course of regulatory action. When risk assessments are performed using only one set of procedures, it may be difficult for risk managers to determine how much health protectiveness is built into a particular hazard determination or risk characterization. When there are alternative procedures having significant biological support, the Agency encourages assessments to be performed using these alternative procedures, if feasible, in order to shed light on the uncertainties in the assessment, recognizing that the Agency may decide to give greater weight to one set of procedures than another in a specific assessment or management decision.

Encouraging risk assessors to be receptive to new scientific information, NRC discussed the need for departures from default options when a "sufficient showing" is made. It called on EPA to articulate clearly its criteria for a departure so that decisions to depart from default options would be "scientifically credible and receive public acceptance" (p. 91). It was concerned that *ad hoc* departures would undercut the scientific credibility of a risk assessment. NRC envisioned that principles for choosing and departing from default options would balance several objectives, including "protecting the public health, ensuring scientific validity, minimizing serious errors in estimating risks, maximizing incentives for research, creating an orderly and predictable process, and fostering openness and trustworthiness" (p. 81).

Appendices N-1 and N-2 of NRC (1994) discussed two competing standards for choosing default options articulated by members of the committee. One suggested approach would evaluate a departure in terms of whether "it is scientifically plausible" and whether it "tends to protect public health in the face of scientific uncertainty" (p. 601). An alternative approach "emphasizes scientific plausibility with regard to the use of alternative models" (p. 631). Reaching no consensus on a single approach, NRC recognized that developing criteria for departures is an EPA policy matter.

The basis for invoking a default option depends on the circumstances. Generally, if a gap in basic understanding exists or if agent-specific information is missing, a default option may be used. If agent-specific information is present but critical analysis reveals inadequacies, a default

option may also be used. If critical analysis of agent-specific information is consistent with one or more biologically based models as well as with the default option, the alternative models and the default option are both carried through the assessment and characterized for the risk manager. In this case, the default model not only fits the data, but also serves as a benchmark for comparison with other analyses. This case also highlights the importance of extensive experimentation to support a conclusion about mode of action, including addressing the issue of whether alternative modes of action are also plausible. Section 2.4 provides a framework for critical analysis of mode of action information to address the extent to which the available information supports the hypothesized mode of action, whether alternative modes of action are also plausible, and whether there is confidence that the same inferences can be extended to populations and lifestages that are not represented among the experimental data.

Generally, cancer risk decisions strive to be "scientifically defensible, consistent with the agency's statutory mission, and responsive to the needs of decision-makers" (NRC, 1994). Scientific defensibility would be evaluated through use of EPA's Science Advisory Board, EPA's Office of Pesticide Programs' Scientific Advisory Panel, or other independent expert peer review panels to determine whether a consensus among scientific experts exists. Consistency with the Agency's statutory mission would consider whether the risk assessment overall supports EPA's mission to protect human health and safeguard the natural environment. Responsiveness to the needs of decisionmakers would take into account pragmatic considerations such as the nature of the decision; the required depth of analysis; the utility, time, and cost of generating new scientific data; and the time, personnel, and resources allotted to the risk assessment.

With a multitude of types of data, analyses, and risk assessments, as well as the diversity of needs of decisionmakers, it is neither possible nor desirable to specify step-by-step criteria for decisions to invoke a default option. A discussion of major default options appears in the Appendix. Screening-level assessments may more readily use default parameters, even worst-case assumptions, that would not be appropriate in a full-scale assessment. On the other hand, significant risk management decisions will often benefit from a more comprehensive assessment, including alternative risk models having significant biological support. To the extent practicable, such assessments should provide central estimates of potential risks in conjunction with lower

and upper bounds (e.g., confidence limits) and a clear statement of the uncertainty associated with these estimates.

In the absence of sufficient data or understanding to develop of a robust, biologically based model, an appropriate policy choice is to have a single preferred curve-fitting model for each type of data set. Many different curve-fitting models have been developed, and those that fit the observed data reasonably well may lead to several-fold differences in estimated risk at the lower end of the observed range. In addition, goodness-of-fit to the experimental observations is not by itself an effective means of discriminating among models that adequately fit the data (OSTP, 1985). To provide some measure of consistency across different carcinogen assessments, EPA uses a standard curve-fitting procedure for tumor incidence data. Assessments that include a different approach should provide an adequate justification and compare their results with those from the standard procedure. Application of models to data should be conducted in an open and transparent manner.

1.3.2. Mode of Action

The use of mode of action[2] in the assessment of potential carcinogens is a main focus of these cancer guidelines. This area of emphasis arose because of the significant scientific advances that have developed concerning the causes of cancer induction. Elucidation of a mode of action for a particular cancer response in animals or humans is a data-rich determination. Significant information should be developed to ensure that a scientifically justifiable mode of action underlies the process leading to cancer at a given site. In the absence of sufficiently, scientifically justifiable mode of action information, EPA generally takes public health-protective, default positions regarding the interpretation of toxicologic and epidemiologic data:

[2] The term *"mode of action"* is defined as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. A *"key event"* is an empirically observable precursor step that is itself a necessary element of the mode of action or is a biologically based marker for such an element. Mode of action is contrasted with "*mechanism of action*," which implies a more detailed understanding and description of events, often at the molecular level, than is meant by mode of action. The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here. There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression.

animal tumor findings are judged to be relevant to humans, and cancer risks are assumed to conform with low dose linearity.

Understanding of mode of action can be a key to identifying processes that may cause chemical exposures to differentially affect a particular population segment or lifestage. Some modes of action are anticipated to be mutagenic and are assessed with a linear approach. This is the mode of action of radiation and several other agents that are known carcinogens. Other modes of action may be modeled with either linear or nonlinear[3] approaches after a rigorous analysis of available data under the guidance provided in the framework for mode of action analysis (see Section 2.4.3).

1.3.3. Weight of Evidence Narrative

The cancer guidelines emphasize the importance of weighing all of the evidence in reaching conclusions about the human carcinogenic potential of agents. This is accomplished in a single integrative step after assessing all of the individual lines of evidence, which is in contrast to the step-wise approach in the 1986 cancer guidelines. Evidence considered includes tumor findings, or lack thereof, in humans and laboratory animals; an agent's chemical and physical properties; its structure-activity relationships (SARs) as compared with other carcinogenic agents; and studies addressing potential carcinogenic processes and mode(s) of action, either *in vivo* or *in vitro*. Data from epidemiologic studies are generally preferred for characterizing human cancer hazard and risk. However, all of the information discussed above could provide valuable insights into the possible mode(s) of action and likelihood of human cancer hazard and risk. The cancer guidelines recognize the growing sophistication of research methods,

[3]The term "*nonlinear*" is used here in a narrower sense than its usual meaning in the field of mathematical modeling. In these cancer guidelines, the term "*nonlinear*" refers to threshold models (which show no response over a range of low doses that include zero) and some nonthreshold models (e.g., a quadractic model, which shows some response at all doses above zero). In these cancer guidelines, a nonlinear model is one whose slope is zero at (and perhaps above) a dose of zero. A *low-dose-linear* model is one whose slope is greater than zero at a dose of zero. A low-dose-linear model approximates a straight line only at very low doses; at higher doses near the observed data, a low-dose-linear model can display curvature. The term "*low-dose-linear*" is often abbreviated "linear," although a low-dose-linear model is not linear at all doses. Use of nonlinear approaches does not imply a biological threshold dose below which the response is zero. Estimating thresholds can be problematic; for example, a response that is not statistically significant can be consistent with a small risk that falls below an experiment's power of detection.

particularly in their ability to reveal the modes of action of carcinogenic agents at cellular and subcellular levels as well as toxicokinetic processes.

Weighing of the evidence includes addressing not only the likelihood of human carcinogenic effects of the agent but also the conditions under which such effects may be expressed, to the extent that these are revealed in the toxicological and other biologically important features of the agent.

The weight of evidence narrative to characterize hazard summarizes the results of the hazard assessment and provides a conclusion with regard to human carcinogenic potential. The narrative explains the kinds of evidence available and how they fit together in drawing conclusions, and it points out significant issues/strengths/limitations of the data and conclusions. Because the narrative also summarizes the mode of action information, it sets the stage for the discussion of the rationale underlying a recommended approach to dose-response assessment.

In order to provide some measure of clarity and consistency in an otherwise free-form, narrative characterization, standard descriptors are used as part of the hazard narrative to express the conclusion regarding the weight of evidence for carcinogenic hazard potential. There are five recommended standard hazard descriptors: "*Carcinogenic to Humans*," "*Likely to Be Carcinogenic to Humans*," "*Suggestive Evidence of Carcinogenic Potential*," "*Inadequate Information to Assess Carcinogenic Potential*," and "*Not Likely to Be Carcinogenic to Humans*." Each standard descriptor may be applicable to a wide variety of data sets and weights of evidence and is presented only in the context of a weight of evidence narrative. Furthermore, as described in Section 2.5 of these cancer guidelines, more than one conclusion may be reached for an agent.

1.3.4. Dose-response Assessment

Dose-response assessment evaluates potential risks to humans at particular exposure levels. The approach to dose-response assessment for a particular agent is based on the conclusion reached as to its potential mode(s) of action for each tumor type. Because an agent may induce multiple tumor types, the dose-response assessment includes an analysis of all tumor types, followed by an overall synthesis that includes a characterization of the risk estimates across tumor types, the strength of the mode of action information of each tumor type, and the

anticipated relevance of each tumor type to humans, including susceptible populations and lifestages (e.g., childhood).

Dose-response assessment for each tumor type is performed in two steps: assessment of observed data to derive a point of departure (POD),[4] followed by extrapolation to lower exposures to the extent that is necessary. Data from epidemiologic studies, of sufficient quality, are generally preferred for estimating risks. When animal studies are the basis of the analysis, the estimation of a human-equivalent dose should utilize toxicokinetic data to inform cross-species dose scaling if appropriate and if adequate data are available. Otherwise, default procedures should be applied. For oral dose, based on current science, an appropriate default option is to scale daily applied doses experienced for a lifetime in proportion to body weight raised to the 3/4 power (U.S. EPA, 1992b). For inhalation dose, based on current science, an appropriate default methodology estimates respiratory deposition of particles and gases and estimates internal doses of gases with different absorption characteristics. When toxicokinetic modeling (see Section 3.1.2) is used without toxicodynamic modeling (see Section 3.2.2), the dose-response assessment develops and supports an approach for addressing toxicodynamic equivalence, perhaps by retaining some of the cross-species scaling factor (see Section 3.1.3). Guidance is also provided for adjustment of dose from adults to children (see Section 4.3.1).

Response data on effects of the agent on carcinogenic processes are analyzed (nontumor data) in addition to data on tumor incidence. If appropriate, the analyses of data on tumor incidence and on precursor effects may be used in combination. To the extent the relationship between precursor effects and tumor incidence are known, precursor data may be used to estimate a dose-response function below the observable tumor data. Study of the dose-response function for effects believed to be part of the carcinogenic process influenced by the agent may also assist in evaluating the relationship of exposure and response in the range of observation and at exposure levels below the range of observation.

[4] A "*point of departure*" (POD) marks the beginning of extrapolation to lower doses. The POD is an estimated dose (usually expressed in human-equivalent terms) near the lower end of the observed range, without significant extrapolation to lower doses.

The first step of dose-response assessment is evaluation within the range of observation. Approaches to analysis of the range of observation of epidemiologic studies are determined by the type of study and how dose and response are measured in the study. In the absence of adequate human data for dose-response analysis, animal data are generally used. If there are sufficient quantitative data and adequate understanding of the carcinogenic process, a biologically based model may be developed to relate dose and response data on an agent-specific basis. Otherwise, as a default procedure, a standard model can be used to curve-fit the data.

The POD for extrapolating the relationship to environmental exposure levels of interest, when the latter are outside the range of observed data, is generally the lower 95% confidence limit on the lowest dose level that can be supported for modeling by the data. SAB (1997) suggested that, "it may be appropriate to emphasize lower statistical bounds in screening analyses and in activities designed to develop an appropriate human exposure value, since such activities require accounting for various types of uncertainties and a lower bound on the central estimate is a scientifically-based approach accounting for the uncertainty in the true value of the ED_{10} [or central estimate]." However, the consensus of the SAB (1997) was that, "both point estimates and statistical bounds can be useful in different circumstances, and recommended that the Agency routinely calculate and present the point estimate of the ED_{10} [or central estimate] and the corresponding upper and lower 95% statistical bounds." For example, it may be appropriate to emphasize the central estimate in activities that involve formal uncertainty analysis that are required by OMB Circular A-4 (OMB, 2003) as well as ranking agents as to their carcinogenic hazard. Thus, risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decisionmakers.

The second step of dose-response assessment is extrapolation to lower dose levels, if needed. This extrapolation is based on extension of a biologically based model if supported by substantial data (see Section 3.3.2). Otherwise, default approaches can be applied that are consistent with current understanding of mode(s) of action of the agent, including approaches that assume linearity or nonlinearity of the dose-response relationship, or both. A default approach for linearity extends a straight line from the POD to zero dose/zero response (see

Section 3.3.3). The linear approach is used when: (1) there is an absence of sufficient information on modes of action or (2) the mode of action information indicates that the dose-response curve at low dose is or is expected to be linear. Where alternative approaches have significant biological support, and no scientific consensus favors a single approach, an assessment may present results using alternative approaches. A nonlinear approach can be used to develop a reference dose or a reference concentration (see Section 3.3.4).

1.3.5. Susceptible Populations and Lifestages

An important use of mode of action information is to identify susceptible populations and lifestages. It is rare to have epidemiologic studies or animal bioassays conducted in susceptible individuals. This information need can be filled by identifying the key events of the mode of action and then identifying risk factors, such as differences due to genetic polymorphisms, disease, altered organ function, lifestyle, and lifestage, that can augment these key events. To do this, the information about the key precursor events is reviewed to identify particular populations or lifestages that can be particularly susceptible to their occurrence (see Section 2.4.3.4). Any information suggesting quantitative differences between populations or lifestages is flagged for consideration in the dose-response assessment (see Section 3.5 and U.S. EPA 2002b).

1.3.6. Evaluating Risks from Childhood Exposures

NRC (1994) recommended that "EPA should assess risks to infants and children whenever it appears that their risks might be greater than those of adults." Executive Order 13045 (1997) requires that "each Federal Agency shall make it a high priority to identify and assess environmental health and safety risks that may disproportionately affect children, and shall ensure that their policies, programs, and standards address disproportionate risks that result from environmental health risks or safety risks." In assessing risks to children, EPA considers both effects manifest during childhood and early-life exposures that can contribute to effects at any time later in life.

These cancer guidelines view childhood as a sequence of lifestages rather than viewing children as a subpopulation, the distinction being that a subpopulation refers to a portion of the

population, whereas a lifestage is inclusive of the entire population. Exposures that are of concern extend from conception through adolescence and also include pre-conception exposures of both parents. These cancer guidelines use the term "childhood" in this more inclusive sense.

Rarely are there studies that directly evaluate risks following early-life exposure. Epidemiologic studies of early-life exposure to environmental agents are seldom available. Standard animal bioassays generally begin dosing after the animals are several weeks old, when many organ systems are mature. This could lead to an understatement of risk, because an accepted concept in the science of carcinogenesis is that young animals are usually more susceptible to the carcinogenic activity of a chemical than are mature animals (McConnell, 1992).

At this time, there is some evidence of higher cancer risks following early-life exposure. For radiation carcinogenesis, data indicate that risks for several forms of cancer are highest following childhood exposure (NRC, 1990; Miller, 1995; U.S. EPA, 1999c). These human results are supported by the few animal bioassays that include perinatal (prenatal or early postnatal) exposure. Perinatal exposure to some agents can induce higher incidences of the tumors seen in standard bioassays; some examples include vinyl chloride (Maltoni et al., 1981), diethylnitrosamine (Peto et al., 1984), benzidine, DDT, dieldrin, and safrole (Vesselinovitch et al., 1979). Moreover, perinatal exposure to some agents, including vinyl chloride (Maltoni et al., 1981) and saccharin (Cohen, 1995; Whysner and Williams, 1996), can induce different tumors that are not seen in standard bioassays. Surveys comparing perinatal carcinogenesis bioassays with standard bioassays for a limited number of chemicals (McConnell, 1992; U.S. EPA, 1996b) have concluded that

- the same tumor sites are usually observed following either perinatal or adult exposure, and

- perinatal exposure in conjunction with adult exposure usually increases the incidence of tumors or reduces the latent period before tumors are observed.

The risk attributable to early-life exposure often appears modest compared with the risk from lifetime exposure, but it can be about 10-fold higher than the risk from an exposure of similar duration occurring later in life (Ginsberg, 2003). Further research is warranted to investigate the extent to which these findings apply to specific agents, chemical classes, and modes of action or in general.

These empirical results are consistent with current understanding of the biological processes involved in carcinogenesis, which leads to a reasonable expectation that children can be more susceptible to many carcinogenic agents (Anderson et al., 2000; Birnbaum and Fenton, 2003; Ginsberg, 2003; Miller et al., 2002; Scheuplein et al., 2002). Some aspects potentially leading to childhood susceptibility are listed below.

- Differences in the capacity to metabolize and clear chemicals can result in larger or smaller internal doses of the active agent(s).

- More frequent cell division during development can result in enhanced expression of mutations due to the reduced time available for repair of DNA lesions (Slikker et al., 2004).

- Some embryonic cells, such as brain cells, lack key DNA repair enzymes.

- More frequent cell division during development can result in clonal expansion of cells with mutations from prior unrepaired DNA damage (Slikker et al., 2004).

- Some components of the immune system are not fully functional during development (Holladay and Smialowicz, 2000; Holsapple et al., 2003).

- Hormonal systems operate at different levels during different lifestages.

- Induction of developmental abnormalities can result in a predisposition to carcinogenic effects later in life (Anderson et al., 2000; Birnbaum and Fenton, 2003; Fenton and Davis, 2002).

To evaluate risks from early-life exposure, these cancer guidelines emphasize the role of toxicokinetic information to estimate levels of the active agent in children and toxicodynamic information to identify whether any key events of the mode of action are of increased concern early in life. Developmental toxicity studies can provide information on critical periods of exposure for particular targets of toxicity.

An approach to assessing risks from early-life exposure is presented in Figure 1-1. In the hazard assessment, when there are mode of action data, the assessment considers whether these data have special relevance during childhood, considering the various aspects of development listed above. Examples of such data include toxicokinetics that predict a sufficiently large internal dose in children or a mode of action where a key precursor event is more likely to occur during childhood. There is no recommended default to settle the question of whether tumors arising through a mode of action are relevant during childhood; and adequate understanding the mode of action implies that there are sufficient data (on either the specific agent or the general mode of action) to form a confident conclusion about relevance during childhood (see Section 2.4.3.4).

In the dose-response assessment, the potential for susceptibility during childhood warrants explicit consideration in each assessment. These cancer guidelines encourage developing separate risk estimates for children according to a tiered approach that considers what pertinent data are available (see Section 3.5). Childhood may be a susceptible period; moreover, exposures during childhood generally are not equivalent to exposures at other times and may be treated differently from exposures occurring later in life (see Section 3.5). In addition, adjustment of unit risk estimates may be warranted when used to estimate risks from childhood exposure (see Section 4.4).

At this time, several limitations preclude a full assessment of children's risk. There are no generally used testing protocols to identify potential environmental causes of cancers that are

unique to children, including several forms of childhood cancer and cancers that develop from parental exposures, and cases where developmental exposure may alter susceptibility to carcinogen exposure in the adult (Birnbaum and Fenton, 2003). Dose-response assessment is limited by an inability to observe how developmental exposure can modify incidence and latency and an inability to estimate the ultimate tumor response resulting from induced susceptibility to later carcinogen exposures.

To partially address the limitations identified above, EPA developed in conjunction with these cancer guidelines, *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* ("Supplemental Guidance"). The Supplemental Guidance addresses a number of issues pertaining to cancer risks associated with early-life exposures generally, but provides specific guidance on procedures for adjusting cancer potency estimates only for carcinogens acting through a mutagenic mode of action. This Supplemental Guidance recommends, for such chemicals when no chemical-specific data exist, a default approach using estimates from chronic studies (i.e., cancer slope factors) with appropriate modifications to address the potential for differential risk of early-lifestage exposure.

The Agency considered both the advantages and disadvantages to extending the recommended, age dependent adjustment factors for carcinogenic potency to carcinogenic agents for which the mode of action remains unknown. EPA decided to recommend these factors only for carcinogens acting through a mutagenic mode of action based on a combination of analysis of available data and long-standing science policy positions which govern the Agency's overall approach to carcinogen risk assessment. In general, the Agency prefers to rely on analyses of data, rather than general defaults. When data are available for a sensitive lifestage, they would be used directly to evaluate risks for that chemical and that lifestage on a case-by-case basis. In the case of nonmutagenic carcinogens, when the mode of action is unknown, the data were judged by EPA to be too limited and the modes of action too diverse to use this as a category for which a general default adjustment factor approach can be applied. In this situation, a linear low-dose extrapolation methodology (without further adjustment) is recommended. It is the Agency's long-standing science policy position that use of the linear low-dose extrapolation approach

provides adequate public health conservatism in the absence of chemical-specific data indicating differential early-life sensitivity or when the mode of action is not mutagenic.

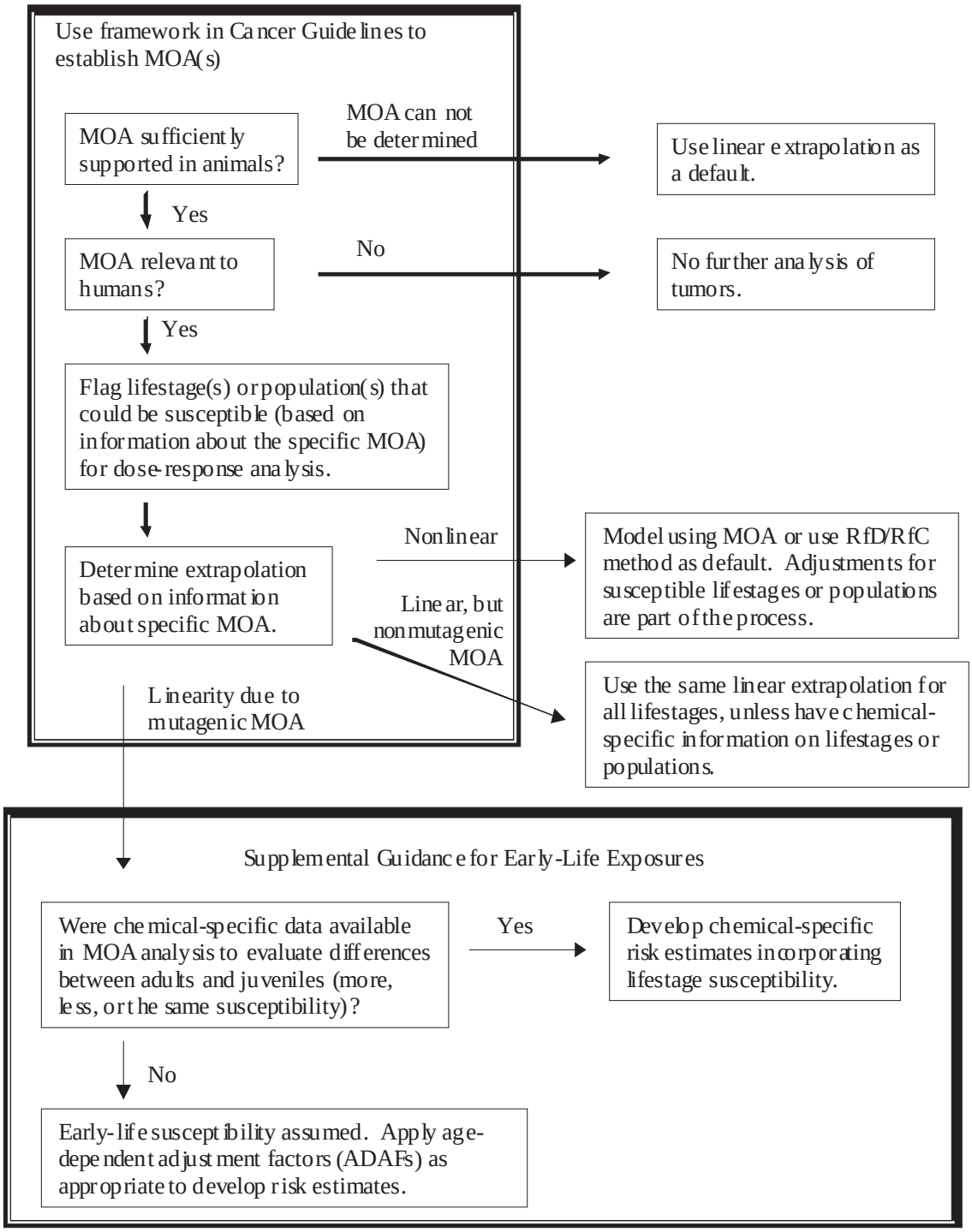
The Agency expects to produce additional supplemental guidance for other modes of action, as data from new research and toxicity testing indicate it is warranted. EPA intends to focus its research, and work collaboratively with its federal partners, to improve understanding of the implications of early life exposure to carcinogens. Development of guidance for estrogenic agents and chemicals acting through other processes resulting in endocrine disruption and subsequent carcinogenesis, for example, might be a reasonable priority in light of the human experience with diethylstilbesterol and the existing early life animal studies. It is worth noting that each mode of action for endocrine disruption will probably require separate analysis.

As the Agency examines additional carcinogenic agents, the age groupings may differ from those recommended for assessing cancer risks from early-life exposure to chemicals with a mutagenic mode of action. Puberty and its associated biological changes, for example, involve many biological processes that could lead to changes in sensitivity to the effects of some carcinogens, depending on their mode of action. The Agency is interested in identifying lifestages that may be particularly sensitive or refractory for carcinogenesis, and believes that the mode of action framework described in these cancer guidelines is an appropriate mechanism for elucidating these lifestages. For each additional mode of action evaluated, the various age groupings determined to be at differential risk may differ from those proposed in the Supplemental Guidance. For example, the age groupings selected for the age-dependent adjustments for carcinogens acting through a mutagenic mode of action were initially selected based on the available data, i.e., for the laboratory animal age range representative of birth to < 2 years in humans. More limited data and information on human biology were used to determine a science-informed policy regarding 2 to < 16 years. Data were not available to refine the latter age group. If more data become available regarding carcinogens with a mutagenic mode of action, consideration may be given to further refinement of these age groups.

1.3.7. Emphasis on Characterization

The cancer guidelines emphasize the importance of a clear and useful characterization narrative that summarizes the analyses of hazard, dose-response, and exposure assessment. These characterizations summarize the assessments to explain the extent and weight of evidence, major points of interpretation and rationale for their selection, strengths and weaknesses of the evidence and the analysis, and discuss alternative conclusions and uncertainties that deserve serious consideration (U.S. EPA, 2000b). They serve as starting materials for the overall risk characterization process that completes the risk assessment.

Figure 1-1. Flow chart for early-life risk assessment using mode of action framework.



2. HAZARD ASSESSMENT

2.1. OVERVIEW OF HAZARD ASSESSMENT AND CHARACTERIZATION

2.1.1. Analyses of Data

The purpose of hazard assessment is to review and evaluate data pertinent to two questions: (1) whether an agent may pose a carcinogenic hazard to human beings, and (2) under what circumstances an identified hazard may be expressed (NRC, 1994). Hazard assessment involves analyses of a variety of data that may range from observations of tumor responses to analysis of structure-activity relationships (SARs). The purpose of the assessment is not simply to assemble these separate evaluations; its purpose is to construct a total analysis examining what the biological data reveal as a whole about carcinogenic effects and mode of action of the agent, and their implications for human hazard and dose-response evaluation. Conclusions are drawn from weight-of-evidence evaluations based on the combined strength and coherence of inferences appropriately drawn from all of the available information. To the extent that data permit, hazard assessment addresses the question of mode of action of an agent as both an initial step in identifying human hazard potential and as a component in considering appropriate approaches to dose-response assessment.

The topics in this chapter include analysis of tumor data, both human and animal, and analysis of other key information about properties and effects that relate to carcinogenic potential. The chapter addresses how information can be used to evaluate potential modes of action. It also provides guidance on performing a weight of evidence evaluation.

2.1.2. Presentation of Results

Presentation of the results of hazard assessment should be informed by Agency guidance as discussed in Section 2.6. The results are presented in a technical hazard characterization that serves as a support to later risk characterization. It includes:

- a summary of the evaluations of hazard data,
- the rationales for its conclusions, and

- an explanation of the significant strengths or limitations of the conclusions.

Another presentation feature is the use of a weight of evidence narrative that includes both a conclusion about the weight of evidence of carcinogenic potential and a summary of the data on which the conclusion rests. This narrative is a brief summary that *in toto* replaces the alphanumerical classification system used in EPA's 1986 cancer guidelines (U.S. EPA, 1986a).

2.2. ANALYSIS OF TUMOR DATA

Evidence of carcinogenicity comes from finding tumor increases in humans or laboratory animals exposed to a given agent or from finding tumors following exposure to structural analogues to the compound under review. The significance of observed or anticipated tumor effects is evaluated in reference to all the other key data on the agent. This section contains guidance for analyzing human and animal studies to decide whether there is an association between exposure to an agent or a structural analogue and occurrence of tumors. Note that the use of the term "tumor" in these cancer guidelines is defined as malignant neoplasms or a combination of malignant and corresponding benign neoplasms.

Observation of only benign neoplasia may or may not have significance for evaluation under these cancer guidelines. Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis. There is a range of possibilities for their overall significance. They may deserve attention because they are serious health problems even though they are not malignant; for instance, benign tumors may be a health risk because of their effect on the function of a target tissue such as the brain. They may be significant indicators of the need for further testing of an agent if they are observed in a short- term test protocol, or such an observation may add to the overall weight of evidence if the same agent causes malignancies in a long-term study. Knowledge of the mode of action associated with a benign tumor response may aid in the interpretation of other tumor responses associated with the same agent. In other cases, observation of a benign tumor response alone may have no significant health hazard implications when other sources of evidence show no suggestion of carcinogenicity.

2.2.1. Human Data

Human data may come from epidemiologic studies or case reports. (Clinical human studies, which involve intentional exposures to substances, may provide toxicokinetic data, but generally not data on carcinogenicity.) The most common sources of human data for cancer risk assessment are epidemiologic investigations. Epidemiology is the study of the distribution of disease in human populations and the factors that may influence that distribution. The goals of cancer epidemiology are to identify distribution of cancer risk and determine the extent to which the risk can be attributed causally to specific exposures to exogenous or endogenous factors (see Centers for Disease Control and Prevention [CDC, 2004]). Epidemiologic data are extremely valuable in risk assessment because they provide direct evidence on whether a substance is likely to produce cancer in humans, thereby avoiding issues such as: species-to-species inference, extrapolation to exposures relevant to people, effects of concomitant exposures due to lifestyles. Thus, epidemiologic studies typically evaluate agents under more relevant conditions. When human data of high quality and adequate statistical power are available, they are generally preferable over animal data and should be given greater weight in hazard characterization and dose-response assessment, although both can be used.

Null results from epidemiologic studies alone generally do not prove the absence of carcinogenic effects because such results can arise either from an agent being truly not carcinogenic or from other factors such as: inadequate statistical power, inadequate study design, imprecise estimates, or confounding factors. Moreover, null results from a well-designed and well-conducted epidemiologic study that contains usable exposure data can help to define upper limits for the estimated dose of concern for human exposure in cases where the overall weight of the evidence indicates that the agent is potentially carcinogenic in humans. Furthermore, data from a well designed and well conducted epidemiologic study that does not show positive results, in conjunction with compelling mechanistic information, can lend support to a conclusion that animal responses may not be predictive of a human cancer hazard.

Epidemiology can also complement experimental evidence in corroborating or clarifying the carcinogenic potential of the agent in question. For example, epidemiologic studies that show elevated cancer risk for tumor sites corresponding to those at which laboratory animals

experience increased tumor incidence can strengthen the weight of evidence of human carcinogenicity. Furthermore, biochemical or molecular epidemiology may help improve understanding of the mechanisms of human carcinogenesis.

2.2.1.1. *Assessment of Evidence of Carcinogenicity from Human Data*

All studies that are considered to be of acceptable quality, whether yielding positive or null results, or even suggesting protective carcinogenic effects, should be considered in assessing the totality of the human evidence. Conclusions about the overall evidence for carcinogenicity from available studies in humans should be summarized along with a discussion of uncertainties and gaps in knowledge. Conclusions regarding the strength of the evidence for positive or negative associations observed, as well as evidence supporting judgments of causality, should be clearly described. In assessing the human data within the overall weight of evidence, determination about the strength of the epidemiologic evidence should clearly identify the degree to which the observed associations may be explained by other factors, including bias or confounding.

Characteristics that are generally desirable in epidemiologic studies include (1) clear articulation of study objectives or hypothesis; (2) proper selection and characterization of comparison groups (exposed and unexposed groups or case and control groups); (3) adequate characterization of exposure; (4) sufficient length of follow-up for disease occurrence; (5) valid ascertainment of the causes of cancer morbidity and mortality; (6) proper consideration of bias and confounding factors; (7) adequate sample size to detect an effect; (8) clear, well-documented, and appropriate methodology for data collection and analysis; (9) adequate response rate and methodology for handling missing data; and (10) complete and clear documentation of results. No single criterion determines the overall adequacy of a study. Practical and resource constraints may limit the ability to address all of these characteristics in a study. The risk assessor is encouraged to consider how the limitations of the available studies might influence the conclusions. While positive biases may be due, for example, to a healthy worker effect, it is also important to consider negative biases, for example, workers who may leave the workforce due to illness caused either by high exposures to the agent or to effects of confounders such as smoking.

The following discussions highlight the major factors included in an analysis of epidemiologic studies.

2.2.1.2. *Types of Studies*

The major types of cancer epidemiologic study designs used for examining environmental causes of cancer are analytical studies and descriptive studies. Each study type has well-known strengths and weaknesses that affect interpretation of results, as summarized below (Lilienfeld and Lilienfeld, 1979; Mausner and Kramer, 1985; Kelsey et al., 1996; Rothman and Greenland, 1998).

Analytical epidemiologic studies, which include case-control and cohort designs, are generally relied on for identifying a causal association between human exposure and adverse health effects. In case-control studies, groups of individuals with (cases) and without (controls) a particular disease are identified and compared to determine differences in exposure. In cohort studies, a group of "exposed" and "nonexposed" individuals are identified and studied over time to determine differences in disease occurrence. Cohort studies can be performed either prospectively or retrospectively from historical records. The type of study chosen may depend on the hypothesis to be evaluated. For example, case-control studies may be more appropriate for rare cancers while cohort studies may be more appropriate for more commonly occurring cancers.

On the other hand, descriptive epidemiologic studies examine symptom or disease rates among populations in relation to personal characteristics such as age, gender, race, and temporal or environmental conditions. Descriptive studies are most frequently used to generate hypotheses about exposure factors, but subsequent analytical designs are necessary to infer causality. For example, cross-sectional designs might be used to compare the prevalence of cancer between areas near and far from a Superfund site. However, in studies where exposure and disease information applies only to the current conditions, it is not possible to infer that the exposure actually *caused* the disease. Therefore, these studies are used to identify patterns or trends in disease occurrence over time or in different geographical locations, but typical

limitations in the characterization of populations in these studies make it difficult to infer the causal agent or degree of exposure.

Case reports describe a particular effect in an individual or group of individuals who were exposed to a substance. These reports are often anecdotal or highly selective in nature and generally are of limited use for hazard assessment. Specifically, cancer causality can rarely be inferred from case reports alone. Investigative follow-up may or may not accompany such reports. For cancer, the most common types of case series are associated with occupational and childhood exposures. Case reports can be particularly valuable for identifying unique features, such as an association with an uncommon tumor (e.g., inhalation of vinyl chloride and hepatic angiosarcoma in workers or ingestion of diethylstilbestrol by mothers and clear-cell carcinoma of the vagina in offspring).

2.2.1.3. ***Exposure Issues***.

For epidemiologic data to be useful in determining whether there is an association between health effects and exposure to an agent, there should be adequate characterization of exposure information. In general, greater weight should be given to studies with more precise and specific exposure estimates.

Questions to address about exposure are: What can one reliably conclude about the exposure parameters including (but not limited to) the level, duration, route, and frequency of exposure of individuals in one population as compared with another? How sensitive are study results to uncertainties in these parameters?

Actual exposure measurements are not available for many retrospective studies. Therefore, surrogates are often used to reconstruct exposure parameters. These may involve attributing exposures to job classifications in a workplace or to broader occupational or geographic groupings. Use of surrogates carries a potential for misclassification, i.e., individuals may be placed in an incorrect exposure group. Misclassification generally leads to reduced ability of a study to detect differences between study and referent populations.

When either current or historical monitoring data are available, the exposure evaluation includes consideration of the error bounds of the monitoring and analytic methods and whether

the data are from routine or accidental exposures. The potential for misclassification and for measurement errors is amenable to both qualitative and quantitative analysis. These are essential analyses for judging a study's results, because exposure estimation is the most critical part of a retrospective study.

2.2.1.4. *Biological Markers*.

Biological markers potentially offer excellent measures of exposure (Hulka and Margolin, 1992; Peto and Darby, 1994). In some cases, molecular or cellular effects (e.g., DNA or protein adducts, mutation, chromosomal aberrations, levels of thyroid-stimulating hormone) can be measured in blood, body fluids, cells, and tissues to serve as biomarkers of exposure in humans and animals (Callemen et al., 1978; Birner et al., 1990). As such, they can act as an internal surrogate measure of chemical dose, representing, as appropriate, either recent exposure (e.g., serum concentration) or accumulated exposure over some period (e.g., hemoglobin adducts). Validated markers of exposure such as alkylated hemoglobin from exposure to ethylene oxide (Van Sittert et al., 1985) or urinary arsenic (Enterline et al., 1987) can improve estimates of dose over the relevant time periods for the markers. Markers closely identified with effects promise to greatly increase the ability of studies to distinguish real effects from bias at low levels of relative risk between populations (Taylor et al., 1994; Biggs et al., 1993) and to resolve problems of confounding risk factors. However, when using molecular or cellular effects as biomarkers of exposure, since many of these changes are often not specific to just one type of exposure, it is important to be aware that changes may be due to exposures unrelated to the exposure of interest and attention must be paid to controlling for potential confounders.

Biochemical or molecular epidemiologic studies may use biological markers of effect as indicators of disease or its precursors. The application of techniques for measuring cellular and molecular alterations due to exposure to specific environmental agents may allow conclusions to be drawn about the mechanisms of carcinogenesis (see section 2.4 for more information on this topic).

2.2.1.5. ***Confounding Factors***.

Control for potential confounding factors is an important consideration in the evaluation of the design and in the analysis of observational epidemiologic studies. A confounder is a variable that is related to both the health outcome of concern (cancer) and exposure. Common examples include age, socioeconomic status, smoking habits, and diet. For instance, if older people are more likely to be exposed to a given contaminant as well as more likely to have cancer because of their age, age is considered a confounder. Adjustment for potentially confounding factors (from a statistical as contrasted with an epidemiologic point of view) can occur either in the design of the study (e.g., individual or group matching on critical factors) or in the statistical analysis of the results (stratification or direct or indirect adjustment). Direct adjustment in the statistical analysis may not be possible owing to the presentation of the data or because needed information was not collected during the study. In this case, indirect comparisons may be possible. For example, in the absence of data on smoking status among individuals in the study population, an examination of the possible contribution of cigarette smoking to increased lung cancer risk may be based on information from other sources, such as the American Cancer Society's longitudinal studies (Hammand, 1966; Garfinkel and Silverberg, 1991). The effectiveness of adjustments contributes to the ability to draw inferences from a study.

Different studies involving exposure to an agent may have different confounding factors. If consistent increases in cancer risk are observed across a collection of studies with different confounding factors, the inference that the agent under investigation was the etiologic factor is strengthened.

There may also be instances where the agent of interest is a risk factor in conjunction with another agent. For instance, interaction as well as effect-measure modification are sometimes construed to be confounding, but they are different than confounding. Interaction is described as a situation in which two or more risk factors modify the effect of each other with regard to the occurrence of a given effect. This phenomenon is sometimes described as effect-measure modification or heterogeneity of effect (Szklo and Nieto, 2000). Effect-measure modification refers to variation in the magnitude of measure exposure effect across levels of another variable (Rothman and Greenland, 1998). The variable across which the effect measure varies and is

called an *effect modifier* (e.g., hepatitis virus B and aflatoxin in hepatic cancer). Interaction, on the other hand, means effect of the exposure on the outcome differs, depending on the presence of another variable (the effect modifier). When the effect of the exposure of interest is accentuated by another variable, it is said to be synergistic interaction. Synergistic interaction can be additive (e.g., hepatitis virus B and aflatoxin in hepatic cancer) or multiplicative (e.g., asbestos and smoking in lung cancer). If the effect of exposure is diminished or eliminated by another variable, it said to be antagonistic interaction (e.g., intake of vitamin E and lower occurrence of lung cancer).

2.2.1.6. ***Statistical Considerations***.

The analysis should apply appropriate statistical methods to ascertain whether the observed association between exposure and effects would be expected by chance. A description of the method or methods used should include the reasons for their selection. Statistical analyses of the bias, confounding, and interaction are part of addressing the significance of an association and the power of a study to detect an effect.

The analysis augments examination of the results for the whole population with exploration of the results for groups with comparatively greater exposure or time since first exposure. This may support identifying an association or establishing a dose-response trend. When studies show no association, such exploration may apply to determining an upper limit on potential human risk for consideration alongside results of animal tumor effects studies.

2.2.1.6.1. ***Likelihood of observing an effect***. The power of a study – the likelihood of observing an effect if one exists – increases with sample size, i.e., the number of subjects studied from a population. (For example, a quadrupling of a background rate in the 1 per 10,000 range would require more subjects who have experienced greater or longer exposure or lengthier follow-up, than a doubling of a background rate in the 1 per 100 range.) If the size of the effect is expected to be very small at low doses, higher doses or longer durations of exposure may be needed to have an appreciable likelihood of observing an effect with a given sample size. Because of the often long latency period in cancer development, the likelihood of observing an effect also

depends on whether adequate time has elapsed since exposure began for effects to occur. Since the design of the study and the choice of analysis, as well as the design level of certainty in the results and the magnitude of response in an unexposed population also affect the likelihood of observing an effect, it is important to carefully interpret the absence of an observed effect. A unique feature that can be ascribed to the effects of a particular agent (such as a tumor type that is seen only rarely in the absence of the agent) can increase sensitivity by permitting separation of bias and confounding factors from real effects. Similarly, a biomarker particular to the agent can permit these distinctions. Statistical re-analyses of data, particularly an examination of different exposure indices, can give insight into potential exposure-response relationships. These are all factors to explore in statistical analysis of the data.

2.2.1.6.2. ***Sampling and other bias issues.*** When comparing cases and controls or exposed and non-exposed populations, it would be preferable for the two populations to differ only in exposure to the agent in question. Because this is seldom the case, it is important to identify sources of sampling and other potential biases inherent in a study design or data collection methods.

Bias is a systematic error. In epidemiologic studies, bias can occur in the selection of cases and controls or exposed and non-exposed populations, as well as the follow up of the groups, or the classification of disease or exposure. The size of the risks observed can be affected by noncomparability between populations of factors such as general health, diet, lifestyle, or geographic location; differences in the way case and control individuals recall past events; differences in data collection that result in unequal ascertainment of health effects in the populations; and unequal follow-up of individuals (Rothman and Greenland, 1998). Other factors worth consideration can be inherent in the available cohorts, e.g., use of occupational studies (the healthy worker effect), absence of one sex, or limitations in sample size for one or more ethnicities.

The mere presence of biases does not invalidate a study, but should be reflected in the judgment of its strengths or weaknesses. Acceptance of studies for assessment depends on identifying their sources of bias and the possible effects on study results.

2.2.1.6.3. ***Combining statistical evidence across studies***. Meta-analysis is a means of integrating the results of multiple studies of similar health effects and risk factors. This technique is particularly useful when various studies yield varying degrees of risk or even conflicting associations (negative and positive). It is intended to introduce consistency and comprehensiveness into what otherwise might be a more subjective review of the literature. The value of such an analysis is dependent upon a systematic review of the literature that uses transparent criteria of inclusion and exclusion. In interpreting such analyses, it is important to consider the effects of differences in study quality, as well as the effect of publication bias. Meta-analysis may not be advantageous in some circumstances. These include when the relationship between exposure and disease is obvious from the individual studies; when there are only a few studies of the key health outcomes; when there is insufficient information from available studies related to disease, risk estimate, or exposure classification to insure comparability; or when there are substantial confounding or other biases that cannot be adjusted for in the analysis (Blair et al., 1995; Greenland, 1987; Peto, 1992).

2.2.1.7. ***Evidence for Causality***

Determining whether an observed association (risk) is causal rather than spurious involves consideration of a number of factors. Sir Bradford Hill (Hill, 1965) developed a set of guidelines for evaluating epidemiologic associations that can be used in conjunction with the discussion of causality such as the 2004 Surgeon General's report on smoking (CDC, 2004) and in other documents (e.g., Rothman and Greenland 1998; IPCS, 1999) . The critical assessment of epidemiologic evidence is conceptually based upon consideration of salient aspects of the evidence of associations so as to reach fundamental judgments as to the likely causal significance of the observed associations. In so doing, it is appropriate to draw from those aspects initially presented in Hill's classic monograph (Hill, 1965) and widely used by the scientific community in conducting such evidence-based reviews. A number of these aspects are judged to be particularly salient in evaluating the body of evidence available in this review, including the aspects described by Hill as strength, experiment, consistency, plausibility, and coherence. Other aspects identified by Hill, including temporality and biological gradient, are also relevant and

considered here (e.g., in characterizing lag structures and concentration-response relationships), but are more directly addressed in the design and analyses of the individual epidemiologic studies included in this assessment. As discussed below, these salient aspects are interrelated and considered throughout the evaluation of the epidemiologic evidence generally reflected in the integrative synthesis of the mode of action framework.

The general evaluation of the strength of the epidemiological evidence reflects consideration not only of the magnitude of reported effects estimates and their statistical significance, but also of the precision of the effects estimates and the robustness of the effects associations. Consideration of the robustness of the associations takes into account a number of factors, including in particular the impact of alternative models and model specifications and potential confounding factors, as well issues related to the consequences of measurement error. Consideration of the consistency of the effects associations involves looking across the results of studies conducted by different investigators in different places and times. Particular weight may be given, consistent with Hill's views, to the presence of "similar results reached in quite different ways, e.g., prospectively and retrospectively" (Hill, 1965). Looking beyond the epidemiological evidence, evaluation of the biological plausibility of the associations observed in epidemiologic studies reflects consideration of both exposure-related factors and toxicological evidence relevant to identification of potential modes of action (MOAs). Similarly, consideration of the coherence of health effects associations reported in the epidemiologic literature reflects broad consideration of information pertaining to the nature of the biological markers evaluated in toxicologic and epidemiologic studies.

In identifying these aspects as being particularly salient in this assessment, it is also important to recognize that no one aspect is either necessary or sufficient for drawing inferences of causality. As Hill (1965) emphasized:

> "None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required as *a sine qua non*. What they can do, with greater or less strength, is to help us to make up our minds on the fundamental question — is there any other way of explaining the set of facts

> before us, is there any other answer equally, or more, likely than cause and effect?"

While these aspects frame considerations weighed in assessing the epidemiologic evidence, they do not lend themselves to being considered in terms of simple formulas or hard-and-fast rules of evidence leading to answers about causality (Hill, 1965). One, for example, cannot simply count up the numbers of studies reporting statistically significant results or statistically non-significant results for carcinogenesis and related MOAs and reach credible conclusions about the relative strength of the evidence and the likelihood of causality. Rather, these important considerations are taken into account throughout the assessment with a goal of producing an objective appraisal of the evidence (informed by peer and public comment and advice), which includes the weighing of alternative views on controversial issues. Thus, although these guidelines have become known as "causal criteria," it is important to note that they cannot be used as a strictly quantitative checklist. Rather, these "criteria" should be used to determine the strength of the evidence for concluding causality. In particular, the absence of one or more of the "criteria" does not automatically exclude a study from consideration (e.g., see discussion in CDC, 2004). The list below has been adapted from Hill's guidelines as an aid in judging causality.

(a) Consistency of the observed association. An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered.

(b) Strength of the observed association. The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. A modest risk, however, does not preclude a causal association and may reflect a lower level of exposure, an agent of lower potency, or a common disease with a high background level.

(c) Specificity of the observed association. As originally intended, this refers to increased inference of causality if one cause is associated with a single effect or disease (Hill, 1965). Based on our current understanding that many agents cause cancer at multiple sites, and

many cancers have multiple causes, this is now considered one of the weaker guidelines for causality. Thus, although the presence of specificity may support causality, its absence does not exclude it.

(d) Temporal relationship of the observed association. A causal interpretation is strengthened when exposure is known to precede development of the disease. Because a latent period of up to 20 years or longer is often associated with cancer development in adults, the study should consider whether exposures occurred sufficiently long ago to produce an effect at the time the cancer is assessed. This is among the strongest criteria for an inference of causality.

(e) Biological gradient (exposure-response relationship). A clear exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times). There are many possible reasons that an epidemiologic study may fail to detect an exposure-response relationship. For example, an analysis that included decreasing exposures due to improved technology that is combined with higher prior exposure in an initial analysis can require a segmented analysis to apportion exposure. Other reasons for failure to detect a relationship may include a small range of exposures. Thus, the absence of an exposure-response relationship does not exclude a causal relationship.

(f) Biological plausibility. An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A lack of mechanistic data, however, is not a reason to reject causality.

(g) Coherence. An inference of causality may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. Information is considered from animal bioassays, toxicokinetic studies, and short-term studies. The absence of other lines of evidence, however, is not a reason to reject causality.

(h) Experimental evidence (from human populations). Experimental evidence is seldom available from human populations and exists only when conditions of human exposure have occurred to create a “natural experiment” at different levels of exposure. Strong evidence

for causality can be provided when a change in exposure brings about a change in disease frequency, for example, the decrease in the risk of lung cancer that follows cessation of smoking.

(i) Analogy. SARs and information on the agent's structural analogues can provide insight into whether an association is causal. Similarly, information on mode of action for a chemical, as one of many structural analogues, can inform decisions regarding likely causality.

2.2.2. Animal Data

Various whole-animal test systems are currently used or are under development for evaluating potential carcinogenicity. Cancer studies involving chronic exposure for most of the lifespan of an animal are generally accepted for evaluation of tumor effects (Tomatis et al., 1989; Rall, 1991; Allen et al., 1988; but see Ames and Gold, 1990). Other studies of special design are useful for observing formation of preneoplastic lesions or tumors or investigating specific modes of action. Their applicability is determined on a case-by-case basis.

2.2.2.1. *Long-term Carcinogenicity Studies*

The objective of long-term carcinogenesis bioassays is to determine the potential carcinogenic hazard and dose-response relationships of the test agent. Carcinogenicity rodent studies are designed to examine the production of tumors as well as preneoplastic lesions and other indications of chronic toxicity that may provide evidence of treatment-related effects and insights into the way the test agent produces tumors. Current standardized carcinogenicity studies in rodents test at least 50 animals per sex per dose group in each of three treatment groups and in a concurrent control group, usually for 18 to 24 months, depending on the rodent species tested (OECD, 1981; U.S. EPA, 1998c). The high dose in long-term studies is generally selected to provide the maximum ability to detect treatment-related carcinogenic effects while not compromising the outcome of the study through excessive toxicity or inducing inappropriate toxicokinetics (e.g., overwhelming absorption or detoxification mechanisms). The purpose of two or more lower doses is to provide some information on the shape of the dose-response curve. Similar protocols have been and continue to be used by many laboratories worldwide.

All available studies of tumor effects in whole animals should be considered, at least preliminarily. The analysis should discard studies judged to be wholly inadequate in protocol, conduct, or results. Criteria for the technical adequacy of animal carcinogenicity studies have been published and should be used as guidance to judge the acceptability of individual studies (e.g., NTP, 1984; OSTP, 1985; Chhabra et al., 1990). As these criteria, in whole or in part, may be updated by the National Toxicology Program (NTP) and others, the analyst should consult the appropriate sources to determine both the current standards as well as those that were contemporaneous with the study. Care should be taken to include studies that provide some evidence bearing on carcinogenicity or that help interpret effects noted in other studies, even if these studies have some limitations of protocol or conduct. Such limited, but not wholly inadequate, studies can contribute as their deficiencies permit. The findings of long-term rodent bioassays should be interpreted in conjunction with results of prechronic studies along with toxicokinetic studies and other pertinent information, if available. Evaluation of tumor effects takes into consideration both biological and statistical significance of the findings (Haseman, 1984, 1985, 1990, 1995). The following sections highlight the major issues in the evaluation of long-term carcinogenicity studies.

2.2.2.1.1. ***Dosing issues***. Among the many criteria for technical adequacy of animal carcinogenicity studies is the appropriateness of dose selection. The selection of doses for chronic bioassays is based on scientific judgments and sound toxicologic principles. Dose selection should be made on the basis of relevant toxicologic information from prechronic, mechanistic, and toxicokinetic and mechanistic studies. A scientific rationale for dose selection should be clearly articulated (e.g., NTP, 1984; ILSI, 1997). How well the dose selection is made is evaluated after the completion of the bioassay.

Interpretation of carcinogenicity study results is profoundly affected by study exposure conditions, especially by inappropriate dose selection. This is particularly important in studies that do not show positive results for carcinogenicity, because failure to use a sufficiently high dose reduces the sensitivity of the studies. A lack of tumorigenic responses at exposure levels that cause significant impairment of animal survival may also not be acceptable. In addition,

overt toxicity or qualitatively altered toxicokinetics due to excessively high doses may result in tumor effects that are secondary to the toxicity rather than directly attributable to the agent.

With regard to the appropriateness of the high dose, an adequate high dose would generally be one that produces some toxic effects without unduly affecting mortality from effects other than cancer or producing significant adverse effects on the nutrition and health of the test animals (OECD, 1981; NRC, 1993a). If the test agent does not appear to cause any specific target organ toxicity or perturbation of physiological function, an adequate high dose can be specified in terms of a percentage reduction of body weight gain over the lifespan of the animals. The high dose would generally be considered inadequate if neither toxicity nor change in weight gain is observed. On the other hand, significant increases in mortality from effects other than cancer generally indicate that an adequate high dose has been exceeded.

Other signs of treatment-related toxicity associated with an excessive high dose may include (a) significant reduction of body weight gain (e.g., greater than 10%), (b) significant increases in abnormal behavioral and clinical signs, (c) significant changes in hematology or clinical chemistry, (d) saturation of absorption and detoxification mechanisms, or (e) marked changes in organ weight, morphology, and histopathology. It should be noted that practical upper limits have been established to avoid the use of excessively high doses in long-term carcinogenicity studies of environmental chemicals (e.g., 5% of the test substance in the feed for dietary studies or 1 g/kg body weight for oral gavage studies [OECD, 1981]).

For dietary studies, weight gain reductions should be evaluated as to whether there is a palatability problem or an issue with food efficiency; certainly, the latter is a toxic manifestation. In the case of inhalation studies with respirable particles, evidence of impairment of normal clearance of particles from the lung should be considered along with other signs of toxicity to the respiratory airways to determine whether the high exposure concentration has been appropriately selected (U.S. EPA, 2001a). For dermal studies, evidence of skin irritation may indicate that an adequate high dose has been reached (U.S. EPA, 1989).

In order to obtain the most relevant information from a long-term carcinogenicity study, it is important to maximize exposure conditions to the test material. At the same time, caution is appropriate in using excessive high-dose levels that would confound the interpretation of study

results to humans. The middle and lowest doses should be selected to characterize the shape of the dose-response curve as much as possible. It is important that the doses be adequately spaced so that the study can provide relevant dose-response data for assessing human hazard and risk. If the testing of potential carcinogenicity is being combined with an evaluation of noncancer chronic toxicity, the study should be designed to include one dose in addition to the control(s) that is not expected to elicit adverse effects.

There are several possible outcomes regarding the study interpretation of the significance and relevance of tumorigenic effects associated with exposure or dose levels below, at, or above an adequate high dose. The general guidance is given here; for each case, the information at hand should be evaluated and a rationale should be given for the position taken.

- *Adequately high dose.* If an adequately high dose has been used, tumor effects are judged positive or negative depending on the presence or absence of significant tumor incidence increases, respectively.

- *Excessively high dose.* If toxicity or mortality is excessive at the high dose, interpretation depends on whether or not tumors are found.

  – Studies that show tumor effects only at excessive doses may be compromised and may or may not carry weight, depending on the interpretation in the context of other study results and other lines of evidence. Results of such studies, however, are generally not considered suitable for dose-response extrapolation if it is determined that the mode(s) of action underlying the tumorigenic responses at high doses is not operative at lower doses.

  – Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits.

- If a study does not show an increase in tumor incidence at a toxic high dose and appropriately spaced lower doses are used without such toxicity or tumors, the study is generally judged as negative for carcinogenicity.

- *Inadequately high dose*. Studies of inadequate sensitivity where an adequately high dose has not been reached may be used to bound the dose range where carcinogenic effects might be expected.

2.2.2.1.2. ***Statistical considerations***. The main aim of statistical evaluation is to determine whether exposure to the test agent is associated with an increase of tumor development. Statistical analysis of a long-term study should be performed for each tumor type separately. The incidence of benign and malignant lesions of the same cell type, usually within a single tissue or organ, are considered separately but may be combined when scientifically defensible (McConnell et al., 1986).

Trend tests and pairwise comparison tests are the recommended tests for determining whether chance, rather than a treatment-related effect, is a plausible explanation for an apparent increase in tumor incidence. A trend test such as the Cochran-Armitage test (Snedecor and Cochran, 1967) asks whether the results in all dose groups together increase as dose increases. A pairwise comparison test such as the Fisher exact test (Fisher, 1950) asks whether an incidence in one dose group is increased over that of the control group. By convention, for both tests a statistically significant comparison is one for which p is less than 0.05 that the increased incidence is due to chance. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the result.

A statistically significant response may or may not be biologically significant and vice versa. The selection of a significance level is a policy choice based on a trade-off between the risks of false positives and false negatives. A result with a significance level of greater or less than 5% (the most common significance level) is examined to see if the result confirms other scientific information. When the assessment departs from a simple 5% level, this should be

highlighted in the risk characterization. A two-tailed test or a one-tailed test can be used. In either case a rationale is provided.

Statistical power can affect the likelihood that a statistically significant result could reasonably be expected. This is especially important in studies or dose groups with small sample sizes or low dose rates. Reporting the statistical power can be useful for comparing and reconciling positive and negative results from different studies.

Considerations of multiple comparisons should also be taken into account. Haseman (1983) analyzed typical animal bioassays that tested both sexes of two species and concluded that, because of multiple comparisons, a single tumor increase for a species-sex-site combination that is statistically significant at the 1% level for common tumors or 5% for rare tumors corresponds to a 7–8% significance level for the study as a whole. Therefore, animal bioassays presenting only one significant result that falls short of the 1% level for a common tumor should be treated with caution.

2.2.2.1.3. ***Concurrent and historical controls*****.** The standard for determining statistical significance of tumor incidence comes from a comparison of tumors in dosed animals with those in concurrent control animals. Additional insights about both statistical and biological significance can come from an examination of historical control data (Tarone, 1982; Haseman, 1995). Historical control data can add to the analysis, particularly by enabling identification of uncommon tumor types or high spontaneous incidence of a tumor in a given animal strain. Identification of common or uncommon situations prompts further thought about the meaning of the response in the current study in context with other observations in animal studies and with other evidence about the carcinogenic potential of the agent. These other sources of information may reinforce or weaken the significance given to the response in the hazard assessment. Caution should be exercised in simply looking at the ranges of historical responses, because the range ignores differences in survival of animals among studies and is related to the number of studies in the database.

In analyzing results for uncommon tumors in a treated group that are not statistically significant in comparison with concurrent controls, the analyst may be informed by the

experience of historical controls to conclude that the result is in fact unlikely to be due to chance. However, caution should be used in interpreting results. In analyzing results for common tumors, a different set of considerations comes into play. Generally speaking, statistically significant increases in tumors should not be discounted simply because incidence rates in the treated groups are within the range of historical controls or because incidence rates in the concurrent controls are somewhat lower than average. Random assignment of animals to groups and proper statistical procedures provide assurance that statistically significant results are unlikely to be due to chance alone. However, caution should be used in interpreting results that are barely statistically significant or in which incidence rates in concurrent controls are unusually low in comparison with historical controls.

In cases where there may be reason to discount the biological relevance to humans of increases in common animal tumors, such considerations should be weighed on their own merits and clearly distinguished from statistical concerns.

When historical control data are used, the discussion should address several issues that affect comparability of historical and concurrent control data, such as genetic drift in the laboratory strains, differences in pathology examination at different times and in different laboratories (e.g., in criteria for evaluating lesions; variations in the techniques for the preparation or reading of tissue samples among laboratories), and comparability of animals from different suppliers. The most relevant historical data come from the same laboratory and the same supplier and are gathered within 2 or 3 years one way or the other of the study under review; other data should be used only with extreme caution.

2.2.2.1.4. ***Assessment of evidence of carcinogenicity from long-term animal studies***. In general, observation of tumors under different circumstances lends support to the significance of the findings for animal carcinogenicity. Significance is generally increased by the observation of more of the factors listed below. For a factor such as malignancy, the severity of the observed pathology can also affect the significance. The following observations add significance to the tumor findings:

- uncommon tumor types;
- tumors at multiple sites;
- tumors by more than one route of administration;
- tumors in multiple species, strains, or both sexes;
- progression of lesions from preneoplastic to benign to malignant;
- reduced latency of neoplastic lesions;
- metastases;
- unusual magnitude of tumor response;
- proportion of malignant tumors; and
- dose-related increases.

In these cancer guidelines, tumors observed in animals are generally assumed to indicate that an agent may produce tumors in humans. Mode of action may help inform this assumption on a chemical-specific basis. Moreover, the absence of tumors in well-conducted, long-term animal studies in at least two species provides reasonable assurance that an agent may not be a carcinogenic concern for humans.

2.2.2.1.5. ***Site concordance*****.** Site concordance of tumor effects between animals and humans should be considered in each case. Thus far, there is evidence that growth control mechanisms at the level of the cell are homologous among mammals, but there is no evidence that these mechanisms are site concordant. Moreover, agents observed to produce tumors in both humans and animals have produced tumors either at the same site (e.g., vinyl chloride) or different sites (e.g., benzene) (NRC, 1994). Hence, site concordance is not always assumed between animals and humans. On the other hand, certain modes of action with consequences for particular tissue sites (e.g., disruption of thyroid function) may lead to an anticipation of site concordance.

2.2.2.2. ***Perinatal Carcinogenicity Studies***

The objective of perinatal carcinogenesis studies is to determine the carcinogenic potential and dose-response relationships of the test agent in the developing organism. Some

investigators have hypothesized that the age of initial exposure to a chemical carcinogen may influence the carcinogenic response (Vesselinovitch et al., 1979; Rice, 1979; McConnell, 1992). Current standardized long-term carcinogenesis bioassays generally begin dosing animals at 6–8 weeks of age and continue dosing for the lifespan of the animal (18–24 months). This protocol has been modified in some cases to investigate the potential of the test agent to induce transplacental carcinogenesis or to investigate the potential differences following perinatal and adult exposures, but currently there is not a standardized protocol for testing agents for carcinogenic effects following prenatal or early postnatal exposure.

Several cancer bioassay studies have compared adult and perinatal exposures (see McConnell, 1992; U.S. EPA, 1996b). A review of these studies reveals that perinatal exposure rarely identifies carcinogens that are not found in standard animal bioassays. Exposure that is perinatal can increase the incidence of a given type of tumor. The increase may reflect an increased length of exposure and a higher dose for the developing organism relative to the adult or an increase in susceptibility in some cases. Additionally, exposure that is perinatal through adulthood sometimes reduces the latency period for tumors to develop in the growing organism (U.S. EPA, 1996b). EPA evaluates the usefulness of perinatal studies on an agent-by-agent basis (e.g., U.S. EPA, 1997a, b).

Perinatal study data analysis generally follows the principles discussed above for evaluating other long-term carcinogenicity studies. When differences in responses between perinatal animals and adult animals suggest an increased susceptibility of perinatal or postnatal animals, such as the ones below, a separate evaluation of the response should be prepared:

- a difference in dose-response relationship,
- the presence of different tumor types,
- an earlier onset of tumors, or
- an increase in the incidence of tumors.

2.2.2.3. *Other Studies*

Intermediate-term and acute dosing studies often use protocols that screen for carcinogenic or preneoplastic effects, sometimes in a single tissue. Some protocols involve the development of various proliferative lesions, such as foci of alteration in the liver (Goldsworthy et al., 1986). Others use tumor endpoints, such as the induction of lung adenomas in the sensitive strain A mouse (Maronpot et al., 1986) or tumor induction in initiation-promotion studies using various organs such as the bladder, intestine, liver, lung, mammary gland, and thyroid (Ito et al., 1992). In these tests, the selected tissue rather than the whole animal is, in a sense, the test system. Important information concerning the steps in the carcinogenic process and mode of action can be obtained from "start/stop" experiments. In these protocols, an agent is given for a period of time to induce particular lesions or effects and then stopped in order to evaluate the progression or reversibility of processes (Todd, 1986; Marsman and Popp, 1994).

Assays in genetically engineered rodents may provide insight into the chemical and gene interactions involved in carcinogenesis (Tennant et al., 1995). These mechanistically based approaches involve activated oncogenes that are introduced (transgenic) or tumor suppressor genes that are deleted (knocked out). If appropriate genes are selected, not only may these systems provide information on mechanisms, but the rodents typically show tumor development earlier than in the standard bioassay. Transgenic mutagenesis assays also represent a mechanistic approach for assessing the mutagenic properties of agents as well as developing quantitative linkages between exposure, internal dose, and mutation related to tumor induction (Morrison and Ashby, 1994; Sisk et al., 1994; Hayward et al., 1995).

The support that these studies give to a determination of carcinogenicity rests on their contribution to the consistency of other evidence about an agent. For instance, benzoyl peroxide has promoter activity on the skin, but the overall evidence may be less supportive (Kraus et al., 1995). These studies also may contribute information about mode of action. It is important to recognize the limitations of these experimental protocols, such as short duration, limited histology, lack of complete development of tumors, or experimental manipulation of the carcinogenic process, that may limit their contribution to the overall assessment. Generally, their results are appropriate as aids in the interpretation of other toxicological evidence (e.g., rodent

chronic bioassays), especially regarding potential modes of action. On the basis of currently available information, it is unlikely that any of these assays, which are conducted for 6 months with 15 animals per group, will replace all chronic bioassays for hazard identification (Spalding et al., 2000; Gulezian et al., 2000; ILSI, 2001).

2.2.3. Structural Analogue Data

For some chemical classes, there is significant available information, largely from rodent bioassays, on the carcinogenicity of analogues. Analogue effects are instructive in investigating carcinogenic potential of an agent as well as in identifying potential target organs, exposures associated with effects, and potential functional class effects or modes of action. All appropriate studies should be included and analyzed, whether indicative of a positive effect or not. Evaluation includes tests in various animal species, strains, and sexes; with different routes of administration; and at various doses, as data are available. Confidence in conclusions is a function of how similar the analogues are to the agent under review in structure, metabolism, and biological activity. It is important to consider this confidence to ensure a balanced position.

2.3. ANALYSIS OF OTHER KEY DATA

The physical, chemical, and structural properties of an agent, as well as data on endpoints that are thought to be critical elements of the carcinogenic process, provide valuable insights into the likelihood of human cancer risk. The following sections provide guidance for analyses of these data.

2.3.1. Physicochemical Properties

Physicochemical properties affect an agent’s absorption, tissue distribution (bioavailability), biotransformation, and degradation in the body and are important determinants of hazard potential (and dose-response analysis). Properties that should be analyzed include, but are not limited to, molecular weight, size, and shape; valence state; physical state (gas, liquid, solid); water or lipid solubility, which can influence retention and tissue distribution; and potential for chemical degradation or stabilization in the body.

An agent's potential for chemical reaction with cellular components, particularly with DNA and proteins, is also important. The agent's molecular size and shape, electrophilicity, and charge distribution are considered in order to decide whether they would facilitate such reactions.

2.3.2. Structure-Activity Relationships (SARs)

SAR analyses and models can be used to predict molecular properties, surrogate biological endpoints, and carcinogenicity (see, e.g., Richard, 1998a, b; Richard and Williams, 2002; Contrera et al., 2003). Overall, these analyses provide valuable initial information on agents, they may strengthen or weaken concern, and they are part of the weight of evidence.

Currently, SAR analysis is most useful for chemicals and metabolites that are believed to initiate carcinogenesis through covalent interaction with DNA (i.e., DNA-reactive, mutagenic, electrophilic, or proelectrophilic chemicals) (Ashby and Tennant, 1991). For organic chemicals, the predictive capability of SAR analysis combined with other toxicity information has been demonstrated (Ashby and Tennant, 1994). The following parameters are useful in comparing an agent to its structural analogues and congeners that produce tumors and affect related biological processes such as receptor binding and activation, mutagenicity, and general toxicity (Woo and Arcos, 1989):

- nature and reactivity of the electrophilic moiety or moieties present;
- potential to form electrophilic reactive intermediate(s) through chemical, photochemical, or metabolic activation;
- contribution of the carrier molecule to which the electrophilic moiety(ies) is attached;
- physicochemical properties (e.g., physical state, solubility, octanol/water partition coefficient, half-life in aqueous solution);

- structural and substructural features (e.g., electronic, stearic, molecular geometric);
- metabolic pattern (e.g., metabolic pathways and activation and detoxification ratio); and
- possible exposure route(s) of the agent.

Suitable SAR analysis of non-DNA-reactive chemicals and of DNA-reactive chemicals that do not appear to bind covalently to DNA should be based on knowledge or postulation of the probable mode(s) of action of closely related carcinogenic structural analogues (e.g., receptor mediated, cytotoxicity related). Examination of the physicochemical and biochemical properties of the agent may then provide the rest of the information needed in order to make an assessment of the likelihood of the agent's activity by that mode of action.

2.3.3. Comparative Metabolism and Toxicokinetics

Studies of the absorption, distribution, biotransformation, and excretion of agents permit comparisons among species to assist in determining the implications of animal responses for human hazard assessment, supporting identification of active metabolites, identifying changes in distribution and metabolic pathway or pathways over a dose range, and making comparisons among different routes of exposure.

If extensive data are available (e.g., blood/tissue partition coefficients and pertinent physiological parameters of the species of interest), physiologically based toxicokinetic models can be constructed to assist in a determination of tissue dosimetry, species-to-species extrapolation of dose, and route-to-route extrapolation (Conolly and Andersen, 1991; see Section 3.1.2). If sufficient data are not available, it may be assumed as a default that toxicokinetic and metabolic processes are qualitatively comparable among species. Discussion of appropriate procedures for quantitative, interspecies comparisons appears in Chapter 3.

The *qualitative* question of whether an agent is absorbed by a particular route of exposure is important for weight of evidence classification, discussed in Section 2.5. Decisions about

whether route of exposure is a limiting factor on expression of any hazard, e.g., absorption does not occur by a specified route, are generally based on studies in which effects of the agent or its structural analogues have been observed by different routes, on physical-chemical properties, or on toxicokinetics studies.

Adequate metabolism and toxicokinetic data can be applied toward the following, as data permit. Confidence in conclusions is enhanced when *in vivo* data are available.

- *Identifying metabolites and reactive intermediates of metabolism and determining whether one or more of these intermediates is likely to be responsible for the observed effects*. Information on the reactive intermediates focuses on SAR analysis, analysis of potential modes of action, and estimation of internal dose in dose-response assessment (D'Souza et al., 1987; Krewski et al., 1987).

- *Identifying and comparing the relative activities of metabolic pathways in animals and in humans, and at different ages*. This analysis can provide insights for extrapolating results of animal studies to humans.

- *Describing anticipated distribution within the body and possibly identifying target organs*. Use of water solubility, molecular weight, and structure analysis can support qualitative inferences about anticipated distribution and excretion. In addition, describing whether the agent or metabolite of concern will be excreted rapidly or slowly or whether it will be stored in a particular tissue or tissues to be mobilized later can identify issues in comparing species and formulating dose-response assessment approaches.

- *Identifying changes in toxicokinetics and metabolic pathways with increases in dose*. These changes may result in important differences between high and low dose levels in disposition of the agent or generation of its active forms. These

studies play an important role in providing a rationale for dose selection in carcinogenicity studies.

- *Identifying and comparing metabolic process differences by age, sex, or other characteristic so that susceptible subpopulations can be recognized*. For example, metabolic capacity with respect to P450 enzymes in newborn children is extremely limited compared to that in adults, so that a carcinogenic metabolite formed through P450 activity will have limited effect in the young, whereas a carcinogenic agent deactivated through P450 activity will result in increased susceptibility of this lifestage (Cresteil, 1998). A variety of changes in toxicokinetics and physiology occur from the fetal stage to post-weaning to young child. Any of these changes may make a difference for risk (Renwick, 1998).

- *Determining bioavailability via different routes of exposure by analyzing uptake processes under various exposure conditions*. This analysis supports identification of hazards for untested routes. In addition, use of physicochemical data (e.g., octanol-water partition coefficient information) can support an inference about the likelihood of dermal absorption (Flynn, 1990).

Attempts should be made in all of these areas to clarify and describe as much as possible the variability to be expected because of differences in species, sex, age, and route of exposure. The analysis takes into account the presence of subpopulations of individuals who are particularly vulnerable to the effects of an agent because of toxicokinetic or metabolic differences (genetically or environmentally determined) (Bois et al., 1995) and is a special emphasis for assessment of risks to children.

2.3.4. Toxicological and Clinical Findings

Toxicological findings in experimental animals and clinical observations in humans are important resources for the cancer hazard assessment. Such findings provide information on

physiological effects and effects on enzymes, hormones, and other important macromolecules as well as on target organs for toxicity. For example, given that the cancer process represents defects in processes such as terminal differentiation, growth control, and cell death, developmental studies of agents may provide an understanding of the activity of an agent that carries over to cancer assessment. Toxicity studies in animals by different routes of administration support comparison of absorption and metabolism by those routes. Data on human variability in standard clinical tests may also provide insight into the range of human susceptibility and the common mechanisms of agents that affect the tested parameters.

2.3.5. Events Relevant to Mode of Carcinogenic Action

Knowledge of the biochemical and biological changes that precede tumor development (which include, but are not limited to, mutagenesis, increased cell proliferation, inhibition of programmed cell death, and receptor activation) may provide important insight for determining whether a cancer hazard exists and may help inform appropriate consideration of the dose-response relationship below the range of observable tumor response. Because cancer can result from a series of genetic alterations in the genes that control cell growth, division, and differentiation (Vogelstein et al., 1988; Hanahan and Weinberg, 2000; Kinzler and Vogelstein, 2002), the ability of an agent to affect genotype (and hence gene products) or gene expression is of obvious importance in evaluating its influence on the carcinogenic process. Initial and key questions to examine are: Does the agent (or its metabolite) interact directly with DNA, leading to mutations that bring about changes in gene products or gene expression? Does the agent bring about effects on gene expression via other nondirect DNA interaction processes?

Furthermore, carcinogenesis involves a complex series and interplay of events that alter the signals a cell receives from its extracellular environment, thereby promoting uncontrolled growth. Many, but not all, mutagens are carcinogens, and some, but not all, agents that induce cell proliferation lead to tumor development. Thus, understanding the range of key steps in the carcinogenic process upon which an agent might act is essential for evaluating its mode of action. Determination of carcinogens that are operating by a mutagenic mode of action, for example, entails evaluation of *in vivo* or *in vitro* short-term testing results for genetic endpoints, metabolic

profiles, physicochemical properties, and structure-activity relationship (SAR) analyses in a weight-of-evidence approach (Dearfield et al., 1991; U.S. EPA, 1986b; Waters et al., 1999). Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA-reactive and/or has the ability to bind to DNA. Also, mutagenic carcinogens usually produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed *in vivo* which generally are supported by positive tests *in vitro*. Additionally, carcinogens may be identified as operating via a mutagenic mode of action if they have similar properties and SAR to mutagenic carcinogens. Endpoints that provide insight into an agent's ability to alter gene products and gene expression, together with other features of an agent's potential mode of carcinogenic action, are discussed below.

2.3.5.1. *Direct DNA-Reactive Effects*

It is well known that many carcinogens are electrophiles that interact with DNA, resulting in DNA adducts and breakage (referred to in these cancer guidelines as direct DNA effects). Usually during the process of DNA replication, these DNA lesions can be converted into and fixed as mutations and chromosomal alterations that then may initiate and otherwise contribute to the carcinogenic process (Shelby and Zeiger, 1990; Tinwell and Ashby, 1991; IARC, 1999). Thus, studies of mutations and other genetic lesions continue to inform the assessment of potential human cancer hazard and in the understanding of an agent's mode of carcinogenic action.

EPA has published testing guidelines for detecting the ability of an agent to damage DNA and produce mutations and chromosomal alterations (as discussed in Dearfield et al., 1991). Briefly, standard tests for gene mutations in bacteria and mammalian cells *in vitro* and *in vivo* and for structural chromosomal aberrations *in vitro* and *in vivo* are important examples of relevant methods. New molecular approaches, such as mouse mutations and cancer transgenic models, are providing a means to examine mutation at tissue sites where the tumor response is observed (Heddle and Swiger, 1996; Tennant et al., 1999). Additionally, continued improvements in fluorescent-based chromosome staining methods (fluorescent *in situ*

hybridization [FISH]) will allow the detection of specific chromosomal abnormalities in relevant target tissues (Tucker and Preston, 1998).

Endpoints indicative of DNA damage but not measures of mutation *per se*, such as DNA adducts or strand breakage, may be detected in relevant target tissues and thus contribute to evaluating an agent's mutagenic potential. Evidence of chemical-specific DNA adducts (e.g., reactions at oxygen sites in DNA bases or with ring nitrogens of guanine and adenine) provides information on a mutagen's ability to directly interact with DNA (La and Swenberg, 1996). Some planar molecules (e.g., 9-aminoacridine) intercalate between base pairs of DNA, which results in a physical distortion in DNA that may lead to mutations when DNA replicates. As discussed below, some carcinogens do not interact directly with DNA, but they can produce increases in endogenous levels of DNA adducts (e.g., 8-hydroxyguanine) by indirect mechanisms.

2.3.5.2. *Indirect DNA Effects or Other Effects on Genes/Gene Expression*

Although some carcinogens may result in an elevation of mutations or cytogenetic anomalies, as detected in standard assays, they may do so by indirect mechanisms. These effects may be brought about by chemical-cell interactions rather than by the chemical (or its metabolite) directly interacting with DNA. An increase in mutations might be due to cytotoxic exposures causing regenerative proliferation or to mitogenic influences (Cohen and Ellwein, 1990). Increased cell division may elevate mutation by clonal expansion of initiated cells or by increasing the number of genetic errors by rapid cell division and reduced time for DNA repair. Some agents might result in an elevation of mutations by interfering with the enzymes involved in DNA repair and recombination (Barrett and Lee, 1992). Damage to certain critical DNA repair genes or other genes (e.g., the p53 gene) may result in genomic instability, which predisposes cells to further genetic alterations and increases the probability of neoplastic progression (Harris and Hollstein, 1993; Levine et al., 1994; Rouse and Jackson, 2002). Likewise, DNA repair processes may be saturated at certain doses of a chemical, leading to an elevation of genetic alterations.

The initiation of programmed cell death (apoptosis) can potentially be blocked by an agent, thereby permitting replication of cells carrying genetic errors that would normally be removed from the proliferative pool. At certain doses an agent may also generate reactive oxygen species that produce oxidative damage to DNA and other macromolecules (Chang et al. 1988; Kehrer, 1993; Clayson et al., 1994). The role of cellular alterations that are attributable to oxidative damage in tumorigenesis (e.g., 8-hydroxyguanine) is currently unclear.

Several carcinogens have been shown to induce aneuploidy (the loss or gain of chromosomes) (Barrett, 1992; Gibson et al., 1995). Aneuploidy can result in the loss of heterozygosity or genomic instability (Cavenee et al., 1986; Fearon and Vogelstein, 1990). Agents that cause aneuploidy typically interfere with the normal process of chromosome segregation by interacting with non-DNA targets such as the proteins needed for chromosome segregation and chromosome movement. Whether this chromosome imbalance is the cause or the effect of tumorigenesis is not clear. Thus, it is important to understand if the agent induces aneuploidy as a key early event in the carcinogenic process.

It is possible for an agent to alter gene expression by transcriptional, translational, or post-translational modifications. For example, perturbation of DNA methylation patterns may cause effects that contribute to carcinogenesis (Jones, 1986; Holliday, 1987; Goodman and Counts, 1993; Chuang et al., 1996; Baylin and Bestor, 2002). Overexpression of genes by DNA amplification has been observed in certain tumors (Vainio et al., 1992). Mechanisms of altering gene expression may involve cellular reprogramming through hormonal or receptor-mediated mechanisms (Barrett, 1992; Ashby et al., 1994).

Both cell proliferation and programmed cell death can be part of the maintenance of homeostasis in many normal tissues, and alterations in the level or rate of either can be important elements of the carcinogenic process. The balance between the two can directly affect the survival and growth of initiated cells as well as preneoplastic and tumor cell populations (i.e., increase in cell proliferation or decrease in cell death) (Moolgavkar, 1986; Cohen and Ellwein, 1990, 1991; Cohen et al., 1991; Bellamy et al., 1995). Thus, measurements of these events can contribute to the weight of the evidence for cancer hazard prediction and to mode of action

understanding. In studies of proliferative effects, distinctions should be made between mitogenesis and regenerative proliferation (Cohen and Ellwein, 1990, 1991; Cohen et al., 1991).

In applying information from studies on cell proliferation and apoptosis to risk assessment, it is important to identify the tissues and target cells involved, to measure effects in both normal and neoplastic tissue, to distinguish between apoptosis and necrosis, and to determine the dose that affects these processes. Gap-junctional intercellular communication is believed to play a role in tissue and organ development and in the maintenance of a normal cellular phenotype within tissues. A growing body of evidence suggests that chemical interference with gap-junctional intercellular communication is a contributing factor in tumor development (Swierenga and Yamasaki, 1992; Yamasaki, 1995).

2.3.5.3. *Precursor Events and Biomarker Information*

Most testing schemes for mutagenicity and other short-term assays were designed for hazard identification purposes; thus, these assays are generally conducted using acute exposures. For data on "precursor steps" to be useful in informing the dose-response curve for tumor induction below the level of observation, it is often useful for data to come from *in vivo* studies and from studies where exposure is repeated or given over an extended period of time. Although consistency of results across different assays and animal models provides a stronger basis for drawing conclusions, it is desirable to have data on the precursor event in the same target organ, sex, animal strain, and species as the tumor data. In evaluating an agent's mode of action, it is usually not sufficient to determine that some event commences upon dosing. It is important to understand whether it is a necessary event that plays a key role in the process that leads to tumor development versus an effect of the cancer process itself or simply an associated event.

Various endpoints can serve as biological markers of effects in biological systems or samples. These may help identify doses at which elements of the carcinogenic process are operating; aid in interspecies extrapolations when data are available from both experimental animal and human cells; and under certain circumstances, provide insights into the possible shape of the dose-response curve below levels where tumor incidences are observed (e.g., Choy, 1993).

Genetic and other findings (such as changes in proto-oncogenes and tumor suppressor genes in preneoplastic and neoplastic tissue or, possibly, measures of endocrine disruption) can indicate the potential for disease and, as such, serve as biomarkers of effect. They, too, can be used in different ways.

- The spectrum of genetic changes in proliferative lesions and tumors following chemical administration to experimental animals can be determined and compared with that in spontaneous tumors in control animals, in animals exposed to other agents of varying structural and functional activities, and in persons exposed to the agent under study.

- Biomarkers of effect and/or precursors may help to identify subpopulations of individuals who may be at an elevated risk for a certain cancer or exposure to a certain agent, e.g., cytochrome P450 2D6/debrisoquine sensitivity for lung cancer (Caporaso et al., 1989) or inherited colon cancer syndromes (Kinzler et al., 1991; Peltomäki et al., 1993).

- As with biomarkers of exposure, it may be justified in some cases to use biomarkers of effect and/or precursors for dose-response assessment or to provide insight into the potential shape of the dose-response curve at doses below those at which tumors are induced experimentally.

In applying biomarker data to cancer assessment an assessment should consider:

- analytical methodology,
- routes of exposure,
- exposure to mixtures,
- time after exposure,
- sensitivity and specificity of biomarkers, and
- dose-response relationships.

2.3.5.4. *Judging Data*

Criteria that are generally applicable for judging the adequacy of mechanistically based data include:

- mechanistic relevance of the data to carcinogenicity,
- number of studies of each endpoint,
- consistency of results in different test systems and different species,
- similar dose-response relationships for tumor and mode of action-related effects,
- conduct of the tests in accordance with generally accepted protocols, and
- degree of consensus and general acceptance among scientists regarding interpretation of the significance and specificity of the tests.

Although important information can be gained from *in vitro* test systems, a higher level of confidence is generally given to data that are derived from *in vivo* systems, particularly those results that show a site concordance with the tumor data.

It is important to remember that when judging and considering the use of any data, the basic standard of quality, as defined by the EPA Information Quality Guidelines, should be satisfied.

2.4. MODE OF ACTION—GENERAL CONSIDERATIONS AND FRAMEWORK FOR ANALYSIS

2.4.1. General Considerations

The interaction between the biology of the organism and the chemical properties of the agent determine whether there is an adverse effect. Thus, mode of action analysis is based on physical, chemical, and biological information that helps to explain key events in an agent's influence on development of tumors. The entire range of information developed in the assessment is reviewed to arrive at a reasoned judgment. An agent may work by more than one mode of action, both at different sites and at the same tumor site. Thus the mode of action and human relevance cannot necessarily be generalized to other toxic endpoints or tissues or cell types without additional analyses (IPCS, 1999; Meek et al., 2003). At least some information

bearing on mode of action (e.g., SAR, screening tests for mutagenicity) is present for most agents undergoing assessment of carcinogenicity, even though certainty about exact molecular mechanisms may be rare.

Information for mode of action analysis generally includes tumor data in humans and animals and among structural analogues, as well as the other key data. The more complete the data package and the generic knowledge about a given mode of action, the more confidence one has and the more one can rely on assessment of available data rather than reverting to default options to address the absence of information on mode of action. Reasoned judgments are generally based on a data-rich source of chemical, chemical class, and tumor type-specific information. Many times there will be conflicting data and gaps in the information base; it is important to carefully evaluate these uncertainties before reaching any conclusion.

In making decisions about potential modes of action and the relevance of animal tumor findings to humans (Ashby et al., 1990; Ashby and Tennant, 1991; Tennant, 1993; IPCS 1999; Sonich-Mullin et al., 2001; Meek et al., 2003), very often the results of chronic animal studies may give important clues. Some of the important factors to review include:

- tumor types, for example, those responsive to endocrine influence or those produced by DNA-reactive carcinogens;

- number of studies and of tumor sites, sexes, and species affected or unaffected in those studies and if the data present a coherent story;

- similarity of metabolic activation and detoxification for a specific chemical between humans and tested species;

- influence of route of exposure on the spectrum of tumors and whether they occur at point of exposure or systemic sites;

- effect of high dose exposures on the target organ or systemic toxicity that may not reflect typical physiological conditions, for example, urinary chemical changes associated with stone formation, effects on immune surveillance;

- presence of proliferative lesions, for example, hepatic foci, or hyperplasia;

- effect of dose and time on the progression of lesions from preneoplastic to benign tumors, then to malignant;

- ratio of malignant to benign tumors as a function of dose and time;

- time of appearance of tumors after commencing exposure;

- development of tumors that invade locally or systemically, or lead to death;

- tumors at organ sites with high or low background historical incidence in laboratory animals;

- biomarkers in tumor cells, both induced and spontaneous, for example, DNA or protein adducts, mutation spectra, chromosome changes, oncogene activation; and/or

- shape of the dose-response curve in the range of tumor observation, for example, linear versus nonlinear.

Some of the myriad ways in which information from chronic animal studies influences mode of action judgments include, but are not limited to, the following:

- multisite and multispecies tumor effects that are often associated with mutagenic agents;

- tumors restricted to one sex or species suggesting an influence restricted to gender, strain, or species;

- late onset of tumors that are primarily benign, are at sites with a high historical background incidence, or show reversal of lesions on cessation of exposure suggesting a growth-promoting mode of action;

- the possibility that an agent acting differently in different tissues; or

- the possibility that has more than one mode of action in a single tissue.

Simple knowledge of sites of tumor increase in rodent studies can give preliminary clues as to mode of action. Experience at the National Toxicology Program (NTP) indicates that substances that are DNA reactive and that produce gene mutations may be unique in producing tumors in certain anatomical sites, whereas tumors at other sites may arise from both mutagenic or nonmutagenic influences (Ashby and Tennant, 1991; Huff et al., 1991).

The types of data and their influence on judgments regarding mode of action are expected to evolve, both as science advances and as the risk assessment community gains more experience with these analyses. This section contains a framework for evaluating hypothesized mode(s) of action. This framework has similarities to and differences with the concepts presented in other MOA frameworks (e.g., IPCS, 1999; Sonich-Mullin et al., 2001; Meek et al., 2003). Differences are often due to the context of the use for the framework. For example, the Meek et al. (2003) presents a stand-alone document for addressing mode of action issues; thus, it recommends that conclusions concerning MOA be rendered separately. In these cancer guidelines, however, they are incorporated into the context of all of the data regarding weight of the evidence for carcinogenicity.

2.4.2. Evaluating an Hypothesized Mode of Action

2.4.2.1. *Peer Review*

In reaching conclusions, the question of "general acceptance" of a mode of action should be tested as part of the independent peer review that EPA obtains for its assessment and conclusions. In some cases the mode of action may already have been established by development of a large body of research information and characterization of the phenomenon over time. In some cases there will have been development of an Agency policy (e.g., mode of action involving alpha-2u-globulin in the male rat [U.S. EPA, 1991b]) or a series of previous assessments in which both the mode of action and its applicability to particular cases has been explored. If so, the assessment and its peer review can focus on the evidence that a particular agent acts in this mode. The peer review should also evaluate the strengths and weaknesses of competing modes of action.

In other cases, the mode of action may not have previously been the subject of an Agency document. If so, the data to support both the mode of action and the associated activity of the agent should undergo EPA assessment and subsequent peer review.

2.4.2.2. *Use of the Framework*

The framework supports a full analysis of mode of action information, but it can also be used as a screen to decide whether sufficient information is available to evaluate or whether the data gaps are too substantial to justify further analysis. Mode of action conclusions are used to address the question of human relevance of animal tumor responses, to address differences in anticipated response among humans, such as between children and adults or men and women; and as the basis of decisions about the anticipated shape of the dose-response relationship. Guidance on the latter appears in Section 3.

This framework is intended to provide an analytical approach for evaluating the mode of action. It is neither a checklist nor a list of required criteria. As the type and amount of information will depend on the mode of action postulated, scientific judgment is important to determine if the weight of evidence is sufficient.

2.4.3. Framework for Evaluating Each Hypothesized Carcinogenic Mode of Action

This framework is intended to be an analytic tool for judging whether available data support a mode of carcinogenic action hypothesized for an agent. It is based upon considerations for causality in epidemiologic investigations originally articulated by Hill (1965) but later modified by others and extended to experimental studies. The original Hill criteria were applied to epidemiologic data, whereas this framework is applied to a much wider assortment of experimental data, so it retains the basic principles of Hill but is much modified in content.

The modified Hill criteria can be useful for organizing thinking about aspects of causation, and they are consistent with the scientific method of developing hypotheses and testing those hypotheses experimentally. During analysis by EPA, and as guidance for peer review, a key question is whether the data to support a mode of action meet the standards generally applied in experimental biology regarding inference of causation.

All pertinent studies are reviewed in analyzing a mode of action, and an overall weighing of evidence is performed, laying out the strengths, weaknesses, and uncertainties of the case as well as potential alternative positions and rationales. Identifying data gaps and research needs is also part of the assessment.

To evaluate whether an hypothesized mode of action is operative, an analysis starts with an outline of the scientific findings regarding the hypothesized key events leading to cancer, and then weighing information to determine whether there is a causal relationship between these events and cancer formation, i.e., that the effects are critical for induction of tumors. It is not generally expected that the complete sequence will be known at the molecular level. Instead, empirical observations made at different levels of biological organization—biochemical, cellular, physiological, tissue, organ, and system—are analyzed.

Several important points should be considered when working with the framework:

- The topics listed for analysis should *not* be regarded as a checklist of necessary "proofs." The judgment of whether an hypothesized mode of action is supported by available data takes account of the analysis as a whole.

- The framework provides a structure for organizing the facts upon which conclusions as to mode of action rest. The purpose of using the framework is to make analysis transparent and to allow the reader to understand the facts and reasoning behind a conclusion.

- The framework does not dictate an answer. The weight of evidence that is sufficient to support a decision about a mode of action may be less or more, depending on the purpose of the analysis, for example, screening, research needs identification, or full risk assessment. To make the reasoning transparent, the purpose of the analysis should be made apparent to the reader.

- Toxicokinetic studies may contribute to mode of action analysis by contributing to identifying the active form(s) of an agent that is central to the mode of action. Apart from contributing in this way, toxicokinetics studies may reveal effects of saturation of metabolic processes. These may not be considered key events in a mode of action, but they are given separate consideration in assessing dose metrics and potential nonlinearity of the dose-response relationship.

- Generally, “sufficient” support is a matter of scientific judgment in the context of the requirements of the decisionmaker or in the context of science policy guidance regarding a certain mode of action.

- Even when an hypothesized mode of action is supported for a described response in a specific tissue, it may not explain other tumor responses observed, which should get separate consideration in hazard and dose-response assessment.

For each tumor site being evaluated, the mode of action analysis should begin with a description of the relevant data and key events that may be associated with an hypothesized mode of action and its sequence of key events (see Section 2.4.3.1). This can be followed by a

discussion of various aspects of the experimental support for hypothesized mode(s) of action in animals and humans (see Section 2.4.3.2). The possibility of other modes of action also should be considered and discussed (see Section 2.4.3.3); if there is evidence for more than one mode of action, each should receive a separate analysis. Conclusions about each hypothesized mode of action should address whether the mode of action is supported in animals and is relevant to humans and which populations or lifestages can be particularly susceptible (see Section 2.4.3.4). In a risk assessment document, the analysis of an hypothesized mode of action can be presented before or with the characterization of an agent's potential hazard to humans.

2.4.3.1. *Description of the Hypothesized Mode of Action*

Summary description of the hypothesized mode of action. For each tumor site, the mode of action analysis begins with a description of the hypothesized mode of action and its sequence of key events. If there is evidence for more than one mode of action, each receives a separate analysis.

Identification of key events. In order to judge how well data support involvement of a key event in carcinogenic processes, the experimental definition of the event or events should be clear and reproducible. To support an association, experiments should define and measure an event consistently.

- Can a list of events be identified that are key to the carcinogenic process?

- Are the events well defined?

Pertinent observations may include, but are not limited to, receptor-ligand changes, cytotoxicity, cell cycle effects, increased cell growth, organ weight differences, histological changes, hormone or other protein perturbations, or DNA and chromosome effects.

2.4.3.2. *Discussion of the Experimental Support for the Hypothesized Mode of Action*

The experimental support for the hypothesized mode of action should be discussed from several viewpoints patterned after the Hill criteria (see Section 2.2.1.7). For illustration, the explanation of each topic includes typical questions to be addressed to the available empirical data and experimental observations anticipated to be pertinent. The latter will vary from case to case. For a particular mode of action, certain observations may be established as essential in practice or policy, for example, measures of thyroid hormone levels in supporting thyroid hormone elevation as a key event in carcinogenesis.

Strength, consistency, specificity of association. A statistically significant association between events and a tumor response observed in well-conducted studies is generally supportive of causation. Consistent observations in a number of such studies with differing experimental designs increase that support, because different designs may reduce unknown biases. Studies showing "recovery," i.e, absence or reduction of carcinogenicity when the event is blocked or diminished, are particularly useful tests of the association. Specificity of the association, without evidence of other modes of action, strengthens a causal conclusion. A lack of strength, consistency, and specificity of association weakens the causal conclusions for a particular mode of action.

- What is the level of statistical and biological significance for each event and for cancer?

- Do independent studies and different experimental hypothesis-testing approaches produce the same associations?

- Does the agent produce effects other than those hypothesized?

- Is the key event associated with precursor lesions?

Pertinent observations include tumor response associated with events (site of action logically relates to event[s]), precursor lesions associated with events, initiation-promotion studies, and stop/recovery studies.

Dose-response concordance. If a key event and tumor endpoints increase with dose such that the key events forecast the appearance of tumors at a later time or higher dose, a causal association can be strengthened. Dose-response associations of the key event with other precursor events can add further strength. Difficulty arises when an event is not causal but accompanies the process generally. For example, if tumors and the hypothesized precursor both increase with dose, the two responses will be correlated regardless of whether a causal relationship exists. This is similar to the issue of confounding in epidemiologic studies. Dose-response studies coupled with mechanistic studies can assist in clarifying these relationships.

- What are the correlations among doses producing events and cancer?

Pertinent observations include, but are not limited to, 2-year bioassay observation of lesions correlated with observations of hormone changes and the same lesions in shorter term studies or in interim sacrifice.

Temporal relationship. If an event is shown to be causally linked to tumorigenesis, it will precede tumor appearance. An event may also be observed contemporaneously or after tumor appearance; these observations may add to the strength of association but not to the temporal association.

- What is the ordering of events that underlie the carcinogenic process?

- Is this ordering consistent among independent studies?

Pertinent observations include studies of varying duration observing the temporal sequence of events and development of tumors.

Biological plausibility and coherence. It is important that the hypothesized mode of action and the events that are part of it be based on contemporaneous understanding of the biology of cancer to be accepted. If the body of information under scrutiny is consistent with other examples (including structurally related agents) for which the hypothesized mode of action is accepted, the case is strengthened. Because some modes of action can be anticipated to evoke effects other than cancer, the available toxicity database on noncancer effects, for example, reproductive effects of certain hormonal disturbances, can contribute to this evaluation.

- Is the mode of action consistent with what is known about carcinogenesis in general and for the case specifically?

- Are carcinogenic effects and events consistent across structural analogues?

- Is the database on the agent internally consistent in supporting the purported mode of action, including relevant noncancer toxicities?

Pertinent observations include the scientific basis for considering an hypothesized mode of action generally, given the contemporaneous state of knowledge of carcinogenic processes; previous examples of data sets showing the mode of action; data sets on analogues; and coherence of data in this case from cancer and noncancer toxicity studies.

2.4.3.3. *Consideration of the Possibility of Other Modes of Action*

The possible involvement of more than one mode of action at the tumor site should be considered. Pertinent observations that are not consistent with the hypothesized mode of action can suggest the possibility of other modes of action. Some pertinent observations can be consistent with more than one mode of action. Furthermore, different modes of action can operate in different dose ranges; for example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity may not occur.

If there is evidence for more than one mode of action, each should receive a separate analysis. There may be an uneven level of experimental support for the different modes of action. Sometimes this can reflect disproportionate resources spent on investigating one particular mode of action and not the validity or relative importance of the other possible modes of action. Ultimately, however, the information on all of the modes of action should be integrated to better understand how and when each mode acts, and which mode(s) may be of interest for exposure levels relevant to human exposures of interest.

2.4.3.4. *Conclusions About the Hypothesized Mode of Action*

Conclusions about the hypothesized mode of action should address the issues listed below. For those agents for which the mode of action is considered useful for the risk assessment, the weight of the evidence concerning mode of action in animals as well as its relevance for humans would be incorporated into the weight of evidence narrative (Section 2.5).

(a) Is the hypothesized mode of action sufficiently supported in the test animals? Associations observed between key events and tumors may or may not support an inference of causation. The conclusion that the agent causes one or more key events that results in tumors is strengthened as more aspects of causation are satisfied and weakened as fewer are satisfied. Consistent results in different experiments that test the hypothesized mode of action build support for that mode of action. Replicating results in a similar experiment does not generally meaningfully strengthen the original evidence, and discordant results generally weaken that support. Experimental challenge to the hypothesized mode of action, where interrupting the sequence of key events suppresses the tumor response or enhancement of key events increases the tumor response, creates very strong support for the mode of action.

(b) Is the hypothesized mode of action relevant to humans? If an hypothesized mode of action is sufficiently supported in the test animals, the sequence of key precursor events should be reviewed to identify critical similarities and differences between the test animals and humans. The question of concordance can be complicated by cross-species differences in toxicokinetics or

toxicodynamics. For example, the active agent can be formed through different metabolic pathways in animals and humans. Any information suggesting quantitative differences between animals and humans is flagged for consideration in the dose-response assessment. This includes the potential for different internal doses of the active agent or for differential occurrence of a key precursor event.

"Relevance" of a potential mode of action is considered in the context of characterization of hazard, not level of risk. Anticipated levels of human exposure are not used to determine whether the hypothesized mode of action is relevant to humans. Exposure information is integrated into the overall risk characterization.

The question of relevance considers all populations and lifestages. It is possible that the conditions under which a mode of action operates exist primarily in a particular population or lifestage, for example, in those with a pre-existing hormonal imbalance. Other populations or lifestages may not be analogous to the test animals, in which case the question of relevance would be decided by inference.

Special attention should be paid to whether tumors can arise from childhood exposure, considering various aspects of development during these lifestages. Because the studies that support a mode of action are typically conducted in mature animals, conclusions about relevance during childhood generally rely on inference. There is currently no standard Agency position regarding the issue of whether tumors arising through the hypothesized mode of action are relevant during childhood; understanding the mode of action implies that there are sufficient data (on either the specific agent or the general mode of action) to form a confident conclusion about relevance during childhood.

(c) Which populations or lifestages can be particularly susceptible to the hypothesized mode of action? If an hypothesized mode of action is judged relevant to humans, information about the key precursor event(s) is reviewed to identify populations or lifestages that might reasonably expected to be particularly susceptible to their occurrence. Although agent-specific data would provide the strongest indication of susceptibility, this review may also rely on general knowledge about the precursor events and characteristics of individuals susceptible to these

events. Any information suggesting quantitative differences between populations or lifestages should be flagged for consideration in the dose-response assessment (see Section 3.5). This includes the potential for a higher internal dose of the active agent or for an increased occurrence of a key precursor event. Quantitative differences may result in separate risk estimates for susceptible populations or lifestages.

The possibility that childhood is a susceptible period for exposure should be explicitly addressed. Generic understanding of the mode of action can be used to gauge childhood susceptibility, and this determination can be refined through analysis of agent-specific data.

2.4.4 Evolution with Experience

Several groups have proposed or incorporated mode of action into their risk assessments (see, e.g., U.S. EPA , 1991b; Sonich-Mullin et al., 2001; Meek et al., 2003). As the frameworks and mandates under which these evaluations were produced differ, the specific procedures described in and conclusions drawn may also differ. Nevertheless, the number of case studies from all venues remains limited. More experience with differing modes of action are expected to highlight and illustrate the strengths and limitations of the general framework proposed in these cancer guidelines. Moreover, additional toxicological techniques may expand or change scientific judgments regarding which information is useful for mode of action determinations. As warranted, additional guidance may be proposed as experience is gained and/or as toxicological knowledge advances.

2.5. WEIGHT OF EVIDENCE NARRATIVE

The *weight of evidence narrative* is a short summary (one to two pages) that explains an agent's human carcinogenic potential and the conditions that characterize its expression. It should be sufficiently complete to be able to stand alone, highlighting the key issues and decisions that were the basis for the evaluation of the agent’s potential hazard. It should be sufficiently clear and transparent to be useful to risk managers and non-expert readers. It may be useful to summarize all of the significant components and conclusions in the first paragraph of the narrative and to explain complex issues in more depth in the rest of the narrative.

The weight of the evidence should be presented as a narrative laying out the complexity of information that is essential to understanding the hazard and its dependence on the quality, quantity, and type(s) of data available, as well as the circumstances of exposure or the traits of an exposed population that may be required for expression of cancer. For example, the narrative can clearly state to what extent the determination was based on data from human exposure, from animal experiments, from some combination of the two, or from other data. Similarly, information on mode of action can specify to what extent the data are from *in vivo* or *in vitro* exposures or based on similarities to other chemicals. The extent to which an agent's mode of action occurs only on reaching a minimum dose or a minimum duration should also be presented. A hazard might also be expressed disproportionately in individuals possessing a specific gene; such characterizations may follow from a better understanding of the human genome. Furthermore, route of exposure should be used to qualify a hazard if, for example, an agent is not absorbed by some routes. Similarly, a hazard can be attributable to exposures during a susceptible lifestage on the basis of our understanding of human development.

The weight of evidence-of-evidence narrative should highlight:

- the quality and quantity of the data;
- all key decisions and the basis for these major decisions; and
- any data, analyses, or assumptions that are unusual for or new to EPA.

To capture this complexity, a weight of evidence narrative generally includes

- conclusions about human carcinogenic potential (choice of descriptor(s), described below),

- a summary of the key evidence supporting these conclusions (for each descriptor used), including information on the type(s) of data (human and/or animal, *in vivo* and/or *in vitro*) used to support the conclusion(s),

- available information on the epidemiologic or experimental conditions that characterize expression of carcinogenicity (e.g., if carcinogenicity is possible only by one exposure route or only above a certain human exposure level),

- a summary of potential modes of action and how they reinforce the conclusions,

- indications of any susceptible populations or lifestages, when available, and

- a summary of the key default options invoked when the available information is inconclusive.

To provide some measure of clarity and consistency in an otherwise free-form narrative, the weight of evidence descriptors are included in the first sentence of the narrative. Choosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence. These descriptors and narratives are intended to permit sufficient flexibility to accommodate new scientific understanding and new testing methods as they are developed and accepted by the scientific community and the public. Descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases that are clarified by the full narrative. Descriptors, as well as an introductory paragraph, are a short summary of the complete narrative that preserves the complexity that is an essential part of the hazard characterization. **Users of these cancer guidelines and of the risk assessments that result from the use of these cancer guidelines should consider the entire range of information included in the narrative rather than focusing simply on the descriptor.**

In borderline cases, the narrative explains the case for choosing one descriptor and discusses the arguments for considering but not choosing another. For example, between "suggestive" and "likely" or between "suggestive" and "inadequate," the explanation clearly communicates the information needed to consider appropriately the agent's carcinogenic potential in subsequent decisions.

Multiple descriptors can be used for a single agent, for example, when carcinogenesis is dose- or route-dependent. For example, if an agent causes point-of-contact tumors by one exposure route but adequate testing is negative by another route, then the agent could be described as likely to be carcinogenic by the first route but not likely to be carcinogenic by the second. Another example is when the mode of action is sufficiently understood to conclude that a key event in tumor development would not occur below a certain dose range. In this case, the agent could be described as likely to be carcinogenic above a certain dose range but not likely to be carcinogenic below that range.

Descriptors can be selected for an agent that has not been tested in a cancer bioassay if sufficient other information, e.g., toxicokinetic and mode of action information, is available to make a strong, convincing, and logical case through scientific inference. For example, if an agent is one of a well-defined class of agents that are understood to operate through a common mode of action and if that agent has the same mode of action, then in the narrative the untested agent would have the same descriptor as the class. Another example is when an untested agent's effects are understood to be caused by a human metabolite, in which case in the narrative the untested agent could have the same descriptor as the metabolite. As new testing methods are developed and used, assessments may increasingly be based on inferences from toxicokinetic and mode of action information in the absence of tumor studies in animals or humans.

When a well-studied agent produces tumors only at a point of initial contact, the descriptor generally applies only to the exposure route producing tumors unless the mode of action is relevant to other routes. The rationale for this conclusion would be explained in the narrative.

When tumors occur at a site other than the point of initial contact, the descriptor generally applies to all exposure routes that have not been adequately tested at sufficient doses. An

exception occurs when there is convincing information, e.g., toxicokinetic data that absorption does not occur by another route.

When the response differs qualitatively as well as quantitatively with dose, this information should be part of the characterization of the hazard. In some cases reaching a certain dose range can be a precondition for effects to occur, as when cancer is secondary to another toxic effect that appears only above a certain dose. In other cases exposure duration can be a precondition for hazard if effects occur only after exposure is sustained for a certain duration. These considerations differ from the issues of relative absorption or potency at different dose levels because they may represent a discontinuity in a dose-response function.

When multiple bioassays are inconclusive, mode of action data are likely to hold the key to resolution of the more appropriate descriptor. When bioassays are few, further bioassays to replicate a study's results or to investigate the potential for effects in another sex, strain, or species may be useful.

When there are few pertinent data, the descriptor makes a statement about the database, for example, “Inadequate Information to Assess Carcinogenic Potential,” or a database that provides “Suggestive Evidence of Carcinogenic Potential.” With more information, the descriptor expresses a conclusion about the agent’s carcinogenic potential to humans. If the conclusion is positive, the agent could be described as “Likely to Be Carcinogenic to Humans” or, with strong evidence, “Carcinogenic to Humans.” If the conclusion is negative, the agent could be described as “Not Likely to Be Carcinogenic to Humans.”

Although the term “likely” can have a probabilistic connotation in other contexts, its use as a weight of evidence descriptor does not correspond to a quantifiable probability of whether the chemical is carcinogenic. This is because the data that support cancer assessments generally are not suitable for numerical calculations of the probability that an agent is a carcinogen. Other health agencies have expressed a comparable weight of evidence using terms such as “Reasonably Anticipated to Be a Human Carcinogen” (NTP) or “Probably Carcinogenic to Humans” (International Agency for Research on Cancer).

The following descriptors can be used as an introduction to the weight of evidence narrative. The examples presented in the discussion of the descriptors are illustrative. The

examples are neither a checklist nor a limitation for the descriptor. The complete weight of evidence narrative, rather than the descriptor alone, provides the conclusions and the basis for them.

"Carcinogenic to Humans"

This descriptor indicates strong evidence of human carcinogenicity. It covers different combinations of evidence.

- This descriptor is appropriate when there is convincing epidemiologic evidence of a causal association between human exposure and cancer.

- Exceptionally, this descriptor may be equally appropriate with a lesser weight of epidemiologic evidence that is strengthened by other lines of evidence. It can be used when all of the following conditions are met: (a) there is strong evidence of an association between human exposure and either cancer or the key precursor events of the agent's mode of action but not enough for a causal association, and (b) there is extensive evidence of carcinogenicity in animals, and (c) the mode(s) of carcinogenic action and associated key precursor events have been identified in animals, and (d) there is strong evidence that the key precursor events that precede the cancer response in animals are anticipated to occur in humans and progress to tumors, based on available biological information. In this case, the narrative includes a summary of both the experimental and epidemiologic information on mode of action and also an indication of the relative weight that each source of information carries, e.g., based on human information, based on limited human and extensive animal experiments.

"Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor

"Carcinogenic to Humans." Adequate evidence consistent with this descriptor covers a broad spectrum. As stated previously, the use of the term "likely" as a weight of evidence descriptor does not correspond to a quantifiable probability. The examples below are meant to represent the broad range of data combinations that are covered by this descriptor; they are illustrative and provide neither a checklist nor a limitation for the data that might support use of this descriptor. Moreover, additional information, e.g., on mode of action, might change the choice of descriptor for the illustrated examples. Supporting data for this descriptor may include:

- an agent demonstrating a plausible (but not definitively causal) association between human exposure and cancer, in most cases with some supporting biological, experimental evidence, though not necessarily carcinogenicity data from animal experiments;

- an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans;

- a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset;

- a rare animal tumor response in a single experiment that is assumed to be relevant to humans; or

- a positive tumor study that is strengthened by other lines of evidence, for example, either plausible (but not definitively causal) association between human exposure and cancer or evidence that the agent or an important metabolite causes events generally known to be associated with tumor formation (such as DNA reactivity or effects on cell growth control) likely to be related to the tumor response in this case.

"Suggestive Evidence of Carcinogenic Potential"

This descriptor of the database is appropriate when the weight of evidence is suggestive of carcinogenicity; a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion. This descriptor covers a spectrum of evidence associated with varying levels of concern for carcinogenicity, ranging from a positive cancer result in the only study on an agent to a single positive cancer result in an extensive database that includes negative studies in other species. Depending on the extent of the database, additional studies may or may not provide further insights. Some examples include:

- a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor "Likely to Be Carcinogenic to Humans." The study generally would not be contradicted by other studies of equal quality in the same population group or experimental system (see discussions of *conflicting evidence* and *differing results*, below);

- a small increase in a tumor with a high background rate in that sex and strain, when there is some but insufficient evidence that the observed tumors may be due to intrinsic factors that cause background tumors and not due to the agent being assessed. (When there is a high background rate of a specific tumor in animals of a particular sex and strain, then there may be biological factors operating independently of the agent being assessed that could be responsible for the development of the observed tumors.) In this case, the reasons for determining that the tumors are not due to the agent are explained;

- evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion (but does not make the study fatally

flawed), but where the carcinogenic potential is strengthened by other lines of evidence (such as structure-activity relationships); or

- a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend.

"Inadequate Information to Assess Carcinogenic Potential"

This descriptor of the database is appropriate when available data are judged inadequate for applying one of the other descriptors. Additional studies generally would be expected to provide further insights. Some examples include:

- little or no pertinent information;

- conflicting evidence, that is, some studies provide evidence of carcinogenicity but other studies of equal quality in the same sex and strain are negative. *Differing results*, that is, positive results in some studies and negative results in one or more different experimental systems, do not constitute *conflicting evidence*, as the term is used here. Depending on the overall weight of evidence, differing results can be considered either suggestive evidence or likely evidence; or

- negative results that are not sufficiently robust for the descriptor, "Not Likely to Be Carcinogenic to Humans."

"Not Likely to Be Carcinogenic to Humans"

This descriptor is appropriate when the available data are considered robust for deciding that there is no basis for human hazard concern. In some instances, there can be positive results in experimental animals when there is strong, consistent evidence that each mode of action in experimental animals does not operate in humans. In other cases, there can be convincing

evidence in both humans and animals that the agent is not carcinogenic. The judgment may be based on data such as:

- animal evidence that demonstrates lack of carcinogenic effect in both sexes in well-designed and well-conducted studies in at least two appropriate animal species (in the absence of other animal or human data suggesting a potential for cancer effects),

- convincing and extensive experimental evidence showing that the only carcinogenic effects observed in animals are not relevant to humans,

- convincing evidence that carcinogenic effects are not likely by a particular exposure route (see Section 2.3), or

- convincing evidence that carcinogenic effects are not likely below a defined dose range.

A descriptor of “not likely” applies only to the circumstances supported by the data. For example, an agent may be “Not Likely to Be Carcinogenic” by one route but not necessarily by another. In those cases that have positive animal experiment(s) but the results are judged to be not relevant to humans, the narrative discusses why the results are not relevant.

Multiple Descriptors

More than one descriptor can be used when an agent's effects differ by dose or exposure route. For example, an agent may be “Carcinogenic to Humans” by one exposure route but “Not Likely to Be Carcinogenic” by a route by which it is not absorbed. Also, an agent could be “Likely to Be Carcinogenic” above a specified dose but “Not Likely to Be Carcinogenic” below that dose because a key event in tumor formation does not occur below that dose.

2.6. HAZARD CHARACTERIZATION

The *hazard characterization* contains the hazard information needed for a full risk characterization (U.S. EPA, 2000b). It presents the results of the hazard assessment and explains how the weight of evidence conclusion was reached. The hazard characterization summarizes, in plain language, conclusions about the agent's potential effects, whether they can be expected to depend qualitatively on the circumstances of exposure, and if anyone can be expected to be especially susceptible. It discusses the extent to which these conclusions are supported by data or are the result of default options invoked because the data are inconclusive. It explains how complex cases with differing results in different studies were resolved. The hazard characterization highlights the major issues addressed in the hazard assessment and discusses alternative interpretations of the data and the degree to which they are supportable scientifically and are consistent with EPA guidelines.

When the conclusion is supported by mode of action information, the hazard characterization also provides a clear summary of the mode of action conclusions (see Section 2.4.3.4), including the completeness of the data, the strengths and limitations of the inferences made, the potential for other modes of action, and the implications of the mode of action for selecting viable approaches to the dose-response assessment. The hazard characterization also discusses the extent to which mode of action information is available to address the potential for disproportionate risks in specific populations or lifestages or the potential for enhanced risks on the basis of interactions with other agents or stressors, if anticipated.

Topics that can be addressed in a hazard characterization include:

- summary of the results of the hazard assessment;

- identification of any likely susceptible populations and lifestages, especially attending to children, infants, and fetuses;

- conclusions about the agent's mode of action, and implications for selecting approaches to the dose-response assessment;

- identification of the available lines of evidence (e.g., animal bioassays, epidemiologic studies, toxicokinetic information, mode of action studies, and information about structural analogues or metabolites), highlighting data quality and coherence of results from different lines of evidence; and

- strengths and limitations of the hazard assessment, highlighting significant issues in interpreting the data, alternative interpretations that are considered equally plausible, critical data gaps, and default options invoked when the available information is inconclusive.

3. DOSE-RESPONSE ASSESSMENT

Dose-response assessment estimates potential risks to humans at exposure levels of interest. Dose-response assessments are useful in many applications: estimating risk at different exposure levels, estimating the risk reduction for different decision options, estimating the risk remaining after an action is taken, providing the risk information needed for benefit-cost analyses of different decision options, comparing risks across different agents or health effects, and setting research priorities. The purpose of the assessment should consider the quality of the data available, which will vary from case to case.

A dose-response analysis is generally developed from each study that reports quantitative data on dose and response. Alternative measures of dose are available for analyzing human and animal studies (see Section 3.1). A two-step approach distinguishes analysis of the dose-response data from inferences made about lower doses. The first step is an analysis of dose and response in the range of observation of the experimental or epidemiologic studies (see Section 3.2). Modeling is encouraged to incorporate a wide range of experimental data into the dose-response assessment (see Sections 3.1.2, 3.2.1, 3.2.2, 3.2.3). The modeling yields a point of departure (POD) near the lower end of the observed range, without significant extrapolation to lower doses (see Sections 3.2.4, 3.2.5). The second step is extrapolation to lower doses (see Section 3.3). The extrapolation approach considers what is known about the agent's mode of action (see Section 3.3.1). Both linear and nonlinear approaches are available (see Sections 3.3.3, 3.3.4). When multiple estimates can be developed, the strengths and weaknesses of each are presented. In some cases, they may be combined in a way that best represents human cancer risk (see Section 3.3.5). Special consideration is given to describing dose-response differences attributable to different human exposure scenarios (see Section 3.4) and to susceptible populations and lifestages (see Section 3.5). It is important to discuss significant uncertainties encountered in the analysis (see Section 3.6) and to characterize other important aspects of the dose-response assessment (see Section 3.7).

The scope, depth, and use of a dose-response assessment vary in different circumstances. Although the quality of dose-response data is not necessarily related to the weight of evidence

descriptor, dose-response assessments are generally completed for agents considered "Carcinogenic to Humans" and "Likely to Be Carcinogenic to Humans." When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates. Dose-response assessments are generally not done when there is inadequate evidence, although calculating a bounding estimate from an epidemiologic or experimental study that does not show positive results can indicate the study's level of sensitivity and capacity to detect risk levels of concern.

Cancer is a collection of several diseases that develop through cell and tissue changes over time. Dose-response assessment procedures based on tumor incidence have seldom taken into account the effects of key precursor events within the whole biological process due to lack of empirical data and understanding about these events. In this discussion, response data include measures of key precursor events considered integral to the carcinogenic process in addition to tumor incidence. These responses may include changes in DNA, chromosomes, or other key macromolecules; effects on growth signal transduction, including induction of hormonal changes; or physiological or toxic effects that include proliferative events diagnosed as precancerous but not pathology that is judged to be cancer. Analysis of such responses may be done along with that of tumor incidence to enhance the tumor dose-response analysis. If dose-response analysis of nontumor key events is more informative about the carcinogenic process for an agent, it can be used in lieu of, or in conjunction with, tumor incidence analysis for the overall dose-response assessment.

As understanding of mode of action improves and new types of data become available, dose-response assessment will continue to evolve. These cancer guidelines encourage the development and application of new methods that improve dose-response assessment by reflecting new scientific understanding and new sources of information.

3.1. ANALYSIS OF DOSE

For each effect observed, dose-response assessment should begin by determining an appropriate *dose metric*. Several dose metrics have been used, e.g., delivered dose, body burden, and area under the curve, and others may be appropriate depending on the data and mode of action.

Selection of an appropriate dose metric considers what data are available and what is known about the agent's mode of action at the target site, and uncertainties involved in estimation and application of alternative metrics. The dose metric specifies:

- the agent measured, preferably the active agent (administered agent or a metabolite);
- proximity to the target site (exposure concentration, potential dose, internal dose, or delivered dose,[5] reflecting increasing proximity); and

- the time component of the effective dose (cumulative dose, average dose, peak dose, or body burden).

Analyses can be based on estimates of animal dose metrics or human dose metrics. The assessment should describe the approach used to select a dose metric and the reasons for this approach. The final analysis, however, should determine a human equivalent dose metric. This facilitates comparing results from different datasets and effects by using human equivalent dose/concentrations as common metrics. When appropriate, it may be necessary to convert dose metrics across exposure routes. When route-to-route extrapolations are made, the underlying data, algorithms, and assumptions are clearly described.

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[5] *Exposure* is contact of an agent with the outer boundary of an organism. *Exposure concentration* is the concentration of a chemical in its transport or carrier medium at the point of contact. *Dose* is the amount of a substance available for interaction with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. *Potential dose* is the amount ingested, inhaled, or applied to the skin. *Applied dose* is the amount of a substance presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer boundary of the organism). *Absorbed dose* is the amount crossing a specific absorption barrier (e.g., the exchange boundaries of skin, lung, and digestive tract) through uptake processes. *Internal dose* is a more general term, used without respect to specific absorption barriers or exchange boundaries. *Delivered dose* is the amount of the chemical available for interaction by any particular organ or cell (U.S. EPA, 1992a).

Timing of exposure can also be important. When there is a susceptible lifestage, doses during the susceptible period are not equivalent to doses at other times, and they would be analyzed separately.

3.1.1. Standardizing Different Experimental Exposure Regimens

Complex exposure or dosing regimens are often present in experimental and epidemiologic studies. The resulting internal dose depends on many variables, including concentration, duration, frequency of administration, and duration of recovery periods between administrations. Internal dose also depends on variables that are intrinsic to the exposed individual, such as lifestage and rates of metabolism and clearance. To facilitate comparing results from different study designs and to make inferences about human exposures, a summary estimate of the dose metric, whether the administered dose or inhalation exposure concentration or an internal metric, may be derived for a complex exposure regimen.

Toxicokinetic modeling is the preferred approach for estimating dose metrics from exposure. Toxicokinetic models generally describe the relationship between exposure and measures of internal dose over time. More complex models can reflect sources of intrinsic variation, such as polymorphisms in metabolism and clearance rates. When a robust model is not available, or when the purpose of the assessment does not warrant developing a model, simpler approaches may be used.

For chronic exposure studies, the cumulative exposure or dose administered often is expressed as an average over the duration of the study, as one consistent dose metric. This approach implies that a higher dose administered over a short duration is equivalent to a commensurately lower dose administered over a longer duration. Uncertainty usually increases as the duration becomes shorter relative to the averaging duration or the intermittent doses become more intense than the averaged dose. Moreover, doses during any specific susceptible or refractory period would not be equivalent to doses at other times. For these reasons, cumulative exposure or potential dose may be replaced by a more appropriate dose metric when indicated by the data.

For mode of action studies, the dose metric should be calculated over a duration that reflects the time to occurrence of the key precursor effects. Mode of action studies are often of limited duration, as the precursors can be observed after less-than-chronic exposures. When the experimental exposure regimen is specified on a weekly basis (for example, 4 hours a day, 5 days a week), the daily exposure may be averaged over the week, where appropriate.

Doses in studies at the cellular or molecular level can be difficult to relate to organ- or organism-level dose metrics. Toxicokinetic modeling can sometimes be used to relate doses at the cellular or molecular level to doses or exposures at higher levels of organization.

3.1.2. Toxicokinetic Data and Modeling

In the absence of chemical-specific data, physiologically based toxicokinetic modeling is potentially the most comprehensive way to account for biological processes that determine internal dose. Physiologically based models commonly describe blood flow between physiological compartments and simulate the relationship between applied dose and internal dose. Toxicokinetic models generally need data on absorption, distribution, metabolism, and elimination of the administered agent and its metabolites.

Additionally, in the case of inhalation exposures, models can explicitly characterize the geometry of the respiratory tract and the airflow through it, as well as the interaction of this airflow with the entrained particles or fibers and gases (Kimbell et al., 2001; Subramaniam et al., 2003). Because of large interspecies differences in airway morphometry such models can be particularly useful in interspecies extrapolations. When employed, however, the potential for large inter-individual differences in airway morphometry, are considered to ensure that the models provide information representative of human populations.

Toxicokinetic models can improve dose-response assessment by revealing and describing nonlinear relationships between applied and internal dose. Nonlinearity observed in a dose-response curve often can be attributed to toxicokinetics (Hoel et al., 1983; Gaylor et al., 1994), involving, for example, saturation or induction of enzymatic processes at high doses. In some cases, toxicokinetic processes tend to become linear at sufficiently low doses (Hattis, 1990).

A discussion of confidence should accompany the presentation of model results and include consideration of model validation and sensitivity analysis, stressing the predictive performance of the model and whether the model is sufficient to support decision-making. Quantitative uncertainty analysis is important for evaluating the performance of a model, whether the model is based primarily on default assumptions or chemical-specific data. The uncertainty analysis covers questions of *model uncertainty* (e.g., Is the model based on the appropriate biology and how does that affect estimates of dose metrics?) and *parameter uncertainty* (e.g., Do the data support unbiased and stable estimates of the model parameters?). When a delivered dose measure is used in animal-to-human extrapolation, the assessment discusses the confidence of the target tissue and its toxicodynamics being the same in both species (see Section 3.6). Toxicokinetic modeling results may be presented alone as the preferred method of estimating human equivalent exposures or doses, or these results may be presented in parallel with default procedures (see Section 3.1.3), depending on the confidence in the modeling.

3.1.3. Cross-species Scaling Procedures

Standard cross-species scaling procedures are available when the data are not sufficient to support a toxicokinetic model or when the purpose of the assessment does not warrant developing one. The aim is to define exposure levels for humans and animals that are expected to produce the same degree of effect (U.S. EPA, 1992b), taking into account differences in scale between test animals and humans, such as size and lifespan.

3.1.3.1. Oral Exposures

For oral exposures, administered doses should be scaled from animals to humans on the basis of equivalence of $mg/kg^{3/4}$-d (milligrams of the agent normalized by the 3/4 power of body weight per day) (U.S. EPA, 1992b). The 3/4 power is consistent with current science, including empirical data that allow comparison of potencies in humans and animals, and it is also supported by analysis of the allometric variation of key physiological parameters across mammalian species. It is generally more appropriate at low doses, where sources of nonlinearity such as saturation of enzyme activity are less likely to occur. This scaling is intended as an

unbiased estimate rather than a conservative one. Equating exposure concentrations in food or water is an alternative version of the same approach, because daily intakes of food or water are approximately proportional to the 3/4 power of body weight.

The aim of these cross-species scaling procedures is to estimate administered doses in animals and humans that result in equal lifetime risks. It is useful to recognize two components of this equivalence: *toxicokinetic equivalence*, which determines administered doses in animals and humans that yield equal tissue doses, and *toxicodynamic equivalence*, which determines tissue doses in animals and humans that yield equal lifetime risks (U.S. EPA, 1992b). Toxicokinetic modeling (see Section 3.1.2) addresses factors associated with toxicokinetic equivalence, and toxicodynamic modeling (see Section 3.2.2) addresses factors associated with toxicodynamic equivalence. When toxicokinetic modeling is used without toxicodynamic modeling, the dose-response assessment develops and supports an approach for addressing toxicodynamic equivalence, perhaps by retaining some of the cross-species scaling factor (e.g., using the square root of the cross-species scaling factor or using a factor of 3 to cover toxicodynamic differences between animals and humans, as is currently done in deriving inhalation reference concentrations [U.S. EPA, 1994]).

When assessing risks from childhood exposure, the $mg/kg^{3/4}$-d scaling factor does not use the child's body weight (U.S. EPA, 1992b). This reflects several uncertainties in extrapolating risks to children:

- The data supporting the $mg/kg^{3/4}$-d scaling factor were derived for differences across species and may not apply as well to differently sized individuals of the same species or to different lifestages.

- In addition to metabolic differences, there are also important toxicodynamic differences; for example, children have faster rates of cell division than do adults, so scaling across different lifestages and species simultaneously may be particularly uncertain.

3.1.3.2. Inhalation Exposures

For inhalation exposures experimental exposure concentrations are replaced with human equivalent concentrations calculated using EPA's methods for deriving inhalation reference concentrations (U.S. EPA, 1994), which give preference to the use of toxicokinetic modeling. When toxicokinetic models are unavailable, default dosimetry models are employed to extrapolate from experimental exposure concentrations to human equivalent concentrations. When toxicokinetic modeling or dosimetry modeling is used without toxicodynamic modeling, the dose-response assessment develops and supports an approach for addressing toxicodynamic equivalence.

The default dosimetry models typically involve the use of species-specific physiologic and anatomic factors relevant to the form of the agent (e.g., particle or gas) and categorized with regard to whether the response occurs either locally (i.e., within the respiratory tract) or remotely. For example, current default models (U.S. EPA, 1994) use parameters such as:

- inhalation rate and surface area of the affected part of the respiratory tract for gases eliciting the response locally,

- blood:gas partition coefficients for remote acting gases,

- fractional deposition with inhalation rate and surface area of the affected part of the respiratory tract for particles eliciting the response locally, and

- fractional deposition with inhalation rate and body weight for particles eliciting the response remotely.

The current default values for some parameters used in the default models (e.g., breathing rate and respiratory tract surface area) are based on data from adults (U.S. EPA, 1994). The human respiratory system passes through several distinct stages of maturation and growth during the first several years of life and into adolescence (Pinkerton and Joad, 2000), during which

characteristics important to disposition of inhaled toxicants may vary. Children and adults breathing the same concentration of an agent may receive different doses to the body or lungs (U.S. EPA, 2002b). Consequently, it may be appropriate to evaluate the default models by considering physiologic and anatomic factors representative of early lifestages, for example through the substitution of child-specific parameters (U.S. EPA, 2002b). Such evaluation uses the default model and dosimetric adjustment in use at the time of the assessment coupled with the best understanding of child-specific parameters at that time (e.g., drawn from the scientific literature). This analysis is undertaken with caution: (1) because of the correlations between activity level, breathing rate, respiratory tract dimensions, and body weight and (2) to avoid the possibility of mismatching the type of agent (gas or particle) and its site of response (within the respiratory tract or remote from the respiratory tract) with the relevant dosimetry factors in use at the time of the assessment. Analyses of children's inhalation dosimetry are also considered when using model structures beyond the default models (e.g., physiologically based toxicokinetic models).

When using dosimetry modeling, the comparison of human-equivalent concentrations for different lifestages (e.g., for an adult and a child) can indicate whether it is important to carry both concentrations forward in the dose-response assessment or whether a verbal characterization of any findings will suffice.

3.1.4. Route Extrapolation

In certain situations, an assessment based on studies of one exposure route may be applied to another exposure route. Route-to-route extrapolation has both qualitative and quantitative aspects. For the qualitative aspect, the assessor should weigh the degree to which positive results by one exposure route support a judgment that similar results would be expected by another route. In general, confidence in making such a judgment is strengthened when tumors are observed at a site distant from the portal of entry and when absorption is similar through both routes. In the absence of contrary data, a qualitative default option can be used: if the agent is absorbed through an exposure route to give an internal dose, it may be carcinogenic by that route.

When a qualitative extrapolation can be supported, quantitative extrapolation may still be problematic due to the absence of adequate data. The differences in biological processes among routes of exposure (oral, inhalation, dermal) can be great because of, for example, first-pass effects and different results from different exposure patterns. There is no generally applicable method for accounting for these differences in uptake processes in a quantitative route-to-route extrapolation of dose-response data in the absence of good data on the agent of interest. Therefore, route-to-route extrapolation of dose data relies on a case-by-case analysis of available data. When good data on the agent itself are limited, an extrapolation analysis can be based on expectations from physical and chemical properties of the agent, properties and route-specific data on structurally analogous compounds, or *in vitro* or *in vivo* uptake data on the agent.

Route-to-route uptake models may be applied if model parameters are suitable for the compound of interest. Such models are currently considered interim methods; further model development and validation is awaiting the development of more extensive data. For screening or hazard ranking, route-to-route extrapolation may be based on assumed quantitative comparability as a default, as long as it is reasonable to assume absorption by compared routes. When route-to-route extrapolation is used, the assessor’s degree of confidence in both the qualitative and quantitative extrapolation is discussed in the assessment and highlighted in the dose-response characterization.

Toxicokinetic modeling can be used to compare results of studies by different exposure routes. Results can also be compared on the basis of internal dose for effects distant from the point of contact.

Route extrapolation can be used to understand how internal dose and subsequent effects depend on exposure route. If testing by different exposure routes is available, the observation of similar or dissimilar internal doses can be important in determining whether and what conclusions can be made concerning the dose-response function(s) for different routes of exposure.

3.2. ANALYSIS IN THE RANGE OF OBSERVATION

The principle underlying these cancer guidelines is to use approaches that include as much information as possible. Quantitative information about key precursor events can be used to develop a toxicodynamic model. Alternatively, such information can be fitted by empirical models to extend the dose-response analysis of tumor incidence to lower doses and response levels. The analysis in the range of observation is used to establish a POD near the lower end of the observed range (see Section 3.3).

3.2.1. Epidemiologic Studies

Ideally, epidemiologic data would be used to select the dose-response function for human exposures. Because epidemiologic data are usually limited and many models may fit the data (Samet et al.,1998), other factors may influence model choice. For epidemiologic studies, including those with grouped data, analysis by linear models in the range of observation is generally appropriate unless the fit is poor. The relatively small exposure range observed in many epidemiologic studies, for example, makes it difficult to discern the shape of the exposure- or dose-response curve. Exposure misclassification and errors in exposure estimation also obscure the shape of the dose-response curve. When these errors are unsystematic or random, the result is frequently to bias the risk estimates toward zero. When a linear model fits poorly, more flexible models that allow for low-dose linearity, for example, a linear-quadratic model or a Hill model (Murrell et al., 1998), are often considered next.

Analysis of epidemiologic studies depends on the type of study and quality of the data, particularly the availability of quantitative measures of exposure. The objective is to develop a dose-response curve that estimates the incidence of cancer attributable to the dose (as estimated from the exposure) to the agent. In some cases, e.g., tobacco smoke or occupational exposures, the data are in the range of the exposures of interest. In other cases, as with data from animal experiments, information from the observable range is extrapolated to exposures of interest.

Analysis of effects raises additional issues:

- Many studies collect information from death certificates, which leads to estimates of mortality rather than incidence. Because survival rates vary for different cancers, the analysis may be improved by adjusting mortality figures to reflect the relationship between incidence and mortality.

- Epidemiologic studies, by their nature, are limited in the extent to which they can control for effects due to exposures from other agents. In some cases, the agent can have discernible interactive effects with another agent, making it possible to estimate the contribution of each agent as a risk factor for the effects of the other. For example, competing risks in a study population can limit the observed occurrence of cancer, while additive effects may lead to an increase occurrence of cancer. In the case of rates not already so adjusted, the analysis can be improved by correcting for competing or additive risks that are not similar in exposed and comparison groups.

- Comparison groups that are not free from exposure to the agent can bias the risk estimates toward zero. The analysis can be improved by considering background exposures in the exposed and comparison groups.

- The latent period for most cancers implies that exposures immediately preceding the detection of a tumor would be less likely to have contributed to its development and, therefore, may count less in the analysis. Study subjects who were first exposed near the end of the study may not have had adequate time since exposure for cancer to develop; therefore, analysis of their data may be similar to analysis of data for those who were not exposed. However, for carcinogens that act on multiple stages of the carcinogenic process, especially the later stages, all periods of exposure. including recent exposures, may be important.

Some study designs can yield only a partial characterization of the overall hazard and therefore risk as, for example, in studies that: (1) investigate only one effect (typical of many

case-control studies), (2) include only one population segment (e.g., male workers or workers of one socioeconomic class), or (3) include only one lifestage (e.g., childhood leukemia following maternal exposure to contaminated drinking water). To obtain a more complete characterization that includes risks of other cancers, estimates from these studies can be supplemented with estimates from other studies that investigated other cancers, population segments, or lifestages (see Section 3.5).

When several studies are available for dose-response analysis, *meta-analysis* can provide a systematic approach to weighing positive studies and those studies that do not show positive results, and calculating an overall risk estimate with greater precision. Issues considered include the comparability of studies, heterogeneity across studies, and the potential for a single large study to dominate the analysis. Confidence in a meta-analysis is increased when it considers study quality, including definition of the study population and comparison group, measurement of exposure, potential for exposure misclassification, adequacy of follow-up period, and analysis of confounders (see Section 2.2.1.3).

3.2.2. Toxicodynamic ("Biologically Based") Modeling

Toxicodynamic modeling can be used when there are sufficient data to ascertain the mode of action (see Section 2.4) and quantitatively support model parameters that represent rates and other quantities associated with the key precursor events of the mode of action. Toxicodynamic modeling is potentially the most comprehensive way to account for the biological processes involved in a response. Such models seek to reflect the sequence of key precursor events that lead to cancer. Toxicodynamic models can contribute to dose-response assessment by revealing and describing nonlinear relationships between internal dose and cancer response. Such models may provide a useful approach for analysis in the range of observation, provided the purpose of the assessment justifies the effort involved.

If a new model is developed for a specific agent, extensive data on the agent are important for identifying the form of the model, estimating its parameters, and building confidence in its results. Conformance to the observed tumor incidence data alone does not establish a model's validity, as a model can be designed with a sufficiently large number of parameters so as to fit

any given dataset. Peer review, including both an examination of the scientific basis supporting the model and an independent evaluation of the model's performance, is an essential part of evaluating the new model.

If a standard model already exists for the agent's mode of action, the model can be adapted for the agent by using agent-specific data to estimate the model's parameters. An example is the two-stage clonal expansion model developed by Moolgavkar and Knudson (1981) and Chen and Farland (1991). These models continue to be improved as more information becomes available.

It is possible for different models to provide equivalent fits to the observed data but to diverge substantially in their projections at lower doses. When model parameters are estimated from tumor incidence data, it is often the case that different combinations of parameter estimates can yield similar results in the observed range. For this reason, critical parameters (e.g., mutation rates and cell birth and death rates) are estimated from laboratory studies and not by curve-fitting to tumor incidence data (Portier, 1987). This approach reduces model uncertainty (see Section 3.6) and ensures that the model does not give answers that are biologically unrealistic. This approach also provides a robustness of results, where the results are not likely to change substantially if fitted to slightly different data.

Toxicodynamic modeling can provide insight into the relationship between tumors and key precursor events. For example, a model that includes cell proliferation can be used to explore the extent to which small increases in the cell proliferation rate can lead to large lifetime tumor incidences (Gaylor and Zheng, 1996). In this way, toxicodynamic modeling can be used to select and characterize an appropriate precursor response level (see Section 3.2.2, 3.2.5).

3.2.3. Empirical Modeling (“Curve Fitting”)

When a toxicodynamic model is not available or when the purpose of the assessment does not warrant developing such a model, empirical modeling (sometimes called “curve fitting”) should be used in the range of observation. A model can be fitted to data on either tumor incidence or a key precursor event. Goodness-of-fit to the experimental observations is not by itself an effective means of discriminating among models that adequately fit the data (OSTP,

1985). Many different curve-fitting models have been developed, and those that fit the observed data reasonably well may lead to several-fold differences in estimated risk at the lower end of the observed range. Another problem occurs when a multitude of alternatives are presented without sufficient context to make a reasoned judgment about the alternatives. This form of model uncertainty reflects primarily the availability of different computer models and not biological information about the agent being assessed or about carcinogenesis in general. In cases where curve-fitting models are used because the data are not adequate to support a toxicodynamic model, there generally would be no biological basis to choose among alternative curve-fitting models. However, in situations where there are alternative models with significant biological support, the decisionmaker can be informed by the presentation of these alternatives along with their strengths and uncertainties.

Quantitative data on precursors can be used in conjunction with, or in lieu of, data on tumor incidence to extend the dose-response curve to lower doses. Caution is used with rates of molecular events such as mutation or cell proliferation or signal transduction. Such rates can be difficult to relate to cell or tissue changes overall. The timing of observations of these phenomena, as well as the cell type involved, is linked to other precursor events to ensure that the measurement is truly a key event (Section 2.4).

For incidence data on either tumors or a precursor, an established empirical procedure is used to provide objectivity and consistency among assessments. The procedure models incidence, corrected for background, as an increasing function of dose. The models are sufficiently flexible in the observed range to fit linear and nonlinear datasets. Additional judgments and perhaps alternative analyses are used when the procedure fails to yield reliable results. For example, when a model's fit is poor, the highest dose is often omitted in cases where it is judged that the highest dose reflects competing toxicity that is more relevant at high doses than at lower doses. Another example is when there are large differences in survival across dose groups; here, models that includes time-to-tumor or time-to-event information may be useful.

For continuous data on key precursor effects, empirical models can be chosen on the basis of the structure of the data. The rationale for the choice of model, the alternatives

considered and rejected, and a discussion of model uncertainty are included in the dose-response characterization.

3.2.4. Point of Departure (POD)

For each tumor response, a POD from the observed data should be estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses.

The POD is used as the starting point for subsequent extrapolations and analyses. For linear extrapolation, the POD is used to calculate a *slope factor* (see Section 3.3.3), and for nonlinear extrapolation the POD is used in the calculation of a *reference dose* or *reference concentration* (see Section 3.3.4). In a risk characterization, the POD is part of the determination of a *margin of exposure* (see Section 5.4). With appropriate adjustments, it can also be used as the basis for *hazard rankings* that compare different agents or health effects.

The lowest POD is used that is adequately supported by the data. If the POD is above some data points, it can fail to reflect the shape of the dose-response curve at the lowest doses and can introduce bias into subsequent extrapolations (see Figure 3-1). On the other hand, if the POD is far below all observed data points, it can introduce model uncertainty and parameter uncertainty (see Section 3.6) that increase with the distance between the data and the POD. Use of a POD at the lowest level supported by the data seeks to balance these considerations. It uses information from the model(s) a small distance below the observed range rather than discarding this information and using extrapolation procedures in a range where the model(s) can provide some useful information. Statistical tests involving the ratio of the central estimate and its lower bound (i.e., ED_{xx}/LED_{xx}) can be useful for evaluating how well the data support a model's estimates at a particular response level. (Note that the ability to model at a particular response level is not the same as the study's ability to identify an increase at that response level as statistically significant.)

The POD for extrapolating the relationship to environmental exposure levels of interest, when the latter are outside the range of observed data, is generally the lower 95% confidence

limit on the lowest dose level that can be supported for modeling by the data. SAB (1997) suggested that, "it may be appropriate to emphasize lower statistical bounds in screening analyses and in activities designed to develop an appropriate human exposure value, since such activities require accounting for various types of uncertainties and a lower bound on the central estimate is a scientifically-based approach accounting for the uncertainty in the true value of the ED_{10} [or central estimate]." However, the consensus of the SAB (1997) was that, "both point estimates and statistical bounds can be useful in different circumstances, and recommended that the Agency routinely calculate and present the point estimate of the ED_{10} [or central estimate] and the corresponding upper and lower 95% statistical bounds." For example, it may be appropriate to emphasize the central estimate in activities that involve formal uncertainty analysis that are required by OMB Circular A-4 (OMB, 2003) as well as ranking agents as to their carcinogenic hazard. Thus, risk assessors should calculate, to the extent practicable, and present the central estimate and the corresponding upper and lower statistical bounds (such as confidence limits) to inform decisionmakers.

When tumor data are used, a POD is obtained from the modeled tumor incidences. Conventional cancer bioassays, with approximately 50 animals per group, generally can support modeling down to an increased incidence of 1–10%; epidemiologic studies, with larger sample sizes, below 1%. Various models commonly used for carcinogens yield similar estimates of the POD at response levels as low as 1% (Krewski and Van Ryzin, 1981; Gaylor et al., 1994). Consequently, response levels at or below 10% can often be used as the POD. As a modeling convention, the lower bound on the doses associated with standard response levels of 1, 5, and 10% can be analyzed, presented, and considered. For making comparisons at doses within the observed range, the ED_{10} and LED_{10} are also reported and can be used, with appropriate adjustments, in hazard rankings that compare different agents or health effects (U.S. EPA, 2002c). A *no-observed-adverse-effect level* (NOAEL) generally is not used for assessing the potential for carcinogenic response when one or more models can be fitted to the data.

When good quality precursor data are available and are clearly tied to the mode of action of the compound of interest, models that include both tumors and their precursors may be advantageous for deriving a POD. Such models can provide insight into quantitative

relationships between tumors and precursors (see Section 3.2.2), possibly suggesting the precursor response level that is associated with a particular tumor response level. The goal is to use precursor data to extend the observed range below what can be observed in tumor studies. EPA is continuing to examine this issue and anticipates that findings and conclusions may result in supplemental guidance to these cancer guidelines. If the precursor data are drawn from small samples or if the quantitative relationship between tumors and precursors is not well defined, then the tumor data will provide a more reliable POD. Precursor effects may or may not be biologically adverse in themselves; the intent is to consider not only tumors but also damage that can lead to subsequent tumor development by the agent. Analysis of continuous data may differ from discrete data; Murrell et al. (1998) discuss alternative approaches to deriving a POD from continuous data.

3.2.5. Characterizing the POD: The POD Narrative

As a single-point summary of a single dose-response curve, the POD alone does not convey all the critical information present in the data from which it is derived. To convey a measure of uncertainty, the POD should be presented as a central estimate with upper and lower bounds. A POD narrative summarizes other important features of the database and the POD that are important to account for in low-dose extrapolations or other analyses.

(a) Nature of the response. Is the POD based on tumors or a precursor? If on tumors, does the POD measure incidence or mortality? Is it a lifetime measure or was the study terminated early? The relationships between precursors and tumors, incidence and mortality, and lifetime and early-termination results vary from case to case. Modeling can provide quantitative insight into these relationships, for example, linking a change in a precursor response to a tumor incidence (see Section 3.2.2). This can aid in evaluating the significance of the response at the POD and adjusting different PODs to make them comparable.

(b) Level of the response. What level of response is associated with the POD, for example, 1% cancer risk, 10% cancer risk, or 10% change in a precursor measure?

(c) Nature of the study population. Is the POD based on humans or animals? How large is the effective sample size? Is the study group representative of the general population, of healthy adult workers, or of a susceptible group? Are both sexes represented? Did exposure occur during a susceptible lifestage?

(d) Slope of the dose-response curve at the POD. How does response change as dose is reduced below the POD? A steep slope indicates that risk decreases rapidly as dose decreases. On the other hand, a steep slope also indicates that errors in an exposure assessment can lead to large errors in estimating risk. Both aspects of the slope are important. The slope also indicates whether dose-response curves for different effects are likely to cross below the POD. For example, in the ED_{01} study where 2-acetylaminofluorene caused bladder carcinomas and liver carcinomas in mice (Littlefield et al., 1980), the dose-response curves for these tumors cross between 10% and 1% response (see Figure 3-2). This crossing, which can be inferred from the slopes of the curves at a 10% response, shows how considering the slope can lead to better inferences about the predominant effects expected at lower doses. Mode of action data can also be useful; quantitative information about key precursor events can be used to describe how risk decreases as dose decreases below the POD.

(e) Relationship of the POD with other cancers. How does the POD for this cancer relate to PODs for other cancers observed in the database? For example, a POD based on male workers would not reflect the implications of mammary tumors in female rats or mice.

(f) Extent of the overall cancer database. Have potential cancer responses been adequately studied (e.g., were all tissues examined), or is the database limited to particular effects, population segments, or lifestages? Do the mode of action data suggest a potential for cancers not observed in the database (e.g., disruption of particular endocrine pathways leading to related cancers)?

3.2.6. Relative Potency Factors

Relative potency factors (of which toxicity equivalence factors are a special case) can be used for a well-defined class of agents that operate through a common mode of action for the same toxic endpoint. A complete dose-response assessment is conducted for one well-studied member of the class that serves as the *index chemical* for the class. The other members of the class are tied to the index chemical by relative potency factors that are based on characteristics such as relative toxicological outcomes, relative metabolic rates, relative absorption rates, quantitative SARs, or receptor binding characteristics (U.S. EPA, 2000c). Examples of this approach are the *toxicity equivalence factors* for dioxin-like compounds and the relative potency factors for some carcinogenic polycyclic aromatic hydrocarbons. Whenever practicable, toxicity equivalence factors should be validated and accompanied by quantitative uncertainty analysis.

3.3. EXTRAPOLATION TO LOWER DOSES

The purpose of low-dose extrapolation is to provide as much information as possible about risk in the range of doses below the observed data. The most versatile forms of low-dose extrapolation are dose-response models that characterize risk as a probability over a range of environmental exposure levels. These risk probabilities allow estimates of the risk reduction under different decision options and estimates of the risk remaining after an action is taken and provide the risk information needed for benefit-cost analyses of different decision options.

When a dose-response model is not developed for lower doses, another form of low-dose extrapolation is a safety assessment that characterizes the safety of one lower dose, with no explicit characterization of risks above or below that dose. Although this type of extrapolation may be adequate for evaluation of some decision options, it may not be adequate for other purposes (e.g., benefit-cost analyses) that require a quantitative characterization of risks across a range of doses. At this time, safety assessment is the default approach for tumors that arise through a nonlinear mode of action; however, EPA continues to explore methods for quantifying dose-response relationships over a range of environmental exposure levels for tumors that arise through a nonlinear mode of action (U.S. EPA, 2002c). EPA program offices that need this more

explicit dose-response information may develop and apply methods that are informed by the methods described in these cancer guidelines.

3.3.1. Choosing an Extrapolation Approach

The approach for extrapolation below the observed data considers the understanding of the agent's mode of action at each tumor site (see Section 2.4). Mode of action information can suggest the likely shape of the dose-response curve at lower doses. The extent of inter-individual variation is also considered, with greater variation spreading the response over a wider range of doses.

Linear extrapolation should be used when there are MOA data to indicate that the dose-response curve is expected to have a linear component below the POD. Agents that are generally considered to be linear in this region include:

- agents that are DNA-reactive and have direct mutagenic activity, or

- agents for which human exposures or body burdens are high and near doses associated with key precursor events in the carcinogenic process, so that background exposures to this and other agents operating through a common mode of action are in the increasing, approximately linear, portion of the dose-response curve.

When the weight of evidence evaluation of all available data are insufficient to establish the mode of action for a tumor site and when scientifically plausible based on the available data, linear extrapolation is used as a default approach, because linear extrapolation generally is considered to be a health-protective approach. Nonlinear approaches generally should not be used in cases where the mode of action has not been ascertained. Where alternative approaches with significant biological support are available for the same tumor response and no scientific consensus favors a single approach, an assessment may present results based on more than one approach.

A *nonlinear approach* should be selected when there are sufficient data to ascertain the mode of action and conclude that it is not linear at low doses and the agent does not demonstrate mutagenic or other activity consistent with linearity at low doses. Special attention is important when the data support a nonlinear mode of action but there is also a suggestion of mutagenicity. Depending on the strength of the suggestion of mutagenicity, the assessment may justify a conclusion that mutagenicity is not operative at low doses and focus on a nonlinear approach, or alternatively, the assessment may use both linear and nonlinear approaches.

Both linear and nonlinear approaches may be used when there are multiple modes of action. If there are multiple tumor sites, one with a linear and another with a nonlinear mode of action, then the corresponding approach is used at each site. If there are multiple modes of action at a single tumor site, one linear and another nonlinear, then both approaches are used to decouple and consider the respective contributions of each mode of action in different dose ranges. For example, an agent can act predominantly through cytotoxicity at high doses and through mutagenicity at lower doses where cytotoxicity does not occur. Modeling to a low response level can be useful for estimating the response at doses where the high-dose mode of action would be less important.

3.3.2. Extrapolation Using a Toxicodynamic Model

The preferred approach is to develop a toxicodynamic model of the agent's mode of action and use that model for extrapolation to lower doses (see Section 3.2.2). The extent of extrapolation is governed by an analysis of *model uncertainty*, where alternative models that fit similarly in the observed range can diverge below that range (see Section 3.6). Substantial divergence is likely when model parameters are estimated from tumor incidence data, so that different combinations of parameter estimates yield similar fits in the observed range but have different implications at lower doses. An analysis of model uncertainty can be used to determine the range where extrapolation using the toxicodynamic model is supported and where further extrapolation would be based on either a linear or a nonlinear default, as appropriate (see Sections 3.3.3, 3.3.4).

3.3.3. Extrapolation Using a Low-dose, Linear Model

Linear extrapolation should be used in two distinct circumstances: (1) when there are data to indicate that the dose-response curve has a linear component below the POD, or (2) as a default for a tumor site where the mode of action is not established (see Section 3.3.1). For linear extrapolation, a line should be drawn from the POD to the origin, corrected for background. This implies a proportional (linear) relationship between risk and dose at low doses. (Note that the dose-response curve generally is not linear at higher doses.)

The slope of this line, known as the *slope factor*, is an upper-bound estimate of risk per increment of dose that can be used to estimate risk probabilities for different exposure levels. The slope factor is equal to $0.01/LED_{01}$ if the LED_{01} is used as the POD.

Unit risk estimates express the slope in terms of μg/L drinking water or $\mu g/m^3$ or ppm air. In general, the drinking water unit risk is derived by converting a slope factor from units of mg/kg-d to units of μg/L, whereas an inhalation unit risk is developed directly from a dose-response analysis using equivalent human concentrations already expressed in units of $\mu g/m^3$. Unit risk estimates often assume a standard intake rate (L/day drinking water or m^3/day air) and body weight (kg), which may need to be reconciled with the exposure factors for the population of interest in an exposure assessment (see Section 4.4). Alternatively, when the slope factor for inhalation is in units of ppm, it may sometimes be termed the inhalation unit risk. Although unit risks have not been calculated in the past for dermal exposures, both exposures that are absorbed into the systemic circulation and those that remain in contact with the skin are also important.

Risk-specific doses are derived from the slope factor or unit risk to estimate the dose associated with a specific risk level, for example, a one-in-a-million increased lifetime risk.

3.3.4. Nonlinear Extrapolation to Lower Doses

A nonlinear extrapolation method can be used for cases with sufficient data to ascertain the mode of action and to conclude that it is not linear at low doses but with not enough data to support a toxicodynamic model that may be either nonlinear or linear at low doses. Nonlinear extrapolation having a significant biological support may be presented in addition to a linear approach when the available data and a weight of evidence evaluation support a nonlinear

approach, but the data are not strong enough to ascertain the mode of action applying the Agency's mode of action framework. If the mode of action and other information can support chemical-specific modeling at low doses, it is preferable to default procedures.

For cases where the tumors arise through a nonlinear mode of action, an oral *reference dose* or an inhalation *reference concentration*, or both, should be developed in accordance with EPA's established practice for developing such values, taking into consideration the factors summarized in the characterization of the POD (see Section 3.2.5). This approach expands the past focus of such reference values (previously reserved for effects other than cancer) to include carcinogenic effects determined to have a nonlinear mode of action. As with other health effects of concern, it is important to put cancer in perspective with the overall health impact of an exposure by comparing reference value calculations for cancer with those for other health effects.

For effects other than cancer, reference values have been described as being based on the assumption of biological thresholds. The Agency's more current guidelines for these effects (U.S. EPA, 1996a, 1998b), however, do not use this assumption, citing the difficulty of empirically distinguishing a true threshold from a dose-response curve that is nonlinear at low doses.

Economic and policy analysts need to know how the probability of cancer varies at exposures above the reference value and whether, and to what extent, there are health benefits from reducing exposures below the reference value. The risk assessment community is working to develop better methods to provide more useful information to economic and policy analysts.

3.3.5. Comparing and Combining Multiple Extrapolations

When multiple estimates can be developed, all datasets should be considered and a judgment made about how best to represent the human cancer risk. Some options for presenting results include:

- adding risk estimates derived from different tumor sites (NRC, 1994),

- combining data from different datasets in a joint analysis (Putzrath and Ginevan, 1991; Stiteler et al., 1993; Vater et al., 1993),

- combining responses that operate through a common mode of action,

- representing the overall response in each experiment by counting animals with any tumor showing a statistically significant increase,

- presenting a range of results from multiple datasets (in this case, the dose-response assessment includes guidance on how to choose an appropriate value from the range),

- choosing a single dataset if it can be justified as most representative of the overall response in humans, or

- a combination of these options.

Cross-comparison of estimates from human and animal studies can provide a valuable risk perspective.

- Calculating an animal-derived slope factor and using it to estimate the risk expected in a human study can provide information with which to evaluate the human study design, for example, adequacy of exposure level and sample size.

- Calculating an upper-bound slope factor from a human study that does not show positive results but that has good exposure information, and comparing it to an animal-derived slope factor can indicate whether the animal and humans studies are consistent.

3.4. EXTRAPOLATION TO DIFFERENT HUMAN EXPOSURE SCENARIOS

As described in the previous cancer guidelines, special problems arise when the human exposure situation of concern suggests exposure regimens, e.g., route and dosing schedule, that are substantially different from those used in the relevant animal studies. Unless there is evidence to the contrary in a particular case, the cumulative dose received over a lifetime, expressed as average daily exposure prorated over a lifetime, is recommended as an appropriate measure of exposure to a carcinogen. That is, the assumption is made that a high dose of a carcinogen received over a short period of time is equivalent to a corresponding low dose spread over a lifetime. This approach becomes more problematical as the exposures in question become more intense but less frequent, especially when there is evidence that the agent has shown dose-rate effects (U.S. EPA 1986a).

Accordingly, *for lifetime human exposure scenarios* that involve intermittent or varying levels of exposure, the prevailing practice has been to assess exposure by calculating a *lifetime average daily exposure or dose* (U.S. EPA, 1992a).

For less-than-lifetime human exposure scenarios, too, the lifetime average daily exposure or dose has often been used. The use of these lifetime average exposure metrics was adopted with low-dose linear cancer assessments in mind. The lifetime averaging implies that less-than-lifetime exposure is associated with a linearly proportional reduction of the lifetime risk, regardless of when exposures occur. Such averaging may be problematic in some situations. This can be illustrated using both the multistage model and the two-stage clonal expansion model that predict that short-duration risks are not necessarily proportional to exposure duration and can depend on the nature of the carcinogen and the timing of exposure (Goddard et al., 1995; Murdoch et al., 1992). These examples indicate some circumstances in which use of a lifetime average daily dose (LADD) would underestimate cancer risk by two- to fivefold, and others in which it might overestimate risk (Murdoch et al., 1992). Thus, averaging over the duration of a lifestage or a critical window of exposure may be appropriate. As methodological research focuses on new approaches for estimating risks from less-than-lifetime exposures, methods and defaults can be expected to change.

This highlights the importance for each dose-response assessment to critically evaluate all information pertaining to less-than-lifetime exposure. For example, detailed stop-exposure studies can provide information about the relationship between exposure duration, precursor effects, potential for reversibility, and tumor development. Toxicokinetic modeling can investigate differences in internal dose between short-term and long-term exposure or between intermittent and constant exposure. Persistence in the body can be useful in explaining long-term effects resulting from shorter-term exposures.

For nonlinear cancer analyses, it may be appropriate to assess exposure by calculating a *daily dose* that is averaged over the exposure duration for the study (see Section 3.1.1). For example, when the analysis is based on precursor effects that result from less than a lifetime exposure, that exposure period may be used. This reflects an expectation that the precursor effects on which the analysis is based can result from less-than-lifetime exposure, bringing consistency to the methods used for dose-response assessment and exposure assessment in such cases. The dose-response assessment can provide a recommendation to exposure assessors about the averaging time that is appropriate to the mode of action and to the exposure duration of the scenario.

3.5. EXTRAPOLATION TO SUSCEPTIBLE POPULATIONS AND LIFESTAGES

The dose-response assessment strives to derive separate estimates for susceptible populations and lifestages so that these risks can be explicitly characterized. For a susceptible population, higher risks can be expected from exposures anytime during life, but this applies to only a portion of the general population (e.g., those bearing a particular genetic susceptibility). In contrast, for a susceptible lifestage, higher risks can be expected from exposures during only a portion of a lifetime, but everyone in the population may pass through those lifestages. Effects of exposures during a susceptible period are not equivalent to effects of exposures at other times; consequently, it is useful to estimate the risk attributable to exposures during each period.

Depending on the data available, a tiered approach should be used to address susceptible populations and lifestages.

- When there is an epidemiologic study or an animal bioassay that reports quantitative results for susceptible individuals, the data should be analyzed to provide a separate risk estimate for those who are susceptible. If susceptibility pertains to a lifestage, it is useful to characterize the portion of the lifetime risk that can be attributed to the susceptible lifestage.

- When there are data on some risk-related parameters that allow comparison of the general population and susceptible individuals, the data should be analyzed with an eye toward adjusting the general population estimate for susceptible individuals. This analysis can range from toxicokinetic modeling that uses parameter values representative of susceptible individuals to more simply adjusting a general population estimate to reflect differences in important rate-governing parameters. Care is taken to not make parameter adjustments in isolation, as the appropriate adjustment can depend on the interactions of several parameters; for example, the ratio of metabolic activation and clearance rates can be more appropriate than the activation rate alone (U.S. EPA, 1992b).
- In the absence of such agent-specific data, there is some general information to indicate that childhood can be a susceptible lifestage for exposure to some carcinogens (U.S. EPA, 2005); this warrants explicit consideration in each assessment. The potential for susceptibility from early-life exposure is expected to vary among specific agents and chemical classes. In addition, the concern that the dose-averaging generally used for assessing less-than-lifetime exposure is more likely to understate than overstate risk (see Section 3.4) contributes to the suggestion that alternative approaches be considered for assessing risks from less-than-lifetime exposure that occurs during childhood. Accompanying these cancer guidelines is the Supplemental Guidance that the Agency will use to assess risks from early-life exposure to potential carcinogens (U.S. EPA, 2005). The Supplemental Guidance may be updated to reflect new data and new understanding that may become available in the future.

3.6. UNCERTAINTY

The NRC (1983, 1994, 1996, 2002) has repeatedly advised that proper characterization of uncertainty is essential in risk assessment. An assessment that omits or underestimates uncertainty can leave decisionmakers with a false sense of confidence in estimates of risk. On the other hand, a high level of uncertainty does not imply that a risk assessment or a risk management action should be delayed (NRC, 2002). Uncertainty in dose-response assessment can be classified as either *model uncertainty* or *parameter uncertainty*. A related concept, *human variation*, is discussed below. Assessments should discuss the significant uncertainties encountered in the analysis, distinguishing, if possible, between model uncertainty, parameter uncertainty, and human variation. Origins of these uncertainties can span a range, from a single causal thread supported by sparse data, to abundant information that presents multiple possible conclusions or that does not coalesce. As described in Section 2.6 and in Section 5.1, all contributing features should be noted.

Model uncertainty refers to a lack of knowledge needed to determine which is the correct scientific theory on which to base a model. In risk assessment, model uncertainty is reflected in alternative choices for model structure, dose metrics, and extrapolation approaches. Other sources of model uncertainty concern whether surrogate data are appropriate, for example, using data on adults to make inferences about children. The full extent of model uncertainty usually cannot be quantified; a partial characterization can be obtained by comparing the results of alternative models. Model uncertainty is expressed through comparison of separate analyses from each model, coupled with a subjective probability statement, where feasible and appropriate, of the likelihood that each model might be correct (NRC, 1994).

Some aspects of model uncertainty that should be addressed in an assessment include the use of animal models as a surrogate for humans, the influence of cross-species differences in metabolism and physiology, the use of effects observed at high doses as an indicator of the potential for effects at lower doses, the effect of using linear or nonlinear extrapolation to estimate risks, the use of using small samples and subgroups to make inferences about entire human populations or subpopulations with differential susceptibilities, and the use of

experimental exposure regimens to make inferences about different human exposure scenarios (NRC, 2002).

Toxicokinetic and toxicodynamic models are generally premised on *site concordance* across species, modeling, for example, the relationship between administered dose and liver tissue concentrations to predict increased incidences of liver cancer. This relationship, which can be observed in animals, is typically only inferred for humans. There are, however, numerous examples of an agent causing different cancers in different species. The assessment should discuss the relevant data that bear on this form of model uncertainty.

Parameter uncertainty refers to a lack of knowledge about the values of a model's parameters. This leads to a distribution of values for each parameter. Common sources of parameter uncertainty include random measurement errors, systematic measurement errors, use of surrogate data instead of direct measurements, misclassification of exposure status, random sampling errors, and use of an unrepresentative sample. Most types of parameter uncertainty can be quantified by statistical analysis.

Human variation refers to person-to-person differences in biological susceptibility or in exposure. Although both human variation and uncertainty can be characterized as ranges or distributions, they are fundamentally different concepts. Uncertainty can be reduced by further research that supports a model or improves a parameter estimate, but human variation is a reality that can be better characterized, but not reduced, by further research. Fields other than risk assessment use “variation” or “variability” to mean dispersion about a central value, including measurement errors and other random errors that risk assessors address as uncertainty.

Probabilistic risk assessment, informed by expert judgment, has been used in exposure assessment to estimate human variation and uncertainty in lifetime average daily exposure concentration or dose. Probabilistic methods can be used in this exposure assessment application because the pertinent variables (for example, concentration, intake rate, exposure duration, and body weight) have been identified, their distributions can be observed, and the formula for combining the variables to estimate the lifetime average daily dose is well defined (see U.S. EPA, 1992a). Similarly, probabilistic methods can be applied in dose-response assessment when there is an understanding of the important parameters and their relationships, such as

identification of the key determinants of human variation (for example, metabolic polymorphisms, hormone levels, and cell replication rates), observation of the distributions of these variables, and valid models for combining these variables. With appropriate data and expert judgment, formal approaches to probabilistic risk assessment can be applied to provide insight into the overall extent and dominant sources of human variation and uncertainty. In doing this, it is important to note that analyses that omit or underestimate some principal sources of variation or uncertainty could provide a misleadingly narrow description of the true extent of variation and uncertainty and give decisionmakers a false sense of confidence in estimates of risk. Specification of joint probability distributions is appropriate when variables are not independent of each other. In each case, the assessment should carefully consider the questions of uncertainty and human variation and discuss the extent to which there are data to address them.

Probabilistic risk assessment has also been used in dose-response assessment to determine and distinguish the degree of uncertainty and variability in toxicokinetic and toxicodynamic modeling. Although this field is less advanced that probabilistic exposure assessment, progress is being made and these cancer guidelines are flexible enough to accommodate continuing advances in these approaches.

Advances in uncertainty analysis are expected as the field develops. The cancer guidelines are intended to be flexible enough to incorporate additional approaches for characterizing uncertainty that have less commonly been used by regulatory agencies. In all scientific and engineering fields, data and research limitations often limit the application of established methods. A dearth of data is a particular problem when quantifying the *probability distribution* of model outputs. In many of these scientific and engineering disciplines, researchers have used rigorous expert elicitation methods to overcome the lack of peer-reviewed methods and data. Although expert elicitation has not been widely used in environmental risk assessment, several studies have applied this methodology as a tool for understanding quantitative risk. For example, expert elicitation has been used in chemical risk assessment and its associated uncertainty (e.g., Richmond, 1981; Renn, 1999; Florig et al., 2001; Morgan et al., 2001; Willis et al., 2004), components of risk assessment such as hazard assessment and dose-response

evaluation (e.g., Hawkins and Graham 1988; Jelovsek et al., 1990; Evans et al., 1994; IEc, 2004; U.S. EPA 2004) and exposure assessment (e.g., Whitfield and Wallsten, 1989; Hawkins and Evans, 1989; Winkler et al., 1995; Stiber et al., 1999; Walker et al., 2001, 2003; Van Der Fels-Klerx et al., 2002), and for evaluating other types of risks (e.g., North and Merkhofer, 1976; Fos and McLin, 1990). These cancer guidelines are flexible enough to accommodate the use of expert elicitation to characterize cancer risks, as a complement to the methods presented in the cancer guidelines. According to NRC (NRC, 2002), the rigorous use of expert elicitation for the analyses of risks is considered to be quality science.

3.7. DOSE-RESPONSE CHARACTERIZATION

A *dose-response characterization* extracts the dose-response information needed in a full risk characterization (U.S. EPA, 2000b), including:

- presentation of the recommended estimates (slope factors, reference doses, reference concentrations) and alternatives with significant biological support,
- a summary of the data supporting these estimates,
- a summary and explanation of the modeling approaches used,
- a description of any special features such as the development and consolidation of multiple estimates as detailed in Section 3.3.5,
- the POD narrative (see Section 3.2.5),
- a summary of the key defaults invoked,
- identification of susceptible populations or lifestages and quantification of their differential susceptibility, and

- a discussion of the strengths and limitations of the dose-response assessment, highlighting significant issues in developing risk estimates, alternative approaches considered equally plausible, and how these issues were resolved.

All estimates should be accompanied by the weight of evidence descriptor and its narrative (see Section 2.5) to convey a sense of the qualitative uncertainty about whether the agent may or may not be carcinogenic.

Slope factors generally represent an upper bound on the average risk in a population or the risk for a randomly selected individual but not the risk for a highly susceptible individual or group. Some individuals face a higher risk and some face a lower risk. The use of upper bounds generally is considered to be a health-protective approach for covering the risk to susceptible individuals, although the calculation of upper bounds is not based on susceptibility data. Similarly, exposure during some lifestages can contribute more or less to the total lifetime risk than do similar exposures at other times. The dose-response assessment characterizes, to the extent possible, the extent of these variations.

Depending on the supporting data and modeling approach, a slope factor can have a mix of traits that tend to either estimate, overestimate, or underestimate risk.

Some examples of traits that tend to overestimate risk include the following.

- The slope factor is derived from data on a highly susceptible animal strain.

- Linear extrapolation is used as a default and extends over several orders of magnitude.

- The largest of several slope factors is chosen.

Some examples of traits that tend to underestimate risk include the following.

- Several tumor types were observed, but the slope factor is based on a subset of them.

- The study design does not include exposure during a susceptible lifestage, for example, perinatal exposure.

- The study population is of less-than-average susceptibility, for example, healthy adult workers.

- There is random exposure misclassification or random exposure measurement error in the study from which the slope factor is derived.

Some examples of traits that inherently neither overestimate nor underestimate risk include the following.

- The slope factor is derived from data in humans or in an animal strain that responds like humans.

- Linear extrapolation is appropriate for the agent’s mode of action.

- Environmental exposures are close to the observed data.

- Several slope factors for the same tumor are averaged or a slope factor is derived from pooled data from several studies.

- The slope factor is derived from the only suitable study.

Figure 3-1. Compatibility of alternative points of departure with observed and modeled tumor incidences

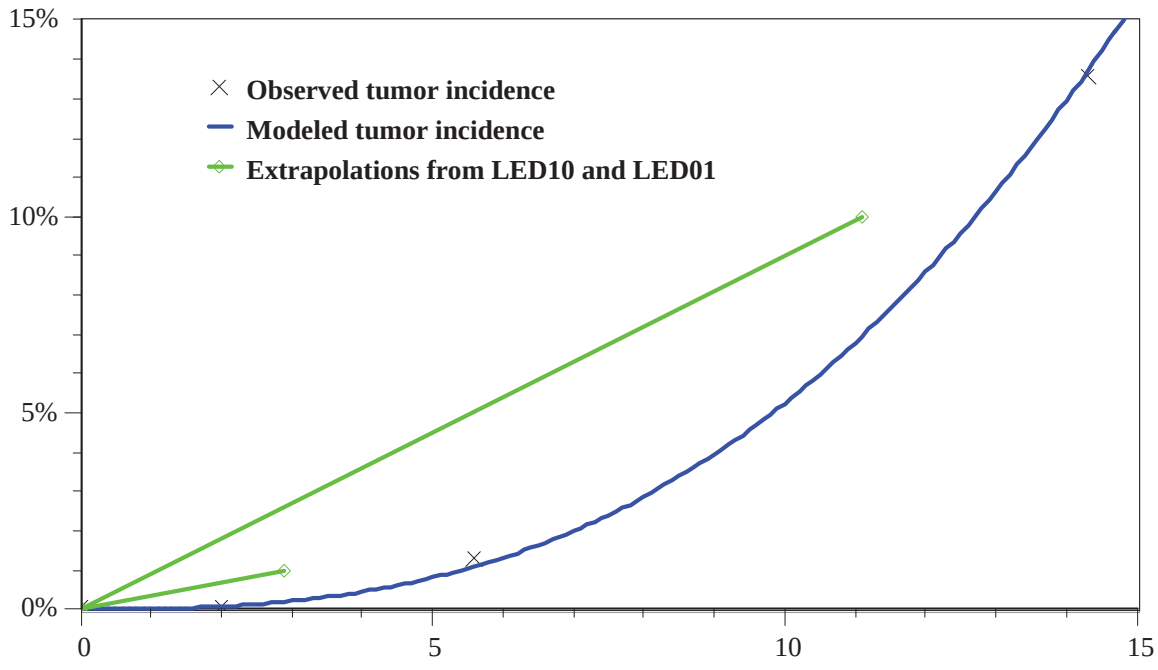
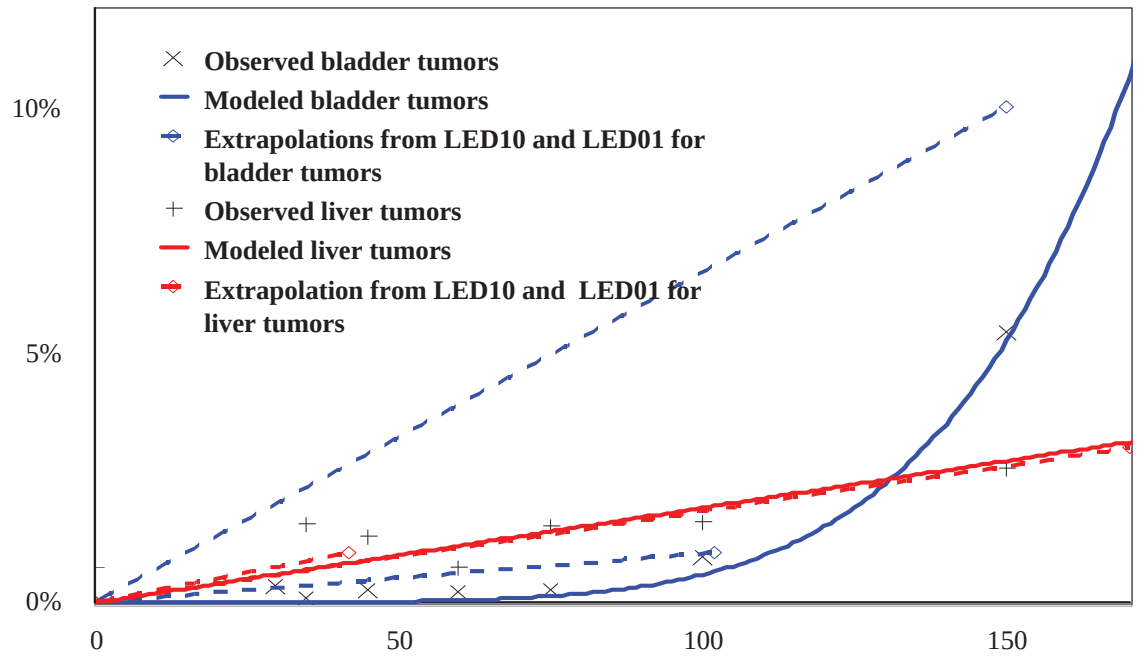


Figure 3-2. Crossing--between 10% and 1%--of dose-response curves for bladder carcinomas and liver carcinomas induced by 2-AAF



4. EXPOSURE ASSESSMENT

Exposure assessment is the determination (qualitative and quantitative) of the magnitude, frequency, and duration of exposure and internal dose (U.S. EPA, 1992a). This section provides a brief overview of exposure assessment principles, with an emphasis on issues related to carcinogenic risk assessment. The information presented here should be used in conjunction with other guidance documents, including *Guidelines for Exposure Assessment* (U.S. EPA, 1992a), *Science Policy Council Handbook: Risk Characterization* (U.S. EPA, 2000b), *Exposure Factors Handbook* (U.S. EPA, 1997c), the 1997 *Policy for Use of Probabilistic Analysis in Risk Assessments* (U.S. EPA, 1997d), and the 1997 *Guiding Principles for Monte Carlo Analysis* (U.S. EPA, 1997e). In addition, program-specific guidelines for exposure assessment should be consulted.

Exposure assessment generally consists of four major steps: defining the assessment questions, selecting or developing the conceptual and mathematical models, collecting data or selecting and evaluating available data, and exposure characterization. Each of these steps is briefly described below.

4.1. DEFINING THE ASSESSMENT QUESTIONS

In providing a clear and unambiguous statement of the purpose and scope of the exposure assessment (U.S. EPA, 1997e), consider the following.

- The management objectives of the assessment will determine whether deterministic screening level analyses are adequate or whether full probabilistic exposure characterization is needed.

- Identify and include all important sources (e.g., pesticide applications), pathways (e.g., food or water), and routes (e.g., ingestion, inhalation, and dermal) of exposure in the assessment. If a particular source, pathway, or route is omitted, a clear and transparent explanation should be provided.

- Separate analyses should be conducted for each definable subgroup within the population of interest. In particular, subpopulations or lifestages that are believed to be highly exposed or susceptible to a particular health effect should be studied. These include people with certain diseases or genetic susceptibilities and others whose behavior or physiology may lead to higher exposure or susceptibility. Consider the following examples:

  — Physiological differences between men and women (e.g., body weight and inhalation rate) may lead to important differences in exposures. See, for example, the discussion in *Exposure Factors Handbook* (U.S. EPA, 1997c, Appendix 1A).

  — Pregnant and lactating women may have exposures that differ from the general population (e.g., slightly higher water consumption) (U.S. EPA, 1997c). Further, exposure to pregnant women may result in exposure to the developing fetus (NRC, 1993b).

  — Children consume more food per body weight than do adults while consuming fewer types of foods, i.e., have a more limited diet (ILSI, 1992; NRC, 1993b; U.S. EPA, 1997c). In addition, children engage in crawling and mouthing (i.e., putting hands and objects in the mouth) behaviors, which can increase their exposures.

  — The elderly and disabled may have important differences in their exposures due to a more sedentary lifestyle (U.S. EPA, 1997c). In addition, the health status of this group may affect their susceptibility to the detrimental effects of exposure.

For further guidance, see *Guidelines for Exposure Assessment* (U.S. EPA, 1992a, § 3).

4.2. SELECTING OR DEVELOPING THE CONCEPTUAL AND MATHEMATICAL MODELS

Carcinogen risk assessment models have generally been based on the premise that risk is proportional to cumulative lifetime dose. For lifetime human exposure scenarios, therefore, the exposure metric used for carcinogenic risk assessment has been the lifetime average daily dose (LADD) or, in the case of inhalation exposure, the lifetime average exposure concentration. These metrics are typically used in conjunction with the corresponding slope factor to calculate individual excess cancer risk. The LADD is typically an estimate of the daily intake of a carcinogenic agent throughout the entire life of an individual, while the lifetime average exposure concentration is the corresponding estimate of average exposure concentration for the carcinogenic agent over the entire life of an individual. Depending on the objectives of the assessment, the LADD or lifetime average exposure concentration may be calculated deterministically (using point estimates for each factor to derive a point estimate of the exposure) or stochastically (using probability distributions to represent each factor and such techniques as Monte Carlo analysis to derive a distribution of the LADD) (U.S. EPA, 1997e). Stochastic analyses may help to identify certain population segments or lifestages that are highly exposed and may need to be assessed as a special subgroup. For further guidance, see *Guidelines for Exposure Assessment* (U.S. EPA, 1992a, § 5.3.5.2). As methodological research focuses on new approaches for estimating risks from less-than-lifetime exposures, methods and defaults can be expected to change.

There may be cases where the mode of action indicates that dose rates are important in the carcinogenic process. In these cases, short-term, less-than-lifetime exposure estimates may be more appropriate than the LADD for risk assessment. This may be the case when a nonlinear dose-response approach is used (see Section 3.3.4).

4.3. COLLECTING DATA OR SELECTING AND EVALUATING AVAILABLE DATA

After the assessment questions have been defined and the conceptual and mathematical models have been developed, it is important to compile and evaluate existing data or, if necessary, to collect new data. Depending on the exposure scenario under consideration, data on

a wide variety of exposure factors may be needed. EPA's *Exposure Factors Handbook* (U.S. EPA, 1997c) contains a large compilation of exposure data, with some analysis and recommendations. Some of these data are organized by age groups to assist with assessing such subgroups as children. See, for example, *Exposure Factors Handbook* (U.S. EPA, 1997c, Volume 1, Chapter 3). When using these existing data, it is important to evaluate the quality of the data and the extent to which the data are representative of the population under consideration. EPA's (U.S. EPA, 2000d) and OMB's (OMB 2002) guidance on information quality, as well as program-specific guidances can provide further assistance for evaluating existing data.

When existing data fail to provide an adequate surrogate for the needs of a particular assessment, it is important to collect new data. Such data collection efforts should be guided by the references listed above (e.g., *Guidance for Data Quality Assessment* and program-specific guidance). Once again, subpopulations or lifestages of concern are an important consideration in any data collection effort.

4.3.1. Adjusting Unit Risks for Highly Exposed Populations and Lifestages

Unit risk estimates that have been developed in the dose-response assessment often assumed standard adult intake rates. When an exposure assessment focuses on a population or lifestage with differential exposure, good exposure assessment practice would replace the standard intake rates with values representative of the exposed population. Small changes in exposure assessments can be approximated by using linearly proportional adjustments of exposure parameters, but a more accurate integrative analysis may require an analysis stratified by exposure duration (see Section 5.1) .

> *For example*, to adjust the drinking water unit risk for an active population that drinks 4 L/day (instead of 2 L/day), multiply the unit risk by 2.

Because children drink more water relative to their body weight than do adults (U.S. EPA, 2002d), adjustments to unit risk estimates are warranted whenever they are applied in an assessment of childhood exposure.

> *For example*, to adjust the drinking water unit risk for a 9-kg infant who drinks 1 L/day (instead of a 70-kg adult who drinks 2 L/day), multiply the unit risk by [(1 L/day) / (9 kg)] / [(2 L/day) / (70 kg)] = 3.9.

Inhalation dosimetry is employed to derive the human equivalent exposure concentrations on which inhalation unit risks, and reference concentrations, are based (U.S. EPA, 1994). As described previously (see Sections 3.1.2, 3.1.3), different dosimetry methods may be employed depending on the availability of relevant data and chemical-specific characteristics of the pollutant. Consideration of lifestage-particular physiological characteristics in the dosimetry analysis may result in a refinement to the human equivalent concentration (HEC) to insure relevance in risk assessment across lifestages, or might conceivably conclude with multiple HECs, and corresponding inhalation unit risk values (e.g., separate for childhood and adulthood).

The dose-response assessment discusses the key sources of uncertainty in estimating dosimetry, including any related to lifestage. Review of this discussion and of the dosimetric analysis performed in deriving the HEC and resultant unit risk will assist in the appropriate application of inhalation unit risk values to exposure across lifestages.

4.4. EXPOSURE CHARACTERIZATION

The exposure characterization is a technical characterization that presents the assessment results and supports the risk characterization. It provides a statement of the purpose, scope, and approach used in the assessment, identifying the exposure scenarios and population subgroups covered. It provides estimates of the magnitude, frequency, duration, and distribution of exposures among members of the exposed population as the data permit. It identifies and compares the contribution of different sources, pathways, and routes of exposure. In particular, a

qualitative discussion of the strengths and limitations (uncertainties) of the data and models are presented.

The discussion of uncertainties is a critical component of the exposure characterization. Uncertainties can arise out of problems with the conceptual and mathematical models. Uncertainties can also arise from poor data quality and data that are not quite representative of the population or scenario of interest. Consider the following examples of uncertainties.

- National data (i.e., data collected to represent the entire U.S. population) may not be representative of exposures occurring within a regional or local population.

- Use of short-term data to infer chronic, lifetime exposures should be done with caution. Use of short-term data to estimate long-term exposures has the tendency to underestimate the number of people exposed while overestimating the exposure levels experienced by those in the upper end (i.e., above the 90^{th} percentile) of the exposure distribution. For further guidance, refer to *Guidelines for Exposure Assessment* (U.S. EPA, 1992a, § 5.3.1).

- Children's behavior, including their more limited diet, may lead to relatively high but intermittent exposures. This pattern of exposure, "one that gradually declines over the developmental period and which remains relatively constant thereafter" is not accounted for in the LADD model (ILSI, 1992). Further, the physiological characteristics of children may lead to important differences in exposure. Some of these differences can be accounted for in the LADD model. For further guidance, see *Guidelines for Exposure Assessment* (U.S. EPA, 1992a, § 5.3.5.2).

Overall, the exposure characterization should provide a full description of the sources, pathways, and routes of exposure. The characterization also should include a full description of the populations assessed. In particular, highly exposed or susceptible subpopulation or lifestage should be discussed. For further guidance on the exposure characterization, consult *Guidelines*

for Exposure Assessment (U.S. EPA, 1992a), the *Policy and Guidance for Risk Characterization* (U.S. EPA, 2000b,1995) and EPA's *Rule Writer's Guide to Executive Order 13045* (especially Attachment C: Technical Support for Risk Assessors—Suggestions for Characterizing Risks to Children [U.S. EPA, 1998d]).

5. RISK CHARACTERIZATION

5.1. PURPOSE

EPA has developed general guidance on risk characterization for use in its risk assessment activities. The core of EPA's risk characterization policy (U.S. EPA, 2000b, 1995) includes the following.

> Each risk assessment prepared in support of decision making at EPA should include a risk characterization that follows the principles and reflects the values outlined in this policy. A risk characterization should be prepared in a manner that is clear, transparent, reasonable, and consistent with other risk characterizations of similar scope prepared across programs in the Agency. Further, discussion of risk in all EPA reports, presentations, decision packages, and other documents should be substantively consistent with the risk characterization. The nature of the risk characterization will depend upon the information available, the regulatory application of the risk information, and the resources (including time) available. In all cases, however, the assessment should identify and discuss all the major issues associated with determining the nature and extent of the risk and provide commentary on any constraints limiting fuller exposition.

Risk characterization should be carried out in accordance with the EPA (U.S. EPA, 2002a) and OMB (2002) information quality guidelines. EPA's risk characterization handbook (U.S. EPA, 2000b) provides detailed guidance to Agency staff. The discussion below does not attempt to duplicate this material, but it summarizes its applicability to carcinogen risk assessment.

The risk characterization includes a summary for the risk manager in a nontechnical discussion that minimizes the use of technical terms. It is an appraisal of the science that informs the risk manager in public health decisions, as do other decision-making analyses of economic,

social, or technology issues. It also serves the needs of other interested readers. The summary is an information resource for preparing risk communication information, but being somewhat more technical than desired for communication with the general public, is not itself the usual vehicle for communication with every audience.

The risk characterization also brings together the assessments of hazard, dose response, and exposure to make risk estimates for the exposure scenarios of interest. This analysis that follows the summary is generally much more extensive. It typically will identify exposure scenarios of interest in decision making and present risk analyses associated with them. Some of the analyses may concern scenarios in several media; others may examine, for example, only drinking water risks. As these cancer guidelines allow different hazard characterizations and different potencies for specified conditions, e.g., exposure level, route of exposure, or lifestage, some of the integrative analyses may need to be stratified to accommodate the appropriate combinations of parameters across relevant exposure durations.

In constructing high end estimates of risk, the assessor should bear in mind that the high-end risk is a plausible estimate of the risk for those persons at the upper end of the risk distribution (U.S. EPA, 1992a). The intent of this approach is to convey an estimate of risk in the upper range of the distribution, but to avoid estimates that are beyond the true distribution. Overly conservative assumptions, when combined, can lead to unrealistic estimates of risk. This means that when constructing estimates from a series of factors (e.g., emissions, exposure, and unit risk estimates) not all factors should be set to values that maximize exposure, dose, or effect, since this will almost always lead to an estimate that is above the 99th-percentile confidence level and may be of limited use to decisionmakers. This is particularly problematic when using unbounded lognormal factor distributions.

While it is an appropriate aim to assure protection of health and the environment in the face of scientific uncertainty, common sense, reasonable applications of assumptions and policy, and transparency are essential to avoid unrealistically high estimates. It is also important to inform risk managers of the final distribution of risk estimates (U.S. EPA, 2000b; 1995). Otherwise, risk management decisions may be made on varying levels of conservatism, leading

to misplaced risk priorities and potentially higher overall risks. (Nichols and Zeckhauser,1986; Zeckhauser and Viscusi,1990).

The risk characterization presents an integrated and balanced picture of the analysis of the hazard, dose-response, and exposure. The risk analyst should provide summaries of the evidence and results and describe the quality of available data and the degree of confidence to be placed in the risk estimates. Important features include the constraints of available data and the state of knowledge, significant scientific issues, and significant science and science policy choices that were made when alternative interpretations of data exist (U.S. EPA, 1995, 2000b). Choices made about using data or default options in the assessment are explicitly discussed in the course of analysis, and if a choice is a significant issue, it is highlighted in the summary. In situations where there are alternative approaches for a risk assessment that have significant biological support, the decisionmaker can be informed by the presentation of these alternatives along with their strengths and uncertainties.

5.2. APPLICATION

Risk characterization is a necessary part of generating any Agency report on risk, whether the report is preliminary — to support allocation of resources toward further study — or comprehensive — to support regulatory decisions. In the former case, the detail and sophistication of the characterization are appropriately small in scale; in the latter case, appropriately extensive. Even if a document covers only parts of a risk assessment (hazard and dose-response analyses, for instance), the results of these are characterized.

Risk assessment is an iterative process that grows in depth and scope in stages from screening for priority making to preliminary estimation to fuller examination in support of complex regulatory decision making. Default options may be used at any stage, but they are predominant at screening stages and are used less as more data are gathered and incorporated at later stages. Various provisions in EPA-administered statutes require decisions based on differing findings for which differing degrees of analysis are appropriate. There are close to 30 provisions within the major statutes that require decisions based on risk, hazard, or exposure assessment. For example, Agency review of pre-manufacture notices under Section 5 of the Toxic Substances

Control Act relies on screening analyses, whereas requirements for industry testing under Section 4 of that Act rely on preliminary analyses of risk or simply of exposure. In comparison, air quality criteria under the Clean Air Act rest on a rich data collection and are required by statute to undergo periodic reassessment. There are provisions that require ranking of hazards of numerous pollutants — which may be addressed through a screening level of analysis — and other provisions for which a full assessment of risk is more appropriate.

Given this range in the scope and depth of analyses, not all risk characterizations can or should be equal in coverage or depth. The risk assessor should carefully decide which issues in a particular assessment are important to present, choosing those that are noteworthy in their impact on results. For example, health effect assessments typically rely on animal data because human data are rarely available. The objective of characterization of the use of animal data is not to recount generic issues about interpreting and using animal data; Agency guidance documents cover these issues. Rather, the objective is to highlight any significant issues that arose within the particular assessment being characterized and inform the reader about significant uncertainties that affect conclusions.

5.3. PRESENTATION OF THE RISK CHARACTERIZATION SUMMARY

The presentation is a nontechnical discussion of important conclusions, issues, and uncertainties that uses the hazard, dose response, exposure, and integrative analyses for technical support. The primary technical supports within the risk assessment are the hazard characterization, dose-response characterization, and exposure characterization described in these cancer guidelines. The risk characterization is derived from these. The presentation should fulfill the aims outlined in the purpose section above.

5.4. CONTENT OF THE RISK CHARACTERIZATION SUMMARY

Specific guidance on hazard, dose-response, and exposure characterization appears in previous sections. Overall, the risk characterization routinely includes the following, capturing the important items covered in hazard, dose response, and exposure characterization:

- primary conclusions about hazard, dose response, and exposure, including alternatives with significant biological support;

- nature of key supporting information and analytic methods;

- risk estimates and their attendant uncertainties, including key uses of default options when data are missing or uncertain.

  — With linear extrapolations, risk below the POD is typically approximated by multiplying the slope factor by an estimate of exposure, i.e., Risk = Slope Factor x Exposure. For exposure levels above the POD, the dose-response model is used instead of this approximation.

  — With nonlinear extrapolations, the method of risk assessment depends on the procedure used. If a nonlinear dose-response function has been determined, it can be used with the expected exposure to estimate a risk. If an RfD or RfC was calculated, the hazard can be expressed as a *hazard quotient* (HQ), defined as the ratio of an exposure estimate over the reference dose (RfD) or reference concentration (RfC), i.e., HQ = Exposure / (RfD or RfC). From the hazard quotient, it can generally be inferred whether the nonlinear mode of action is relevant at the environmental exposure level in question;

- statement of the extent of extrapolation of risk estimates from observed data to exposure levels of interest and its implications for certainty or uncertainty in quantifying risk. The extent of extrapolation can be expressed as a *margin of exposure* (MOE), defined as the ratio of the POD over an exposure estimate (MOE = POD / Exposure);

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- significant strengths and limitations of the data and analyses, including any major peer review issues;

- appropriate comparison with similar EPA risk analyses or common risks with which people may be familiar; and

- comparison with all appropriate assessments of the same problem by others.

It is often difficult to know *a priori* when or how different results of a cancer risk assessment are likely to be used by Agency economists, policy analysts, and decisionmakers, so it is important that the resulting characterizations include the necessary information for these analyses to the extent practicable. OMB and EPA guidelines for benefit-cost analysis require expected or central estimates of risk and information on the uncertainty of the estimate when it is possible or practicable. The extent of the uncertainty information needed for analysis depends, in part, on the scale of the policy being considered, with formal quantitative analysis of uncertainty being required in some cases.[6] OMB Circular A-4 (OMB, 2003) emphasizes that agencies "should try to provide some estimate of the probability distribution of regulatory benefits and costs." These OMB guidelines note, "Whenever it is possible to characterize quantitatively the probability distribution, some estimates of expected value ... must be provided in addition to ranges, variances, specified low-end and high-end percentile estimates, and other characteristics of the distribution." The risk characterization should therefore include, where practicable, expected or central estimates of risk, as well as upper and lower bounds, e.g., confidence limits, based on the POD, if not a full characterization of uncertainty of the risk. As discussed in EPA's *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency* (Appendix B), statutory mandates, such as the Safe Drinking Water Act, the Food Quality Protection Act, and the Clean

6 Specifically, OMB guidelines state: "For rules that exceed the $1 billion annual [economic effects] threshold, a formal quantitative analysis of uncertainty is required. For rules with annual benefits and/or costs in the range from 100 million to $1 billion, you should seek to use more rigorous approaches with higher consequence rules" (OMB, 2003, page 158)

Air Act, call for the Agency to generate specific kinds of risk information, and thus these updated cancer assessment guidelines should be read in conjunction with the Agency's statutory mandates regarding risk assessment.

APPENDIX A: MAJOR DEFAULT OPTIONS

This discussion covers the major default options commonly employed when data are missing or sufficiently uncertain in a cancer risk assessment, as adopted in these cancer guidelines. These options are predominantly inferences that help use the data observed under empirical conditions in order to estimate events and outcomes under environmental conditions. Several inferential issues arise when effects seen in a subpopulation of humans or animals are used to infer potential effects in the population of environmentally exposed humans. Several more inferential issues arise in extrapolating the exposure-effect relationship observed empirically to lower-exposure environmental conditions. The following issues cover the major default areas.

- Is the presence or absence of effects observed in a human population predictive of effects in another exposed human population?

- Is the presence or absence of effects observed in an animal population predictive of effects in exposed humans?

- How do metabolic pathways relate across species and among different age groups and between sexes in humans?

- How do toxicokinetic processes relate across species and among different age groups and between sexes in humans?

- What is the relationship between the observed dose-response relationship to the relationship at lower doses?

Is the Presence or Absence of Effects Observed in a Human Population Predictive of Effects in Another Exposed Human Population?

When cancer effects in exposed humans are attributed to exposure to an agent, the default option is that the resulting data are predictive of cancer in any other exposed human population. Most studies investigating cancer outcomes in humans from exposure to agents are often studies of occupationally exposed humans. By sex, age, and general health, workers may not be representative of the general population exposed environmentally to the same agents. In such studies there is no opportunity to observe subpopulations who are likely to be under represented, such as fetuses, infants and children, women, or people in poor health, who may respond differently from healthy workers. Therefore, it is understood that this option could still underestimate the response of certain human subpopulations (NRC, 1993b, 1994).

When cancer effects are not found in an exposed human population, this information by itself is not generally sufficient to conclude that the agent poses no carcinogenic hazard to this or other populations of potentially exposed humans, including susceptible subpopulations or lifestages. This is because epidemiologic studies often have low power to detect and attribute responses and typically evaluate cancer potential in a restricted population (e.g., by age, healthy workers). The topic of susceptibility and variation is addressed further in the discussion below of quantitative default options about dose-response relationships. Well-conducted studies that fail to detect a statistically significant positive association, however, may have value and should be judged on their merits, including population size, duration of the study, the quality of the exposure characterization and measures of outcome, and the magnitude and duration of the exposure.

There is not yet enough knowledge to form a basis for any generally applicable qualitative or quantitative inference to compensate for the gap in knowledge concerning other populations. In these cancer guidelines, this problem is left to analysis in individual cases, to be attended to with further general guidance as future research and information allow. When information on a susceptible subpopulation or lifestage exists, it will be used. For example, an agent such as diethylstilbestrol (DES) causes a rare form of vaginal cancer (clear-cell adenocarcinoma) (Herbst

et al., 1971) in about 1 per 1000 of adult women whose mothers were exposed during pregnancy (Hatch et al., 1998).

Is the Presence or Absence of Effects Observed in an Animal Population Predictive of Effects in Exposed Humans? *The default option is that positive effects in animal cancer studies indicate that the agent under study can have carcinogenic potential in humans.* Thus, if no adequate human or mode of action data are present, positive effects in animal cancer studies are a basis for assessing the carcinogenic hazard to humans. This option is a public health-protective policy, and it is both appropriate and necessary, given that we do not test for carcinogenicity in humans. The option is supported by the fact that nearly all of the agents known to cause cancer in humans are carcinogenic in animals in tests that have adequate protocols (IARC, 1994; Tomatis et al., 1989; Huff, 1994). Moreover, almost one-third of human carcinogens were identified subsequent to animal testing (Huff, 1993). Further support is provided by research on the molecular biology of cancer processes, which has shown that the mechanisms of control of cell growth and differentiation are remarkably homologous among species and highly conserved in evolution. Nevertheless, the same research tools that have enabled recognition of the nature and commonality of cancer processes at the molecular level also have the power to reveal differences and instances in which animal responses are not relevant to humans (Lijinsky, 1993; U.S. EPA, 1991b). Under these cancer guidelines, available mode of action information is studied for its implications in both hazard and dose-response assessment and its ability to obviate default options.

There may be instances in which the use of an animal model would identify a hazard in animals that is not truly a hazard in humans (e.g., the alpha-2u-globulin association with renal neoplasia in male rats [U.S. EPA, 1991b]). The extent to which animal studies may yield false positive indications for humans is a matter of scientific debate. To demonstrate that a response in animals is not relevant to any human situation, adequate data to assess the relevancy issue are important.

In general, while effects seen at the highest dose tested are assumed to be appropriate for assessment, it is necessary that the experimental conditions be scrutinized. Animal studies

are conducted at high doses in order to provide statistical power, the highest dose being one that is minimally toxic (maximum tolerated dose or MTD). Consequently, the question often arises of whether a carcinogenic effect at the highest dose may be a consequence of cell killing with compensatory cell replication or of general physiological disruption rather than inherent carcinogenicity of the tested agent. There is little doubt that this may happen in some cases, but skepticism exists among some scientists that it is a pervasive problem (Ames and Gold, 1990; Melnick et al., 1993; Barrett, 1993). If adequate data demonstrate that the effects are solely the result of excessive toxicity rather than carcinogenicity of the tested agent *per se*, then the effects may be regarded as not appropriate to include in assessment of the potential for human carcinogenicity of the agent. This is a matter of expert judgment, with consideration given to all of the data available about the agent, including effects in other toxicity studies, structure-activity relationships, and effects on growth control and differentiation.

When cancer effects are not found in well-conducted animal cancer studies in two or more appropriate species and other information does not support the carcinogenic potential of the agent, these data provide a basis for concluding that the agent is not likely to possess human carcinogenic potential, in the absence of human data to the contrary. This default option about lack of cancer effects has limitations. It is recognized that animal studies (and epidemiologic studies as well) have very low power to detect cancer effects. Detection of a 10% tumor incidence is generally the limit of power with standard protocols for animal studies (with the exception of rare tumors that are virtually markers for a particular agent, e.g., angiosarcoma caused by vinyl chloride). In some situations, the tested animal species may not be predictive of effects in humans; for example, arsenic shows only minimal or no effect in animals, whereas it is clearly positive in humans. Therefore, it is important to consider other information as well; absence of mutagenic activity or absence of carcinogenic activity among structural analogues can increase the confidence that negative results in animal studies indicate a lack of human hazard.

Another limitation is that standard animal study protocols are not yet available for effectively studying perinatal effects. The potential for effects on the very young generally should be considered separately. Under existing Agency policy (U.S. EPA, 1997a, b), perinatal studies

accomplished by modification of existing adult bioassay protocols are important in special circumstances.

Target organ concordance is not a prerequisite for evaluating the implications of animal study results for humans. Target organs of carcinogenesis for agents that cause cancer in both animals and humans are most often concordant at one or more sites (Tomatis et al., 1989; Huff, 1994). However, concordance by site is not uniform. The mechanisms of control of cell growth and differentiation are concordant among species, but there are marked differences among species in the way control is managed in various tissues. For example, in humans, mutations of the tumor suppressor genes p53 and retinoblastoma are frequently observed genetic changes in tumors. These tumor-suppressor genes are also observed to be operating in some rodent tissues, but other growth control mechanisms predominate in other rodent tissues. Thus, an animal response may be due to changes in a control that are relevant to humans but appear in animals in a different way.

However, it is appropriate under these cancer guidelines to consider the influences of route of exposure, metabolism, and, particularly, some modes of action that may either support or not support target organ concordance between animals and humans. When data allow, these influences are considered in deciding whether agent-, species-, or organ-specific situations are appropriate to use in preference to this default assumption (NRC, 1994). In contrast, use of toxicokinetic modeling inherently assumes site concordance, as these models are used to estimate delivered dose to a particular tissue or organ in humans on the basis of the same tissue or organ from animal data.

The default is to include benign tumors observed in animal studies in the assessment of animal tumor incidence, if such tumors have the capacity to progress to the malignancies with which they are associated. This default is consistent with the approach of the National Toxicology Program and the International Agency for Research on Cancer and is more protective of public health than not including benign tumors in the assessment; benign and malignant tumors are treated as representative of related responses to the test agent (McConnell et al., 1986), which is scientifically appropriate. Nonetheless, in assessing findings from animal studies, a greater proportion of malignancy is weighed more heavily than is a response with a

greater proportion of benign tumors. Greater frequency of malignancy of a particular tumor type in comparison with other tumor responses observed in an animal study is also a factor to be considered in selecting the response to be used in dose-response assessment.

Benign tumors that are not observed to progress to malignancy are assessed on a case-by-case basis. There is a range of possibilities for the overall significance of benign tumors. They may deserve attention because they are serious health problems even though they are not malignant; for instance, benign tumors may be a health risk because of their effect on the function of a target tissue, such as the brain. They may be significant indicators of the need for further testing of an agent if they are observed in a short-term test protocol, or such an observation may add to the overall weight of evidence if the same agent causes malignancies in a long-term study. Knowledge of the mode of action associated with a benign tumor response may aid in the interpretation of other tumor responses associated with the same agent.

How Do Metabolic Pathways Relate Across Species and Among Different Age Groups and Between Sexes in Humans?

The default option is that there is a similarity of the basic pathways of metabolism and the occurrence of metabolites in tissues in regard to the species-to-species extrapolation of cancer hazard and risk. If comparative metabolism studies were to show no similarity between the tested species and humans and a metabolite(s) was the active form, there would be less support for an inference that the animal response(s) relates to humans. In other cases, parameters of metabolism may vary quantitatively between species; this becomes a factor in deciding on an appropriate human-equivalent dose based on animal studies, optimally in the context of a toxicokinetic model. Although the basic pathways are assumed to be the same among humans, the presence of polymorphisms in the general population and factors such as the maturation of the pathways in infants should be considered. The active form of an agent may be present to differing degrees, or it may be completely absent, which may result in greater or lesser risk for subpopulations.

How Do Toxicokinetic Processes Relate Across Species and Among Different Age Groups and Between Sexes in Humans?

A major issue is how to estimate human-equivalent doses in extrapolating from animal studies. *As a default for oral exposure, a human equivalent dose for adults is estimated from data on another species by an adjustment of animal applied oral dose by a scaling factor based on body weight to the 3/4 power. The same factor is used for children because it is slightly more protective than using children's body weight (see Section 3.1.3).* This adjustment factor is used because it represents scaling of metabolic rate across animals of different size. Because the factor adjusts for a parameter that can be improved on and brought into more sophisticated toxicokinetic modeling when such data become available, they are usually preferable to the default option.

For inhalation exposure, a human equivalent dose for adults is estimated by default methodologies that provide estimates of lung deposition and internal dose (U.S. EPA, 1994). The methodologies can be refined to more sophisticated forms with data on toxicokinetic and metabolic parameters of the specific agent. This default option, like the one for oral exposure, is selected in part because it lays a foundation for incorporating better data. The use of information to improve dose estimation from applied to internal to delivered dose is encouraged, including use of toxicokinetic modeling instead of any default, where data are available.

There are important differences between infants, adults, and older adults in the processes of absorption, distribution, and elimination; for example, infants tend to absorb metals through the gut more rapidly and more efficiently than do older children or adults (Calabrese, 1986). Renal elimination is also not as efficient in infants. Although these processes reach adult competency at about the time of weaning, they may have important implications, particularly when the dose-response relationship for an agent is considered to be nonlinear and there is an exposure scenario disproportionately affecting infants, because in these cases the magnitude of dose is more pertinent than the usual approach in linear extrapolation of averaging dose across a lifetime. Efficiency of intestinal absorption in older adults tends to be generally less overall for most chemicals. Another notable difference is that, post-weaning (about 1 year), children have a

higher metabolic rate than do adults (Renwick, 1998), and they may toxify or detoxify agents at a correspondingly higher rate.

For a route-to-route exposure extrapolation, *the default option is that an agent that causes internal tumors by one route of exposure will be carcinogenic by another route if it is absorbed by the second route to give an internal dose.* This is a qualitative option and is considered to be public-health protective. The rationale is that for internal tumors an internal dose is significant no matter what the route of exposure. Additionally, the metabolism of the agent will be qualitatively the same for an internal dose. The issue of quantitative extrapolation of the dose-response relationship from one route to another is addressed case by case. Quantitative extrapolation is complicated by considerations such as first-pass metabolism.

What Is the Correlation of the Observed Dose-Response Relationship to the Relationship at Lower Doses?

If sufficient data are available, a biologically based model for both the observed range and extrapolation below that range may be used. Although no standard biologically based models are in existence, an agent-specific model may be developed if extensive data exist in a particular case and the purpose of the assessment justifies the investment of the resources needed. *The default procedure for the observed range of data when a biologically based model is not used is to use a curve-fitting model for incidence data.*

In the absence of data supporting a biologically based model for extrapolation outside of the observed range, the choice of approach is based on the view of mode of action of the agent arrived at in the hazard assessment. If more than one approach (e.g., both a nonlinear and linear approach) are supported by the data, they should be used and presented to the decisionmaker.

A linear extrapolation approach is used when the mode of action information is supportive of linearity or mode of action is not understood. The linear approach is used when a view of the mode of action indicates a linear response, for example, when a conclusion is made that an agent directly causes alterations in DNA, a kind of interaction that not only theoretically requires one reaction but also is likely to be additive to ongoing, spontaneous gene mutation. Other kinds of activity may have linear implications, for example, linear rate-limiting steps

would also support a linear procedure. The linear approach is to draw a straight line between a point of departure from observed data, generally as a default, an LED chosen to be representative of the lower end of the observed range, and the origin (zero incremental dose, zero incremental response). This approach is generally considered to be public-health protective.

The linear default is thought to generally provide an upper-bound calculation of potential risk at low doses, for example, a 1/100,000 to 1/1,000,000 risk. This upper bound is thought to be public-health protective at low doses for the range of human variation, considering the typical Agency target range for risk management of 1/1,000,000 to 1/10,000, although it may not completely be so (Bois et al., 1995) if pre-existing disease or genetic constitution place a percentage of the population at greater risk from exposure to carcinogens. The question of what may be the actual variation in human susceptibility is one that was discussed in general in the NRC (1994) report, as well as the NRC report on pesticides in children and infants (NRC, 1993b). NRC has recommended research on the question, and EPA and other agencies are conducting such research. Given the current state of knowledge, EPA will assume that the linear default procedure adequately accounts for human variation unless there is case-specific information for a given agent or mode of action that indicates a particularly susceptible subpopulation or lifestage, in which case the special information will be used.

When adequate data on mode of action provide sufficient evidence to support a nonlinear mode of action for the general population and/or any subpopulations of concern, a different approach — a reference dose/reference concentration that assumes that nonlinearity – is used. The POD is again generally an BMDL when incidence data are modeled. A sufficient basis to support this nonlinear procedure is likely to include data on responses that are key events integral to the carcinogenic process. This means that the POD may be based on these precursor response data, for example, hormone levels or mitogenic effects rather than tumor incidence data.

When the mode of action information indicates that the dose-response function may be adequately described by both a linear and a nonlinear approach, then the results of both the linear and the nonlinear analyses are presented. An assessment may use both linear and nonlinear approaches if different responses are thought to result from different modes of action or a response appears to be very different at high and low doses due to influence of separate

modes of action. The results may be needed for assessment of combined risk from agents that have common modes of action.

Absent data to the contrary, the default assumption is that the cumulative dose received over a lifetime, expressed as a lifetime average daily dose or lifetime average daily exposure, is an appropriate measure of dose or exposure. This assumes that a high dose of such an agent received over a shorter period of time is equivalent to a low dose spread over a lifetime. This is thought to be a relatively public-health-protective option and has some empirical support (Monro, 1992). A counter example, i.e., effects of short-term, high exposure levels that result in subsequent cancer development, is treatment of cancer patients with certain chemotherapeutic agents. When sufficient information is available to support a different approach, it can be used. For example, short-term exposure estimates (several days to several months) may be more appropriate than the lifetime average daily dose. In these cases, both agent concentration and duration are likely to be important, because such effects may be reversible at cessation of very short-term exposures.

APPENDIX B: EPA's GUIDANCE FOR DATA QUALITY ASSESSMENT

U.S. EPA (U.S. Environmental Protection Agency). (2000d) Guidance for data quality assessment: practical methods for data analysis. Office of Environmental Information, Washington, DC. EPA/600/R-96/084. Available from: http://www.epa.gov/quality/qs-docs/g9-final.pdf.

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Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens

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PREFACE

U.S. Environmental Protection Agency (EPA or the Agency) cancer risk assessments may be conducted differently than envisioned in this Supplemental Guidance for many reasons including, for example, new information, new scientific understanding, or different science policy judgment. The practice of risk assessment with respect to accounting for early-life exposures to toxicants continues to develop, and specific components of this Supplemental Guidance may become outdated or may otherwise require modification in individual settings. It is EPA's intent to use, to the extent practicable and consistent with Agency statutes and regulations, the best available science in its risk assessments and regulatory actions, and this Supplemental Guidance is not intended to provide any substantive or procedural obstacle in achieving that goal. Therefore, the Supplemental Guidance has no binding effect on EPA or on any regulated entity. Where EPA does use the approaches in the Supplemental Guidance in developing risk assessments, it will be because EPA has decided in the context of that risk assessment that the approaches from the Supplemental Guidance are suitable and appropriate. This judgment will be tested through peer review, and the risk assessment will be modified to use different approaches if appropriate.

This Supplemental Guidance is intended for guidance only. It does not establish any substantive "rules" under the Administrative Procedure Act or any other law and has no binding effect on EPA or any regulated entity, but instead represents a non-binding statement of policy.

The Supplemental Guidance addresses a number of issues pertaining to cancer risks associated with early-life exposures generally, but provides specific guidance on potency adjustment only for carcinogens acting through a mutagenic mode of action. This guidance recommends for such chemicals, a default approach using estimates from chronic studies (i.e., cancer slope factors) with appropriate modifications to address the potential for differential risk of early-lifestage exposure. Default adjustment factors are meant to be used only when no chemical-specific data are available to assess directly cancer susceptibility from early-life exposure to a carcinogen acting through a mutagenic mode of action.

The Agency considered both the advantages and disadvantages of extending the recommended, age dependent adjustment factors for carcinogenic potency to carcinogenic agents for which the mode of action remains unknown. EPA recommends these factors only for carcinogens acting through a mutagenic mode of action based on a combination of analysis of available data and long-standing science policy positions that set out the Agency's overall approach to carcinogen risk assessment, e.g., the use of a linear, no threshold extrapolation procedure in the absence of data in order to be health protective. In general, the Agency prefers

to rely on analyses of data rather than on general defaults. When data are available for a susceptible lifestage, they should be used directly to evaluate risks for that chemical and that lifestage on a case-by-case basis. In the case of nonmutagenic carcinogens, when the mode of action is unknown, the data were judged by EPA to be too limited and the modes of action too diverse to use this as a category for which a general default adjustment factor approach can be applied. In this situation per the Agency's *Guidelines for Carcinogen Risk Assessment*, a linear low-dose extrapolation methodology is recommended. It is the Agency's long-standing science policy position that use of the linear low-dose extrapolation approach (without further adjustment) provides adequate public health conservatism in the absence of chemical-specific data indicating differential early-life susceptibility or when the mode of action is not mutagenicity.

The Agency expects to produce additional supplemental guidance for other modes of action, as data from new research and toxicity testing indicate it is warranted. EPA intends to focus its research, and to work collaboratively with its federal partners, to improve understanding of the implications of early life exposure to carcinogens. Development of guidance for estrogenic agents and chemicals acting through other processes resulting in endocrine disruption and subsequent carcinogenesis, for example, might be a reasonable priority in light of the human experience with diethylstilbesterol and the existing early-life animal studies. It is worth noting that each mode of action for endocrine disruption will probably require separate analysis.

As the Agency examines additional carcinogenic agents, the age groupings may differ from those recommended for assessing cancer risks from early-life exposure to chemicals with a mutagenic mode of action. Puberty and its associated biological changes, for example, involve many biological processes that could lead to changes in susceptibility to the effects of some carcinogens, depending on their mode of action. The Agency is interested in identifying lifestages that may be particularly sensitive or refractory for carcinogenesis, and believes that the mode of action framework described in the Agency's *Guidelines for Carcinogen Risk Assessment* is an appropriate mechanism for elucidating these lifestages. For each additional mode of action evaluated, the various age groupings determined to be at differential risk may differ from those described in this Supplemental Guidance. For example, the age groupings selected for the age-dependent adjustments were initially selected based on the available data, i.e., for the laboratory animal age range representative of birth to < 2 years in humans. More limited data and information on human biology are being used to determine a science-informed policy regarding 2 to < 16 years. Data were not available to refine the latter age group. If more data become available regarding carcinogens with a mutagenic mode of action, consideration may be given to further refinement of these age groups.

Access to data and other information relating to the Cancer Guidelines (U.S. EPA, 2005) and this Supplemental Guidance will be through EPA's Risk Assessment Forum website, under Publications, Guidelines, Guidelines for Cancer Risk Assessment. The URL is http://www.epa.gov/cancerguidelines. The data and results of analyses are available in spreadsheets.

1. INTRODUCTION

Cancer risk to children in the context of the U.S. Environmental Protection Agency's cancer guidelines (U.S. EPA, 2005) includes both early-life exposures that may result in the occurrence of cancer during childhood and early-life exposures that may contribute to cancers later in life. The National Research Council (NRC, 1994) recommended that "EPA should assess risks to infants and children whenever it appears that their risks might be greater than those of adults." This document focuses on cancer risks from early-life exposure compared with those from exposures occurring later in life. Evaluating childhood cancer and childhood exposures resulting in cancer later in life are related, but separable, issues.

Historically, the focus on cancer has been as a disease associated with aging, resulting from extended exposure duration with prolonged latency periods before the cancers appear. Because much of cancer epidemiology addresses occupational exposures and because rodent cancer studies are designed to last approximately a lifetime (two years) beginning after sexual maturity, the cancer database used by EPA and other agencies for risk assessment focuses on adults. However, extensive literature demonstrates that exposures early in life (i.e., transplacental or *in utero*, early postnatal, lactational) in animals can result in the development of cancer (reviewed in Toth, 1968; Della Porta and Terracini, 1969; Druckery, 1973; Rice, 1979; Vesselinovitch et al., 1979; Rice and Ward, 1982; Vesselovitch et al., 1983; Anderson et al., 2000). Thus, one element in extending analyses to children is to evaluate the extent to which exposures early in life would alter the incidence of cancers observed later in life, compared with the incidence observed with adult-only exposures (Anderson et al., 2000; NRC, 1993).

The causes of cancer encompass a variety of possible risk factors, including genetic predisposition (Tomlinson et al., 1997), diet, lifestyle, associations with congenital malformations (Bosland, 1996), and exposure to biological and physical agents and chemicals in the environment. In some cases, tumors in adults and children have been compared (Anderson et al., 2000; Ginsberg et al., 2002). Children and adults generally develop the same spectrum of tumors when they have inherited gene and chromosomal mutations, such as Li-Fraumeni syndrome (Birch et al., 1998). With ionizing radiation, which operates through a mutagenic mode of action, both the young and the old develop many of the same tumors, with the difference being that children are more susceptible for a number of tumor types (NRC, 1990; U.S. EPA, 1994; UNSCEAR, 2000). Studies with anticancer drugs (cytotoxic and immunosuppressive) demonstrate a similar spectrum of tumors (Hale et al., 1999; Kushner et al., 1998; Larson et al., 1996; Nyandoto et al., 1998). Various viral infections, such as Epstein Barr and hepatitis B, lead to lymphoma and liver cancer, respectively, in both age groups (Lindahl et

al., 1974; Mahoney, 1999). These observations in humans indicate that the mode of action for these agents would be the same or similar for adults and children.

Although there are similarities between childhood and adult tumors, significant differences are also known to exist (Grufferman, 1998; Israel, 1995). Tumors of childhood generally consist more of embryonic cell tumors, while adults have more carcinomas. Leukemias, brain and other nervous system tumors, lymphomas (lymph node cancers), bone cancers, soft tissue sarcomas, kidney cancers, eye cancers, and adrenal gland cancers are the most common cancers of children, while skin, prostate, breast, lung, and colorectal cancers are the most common in adults (Ries et al., 1999; U. S. Cancer Statistics Working Group, 2002). Some tumors are unique to the young, including several with well established genetic bases, such as tumors of the kidney (Wilms' tumor) or eye (retinoblastoma) (Anderson et al., 2000; Israel, 1995).

The relative rarity in the incidence of childhood cancers and a lack of animal testing guidelines with perinatal[1] exposure impede a full assessment of children's cancer risks from exposure to chemicals in the environment. Unequivocal evidence of childhood cancer in humans occurring from chemical exposures is limited (Anderson et al., 2000). Established risk factors for the development of childhood cancer include radiation and certain pharmaceutical agents used in chemotherapy (Reise, 1999). There is some evidence in humans for adult tumors resulting from perinatal exposure. Pharmacological use of diethylstilbesterol (DES) during pregnancy to prevent miscarriages induced clear cell adenocarcinoma of the vagina in a few daughters exposed *in utero* though this tumor was not observed in exposed mothers (Hatch et al., 1998; Robboy et al., 1984; Vessey, 1989). In addition to the limited human data, there are examples of transplacental carcinogens in animal studies, such as recent studies with nickel and arsenic (Diwan et al., 1992; Waalkes et al., 2003), as well as studies suggesting that altered development can affect later susceptibility[2] to cancer induced by exposure to other chemicals (Anderson et al., 2000; Birnbaum and Fenton, 2003).

Infrequently, perinatal exposure in animals has been shown to induce tumors of different types than those observed with adult exposures. Studies with saccharin (Cohen et al., 1995; Whysner and Williams, 1996; IARC, 1999) and ascorbate (Cohen et al., 1998; Cohen et al., 1995; NTP, 1983) found cancer when exposures were initiated in the perinatal period. In

[1] Perinatal is defined as the time around birth and may include both prenatal (prior to birth) and postnatal (after birth) periods.

[2] Susceptibility is defined here as an increased likelihood of an adverse effect, often discussed in terms of relationship to a factor that can be used to describe a human subpopulation (e.g., lifestage, demographic feature, or genetic characteristic). The terms "susceptibility" and "sensitivity" are used with a variety of definitions in published literature making it essential that readers are aware of these differences in terminology across documents.

contrast, studies submitted to the Food and Drug Administration of approximately a dozen other food additives and colorings that were not adult carcinogens did not indicate cancer, even when perinatal exposures occurred (U.S. EPA, 1996). When observed, the differences between childhood and adult cancers suggest the importance of evaluating the impacts of maternal exposures during pregnancy as well as exposures to children (Anderson et al., 2000). The effects of maternal exposures and transplacental carcinogens require separate evaluation and are not quantitatively evaluated in the analysis presented below.

The limited human information described briefly above is supported by a number of animal bioassays that include both perinatal and adult exposures to chemicals. Standard animal bioassays generally begin dosing after the animals are 6-8 weeks old, when many organs and systems are almost fully developed, though substantial growth in body size continues thereafter (as more fully discussed in Hattis et al., 2005). The literature can be divided roughly into three types of exposure scenarios: those that include *repeated* exposures for the early postnatal to juvenile period, as compared with chronic later-life dosing; *lifetime* (i.e., combined perinatal and adult) exposure as compared with chronic later-life dosing; and those that include more *acute* exposures, such as a single intraperitoneal (ip) or subcutaneous injection, for both early-life and later-life dosing. In the early-life exposure studies that are available, perinatal exposure usually induces higher incidence of tumors later in life than the incidence seen in standard bioassays where adult animals only were exposed; some examples include diethylnitrosamine (DEN) (Peto et al., 1984), benzidine (Vesselinovitch et al., 1979), DDT (Vesselinovitch et al., 1979), and polybrominated biphenyls (PCBs) (Chhabra et al., 1993a). Reviews comparing early-life carcinogenesis bioassays with standard bioassays for a limited number of chemicals (McConnell, 1992; Miller et al., 2002; U.S. EPA, 1996) have concluded:

- The same tumor sites usually are observed following either perinatal or adult exposure.
- Perinatal exposure in conjunction with adult exposure usually increases the incidence of tumor bearing animals or reduces the latent period before tumors are observed.

There is limited evidence to inform the mode(s) of action leading to differences in tumor type and tumor incidence following early-life exposure and exposure later in life. Differences in the capacity to metabolize and clear chemicals at different ages can result in larger or smaller internal doses of the active agent(s), either increasing or decreasing risk (Ginsberg et al., 2002; Renwick, 1998). There is reason to surmise that some chemicals with a mutagenic mode of action, which would be expected to cause irreversible changes to DNA, would exhibit a greater effect in early-life versus later-life exposure. Several studies have shown increased susceptibility

of weanling animals to the formation of DNA adducts following exposure to vinyl chloride (Laib et al., 1989; Morinello et al., 2002a; Morinello et al., 2002b). Additionally, even though not used quantitatively in the analyses in this document, a recent analysis of *in vivo* transplacental micronucleus assays indicated that fetal tissues generally are more sensitive than maternal tissues for induction of micronuclei from mutagenic chemicals (Hayashi et al., 2000), providing qualitative support for the early-life susceptibility. Similarly, the neonatal mouse model for carcinogenesis, which uses two doses prior to weaning followed by observation of tumors at one year, shows carcinogenic responses for mutagenic agents (Flammang et al., 1997; McClain et al., 2001). These results are consistent with the current understanding of biological processes involved in carcinogenesis, which leads to a reasonable expectation that children can be more susceptible to carcinogenic agents than adults (Anderson et al., 2000; Birnbaum and Fenton, 2003; Ginsberg, 2003; Miller et al., 2002; Scheuplein et al., 2002). Some aspects potentially leading to childhood susceptibility include the following issues.

- More frequent cell division during development can result in enhanced fixation of mutations due to the reduced time available for repair of DNA lesions and clonal expansion of mutant cells gives a larger population of mutants (Slikker et al, 2004).
- Some embryonic cells, such as brain cells, lack key DNA repair enzymes.
- Some components of the immune system are not fully functional during development (Holladay and Smialowicz, 2000; Holsapple et al., 2003).
- Hormonal systems operate at different levels during different lifestages (Anderson et al., 2000).
- Induction of developmental abnormalities can result in a predisposition to carcinogenic effects later in life (Anderson et al., 2000; Birnbaum and Fenton, 2003; Fenton and Davis, 2002).

The methodology that has been generally used by the U.S. EPA to estimate cancer risk associated with oral exposures relies on estimation of the lifetime average daily dose, which can account for differences between adults and children with respect to exposure factors such as eating habits and body weight. However, susceptibility differences with respect to early lifestages are not taken into consideration because cancer slope factors[3] are based upon effects

[3] Cancer slope factor – An upper bound estimate of the increased cancer risk from a lifetime exposure to an agent. This estimate, usually expressed in units of proportion (of a population) affected per unit exposure (e.g., mg/kg-day or ug/m^3), is generally reserved for use in the low-dose region of the dose-response relationship. It is often the statistical upper bound on the potency and therefore the risk. “Upper bound” in this context is a plausible

observed following exposures to adult humans or sexually mature animals. Since a much larger database exists for chemicals inducing cancer in adult humans or sexually mature animals, it is necessary to determine whether adjustment of such adult-based cancer slope factors would be appropriate when assessing cancer risks associated with exposures early in life. The analysis undertaken here addresses this issue, focusing upon studies that define the potential duration and degree of increased susceptibility that may arise from childhood, defined as early-life (typically postnatal and juvenile animal) exposures. Some of these analyses, along with a more complete description of the procedures used, have been published (Barton et al., 2005). The analysis presented in this Supplemental Guidance and in the published article form the basis for developing Supplemental Guidance for evaluating cancer susceptibility associated with early-life exposures.

upper limit to the true probability.

2. PROCEDURES

This section describes the steps taken to assess potential susceptibility to early-life exposure to carcinogenic compounds compared with adult and whole-life exposure. The readily available literature was reviewed to identify animal studies that compared tumor incidence between early-life and adult-only exposures or between early-life-and-adult and adult-only exposures. Studies were categorized by length of exposure; those studies with quantitative information to estimate tumor incidence over time for early-life and adult exposures were identified. These studies provided the basis for quantitatively estimating the difference in susceptibility between early-life and adult exposures, as described below. Finally, summaries of available human data for radiation exposure were reviewed in the context of tumor incidence from early-life versus later-in-life exposure.

2.1. DATA SOURCES FOR ANIMAL STUDIES

Studies in the literature included in this analysis are those that report tumor response from experiments that included both early-life and adult exposure as separate experimental groups. Initial studies for consideration were identified through review articles and a search of the National Toxicology Program (NTP) database. Reviews of the literature regarding cancer susceptibility from early-life exposure in animals include McConnell (1992), Ginsberg (2003), Anderson et al. (2000), Miller et al. (2002) and U.S. EPA (1996). A literature search was conducted utilizing key words and MeSH headings (Medline) from studies identified in the available reviews. The list of chemicals included in this analysis for quantitative evaluation is shown in Table 1a and 1b.

Abstracts or papers were reviewed to determine if a study provided information that could be used for quantitative analysis. The criteria used to decide if a study could be included in the quantitative analysis were:

- Exposure groups at different post-natal ages in the same study or same laboratory, if not concurrent (to control for a large number of potential cross-laboratory experimental variables including pathological examinations),

- Same strain/species (to eliminate strain-specific responses confounding age-dependent responses),

- Approximately the same dose within the limits of diets and drinking water intakes that obviously can vary with age (to eliminate dose-dependent responses confounding age-dependent responses),

- Similar latency period following exposures of different ages (to control for confounding latency period for tumor expression with age-dependent responses), arising from sacrifice at >1 year for all groups exposed at different ages, where early-life exposure can occur up to about 7 weeks. Variations of around 10 to 20% in latency period are acceptable,

- Postnatal exposure for juvenile rats and mice at ages younger than the standard 6 to 8 week start for bioassays; prenatal (*in utero*) exposures are not part of the current analysis. Studies that have postnatal exposure were included (without adjustment) even if they also involved prenatal exposure,

- "Adult" rats and mice exposure beginning at approximately 6 to 8 weeks old or older, i.e. comparable to the age at initiation of a standard cancer bioassay (McConnell, 1992). Studies with animals only at young ages do not provide appropriate comparisons to evaluate age-dependency of response (e.g., the many neonatal mouse cancer studies). Studies in other species were used a supporting evidence, because they are relatively rare and the determination of the appropriate comparison ages across species is not simple, and

- Number of affected animals and total number of animals examined are available or reasonably reconstructed for control, young, and adult groups (i.e., studies reporting only percent response or not including a control group would be excluded unless a reasonable estimate of historical background for the strain was obtainable).

Tables 2 and 3 include information on the methods and results from the animal studies identified in Table 1b. Pertinent information on species, sex, dosing regimen, and tumor incidence is given. Additionally, the "Notes" column includes general information about the relationship between tumor incidence, animal age at first dosing, and sex. The data in Tables 2 and 3 were used for the calculations, described below, for estimating potentially increased cancer risk from early-life exposure.

The available literature includes a wide range of exposure scenarios. This range is due in part to the lack of a defined protocol for early-life testing and the difficulty of standardizing and administering doses preweaning. As noted previously, the literature can be divided roughly into three types of exposure scenarios: those that include repeated exposures for the early postnatal to juvenile period, as compared with chronic later-life dosing; lifetime (i.e., combined perinatal and adult) exposure as compared with chronic later-life dosing; and those that include more acute exposures, such as a single intraperitoneal (ip) or subcutaneous injection, for both early-life and later-life dosing. Table 2 includes the studies that had early postnatal to juvenile exposures, adult chronic exposures, and lifetime exposures. Table 3 includes studies with acute exposures. A discussion of the implications of the different exposure scenarios is included in Section 3.

Studies were identified for more than 50 chemicals not included in Tables 2 and 3 that demonstrated carcinogenesis following perinatal exposure, but did not directly compare exposures at different ages. A large number of studies address *in utero* exposures only. More than 100 chemicals (with both negative and positive findings) have been studied in the neonatal mouse assay, but this assay does not have a comparable adult exposure (Flammang et al., 1997; McClain et al., 2001; Fujii, 1991). Studies across laboratories often varied in their use of animal strains (e.g., for AZT studies, Diwan et al., 1999 used CD-1 mice, while NTP, 1999 used $B6C3F_1$ mice). Studies of tamoxifen use two Wistar-derived strains and had very different periods for tumor expression, i.e., sacrifice at 20 months for adult-exposed rats and natural death up to 35 months for juvenile-exposed rats, with uterine tumors observed in animals dying after 22 months (Carthew et al., 2000; Carthew et al., 1996; Carthew et al., 1995). Due to these factors, the chemicals that belong to this group were not evaluated quantitatively. In addition, there were studies assessing radiation in animals (Covelli et al., 1984; Di et al., 1990; Sasaki et al., 1978). The radiation data were not analyzed in depth, in part because there are recognized differences in toxicokinetics and toxicodynamics between radiation and chemicals with a mutagenic mode of action for carcinogenesis. Even though the data on A-bomb survivors provide information for many different cancer sites in humans with a single exposure involving all ages, a number of national and international committees of experts have analyzed and modeled these data to develop risk estimates for various specific applications. Furthermore, lack of uniformity regarding radiation doses, gestational age at exposure, and the animal strains used make it difficult to make comparisons across studies (Preston et al., 2000).

2.2. EVALUATING THE MODE OF ACTION OF CARCINOGENS

Evaluation of the mode of action of a carcinogen was based upon a weight-of-evidence approach. Multiple modes of action are associated with the chemicals in this database, but a number are associated with mutagenicity (i.e., benzo(a)pyrene, benzidine, dibenzanthracene, diethylnitrosamine, dimethylbenz(a)anthracene, dimethylnitrosamine, ethylnitrosourea, 3-methylcholanthrene, methylnitrosourea, safrole, urethane, and vinyl chloride). Determination of carcinogens that are operating by a mutagenic mode of action entails evaluation of short-term testing results for genetic endpoints, metabolic profiles, physicochemical properties, and structure-activity relationship (SAR) analyses in a weight-of-evidence approach (Dearfield et al., 1991; U.S. EPA, 1986, 1991; Waters et al., 1999), as has been done for several chemicals (e.g., Dearfield et al., 1999; McCarroll et al., 2002; U.S. EPA, 2000a). Key data for a mutagenic mode of action may be evidence that the carcinogen or a metabolite is DNA reactive and/or has the ability to bind to DNA. Also, such carcinogens usually produce positive effects in multiple test

systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed *in vivo* which generally are supported by positive tests *in vitro*. Additionally, carcinogens may be identified as operating via a mutagenic mode of action if they have similar properties and SAR to established mutagenic mode of action.

2.3. QUANTITATIVE METHODS

To estimate the potential difference in susceptibility between early-life and adult exposure, we calculated the estimated ratio of the cancer potency from early-life exposure compared to the estimated cancer potency from adult exposure. The cancer potency was estimated from a one-hit model, or a restricted form of the Weibull model, which is commonly used to estimate cumulative incidence for tumor onset. The general form of the equation is:

P(dose) = 1-[1-P(0)]exp(-cancer potency*dose)

The ratio of juvenile to adult cancer potencies were calculated by fitting this model to the data for each age group. The model fit depended upon the design of the experiment that generated the data. Two designs should be handled separately: experiments in which animals are exposed either as juveniles or as adults (with either a single or multiple dose in each period), and experiments in which exposure begins either in the juvenile or in the adult period, but once begun, continues through life.

For the first case, the model equations are:

$$P_A = P_0 + (1 - P_0)(1 - \mathrm{e}^{-m_A \delta_A}) \qquad (1)$$
$$P_J = P_0 + (1 - P_0)\left(1 - \mathrm{e}^{-m_A \mathrm{e}^{\lambda} \delta_J}\right)$$

where:

subscripts *A* and *J* refer to the adult and juvenile period, respectively,
λ is the natural logarithm of the juvenile:adult cancer potency ratio,
P_0 is the fraction of control animals with the particular tumor type being modeled,
P_x is the fraction of animals exposed in age period *x* with the tumor,
m_A is the rate of accumulation of "hits" per unit of time for adults, i.e., the cancer potency, and
δ_x is the duration or number of exposures during age period *x*.

For a substantial number of data sets (acute exposures), $\delta_J = \delta_A = 1$. We are interested in

determining λ, which is the logarithm of the estimated ratio of juvenile to adult cancer potencies, a measure of potential susceptibility for early-life exposure.

For the second kind of design, the model equations should take into account that exposures that were initiated in the juvenile period continue through the adult period. The model equations for the fraction of animals exposed only as adults with tumors in this design are the same as in the first design, but the fraction of animals whose first exposure occurred in the juvenile period is:

$$P_J = P_0 + (1 + P_0)\left(1 - e^{-m_A e^{\lambda}(\delta_J - \delta_A) - m_A \delta_A}\right) \quad (2)$$

All symbols in (eq. 2) have the same interpretation as their counterparts in (eq. 1), but now δ_J includes the duration of exposure during the juvenile period as well as the subsequent adult period.

Parameters in these models were estimated using Bayesian methods (see, for example, Carlin and Louis, 2000), and all inferences about the ratios were based on the marginal posterior distribution of λ. Some of these analyses, including a more complete description of the procedures (including the potential effect of alternative Bayesian priors that have been examined) have been published (Barton et al., 2005). The data for estimating each ratio were in the form of numbers of animals tested and number affected for each of control, juvenile-exposed, and adult-exposed animals, and duration of exposure for each of the juvenile-exposed and adult-exposed groups. A few data sets had separate control groups for the juvenile-exposed and adult-exposed groups, and equations 1 and 2 were modified accordingly. The likelihood for the parameters in the model was the product of three (or four, if there were two control groups) binomial probabilities: for the number of animals with tumors in the control group(s), for the juvenile-exposed group, and for the adult-exposed group. The prior for P_0 (the fraction of control animals with a particular tumor) was right triangular (right angle at the origin), based on the assumption that control incidences should be relatively low. (The base of the distribution is one, as P_0 can not exceed one. As this is a probability distribution, the area of the triangle is one. Therefore, its height at the origin must be 2.) The effect of exposure in adults is quantified by the extra risk, Q, where the probability that an animal has a tumor is $P_0 + (1 - P_0)Q$. So, from equations 1, $Q = 1 - e^{-m_A \delta_A}$, Q was given a uniform prior on the interval (0,1), reflecting total ignorance about the extra risk of adult exposure. Finally, the prior for λ was Gaussian with mean 0 (corresponding to a median or geometric mean ratio of one) and standard deviation 3. The prior for the log ratio of juvenile to adult cancer potency has some influence over the posterior estimates for the ratio of juvenile to adult potency. The magnitude of that influence depends on

the amount of support in the data for different values of the log ratio. The prior also effectively downweights extremely large or small values for the juvenile to adult potency ratio. Three priors for the standard deviation were evaluated (Barton et al., 2005, see Appendix), with the intent of finding the largest prior, i.e., one that would contain the least informative assumption for the prior. A standard deviation of 9 was tried, but some of the intervals would not converge. A standard deviation of 3 worked well, allowed ratio estimates to be derived, with all of the data of interest. An intermediate value of 6 was also examined to ascertain if a less informative prior could be used. While the intervals converged, a sensitivity analysis showed that this value for the standard deviation resulted in sufficient down-weighting of the ratios with limited information that these data would not influence the result. This was considered an unreasonable bias, so a standard deviation of 3 was used for the further analyses. A further discussion of these analyses can be found in Barton et al. (2005).

The posterior distribution for the unknown parameters in these models is the product of the likelihood from the data and the priors (the "unnormalized" prior), divided by a normalization constant that is the integral of the unnormalized prior over the ranges of all the parameters. This normalization constant was computed using numerical integration, as were posterior means and variances and marginal posterior quantiles for the log-ratio λ. All numerical computations were carried out in the R statistical programming language (version 1.8.1; R Development Core Team, 2003).

This method produced a posterior mean ratio of the early-life to adult cancer potency, which is an estimate of the potential susceptibility of early-life exposure to carcinogens. If the ratio was greater than one, this indicated that the experiment found that there was greater susceptibility from early-life exposure. If the ratio was less than one, this indicated that the experiment found that there was less susceptibility from early-life exposure. Summaries of the individual ratios from each of the dose groups from the different experiments for different groupings were also calculated (for example for all acute exposures of chemicals that are carcinogenic by a mutagenic mode of action). The summary ratios were constructed from the individual ratios within a group, by variance-weighting the means of each ratio. The individual, posterior means were weighted by using reciprocals of their posterior variance. This weighting procedure is commonly used because it gives greater weight to those studies for which the variances, i.e., the uncertainties, are smaller. Because the ratios were calculated as log ratios (see eq. 1), exponentiating the resulting inverse-variance-weighted mean yielded inverse-variance-weighted geometric means of ratios.

2.4. IONIZING RADIATION

A supporting role was assigned to the available human radiation data, where cancer incidence in adults who were children at the time of the atomic bomb (A-bomb) exposure was compared with cancer incidence in adults who were older at the time of exposure. Although there are recognized differences in toxicokinetics and toxicodynamics between radiation and chemical carcinogens with a mutagenic mode of action, the data on A-bomb survivors provide information for many different cancer sites in humans with a single exposure involving all ages. In addition to the richness of the data, a number of national and international committees of experts have analyzed and modeled these data to develop risk estimates for various specific applications.

The report of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2000, with Scientific Annexes) lists more than 80 studies, in addition to the reports of the Japanese A-bomb survivors, in which at least one type of cancer was measured in humans who were exposed either intentionally or accidentally to some form of ionizing radiation. However only the A-bomb survivor reports have relevant information on incidence of early-life exposures. One of the more recent papers cited in the UNSCEAR report, by Thompson et al. (1994), contains detailed data on the incidence of 21 different cancers in 37,270 exposed A-bomb survivors (42,702 unexposed). Also, EPA has used data from the A-bomb survivors to develop age-specific relative risk coefficients using various methods for transporting the risk from the Japanese population to the U.S. population (U.S. EPA, 1994). It is beyond the scope of this effort to present all of the radiation data or a discussion of the various analyses and modeling efforts. Rather, information relevant to comparing cancer risks from juvenile versus adult exposure from UNSCEAR (2000) and U.S. EPA (1994; 1999) is presented as representative findings to determine whether the radiation data are similar qualitatively to the chemical findings. More detailed data on the A-bomb survivors can be found in Delongchamp et al. (1997) and Preston et al. (2000).

As previously noted, several studies have assessed radiation in animal studies (Covelli et al., 1984; Di et al., 1990; Sasaki et al., 1978). However, lack of uniformity regarding radiation doses, gestational age at exposure, and the animal strains used make it difficult to compare the experimental data on cancer induction after prenatal irradiation (Preston et al., 2000).

3. RESULTS

3.1. QUALITATIVE EVALUATION OF THE DATABASE

The question addressed in this analysis was whether, and how, available quantitative scientific data could inform risk assessment policy choices for adjusting cancer slope factors when they are used in the assessment of cancer risk from childhood exposure. Cancer slope factors are, with few exceptions, based on adult human epidemiology or standard chronic adult rodent bioassays, which do not address the impacts of early-life exposures. Thus, the critical data are either human epidemiological data on childhood exposures resulting in adult cancer or research studies with rodents involving early postnatal exposures. The major human data available are from radiation exposures (studies summarized in Tables 9-11), with very limited data available for humans exposed during childhood to chemicals (reviewed in Anderson et al., 2000; Miller et al., 2002).

A review of the literature identified several hundred references reporting more than 50 chemicals that have been shown to be able to cause cancer following perinatal exposure (Table 1a) (reviewed in Toth, 1968; Della Porta and Terracini, 1969; Druckery, 1973, Rice, 1979; Vesselinovitch et al., 1979; Rice and Ward, 1982; Vesselovitch et al.; 1983; Fujii, 1991; Anderson et al., 2000). Studies (or groups of studies from a single laboratory on a given chemical) that directly provided quantitative data on carcinogenesis following early postnatal exposures and adult exposures to chemicals in animals were identified for 18 chemicals, listed in Table 1b, 2, and 3. Of the identified studies, there were 11 chemicals involving repeated exposures during early postnatal and adult lifestages (Table 1b) and 8 chemicals using acute exposures (typically single doses) at different ages (Table 1b). Some of the studies evaluated single tissues or organs for tumors (e.g., only liver), while others evaluated multiple tissues and organs (Tables 2 and 3). Mice, rats, or both species and sometimes multiple strains were tested. These studies serve as the basis for the quantitative analyses presented later in the results.

In addition to the studies identified in Table 1b, studies were identified with early postnatal and early-life exposures that were evaluated qualitatively but not quantitatively. Some of these studies are notable and provide important supporting information. Two recent studies used transgenic mouse models for human tumors. Increased multiplicity of colon tumors was observed following earlier versus later azoxymethane exposures (Paulsen et al., 2003). Shortened mammary tumor latency following estradiol exposure occurred when exposures occurred between 8 and 18 weeks as opposed to earlier or later, which is generally consistent with the incidence results analyzed for DMBA (Yang et al., 2003). Several notable examples exist of developmental windows leading to cancer susceptibilities that were not observable in

adults. Several potent estrogenic chemicals including DES, tamoxifen, and genistein produce uterine tumors with early postnatal exposures of mice, though there also appear to be strain-dependent differences in the tumor sites in adult mice (Gass et al., 1964; Greenman et al., 1990; Newbold et al., 1990, 1997, 1998, 2001). Developmental susceptibilities are believed to play a key role in effects observed with saccharin (Cohen et al., 1995; Whysner and Williams, 1996) and ascorbate (Cohen et al., 1998; NTP, 1983), with bladder tumors arising when early-life exposures occurred. Studies with several species, including rat, mouse, and opossum, indicate that nervous systems tumors associated with exposures to ENU and several other chemicals appear to be highly dependent upon exposures occurring within certain windows, particularly prenatal ones (Rice, 1979; Rice and Ward, 1982; Jurgelski et al., 1979).

Analyses of the difference in cancer risk from exposures during different lifetime periods ideally should address both the period of potential susceptibility and the magnitude of the susceptibility. Available studies used a variety of study designs (see Tables 2 and 3), which can be valuable because they provide different information (Figure 1). However, variations in study design can result in a lack of comparability across chemicals, and can limit information on the consistency of effects with different chemicals acting through different modes of action. The acute dosing (largely single dose) studies (Table 3) are valuable because they involve identical exposures with explicitly defined doses and time periods demonstrating that differential tumor incidences arise exclusively from age-dependent susceptibility. These studies address both the period and magnitude of susceptibility. They were not as appropriate for quantitative adjustments for the cancer potency estimates because of their limitations, including that most used subcutaneous or ip injection that historically have not been considered quantitatively relevant routes of environmental exposure for human cancer risk assessment by EPA, and that these routes of exposure are expected to have only partial or a complete absence of first pass metabolism that is likely to affect potency estimates.

The repeated dosing studies with exposures during early postnatal or adult lifetime provide useful information on the relative impact of repeated exposures at different lifestages and may be more likely to have exposure occur during a window of susceptibility, if there is one. One notable difference in study designs was that studies with repeated early postnatal exposure were included in the analysis even if they also involved earlier maternal and/or prenatal exposure, while studies addressing only prenatal exposure were not otherwise a part of this analysis. Another notable difference among studies involved the tissues that were evaluated for tumors: some studies focused on a single tissue, particularly liver, while others evaluated multiple tissues.

Comparisons within a single repeated dosing study may have limitations for evaluating

differential susceptibility because exposures to the chemical can differ during the different lifestages, particularly when dietary or drinking water exposures are involved. A notable example is the PCB study (Chhabra et al., 1993a), in which mobilization of such lipid-soluble chemicals into mother's milk would be expected to result in infants receiving much larger exposures than other lifestages. While lactational transfer is just as relevant to human nursing offspring, this difference in exposure obscures the extent to which the early lifestage is quantitatively more susceptible (i.e., part of the increased early-life cancer risk arises from higher exposure than during the adult period). Maternal metabolism of compounds such as diphenylhydantoin (DPH) (Chhabra et al., 1993b) also may result in lower exposure during lactation, potentially underestimating the early-lifestage risk, if the parent compound is the active form of the chemical. Similar issues exist due to normal age-dependent changes in food and water consumption. Ascribing differential effects observed in animal studies solely to lifestage susceptibility must be done carefully as there may also be differences in the exposures. There are substantial and clear benefits, therefore, from experimental consistency when comparisons are made directly within a study (e.g., same species and strain, consistent pathological evaluation).

One issue to note is the rationale for the organization of the available data. It was observed that the results across a broad range of chemicals with a variety of modes of action were somewhat variable. Therefore, consistent with the approach of the EPA cancer guidelines (U.S. EPA, 2005), an approach based on mode of action appeared to be a common framework for analysis. Variability in lifestage-dependent susceptibility and susceptibility across a range of modes of action was further supported by theoretical analyses using multistage and two-stage models of carcinogenesis (Goddard and Krewski, 1995; Murdoch et al., 1992).

3.2. QUANTITATIVE EVALUATION OF THE DATABASE

As described in the Section 2.3, the potential difference in susceptibility between early-life and adult exposure was calculated as the estimated ratio of cancer potency from early-life exposure over the cancer potency from adult exposure. Tables 4-7 present the results of the quantitative analysis using the studies that were determined qualitatively to have appropriate study designs (Tables 2 and 3) containing sufficient information to analyze. Based on the studies available, the calculations were organized into four tables: (1) compounds acting through a primarily mutagenic mode of action, where the compound was administered by a chronic dosing regimen to adults and repeated dosing in the early postnatal period (Table 4); (2) compounds acting through a primarily nonmutagenic mode of action, where the compound was administered by a chronic dosing regimen to adults and repeated dosing in the early postnatal period (Table 5);

(3) compounds acting through a primarily mutagenic mode of action, where the compounds were administered by an acute dosing regimen (Table 6); and (4) compounds acting primarily through either a mutagenic or nonmutagenic mode of action with chronic adult dosing and repeated early postnatal dosing (Table 7). In these tables, the 2.5% and 97.5% are percentiles of the posterior distribution. For a Bayesian distribution, these percentiles function in a manner similar to the 95% confidence limits for other types of statistical analyses. The results are discussed below, followed by a description of results from analyses of studies of humans exposed to radiation.

3.2.1. Carcinogens with a Mutagenic Mode of Action

The most informative database on early-lifestage susceptibility exists for chemicals with a well-accepted mutagenic mode of action (e.g., diethylnitrosamine, vinyl chloride). This database includes both single-dose studies and repeated-dose studies involving periods of postnatal and/or chronic exposure. These studies help define the periods of increased vulnerability and the magnitude of the susceptibility. The acute dosing studies demonstrate that the age-dependent responses are not due to differences in exposure, because these studies explicitly control the exposure.

3.2.1.1. *Early Postnatal, Juvenile, and Adult Repeated Dosing Studies of Chemicals with a Mutagenic Mode of Action*

Studies comparing repeated dosing for early-life, adult, or lifetime exposures exist for six carcinogens with a mutagenic mode of action [benzidine, diethylnitrosamine (DEN), 3-methylcholanthrene, safrole, urethane, and vinyl chloride]; DEN also had acute dosing studies. Lifetime (i.e., combined juvenile and adult) compared to adult exposure studies were analyzed for DEN, safrole, and urethane, while studies comparing juvenile with adult exposures were analyzed for benzidine, 3-methylcholanthrene, safrole, and vinyl chloride. These chemicals all require metabolic activation to the active carcinogenic form. Analysis of the tumors arising per unit time of exposure found that juvenile exposures with each chemical could be more effective than adult exposures were at inducing tumors (Tables 4 and 7; Figure 2, a graphic representation of the posterior, unweighted geometric means and their 95% confidence intervals, for the ratios of juvenile to adult cancer potency for carcinogens acting through a mutagenic mode of action). The weighted geometric mean for repeat and lifetime exposures is 10.4; for acute exposures the weighted geometric mean value is 1.5. For benzidine and safrole, there was a notable sex difference, with high liver tumor incidence observed for early postnatal exposures of male, but not female, mice. For both the acute and the repeated/lifetime data, the 95th percentile of the individual, unweighted geometric means is above 10 (Figure 2).

This analysis focused upon the duration of exposure as a surrogate for dose, essentially assuming that the doses animals received during the different periods of these studies were similar. This assumption is a limitation of the analysis because these studies involved exposures via lactation (i.e., dosing the mother prior to weaning), drinking water, diet, or inhalation, which have the potential to deliver different doses at different lifestages. However, the range of the magnitudes of the tumor incidence ratios of juvenile to adult exposures is similar (Table 8) for the repeated dosing studies (0.12 – 111, weighted geometric mean 10.5, 42% of ratios greater than 1), lifetime dosing studies (0.18 – 79, weighted geometric mean 8.7, 67% of ratios greater than 1), and acute dosing studies (0.01 – 178, weighted geometric mean 1.5, 55% of ratios greater than 1), suggesting that these differences in dosing are not the sole determinant of the increased incidence of early tumors, i.e., uncertainty and variability remain. Because these comparisons include different chemicals with different tissue specificities, it may be informative to consider liver as a target organ affected by all of these chemicals. The range of the magnitudes of the liver tumor incidence ratios of juvenile to adult exposures is similar for the repeated dosing studies (0.12 – 111, weighted geometric mean 41.8, 86% of ratios greater than 1, Table 4), lifetime dosing studies (0.47 – 79, weighted geometric mean 14.9, 80% of ratios greater than 1, Table 7), and acute dosing studies (0.1 – 40, weighted geometric mean 8.1, 77% of ratios greater than 1, Table 8). Thus, the repeated dose studies support the concept that early-lifestage exposure to carcinogenic chemicals with a mutagenic mode of action would lead to an increased tumor incidence compared with adult exposures of a similar duration and dose.

3.2.1.2. *Acute Dosing Studies of Chemicals with a Mutagenic Mode of Action*

Acute dosing studies are available for eight carcinogens with a mutagenic mode of action that were administered to mice or rats [benzo[a]pyrene (BaP), dibenzanthracene (DBA), Diethylnitrosamine (DEN), dimethylbenzanthracene (DMBA), dimethylnitrosamine (DMN), ethylnitrosourea (ENU), methylnitrosourea (NMU), and urethane (also known as ethyl carbamate)] (Table 1b). Except for ENU and NMU, these compounds require metabolic activation to their active carcinogenic forms. These acute dosing studies generally compared a single exposure during the first few weeks of life with the identical or similar exposure in young adult animals (Tables 3 and 6). Many of these studies compared exposures during the preweaning period (i.e., approximately day 21 for rats and mice) with effects around week 6, which is approximately the age at which typical chronic bioassays begin dosing animals. These studies largely were by subcutaneous or ip injection, which historically have not been considered quantitatively relevant routes of environmental exposure for human cancer risk assessment by EPA. For purposes of comparing age-dependent susceptibilities to tumor development, these

data are highly relevant. The injection route typically alters the pharmacokinetic time courses of the parent compound and the metabolites compared with oral or other exposures due to altered kinetics of absorption and metabolism. However, for these compounds and the systemic organ effects observed, there are several pharmacokinetic reasons to believe that the age-dependent trends would be similar with other routes of exposure. These compounds are expected to be reasonably well absorbed orally, comparable with injection routes, and largely require metabolic activation, so partial or complete absence of first pass metabolism in the injection studies would be similar to or underestimate metabolic activation when compared with oral exposure.

The early exposures often resulted in higher incidence of tumors than later exposures, with increased early susceptibilities up to 178-fold (unweighted ratios in Table 6 range from 0.011 to 178, with a weighted geometric mean of 1.5, and 55% of ratios greater than 1, Figure 2, Table 8). Examples of the general age-dependent decline in susceptibility of tumor response include BaP (liver tumors), DEN (liver tumors), ENU (liver and nervous system tumors), and urethane (liver and lung tumors). While generally the Day 1 and Day 15 time points were higher than later time points, in several cases similar tumor incidence was observed at both these early times (e.g., ENU-induced kidney tumors, Tables 6 and 8).

While the degree of susceptibility generally declines during the early postnatal period through puberty into early adulthood, there are exceptions due perhaps to pubertal periods of tissue development (e.g., mammary tissues) or very early development of xenobiotic metabolizing enzymes. One such exception was the increased incidence of mammary tumors in 5-8 week old rats given DMBA, compared with older or younger rats (Meranze et al., 1969; Russo et al., 1979). Meranze et al. (1969) reported 8% mammary tumors following a single dose of DMBA at less than two weeks, 56% if given once to animals between 5 and 8 weeks old, and 15% when given once to 26 week old rats. Thus, a ratio of 7.1 is obtained when comparing susceptibilities of 5–8 week and 26-week-old rats (Table 6) compared to a ratio of 0.2 when comparing the exposure at 2 weeks versus 26 weeks. A similar effect was observed by Russo et al. (1979); see Table 3. This observation corresponds well with pubertal development of the mammary tissue, with ovarian function commencing between 3 and 4 weeks (after the < 2 week time point in the Meranze et al., 1969 study), and mammary ductal growth and branching occurring such that it is approximately two-thirds complete by week 5, consistent with the 5–8 week susceptible period of Meranze et al. (Silberstein, 2001). While this differs from the general trend previously discussed, it indicates susceptibility later in the juvenile period rather than earlier. Another example of deviation from the general trend toward an age-dependent decline is DEN-induced lung tumors that were somewhat lower in incidence following exposure on day 1 than observed for the day 15 or day 42 exposures (Vesselinovitch et al., 1975) (Tables 3 and 6).

There are substantial differences in the early-life susceptibility of different tissues observed in the acute studies (Table 8). It should be noted that the target tissues vary with chemical, so the number of chemicals for which data are available varies for each tissue. Several tissues have weighted geometric mean ratios of greater than 1 including kidney, leukemia, liver, lymph, mammary, nerve, reticular tissue, thymic lymphoma, and uterus/vagina. Some of these, such as the nerve and mammary tumors, appear to have a very specific window of susceptibility, as noted above, and the ratios were much higher if the exposure occurred during this window. Tissues with weighted mean ratios less than 1 include forestomach, harderian gland, ovaries, and thyroid. Lung has a weighted geometric mean of 1. Many of the studies produced very high lung tumor responses regardless of age, so the results are difficult to interpret, as illustrated by the dose-response data with urethane in Rogers (1951) in which the increased early susceptibility is only apparent when the dose is low. The large numbers of studies with high lung tumor responses at all ages contribute to the differences in the weighted geometric means for the acute and for the repeated dosing studies.

Overall, the acute dosing studies support the concept that early-lifestage exposure to carcinogenic chemicals with a mutagenic mode of action would lead to an increased incidence of tumors compared with adult exposures of a similar dose and duration. These studies generally use the same dose and duration at all ages, and thus do not have the type of issues discussed for the repeated dosing studies. On the other hand, the acute dosing studies have limitations that were sufficient to decide that they should not be included in the quantitative adjustment of cancer potency. First, as mentioned in the previous paragraph, the large number of studies of lung tumors with almost 100% response observed at all doses and all ages would significantly bias the median ratio toward unity for a reason based on study design rather than biology. Second, cancer potency estimates are usually derived from chronic exposures. Therefore, any adjustment to those potencies should be, if possible, from similar exposures. Third, most exposures of concern to the Agency are from repeated or chronic exposures rather than acute exposures. Finally, many of the acute studies used ip exposures, which is not the usual route of exposure for environmental chemicals. Thus, the repeated and lifetime studies are more appropriate for the purpose of this analysis.

3.2.2. Carcinogens With Modes of Action Other Than Mutagenicity

Studies comparing tumors observed at the same sites following early postnatal and chronic adult exposures in a single protocol were available for six chemicals that do not act through a mutagenic mode of action [amitrole, dichlorodiphenyltrichloroethane (DDT), dieldrin, ethylene thiourea (ETU), diphenylhydantoin (DPH), polybrominated biphenyls (PBB)] (Table 5).

These chemicals cause tumors through several different, not necessarily well defined, modes of action. For example, thyroid hormone disruption by ETU causes thyroid tumors; some PBBs act through aryl hydrocarbon (Ah) receptors, while others are phenobarbital-like pleiotrophic inducers of liver enzymes and liver tumors. Three of these studies evaluated only mouse liver tumors (amitrole, DDT, dieldrin), while the other three evaluated a large number of tissues in both mice and rats (ETU, DPH, PBB). These studies generally included a combined perinatal and adult exposure as well as the separate perinatal or adult-only groups. It should be noted that no acute perinatal dosing studies of carcinogenesis were identified for these agents; such protocols are generally considered largely non-responsive for modes of action other than mutagenicity and potent estrogenicity (e.g., DES).

For five chemicals (amitrole, DDT, dieldrin, PBB and DPH), the same tumors were observed from early and/or adult exposures, though the studies for amitrole, DDT, and dieldrin only evaluates the animals for liver tumors. With ETU, no tumors in mice or rats were observed following perinatal exposure alone (except a small, not-statistically-significant increase in male rat thyroid tumors), while thyroid tumors were observed in adult rats and thyroid, liver, and pituitary tumors in adult mice. Analysis of the incidence of tumors per time of exposure shows early-lifestage susceptibilities. The range of the magnitudes of the tumor incidence ratios of juvenile to adult exposures is similar for the repeated dosing studies (0.06–13.3, weighted geometric mean 2.2, 27% of ratios greater than 1, Tables 5 and 8) and lifetime dosing studies (0.15–36, weighted geometric mean 3.4, 21% of ratios greater than 1, Tables 7 and 8). These ranges and means are similar to those for chemicals with a mutagenic mode of action, though the means and maximums are somewhat lower. Again, liver tumors are common to these chemicals. The range of the magnitudes of liver tumor incidence ratios of juvenile to adult exposures also is similar for the repeated dosing studies (0.06–13.3, weighted geometric mean 2.6, 43% of ratios greater than 1, Tables 5 and 8) and lifetime dosing studies (0.15–36, weighted geometric mean 5.8, 33% of ratios greater than 1, Tables 7 and 8).

The major factor that complicates the interpretation of the results is that these studies, except with DDT and dieldrin, involved dietary feeding initially to the mother, which potentially could increase or decrease the dose received by the pups. Due to the maternal dosing during pregnancy and lactation, the extent to which offspring received similar doses during different early and adult lifestages is particularly uncertain for DPH, ETU, and PBBs. Oral gavage doses in young animals were selected to approximate the average daily dose in adult dietary studies based on standard estimates of feed consumption in the studies with DDT and dieldrin, while the amitrole study involved dietary feeding postnatally to the mother so the young were dosed via lactation. In addition, DDT, dieldrin, and some PBBs are more persistent in the body than are

most chemicals, leading to a prolonged exposure even following limited dosing. Thus, these studies provide evidence that early lifestages can be more susceptible to exposures to chemicals causing cancer through a variety of modes of action other than mutagenicity. However, the studies with ethylene thiourea, which acts via thyroid disruption, indicate that this is not necessarily the case for all modes of action.

3.2.3. Ionizing Radiation

As mentioned previously, the UNSCEAR, Annex I (2000) includes information derived from a wide range of both intentional (generally diagnostic or therapeutic medical) and accidental radiation exposures. Only information derived from the Japanese population (referred to as the Life Span Study in the UNSCEAR Annex I) is presented here. A statistically significant excess cancer mortality associated with radiation has been found among the bomb survivors for the following types of cancer: esophagus, stomach, colon, liver, lung, bone and connective tissue, skin, breast, urinary tract, and leukemia. Tables 9 and 10 are extracted from the tables in UNSCEAR, Annex I. The excess relative risk (ERR) is the increased cancer rate relative to an unexposed population; an ERR of 1 corresponds to a doubling of the cancer rate. Because of the low numbers of cancers in individual sites within narrow age groups, the ERRs for the various solid tumors and leukemia were presented only as less than or greater than 20 years of age at the time of exposure. The larger number of thyroid tumors enable a more detailed breakout shown in Table 10. Most sites show greater risks in the younger than in the older ages.

The U.S. EPA (1994) document presents a methodology for estimation of cancer risks in the U.S. population due to low-LET (linear energy transfer) radiation exposures using data from the Atomic Bomb Survivor Study (ABSS) as well as from selected medical exposures. The report developed mortality risk coefficients using several models that took into account age and gender dependence of dosimetry, radiogenic risk, and competing causes of death as well as transporting of risks across populations. The risk projections were updated using more recent vital statistics in a report that also included an uncertainty analysis (U.S. EPA, 1999). Details of the derivation of these coefficients are available at http://www.epa.gov/radiation/docs/rad_risk.pdf .

Table 11 contains the calculated age-specific risk coefficients derived from the application of the various models to the ABSS data. For most of the sites in the table, the risk coefficients are higher in the earlier age groups; liver, bone, skin, and kidney coefficients are age-independent and only esophageal cancer coefficients increase with increasing age. Also of note is that the coefficients generally are higher for females. Similar to the information from the UNSCEAR (2000) Annex, most sites show greater risks in the younger ages than the older ages.

However, a comparison of the two tables seems to show reversal of risks for some sites as a function of age at exposure. While the high sampling variability in the epidemiological data for some ages may contribute to this apparent reversal, the choice of risk models and associated parameters also is a factor.

4. DISCUSSION

The challenge for this analysis was how to use the existing, but limited, scientific database on early postnatal and juvenile exposures to carcinogens to inform a science policy decision on whether, and if so how, to assess the risk from childhood exposures to chemicals for which we have evidence of carcinogenicity only in adult humans or sexually mature laboratory animals. The database overall is of limited size (particularly compared with the number of chemicals that have been studied in adult occupational epidemiological studies or chronic bioassays). The majority of the human data involves exposures to ionizing radiation or DES (Anderson et al., 2000). More than 50 chemicals have been demonstrated to cause cancer following perinatal exposures in animals (without adult exposures), but only a subset of the chemicals have comparative studies across ages. The comparative experimental studies used 18 chemicals, 12 of which had mutagenic modes of action and 6 of which had data from repeated or lifetime exposures. Other analyses of similar data have found similar results (Hattis et al. 2005), but have focused on other aspects of the data, e.g., gender differences.

Previously published or internal U.S. EPA analyses have concluded that the standard animal bioassay protocols usually do not miss chemicals that would have been identified as carcinogens if perinatal exposures had been undertaken (McConnell, 1992; Miller et al., 2002; U.S. EPA, 1996). Given the increased complexity and costs of chronic bioassays with perinatal exposures, a limited number of such studies have been performed. However, these are the studies that largely constitute the available database for this analysis. In addition to the chronic bioassays with perinatal exposures, there are studies with acute dosing at different lifestages and a large number of studies with perinatal exposures without a directly comparative adult study.

Two other kinds of information can contribute toward developing a scientifically informed policy: theoretical analyses and analyses of stop studies.[4] Theoretical analyses suggest that the differential susceptibility would depend in part on the mode of action (i.e., at what step in the cancer process(s) the chemical was acting) and that the use of the average daily exposure prorated over a lifetime may underestimate or overestimate the cancer risk when exposures are time-dependent (Goddard and Krewski, 1995; Murdoch et al., 1992). Evidence for old-age-dependent promotion of basophilic foci in rats by peroxisome proliferators appears to provide a concrete example consistent with these theoretical analyses (Cattley et al., 1991; Kraupp-Grasl et al., 1991). The stop studies performed by the National Toxicology Program began exposure at the standard post-weaning age, but stopped exposure after varying periods of months. Other groups of animals were exposed for a full two years; all animals were evaluated

[4] Stop studies are studies in which exposure is halted after a predetermined period.

for tumors at the end of two years regardless of the duration of exposure (Halmes et al., 2000). Related data also are available from the stop studies with vinyl chloride (Drew et al., 1983). Analysis by Halmes et al. (2000) showed that, for six of the eleven chemicals and half the tumor sites, the assumption that the cancer risk would be equal when the product of concentration and time (i.e., C x T) was constant was incorrect, and usually underestimated risk, as more of the risk came from the beginning of the exposure rather than the end. This dependence of risk on both duration and intensity of exposure did not appear to be correlated with mutagenicity. It should be noted that these stop studies all involved exposures early in the life of the animal (as opposed to a limited number of cancer studies that looked at later periods of life; e.g., Drew et al., 1983), but the extent to which the differences in tumor outcome result from increased susceptibility in these early periods or the extended period for expression of the cancer cannot be evaluated. These stop studies also used doses as high as or higher than the highest dose used in the two-year exposure. This latter factor clearly had a significant effect for two chemicals, causing tumors at higher doses that were not observed at lower doses. These results suggest that pharmacokinetic or other dose-rate dependencies can make the effects of exposures at high doses different from those exposures at lower doses. While not directly informative about early childhood exposures, these studies provide a perspective on the common cancer risk assessment practice of averaging exposures over a lifetime, especially those that include earlier lifestages. Thus, alternative methods for estimating risks from short-term exposures during childhood should be considered.

Information on different lifestage susceptibilities to cancer risks for humans exists for ionizing radiation. The effects of chemical mutagens at different lifestages on cancer induction are derived from laboratory animal studies. While the induction of cancer by ionizing radiation and the induction of cancer by chemical mutagens are not identical processes, both involve direct damage to DNA as critical causal steps in the process. In both cases, the impacts of early exposure can be greater than the impacts of later exposures, probably due to some combination of early-lifestage susceptibility and the longer periods for observation of effects. As indicated in Tables 9 and 10, A-bomb survivors exhibited different lifestage dependencies at different tumor sites, though the total radiation-related incidence of tumors showed a general slow decline with age at exposure. However, as previously noted, there are apparent differences at some sites between the two tables. In addition to the sampling and modeling differences, the excess risk values in Table 9 are based on Japanese baselines while the coefficients in Table 10 reflect UNSCEAR’s effort to transport the risks from the Japanese population to that of the United States. However, it is clear that the total radiation-related tumor incidence showed a general slow decline with age at exposure.

The studies in rodents of chemicals with mutagenic modes of action similarly support a

general decline in induced cancer risk with age at exposure and similarly show some differences for individual tumor sites. In general, the earliest two or three postnatal weeks in mice and rats appeared to be the most susceptible, though some degree of increased susceptibility through puberty in rats (beginning around 5–7 weeks) and mice (beginning around 4–6 weeks) for some types of tumors exists.

All the acute dosing studies that demonstrated carcinogenicity with animals of different ages used chemicals with a mutagenic mode of action (Tables 4 and 6). These studies provide the clearest demonstrations of periods of differential susceptibility because the exposure rate is constant at the different ages. The repeated dose studies also include several of the most informative studies for assessing perinatal carcinogenesis, notably those on vinyl chloride and DEN (Tables 2 and 4). The vinyl chloride studies by Maltoni and colleagues are part of a large series of studies on this compound that included exposures to different concentrations for varying durations, including some at early lifestages (Maltoni et al., 1984). The DEN study by Peto et al. (1984) used a unique chronic study design in which groups of rats were exposed to multiple drinking water concentrations starting at 3, 6, or 20 weeks of life. This design provides information on the susceptibility of early exposure periods within a nearly lifetime exposure.

Beyond the analysis described here, there are conceptual biological rationales that would suggest DNA-damaging agents would have greater impacts on early lifestages. Growth involves substantial levels of cell replication, even in organs that in adults are only very slowly replicating, thus increasing the likelihood that a cell will undergo division before the DNA damage caused by the mutagen has been repaired. Increased replication also can lead to a greater division of initiated cells, leading to a larger number of initiated cells per specified dose. These periods of cell replication can vary for different tissues. For example, DMBA appears to be more effective at initiating mammary tumors in 6-8 week old rats, which are undergoing development of that tissue, than during earlier or later periods (Meranze et al., 1969). While tumor promotion processes can be very dependent upon the duration of promotion, initiation processes can occur in relatively brief periods (e.g., the single-dose studies in animals or radiation exposure in humans). Most tumors take extended periods to develop, making damage that occurs earlier in life more likely to result in tumors prior to death than would exposures that occur later in life. While some of these observations may also pertain to other modes, all of them (with some differences among tumor sites) appear to be potentially relevant to a greater susceptibility to mutagenic modes of action during early-life stages (vs. later-life stages).

The information on lifestage susceptibility for chemicals inducing cancers through modes of action other than direct DNA interaction is more varied, showing an increase in tumor incidence during perinatal exposure versus exposures of mature animals (e.g., polybrominated

biphenyls induced liver tumors), no tumors from perinatal exposure (e.g., ethylene thiourea induced thyroid tumors), no effect of combined perinatal and adult exposure (e.g., DPH liver tumors in rats and female mice), and different tumors from perinatal exposure versus adult exposure (e.g., DES, ascorbate). These variations are likely a result of the modes of action of these chemicals and the pharmacokinetic differences in doses during different periods of life. No studies were evaluated that were directly comparable to the single-dose studies with mutagens, which clearly show significant differences in tumor responses after explicitly controlled doses at different lifestages.

Some evidence for an effect of early-lifestage exposures on tumor incidence was observed in studies with polybrominated biphenyls, amitrole, DDT, dieldrin, and diphenylhydantoin. These studies show increased incidence of tumors in mice from perinatal exposure, though only those for polybrominated biphenyls were statistically significant. (A nonstatistically significant increase also was observed in male rats with polybrominated biphenyls.) Combined perinatal and adult exposures generally gave statistically significant increases, though not necessarily for each sex and species (rat and mice) in the diphenylhydantoin and polybrominated biphenyl studies.

There are important demonstrations of chemicals acting through modes of action other than mutagenic to cause different tumor types with early-lifestage exposures compared with exposures for adults, e.g., tamoxifen and DES (Carthew et al., 2000; Carthew et al., 1996, Gass et al., 1964; Newbold et al., 1990, 1997, 1998). In addition, studies with *in utero* exposure to atrazine (Fenton and Davis, 2002), DES, and arsenic (Waalkes et al., 2003) indicate that early-life exposures to compounds can alter susceptibility of endocrine and reproductive organs. Three of these compounds (i.e., DES, genistein, and tamoxifen) bind to the estrogen receptor. Ongoing studies on ethinyl estradiol, nonylphenol, and genistein by the National Toxicology Program will add to this database for estrogens (Laurenzana et al., 2002; Newbold et al., 2001). These studies will evaluate cancer incidence in offspring exposed *in utero*, during lactation, and through adulthood via diet. A study with genistein found uterine tumor development to be dependent upon early-lifestage exposures (Newbold et al., 2001). Another recent study of estrogen found a shorter latency for mammary tumors in mice exposed at 8 and 12 weeks as compared to mice exposed at 4 or 18 weeks, indicating a susceptible period between 8 to 12 weeks of exposure (Yang, 2003). Thus, there is an actively growing database from which to consider issues of childhood exposure and cancer for compounds acting through the estrogen receptor or other mechanisms of endocrine disruption.

The ability to estimate with any accuracy the juvenile to adult cancer potency ratio depends very much on the experimental design used. The lifetime design has less ability to

distinguish increased susceptibility from early-life exposure than the other types of designs. Consider two different experimental designs. In the first, the "lifetime" design, a group of animals are exposed starting as juveniles, and exposure continues through adulthood. A second group are exposed only in adulthood, and the juvenile:adult ratio results from a comparison of tumor incidences in the two groups. In the second, the "repeated" design, one group of animals is exposed only during the juvenile period, and is then followed through adulthood to assess tumor incidence, and a second group of animals is exposed only through adulthood. The lifetime design turns out to be a particularly insensitive design for estimating the juvenile:adult ratio.

The following example demonstrates the magnitude of the problem: Suppose the risk per day of exposure of a chemical is ten fold greater in the juvenile period as in the adult period, and animals exposed through adulthood at a particular dose level have an extra risk of 60% for having at least one tumor, while 1% of control animals have tumors. The adult exposure period is 94 weeks, while the juvenile exposure period is 4 weeks. Thus, in the lifetime design, the group of animals exposed as juveniles will receive a total of 98 weeks of exposure, (4 in juvenile and 94 in adult), while those receiving the adult-only exposure receive 94 weeks of exposure. In the repeated design, animals exposed as juveniles receive only 4 weeks of exposure, while the adults receive 94 weeks, just as in the lifetime design. Each group starts with 50 animals. Under these assumptions, using equations (1) and (2) from Section 2.3, the expected number of animals with tumors in the three treatment groups (control, juvenile-exposed, adult-exposed groups) in the two designs is:

	Number of animals with tumors		
	Control	**Early-life exposure**	**Adult exposure**
Lifetime	1	36	30
Repeated	1	16	30

Notice that in the "lifetime" design, only six more juvenile-exposed animals have tumors than in the adult-exposed group, whereas in the "repeated" design, 16 juvenile-exposed animals have tumors. The data in the lifetime design are consistent with the hypothesis of no tumors being induced during the juvenile period: the ratios 36/50 and 30/50 are not statistically significantly different. In other words, the data from the lifetime design are statistically consistent with the hypothesis of *no risk at all* during the juvenile period, even though the real response is a 10 times greater risk from early-life exposure. The difference between the results from the two different study designs is due to the one-hit model: each additional week of a long exposure contributes less than the previous week to the total number of animals with tumors.

Note that, even if the one-hit model is not correct, chronic exposure probably results in a non-statistically significant increase for the lifetime exposure including juveniles as compared with only adult exposure.

The proper measure of relative potency of an exposure in the juvenile period relative to an exposure in the adult period is the ratio of doses in the two periods that give the same incidence of tumors. However, most of the data sets used in this report contained only one non-control dose, precluding the extensive dose-response modeling that would be required to estimate this ratio of doses. However, this document largely considered chemicals for which a mutagenic mode of action has been established and for which a linear, no-threshold dose-response function is assumed for the low-dose range being considered for risk assessment. In the case of the linear dose-response function, the analysis of the relative response from the same dose will produce the same value as ratio of doses that produces the same incidence of tumors.

For a one-hit dose-response equation, the probability of developing a tumor after the same dose and duration in the juvenile or adult period is

$$P_a = 1 - (1 - P_0)e^{-m_a x}$$
$$P_j = 1 - (1 - P_0)e^{-m_j x}$$

for dose x. Suppose we want to calculate the dose D_a or D_j that results in a given incidence of tumors after an adult or juvenile exposure. From equation 1, D_a and D_j equal:

$$D_a = \frac{-\ln\left(\frac{1-P_c}{1-P_0}\right)}{m_a}$$
$$D_j = \frac{-\ln\left(\frac{1-P_c}{1-P_0}\right)}{m_j}$$

Thus, the ratio $D_a/D_j = m_j/m_a$, the ratio calculated in this document.

In summary, this analysis supports the conclusion that there can be greater susceptibility for the development of tumors as a result of exposures to chemicals acting through a mutagenic mode of action, when the exposures occur in early lifestages as compared with later lifestages. Thus, this Supplemental Guidance recommends for chemicals with a mutagenic mode of action for carcinogenesis when chemical-specific data on early-life exposure are absent, a default

approach using estimates from chronic studies (i.e., cancer slope factors) with appropriate modifications to address the potential for differential risk of early-lifestage exposure. For chemicals acting through a non-mutagenic mode of action, e.g., hormonally mediated carcinogens, the available data suggest that other approaches may need to be developed for addressing cancer risk estimates from childhood exposures. This is a particular concern because the tumors arising from hormonally active chemicals appear to involve different sites when exposure is during early-life versus adulthood, an effect that has been observed relatively infrequently. Development of such approaches would require additional research to provide an expanded scientific basis for their support, including additional research and the possible development of new toxicity testing protocols that consider early lifestage dosing.

The current data do also not allow analysis of some issues of potential interest for risk assessment, e.g., potential increased risk of childhood cancer, from *in utero* or childhood exposures. Assessing the role of environmental exposures on childhood cancers is difficult, but additional research could include epidemiological studies or experimental studies with animals genetically designed to express cancers analogous to human childhood cancers. Rigorous quantification of exposure doses at different lifestages and in rodent pups in experimental studies would be useful for evaluating whether there is greater childhood susceptibility. Pharmacokinetic modeling could better define the internal doses to improve determination of the magnitude of increased susceptibility.

5. GUIDANCE FOR ASSESSING CANCER RISKS FROM EARLY-LIFE EXPOSURE

Consistent with the approach and recommendations of the U.S. EPA cancer risk assessment guidelines (U.S. EPA, 2004), any assessment of cancer susceptibility will begin with a critical analysis of the available information. Figure 3 shows the proposed steps in the process. The potential for increased susceptibility to cancer from early-life exposure, relative to comparable exposure later in life, generally warrants explicit consideration for each assessment.

When developing quantitative estimates of cancer risk, the Agency recommends integration of age-specific values for both exposure and toxicity/potency where such data are available and appropriate. Children, in general, are expected to have some exposures that differ from those of adults (either higher or lower), due to differences in size, physiology, and behavior. For example, children are generally assumed to eat more food and drink more water relative to their body weight than adults. Children's normal activities, such as putting their hands into their mouths or playing on the ground, can result in exposures to contaminants that adults do not encounter. Moreover, children and adults exposed to the same concentration of an agent in food, water, or air may receive different (higher or lower) internal doses due to differences, for example, in intake, metabolism, or absorption rates. Children are less likely than adults to be exposed to products typically used in industrial settings and often have more limited diets than adults. When assessing risks, if the data are available and relevant, it is important to include exposure that is measured or modeled for all lifestages, including exposures during childhood and during adulthood. EPA continues to develop better tools for assessing childhood exposure differences, such as the *Child-Specific Exposure Factors Handbook* (U.S. EPA, 2002a), and models, such as Stochastic Human Exposure and Dose Simulation (SHEDS) and Consolidated Human Activity Database (CHAD) (McCurdy et al., 2000; Zartarian et al., 2000)

Mode-of-action studies can be a source of data on quantitative differences between children and adults (Figure 3, Box 1). If the available information is sufficient to establish the agent's mode of action for early-life and adult exposures, then the implications for early-life exposure of that mode of action are used to develop separate risk estimates for childhood exposure. Pertinent information can be obtained both from agent-specific studies and from other

studies that investigate the general properties of the particular mode of action. All data indicating quantitative differences between children and adults are considered in developing those portion(s) of the risk estimates for exposure estimates that include childhood exposure. Some examples include the potential for children to have a different internal dose of the active agent or a change in a key precursor event (see Section 2.4.3.4 of the *Guidelines for Cancer Risk Assessment*).

When the mode of action cannot be established (Figure 3, Box 2), the policy choice would be to use linear extrapolation to lower doses such that risk estimates are based on a lifetime average daily exposure without further adjustment. No general adjustment is recommended at this time. This policy choice is consistent with past U.S. EPA practice that has been favorably evaluated over the years. The result would be expected to produce plausible upper bound risk estimates, based on the use of linear extrapolation as a default in the absence of information on the likely shape of the dose-response curve.

When a mode of action other than mutagenicity is established, if it is nonlinear (Figure 3, Box 3) or linear (Figure 3, Box 4), no general adjustment is recommended at this time. Although the available studies (discussed previously) indicates that higher or lower cancer risks may result from early-life exposure, there is insufficient information or analyses currently available to determine a general adjustment at this time. As other modes of action become better understood, this information may include data on quantitative differences between children and adults. If such data are available, an analysis of the differences could be used to adjust risk estimates for childhood exposure. EPA expects to expand this Supplemental Guidance to specifically address modes of action other than mutagenicity when sufficient data are available and analyzed.

When the data indicate a mutagenic mode of action,[5] the available studies (discussed

5 Determination of chemicals that are operating by a mutagenic mode of action entails evaluation of test results for genetic endpoints, metabolic profiles, physicochemical properties, and structure-activity analyses in a weight-of-evidence approach (Waters et al., 1999). Established protocols are used to generate the data (Cimino, 2001; OECD, 1998; U.S. EPA, 2002b); however, it is recognized that newer methods and technologies such as those arising from genomics can provide useful data and insights to a mutagenic mode of action. Carcinogens acting through a mutagenic mode of action generally interact with DNA and can produce such effects as DNA adducts and/or breakage. Carcinogens with a mutagenic mode of action often produce positive effects in multiple test systems for different genetic endpoints, particularly gene mutations and structural chromosome aberrations, and in tests performed *in vivo*, which generally are supported by those performed *in vitro*. This mode of action is addressed in more detail in Section 2.3.5 of EPA's cancer guidelines (U.S. EPA, 2005).

above) indicate higher cancer risks resulting from a given exposure occurring early in life when compared with the same amount of exposure during adulthood. However, chemical-specific data relating to mode of action (e.g., toxicokinetic or toxicodynamic information) may suggest that even though a compound has a mutagenic mode of action, higher cancer risks may not result. Such data should be considered before applying the age-dependent adjustment factors.

If the available, chemical-specific information includes an epidemiologic study of the effects of childhood exposure or an animal bioassay involving early-life exposure (Figure 3, Box 5), then these studies are analyzed to develop risk estimates (i.e., cancer slope factors) that specifically address any potential for differential potency in early lifestages. An example is the IRIS assessment of vinyl chloride (U.S. EPA, 2000b; c).

In the absence of early-life studies on a specific chemical under consideration (Figure 3, Box 6), the extrapolation from the point of departure to lower doses employs linear extrapolation (see Section 3.3.1 of the U.S. EPA [2005] cancer guidelines). This choice is based on mode-of-action data indicating that mutagens can give rise to cancers with an apparently low-dose linear response. Adjustments to the resultant risk estimates are specified with regard to childhood exposures. This approach is adopted because risk estimates based on an average daily exposure prorated over a lifetime do not consider the potential for higher cancer risks from early-life exposure.

The adjustments described below reflect the potential for early-life exposure to make a greater contribution to cancers appearing later in life. The 10-fold adjustment represents an approximation of the weighted geometric mean tumor incidence ratio from juvenile or adult exposures in the repeated dosing studies (see Table 8). This adjustment is applied for the first 2 years of life, when toxicokinetic and toxicodynamic differences between children and adults are greatest (Ginsberg et al., 2002; Renwick, 1998). Toxicokinetic differences from adults, which are greatest at birth, resolve by approximately 6 months to 1 year, while higher growth rates extend for longer periods. The 3-fold adjustment represents an intermediate level of adjustment that is applied after 2 years of age through <16 years of age. This upper age limit represents middle adolescence following the period of rapid developmental changes in puberty and the conclusion of growth in body height in NHANES data (Hattis et al., 2005). Efforts to map the approximate start of mouse and rat bioassays (i.e., 60 days) to equivalent ages in humans ranged from 10.6 to 15.1 years (Hattis et al., 2005). Data are not available to calculate a specific dose-response adjustment factor for the 2 to <16-year age range, so EPA selected the 3-fold

adjustment because it reflects a midpoint, i.e., approximately half the difference between 1 and 10 on a logarithmic scale ($10^{1/2}$), between the 10-fold adjustment for the first two years of life and no adjustment (i.e., 1-fold) for adult exposure. EPA also recognizes that exposures occurring near the end of life may have little effect on lifetime cancer risk, but lacks adequate data at present to provide an adjustment for this "wasted dose" effect. Similarly, since most of the studies involved only one latency period, the potential effect of early-life exposure on latency for the observed tumors could not be evaluated. The lack of data on effect on latency also limited the types of analyses that could be performed, e.g., more complex dose-response functions, such as multi-stage or clonal expansion models, could not be evaluated. Thus, the potential effects of early-life exposures on latency were not evaluated. Finally, as the adjustment factors are derived from a weighted geometric mean of the data evaluated, these adjustment will both over-estimate and under-estimate the potential potency for early-life exposure for chemicals with a mutagenic mode of action for carcinogenesis. An examination of the data in the tables demonstrates that some of the ratios were less than one, while others exceeded 10. For this reason, the Supplemental Guidance emphasizes that chemical-specific data should be used in preference to these default adjustment factors whenever such data are available.

The following adjustments represent a practical approach that reflects the results of the preceding analysis, which concluded that cancer risks generally are higher from early-life exposure than from similar exposure durations later in life:

- For exposures before 2 years of age (i.e., spanning a 2-year time interval from the first day of birth up until a child’s second birthday), a 10-fold adjustment.

- For exposures between 2 and <16 years of age (i.e., spanning a 14-year time interval from a child’s second birthday up until their sixteenth birthday), a 3-fold adjustment.

- For exposures after turning 16 years of age, no adjustment.

Clearly other age groups, such as an age group experiencing pubertal changes in physiology, or approximately ages 9 - 15, may experience changes in biological processes that could lead to modifications in the susceptibility to the effects of some carcinogens, depending on the mode of action. This Supplemental Guidance focuses on carcinogens with a mutagenic mode

of action. For any mode of action, the Agency is interested in identifying lifestages that may be particularly sensitive or refractory for carcinogenesis, and believes that the mode of action framework as described by EPA's cancer guidelines (U.S. EPA, 2005), is an appropriate mechanism for elucidating these lifestages. In general, the Agency's analyses of lifestages that may be susceptible will depend on three factors: (1) establishing the mode of action for carcinogenesis; (2) using knowledge about the biological and toxicological key events in that mode of action that are likely to be affected by lifestages; and (3) the availability, or development, of data that allow analysis of the effects of chemicals acting by that mode of action during the relevant ages. For each mode of action evaluated, therefore, the various age groupings determined to be at a differential risk, which may differ significantly from those proposed for the mutagenic mode of action, are expected to be evaluated independently of other modes of action. When data, including well established mode of action data, are available that allow specific evaluation of lifestage differences in toxicokinetics or toxicodynamics that would lead to lesser or greater susceptibility from early-life exposures to carcinogens, then those data should be used, as generally discussed in EPA's cancer guidelines (U.S. EPA, 2005), in preference to the default procedures described in this Supplemental Guidance.

The 10-fold and 3-fold adjustments in slope factor are to be combined with age-specific exposure estimates when estimating cancer risks from early life exposure to carcinogens that act through a mutagenic mode of action. It is important to emphasize that these adjustments are combined with corresponding age-specific estimates of exposure to assess cancer risk. For example, for a 70-year lifetime, where there are data showing negligible exposure to children, the estimated cancer risk from childhood exposure would be also negligible and the lifetime cancer risk would be reduced to that resulting from the relevant number of years of adult exposure (in the absence of specific information, 55 years). Where there are data (measured or modeled) for childhood exposures, the age-group specific exposure values are used along with the corresponding adjustments to the slope factor. Where there are no relevant data or models for childhood exposures and only lifetime average exposure data are available, the lifetime exposure data are used with the adjustments to the slope factor for each age segment.

It is recognized that, when the exposure is fairly uniform over a lifetime, the effect of these adjustments on estimated lifetime cancer risk are small relative to the overall uncertainty of

such estimates. These adjustments can be applied when estimating the cancer risk resulting from childhood exposure. These adjustments are applied when developing risk estimates from conventional animal bioassays or epidemiologic studies of effects of adult exposure. Some examples follow in the next section.

The Agency has also carefully considered both the advantages and disadvantages to extending the default potency adjustment factors to carcinogenic chemicals for which the mode of action remains unknown. It is the Agency's long-standing science policy position that use of the linear low-dose extrapolation approach (without further adjustment) provides adequate public health conservatism in the absence of chemical-specific data indicating differential early-life susceptibility. At the present time, therefore, EPA is recommending these age-dependent adjustment factors only for carcinogens acting through a mutagenic mode of action based on a combination of analysis of available data and the above-mentioned science policy position. In general, the Agency prefers to rely on analyses of data, rather than general defaults. When data are available for a susceptible lifestage, they should be used directly to evaluate risks for that chemical and that lifestage on a case-by-case basis. In this analysis, the data for non-mutagenic carcinogens, when the mode of action is unknown, were judged to be too limited and the modes of action too diverse to use this as a category for which a general default adjustment factor approach can be applied.

6 COMBINING LIFESTAGE DIFFERENCES IN EXPOSURE AND DOSE-RESPONSE WHEN ASSESSING CARCINOGEN RISK - SOME EXAMPLES FOR CARCINOGENS THAT ACT THROUGH A MUTAGENIC MODE OF ACTION

It is important for the risk assessor to consider lifestage differences in both exposure and dose-response when assessing cancer risk resulting from early-life exposures. As discussed in Section 5, age dependent adjustments factors (ADAFs) in dose response (i.e., slope factors) are combined with age specific exposure estimates when assessing cancer risks. This is a departure from the way cancer risks have historically been based upon the premise that risk is proportional to the daily average of lifetime dose. This Supplemental Guidance recommends an integrative approach that can be used to assess total lifetime risk resulting from lifetime or less-than-lifetime exposure during a specific portion of a lifetime.

The following examples can help demonstrate how to apply this guidance by integrating potential lifestage differences in exposure and/or dose-response (potency), and also demonstrate what the resulting impacts are on calculated risks. These hypothetical examples consider risks from both lifetime, as well as less-than-lifetime oral exposures. Risks associated with inhalation exposure to carcinogens that act via a mutagenic mode of action are calculated in similar fashion by applying the appropriate ADAF(s) along with the corresponding inhalation unit risk estimate, using pertinent estimates of exposure concentration.

Note again, ADAFs are only to be used for agents with a mutagenic mode of action for carcinogenesis when chemical-specific data are absent. For all modes of action, when chemical-specific data are available for early-life exposure, those data should be used.

6.1 CALCULATING LIFETIME RISKS ASSOCIATED WITH LIFETIME EXPOSURES

Example 1: Consider a scenario of exposure to a carcinogen with a **nonmutagenic** mode of action. Suppose the oral cancer slope factor derived from a typical animal study (i.e., where dosing begins after puberty) is estimated to be 2 per mg/kg-d, and the exposure rate remains constant throughout life at 0.0001 mg/kg-d (this is equivalent to saying the daily average of lifetime dose rate is equal to 0.0001 mg/kg-d). The risk from lifetime exposure is calculated by multiplying the slope factor and the exposure rate:

$$\text{Risk} = (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d})$$

$= 2 \times 10^{-4}$

Example 2: Now consider the same exposure scenario for a carcinogen with a **mutagenic** mode of action for which the oral cancer slope factor, derived from a typical animal study where dosing begins after puberty, is also estimated to be 2 per mg/kg-d. In this case, ADAFs are used, as follows.

a. To calculate lifetime risk for a population with average life expectancy of 70 years, sum the risk associated with each of the three relevant time periods:

- Risk during the first 2 years of life (where the ADAF = 10);
- Risk for ages 2 through < 16 (ADAF = 3); and
- Risk for ages 16 until 70 years (ADAF = 1).

Thus, risk equals the sum of:

- Risk for birth through < 2 yr = (2 per mg/kg-d) x 10 (ADAF) x (0.0001 mg/kg-d) x 2yr/70yr
  $= 0.6 \times 10^{-4}$
- Risk for ages 2 through < 16 = (2 per mg/kg-d) x 3 (ADAF) x (0.0001 mg/kg-d) x (13yr/70yr)
  $= 1.1 \times 10^{-4}$
- Risk for ages 16 until 70 = (2 per mg/kg-d) x 1 (ADAF) x (0.0001 mg/kg-d) x (55yr/70yr)
  $= 1.6 \times 10^{-4}$

Risk $= 0.6 \times 10^{-4} + 1.1 \times 10^{-4} + 1.6 \times 10^{-4}$
$= 3.3 \times 10^{-4}$

b. If exposure varies with age, then such differences are also included. Now suppose the same example as immediately above, except that exposure for ages 1 through <12 was twice as high as exposure for all other ages. In this case, sum the risk associated with each of the five relevant time periods in which exposure rates and/or potencies (slope

factors) vary:

Risk equals the sum of:

- Risk for birth through < 1 yr (1yr) = (2 per mg/kg-d) x 10 (ADAF) x 0.0001 mg/kg-d x 1yr/70yr
  $= 0.3 \times 10^{-4}$
- Risk for ages 1 through < 2 (1yr) = (2 per mg/kg-d) x 10 (ADAF) x 0.0002 mg/kg-d x 1yr/70 yr
  $= 0.6 \times 10^{-4}$
- Risk for ages 2 through < 12 (10yr) = (2 per mg/kg-d) x 3 (ADAF) x 0.0002 mg/kg-d x 10yr/70yr
  $= 1.7 \times 10^{-4}$
- Risk for ages 12 through < 16 (4yr) = (2 per mg/kg-d) x 3 (ADAF) x 0.0001 mg/kg-d x 4yr/70yr
  $= 0.3 \times 10^{-4}$
- Risk for ages 16 until 70 years (55yr) = (2 per mg/kg-d) x 1 (ADAF) x 0.0001 mg/kg-d x 55yr/70yr
  $= 1.6 \times 10^{-4}$

Risk $= 0.3 \times 10^{-4} + 0.6 \times 10^{-4} + 1.7 \times 10^{-4} + 0.3 \times 10^{-4} + 1.6 \times 10^{-4}$
$= 4.5 \times 10^{-4}$

6.2 CALCULATING LIFETIME RISKS ASSOCIATED WITH LESS THAN LIFETIME EXPOSURES

If exposure only occurs for a limited number of years (for example, consider a family that lives near a source of exposure for a five-year period of time before moving away), it is critical to combine lifestage differences in exposure and dose-response for the relevant time interval. The examples presented below demonstrate how adjusting potency and/or exposure can affect the assessment of cancer risk.

Example 3: If exposure to a carcinogen with a mutagenic mode of action with an oral slope factor equal to 2 per mg/kg-d occurs during adulthood for only 5 years, the daily average of lifetime dose is time weighted to apportion risk for the number of years of exposure by a factor of 5/70:

$$\text{Risk} = (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (5\text{yr}/70\text{yr})$$
$$= 1.4 \times 10^{-5}$$

Example 4: If this 5-year exposure occurs during childhood, the risk calculations are adjusted to consider the potential for higher potency from early-life exposure. Assessors should remember that the age dependent adjustment factors for carcinogens with a mutagenic mode of action are applied only to exposure periods occurring up to age 16.

a. For a child exposed between ages 5 and 10, only a 3-fold ADAF is applied because the exposure occurs entirely between ages 2 and <16 years:

$$\text{Risk} = 3 \text{ (ADAF)} \times (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (5 \text{ yr}/70 \text{ yr})$$
$$= 4.3 \times 10^{-5}$$

b. For an exposure between ages 13 and <18, a 3-fold ADAF is applied only to the 3-year portion occurring before age 16:

Risk equals the sum of:

- Risk for ages 13 through < 16 (3yr) $= 3 \text{ (ADAF)} \times (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (3 \text{ yr}/70 \text{ yr})$
  $= 2.6 \times 10^{-5}$
- Risk for ages 16 through < 18 (2yr) $= 1 \text{ (ADAF)} \times (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (2 \text{ yr}/70 \text{ yr})$
  $= 0.6 \times 10^{-5}$

$$\text{Risk} = 2.6 \times 10^{-5} + 0.6 \times 10^{-5}$$

$= 3.2 \times 10^{-5}$

c. For a child exposed from birth through age 5, different ADAFs are applied to the periods before and after age 2:

Risk equals the sum of:

- Risk for birth through < 2 (2yr) $= 10 \text{ (ADAF)} \times (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (2 \text{ yr}/70 \text{ yr})$
  $= 5.7 \times 10^{-5}$
- Risk for ages 2 through < 5 (3yr) $= 3 \text{ (ADAF)} \times (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times (3 \text{ yr}/70 \text{ yr})$
  $= 2.6 \times 10^{-5}$

Risk $= 5.7 \times 10^{-5} + 2.6 \times 10^{-5}$
$= 8.3 \times 10^{-5}$

Example 5: Lifetime risk calculations based on less-than-lifetime exposure to a carcinogen with a mutagenic mode of action include any lifestage changes in potency as well as exposure. In this example, again consider a scenario of 5 years of exposure to a carcinogen with a mutagenic mode of action, but suppose that the exposure rate is found to vary from 0.0002 mg/kg-d during the first 2 years of life, to 0.0001 mg/kg-d during the last 3 years.

a. For a child exposed between birth and age 5, sum the risk associated with the two relevant time periods:

Risk equals the sum of:

- Risk for birth through < 2 (2yr) = 10 (ADAF) x (2 per mg/kg-d) x (0.0002 mg/kg-d) x (2 yr/70 yr)
  $= 11.4 \times 10^{-5}$
- Risk for ages 2 through < 5 (3yr) = 3 (ADAF) x (2 per mg/kg-d) x (0.0001 mg/kg-d) x (3 yr/70 yr)
  $= 2.6 \times 10^{-5}$

Risk $= 11.4 \times 10^{-5} + 2.6 \times 10^{-5}$
$= 1.4 \times 10^{-4}$

b. For comparison, a similar risk calculation for 5 years of exposure later in life (after age 16) in which the first 2 years of exposure are double that of the next 3 years are carried out without any adjustment for potency:

Risk equals the sum of:

- Risk for first 2 years of adult exposure = 1 (ADAF) x (2 per mg/kg-d) x (0.0002 mg/kg-d) x (2yr/70yr)
  $= 1.1 \times 10^{-5}$
- Risk for final 3 years of adult exposure = 1 (ADAF) x (2 per mg/kg-d) x (0.0001 mg/kg-d) x (3yr/70yr)
  $= 0.9 \times 10^{-5}$

Risk $= 1.1 \times 10^{-5} + 0.9 \times 10^{-5}$
$= 2 \times 10^{-5}$

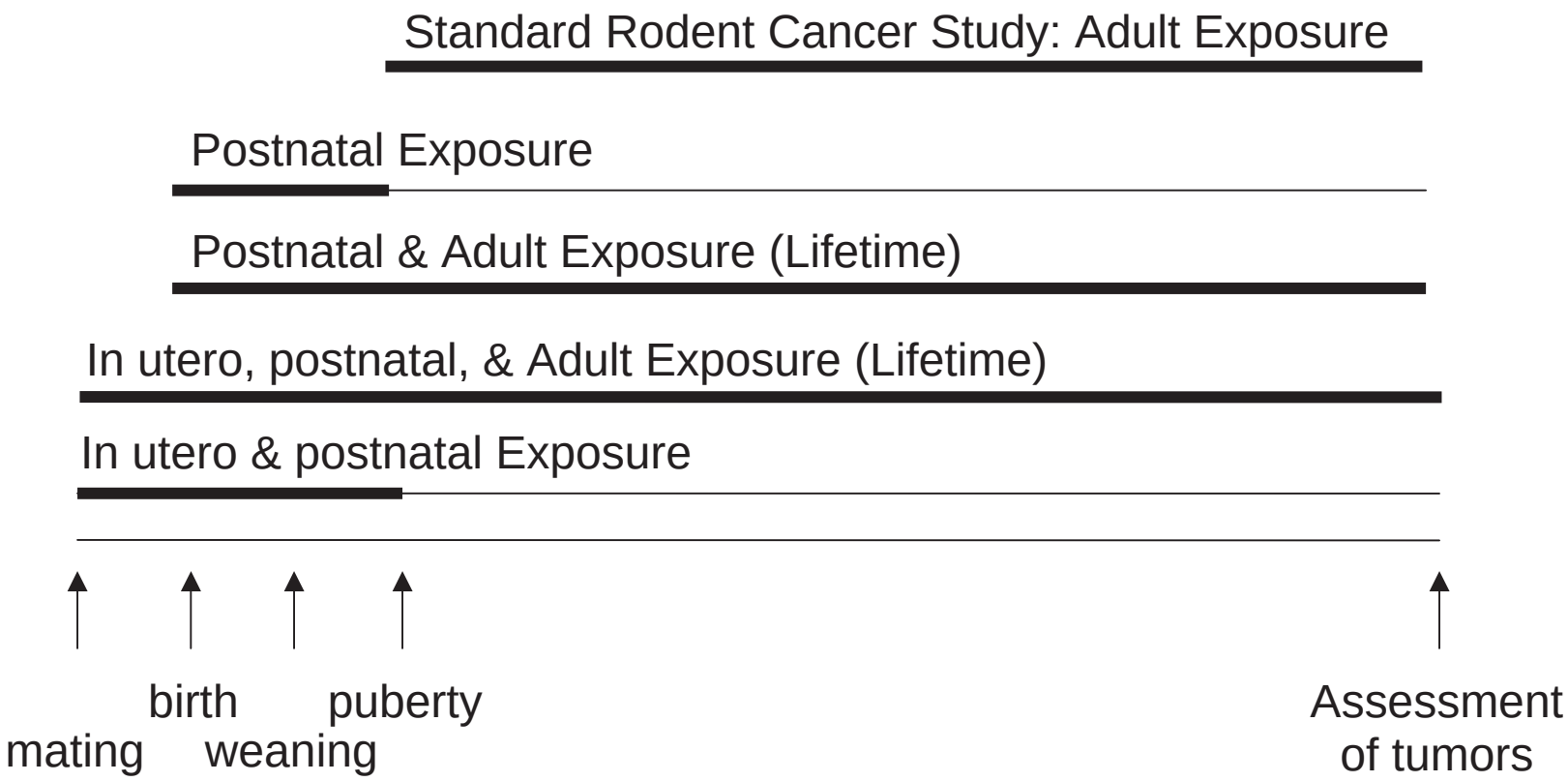

Figure 1. Study designs.

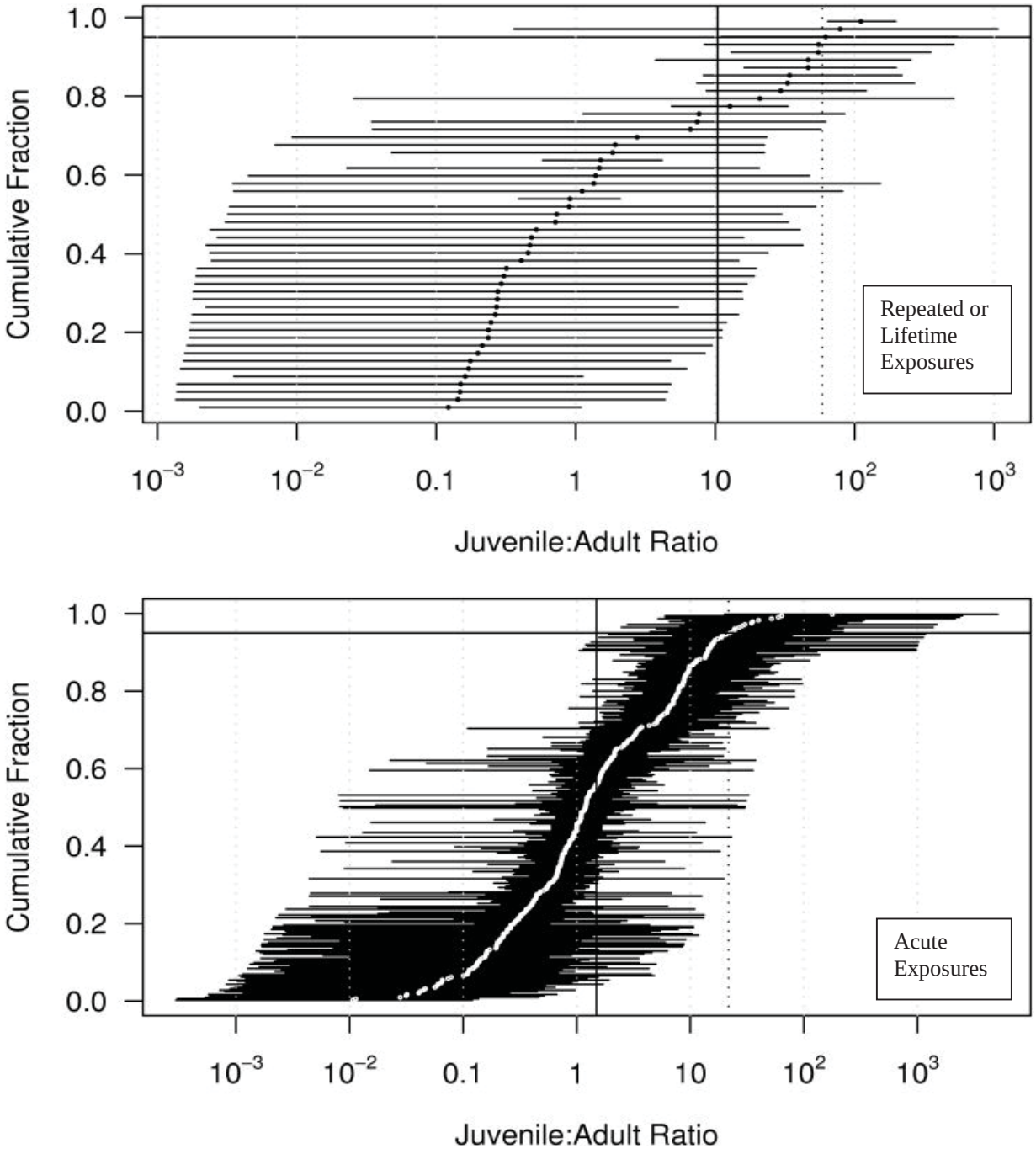

Figure 2: Posterior, unweighted geometric means and 95% confidence intervals for the ratios of juvenile to adult cancer potency for carcinogens acting primarily through a mutagenic mode of action. The top panel is for repeated and lifetime exposure studies (geometric mean in black), the bottom panel is for acute exposure studies mutagens (geometric mean in white). The horizontal lines to the left and right of each geometric mean correspond to 95% confidence limits. The vertical dark line represents the inverse-variance weighted geometric mean of the posterior geometric means. The horizontal dark line represents the 95th percentile of the unweighted distribution, with the vertical, dotted line establishing it value.

Figure 3. Flow chart for early-life risk assessment using mode of action framework

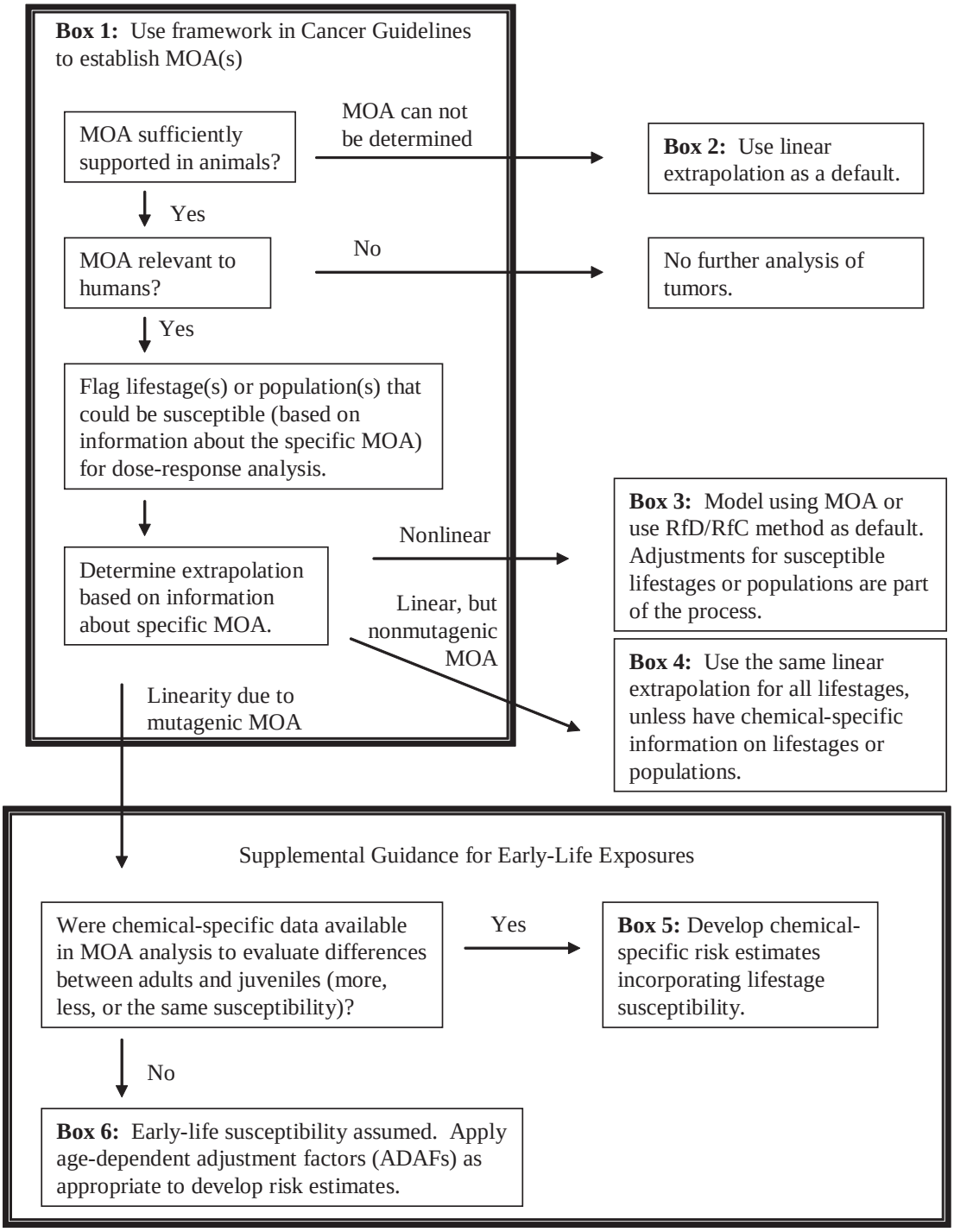

Table 1a. Chemicals that have been found to have carcinogenic effects from prenatal or postnatal exposure in animals as identified in different review articles

Chemical name	Review articles including prenatal and postnatal exposure					Chemicals selected for quantitative analysis
	Fujii (1991)	McClain et al. (2001)	Anderson et al. (2000)	Della Porta and Terracini (1969)	Other literature	
4-Acetylaminobiphenyl (AAB)	X					
4-Aminoazobenzene (AB)	X					
3-Amino-1,4,-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1)	X					
2-Aminodipyridol[1,2-a:3',2'-d]imidazole (Glu-P-2)	X					
2-Amino-6-methyldipyridol[1,2-a:3',2'-d]imidazole (Glu-P-1)	X					
3-Amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2)	X					
Amitrole						X
Arsenic					X	
5-Azacytidine			X			
3'-Azido-3'-deoxythymidine (AZT)			X			
Azoxymethane			X			
Benz[*a*]anthracene				X		
Benzidine			X			X
Benzo[*a*]pyrene (BaP)	X			X		X
1-(4'Bromophenylazo)-1-phenyl-1-hydroperoxymethane (BPH)	X					
N-Butyl-N-(3-carboxypropyl)nitrosamine (BCPN)	X					
N-Butyl-N-(3 hydroxbutyl)nitrosamine (BBN)	X					
Butylnitrosourea (BNU)	X					
Cyclophosphamide		X				
Dibenz[*a*,*h*]anthracene (DBA)				X		X
Dibutylnitrosamine (DBN)	X					
Dichlorodiphenyltrichloroethane (DDT)						X
Dieldrin						X
2-Diethylaminoethyl-2,2-dephenylvalerate hydrochloride (SKF 525A)	X					

Table 1a. Chemicals that have been found to have carcinogenic effects from prenatal or postnatal exposure in animals as identified in different review articles (continued)

Chemical name	Review articles including prenatal and postnatal exposure					Chemicals selected for quantitative analysis
	Fujii (1991)	McClain et al. (2001)	Anderson et al. (2000)	Della Porta and Terracini (1969)	Other literature	
Diethylnitrosamine (DEN)	X		X			X
Diethylstilbesterol (DES)			X			
4-Dimethylaminoazobenzene				X		
1,2-Dimethylhydrazine (DMH)	X					
7,12-Dimethylbenz[*a*]anthracene (DMBA)	X		X	X		X
Dimethylnitrosamine (DMN)	X		X	X		X
5',5'-Diphenylhydantoin (DPH)						X
Estradiol	X	X				
6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (Santoquin)	X					
Ethylene thiourea (ETU)						X
Ethyl methane sulphonate				X		
Ethylnitrosobiuret			X			
Ethylnitrosourea (ENU)			X			X
N-2-Fluorenylacetamide (FAA)	X			X		
Genistein					X	
3-Hydroxyl-4-acetylaminobiphenyl (N-OH-AAB)	X					
N-2-Hydroxy-N-2-fluorenylacetamide (N-OH-FAA)	X					
2-Hydroxypropyl-propylnitrosamine			X			
9-Methylanthracene				X		
Methyl-2-benzylhydrazine			X			
Methylcholanthrene			X	X		
3-Methyl-4-dimethylaminoabenzene (3'ME-DAB)	X					
4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)			X			
Methylnitrosourea (NMU)			X			
Methylnitrosourethane			X			
1-Methyl-3-nitro-1-nitrosoguanidine (MNNG)	X					

Table 1a. Chemicals that have been found to have carcinogenic effects from prenatal or postnatal exposure in animals as identified in different review articles (continued)

Chemical name	Review articles including prenatal and postnatal exposure					Chemicals selected for quantitative analysis
	Fujii (1991)	McClain et al. (2001)	Anderson et al. (2000)	Della Porta and Terracini (1969)	Other literature	
2-Naphthylamine				X		
2-Naphthylhydroxyamine				X		
Nickel acetate			X			
N-Nitrosobuylamine			X			
4-Nitroquinoline-1-oxide			X	X		
N-Nitrosomethyl(2-oxopropyl)amine			X			
2-Oxopropyl-propylnitrosamine			X			
1-Phenyl-3,3',-dimethylhydrzine			X			
1-Phenyl-3,3,-dimethyltriazene			X			
Polybrominated biphenyls (PBBs)						X
Safrole (3,4-methylenedioxyally benzene)	X		X			X
Soot	X					
Sterigmatocystin	X					
Tamoxifen					X	
1,3,5-Trimethyl-2,4,6-tris[3,5-di-tert-butyl-4-hydroxybenzyl]benzene (Ionox 33)	X					
Urethane (ethyl carbamate)			X	X		X
Vinyl chloride						X

Table 1b. List of chemicals considered in this analysis. (These are chemicals for which both early-life and adult exposure are reported in the same animal experiment.)

Chemical	References	Study type	Mutagenic mode of action
Amitrole	Vesselinovitch (1983)	Repeat dosing	
Benzidine	Vesselinovitch et al. (1975b)	Repeat dosing	X
Benzo[*a*]pyrene (BaP)	Vesselinovitch et al. (1975a)	Acute exposure	X
Dibenzanthracene (DBA)	Law (1940)	Acute exposure	X
Dichlorodiphenyltrichloroethane (DDT)	Vesselinovitch et al. (1979)	Repeat dosing Lifetime exposure	
Dieldrin	Vesselinovitch et al. (1979)	Repeat dosing Lifetime exposure	
Diethylnitrosamine (DEN)	Peto et al. (1984)	Lifetime exposure	X
	Vesselinovitch et al. (1984)	Acute exposure	
Dimethylbenz[*a*]anthracene (DMBA)	Meranze et al. (1969)	Acute exposure	X
	Pietra et al. (1961)	Acute exposure	
	Walters (1966)	Acute exposure	
Dimethylnitrosamine (DMN)	Hard (1979)	Acute exposure	X
Diphenylhydantoin, 5,5- (DPH)	Chhabra et al. (1993b)	Repeat dosing Lifetime exposure	
Ethylnitrosourea (ENU)	Naito et al. (1981)	Acute exposure	X
	Vesselinovitch et al. (1974)	Acute exposure	
	Vesselinovitch (1983)	Acute exposure	
Ethylene thiourea (ETU)	Chhabra et al. (1992)	Repeat dosing Lifetime exposure	
3-Methylcholanthrene (3-MU)[a]	Klein (1959)	Repeat dosing	X
Methylnitrosourea (NMU)	Terracini and Testa (1970) Terracini et al. (1976)	Acute exposure Acute exposure	X
Polybrominated biphenyls (PBBs)	Chhabra et al. (1993a)	Repeat dosing Lifetime exposure	
Safrole	Vesselinovitch et al. (1979)	Repeat dosing Lifetime exposure	X
Urethane	Chieco-Bianchi et al. (1963) Choudari Kommineni et al. (1970) De Benedictis et al. (1962) Fiore-Donati et al. (1962)	Acute exposure Acute exposure Acute exposure Acute exposure	X
	Klein (1966)	Acute exposure Lifetime exposure	
	Liebelt et al. (1964)	Acute exposure	
	Rogers (1951)	Acute exposure	
Vinyl chloride (VC)	Maltoni et al. (1984)	Repeat dosing	X

[a] Formerly known as 20-methylcholanthrene.

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Amitrole	Mice ($B6C3F_1$)	liver	Control	None	Control: 0 ppm	N/A	90 weeks	1/98 (1%)	0/96 (0%)	Incidences are mice with adenomas or carcinomas.	Vesselinovitch (1983)
			Gestation day 12	Diet, to mothers	500 ppm	Gestation day 12 to delivery		6/74 (8%)[b]	0/83 (0%)[b]		
			Newborn	Diet, to mothers	500 ppm	Birth until weaning		10/45 (22%)[b]	0/55 (0%)[b]		
			At weaning	Diet, to offspring	500 ppm	From weaning to 90 weeks		20/55 (36%)[b]	9/49 (18%)[b]		
Benzidine	Mice ($B6C3F_1$)	liver	Control	None	Control: 0 ppm	N/A	90 weeks	1/98 (1%)	0/100 (0%)	Higher sensitivity in males during perinatal period, in females during adulthood. Incidences are mice with adenomas or carcinomas.	Vesselinovitch et al. (1975b) Vesselinovitch et al. (1979a)
			Gestation day 12	Diet, to mothers	150 ppm	Gestation day 12 to delivery		17/55 (31%)[c]	2/62 (3%)[d]		
			Newborn	Diet, to mothers	150 ppm	Birth until weaning		62/65 (95%)[c]	2/43 (5%)[d]		
			At weaning	Diet, to offspring	150 ppm	From weaning to 90 weeks		22/50 (44%)[c]	47/50 (94%)[c]		
			Gestation day 12	Diet, to mothers	150 ppm	Gestation day 12 until weaning		49/49 (100%)[c]	12/48 (25%)[c]		
			Gestation day 12	Diet, to mothers	150 ppm	Gestation day 12 until 90 weeks		50/50 (100%)[c]	47/50 (94%)[c]		
DDT Dichlorodiphenyl-trichloroethane	Mice ($B6C3F_1$)	liver	Control	None	Control: 0 ppm	N/A	90 weeks	1/50 (2%)	—		Vesselinovitch et al. (1979b)
			Week 1	Gavage, daily	230 µg	Weeks 1–4		5/49 (10%)[d]	—		
			Week 5	Diet, daily	150 ppm	Weeks 5–90		8/49 (16%)[d]	—		
			Week 1	Gavage, daily until 4 weeks, then in diet	230 µg 150 ppm (diet)	Weeks 1–90		10/50 (20%)[c]	—		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Dieldrin	Mice ($B6C3F_1$)	liver	Control	None	Control: 0 ppm	N/A	90 weeks	1/58 (2%)	—		Vesselinovitch et al. (1979b)
			Week 1	Gavage, daily	12.5 µg	Weeks 1–4		3/46 (7%)[b]	—		
			Week 5	Diet, daily	10 ppm	Weeks 5–90		7/60 (12%)[b]	—		
			Week 1	Gavage, daily until 4 weeks, then in diet	12.5 µg 10 ppm	Weeks 1–90		21/70 (30%)[a]	—		
DEN[e] Diethylnitrosamine	Rats (Colworth)	liver	Control		Control	N/A		29/384 (8%)		Highest tumor rate when dosed at earlier ages. Incidents are rats with adenomas or carcinomas.	Peto et al. (1984)
			Week 3	Diet (in drinking water), daily	16 different doses combined[f]	From week 3 until death	6 months–3 years	105/180 (58%)[b]			
			Week 6			From week 6 until death		714/1440 (50%)[b]			
			Week 20			From week 20 until death		76/180 (42%)[b]			
		esophagus	Control		Control	N/A		0/384 (0%)			
			Week 3	Diet (in drinking water), daily	16 different doses combined[g]	From week 3 until death		77/180 (43%)[b]			
			Week 6			From week 6 until death		663/1440 (46%)[b]			
			Week 20			From week 20 until death		88/180 (49%)[b]			

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DPH Diphenylhydantoin, 5,5-	Rats (F344/N)	liver	Control	Control	0 ppm	N/A	2 years	0/50 (0%)	0/50 (0%)	In rats, perinatal exposure ranged from 63 to 630 ppm, and adult exposures ranged from 240 to 2,400 ppm. In mice, perinatal exposure ranged from 21 to 210 ppm. Adult exposure ranged from 30 to 300 ppm in males and 60 to 600 ppm in females. Tumor incidences are animals with adenomas or carcinomas.	Chhabra et al. (1993b)
			Perinatal	Diet, daily	630 ppm	Perinatal through 8 weeks		1/50 (2%)[d]	0/49 (0%)[d]		
			8 weeks		800 ppm	8 weeks–2 years		2/50 (4%)[d]	1/50 (2%)[d]		
			8 weeks		2,400 ppm	8 weeks–2 years		4/50 (8%)[d]	1/50 (2%)[d]		
			Perinatal		630–800	Perinatal through 2 years		1/49 (2%)[d]	0/50 (0%)[d]		
			Perinatal		630–2,400 ppm	Perinatal through 2 years		5/49 (10%)[c]	0/50 (0%)[d]		
	Mice ($B6C3F_1$)	liver	Control	Control male	0 ppm	N/A	2 years	29/50 (58%)			
			Perinatal	Diet, male	210 ppm	Perinatal through 8 weeks		33/50 (66%)[d]			
			8 weeks		100 ppm	8 weeks–2 years		29/49 (59%)[d]			
			8 weeks		300 ppm	8 weeks–2 years		26/49 (53%)[d]			
			Perinatal		210–100 ppm	Perinatal through 2 years		35/49 (71%)[d]			
			Perinatal		210–300 ppm	Perinatal through 2 years		41/50 (82%)[c]			
			Control	Control female	0 ppm	N/A	2 years		5/48 (10.4%)[d]		
			Perinatal	Diet, female	210 ppm	Perinatal through 8 weeks			12/49 (24.5%)[d]		
			8 weeks		200 ppm	8 weeks–2 years			14/49 (28%)[c]		
			8 weeks		600 ppm	8 weeks–2 years			30/50 (60%)[c]		
			Perinatal		210–200 ppm	Perinatal through 2 years			16/50 (32%)[c]		
			Perinatal		210–600 ppm	Perinatal through 2 years			34/50 (68%)[c]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ETU Ethylene thiourea	Rats (F344/N)	thyroid	Control	Control	0 ppm	N/A	2 years	1/49 (2%)	3/50 (6%)	Tumor incidences are animals with adenomas or carcinomas.	Chhabra et al. (1992)
			Perinatal	Diet, daily	90 ppm	Perinatal through 8 weeks		4/49 (8%)[d]	3/50 (6%)[d]		
			8 weeks		83 ppm	8 weeks–2 years		12/46 (26%)[c]	7/44 (16%)[d]		
			8 weeks		250 ppm	8 weeks–2 years		37/50 (74%)[c]	30/49 (61%)[c]		
			Perinatal		90–83 ppm	Perinatal through 2 years		13/50 (26%)[c]	9/47 (19%)[d]		
			Perinatal		90–250 ppm	Perinatal through 2 years		48/50 (96%)	37/50 (74%)		
	Mice ($B6C3F_1$)	liver	Control	Control	0 ppm	N/A	2 years	20/49 (41%)	4/50 (8%)		
			Perinatal	Diet, daily	330 ppm	Perinatal through 8 weeks		13/49 (26.5%)[d]	5/49 (10%)[d]		
			8 weeks		330 ppm	8 weeks–2 years		32/50 (64%)[c]	44/50 (88%)[c]		
			8 weeks		1,000 ppm	8 weeks–2 years		46/50 (92%)[c]	48/50 (96%)[c]		
			Perinatal		330–330 ppm	Perinatal through 2 years		34/49 (69%)[c]	46/50 (92%)[c]		
			Perinatal		330–1,000 ppm	Perinatal through 2 years		47/49 (6%)[c]	49/50 (98%)[c]		
		thyroid	Control	Control	0 ppm	N/A		1/50 (2%)	0/50 (0%)		
			Perinatal	Diet, daily	330 ppm	Perinatal through 8 weeks		1/46 (2%)[d]	1/49 (2%)[d]		
			8 weeks		330 ppm	8 weeks–2 years		1/49 (2%)[d]	2/50 (4%)[d]		
			8 weeks		1,000 ppm	8 weeks–2 years		29/50 (58%)[c]	38/50 (76%)[c]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ETU Ethylene thiourea (continued)			Perinatal		330–330 ppm	Perinatal through 2 years		2/48 (4%)[d]	10/49 (20%)[c]		
			Perinatal		330–1,000 ppm	Perinatal through 2 years		35/49 (71%)[c]	38/50 (76%)[c]		
		pituitary	Control	Control	0 ppm	N/A		0/44 (0%)	11/47 (23%)		
			Perinatal	Diet, daily	330 ppm	Perinatal through 8 weeks		0/42 (0%)[d]	11/48 (23%)[d]		
			8 weeks		330 ppm	8 weeks–2 years		0/42 (0%)[d]	19/49 (39%)[d]		
			8 weeks		1,000 ppm	8 weeks–2 years		8/41 (19.5%)[c]	26/49 (53%)[c]		
			Perinatal		330–330 ppm	Perinatal through 2 years		0/45 (0%)[d]	26/47 (55%)[c]		
			Perinatal		330–1,000 ppm	Perinatal through 2 years		4/39 (10%)[d]	24/47 (51%)[c]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death		Tumor incidence		Reference
							M	F	M	F	
3-Methylcholanthrene (formerly known as 20-methylcholanthrene)	Mice (Albino)	liver	Control	gavage, 3× per week	NA	NA	475 days	480 days	3/39 (7.7%)	0/36 (0%)	Klein (1959)
			8 days		0.25 mg/g	10×	311 days	321 days	21/25 (84%)[b]	7/30 (23.3%)[b]	
			90 days		0.25 mg/g	10×	330 days	366 days	1/26 (3.8%)[b]	0/29 (0%)[d]	
		lung	Control		NA	NA	475 days	480 days	17/39 (43.6%)	14/36 (38.9%)	
			8 days		0.25 mg/g	10×	311 days	321 days	25/25 (100%)[b]	28/30 (93.3%)[b]	
			90 days		0.25 mg/g	10×	330 days	366 days	25/26 (96.2%)[b]	27/29 (93.1%)[b]	
		fore-stomach	Control		NA	NA	475 days	480 days	0/39 (0%)	0/36 (0%)	
			8 days		0.25 mg/g	10×	311 days	321 days	12/25 (48%)[b]	12/30 (40%)[b]	
			90 days		0.25 mg/g	10×	330 days	366 days	13/26 (50%)[b]	8/29 (27.6%)[b]	
		skin	Control		NA	NA	475 days	480 days	0/39 (0%)	0/36 (0%)	
			8 days		0.25 mg/g	10×	311 days	321 days	4/25 (16%)[b]	4/30 (13.3%)[b]	
			90 days		0.25 mg/g	10×	330 days	366 days	1/26 (3.8%)[b]	1/25 (4%)[b]	

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
PBBs Polybrominated biphenyls	Rats (F344/N)	liver[g]	Control	Control	0 ppm	N/A	2 years	1/50 (2%)	0/50 (0%)	Findings suggest that combined perinatal and adult exposure increases PBB-related hepatocellular carcinogenicity relative to adult-only exposure in mice and female rats. Apparent association between increasing incidences of MCL and exposure to PBB in male and female rats. Tumor incidences are animals with adenomas or carcinomas.	Chhabra et al. (1993a)
			Perinatal	Diet	10 ppm	Perinatal–8 weeks		5/50 (10%)[d]	0/50 (0%)[d]		
			8 weeks		10 ppm	8 weeks–2 years		12/49 (24%)[c]	12/50 (24%)[c]		
			8 weeks		30 ppm	8 weeks–2 years		41/50 (82%)[c]	39/50 (78%)[c]		
			Perinatal		10–10 ppm	Perinatal–2 years		16/50 (32%)[c]	39/50 (78%)[c]		
			Perinatal		10–30 ppm	Perinatal–2 years		41/50 (82%)[c]	47/50 (94%)[c]		
		Mono-nuclear cell leukemia (MCL)	Control	Control	0 ppm	N/A	2 years	25/50 (50%)	14/50 (28%)		
			Perinatal	Diet	10 ppm	Perinatal–8 weeks		31/50 (62%)[d]	13/50 (26%)[d]		
			8 weeks		10 ppm	8 weeks–2 years		33/50 (66%)[c]	22/50 (44%)[d]		
			8 weeks		30 ppm	8 weeks–2 years		31/50 (62%)[d]	23/50 (46%)[c]		
			Perinatal		10–10 ppm	Perinatal–2 years		37/50 (74%)[c]	27/50 (54%)[c]		
			Perinatal		10–30 ppm	Perinatal–2 years		37/50 (74%)[c]	25/50 (50%)[c]		
	Mice ($B6C3F_1$)	liver[g]	Control	Control	0 ppm	N/A	2 years	16/50 (32%)	5/50 (10%)		
			Perinatal	Diet	30 ppm	Perinatal–8 weeks		40/50 (80%)[c]	21/50 (42%)[c]		
			8 weeks		10 ppm	8 weeks–2 years		48/49 (98%)[c]	42/50 (84%)[c]		
			8 weeks		30 ppm	8 weeks–2 years		48/50 (96%)[c]	47/48 (98%)[c]		
			Perinatal		10 ppm	Perinatal–2 years		46/49 (94%)[c]	44/50 (88%)[c]		
			Perinatal		30–30 ppm	Perinatal–2 years		50/50 (100%)[c]	47/47 (100%)[c]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Safrole	Mice ($B6C3F_1$)	liver	Control	None	None	N/A	90 weeks	3/100 (3%)	0/100 (0%)	Highest tumor rate in males due to preweaning treatment. Highest tumor rate in females due to susceptibility in adulthood. Tumor incidences are mice with adenomas or carcinomas.	Vesselinovitch et al. (1979b)
			Day 12 of gestation	Gavage, to mothers	120 µg/g body weight	4× (days 12, 14, 16, 18)		2/61 (3%)[d]	0/65 (0%)[d]		
			Newborn	Gavage, to mothers, on alternate days	120 µg/g body weight	From birth until weaning		28/83 (34%)[c]	2/80 (3%)[d]		
			At weaning	Gavage, to offspring, 2× weekly	120 µg/g body weight	From weaning until 90 weeks		4/35 (11%)[d]	22/36 (61%)[c]		
			Day 12 of gestation	Gavage, to mothers, alternate days	120 µg/g body weight	From gestation until weaning		22/68 (32%)[b]	1/72 (1%)[b]		
			Day 12 of gestation	Gavage, to mothers, alternate days until weaning; Gavage, to offspring, 2× weekly	120 µg/g body weight	From gestation until 90 weeks		19/37 (51%)[b]	37/46 (80%)[b]		
Urethane	Mice ($B6AF_1/J$)	liver	1 week	gavage	2.5 mg/pup	1×	39–40 weeks	Tumor incidence[a]		No tumor data for controls.	Klein (1966)
								M	F		
								12/37 (33%)[b]	0/40 (0%)[b]		
			1 week		2.5 mg/pup	16× (1× at 1 week; 3× weekly for 5 weeks beginning at 4 wks of age)	39 weeks	11/33 (33%)[b]	0/31 (0%)[b]		
			4 weeks		2.5 mg/pup	15× (3× weekly for 5 weeks beginning at 4 weeks of age)	41 weeks	0/37 (0%)[b]	0/31 (0%)[b]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
VC Vinyl chloride	Rats (Sprague-Dawley)	liver angio-sarcoma	Control	Control	0 ppm	N/A	135 weeks	0/22 (0%)	0/29 (0%)	Higher tumor risk when exposed at birth, higher for females.	Maltoni et al. (1984)
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	5/18 (28%)[b]	12/24 (50%)[b]		
					10,000 ppm			6/24 (25%)[b]	9/20 (45%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	3/17 (18%)[b]	10/25 (40%)[b]		
					10,000 ppm			3/21 (14%)[b]	4/25 (16%)[b]		
		zymbal gland	Control	Control	0 ppm	N/A	135 weeks	0/28 (0%)	0/29 (0%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	1/12 (8%)[b]	1/17 (6%)[b]		
					10,000 ppm			1/17 (6%)[b]	0/17 (0%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	3/29 (10%)[b]	4/30 (13%)[b]		
					10,000 ppm			10/30 (33%)[b]	6/30 (20%)[b]		
		leukemia	Control	Control	0 ppm	N/A	135 weeks	0/27 (0%)	1/29 (3%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	N/A	1/7 (14%)[b]		
					10,000 ppm			2/6 (33%)[b]	0/15 (0%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	N/A	0/29 (0%)[b]		
					10,000 ppm			0/27 (0%)[b]	2/29 (7%)[b]		
		nephro-blastoma	Control	Control	0 ppm	N/A	135 weeks	0/22 (0%)	0/29 (0%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	0/15 (0%)[b]	0/21 (0%)[b]		
					10,000 ppm			0/19 (0%)[b]	0/17 (0%)[b]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
VC Vinyl chloride (continued)			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	4/18 (22%)[b]	1/26 (4%)[b]		
					10,000 ppm			3/21 (14%)[b]	2/25 (8%)[b]		
		angio-sarcomas: other sites	Control	Control	0 ppm	N/A	135 weeks	0/29 (0%)	0/29 (0%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	1/15 (7%)[b]	0/21 (0%)[b]		
					10,000 ppm			0/19 (0%)	0/17 (0%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	1/29 (3%)[b]	2/30 (7%)[b]		
					10,000 ppm			2/30 (7%)[b]	1/30 (3%)[b]		
		angiomas and fibromas: other sites	Control	Control	0 ppm	N/A	135 weeks	0/28 (0%)	2/29 (7%)[b]		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	1/15 (7%)[b]	0/21 (0%)[b]		
					10,000 ppm			2/19 (11%)[b]	1/17 (6%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	2/29 (7%)[b]	2/30 (7%)[b]		
					10,000 ppm			2/29 (7%)[b]	1/29 (3%)[b]		
		hepatoma	Control	Control	0 ppm	N/A	135 weeks	0/19 (0%)	0/28 (0%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	9/18 (50%)[b]	11/24 (46%)[b]		
					10,000 ppm			13/24 (54%)[b]	7/20 (35%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	0/10 (0%)[b]	1/17 (6%)[b]		
					10,000 ppm			1/8 (13%)[b]	0/16 (0%)[b]		

Table 2. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult repeated exposures (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
VC Vinyl chloride (continued)		skin carcinomas	Control	Control	0 ppm	N/A	135 weeks	0/20 (0%)	1/29 (3%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	1/10 (10%)[b]	1/14 (7%)[b]		
					10,000 ppm			1/16 (6%)[b]	0/15 (0%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	0/15 (0%)[b]	2/19 (11%)[b]		
					10,000 ppm			2/13 (15%)[b]	1/21 (5%)[b]		
		neuro-blastoma	Control	Control	0 ppm	N/A	135 weeks	0/22 (0%)	0/29 (0%)		
			Newborn	Inhalation	6,000 ppm	4 hrs/day, 5 days/wk, 5 weeks	124 weeks	0/18 (0%)[b]	0/29 (0%)[b]		
					10,000 ppm			0/22 (0%)[b]	0/19 (0%)[b]		
			Week 13		6,000 ppm	4 hrs/day, 5 days/wk, 52 weeks	135 weeks	2/21 (10%)[b]	1/27 (4%)[b]		
					10,000 ppm			2/22 (9%)[b]	5/26 (19%)[b]		

[a] Where not delineated by gender, data combined by study authors or gender not specified. Where percentages only are given, number of subjects not specified.
[b] Not evaluated by authors.
[c] Significant compared with controls.
[d] Evaluated but not significant compared with controls.
[e] Reported as NDEA (N-nitrosodiethylamine) in the original document.
[f] Results from each dose are not available.
[g] Tumors were adenomas or carcinomas.

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
BaP Benzo[*a*]pyrene	Mice ($B6C3F_1$)	liver	Control	Control	None	N/A	142 weeks	7/100 (7%)	1/100 (1%)	In general, hepatomas developed with significantly higher incidence ($p<0.01$) in mice that were treated within 24 hours of birth or at 15 days of age than they did in similarly treated animals at 42 days of age. + higher for males.	Vesselinovitch et al. (1975a)
			Day 1	i.p.	75 µg/g body weight	1×	86 weeks (m) 129 weeks (f)	26/47 (55%)[b]	3/45 (7%)[b]		
					150 µg/g body weight	1×	81 weeks (m) 121 weeks (f)	51/63 (81%)[b]	8/45 (18%)[b]		
			Day 15	i.p.	75 µg/g body weight	1×	93 weeks (m) 116 weeks (f)	36/60 (60%)[b]	4/55 (7%)[b]		
					150 µg/g body weight	1×	81 weeks (m) 90 weeks (f)	32/55 (58%)[b]	4/55 (7%)[b]		
			Day 42	i.p.	75 µg/g body weight	1×	108 weeks(m)	7/55 (13%)[b]	0/47 (0%)[b]		
					150 µg/g body weight	1×	87 weeks (m)	4/47 (9%)[b]	0/46 (0%)[b]		
	Mice ($C3AF_1$)	liver	Control	Control	None	N/A	142 weeks	8/100 (8%)	1/100 (1%)	+ higher for males. “Age at death” is the average age at which tumors were observed.	
			Day 1	i.p.	75 µg/g body weight	1×	80 weeks (m) 91 weeks (f)	21/62 (34%)[b]	1/45 (2%)[b]		
					150 µg/g body weight	1×	69 weeks (m) 701 weeks (f)	24/52 (46%)[b]	1/56 (2%)[b]		
			Day 15	i.p.	75 µg/g body weight	1×	90 weeks (m) 102 weeks (f)	15/56 (27%)[b]	1/49 (2%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
BaP Benzo[*a*]pyrene (continued)					150 µg/g body weight	1×	77 weeks (m) 62 weeks (f)	12/53 (23%)[b]	1/57 (2%)[b]		
			Day 42	i.p.	75 µg/g body weight	1×		0/30 (0%)[b]	0/32 (0%)[b]		
					150 µg/g body weight	1×	79 weeks (m)	1/32 (3%)[c]	0/40 (0%)[b]		
	Mice ($B6C3F_1$)	lung	Control	Control	Control	N/A	142 weeks	13/100 (13%)	9/100 (9%)	Both sexes developed lung tumors with higher incidence when treated with BaP at birth than at 15 or 42 days of age ($p<0.05$).	
			Day 1	i.p.	75 µg/g body weight	1×	103 weeks (m) 126 weeks (f)	20/47 (43%)[b]	22/45 (49%)[b]		
					150 µg/g body weight	1×	84 weeks (m) 112 weeks (f)	37/63 (59%)[b]	28/45 (62%)[b]		
			Day 15	i.p.	75 µg/g body weight	1×	103 weeks (m) 122 weeks (f)	15/60 (25%)[b]	18/55 (33%)[b]		
					150 µg/g body weight	1×	82 weeks (m) 101 weeks (f)	20/55 (36%)[b]	18/45 (40%)[b]		
			Day 42	i.p.	75 µg/g body weight	1×	119 weeks (m) 131 weeks (f)	20/55 (36%)[b]	12/47 (26%)[b]		
					150 µg/g body weight	1×	95 weeks (m) 118 weeks (f)	18/47 (38%)[b]	8/46 (17%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
BaP Benzo[*a*]pyrene (continued)	Mice ($C3AF_1$)	lung	Control	Control	None	N/A	142 weeks	60/100 (60%)	50/100 (50%)	Of the two mouse strains tested, $C3AF_1$ mice developed significantly more tumors than did the $B6C3F_1$ mice ($p<0.001$).	Vesselinovitch et al. (1975a)
			Day 1	i.p.	75 µg/g body weight	1×	78 weeks (m) 82 weeks (f)	58/62 (93%)[b]	42/45 (93%)[b]		
					150 µg/g body weight	1×	70 weeks (m) 73 weeks (f)	48/52 (92%)[b]	52/56 (93%)[b]		
			Day 15	i.p.	75 µg/g body weight	1×	87 weeks (m) 98 weeks (f)	52/56 (93%)[b]	46/49 (94%)[b]		
					150 µg/g body weight	1×	75 weeks (m) 79 weeks (f)	50/53 (94%)[b]	52/57 (91%)[b]		
			Day 42	i.p.	75 µg/g body weight	1×	91 weeks (m) 93 weeks (f)	28/30 (93%)[b]	28/32 (87%)[b]		
					150 µg/g body weight	1×	85 weeks (m) 83 weeks (f)	28/32 (87%)[b]	36/40 (90%)[b]		
DBA Dibenzanthracene	Mice (Caracul × P stock)	lung	Control	Control	None	N/A	228 days	1/31 (3.2%)			Law (1940)
			Day 1	i.p.	4 mg per cm^3 vehicle	1×	181 days	24/24 (100%)[b]			
			2 months	s.c.	4 mg per cm^3 vehicle	1×	189 days	2/29 (6.9%)[b]			

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DEN Diethylnitrosamine	Mice ($B6C3F_1$)	liver	Control	Control	Vehicle (0.01 mL trioctanoin/g body weight)	4×	142 weeks (m) 137 weeks (f)	7/98 (7%)	1/100 (1%)	Animals treated as newborns and infants developed significantly more liver tumors than animals that were treated as young adults. Newborns and infant females developed liver tumors at a later age than similarly treated males. Incidences for malignant tumors only.	Vesselinovitch et al. (1984)
			Day 1	i.p. (3-, 6- and 6-day intervals)	1.5 µg/g body weight	4×	67 weeks (m) 90 weeks (f)	37/51 (73%)[b]	45/64 (70%)[b]		
					3 µg/g body weight	4×	65 weeks (m) 80 weeks (f)	40/58 (69%)[b]	44/65 (68%)[b]		
			Day 15		1.5 µg/g body weight	4×	86 weeks (m) 117 weeks (f)	41/57 (72%)[b]	40/71 (56%)[b]		
					3 µg/g body weight	4×	76 weeks (m) 96 weeks (f)	48/69 (70%)[b]	46/62 (74%)[b]		
			Day 42		1.5 µg/g body weight	4×	117 weeks (m) 135 weeks (f)	9/49 (18%)[b]	1/47 (2%)[b]		
					3 µg/g body weight	4×	123 weeks (m) 133 weeks (f)	6/38 (16%)[b]	4/57 (7%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DEN Diethylnitrosamine (continued)	Mice ($C3AF_1$)	liver	Control	Control	Vehicle (0.1 trioctanoin/g body weight)	4×	123 weeks (m) 131weeks (f)	8/99 (8%)	1/97 (1%)	Highest tumor rate when dosed at early ages. Newborns and infant females developed liver tumors at a lower incidence than similarly treated males. + higher for males.	Vesselinovitch et al. (1984)
			Day 1	i.p. (3-, 6- and 6-day intervals)	1.5 µg/g body weight	4×	64 weeks (m) 84 weeks (f)	23/32 (72%)[b]	11/39 (28%)[b]		
					3 µg/g body weight	4×	59 weeks (m) 76 weeks (f)	39/58 (67%)[b]	26/50 (52%)[b]		
			Day 15		1.5 µg/g body weight	4×	82 weeks (m) 102 weeks (f)	22/46 (48%)[b]	8/65 (12%)[b]		
					3 µg/g body weight	4×	74 weeks (m) 94 weeks (f)	35/54 (65%)[b]	22/62 (35%)[b]		
			Day 42		1.5 µg/g body weight	4×	105 weeks (m) 106 weeks (f)	12/56 (22%)[b]	0/53 (0%)[b]		
					3 µg/g body weight	4×	105 weeks (m) 103 weeks (f)	9/57 (16%)[b]	0/56 (0%)[b]		
	Mice ($B6C3F_1$)	lung	Control	Control	Vehicle (0.1 trioctanoin/g body weight)	4×	142 weeks (m) 137 weeks (f)	13/98 (13%)	9/100 (9%)	The mice treated as newborns showed lung tumors earlier than animals exposed at other times. It is not known whether this was due to actual earlier emergence of tumors or	

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DEN Diethylnitrosamine (continued)			Day 1	i.p. (3-, 6- and 6-day intervals)	1.5 µg/g body weight	4×	70 weeks (m) 91 weeks (f)	29/51 (57%)[b]	49/64 (77%)[b]	to their earlier detection caused by shorter survival.	
					3 µg/g body weight	4×	68 weeks (m) 81 weeks (f)	34/58 (59%)[b]	42/65 (65%)[b]		
			Day 15		1.5 µg/g body weight	4×	87 weeks (m) 115 weeks (f)	51/57 (89%)[b]	61/71 (86%)[b]		
					3 µg/g body weight	4×	77 weeks (m) 97 weeks (f)	51/69 (74%)[b]	53/62 (85%)[b]		
			Day 42		1.5 µg/g body weight	4×	123 weeks (m) 129 weeks (f)	38/49 (78%)[b]	38/47 (81%)[b]		
					3 µg/g body weight	4×	121 weeks (m) 127 weeks (f)	33/38 (87%)[b]	43/57 (75%)[b]		
	Mice ($C3AF_1$)	lung	Control	Control	Vehicle (0.1 trioctanoin/g body weight)	4×	142 weeks (m) 137weeks (f)	60/99 (61%)	50/97 (52%)	Of the two strains, $C3AF_1$ mice developed lung tumors with a higher incidence and multiplicity than $B6C3F_1$ hybrids.	
			Day 1	i.p. (3-, 6- and 6-day intervals)	1.5 µg/g body weight	4×	65 weeks (m) 84 weeks (f)	30/32 (94%)[b]	38/39 (97%)[b]		
					3 µg/g body weight	4×	59 weeks (m) 76 weeks (f)	49/58 (84%)[b]	46/50 (92%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DEN Diethylnitrosamine (continued)			Day 15		1.5 µg/g body weight	4×	80 weeks (m) 101 weeks (f)	42/46 (91%)[b]	61/65 (94%)[b]		
					3 µg/g body weight	4×	74 weeks (m) 92 weeks (f)	50/54 (93%)[b]	57/62 (92%)[b]		
			Day 42		1.5 µg/g body weight	4×	104 weeks (m) 110 weeks (f)	55/56 (98%)[b]	52/53 (98%)[b]		
					3 µg/g body weight	4×	101 weeks (m) 102 weeks (f)	56/57 (98%)[b]	54/56 (96%)[b]		
	Mice ($B6C3F_1$)	liver	Control	Control	None	N/A	90 weeks	1/98 (1%)	0/96 (0%)	Infant animals of both sexes (Day 15) were more sensitive than similarly exposed adults.	Vesselinovitch and Mihailovich (1983)
			Gestation day 18	i.p.	1.5 µg/g body weight	1×		2/50 (4%)[b]	1/51 (2%)[b]		
			Day 15	i.p. (3-, 6- and 6-day intervals)	1.5 µg/g body weight	4×		47/51 (92%)[b]	60/64 (94%)[b]		
			Day 42		1.5 µg/g body weight	4×		13/49 (26%)[b]	3/47 (6%)[b]		
			Day 1	i.p.	1.5 µg/g body weight	1×	73 weeks	15/59 (25%)[b]	—	At the 1.5-µg dose level, 1-day-old mice developed significantly fewer liver tumors than similarly treated infants (Day 15) ($p<0.025$). Tumor incidence in treated groups versus controls was not evaluated.	Vesselinovitch et al. (1979a)
					5 µg/g body weight	1×		29/45 (64%)[b]	—		
					10 µg/g body weight	1×		24/25 (96%)[b]	—		
			Day 15	i.p.	1.5 µg/g body weight	1×		13/24 (54%)[b]	—		
					5 µg/g body weight	1×		40/54 (74%)[b]	—		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DEN Diethylnitrosamine (continued)					10 µg/g body weight	1×		25/25 (100%)[b]	—		
DMBA Dimethyl-benz[*a*]anthracene	Rats (Sprague-Dawley)	mammary adeno-sarcoma	Day 20	Gavage	10 mg/100 g body weight	1×	Week 25	—	3/6 (50%)[b]	36 of 42 (86%) animals dosed at age 20 days died soon after. Highest number of tumors per animal was in the 46-day group, with decreasing numbers in the older animals. Animals were sacrificed 22 weeks after treatment.	Russo et al. (1979)
			Day 30		10 mg/100 g body weight	1×	Week 26	—	14/15 (93%)[b]		
			Day 40		10 mg/100 g body weight	1×	Week 27	—	8/9 (89%)[b]		
			Day 46		10 mg/100 g body weight	1×	Week 28	—	8/8 (100%)[b]		
			Day 55		10 mg/100 g body weight	1×	Week 29	—	33/34 (97%)[b]		
			Day 70		10 mg/100 g body weight	1×	Week 32	—	5/8 (63%)[b]		
			Day 140		10 mg/100 g body weight	1×	Week 42	—	10/15 (67%)[b]		
			Day 180		10 mg/100 g body weight	1×	Week 47	—	14/26 (54%)[b]		
	Rats (Wistar)	mammary carcinoma[d]	Control 5–8 weeks	Control	None	N/A	17 months	0/22 (0%)	0/25 (0%)	Highest tumor rate in females exposed at 5–8 weeks. Animals were observed for 16 months following treatment.	Meranze et al. (1969)
			Control 26 weeks	Control	None	N/A	20 months	0/31 (0%)	2/20 (10%)		
			< Week 2	Gavage	0.5–1.0 mg	1×	Week 40–56	0/23 (0%)[b]	4/50 (8%)[b]		
			Week 5–8		15 mg	1×	Week 14–55	0/23 (0%)[b]	14/25 (56%)[b]		
			Week 26		15 mg	1×	Week 32–73	0/34 (0%)[b]	4/26 (15%)[b]		
	Rats (Wistar, castrated)	mammary carcinoma	Week 5–8	Gavage	15 mg	1×	Week 14–55	0/21 (0%)[b]	0/22 (0%)[b]		
			Week 26		15 mg	1×	Week 32–73	0/33 (0%)[b]	0/26 (0%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DMBA Dimethyl-benz[*a*]anthracene (continued)	Rats (Wistar)	Total tumors	Control 5–8 weeks	Control	None	N/A	17 months	0/22 (0%)	0/25 (0%)	Total tumors includes leukemia.	
			Control 26 weeks	Control	None	N/A	20 months	2/31 (6%)	5/20 (25%)		
			< Week 2	Gavage	0.5–1.0 mg	1×	Week 40–56	16/23 (70%)[b]	36/50 (72%)[b]		
			Week 5–8		15 mg	1×	Week 14–55	7/23 (30%)[b]	16/25 (64%)[b]		
			Week 26		15 mg	1×	Week 32–73	12/34 (35%)[b]	13/26 (50%)[b]		
	Mice (BALB/c)	lung	Control: Day 1	Control s.c.	Aqueous gelatine	1×	40 weeks	0/12 (0%)	7/23 (30%)	15 µg DMBA gave rise to a significantly greater incidence of lung tumors when administered to newborn mice than to suckling or young adults.	Walters (1966)
			Day 1	s.c.	15 µg	1×	40 weeks[f]	14/14 (100%)[b]	24/24 (100%)[b]		
			Week 2–3 (suckling)	s.c.	15 µg	1×	42–43 weeks	12/23 (52%)[b]	16/22 (73%)[b]		
				s.c.	30 µg (60 µg total)	2×	42–43 weeks	14/14 (100%)[b]	24/24 (100%)[b]		
			Adult[e]	s.c.	15 µg	1×	48-49 weeks	6/12 (50%)[b]	15/33 (45%)[b]		
				s.c.	30 µg (60 µg total)	2×	48-49 weeks	9/10 (90%)[b]	21/23 (91%)[b]		
				s.c.	30 µg (180 µg total)	6×	48-49 weeks	12/12 (100%)[b]	13/13 (100%)[b]		
	Mice (Swiss)	lymphoma	Control	Control	None	N/A	31–52 weeks	3/408 (0.7%)		Higher tumor rates at younger age of exposure. Only one treatment group was exposed i.p.; others were exposed by s.c. injection..	Pietra et al. (1961)
			Day 1	i.p.	30–40 µg	1×	13–33 weeks	6/31 (19%)[b]			
			Day 1	s.c.	30–40 µg	1×	12–27 weeks	8/27 (30%)[b]			
			Week 8	s.c.	900 µg	1×	30 weeks	1/13 (8%)[b]			

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a] M	Tumors[a] F	Comments	Reference
DMBA Dimethyl-benz[*a*]anthracene (continued)	Mice (Swiss)	lung	Control	Control	None	N/A	31–52 weeks	4/408 (0.9%)			
			Day 1	i.p.	30–40 µg	1×	13–33 weeks	24/31 (77%)[b]			
			Day 1	s.c.	30–40 µg	1×	12–27 weeks	23/27 (85%)[b]			
			Week 8	s.c.	900 µg	1×	30 weeks	2/13 (15%)[b]			
DMN Dimethyl-nitrosamine	Rats (Wistar)	kidney carcinoma	Day 1	i.p.	20 mg/kg	1×	≥5 months	1/33 (3)[b]		In the neonatal group, the dose was reduced to 20 mg/kg to achieve approximately equivalent numbers of survivors. No control group.	Hard (1979)
			Day 21		30 mg/kg	1×		5/39 (13)[b]			
			Month 1		30 mg/kg	1×		2/33 (6)[b]			
			Month 1.5		30 mg/kg	1×		1/28 (4)[b]			
			Month 2		30 mg/kg	1×		1/26 (4)[b]			
			Month 3		30 mg/kg	1×		10/27 (37)[b]			
			Month 4		30 mg/kg	1×		7/32 (22)[b]			
			Month 5		30 mg/kg	1×		0/14 (0)[b]			
	Rats (Wistar)	kidney adenoma	Day 1	i.p.	20 mg/kg	1×	≥5 months	1/33 (3)[b]			
			Day 21		30 mg/kg	1×		13/39 (33)[b]			
			Month 1		30 mg/kg	1×		11/33 (33)[b]			
			Month 1.5		30 mg/kg	1×		13/28 (48)[b]			
			Month 2		30 mg/kg	1×		11/26 (42)[b]			
			Month 3		30 mg/kg	1×		18/27 (67)[b]			
			Month 4		30 mg/kg	1×		17/32 (53)[b]			
			Month 5		30 mg/kg	1×		6/14 (43)[b]			

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
DMN Dimethyl-nitrosamine (continued)	Rats (Wistar)	kidney mesenchymal tumors	Day 1	i.p.	20 mg/kg	1×	≥5 months	8/33 (24)[b]		Mesenchymal tumors were most frequent in the three youngest age groups (z test, $p < 0.001$).	
			Day 21		30 mg/kg	1×		18/39 (46)[b]			
			Month 1		30 mg/kg	1×		23/33 (70)[b]			
			Month 1.5		30 mg/kg	1×		5/28 (19)[b]			
			Month 2		30 mg/kg	1×		2/26 (8)[b]			
			Month 3		30 mg/kg	1×		3/27 (11)[b]			
			Month 4		30 mg/kg	1×		7/32 (22)[b]			
			Month 5		30 mg/kg	1×		0/14 (0)[b]			
	Rats (Wistar)	kidney cortical epithelial tumors	Day 1	i.p.	20 mg/kg	1×	≥5 months	2/33 (6)[b]			Hard (1979)
			Day 21		30 mg/kg	1×		16/39 (41)[b]			
			Month 1		30 mg/kg	1×		12/33 (36)[b]			
			Month 1.5		30 mg/kg	1×		14/28 (52)[b]			
			Month 2		30 mg/kg	1×		11/26 (42)[b]			
			Month 3		30 mg/kg	1×		18/27 (67)[b]			
			Month 4		30 mg/kg	1×		21/32 (66)[b]			
			Month 5		30 mg/kg	1×		6/14 (43)[b]			
	Rats (Wistar)	Total tumors	Day 1	i.p.	20 mg/kg	1×	≥5 months	11/33 (33)[b]			
			Day 21		30 mg/kg	1×		25/39 (64)[b]			
			Month 1		30 mg/kg	1×		25/33 (76)[b]			
			Month 1.5		30 mg/kg	1×		17/28 (63)[b]			
			Month 2		30 mg/kg	1×		13/26 (50)[b]			
			Month 3		30 mg/kg	1×		18/27 (67)[b]			
			Month 4		30 mg/kg	1×		22/32 (69)[b]			
			Month 5		30 mg/kg	1×		7/14 (50)[b]			

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ENU Ethylnitrosourea	Rats	nervous system	Day 1	Injection	20 mg/kg	1×		100%[b]		Susceptibility to neuro-oncogenic effect declined with increasing age.	Maekawa and Mitsumori (1990)
			Day 30	Injection	20 mg/kg	1×		61%[b]			
	Mice ($B6C3F_1$)	liver	Control	Control	None	N/A	90 weeks	1/98 (1%)	0/96 (0%)	Both male and female mice were responsive to exposure during prenatal and infant life.	Vesselinovitch (1983)
			Gestation day 18	i.p.	60 µg/g body weight	1×		28/52 (54%)[b]	18/49 (37%)[b]		
			Day 15		60 µg/g body weight	1×		41/50 (82%)[b]	28/51 (55%)[b]		
			Day 42		60 µg/g body weight	1×		10/50 (20%)[b]	5/50 (10%)[b]		
	Rats (Wistar)	nerve tissue	Control	Control	None	N/A	4–7 months	0/16 (0%)	0/10 (0%)	Highest tumor rate seen when exposed during gestation or soon after birth. Statistically significant decrease in tumor incidence with increasing age of exposure.	Naito et al. (1981)
			Gestation day 16	i.p.	40 mg/kg	1×		26/26 (100%)[b]	18/18 (100%)[b]		
			Day 1	s.c.	40 mg/kg	1×		12/12 (100%)[g]	16/16 (100%)[g]		
			Week 1		40 mg/kg	1×		12/17 (71%)[b]	18/20 (90%)[b]		
			Week 2		40 mg/kg	1×		10/14 (71%)[b]	14/18 (78%)[b]		
			Week 3		40 mg/kg	1×		6/13 (46%)[b]	5/17 (29%)[b]		
			Week 4		40 mg/kg	1×		8/15 (53%)[b]	2/10 (20%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ENU Ethylnitrosourea (continued)	Mice ($B6C3F_1$)	lung	Day 1	i.p.	60 µg/g body weight	1×		49/55 (89%)[b]	49/50 (98%)[b]		Vesselinovitch et al. (1974)
			Day 15			1×		50/55 (91%)[b]	47/55 (85%)[b]		
			Day 42			1×		53/59 (90%)[b]	44/51 (86%)[b]		
			Day 1		120 µg/g body weight	1×		36/38 (95%)[b]	54/60 (90%)[b]		
			Day 15			1×		45/49 (92%)[b]	43/50 (86%)[b]		
			Day 42			1×		52/54 (96%)[b]	50/57 (88%)[b]		
	Mice ($C3AF_1$)	lung	Day 1		60 µg/g body weight	1×		46/47 (98%)[g]	51/51 (100%)[g]		
			Day 15			1×		49/49 (100%)[g]	57/59 (97%)[g]		
			Day 42			1×		59/59 (100%)[g]	57/57 (100%)[g]		
			Day 1		120 µg/g body weight	1×		63/64 (98%)[g]	53/57 (93%)[g]		
			Day 15			1×		54/56 (96%)[g]	50/56 (89%)[g]		
			Day 42			1×		59/59 (100%)[g]	48/48 (100%)[g]		
	Mice ($B6C3F_1$)	liver	Day 1	i.p.	60 µg/g body weight	1×		50/54 (93%)[g]	28/43 (65%)[g]		
			Day 15			1×		55/56 (98%)[g]	33/54 (61%)[g]		
			Day 42			1×		12/40 (30%)[b]	6/39 (15%)[b]		
			Day 1		120 µg/g body weight	1×		29/34 (85%)[g]	32/53 (60%)[g]		
			Day 15			1×		45/48 (94%)[g]	29/43 (67%)[g]		
			Day 42			1×		17/49 (35%)[g]	4/50 (8%)[g]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ENU Ethylnitrosourea (continued)	Mice ($C3AF_1$)	liver	Day 1	i.p.	60 µg/g body weight	1×		42/45 (93%)[g]	19/41 (46%)[g]		
			Day 15			1×		42/50 (84%)[g]	19/48 (40%)[g]		
			Day 42			1×		7/29 (24%)[b]	4/50 (8%)[b]		
			Day 1		120 µg/g body weight	1×		55/62 (89%)[g]	19/45 (42%)[g]		
			Day 15			1×		35/45 (78%)[g]	15/35 (43%)[g]		
			Day 42			1×		8/33 (24%)[b]	3/33 (9%)[b]		
	Mice ($B6C3F_1$)	kidney	Day 1	i.p.	60 µg/g body weight	1×		11/48 (23%)[b]	5/49 (10%)[b]		
			Day 15			1×		6/41 (15%)[b]	7/31 (23%)[b]		
			Day 42			1×		4/40 (10%)[b]	3/37 (8%)[b]		
			Day 1		120 µg/g body weight	1×		10/30 (33%)[g]	14/53 (26%)[b]		
			Day 15			1×		17/37 (46%)[g]	19/49 (39%)[b]		
			Day 42			1×		8/40 (20%)[b]	11/39 (28%)[b]		
	Mice ($C3AF_1$)	kidney	Day 1	i.p.	60 µg/g body weight	1×		7/44 (16%)[b]	6/45 (13%)[b]		
			Day 15			1×		7/41 (17%)[b]	8/46 (17%)[b]		
			Day 42			1×		3/42 (42%)[b]	3/43 (7%)[b]		
			Day 1		120 µg/g body weight	1×		4/52 (7%)[b]	6/29 (21%)[g]		
			Day 15			1×		8/35 (23%)[b]	12/29 (41%)[g]		
			Day 42			1×		6/41 (71%)[b]	3/39 (8%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ENU Ethylnitrosourea (continued)	Mice (B6C3F1)	Harderian	Day 1		60 µg/g body weight	1×		7/40 (17%)[b]	5/43 (12%)[b]		
			Day 15			1×		10/51 (20%)[b]	17/59 (29%)[b]		
			Day 42			1×		14/50 (28%)[b]	14/45 (31%)[b]		
			Day 1		120 µg/g body weight	1×		9/30 (30%)[g]	6/52 (12%)[b]		
			Day 15			1×		15/41 (37%)[g]	8/31 (26%)[b]		
			Day 42			1×		25/48 (52%)[g]	14/49 (29%)[b]		
	Mice ($C3AF_1$)	Harderian	Day 1		60 µg/g body weight	1×		3/25 (12%)[b]	4/35 (11%)[b]		
			Day 15			1×		1/9 (11%)[b]	6/38 (16%)[b]		
			Day 42			1×		12/48 (25%)[b]	5/33 (15%)[b]		
			Day 1		120 µg/g body weight	1×		3/52 (6%)[b]	1/25 (4%)[b]		
			Day 15			1×		6/46 (13%)[b]	2/52 (4%)[b]		
			Day 42			1×		5/29 (17%)[b]	2/11 (18%)[b]		
	Mice ($B6C3F_1$)	stomach	Day 1		60 µg/g body weight	1×		3/48 (6%)[b]	4/43 (9%)[b]		
			Day 15			1×		10/42 (24%)[g]	7/45 (16%)[b]		
			Day 42			1×		9/51 (18%)[g]	8/36 (22%)[b]		
			Day 1		120 µg/g body weight	1×		2/29 (7%)[b]	9/53 (17%)[b]		
			Day 15			1×		10/35 (29%)[g]	12/33 (36%)[b]		
			Day 42			1×		12/53 (23%)[g]	12/50 (24%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
ENU Ethylnitrosourea (continued)	Mice ($C3AF_1$)	stomach	Day 1		60 µg/g body weight	1×		2/39 (5%)[b]	7/45 (16%)[b]		
			Day 15			1×		7/45 (16%)[g]	7/38 (18%)[b]		
			Day 42			1×		14/55 (25%)[g]	7/49 (14%)[b]		
			Day 1		120 µg/g body weight	1×		8/60 (13%)[b]	9/44 (20%)[b]		
			Day 15			1×		16/51 (31%)[g]	11/42 (26%)[b]		
			Day 42			1×		19/48 (40%)[g]	13/37 (35%)[b]		
	Mice ($B6C3F_1$)	malignant lymphomas	Day 1		60 µg/g body weight	1×		2/55 (4%)[b]	6/52 (12%)[g]		
			Day 15			1×		3/56 (5%)[b]	14/59 (24%)[g]		
			Day 42			1×		9/59 (15%)[b]	17/59 (29%)[g]		
			Day 1		120 µg/g body weight	1×		8/39 (20%)[b]	15/65 (23%)[g]		
			Day 15			1×		14/60 (23%)[b]	17/58 (29%)[g]		
			Day 42			1×		12/59 (20%)[b]	14/60 (23%)[g]		
	Mice ($C3AF_1$)	malignant lymphomas	Day 1		60 µg/g body weight	1×		6/49 (12%)[b]	8/49 (16%)[g]		
			Day 15			1×		3/49 (6%)[b]	13/61 (21%)[g]		
			Day 42			1×		6/60 (10%)[b]	9/55 (16%)[g]		
			Day 1		120 µg/g body weight	1×		3/66 (5%)[b]	10/58 (17%)[g]		
			Day 15			1×		10/56 (18%)[b]	18/60 (30%)[g]		
			Day 42			1×		3/49 (6%)[b]	13/50 (26%)[g]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumor incidence[a]		Comments	Reference
								M	F		
NMU Methylnitrosourea	Mice ($BC3F_1$)	Total tumors	Control	Control	N/A	N/A	60 weeks	1/20 (5%)	0%	Control mice did not exhibit tumors in target sites except a single hepatoma in a male control mouse.	Terracini and Testa (1970)
		lung	Day 1	i.p.	50 µg/g body weight	1×	60 weeks	12/15 (80%)[b]	16/19 (84%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	10/26 (39%)[b]	10/35 (29%)[b]		
		lympho-sarcoma	Day 1		50 µg/g body weight	1×	60 weeks	23/39 (59%)[b]	23/45 (51%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	11/35 (31%)[b]	21/45 (47%)[b]		
		liver	Day 1		50 µg/g body weight	1×	60 weeks	10/12 (83%)[b]	1/17 (6%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	0%[b]	0%[c]		
		kidney	Day 1		50 µg/g body weight	1×	60 weeks	3/15 (20%)[b]	3/18 (17%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	2/21 (10%)[b]	0%[c]		
		fore-stomach	Day 1		50 µg/g body weight	1×	60 weeks	0%[b]	4/17 (24%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	8/22 (36%)[b]	12/18 (67%)[b]		
	Rats (Wistar)	mammary	Day 1	i.p.	50 µg/g body weight	1×	60 weeks	0%[b]	4/14 (29%)[b]	Tumor incidence for control rats was based on previous experiments (Della Porta et al., 1968) and was not specifically reported in this paper.	Terracini and Testa (1970)
			5 weeks		50 µg/g body weight	1×	60 weeks	0%[b]	3/5 (60%)[b]		
		lympho-sarcoma	Day 1		50 µg/g body weight	1×	60 weeks	1/10 (10%)[b]	0%[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	2/8 (25%)[b]	1/11 (9%)[b]		
		kidney (ana-plastic)	Day 1		50 µg/g body weight	1×	60 weeks	14/18 (78%)[b]	9/13 (69%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	2/5 (40%)[b]	5/12 (42%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumor incidence[a]		Comments	Reference
								M	F		
NMU Methylnitrosourea (continued)		kidney (adenoma)	Day 1		50 µg/g body weight	1×	60 weeks	3/14 (21%)[b]	2/6 (33%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	¼ (25%)[b]	0%[b]		
		forestomach	Day 1		50 µg/g body weight	1×	60 weeks	4/14 (29%)[b]	3/6 (50%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	0%[c]	0%[b]		
		intestine	Day 1		50 µg/g body weight	1×	60 weeks	3/10 (30%)[b]	2/2 (100%)[b]		
			5 weeks		50 µg/g body weight	1×	60 weeks	2/4 (50%)[b]	0%[b]		
	Mice (C3Hf/Dp)	thymus	control	i.p.	NA	NA	120 wks[**]	0/34 (0%)	0/25 (0%)	[*]Age at death from thymic lymphoma reported specifically for some, but not all, dose groups. [**]Control mice were sacrificed at 120 wks. [***]Age of death for all mice in this dose group, regardless of cancer type.	Terracini et al. (1976)
			Day 1		25 µg NMU/g body weight	1×	29 ± 8.4 wks	2/16 (13%)[b]	5/25 (20%)[b]		
			Day 70		25 µg NMU/g body weight	1×	120 wks (M)[***] 100 wks (F)	0/20 (0%)[c]	1/20 (5%)[b]		
			Day 1		50 µg NMU/g body weight	1×	16.5 ± 0.7 wks	16/24 (67%)[b]	30/44 (68%)[b]		
			Day 21		50 µg NMU/g body weight	1×	24.5 ± 2.5 wks	14/44 (32%)[b]	18/38 (47%)[b]		
			Day 70		50 µg NMU/g body weight	1×	31.4 ± 4.4 wks	9/30 (30%)[b]	6/41 (15%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death		Tumor incidence		Reference
							M	F	M	F	
NMU Methylnitrosourea (continued)	Mice (C3Hf/Dp)	extra-thymic lymphoma	control	i.p.	NA	NA	120 weeks	120 weeks	1/34 (3%)	2/25 (8%)	Terracini et al. (1976)
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	2/16 (13%)[b]	1/25 (4%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	0/20 (0%)[b]	0/20 (0%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	0/24 (0%)[b]	0/44 (0%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	1/44 (2%)[b]	0/38 (0%)[b]	
			Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	1/30 (3%)[b]	0/41 (0%)[b]	
		lung	control	i.p.	NA	NA	120 weeks	120 weeks	4/34 (12%)	6/25 (24%)	
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	7/16 (44%)[b]	13/25 (52%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	12/20 (60%)[b]	8/20 (40%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	5/24 (21%)[b]	11/44 (25%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	23/44 (52%)[b]	15/38 (39%)[b]	
			Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	18/30 (60%)[b]	24/41 (59%)[b]	
		liver	control	i.p.	NA	NA	120 weeks	120 weeks	13/34 (38%)	1/25 (4%)	Terracini et al. (1976)
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	9/16 (56%)[g]	2/25 (8%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	12/20 (60%)[g]	2/20 (10%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	4/24 (17%)[g]	3/44 (7%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	21/44 (48%)[g]	1/38 (2.6%)[b]	

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death		Tumor incidence		Reference
							M	F	M	F	
NMU Methylnitrosourea (continued)	Mice (C3Hf/Dp)		Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	8/30 (27%)[g]	2/41 (5%)[b]	
		stomach	control	i.p.	NA	NA	120 weeks	120 weeks	0/34 (0%)	5/25 (20%)	
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	2/16 (13%)[b]	10/25 (40%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	3/20 (15%)[b]	7/20 (35%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	2/24 (8%)[b]	1/44 (2%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	19/44 (43%)[b]	9/38 (24%)[b]	
			Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	8/30 (27%)[b]	21/41 (51%)[b]	
		kidney	control	i.p.	NA	NA	120 weeks	120 weeks	0/34 (0%)	0/25 (0%)	Terracini et al. (1976)
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	0/16 (0%)[b]	0/25 (0%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	0/20 (0%)[b]	0/20 (0%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	0/24 (0%)[b]	4/44 (9%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	1/44 (2%)[b]	4/38 (11%)[b]	
			Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	5/30 (17%)[b]	7/41 (17%)[b]	

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death		Tumor incidence		Reference
							M	F	M	F	
NMU Methylnitrosourea (continued)	Mice (C3Hf/Dp)	ovary	control	i.p.	NA	NA	120 weeks	120 weeks	NA	3/25 (12%)	
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	NA	2/25 (8%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	NA	4/20 (20%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	NA	0/44 (0%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	NA	9/38 (24%)[b]	
			Day 70		50 µg NMU/g body weight	1×	110 weeks	90 weeks	NA	16/41 (39%)[b]	
		mammary	control	i.p.	NA	NA	120 weeks	120 weeks	NA	2/25 (8%)	Terracini et al. (1976)
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	NA	1/25 (4%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	NA	0/20 (0%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	NA	0/44 (0%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	1/44 (2%)[b]	0/38 (0%)[b]	
			Day 70		50 µg NMU/g body weight		110 weeks	90 weeks	NA	4/41 (9.8%)[b]	
		uterus or vagina	control	i.p.	NA	NA	120 weeks	120 weeks	NA	1/25 (4%)	
			Day 1		25 µg NMU/g body weight	1×	100 weeks	90 weeks	NA	1/25 (4%)[b]	
			Day 70		25 µg NMU/g body weight	1×	120 weeks	100 weeks	NA	6/20 (30%)[b]	
			Day 1		50 µg NMU/g body weight	1×	70 weeks	80 weeks	NA	0/44 (0%)[b]	
			Day 21		50 µg NMU/g body weight	1×	100 weeks	90 weeks	NA	1/38 (3%)[b]	
			Day 70		50 µg NMU/g body weight		110 weeks	90 weeks		7/41 (17%)[b]	

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Urethane	Mice (SWR)	lung adenoma	Newborn	s.c.	0.18 mg/g body weight	1×	10 weeks	100%[b]		The average number of tumors per mouse increased linearly with dose.	Kaye and Trainin (1966)
			11–22 weeks	s.c.	0.25 mg/g body weight	1×	23–34 weeks	0%[b]			
	Mice (C3H/f)	liver	Control	Control	None	N/A	493 days (m) 553 days (f)	14/97 (14%)	1/77 (1%)		Liebelt et al. (1964)
			Day 1	i.p.	0.8 mg/g body weight	1×	481 days (m) 434 days (f)	27/30 (90%)[g]	18/39 (46%)[g]		
			8–10 weeks	i.p.	1 mg/g body weight	1×	321 days (m) -	6/25 (24%)[c]	0/32 (0%)[c]		
		lung	Control	Control	None	N/A	493 days (m) 553 days (f)	0/97 (0%)	0/77 (0%)	The number of lung tumors among the controls was not provided.	
			Day 1	i.p.	0.8 mg/g body weight	1×	401 days (m) 408 days (f)	14/30 (46%)[g]	19/39 (48%)[g]		
			8–10 weeks	i.p.	1 mg/g body weight	1×	506 days (m) -	2/25 (8%)[c]	0/32 (0%)[c]		
		reticular tissue	Control	Control	None	N/A	493 days (m) 553 days (f)	2/97 (2%)	6/77 (8%)		
			Day 1	i.p.	0.8 mg/g body weight	1×	285 days (m) 343 days (f)	4/30 (13%)[c]	22/39 (56%)[g]		
			8–10 weeks	i.p.	1 mg/g body weight	1×	- 453 days (f)	0/25 (25%)[c]	4/32 (13%)[c]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Urethane (continued)	Mice (Swiss)	leukemia	Control	Control	None	N/A	8–10 months	1%		Highest tumor rates when dosed at birth. Exposure to newborns was followed by 21.6% leukemia, occurring at a mean age of 105 days.	Fiore-Donati et al. (1962)
			Day 1	s.c.	2 mg in 0.05 mL aqueous solution	1×		13/60 (22%)[b]			
			Day 5		4 mg in 0.05 mL aqueous solution	1×		7/39 (18%)[b]			
			Day 40		20 mg in 0.1 mL aqueous solution	1×		2/63 (3%)[b]			
	Mice (Swiss)	lung adenoma	Control 2 weeks	Control	None	N/A	9 weeks	0/15 (0%)	—	The proportion of animals with adenomas decreased steadily with age of exposure.	Rogers (1951)
			Control 4 weeks	Control	None	N/A	11 weeks	0/14 (0%)	—		
			Control 6 weeks	Control	None	N/A	13 weeks	1/15 (7%)	—		
			Control 8 weeks	Control	None	N/A	15 weeks	2/15 (13%)	—		
			Control 10 weeks	Control	None	N/A	17 weeks	0/15 (0%)	—		
			2 weeks	i.p.	1 mg/g body weight	1×	9 weeks	24/24 (100%)[b]	—		
			4 weeks	i.p.	1 mg/g body weight	1×	11 weeks	23/25 (92%)[b]	—		
			6 weeks	i.p.	1 mg/g body weight	1×	13 weeks	22/25 (88%)[b]	—		
			8 weeks	i.p.	1 mg/g body weight	1×	15 weeks	21/25 (84%)[b]	—		
			10 weeks	i.p.	1 mg/g body weight	1×	17 weeks	19/25 (76%)[b]	—		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumors[a]		Comments	Reference
								M	F		
Urethane (continued)	Mice (Swiss)	lung adenoma	3 weeks	i.p.	0.25 mg/g body weight	1×	12 weeks	16/19 (84%)[b]	—		
					0.5 mg/g body weight	1×	12 weeks	16/20 (80%)[b]	—		
					1 mg/g body weight	1×	12 weeks	18/20 (90%)[b]	—		
			8 weeks	i.p.	0.25 mg/g body weight	1×	17 weeks	4/17 (24%)[b]	—		
					0.5 mg/g body weight	1×	17 weeks	15/16 (94%)[b]	—		
					1 mg/g body weight	1×	17 weeks	18/18 (100%)[b]	—		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumor incidence[a]		Comments	Reference
								M	F		
Urethane (continued)	Mice (Swiss)	liver	Control	Control	N/A	N/A	360–720 days	10/227 (4.4%)	4/222 (8.22%)		Chieco-Bianchi et al. (1963)
			Day 1	s.c.	1 mg/g body weight	1×	180 days	1/20 (5%)[g]	0/20 (0%)[c]		
			Day 1	s.c.	1 mg/g body weight	1×	240 days	2/17 (12%)[g]	0/12 (0%)[c]		
			Day 1	s.c.	1 mg/g body weight	1×	300 days	5/18 (28%)[g]	0/16 (0%)[c]		
			Day 1	s.c.	1 mg/g body weight	1×	360 days	11/20 (55%)[g]	0/23 (0%)[c]		
			Day 1	s.c.	1 mg/g body weight	1×	420 days	13/15 (87%)[g]	2/22 (9%)[g]		
			Day 1	s.c.	1 mg/g body weight	1×	480 days	17/23 (74%)[c]	2/25 (8%)[c]		
			Day 5	s.c.	1 mg/g body weight	1×	420 days	9/13 (69.2%)[b]	2/11 (18.2%)[b]		
			Day 20	s.c.	1 mg/g body weight	1×	420 days	1/13 (8%)[b]	0/16 (0%)[b]		
			Day 40	s.c.	1 mg/g body weight	1×	420 days	0/11 (0%)[b]	0/9 (0%)[b]		
	Mice (Swiss)	skin	Control	Control	N/A	N/A	180–550 days	30/712 (4.21%)		Croton oil treatment initiated at 40 days of age.	Chieco-Bianchi et al. (1963)
			Day 1	s.c.	1 mg urethane/g body weight; 5% croton oil	single dose urethane, croton oil applied 2×/week for 10 mos	660 days	26/59 (44.1%)[g]			

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumor incidence[a]		Comments	Reference
								M	F		
Urethane (continued)			Day 40	s.c.	1 mg urethane/g body weight; 5% croton oil	single dose urethane, croton oil applied 2×/week for 10 mos	700 days	8/41 (19.5%)[b]			
	Mice ($B6AF_1/J$)	liver	Control	gavage	N/A	N/A	71 weeks	1/25 (4%)	0/25 (0%)		Klein (1966)
			Day 1		1 mg/g body weight	1×	66 weeks	9/20 (45%)[g]	9/26 (35%)[g]		
			Day 7		1 mg/g body weight	1×	67 weeks	20/22 (91%)[g]	20/26 (77%)[g]		
			Day 14		1 mg/g body weight	1×	68 weeks	16/20 (80%)[g]	10/23 (43%)[g]		
			Day 21		1 mg/g body weight	1×	69 weeks	13/23 (57%)[g]	1/20 (5%)[g]		
			Day 28		1 mg/g body weight	1×	70 weeks	4/24 (17%)[g]	1/20 (5%)[g]		
		lung	Control	gavage	1 mg/g body weight	1×	71 weeks	9/25 (36%)	6/25 (24%)		
			Day 1		1 mg/g body weight	1×	66 weeks	20/20 (100%)[b]	25/26 (96%)[b]		
			Day 7		1 mg/g body weight	1×	67 weeks	22/22 (100%)[b]	26/26 (100%)[b]		
			Day 14		1 mg/g body weight	1×	68 weeks	19/20 (95%)[b]	19/23 (83%)[b]		
			Day 21		1 mg/g body weight	1×	69 weeks	23/23 (100%)[b]	19/20 (95%)[b]		
			Day 28		1 mg/g body weight	1×	70 weeks	24/24 (100%)[b]	20/20 (100%)[b]		
	Mice ($B6AF_1/J$)	Harderian gland	Control	gavage	1 mg/g body weight	1×	71 weeks	0/25 (0%)	0/25 (0%)		Klein (1966)
			Day 1		1 mg/g body weight	1×	66 weeks	0/20 (0%)[c]	1/26 (4%)[b]		

Table 3. Methodological information and tumor incidence for animal studies with early postnatal and juvenile and adult acute exposure (continued)

Chemical	Species (strain)	Target site	Age when first dosed	Dose route, # doses	Dose	Duration of exposure	Age at death	Tumor incidence[a]		Comments	Reference
								M	F		
Urethane (continued)			Day 7		1 mg/g body weight	1×	67 weeks	0/22 (0%)[c]	1/26 (4%)[b]		
			Day 14		1 mg/g body weight	1×	68 weeks	0/20 (0%)[c]	2/23 (9%)[b]		
			Day 21		1 mg/g body weight	1×	69 weeks	1/23 (4%)[b]	0/20 (0%)[c]		
			Day 28		1 mg/g body weight	1×	70 weeks	0/24 (0%)[c]	0/20 (0%)[c]		
		forestomach	Control	gavage	1 mg/g body weight	1×	71 weeks	0/25 (0%)	1/25 (4%)		
			Day 1		1 mg/g body weight	1×	66 weeks	0/20 (0%)[c]	3/26 (12%)[b]		
			Day 7		1 mg/g body weight	1×	67 weeks	1/22 (5%)[b]	1/26 (4%)[b]		
			Day 14		1 mg/g body weight	1×	68 weeks	1/20 (5%)[b]	4/23 (17%)[b]		
			Day 21		1 mg/g body weight	1×	69 weeks	0/23 (0%)[c]	1/20 (5%)[b]		
			Day 28		1 mg/g body weight	1×	70 weeks	2/24 (8%)[b]	1/20 (5%)[b]		

[a] Where not delineated by gender, data combined by study authors or gender not specified. Where percentages only are given, number of subjects not specified.
[b] Not evaluated by authors.
[c] Evaluated but not significant compared with controls.
[d] Study also included mammary fibroadenomas and fibromas as well as other types of cancers.
[e] 8–9 weeks old.
[f] Includes survivors up to 40 weeks only.
[g] Significant compared with controls.

i.p. = intraperitoneal injection; s.c. = subcutaneous injection

Table 4. Ratio of early-life to adult cancer potencies for studies with repeated exposures of juvenile and adult animals to carcinogens with a mutagenic mode of action*

Compound	Species (strain)	Sex	Dose	Tumor	Unweighted geometric mean	2.5%	Median	97.5%	Reference
Benzidine	Mice ($B6C3F_1$)	male		liver	111	64	**110**	198	Vesselinovitch et al. (1975b)
		female		liver	0.16	0.004	**0.22**	1.1	
3-MU 3-Methylcholanthrene (formerly known as 20-methylcholanthrene)	Mice (Albino)	male	0.25 mg/g	hepatoma	33	7.4	**30**	268	Klein (1959)
		female	0.25 mg/g	hepatoma	7.7	1.1	**7.1**	85	
		male	0.25 mg/g	forestomach	0.91	0.39	**0.91**	2.1	
		female	0.25 mg/g	forestomach	1.5	0.58	**1.5**	4.2	
		male	0.25 mg/g	skin	1.8	0.048	**2.1**	22	
		female	0.25 mg/g	skin	1.5	0.023	**1.8**	21	
Safrole	Mice ($B6C3F_1$)	male		liver	47	16	**44**	198	Vesselinovitch et al. (1979b)
		female		liver	0.12	0.002	**0.18**	1.1	
VC Vinyl chloride	Rats (Sprague-Dawley)	male	6,000 ppm	liver-angiosarcoma	6.7	0.035	**9.8**	57	Maltoni et al. (1984)
		male	10,000 ppm	liver-angiosarcoma	7.4	0.035	**11**	62	
		female	6,000 ppm	liver-angiosarcoma	13	4.9	**13**	33	
		female	10,000 ppm	liver-angiosarcoma	30	8.7	**29**	121	
		male	6,000 ppm	zymbal gland	0.73	0.0032	**1.1**	30	
		male	10,000 ppm	zymbal gland	0.27	0.0022	**0.4**	5.4	
		female	6,000 ppm	zymbal gland	0.48	0.0027	**0.7**	16	
		female	10,000 ppm	zymbal gland	0.15	0.0014	**0.19**	4.5	
		male	10,000 ppm	leukemia	21	0.026	**37**	514	
		female	6,000 ppm	leukemia	1.3	0.0035	**1.7**	153	
		female	10,000 ppm	leukemia	0.29	0.0019	**0.35**	17	
		male	6,000 ppm	nephroblastomas	0.15	0.0014	**0.19**	4.8	
		male	10,000 ppm	nephroblastomas	0.17	0.0015	**0.21**	6.2	
		female	6,000 ppm	nephroblastomas	0.28	0.0018	**0.33**	16	
		female	10,000 ppm	nephroblastomas	0.24	0.0017	**0.29**	11	
		male	6,000 ppm	angiosarcomas-other sites	0.9	0.0033	**1.26**	53	
		male	10,000 ppm	angiosarcomas-	0.25	0.0017	**0.30**	12	

Table 4. Ratio of early-life to adult cancer potencies for studies with repeated exposures of juvenile and adult animals to mutagenic chemicals (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Unweighted geometric mean	2.5%	Median	97.5%	Reference
				other sites					
VC Vinyl chloride (continued)		female	6,000 ppm	angiosarcomas-other sites	0.24	0.0017	**0.29**	11	
		female	10,000 ppm	angiosarcomas-other sites	0.32	0.0019	**0.38**	20	
		male	6,000 ppm	angiomas & fibromas-other sites	0.72	0.0031	**1.0**	33	
		male	10,000 ppm	angiomas & fibromas-other sites	1.4	0.0045	**2.36**	47	
		female	6,000 ppm	angiomas & fibromas-other sites	0.27	0.0018	**0.33**	16	
		female	10,000 ppm	angiomas & fibromas-other sites	0.52	0.0024	**0.63**	41	
		male	6,000 ppm	hepatoma	62	11	**58**	543	
		male	10,000 ppm	hepatoma	34	8.2	**32**	218	
		female	6,000 ppm	hepatoma	55	13	**51**	352	
		female	10,000 ppm	hepatoma	55	8.4	**53**	513	
		male	6,000 ppm	skin carcinomas	1.1	0.0035	**1.5**	82	
		male	10,000 ppm	skin carcinomas	0.41	0.0024	**0.56**	15	
		female	6,000 ppm	skin carcinomas	0.46	0.0024	**0.59**	24	
		female	10,000 ppm	skin carcinomas	0.31	0.0019	**0.37**	19	
		male	6,000 ppm	neuroblastoma	0.21	0.0016	**0.26**	9.5	
		male	10,000 ppm	neuroblastoma	0.20	0.0016	**0.24**	8.5	
		female	6,000 ppm	neuroblastoma	0.27	0.0018	**0.32**	15	
		female	10,000 ppm	neuroblastoma	0.14	0.0014	**0.18**	4.4	

* The 2.5% and 97.5% are percentiles of the posterior distribution. For a Bayesian distribution, these percentiles function in a manner similar to the 95% confidence limits for other types of statistical analyses.

Table 5. Ratio of early-life to adult cancer potencies for studies with repeated exposures of juvenile and adult animals to chemicals with a nonmutagenic mode of action*

Compound	Species (strain)	Sex	Dose	Tumor	Ratio of juvenile to adult potency				Reference
					Unweighted geometric mean	2.5%	Median	97.5%	
Amitrole	Mice ($B6C3F_1$)	male	NA	liver	13	5.1	**14**	30	Vesselinovitch (1983)
		female	NA	liver	0.14	0.0013	**0.18**	3.9	
DDT	Mice ($B6C3F_1$)	male	NA	liver	1.3	0.0044	**2.5**	25	Vesselinovitch et al. (1979a)
Dieldrin	Mice ($B6C3F_1$)	male	NA	liver	0.75	0.0031	**1.2**	27	Vesselinovitch et al. (1979a)
DPH	Rats (F344/N)	male	630	liver	0.4	0.0024	**0.54**	16	Chhabra et al. (1993b)
		female	630	liver	0.24	0.0017	**0.29**	12	
	Mice ($B6C3F_1$)	male	210	liver	1.5	0.0040	**2.4**	71	
		female	210	liver	1.3	0.0056	**2.6**	15	
ETU	Rats (F344/N)	male	90	thyroid	0.37	0.0029	**0.61**	5.4	Chhabra et al. (1992)
		female	90	thyroid	0.23	0.0018	**0.3**	7.0	
	Mice ($B6C3F_1$)	male	330	liver	0.091	0.0011	**0.12**	1.9	
		female	330	liver	0.057	0.0010	**0.081**	0.65	
		male	330	thyroid	0.41	0.0022	**0.52**	25	
		female	330	thyroid	0.4	0.0024	**0.55**	16	
		male	330	pituitary	0.32	0.0019	**0.38**	22	
		female	330	pituitary	0.24	0.0018	**0.32**	6.9	
PBB	Rats (F344/N)	male	10	liver	0.59	0.0041	**1.1**	6.6	Chhabra et al. (1993a)
		female	10	liver	0.063	0.0009	**0.079**	1.2	
		male	10	mononuclear cell leukemia	0.79	0.0035	**1.4**	18	
		female	10	mononuclear cell leukemia	0.21	0.0017	**0.28**	6.0	
	Mice ($B6C3F_1$)	male	30	liver	3.9	1.9	**3.9**	7.5	
		female	30	liver	1.0	0.37	**1.05**	2.1	

* The 2.5% and 97.5% are percentiles of the posterior distribution. For a Bayesian distribution, these percentiles function in a manner similar to the 95% confidence limits for other types of statistical analyses.

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action*

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
BaP*	Mice ($B6C3F_1$)	male	75 µg/kg	liver	1 day	9.3	2.9	**8.4**	55	Vesselinovitch et al. (1975a)
					15 days	11	3.5	**9.6**	61	
		female	75 µg/kg		1 day	1.2	0.0083	**1.6**	31	
					15 days	1.7	0.015	**2.1**	36	
		male	150 µg/kg		1 day	29	8.2	**26**	194	
					15 days	15	4.1	**13**	109	
		female	150 µg/kg		1 day	8.8	1.4	**8.1**	94	
					15 days	1.2	0.0082	**1.6**	30	
	Mice ($C3AF_1$)	male	75 µg/kg	liver	1 day	11	2.1	**10**	112	
					15 days	7.5	1.1	**7.0**	83	
		female	75 µg/kg		1 day	0.2	0.0018	**0.26**	9.1	
					15 days	0.2	0.0017	**0.24**	8.5	
		male	150 µg/kg		1 day	14	3.0	**12.8**	130	
					15 days	3.6	0.11	**3.8**	49	
		female	150 µg/kg		1 day	0.2	0.0017	**0.24**	8.8	
					15 days	0.2	0.0017	**0.24**	8.7	
	Mice ($B6C3F_1$)	Male	75 µg/kg	lung	1 day	1.2	0.45	**1.2**	3.4	
					15 days	0.2	0.0046	**0.31**	1.4	
		female	75 µg/kg	lung	1 day	2.8	1.096	**2.7**	9.5	
					15 days	1.4	0.41	**1.4**	5.1	
		Male	150 µg/kg	lung	1 day	2.2	1.0	**2.1**	5.4	
					15 days	0.8	0.2	**0.82**	2.3	
		female	150 µg/kg	lung	1 day	7.9	2.6	**7.2**	43	
					15 days	3.7	1.1	**3.4**	22	
	Mice ($C3AF_1$)	male	75 µg/kg	lung	1 day	1.2	0.47	**1.2**	3.2	
					15 days	1.1	0.43	**1.08**	3.1	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
BaP* (continued)		female	75 µg/kg	lung	1 day	1.6	0.66	**1.55**	4.0	
					15 days	1.6	0.71	**1.63**	4.2	
		male	150 µg/kg	lung	1 day	1.5	0.57	**1.5**	5.0	
					15 days	1.9	0.71	**1.8**	6.0	
		female	150 µg/kg	lung	1 day	1.3	0.61	**1.3**	2.9	
					15 days	1.2	0.54	**1.1**	2.6	
DBA	Mice			lung		178	20	**143**	5100	Law (1940)
DEN**	Mice ($B6C3F_1$)	male	6 µg/kg	liver	1 day	9.0	3.5	**8.3**	37	Vesselinovitch et al. (1984)
					15 days	8.9	3.5	**8.2**	36	
		female	6 µg/kg	liver	1 day	35	9.1	**31**	239	
					15 days	25	6.3	**226**	175	
		male	12 µg/kg	liver	1 day	9.6	3.3	**8.8**	50	
					15 days	9.8	3.4	**8.9**	51	
		female	12 µg/kg	liver	1 day	16	5.9	**15**	67	
					15 days	19	7.1	**18**	79	
	Mice ($C3AF_1$)	male	6 µg/kg	liver	1 day	7.3	2.9	**6.9**	26	
					15 days	3.5	1.4	**3.3**	13	
		female	6 µg/kg	liver	1 day	17	3.2	**16**	166	
					15 days	6.4	0.86	**6.0**	73	
		male	12 µg/kg	liver	1 day	11	3.7	**9.5**	53	
					15 days	9.8	3.4	**8.9**	50	
		female	12 µg/kg	liver	1 day	40	8.5	**36**	340	
					15 days	25	5.0	**22**	221	
	Mice ($B6C3F_1$)	male	6 µg/kg	lung	1 day	0.5	0.27	**0.52**	0.93	
					15 days	1.6	0.95	**1.6**	2.7	
		female	6 µg/kg	lung	1 day	0.9	0.54	**0.89**	1.5	
					15 days	1.2	0.76	**1.2**	2.0	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
DEN** (continued)		male	12 µg/kg	lung	1 day	0.4	0.21	**0.40**	0.73	
					15 days	0.7	0.39	**0.66**	1.1	
		female	12 µg/kg	lung	1 day	0.7	0.44	**0.73**	1.2	
					15 days	1.4	0.88	**1.4**	2.3	
	Mice ($C3AF_1$)	male	6 µg/kg	lung	1 day	0.7	0.22	**0.67**	1.7	
					15 days	0.5	0.21	**0.56**	1.3	
		female	6 µg/kg	lung	1 day	1.1	0.45	**1.1**	2.5	
					15 days	0.7	0.36	**0.74**	1.5	
		male	12 µg/kg	lung	1 day	0.3	0.084	**0.33**	0.76	
					15 days	0.6	0.26	**0.62**	1.4	
		female	12 µg/kg	lung	1 day	0.7	0.35	**0.75**	1.6	
					15 days	0.7	0.37	**0.75**	1.5	
DMBA#	Rats (Wistar)	male		total	2 vs 5–8 wks	3.3	1.3	**3.2**	10	Meranze et al. (1969)
					2 vs 26 wks	3.2	1.3	**3.1**	9.7	
		female		total	2 vs 5–8 wks	1.3	0.68	**1.3**	2.5	
					2 vs 26 wks	3.3	1.2	**3.0**	16	
				mammary	2 vs 5–8 wks	0.0	0.0012	**0.056**	0.26	
					2 vs 26 wks	0.2	0.0023	**0.29**	5.3	
					5 vs 26 wks	7.1	1.8	**6.4**	55	
	Mice (Balb/c)	male	15 µg	lung	1 day	30	2.8	**22**	1482	Walters (1966)
					15–19 days	1.0	0.28	**1.0**	3.5	
		male	30 µgx2	lung	15–19 days	14	1.056	**10**	978	
		female	15 µg	lung	1 day	60	6.0	**46**	2350	
					15–19 days	3.1	0.51	**3.0**	22	
		female	30 µgx2	lung	15–19 days	15	1.2	**11**	1004	
	Mice (Swiss)			lymphoma		2.7	0.60	**2.5**	19	Pietra et al. (1961)
				lung		9.1	2.9	**8.7**	40	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
DMN***	Rats (Wistar)		3 wks	total	1 month	0.7	0.41	**0.73**	1.3	Hard (1979)
					1.5 months	1.1	0.58	**1.1**	2.1	
					2 months	1.5	0.75	**1.5**	3.0	
					3 months	0.9	0.50	**0.94**	1.8	
			24 hr		1 month	0.3	0.13	**0.28**	0.6	
					1.5 months	0.4	0.18	**0.42**	0.9	
					2 months	0.6	0.24	**0.56**	1.3	
					3 months	0.4	0.16	**0.36**	0.78	
			1 month		1.5 months	1.5	0.80	**1.52**	3.0	
					2 months	2.0	1.0	**2.0**	4.2	
					3 months	1.3	0.69	**1.3**	2.5	
ENU	Mice ($B6C3F_1$)	male		liver		7.8	3.9	**7.7**	18	Vesselinovitch (1983)
		female				7.1	2.9	**6.9**	21	
	Rats (Wistar)	male		nerve tissue	1 day	27	2.5	**20**	1374	Naito et al. (1981)
					1 week	1.6	0.61	**1.6**	4.6	
					2 weeks	1.6	0.58	**1.6**	4.8	
					3 weeks	0.7	0.12	**0.72**	2.3	
		female			1 day	64	6.0	**50**	2488	
					1 weeks	9.6	2.6	**8.9**	59	
					2 weeks	6.2	1.6	**5.7**	40	
					3 weeks	0.7	0.0090	**0.89**	8.9	
	Mice ($B6C3F_1$)	male	60 µg/g	lung	1	1.0	0.60	**1.0**	1.7	Vesselinovitch et al. (1974)
					15	1.1	0.66	**1.1**	1.8	
		female	60 µg/g	lung	1	2.1	1.17	**2.1**	4.1	
					15	1.0	0.60	**1.0**	1.7	
		male	120 µg/g	lung	1	1.0	0.60	**1.0**	1.7	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
					15	1.1	0.66	**1.0**	1.8	
ENU (continued)		female	120 µg/g	lung	1	2.1	1.2	**2.1**	4.1	
					15	1.0	0.60	**1.0**	1.7	
	Mice ($C3AF_1$)	male	60 µg/g	lung	1	8.7	2.7	**8.0**	48	
					15	52	5.2	**39**	2141	
		female	60 µg/g	lung	15	0.7	0.32	**0.72**	1.6	
		male	120 µg/g	lung	1	0.9	0.38	**0.92**	2.2	
					15	0.7	0.28	**0.67**	1.6	
		female	120 µg/g	lung	1	0.5	0.24	**0.54**	1.2	
					15	0.4	0.18	**0.42**	0.92	
	Mice ($B6C3F_1$)	male	60 µg/g	liver	1	8.8	4.2	**8.5**	22	
					15	14	6.2	**14**	37	
		female	60 µg/g	liver	1	6.3	2.6	**6.1**	18	
					15	5.6	2.4	**5.4**	16	
		male	120 µg/g	liver	1	5.2	2.5	**5.1**	11	
					15	7.6	3.9	**7.5**	17	
		female	120 µg/g	liver	1	11	4.1	**11**	46	
					15	14	4.9	**13**	55	
	Mice ($C3AF_1$)	male	60 µg/g	liver	1	12	4.7	**11**	43	
					15	8.1	3.2	**7.6**	29	
		female	60 µg/g	liver	1	7.5	2.6	**7.0**	32	
					15	4.8	1.8	**4.6**	18	
		male	120 µg/g	liver	1	9.8	4.1	**9.3**	32	
					15	6.6	2.7	**6.3**	23	
		female	120 µg/g	liver	1	5.4	1.7	**5.0**	25	
					15	5.4	1.7	**5.1**	25	
	Mice ($B6C3F_1$)	male	60 µg/g	kidney	1	2.2	0.73	**2.1**	8.0	
					15	1.2	0.29	**1.2**	5.1	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
		female	60 µg/g	kidney	1	0.7	0.024	**0.85**	5.9	
					15	2.6	0.61	**2.5**	15	
ENU (continued)		male	120 µg/g	kidney	1	1.7	0.65	**1.7**	4.4	
					15	2.6	1.14	**2.6**	6.4	
		female	120 µg/g	kidney	1	0.9	0.37	**0.87**	2.0	
					15	1.4	0.67	**1.4**	3.2	
	Mice ($C3AF_1$)	male	60 µg/g	kidney	1	1.8	0.17	**1.9**	15	
					15	2.0	0.25	**2.0**	16	
		female	60 µg/g	kidney	1	1.0	0.016	**1.3**	13	
					15	2.1	0.16	**2.2**	20	
		male	120 µg/g	kidney	1	0.2	0.0029	**0.24**	1.5	
					15	1.5	0.38	**1.5**	5.9	
		female	120 µg/g	kidney	1	2.3	0.17	**2.4**	20	
					15	7.1	1.8	**6.5**	47	
	Mice ($B6C3F_1$)	male	60 µg/g	Harderian	1	0.3	0.018	**0.41**	1.4	
					15	0.5	0.075	**0.52**	1.4	
		female	60 µg/g	Harderian	1	0.1	0.0025	**0.16**	0.74	
					15	0.8	0.35	**0.84**	2.0	
		male	120 µg/g	Harderian	1	0.4	0.13	**0.42**	0.96	
					15	0.6	0.26	**0.57**	1.2	
		female	120 µg/g	Harderian	1	0.1	0.0030	**0.18**	0.85	
					15	0.7	0.17	**0.77**	2.1	
	Mice ($C3AF_1$)	male	60 µg/g	Harderian	1	0.1	0.0023	**0.20**	1.3	
					15	0.1	0.0016	**0.18**	1.8	
		female	60 µg/g	Harderian	1	0.4	0.019	**0.52**	2.5	
					15	0.8	0.15	**0.85**	3.4	
		male	120 µg/g	Harderian	1	0.1	0.0010	**0.086**	1.0	
					15	0.3	0.0050	**0.40**	2.8	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
		female	120 µg/g	Harderian	1	0.1	0.0012	**0.094**	1.2	
					15	0.1	0.0012	**0.081**	0.90	
ENU (continued)	Mice ($B6C3F_1$)	male	60 µg/g	stomach	1	0.3	0.0091	**0.34**	2.4	
					15	1.9	0.61	**1.82**	8.7	
		female	60 µg/g	stomach	1	0.2	0.0083	**0.26**	1.1	
					15	0.2	0.0072	**0.24**	1.0	
		male	120 µg/g	stomach	1	0.2	0.0059	**0.20**	0.90	
					15	1.2	0.50	**1.2**	2.9	
		female	120 µg/g	stomach	1	0.6	0.19	**0.60**	1.5	
					15	1.6	0.67	**1.6**	3.7	
	Mice ($C3AF_1$)	male	60 µg/g	stomach	1	0.0	0.0009	**0.063**	0.51	
					15	0.3	0.023	**0.41**	1.3	
		female	60 µg/g	stomach	1	0.8	0.085	**0.89**	3.5	
					15	1.1	0.19	**1.1**	4.5	
		male	120 µg/g	stomach	1	0.2	0.010	**0.19**	0.56	
					15	0.7	0.32	**0.70**	1.5	
		female	120 µg/g	stomach	1	0.4	0.14	**0.46**	1.2	
					15	0.6	0.24	**0.64**	1.5	
NMU	Mice ($BC3F_1$)	male	50 µg/g	lung adenomas	1	3.4	1.3	**3.3**	9.3	Terracini and Testa (1970)
		female	50 µg/g	lung adenomas	1	6.3	2.4	**6.0**	23	
		male	50 µg/g	lymphosarcoma	1	2.5	1.1	**2.4**	6.4	
		female	50 µg/g	lymphosarcoma	1	1.1	0.49	**1.1**	2.4	
		male	50 µg/g	hepatoma	1	35	6.5	**32**	324	
		female	50 µg/g	hepatoma	1	0.3	0.0023	**0.39**	13	
		male	50 µg/g	renal adenoma	1	0.9	0.0093	**1.2**	13	
		female	50 µg/g	renal adenoma	1	1.3	0.0081	**1.7**	33	
		male	50 µg/g	forestomach	1	0.0	0.0006	**0.039**	0.52	
		female	50 µg/g	forestomach	1	0.1	0.0027	**0.15**	0.69	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
	Mice (C3Hf/Dp)	male	25 µg/g	thymic lymphoma	1	1.9	0.048	**2.1**	23	
NMU (continued)		female	25 µg/g	thymic lymphoma	1	1.2	0.0089	**1.5**	30	
		male	25 µg/g	lung adenomas	1	1.0	0.013	**1.2**	11	
		female	25 µg/g	lung adenomas	1	0.4	0.018	**0.46**	1. 7	
		male	25 µg/g	liver tumor	1	0.2	0.0016	**0.21**	4.6	
		female	25 µg/g	liver tumor	1	0.3	0.0026	**0.39**	4.4	
		male	25 µg/g	Stomach	1	0.5	0.0045	**0.67**	6.8	
		female	25 µg/g	Stomach	1	0.3	0.0046	**0.43**	3.8	
				ovarian	1	0.1	0.0014	**0.17**	3.5	
				uterine/vaginal	1	8.6	1.1	**8.1**	97	
		male	50 µg/g	thymic lymphoma	1	7.9	3.1	**7.4**	30	
		female	50 µg/g	thymic lymphoma	1	3.1	1.3	**3.0**	7.8	
		male	50 µg/g	lung adenomas	1	0.04	0.0008	**0.058**	0.45	
		female	50 µg/g	lung adenomas	1	0.1	0.0012	**0.084**	0.53	
		male	50 µg/g	liver tumor	1	0.2	0.0021	**0.33**	7.8	
		female	50 µg/g	liver tumor	1	0.1	0.0011	**0.13**	4.5	
		male	50 µg/g	Stomach	1	0.01	0.0003	**0.013**	0.12	
		female	50 µg/g	Stomach	1	0.1	0.0022	**0.15**	0.96	
				ovarian	1	0.0	0.0003	**0.014**	0.14	
				uterine/vaginal	1	0.0	0.0005	**0.034**	0.46	
		male	50 µg/g	thymic lymphoma	21	4.3	1. 6	**4.1**	17	
		female	50 µg/g	thymic lymphoma	21	1.0	0.39	**1.0**	2.6	
		male	50 µg/g	lung adenomas	21	0.1	0.0022	**0.22**	1.1	
		female	50 µg/g	lung adenomas	21	0.7	0.30	**0.75**	1.7	

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
		male	50 µg/g	liver tumor	21	0.1	0.0013	**0.15**	4.3	
		female	50 µg/g	liver tumor	21	0.9	0.0051	**1.4**	23	
NMU (continued)		male	50 µg/g	stomach	21	0.1	0.001	**0.08**	0.64	
		female	50 µg/g	stomach	21	1.8	0.77	**1.8**	4.7	
				ovarian	21	0.0	0.0007	**0.055**	0.97	
				uterine/vaginal	21	1.7	0.59	**1.7**	6.4	
Urethane	Mice (Swiss)	male	1 mg/g	liver	1	24	4.4	**21**	220	Chieco-Bianchi et al. (1963)
		female	1 mg/g	liver	1	0.4	0.0044	**0.54**	13	
		male	1 mg/g	liver	5	14	2.4	**13**	137	
		female	1 mg/g	liver	5	1.2	0.017	**1.4**	26	
		male	1 mg/g	liver	20	0.2	0.0018	**0.28**	10	
		female	1 mg/g	liver	20	0.1	0.0011	**0.12**	4.8	
		both	1 mg/g	skin	1	0.2	0.0027	**0.32**	5.4	
Urethane + croton oil	Mice (Swiss)	both	1 mg/g	skin	1	2.9	1.2	**2.8**	8.2	
Urethane	Rats (MRC Wistar-derived)	male/ female	16%×6	neurilemmomas	1	0.2	0.0028	**0.33**	4.5	Choudari Kommineni et al. (1970)
		male/ female	16%×6	neurilemmomas	28	0.4	0.0045	**0.51**	6.3	
		male/ female	16%×6	liver	1	7.9	1.4	**7.1**	82	
		male/ female	16%×6	liver	28	0.2	0.0026	**0.4**	11.7	
		male/ female	16%×6	thyroid	1	0.0	0.0006	**0.039**	0.67	
		male/ female	16%×6	thyroid	28	0.1	0.0011	**0.1**	1.5	
	Mice (Swiss)	male/ female	1 mg/g	lung	1	15	1.2	**11**	997	De Benedictis et al. (1962)
	Mice (Swiss)			leukemia		6.7	1.7	**6.1**	45	Fiore-Donati et al.

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
						5.1	1.1	**4.7**	38	(1962)
Urethane (continued)	Mice ($B6AF_1/J$)	male	1 mg/g	liver	21	5.1	1.4	**4.7**	30	Klein (1966)
		female	1 mg/g	liver	21	0.2	0.0019	**0.26**	6.0	
				Harderian gland	1	0.3	0.0021	**0.33**	11	
					7	0.3	0.0021	**0.33**	11	
					14	0.6	0.0044	**0.85**	20	
		male	1 mg/g	Harderian gland	21	0.3	0.0024	**0.41**	13	
		male	1 mg/g	forestomach	1	0.1	0.0009	**0.079**	1.9	
		female	1 mg/g	forestomach	1	0.4	0.0028	**0.49**	11	
		male	1 mg/g	forestomach	7	0.1	0.0017	**0.19**	3.5	
		female	1 mg/g	forestomach	7	0.1	0.0013	**0.16**	5.0	
		male	1 mg/g	forestomach	14	0.2	0.0018	**0.21**	3.9	
		female	1 mg/g	forestomach	14	0.8	0.0056	**1.1**	18	
		male	1 mg/g	forestomach	21	0.1	0.0008	**0.072**	1.7	
		female	1 mg/g	forestomach	21	0.2	0.0015	**0.2**	6.3	
				lung	1	1.0	0.36	**0.95**	2.5	
		male	1 mg/g	lung	14	0.8	0.26	**0.8**	2.3	
		female	1 mg/g	lung	14	0.4	0.16	**0.45**	1.1	
					21	0.9	0.31	**0.86**	2.4	
	Mice (C3H/f)	male	1 mg/g	liver	1	14	4.0	**12**	81	Liebelt et al. (1964)
		female	1 mg/g	liver	1	16	3.2	**15**	155	
		male	1 mg/g	lung	1	5.9	1.7	**5.6**	28	
		female	1 mg/g	lung	1	22	4.5	**20**	203	
		male	1 mg/g	reticular tissue	1	2.0	0.023	**2.3**	38	
		female	1 mg/g	reticular tissue	1	8.6	2.3	**7.7**	60	
	Mice (Swiss)		1 mg/g	pulmonary adenomas	2 vs 4 weeks	14	1.1	**10.1**	965	Rogers (1951)

Table 6. Ratio of early-life to adult cancer potencies for studies with acute exposures of juveniles and adult animals to carcinogens with a mutagenic mode of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Day	Ratio of juvenile to adult potency				Reference
						Unweighted geometric mean	2.5%	Median	97.5 %	
			1 mg/g	pulmonary adenomas	2 vs 6 weeks	16	1.3	**11.3**	1025	
Urethane (continued)			1 mg/g	pulmonary adenomas	2 vs 8 weeks	19	1.6	**13.3**	1126	
			1 mg/g	pulmonary adenomas	2 vs 10 weeks	21	1.9	**14.5**	1168	
			0.25 mg/g	adenomas	3 vs 8 weeks	7.1	2.3	**6.7**	29	
			0.5 mg/g	adenomas	3 vs 8 weeks	0.7	0.29	**0.67**	1.6	
			1.0 mg/g	adenomas	3 vs 8 weeks	0.7	0.28	**0.68**	1.6	

* The 2.5% and 97.5% are percentiles of the posterior distribution. For a Bayesian distribution, these percentiles function in a manner similar to the 95% confidence limits for other types of statistical analyses.

Table 7. Ratio of early-life to adult cancer potencies for studies with lifetime exposures starting with juvenile and adult animals to carcinogens with mutagenic or nonmutagenic modes of action*

Compound	Species (strain)	Sex	Dose	Tumor	Unweightedg eometric mean	2.5%	Median	97.5%	Reference
Mutagenic compounds									
DEN	Rats (Colworth)		multiple	liver	2.8	0.0093	**5.6**	23	Peto et al. (1984)
				esophagus	0.18	0.0015	**0.23**	4.8	
Safrole	Mice ($B6C3F_1$)	male		liver	50	3.7	**50**	253	Vesselinovitch et al. (1979b)
		female		liver	4.0	0.007	**4.0**	23	
Urethane	Mice ($B6AF_1/J$)	male	2.5 mg/pup	liver	79	0.36	**102**	1,064	Klein (1966)
		female	2.5 mg/pup	liver	0.47	0.0022	**0.55**	42	
Nonmutagenic compounds									
DDT	Mice ($B6C3F_1$)			liver	23	0.0023	**0.58**	23	Vesselinovitch et al. (1979a)
Dieldrin	Mice ($B6C3F_1$)			liver	91	0.014	**14**	91	Vesselinovitch et al. (1979a)
DPH	Rats (F344/N)	male	630:800	liver	0.31	0.0019	**0.37**	18	Chhabra et al. (1993b)
			630:2,400	liver	0.36	0.0021	**0.45**	17	
		female	630:800	liver	0.33	0.0019	**0.39**	21	
			630:2,400	liver	0.33	0.0019	**0.39**	21	
	Mice ($B6C3F_1$)	male	210:100	liver	0.71	0.0028	**0.93**	49	
			210:300	liver	14	0.03	**23**	214	
		female	210:200	liver	0.32	0.002	**0.42**	13	
			210:600	liver	0.35	0.0023	**0.53**	8.8	
ETU	Rats (F344/N)	male	90:83	thyroid	0.23	0.0017	**0.3**	7.3	Chhabra et al. (1992)
			90:250	thyroid	9.1	1.1	**10.5**	27	
		female	90:83	thyroid	0.37	0.0021	**0.46**	19	
			90:250	thyroid	0.61	0.0034	**1.1**	10	

Table 7. Ratio of early-life to adult cancer potencies for studies with lifetime exposures starting with juvenile and adult animals to carcinogens with mutagenic or nonmutagenic modes of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Unweighted geometric mean	2.5%	Median	97.5%	Reference
ETU (continued)	Mice ($B6C3F_1$)	male	330:330	liver	0.37	0.0022	**0.5**	14	
			330:1,000	liver	0.48	0.0027	**0.75**	12	
		female	330:330	liver	0.33	0.0023	**0.5**	7.8	
			330:1,000	liver	0.42	0.0025	**0.65**	11	
		male	330:330	thyroid	0.44	0.0022	**0.52**	34	
			330:1,000	thyroid	0.63	0.0035	**1.12**	10	
		female	330:330	thyroid	5.2	0.011	**10**	108	
			330:1,000	thyroid	0.18	0.0016	**0.24**	4.2	
		male	330:330	pituitary	0.40	0.0021	**0.47**	32	
			330:1,000	pituitary	0.18	0.0015	**0.22**	5.7	
		female	330:330	pituitary	0.21	0.0016	**0.26**	10	
			330:1,000	pituitary	0.27	0.0019	**0.36**	9.0	
PBB	Rats (F344/N)	male	10:10	liver	0.39	0.0023	**0.56**	13	Chhabra et al. (1993a)
			10:30	liver	0.18	0.0016	**0.25**	4.3	
		female	10:10	liver	36	15	**36**	86	
			10:30	liver	3.1	0.023	**4.6**	22	
		male	10:10	mononuclear cell leukemia	0.51	0.0025	**0.69**	23	
		male	10:30	mononuclear cell leukemia	0.77	0.0031	**1.1**	35	
		female	10:10	mononuclear cell leukemia	0.54	0.0026	**0.74**	24	
		female	10:30	mononuclear cell leukemia	0.34	0.0021	**0.45**	15	
	Mice ($B6C3F_1$)	male	30:30	liver	8.9	0.015	**12.2**	1,076	
		female	30:30	liver	4.4	0.0075	**6.2**	786	
		male	10:10	liver	0.15	0.0014	**0.2**	3.9	

Table 7. Ratio of early-life to adult cancer potencies for studies with lifetime exposures starting with juvenile and adult animals to carcinogens with mutagenic or nonmutagenic modes of action (continued)

Compound	Species (strain)	Sex	Dose	Tumor	Unweighted geometric mean	2.5%	Median	97.5%	Reference
		female	10:10	liver	0.29	0.0021	**0.43**	7.0	

* The 2.5% and 97.5% are percentiles of the posterior distribution. For a Bayesian distribution, these percentiles function in a manner similar to the 95% confidence limits for other types of statistical analyses.

Table 8. Summary of quantitative estimates of ratio of early-life to adult cancer potencies

Dose	Tissue	Number of chemicals	Inverse-weighted geometric mean ratio	Unweighted Minimum	Unweighted Maximum	Number of ratios	Percentage >1
Chemicals with mutagenic mode of action							
Repeated		4	10.5	0.12	111	45	42
Lifetime		3	8.7	0.18	79	6	67
	Combined repeated and lifetime	**6**	**10.4**	**0.12**	**111**	**51**	**45**
Acute	**Combined**	11	1.5	0.01	178	268	55
	Forestomach	3	0.076	0.01	1.9	32	16
	Harderian	2	0.48	0.06	0.8	20	0.0
	Kidney	2	1.6	0.17	7.1	18	78
	Leukemia	1	5.9	5.1	6.7	2	100
	Liver	5	8.1	0.10	40	70	77
	Lung	7	1.1	0.04	178	77	56
	Lymph	2	1.8	1.1	2.7	3	100
	Mammary (wk 5 vs wk 26)	1	7.1	NA	NA	1	100
	Mammary (wk 2 vs wk 5–8 or 26)	1	0.071	NA	NA	2	0
	Nerve	2	2.3	0.24	64	8	75
	Nerve (Day 1 comparison)	2	10	0.24	64	3	67
	Ovarian	1	0.033	0.01	0.13	3	0
	Reticular tissue	1	6.5	1.96	8.6	2	100
	Thymic lymphoma	1	2.8	1.01	7.9	6	100
	Thyroid	1	0.05	0.03	0.08	2	0
	Uterine/vaginal	1	1.6	0.03	8.6	3	67
	Day 1	7	1.7	0.01	178	127	55
	Day 15	3	1.5	0.06	52	74	65
Chemicals with nonmutagenic mode of action							
Repeated		6	2.2	0.06	13	22	27
Lifetime		5	3.4	0.15	36	38	21

Table 9. Excess Relative Risk (ERR) estimates for cancer incidence from Life Span Study (Japanese survivors)[a]

Site	Average ERR at 1 Sv	
	<20[b]	>20[b]
Stomach	0.74	0.24
Colon	0.62	0.7
Liver	1.3	0.31
Lung	0.57	1.1
Bone and connective tissue	11	0.42
Skin	5.4	0.39
Breast	3.3	0.98
Urinary bladder	0.71	0.79
Leukemia	6.1	3.7

[a] Information extracted from tables in UNSCEAR, Annex I (2000).
[b] Age at exposure.

Table 10. Excess Relative Risk (ERR) estimates for incidence of thyroid cancer from Life Span Study[a]

Age at exposure	Average ERR at 1 Sv (No. cases)
0–9 yr	10.25 (24)
10–19 yr	4.5 (35)
20–29 yr	0.10 (18)
>30 yr	0.04 (55)

[a] Information extracted from tables in UNSCEAR, Annex I (2000).

Table 11. Coefficients for the Revised Methodology mortality risk model (from U.S. EPA, 1999)[a]

Cancer type	Risk model type[b]	Age group				
		0–9	10–19	20–29	30–39	40+
Male:						
Stomach	R	1.223	1.972	2.044	0.3024	0.2745
Colon	R	2.290	2.290	0.2787	0.4395	0.08881
Liver	R	0.9877	0.9877	0.9877	0.9877	0.9877
Lung	R	0.4480	0.4480	0.0435	0.1315	0.1680
Bone	A	0.09387	0.09387	0.09387	0.09387	0.09387
Skin	A	0.06597	0.06597	0.06597	0.06597	0.06597
Breast	R	0.0	0.0	0.0	0.0	0.0
Ovary	R	0.0	0.0	0.0	0.0	0.0
Bladder	R	1.037	1.037	1.037	1.037	1.037
Kidney	R	0.2938	0.2938	0.2938	0.2938	0.2938
Thyroid	A	0.1667	0.1667	0.1667	0.1667	0.1667
Leukemia	R	982.3	311.3	416.6	264.4	143.6
Female:						
Stomach	R	3.581	4.585	4.552	0.6309	0.5424
Colon	R	3.265	3.265	0.6183	0.8921	0.1921
Liver	R	0.9877	0.9877	0.9877	0.9877	0.9877
Lung	R	1.359	1.359	0.1620	0.4396	0.6047
Bone	A	0.09387	0.09387	0.09387	0.09387	0.09387
Skin	A	0.06597	0.06597	0.06597	0.06597	0.06597
Breast	R	0.7000	0.7000	0.3000	0.3000	0.1000
Ovary	R	0.7185	0.7185	0.7185	0.7185	0.7185
Bladder	R	1.049	1.049	1.049	1.049	1.049
Kidney	R	0.2938	0.2938	0.2938	0.2938	0.2938
Thyroid	A	0.3333	0.3333	0.1667	0.1667	0.1667
Leukemia	R	1,176	284.9	370.06	178.8	157.1

[a] The coefficients were derived using several models applied to data from A-bomb survivors and selected medical exposures.

[b] A = absolute risk with coefficient units of 10^{-4} $(Gy\ y)^{-1}$; R= relative risk with coefficient units of Gy^{-1}.

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Attachment

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HTTAC (non-carcinogenic child (0-6 years) exposure)

$$HTTAC\ (mg/L) = \frac{RSC \bullet (RfD \bullet BW)}{W}$$

Symbol	Value	Units	Parameter
RSC	Chemical-Specific	unitless	Relative Source Contribution
RfD	Chemical-Specific	mg/kg-day	Oral Reference Dose
BW	15	kg	Body Weight- child 0-6 years of age
W	0.78	L/day	Drinking Water Ingestion Rate- child 0-6 years of age

HNTAC (carcinogen)

$$HNTAC\ (mg/L) = \frac{TR \bullet \left(AT \bullet 365 \frac{days}{year}\right)}{SF_o \bullet IFW_{adj}}$$

Symbol	Value	Units	Parameter
TR	1.00E-06	unitless	Target Risk Level (1 in 1 million)
AT	70	years	Averaging Time (averaged over a lifetime)
SF_o	Chemical-Specific	$(mg/kg\text{-}day)^{-1}$	Oral Slope Factor
IFW_{adj}	327.95	L/kg	Age-Adjusted Resident Drinking Water Rate

$$IFW_{adj} = \frac{EF_{child} \bullet ED_{child} \bullet IRW_{child}}{BW_{child}} + \frac{EF_{adult} \bullet ED_{adult} \bullet IRW_{adult}}{BW_{adult}}$$

IFW_{adj} =	327.95	L/kg	
Symbol	**Value**	**Units**	**Parameter**
EF	350	days/year	Exposure Frequency - child
ED_{child}	6	years	Exposure Duration- child 0-6 years of age
IRW_{child}	0.78	L/day	Drinking Water Ingestion Rate- child 0-6 years of age
BW_{child}	15	kg	Body Weight- child 0-6 years of age
EF_{adult}	350	days/year	Exposure Frequency - adult
ED_{adult}	20	years	Exposure Duration- adult
IRW_{adult}	2.5	L/day	Drinking Water Ingestion Rate- adult
BW_{adult}	80	kg	Body Weight- adult

HNTAC (carcinogen with a mutagen mode of action)

$$HNTAC\ (mg/L) = \frac{TR \bullet \left(AT \bullet 365\frac{days}{year}\right)}{SF_o \bullet IFWM_{adj}}$$

Symbol	Value	Units	Parameter
TR	1.00E-06	unitless	Target Risk Level (1 in 1 million)
AT	70	years	Averaging Time (averaged over a lifetime)
SF_o	Chemical-Specific	$(mg/kg\text{-}day)^{-1}$	Oral Slope Factor
$IFWM_{adj}$	1019.9	L/kg	Mutagen Age-Adjusted Resident Drinking Water Rate

$$IFWM_{adj} = \frac{EF_{0-2} \bullet ED_{0-2} \bullet IRW_{0-2} \bullet 10}{BW_{0-2}} + \frac{EF_{2-6} \bullet ED_{2-6} \bullet IRW_{2-6} \bullet 3}{BW_{2-6}} + \frac{EF_{6-16} \bullet ED_{6-16} \bullet IRW_{6-16} \bullet 3}{BW_{6-16}} + \frac{EF_{216-26} \bullet ED_{16-26} \bullet IRW_{16-26} \bullet 1}{BW_{16-26}}$$

$IFWM_{adj}$ =	1,019.9	L/kg	
Symbol	**Value**	**Units**	**Parameter**
$EF_{0\text{-}2}$	350	days/year	Exposure Frequency
$ED_{0\text{-}2}$	2	years	Exposure Duration- child 0-2 years of age
$IRW_{0\text{-}2}$	0.78	L/day	Drinking Water Ingestion Rate- child 0-2 years of age
$BW_{0\text{-}2}$	15	kg	Body Weight- child 0-2 years of age
$EF_{2\text{-}6}$	350	days/year	Exposure Frequency
$ED_{2\text{-}6}$	4	years	Exposure Duration- child 2-6 years of age
$IRW_{2\text{-}6}$	0.78	L/day	Drinking Water Ingestion Rate- child 2-6 years of age
$BW_{2\text{-}6}$	15	kg	Body Weight- child 2-6 years of age
$EF_{6\text{-}16}$	350	days/year	Exposure Frequency
$ED_{6\text{-}16}$	10	years	Exposure Duration- child 6-16 years of age
$IRW_{6\text{-}16}$	2.5	L/day	Drinking Water Ingestion Rate- child 6-16 years of age
$BW_{6\text{-}16}$	80	kg	Body Weight- child 6-16 years of age
$EF_{16\text{-}26}$	350	days/year	Exposure Frequency
$ED_{16\text{-}26}$	10	years	Exposure Duration- adult 16-26 years of age
$IRW_{16\text{-}26}$	2.5	L/day	Drinking Water Ingestion Rate- adult 16-26 years of age
$BW_{16\text{-}26}$	80	kg	Body Weight- adult 16-26 years of age

Attachment

1H

1

WHO/HSE/WSH/10.01/11

Atrazine and Its Metabolites in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

Atrazine and Its Metabolites in Drinking-water
Background document for development of WHO *Guidelines for Drinking-water Quality*

Printed by the WHO Document Production Services, Geneva, Switzerland

Preface

One of the primary goals of the World Health Organization (WHO) and its Member States is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published in 1998, addressing selected chemicals. An addendum on microbiological aspects reviewing selected microorganisms was published in 2002. The third edition of the GDWQ was published in 2004, the first addendum to the third edition was published in 2006 and the second addendum to the third edition was published in 2008. The fourth edition will be published in 2011.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Japan, the United Kingdom and the United States of America (USA) prepared the documents for the fourth edition.

Under the oversight of a group of coordinators, each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

The first draft of Atrazine and Its Metabolites in Drinking-water, Background document for development of WHO *Guidelines for Drinking-water Quality* (GDWQ), was prepared by Mr J.K. Fawell, United Kingdom, to whom special thanks are due. This background document is an update of the background document published in the second edition of the GDWQ.

The work of the following working group coordinators was crucial in the development of this document and others contributing to the fourth edition:

Dr J. Cotruvo, J. Cotruvo Associates, USA (*Materials and chemicals*)
Mr J.K. Fawell, United Kingdom (*Naturally occurring and industrial contaminants* and *Pesticides*)
Ms M. Giddings, Health Canada (*Disinfectants and disinfection by-products*)
Mr P. Jackson, WRc-NSF, United Kingdom (*Chemicals – practical aspects*)
Professor Y. Magara, Hokkaido University, Japan (*Analytical achievability*)
Dr Aiwerasia Vera Festo Ngowi, Muhimbili University of Health and Allied Sciences, United Republic of Tanzania (*Pesticides*)
Dr E. Ohanian, Environmental Protection Agency, USA (*Disinfectants and disinfection by-products*)

The draft text was discussed at the Expert Consultation for the fourth edition of the GDWQ, held on 9–13 November 2009. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants at the meeting is gratefully acknowledged.

The WHO coordinators were Mr R. Bos and Mr B. Gordon, WHO Headquarters. Ms C. Vickers provided a liaison with the International Programme on Chemical Safety, WHO Headquarters. Mr M. Zaim, Public Health and the Environment Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms P. Ward provided invaluable administrative support at the Expert Consultation and throughout the review and publication process. Ms M. Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comments are greatly appreciated.

Acronyms and abbreviations used in the text

ADI	acceptable daily intake
DACT	diaminochlorotriazine
DEA	deethyl-atrazine
DIA	deisopropyl-atrazine
DWLOC	Drinking Water Levels of Comparison (USA)
FAO	Food and Agriculture Organization of the United Nations
GAC	granular activated carbon
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
PAC	powdered activated carbon
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	ultraviolet
WHO	World Health Organization

Table of contents

The atrazine drinking-water guideline prepared for the Third Edition of the WHO Guidelines for Drinking-water Quality has been revised following the recent Joint FAO/WHO Meeting on Pesticide Residues (JMPR) evaluation of atrazine and its environmental metabolites (FAO/WHO, 2007).

This background document is based on and largely extracted from this recent JMPR evaluation. Except for the critical studies on which the guidelines are based, primary references are given only for text that has not been extracted from this report. The interested reader should refer to the toxicological monograph published by FAO/WHO (2009) for more detailed information and primary references.

1. GENERAL DESCRIPTION

1.1 Identity

Chemical Abstracts Service Registry No.: 1912-24-9
Molecular formula: $C_8H_{14}ClN_5$

The International Union of Pure and Applied Chemistry name for atrazine is 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine.

1.2 Physicochemical properties

Some physical and chemical properties of atrazine that are relevant to its environmental fate are summarized in Table 1.

Table 1: Physicochemical properties of atrazine[a]

Property	Value
Melting point	175–177 °C
Density	1.187 g/cm^3 at 20 °C
Water solubility	30 mg/l at 20 °C
Log octanol–water partition coefficient	2.3
Vapour pressure	40×10^{-6} Pa at 20 °C

[a] From Meister (1989); Royal Society of Chemistry (1991); Worthing (1991).

1.3 Major uses and sources in drinking-water

Atrazine is a selective systemic herbicide of the chlorotriazine class, used for the control of annual broadleaf and grassy weeds (Worthing, 1991). Key crops in which atrazine-containing herbicides are used include maize, sorghum and sugarcane, with some other minor uses of local importance. Atrazine and its metabolites have been found in surface water and groundwater as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide. The source of the residue should be considered when interpreting water monitoring data. Assessments should be based on whether diffuse sources (e.g. runoff from an agricultural field) or point sources (e.g. an accidental spill or inappropriate disposal) have contributed to a detection in water. The presence of atrazine and its metabolites in surface water is most likely to be

intermittent, particularly in flowing water, but groundwater contamination will usually be relatively persistent.

1.4 Environmental fate

Atrazine can be degraded in surface water by photolysis and microorganisms via *N*-dealkylation and hydrolysis of the chloro substituent; the corresponding half-lives are greater than 100 days at 20 °C. Hydrolysis and microbial degradation also take place in soil, depending mainly on temperature, moisture and pH. Half-lives of 20–50 days at 20–25 °C have been found under laboratory conditions, increasing at lower temperatures (USEPA, 1988). These are similar to the half-lives found under natural conditions, but longer half-lives have been seen under special conditions (Schoen & Winterlin, 1987). Degradation rates normally decrease with increasing depth, and atrazine can be fairly stable in groundwater (Burnside, Fenster & Wicks, 1963).

Atrazine's degradation products in soil include several of the chloro-*s*-triazine metabolites commonly found in water (Keller, 1978) (see section 2). Unsubstituted amino metabolites and triazine are formed later and may be mineralized completely. Atrazine and its dealkylated metabolites are moderately to very mobile in sandy, silt and clay soils (Ciba-Geigy, 1986). Hydroxytriazines, however, are of low mobility (Helling, 1971) and persist for long periods in the soil (Kahn & Saidak, 1981).

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

Atrazine and its chloro-*s*-triazine metabolites—deethyl-atrazine (DEA), deisopropyl-atrazine (DIA) and diaminochlorotriazine (DACT)—have been found in surface water and groundwater. The metabolite hydroxyatrazine is more commonly detected in groundwater than in surface water.

A number of studies have monitored the concentrations of atrazine in groundwater and surface water over the last two decades. Recent monitoring data show declining levels and incidences of detection of atrazine and its chloro-*s*-triazine metabolites (DIA, DEA and DACT) compared with data collected in the early 1990s; this reflects restrictions on the use of atrazine that were introduced in the late 1990s and early 2000s and the introduction of good agricultural practices in the European Union, the United States of America (USA) and other parts of the world. Therefore, older monitoring data generally represent an overestimate of environmental concentrations likely to arise from current use practices.

In surface water, the concentrations of the chlorotriazine metabolites of atrazine are generally less than those of atrazine itself, whereas the concentrations of these metabolites in rural wells are more similar to those of atrazine. The relative order of concentrations in rural wells in the USA was generally as follows: atrazine ~ DEA ~ DACT > DIA > hydroxyatrazine. However, concentrations of DEA that are several-fold higher than those of the parent compound have been reported.

Monitoring carried out in a number of countries indicates that concentrations of atrazine and its chloro-*s*-triazine metabolites in groundwater and surface water rarely exceed 2 µg/l and are commonly well below 0.1 µg/l, although concentrations may be higher in agricultural areas where large amounts of atrazine are used. In the past, its

use for weed control on non-crop land, such as railway lines and paved areas, gave rise to contamination of groundwater in particular, although this use has largely disappeared worldwide. The concentration of atrazine in public water supplies in the USA does not exceed the United States Environmental Protection Agency's (USEPA) Drinking Water Levels of Comparison (DWLOC)—the maximum concentrations in drinking-water that, when considered together with dietary exposure, do not exceed a level of concern—for any age group These DWLOCs, which account for atrazine plus its three chloro-*s*-triazine metabolites, range from 12.5 to 68 µg/l for intermediate (seasonal) or chronic (annual) exposure. Concentrations of atrazine in drinking-water in the United Kingdom are less than 0.1 µg/l, based on data measured when atrazine was registered and when good agricultural practices had been adopted. In Canada, concentrations of atrazine and its chloro-*s*-triazine metabolites did not exceed the interim guideline level (5 µg/l, includes metabolites) in any samples of drinking-water from 10 Canadian treatment plants in the cereal-growing regions of Ontario and Quebec (from groundwater and surface water). Levels of atrazine were below 0.83 µg/l, concentrations of DEA were below 0.35 µg/l, and DIA and DACT were found less often and at lower levels in treated surface water. Total chloro-*s*-triazine concentrations from raw water were below 0.5 µg/l in groundwater and well below 0.68 µg/l in surface water (Tauber, 2006).

It is expected that exposure of the public will be primarily through drinking-water.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

After oral administration to rats, ^{14}C-labelled atrazine was rapidly and almost completely absorbed, independent of dose and sex. Radioactivity was widely distributed throughout the body. Excretion was more than 93% of the administered dose within 7 days, primarily via the urine (approximately 73%) and to a lesser extent via the faeces (approximately 20%; approximately 7% via bile), with more than 50% being excreted within the first 24 h. The elimination half-life of radiolabel from the whole body was 31.3 h in rats; this prolonged half-life was caused by covalent binding of atrazine to cysteine sulfhydryl groups in the β-chain of rodent haemoglobin. Seven days after administration of a single low dose (1 mg/kg body weight), tissue residues represented 6.5–7.5% of the dose, with the highest concentrations in erythrocytes (≤0.63 mg/kg), liver (≤0.50 mg/kg) and kidneys (≤0.26 mg/kg).

Atrazine was extensively metabolized; more than 25 metabolites have been identified in rats. The major metabolic pathways were stepwise dealkylation via either DIA or DEA to DACT, the major metabolite. Dechlorination involving conjugation with glutathione was a minor pathway. The biotransformation of atrazine in rats and humans was qualitatively similar.

4. TOXICOLOGICAL SUMMARY

4.1 Atrazine

Atrazine was of low acute toxicity in rats exposed orally (median lethal dose [LD_{50}] 1870–3090 mg/kg body weight), dermally (LD_{50} >2000 mg/kg body weight) or by

inhalation (median lethal concentration [LC_{50}] >5.8 mg/l). Atrazine was not a skin irritant or an eye irritant in rabbits.

In short-term studies of toxicity in rats, dogs and rabbits, the consistent toxic effects noted across species included reduced body weight gain and food intake and a slight decrease in erythrocyte parameters. Also, liver weights and splenic haemosiderin deposition were increased in rats, whereas there was marked cardiac toxicity in dogs.

Atrazine was tested for genotoxicity in a large number of studies covering an adequate range of end-points. JMPR agreed that it is unlikely that atrazine is genotoxic.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. As in short-term studies, reduced body weight gain and food intake and a decrease in erythrocyte parameters were noted consistently. Additionally, reduced survival of females and cardiovascular effects (atrial thrombi) in both sexes were observed in mice at high doses.

In three studies of carcinogenicity in mice, no treatment-related carcinogenic effects were observed at dietary concentrations up to about 386 and 483 mg/kg body weight per day in males and females, respectively. Overall, the no-observed-adverse-effect level (NOAEL) was 1.2 mg/kg body weight per day, on the basis of lower body weight/body weight gain at 38.4 mg/kg body weight per day and greater.

In one study of carcinogenicity in Fischer 344 rats fed diets containing atrazine at concentrations up to about 20 mg/kg body weight per day, there was no effect at any dose on the onset or incidence of tumours. The NOAEL was about 3.5 mg/kg body weight per day, on the basis of decreased body weight.

In seven studies of carcinogenicity in Sprague-Dawley rats fed diets containing atrazine at concentrations up to about 42 and 65 mg/kg body weight per day in males and females, respectively, an increased incidence of mammary tumours (adenomas, carcinomas, fibroadenomas) with or without an earlier onset (relative to controls) was observed in four studies, whereas there was an earlier onset of mammary tumours without any increase in their overall lifetime incidence in two studies. An earlier onset of pituitary tumours was also observed in one study, with no increase in incidence at term. Overall, the NOAEL for mammary carcinogenicity was 1.5 mg/kg body weight per day, on the basis of a statistically significant increased incidence in mammary tumours at 3.1 mg/kg body weight per day.

In a study of carcinogenicity in ovariectomized Sprague-Dawley rats, neither increases in mammary gland proliferative changes nor mammary tumours were seen at dietary concentrations up to about 21 mg/kg body weight per day, suggesting that the carcinogenic mode of action of atrazine in Sprague-Dawley rats is related to ovarian function.

In a study designed to evaluate the effects of atrazine on the pre-ovulatory luteinizing hormone (LH) surge and on the estrous cycle, groups of 90 female Sprague-Dawley (Crl:CD BR) rats received diets containing atrazine (purity, 97.1%) at a concentration of 0, 25, 50 or 400 mg/kg (equal to 0, 1.8, 3.65 and 29.44 mg/kg bw per day) for

26 weeks. Attenuation of the LH surge and subsequent disruption of the estrous cycle (characterized by an increase in days in estrus) were observed at and above 3.65 mg/kg body weight per day, with a NOAEL of 1.8 mg/kg body weight per day (Morseth, 1996). The NOAEL and lowest-observed-adverse-effect level (LOAEL) for these effects were comparable to those found in the studies of carcinogenicity. The effects on the LH surge and disruption of the estrous cycle were further supported by a number of short-term mechanistic studies. Additional experiments suggested that the effects of atrazine on LH and prolactin secretion are mediated via a hypothalamic site of action.

The postulated mode of action for atrazine-induced mammary tumours in female Sprague-Dawley rats involved disruption of the hypothalamic–pituitary–ovary axis. Atrazine modifies catecholamine function and the regulation of gonadotropin-releasing hormone pulsatility in the rat hypothalamus, with the consequence that the pulse of LH released from the pituitary gland is of insufficient amplitude or duration to trigger ovulation. The failure to ovulate results in persistent secretion of estrogen, which provides a feedback to the pituitary, leading to increased secretion of prolactin. As a result, atrazine accelerates the normal reproductive ageing process in female Sprague-Dawley rats, whereby reproductive senescence is characterized by persistent exposure to estrogen and prolactin. In contrast, women respond to reduced levels of LH by reductions in levels of estrogen. Thus, JMPR considered that the mode of carcinogenic action in certain susceptible rat strains is not relevant for human risk assessment.

Investigations of other modes of action did not provide any evidence that atrazine had intrinsic estrogenic activity or that it increased aromatase activity in vivo.

JMPR concluded that atrazine is not likely to pose a carcinogenic risk to humans.

Although carcinogenicity in humans was not a concern owing to the rat-specific mode of action, alterations in neurotransmitter and neuropeptide function regulating LH and secretion of prolactin may potentially induce adverse effects during critical periods of development (as found in special studies showing pregnancy loss, delayed puberty in males and females, and decreased suckling-induced prolactin release in lactating dams). Unlike the carcinogenic effects, the developmental effects do not appear to be specific to certain strains of rats, and JMPR therefore considered these effects to be relevant for risk assessment in humans.

In special studies of reproductive toxicity, exposure of rats during early pregnancy (i.e. the LH-dependent period) caused increased pre-implantation or post-implantation losses, including full-litter resorptions. Effects were seen at doses of ≥50 mg/kg body weight per day after treatment on days 6–10 of gestation, with a NOAEL of 25 mg/kg body weight per day. In contrast, exposure on days 11–15 of gestation (after the LH-dependent period of pregnancy) at a dose of 200 mg/kg body weight per day did not induce full-litter resorptions.

Suppression of the suckling-induced prolactin release in lactating rats was seen with atrazine at doses of ≥25 mg/kg body weight per day, with a NOAEL of 12.5 mg/kg body weight per day. Treatment of lactating rats on postnatal days 1–4 affected the development of tuberoinfundibular dopaminergic neurons in the pups (presumably

due to the lack of prolactin derived from the dam's milk), with the consequence of impaired regulation of prolactin secretion, hyperprolactinaemia prior to puberty and prostatitis in the adult male offspring.

A delay in sexual development was observed in female rats after exposure on postnatal days 21–46 at doses of ≥30 mg/kg body weight per day, with a NOAEL of 10 mg/kg body weight per day, and in male rats after exposure on postnatal days 23–53 at doses of ≥12.5 mg/kg body weight per day, with a NOAEL of 6.25 mg/kg body weight per day.

In a standard two-generation study of reproduction (conducted according to earlier guidelines, which did not include end-points such as estrous cyclicity and sexual development) in rats, there was no effect on fertility at 36.1 mg/kg body weight per day, the highest dose tested. The NOAEL for parental toxicity was 3.6 mg/kg body weight per day, on the basis of decreased body weight gains and food consumption. The NOAEL for reproductive toxicity was 3.6 mg/kg body weight per day, on the basis of decreased body weights of male pups at postnatal day 21.

In two studies of prenatal developmental toxicity in rats given atrazine on days 6–15 of gestation, the NOAELs for maternal toxicity were 10 or 25 mg/kg body weight per day on the basis of decreased body weight gain and food intake at 70 or 100 mg/kg body weight per day, respectively. The NOAELs for developmental toxicity were 10 or 25 mg/kg body weight per day on the basis of incomplete ossification at several sites at 70 or 100 mg/kg body weight per day, respectively. In a study of prenatal developmental toxicity in rabbits given atrazine on days 7–19 gestation, the NOAEL for maternal toxicity was 5 mg/kg body weight per day on the basis of clinical signs, abortion and decreased food intake and body weight gain at 75 mg/kg body weight per day. The NOAEL for developmental toxicity was 5 mg/kg body weight per day on the basis of increased resorptions, reduced litter size and incomplete ossification at 75 mg/kg body weight per day. In rats and rabbits, the developmental effects were observed only at maternally toxic doses.

JMPR concluded that atrazine was not teratogenic.

Studies using a variety of test systems in vitro and in vivo indicated that modulation of the immune system occurs after exposure to atrazine. However, effects suggestive of impaired function of the immune system were observed only at doses greater than those shown to affect neuroendocrine function, leading to disruption of the estrous cycle or developmental effects.

A range of epidemiological studies (including cohort studies, case–control studies, and ecological or correlational studies) assessed possible relationships between atrazine or other triazine herbicides and cancer in humans. For some cancer types, such as prostate or ovarian cancer and non-Hodgkin lymphoma, the increased risks reported in single studies either could be explained by the methodology used or had not been confirmed in more reliable studies. Thus, the weight of evidence from the epidemiological studies did not support a causal association between exposure to atrazine and the occurrence of cancer in humans.

4.2 Metabolites of atrazine

4.2.1 Chloro-s-triazine metabolites

The toxicity profiles and mode of action of the chloro-*s*-triazine metabolites were similar to those of atrazine; the potency of these metabolites with regard to their neuroendocrine-disrupting properties appeared to be similar to that of the parent compound.

Like atrazine, the chloro-*s*-triazine metabolites were of moderate or low acute oral toxicity in rats; LD_{50}s were 1110, 1240 and 2310–5460 mg/kg body weight for DEA, DIA and DACT, respectively.

Like atrazine, its chloro-*s*-triazine metabolites delayed sexual development of male rats exposed on postnatal days 23–53 at atrazine molar equivalent doses of ≥25 mg/kg body weight per day (DEA, DIA) and ≥12.5 mg/kg body weight per day (DACT), with NOAELs of 12.5 and 6.25 mg/kg body weight per day, respectively. Exposure of female rats to DACT on postnatal days 22–41 delayed sexual development at atrazine molar equivalent doses of ≥50 mg/kg body weight per day, and the NOAEL was 25 mg/kg body weight per day. Doses at which these effects occurred were similar to those observed for parent atrazine.

In short-term feeding studies in rats, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body weight gain and decreased erythrocyte parameters and also, for DACT, disruption of the estrous cycle. The NOAELs were 3.2 mg/kg body weight per day for DEA and DIA and 7.6 mg/kg body weight per day for DACT.

In a 29/52-week study with DACT in Sprague-Dawley rats, effects comparable to those observed with atrazine (attenuation of the LH surge, increased incidences of mammary tumours) were seen at 270 mg/kg diet; the NOAEL was 48 mg/kg diet, equal to 3.4 mg/kg body weight per day. No long-term studies were performed with DEA or DIA.

In short-term feeding studies in dogs, the main effects of the chlorinated metabolites were similar to those of atrazine and included reduced body weight gain and decreased erythrocyte parameters, whereas DEA and DACT showed cardiac toxicity. The NOAELs were 3.7, 3.8 and 3.5 mg/kg body weight per day for DEA, DIA and DACT, respectively.

DEA, DIA and DACT did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In studies of prenatal developmental toxicity in rats, the chlorinated metabolites induced increased incidences of fused sternebrae and/or incomplete ossification at doses of 25–100 mg/kg body weight per day; the NOAELs for developmental toxicity were 25, 5 and 2.5 mg/kg body weight per day for DEA, DIA and DACT, respectively. The effects were seen only at doses that also produced maternal toxicity.

4.2.2 Hydroxyatrazine

The metabolite hydroxyatrazine does not have the same mode of action or toxicity profile as atrazine and its chlorometabolites. The main effect of hydroxyatrazine was kidney toxicity (owing to its low solubility in water, resulting in crystal formation and a subsequent inflammatory response), and there was no evidence that hydroxyatrazine has neuroendocrine-disrupting properties. Also, the acute oral toxicity of hydroxyatrazine in rats (LD_{50} >5050 mg/kg body weight) was lower than that of atrazine or its chlorometabolites.

In short-term feeding studies, the main effects of hydroxyatrazine in rats included reduced body weight gain, increased water consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions. The overall NOAEL was 6.3 mg/kg body weight per day. In dogs, effects included reduced body weight gain and food consumption, changes in clinical chemistry and urine analysis parameters, and kidney lesions; the NOAEL was 5.8 mg/kg body weight per day.

In a 2-year study of toxicity and carcinogenicity in rats, the effects of hydroxyatrazine included clinical signs and increased mortality, reduced body weight gain and food consumption, increased water consumption, changes in haematological, clinical chemistry and urine analysis parameters, and kidney lesions. The NOAEL was 1.0 mg/kg body weight per day. There was no evidence of carcinogenicity (Chow & Hart, 1995).

Hydroxyatrazine did not show genotoxicity in an adequate range of tests in vitro and in vivo.

In a study of prenatal developmental toxicity in rats, the effects of hydroxyatrazine consisted of reduced food consumption and body weight gain in dams and increased incidences of incomplete and absent ossification in fetuses at 125 mg/kg body weight per day; the NOAEL was 25 mg/kg body weight per day for maternal and developmental toxicity. Exposure of female rats on postnatal days 22–41 at atrazine molar equivalent doses up to 200 mg/kg body weight per day did not delay sexual development.

5. PRACTICAL ASPECTS

5.1 Analytical methods and analytical achievability

Atrazine is determined by gas chromatography with nitrogen–phosphorus detection following extraction with pentane or ethyl acetate. The detection limit in tap water and river water is about 0.1 µg/l (Yokley & Cheung, 2000).

Atrazine is determined by gas chromatography/mass spectrometry with solid-phase extraction. The detection limit in raw water and drinking-water is 10 ng/l (Bruzzoniti et al., 2006). Extraction of the metabolites can be achieved using styrene-divinylbenzene sorbents. Detection limits for triazines determined by capillary gas chromatography with nitrogen thermionic specific detection and high-performance liquid chromatography with photodiode array absorption detection following extraction with styrene-divinylbenzene sorbents and elution with acetone were lower

than 5 ng/l (Drevenkar et al., 2002). Atrazine is also determined by isotope dilution gas chromatography/mass spectrometry with solid-phase extraction. The method detection limit in surface water is 1 ng/l (Planas et al., 2006). In the case of a large-volume injection approach using 50 µl of the 40-fold concentrated extract, the detection limit in river water is 2 ng/l (Schellin & Popp, 2007). Using a liquid chromatography/mass spectrometry method utilizing online solid-phase extraction (Koivunen et al., 2006) or ultra-performance liquid chromatography combined with tandem mass spectrometry (Gervais et al., 2008), the limit of quantification is 50 ng/l or 12 ng/l, respectively.

Other methods are included in the USEPA's (2009) document on *Analytical Methods Approved for Drinking Water Compliance Monitoring of Organic Contaminants.*

5.2 Treatment and control methods and performance

Coagulation and filtration are ineffective at removing atrazine. Only 7% removal was achieved with ferric chloride dosed with iron at at 14 mg/l, with an atrazine concentration of 0.84 µg/l (Normann, Haberer & Oehmichen, 1987). Aluminium sulfate (aluminium dose of 2 mg/l at pH 8) was also completely ineffective at reducing an atrazine concentration of 66 µg/l (Miltner et al., 1989). In laboratory tests, atrazine at 3 µg/l in river water was treated with aluminium sulfate (up to 10 mg/l as aluminium) or ferric sulfate (up 40 mg/l as iron), but no removal was observed (Jiang & Adams, 2006). Lime soda softening was also ineffective. Results from other studies also suggest that conventional coagulation-based water treatment processes are ineffective at reducing atrazine concentrations (Foster, Rachwal & White, 1991; Van Hoof, Ackermans & Celens, 1992).

A full-scale granular activated carbon (GAC) plant treating river water with an atrazine concentration of 0.3–2 µg/l, with an empty bed contact time of 15–20 min, treated 20 000 bed volumes before 0.1 µg/l was reached in the effluent (Normann, Haberer & Oehmichen, 1987). Results from other treatment works incorporating GAC have shown that the atrazine concentration can be reduced to 0.1 µg/l (contact times 19–20 min, bed lifetimes 20–24 months, initial atrazine concentrations 0.16–0.27 µg/l) (Croll, Chadwick & Knight, 1991). One study found that the adsorption capacity of GAC varied between 0.04 and 0.22 mg/g carbon, depending on the type of GAC used (Duguet, 1994). The same study also found that the concentration of natural organic matter present greatly affected the capacity of the carbon to adsorb atrazine (e.g. 20 mg of atrazine per gram carbon for distilled water, reduced to 0.6–0.15 mg/g for dissolved organic carbon in range 2–8 mg/l as carbon). GAC has been applied successfully to pesticide removal (including atrazine) using slow sand filtration, as a layer 75–200 mm thick, sandwiched between conventional sand media. Contact times are 30–60 min (Anon, 1994).

Powdered activated carbon (PAC) has been used to remove atrazine from surface waters, with only a seasonal requirement to reduce pesticide concentrations. A dose of 5 mg/l with 40 min contact in a PAC reactor (coupled with coagulation) reduced the influent concentration of atrazine (0.53 µg/l) to 0.08 µg/l; there was a further reduction to <0.05 µg/l in the final water due to the remaining capacity in the PAC once it was separated at the filtration stage (Haist-Gulde, Baldauf & Brauch, 1993). Tests with a batch reactor showed that PAC (3.3 mg/l) reduced the initial

concentration of atrazine (22.5 µg/l) by 60% after 75 min contact (Qi, 1994). Atrazine spiked into water at 5 µg/l was treated with two PACs at pH 7.5. At a PAC dose of 10 mg/l in deionized water, both achieved ~100% removal. In river water, the removals were reduced to ~95% and ~90%, respectively (Jiang & Adams, 2006). In the same study, desethylatrazine was also ~100% removed from deionized water. In river water, the removals were reduced to ~85% and ~70%, respectively.

At a waterworks, ozonation produced removals of 29% and 42% for concentrations of 0.17 µg/l and 0.19 µg/l, respectively (Brauch & Kuhn, 1988). Ozonation conditions, such as dissolved ozone concentration, contact time and applied ozone dose, affect removal efficiency, as well as factors influencing the decomposition of ozone to form hydroxyl radicals (e.g. pH, temperature, organic matter content and bicarbonate concentration) (Duguet, 1994). A combination of ozone and either ultraviolet (UV) radiation or hydrogen peroxide improves removal efficiency and results in bed life extension if associated with GAC adsorption (Duguet, 1994). At full scale, one treatment works achieved a reduction of approximately 40% (initial concentration 100–600 ng/l) with ozone alone (15 min contact, dose approximately 2.2 mg/l); for the same ozone dose, in combination with hydrogen peroxide (H_2O_2/O_3 = 0.4 by mass), there was an 80% reduction in atrazine concentration (Duguet, 1992). For the treatment of atrazine at 1 mg/l to below 0.1 µg/l, the life of the GAC bed was doubled by ozonation alone and increased nearly 6-fold by the combination of oxidants. Other studies have found removals of 60% using ozone at 2.2 mg/l with a contact time of 5 min on a solution containing atrazine at 3 µg/l (Meijers, Van der Veer & Kruithof, 1993). Pilot plant trials have achieved removals of atrazine between 30% and 60% with ozone doses of 2–4 mg/l and 5–15 min contact time (Foster, Rachwal & White, 1991). In a 9-month pilot study, raw water atrazine concentrations of 7–80 ng/l were hardly affected by aluminium sulfate coagulation treatment but were reduced by 66–96% when ozonation (1.5–2 mg/l, 20 min contact) was applied (Hua, Bennett & Letcher, 2006). A combination of bankside storage, ozonation and finally GAC filtration reduced the concentration of atrazine in river water from 0.32 µg/l to 0.11, 0.09 and <0.01 µg/l, respectively (Haist-Gulde, Baldauf & Brauch, 1993).

Membrane filtration using ultrafiltration techniques does not remove atrazine, but nanofiltration is more successful; one study indicated that removals of between 66% and 98% were achieved for atrazine at an initial concentration of 3–5 µg/l (Hofman, 1992). With nanofiltration, rejection rates varied between 75% and 100% for atrazine (influent atrazine 1 µg/l, combined with five other pesticides); the specific membrane type was an important factor in determining rejection rates. Pilot plant trials involving membrane filtration have achieved removals between 79% and 99% for atrazine (Hofman et al., 1993). Atrazine (10 µg/l) was 85–95% removed by a nanofiltration membrane (Chen et al., 2004). Atrazine (concentration not stated) was 80–85% removed by different nanofiltration membranes and 96% removed by a polyamide reverse osmosis membrane (Kosutic et al., 2005). A concentration of 0.5–1.5 mg/l was 98% and 68% removed by two polyvinyl alcohol/polyamide nanofiltration membranes, but only 15% and 11% removed by two polyethersulfone nanofiltration membranes (Kiso et al., 2000). Removals of 82%, 83% and 84% from a 150–300 µg/l solution were reported for three different nanofiltration membranes (Plakas et al., 2006); fouling of the membrane by calcium and humic acid worsened the performance. A full-scale (140 000 m^3/day) nanofiltration plant at Méry-sur-Oise, France, reduced a concentration of 850 ng/l to <50 ng/l (Cyna et al., 2002). In a pilot

plant study, DEA was spiked at 1 µg/l into distilled water, sand-filtered water and GAC-filtered water, then subjected to nanofiltration through composite polyamide membranes. Removals of 62–95% and 62–75% were obtained with two nanofiltration membranes (Boussahel, Baudu & Montiel, 2001). In another study (Boussahel et al., 2000), one of the membranes gave ~90% removal from a 1 µg/l solution, and the other gave ~70% removal. With both membranes, adsorption onto the membrane accounted for about 35% removal.

UV radiation was effective at reducing atrazine concentrations during laboratory studies. With an irrradiation time of 5 min, the atrazine concentration was reduced by almost 90%. With a longer duration of radiation, there was only a small further decrease in the atrazine concentration (Cermak & Cermakova, 1992). UV irradiation, at pilot plant scale, was found to be effective in decomposing atrazine in a chalk borehole water, up to 70% removal being achieved with UV doses of up to 700 Wh/m^3. Removal efficiency was constant over an influent concentration range of 0.1–0.5 µg/l (Bourgine et al., 1995). UV irradiation causes substantial photodecomposition but not complete mineralization (Le Brun et al., 1993).

A laboratory comparison of UV, hydrogen peroxide, ozone, ozone/UV, ozone/hydrogen peroxide, hydrogen peroxide/UV and ozone/hydrogen peroxide/UV demonstrated that all processes destroyed atrazine (hydrogen peroxide only at pH >10) and that UV/hydrogen peroxide gave the fastest degradation rate (Prado & Esplusgas, 1999). Treatment of 3 µg/l solutions of atrazine and DEA with 2 mg/l free chlorine and a 30 min contact time gave no removal (Jiang & Adams, 2006).

A number of point-of-use and point-of-entry water treatment systems based on activated carbon are available and are suitable for treating water that may be contaminated with atrazine. These systems should be used only on microbially safe drinking-water, they should be well flushed before each use and the filters should be changed frequently. Point-of-use and point-of-entry reverse osmosis systems could also be used for removal of atrazine.

Although specific published data on the removal of the atrazine metabolites are limited, what data there are suggest that these will behave in a manner similar to atrazine.

6. GUIDELINE VALUES

Drinking-water may contain metabolites of atrazine as well as atrazine itself. The chloro-*s*-triazine metabolites DEA, DIA and DACT share the same mode of action as atrazine and have a similar toxicological profile. Hence, JMPR decided to establish a group acceptable daily intake (ADI). Hydroxyatrazine, the plant and soil degradate, was not included because its mode of action and toxicological profile are different from those of atrazine and its chloro-*s*-triazine metabolites.

JMPR established a group ADI of 0–0.02 mg/kg body weight based on the NOAEL for atrazine of 1.8 mg/kg body weight per day identified on the basis of LH surge suppression and subsequent disruption of the estrous cycle seen at 3.6 mg/kg body weight per day in a 6-month study in rats, using a safety factor of 100 for interspecies and intraspecies variation. JMPR considered that this NOAEL is protective for the

consequences of neuroendocrine and other adverse effects caused by prolonged exposure to atrazine and its chloro-*s*-triazine metabolites.

Applying this group ADI to a 60 kg adult drinking 2 litres of water per day and allocating 20% of the total daily intake to drinking-water, as exposure to atrazine from other sources it expected to be low, a guideline value of 0.1 mg/l can be derived for atrazine and its chloro-*s*-triazine metabolites in drinking-water. This 20% allocation will be very conservative in most countries, as it is expected that exposure of the public will be primarily through drinking-water.

For hydroxyatrazine, JMPR established an ADI of 0–0.04 mg/kg body weight based on the NOAEL of 1.0 mg/kg body weight per day identified on the basis of kidney toxicity (caused by low solubility in water, resulting in crystal formation and a subsequent inflammatory response) at 7.8 mg/kg body weight per day in a 24-month study in rats, and using a safety factor of 25. A modified safety factor on the basis of kinetic considerations was deemed appropriate, as the critical effect of hydroxyatrazine is dependent on its physicochemical properties, and the interspecies variability for such effects is lower than for effects dependent on the area under the curve.

Applying this ADI to a 60 kg adult drinking 2 litres of water per day and allocating 20% of the total daily intake to drinking-water, a guideline value of 0.2 mg/l can be derived for hydroxyatrazine in drinking-water. Again, this 20% allocation will be very conservative in most countries, as it is expected that exposure of the public will be primarily through drinking-water.

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Attachment

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Herbicides and Their Transformation Products in Source-Water Aquifers Tapped by Public-Supply Wells in Illinois, 2001-02

Water-Resources Investigations Report 03-4226

U.S. Department of the Interior
U.S. Geological Survey

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By Patrick C. Mills (U.S. Geological Survey) and William D. McMillan
(Illinois Environmental Protection Agency)

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Cover photographs: Suction-lift pump, dating from about 1924, standby well 11561, Franklin Grove, Ill. (Photographs courtesy of Kelly Mills, Atlanta, Georgia. Reproduced with permission.)

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Conversion Factors, Vertical Datums, and Abbreviated Water-Quality Units

Multiply	By	To obtain
	Length	
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
square mile (mi^2)	2.590	square kilometer (km^2)
	Flow rate	
gallon per minute (gal/min)	3.785	liter per minute (L/m)
	Mass	
pound (lb)	0.4536	kilogram (kg)
	Yield	
gallon per day per square mile ($gal/d/mi^2$)	0.00144	cubic meter per day per square kilomter

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Horizontal coordinate information is referenced to the North American Datum of 1927 (NAD27).

Abbreviated water-quality units used in this report: Organic- and inorganic-constituent concentrations, water temperature, and other water-quality measures are given in metric units. Constituent concentrations are given in milligrams per liter (mg/L) or micrograms per liter (µg/L). Milligrams per liter are considered equivalent to parts per million at the reported concentrations. Micrograms per liter are considered equivalent to parts per billion at the reported concentrations.

Specific conductance (SC) of water is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25 °C). The unit is equivalent to micromhos per centimeter at 25 °C (µmho/cm), formerly used by the U.S. Geological Survey.

Turbidity is not quantified; determined from visual inspection of water clarity.

pH of water is given in standard units.

Additional abbreviations: milliliter (ml), millimeter (mm).

Herbicides and Their Transformation Products in Source-Water Aquifers Tapped by Public-Supply Wells in Illinois, 2001-02

By Patrick C. Mills (U.S. Geological Survey) and William D. McMillan (Illinois Environmental Protection Agency)

Abstract

During 2001-02, ground-water samples were collected from 117 public-supply wells distributed throughout Illinois to evaluate the occurrence of herbicides and their transformation products in the State's source-water aquifers. Wells were selected using a stratified-random method to ensure representation of the major types of source-water aquifers in the State. Samples were analyzed for 18 herbicides and 18 transformation products, including 3 triazine and 14 chloroacetanilide products. Herbicide compounds (field-applied parent herbicides and their transformation products) were detected in 34 percent of samples. A subset of samples was collected unfiltered to determine if analytical results for herbicides in unfiltered samples are similar to those in paired filtered samples and, thus, can be considered equally representative of herbicide concentrations in ground water supplied to the public. The study by the U.S. Geological Survey was done in cooperation with the Illinois Environmental Protection Agency.

Parent herbicides were detected in only 4 percent of all samples. The six most frequently detected herbicide compounds (from 5 to 28 percent of samples) were chloroacetanilide transformation products. The frequent occurrence of transformation products and their higher concentrations relative to those of most parent herbicides confirm the importance of obtaining information on transformation products to understand the mobility and fate of herbicides in ground-water systems. No sample concentrations determined during this study exceeded current (2003) Federal or State drinking-water standards; however, standards are established for only seven parent herbicides.

Factors related to the occurrence of herbicide compounds in the State's source-water aquifers include unconsolidated and unconfined conditions, various hydrogeologic characteristics and well-construction aspects at shallow depths, and proximity to streams. Generally, the closer an aquifer (or well location) is to a recharge area and (or) the stronger the hydraulic connection between an aquifer and a recharge area, the younger the ground water and the more vulnerable the aquifer will be to contamination by herbicide compounds. The weak relation between current (2001) statewide application rates of herbicides and current (2001-02) occurrence of herbicide compounds in source-water aquifers indicates that additional factors must be considered when relating herbicide-application rates to occurrence. These factors include historical application rates and the mobility and persistence of the various herbicide compounds in ground-water systems. Frequency of detection and concentrations of herbicides compounds in the State's source-water aquifers are indicated to be highest during the spring, when crops are planted and herbicides primarily are applied. Excess nitrate (concentrations of nitrate, as nitrogen, higher than 3 milligrams per liter) in ground water strongly indicates the co-occurrence of herbicide compounds. However, nitrate concentrations are not a reliable indicator of herbicide-compound concentrations. The inverse relation found between current use of land for corn and soybean production and current occurrence of herbicide compounds in underlying aquifers indicates that various factors, along with current agricultural land use, contribute to herbicide occurrence. These factors include, among others, land-use history, ground-water age, ground-water-flow patterns, geology, soil microbiology, and chemistry and persistence of the herbicide compounds. Detection of agriculture-

specific herbicide compounds in 71 percent of samples from urban areas with no current or recent agricultural land use near the sampled wells indicates that recharge to certain high-capacity supply wells may originate at considerable distances (up to about 10 miles) from the wells. Essentially no difference was found between the analytical results for herbicides in paired unfiltered and filtered samples, although additional study of this issue is warranted.

Introduction

Herbicides are used extensively in agricultural regions of the United States for control of competing grass and broadleaf weeds. Substantial advances in agricultural production during the past 35 years are associated directly with the increased use of herbicides. However, this increased use of herbicides has introduced concerns about their occurrence in water resources and their possible adverse effect on human health and the environment. Numerous studies have documented the occurrence of herbicides in ground water at the State (Detroy and others, 1988; Risch, 1994; Mehnert and others, 1995; Kolpin and others, 1997, 1998b, 2000b; Kalkhoff and others, 1998; Dana W. Kolpin, U.S. Geological Survey, written commun., 2004), regional (Kolpin and others, 1994, 1995, 1996, 1998a), and national (U.S. Environmental Protection Agency, 1990, 1992; Holden and others, 1992; Kolpin and others, 2000a, 2002) scales. Concerns about ground-water contamination by herbicides are high in many agricultural regions, particularly in Midwestern States, where ground water typically is the principal source of drinking water. The toxic effects of herbicides on humans and animals, and the disruption of reproductive cycles and food chains have been documented in various studies (Blaustein and Wake, 1990; Carbonell and other, 1995; Nemes-Kosa and Cserhati, 1995; Bain and LeBlanc, 1996; Longley and Sotherton, 1997; Ribas and others, 1997; Russell and others, 1997; Spawn and others, 1997; Andersen and others, 2002; Anderson and others, 2002; Crump and others, 2002; Sinclair and Boxall, 2003).

Despite the number of studies regarding the occurrence of herbicides in ground water, there remains an incomplete understanding of the factors that affect their distribution. Additionally, study of herbicide transformation products (also referred to as degradates, metabolites, or breakdown products) in ground water is comparatively limited. These transformation products result from microbial, physical, and chemical processes that alter the field-applied (parent) herbicides and commonly the products occur more frequently and at higher concentrations in ground water than their parent compounds (Kolpin and others, 1998b). Transformation products can, in some cases, have toxicity similar to their parent herbicide compounds (Belfroid and others, 1998; Tessier and Clark, 1995; Tixier and others, 2001), particularly if their chemical structure remains chlorinated.

Because herbicides are used heavily in Illinois corn and soybean production, various statewide studies have been conducted that evaluate the occurrence of herbicides and, in some cases, their transformation products in ground water (Mehnert and others, 1995; Kelly L. Warner and William S. Morrow, U.S. Geological Survey, written commun., 2003; Dana W. Kolpin, U.S. Geological Survey, written commun., 2004). However, none of these studies specifically address herbicide occurrence in the State's source-water aquifers – that is, aquifers that provide untreated water that is used to supply private wells and public drinking water (U.S. Environmental Protection Agency, 2003a) (figs. 1, 2). In an attempt to better understand the occurrence of herbicides in Illinois' source-water aquifers and the factors that affect their occurrence, 117 public-supply wells distributed throughout the State (figs. 1, 3) were sampled by the U.S. Geological Survey (USGS) from October 2001 through September 2002. The study, done in cooperation with the Illinois Environmental Protection Agency (IEPA), was part of an ongoing IEPA program to assess ambient ground-water quality in the State's source-water aquifers tapped for public supply (Illinois Environmental Protection Agency, 2003a). Since 1998, IEPA periodically has collected samples from a subset of the 360 public-supply wells in its ambient ground-water-quality network for analysis of selected triazine and chloroacetanilide herbicides. These analyses do not include every herbicide reportedly (U.S. Department of Agriculture, 2003) used in Illinois, including heavily used glyphosate (the active ingredient in Roundup), nor their transformation products. The present USGS-IEPA study provides analyses of all herbicides reportedly used in Illinois corn and soybean production during the past decade (1991-2001) (U.S. Department of Agriculture, 2003), some herbicides that may not have been used, and various transformation products of these herbicides.

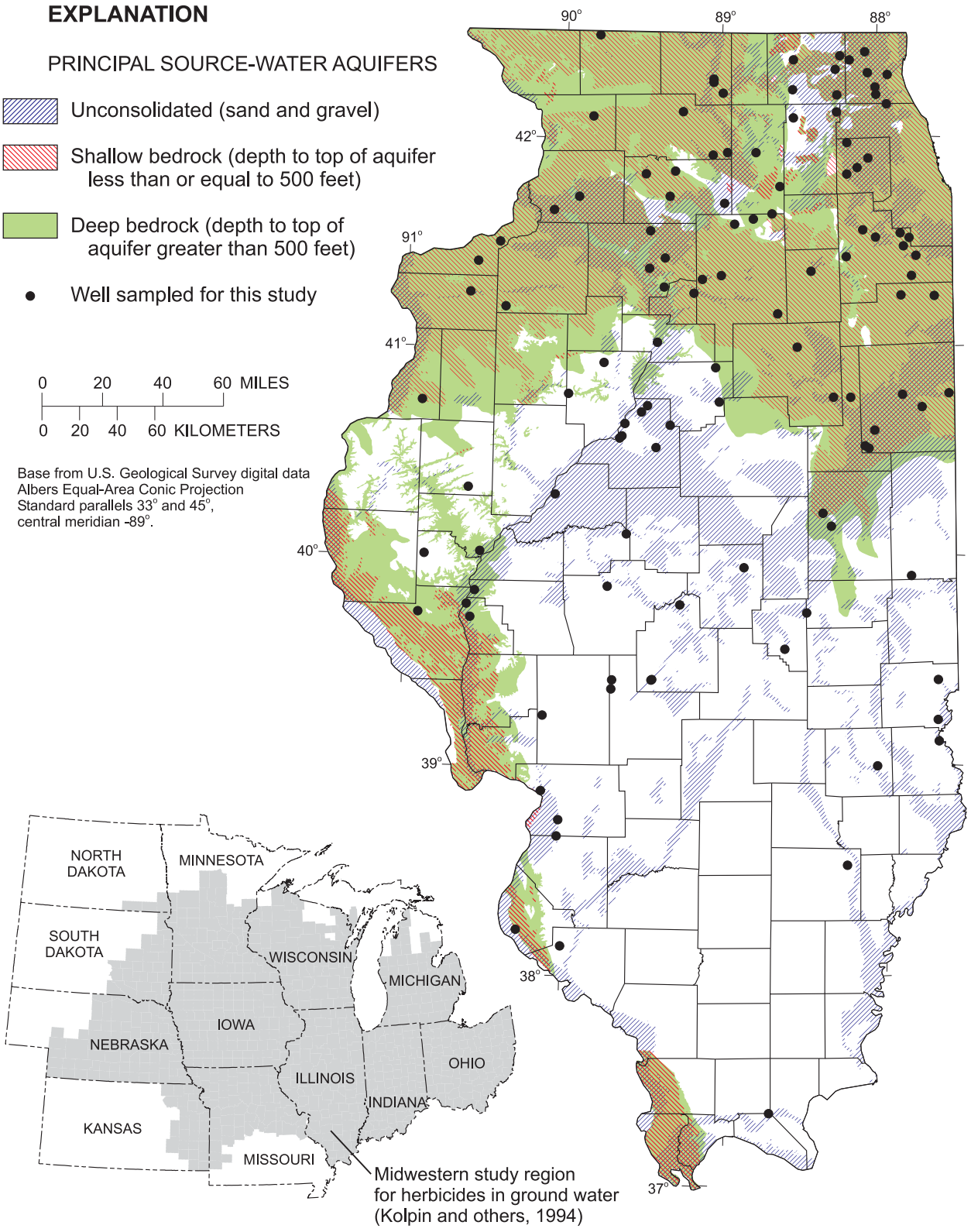

Figure 1. Locations of principal source-water aquifers tapped by most public-supply wells in Illinois (modified from O'Hearn and Schock,1984, fig. 1 and Joseph Konczyk, Illinois Environmental Protection Agency, written commun., 2003).

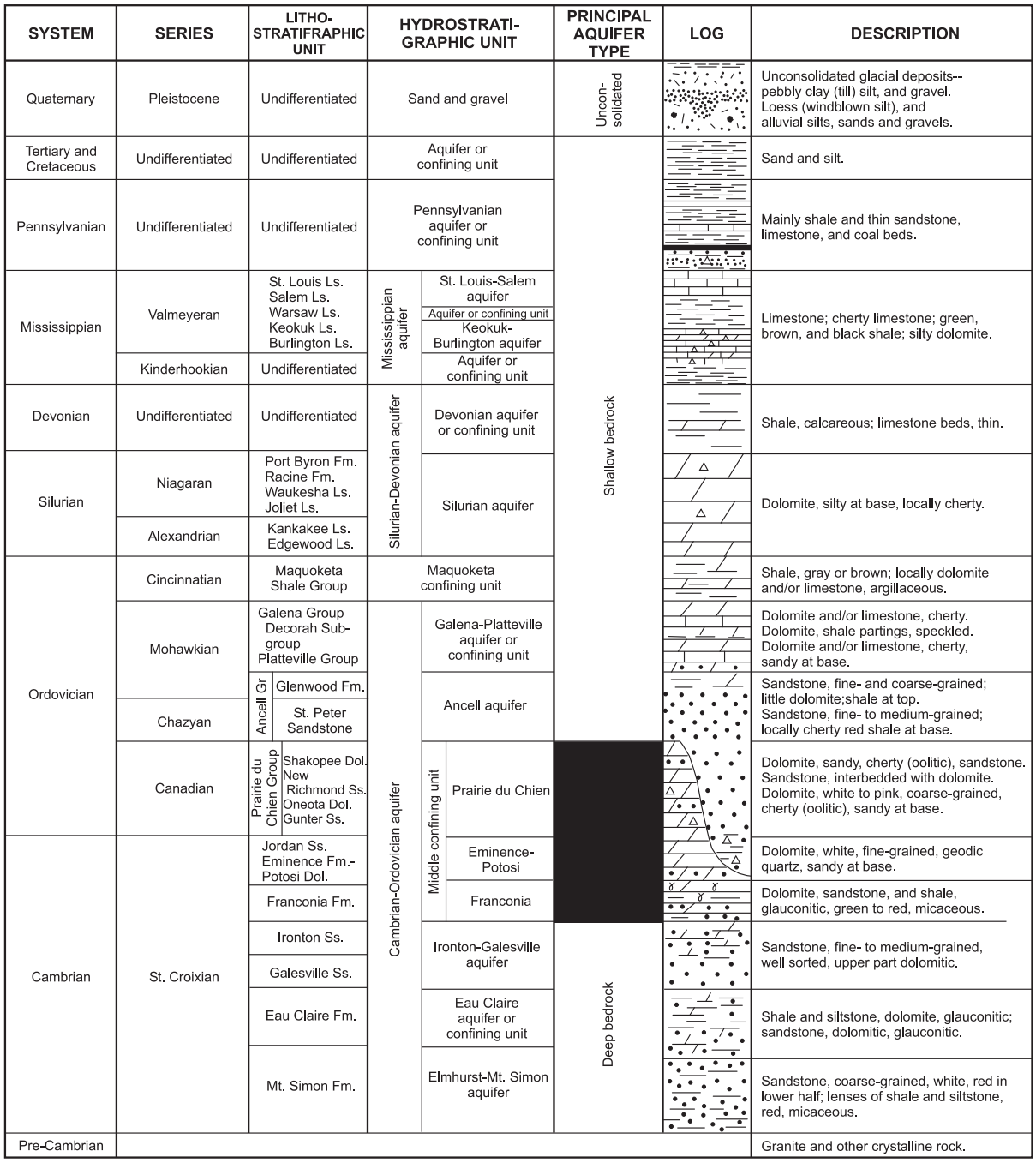

Figure 2. General classification of lithostratigraphic and hydrostratigraphic units that compose source-water aquifers in Illinois (lithostratigraphic and hydrostratigraphic classifications generally follow the use of the Illinois State Geological Survey; modified from Visocky, 1997, fig. 2).

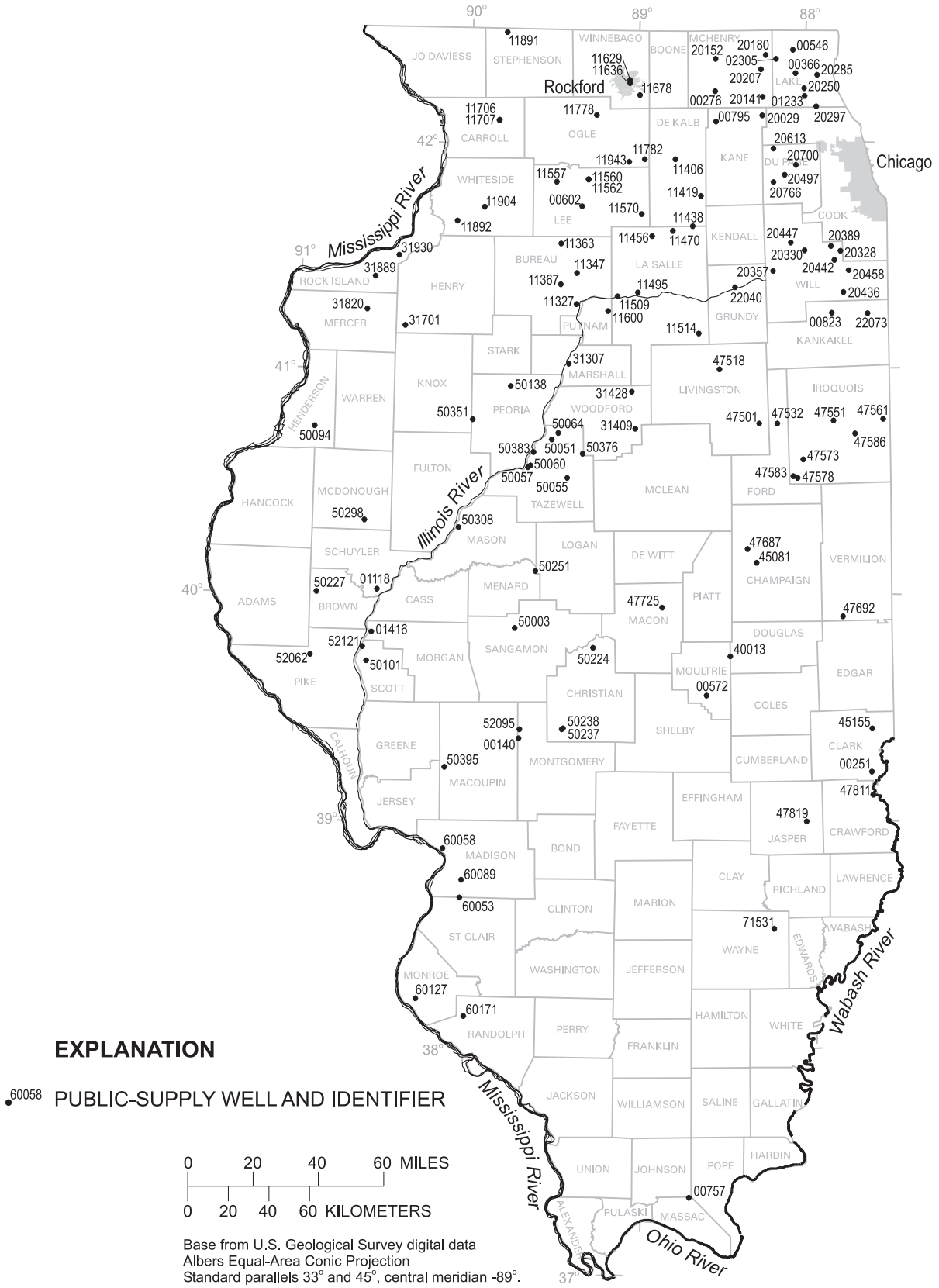

Figure 3. Locations of selected public-supply wells that tap source-water aquifers in Illinois.

Purpose and Scope

This report presents (1) analytical results for selected herbicides and their transformation products in samples collected from 117 public-supply wells in Illinois from October 2001 through September 2002, (2) analysis of selected factors that may relate to the occurrence of herbicides in the State's source-water aquifers or indicate their possible occurrence, and (3) results of herbicide analyses in paired unfiltered and filtered samples to determine if these two types of samples can be considered equally representative of herbicide concentrations in ground water supplied to the public. Analytical results are included for 18 herbicides and 18 transformation products, representing triazine, chloroacetanilide, and glyphosate compounds. Relational factors that were evaluated include herbicide use, hydrogeologic characteristics, well-construction aspects, land use, and occurrence of excess nitrate (concentrations of nitrate, as nitrogen, higher than 3 mg/L) in ground water.

Acknowledgments

Various persons and agencies are acknowledged for their contributions to the study. Wade Boring, Alan Fuhrmann, Laurie Moyer, Gregory White, Edward Wagner, Joseph Konczyk, and Matthew Campbell of the IEPA, Division of Public Water Supplies, scheduled sampling visits with well owners; provided location maps, routing instructions, well-construction details, and other study-related information; calibrated field water-quality meters; prepared wells for sampling; and collected ancillary water-quality data. Well operators and owners freely provided ancillary well information and access to the wells for sampling. Paul Terrio, Robert Kay, and Steven Stammer, USGS, Illinois District, assisted in collecting water samples.

Study Methods

The methods used for well selection, land-use determination, sample collection, laboratory analysis, quality assurance, and data analysis are described in the following sections of the report. Quality-assurance methods include on-site (sampling) and laboratory methods.

Well Selection and Land-Use Determination

Water samples collected in this study for analysis of herbicide compounds were obtained from wells selected by a stratified-random method (Illinois Environmental Protection Agency, 2003a). Criteria used for stratification included aquifer type, geologic-based vulnerability to contamination, and well depth. The selected wells tap major types of source-water aquifers in Illinois – that is, aquifers composed of sand and gravel or of Pennsylvanian- to Cambrian-age bedrock (generally classified as sand and gravel, Pennsylvanian, Mississippian, Silurian-Devonian, and Cambrian-Ordovician aquifers) (fig. 2). Most of the source-water aquifers tapped by the wells in this study (and other public-supply wells in this State) are principal aquifers, as classified by O'Hearn and Schock (1984) (fig. 1). These aquifers have a potential yield of 100,000 gal/d/mi^2, an area of at least 50 mi^2, and are categorized here as unconsolidated (sand and gravel), shallow bedrock (depth to top of aquifer is less than or equal to 500 ft), and deep bedrock (depth to top of aquifer is greater than 500 ft) aquifers (figs. 1, 2). The sand and gravel of the unconsolidated aquifers are deposits from glacial meltwater or streams. The bedrock aquifers generally consist of carbonate or sandstone. Fifty-four of the selected wells tap unconsolidated aquifers (well depths 28-404 ft), 54 tap shallow bedrock aquifers (well depths 71-1,870 ft), and 9 tap deep bedrock aquifers (well depths 820-2,591 ft).

As part of the study, extent of land use for corn and soybean (row-crop) production near the sampled wells was determined. On-site inspection, aerial photographs, and topographic maps were used to identify and estimate percentages of land used for row-crop production within radial distances of 1 and 2 mi of the wells. Trace percentages were estimated to be within the range from greater than 0 to 5 percent of land use. Other percentages were estimated within ranges of from greater than 5 to 25, greater than 25 to 50, greater than 50 to 75, and greater than 75 to 100 percent.

Sample Collection and Laboratory Analysis

Water samples for herbicide analysis were collected by USGS personnel using methods detailed in Kolpin and Burkart (1991) and Wilde and others (1997 to present). Prior to collection of these samples, the wells were purged and other water-quality samples, including

samples for nitrate analysis, were collected by IEPA personnel. Wells were purged through a raw-water tap (before chlorination or other chemical treatment) for 15 minutes or longer to remove water stored in the well casing and delivery piping (fig. 4). Using a calibrated field water-quality meter with an attached flow-through cell, values of water temperature, pH, specific conductance, and oxidation-reduction potential were monitored to ensure stabilization of these field water-quality characteristics and, thus, that the sample would be representative of aquifer water quality. Samples for herbicide analysis were passed through a 0.7 micron, baked, glass-fiber filter (fig. 5a) at a rate of 0.25 gal/min or less and collected in 125-ml, amber, baked-glass bottles (fig. 5b). To limit exposure of the sample to the atmosphere, the filtering equipment was connected directly to the sample tap at the well; more than 90 percent of the wells and sample taps were enclosed in a well house. The samples were shipped chilled (4° C or less) to the analytical laboratory within 2 days of collection.

The filtering equipment, including tubing, connectors, and filter plate, was constructed of Teflon or stainless steel to avoid leaching or adsorption of the organic herbicides. Before sampling, all equipment was cleaned thoroughly with a mixture of laboratory-grade (non-phosphate) soap and tap water and rinsed with deionized water. The water, obtained from the USGS, Illinois District office, has been tested periodically and found free of herbicide compounds.

Selected samples were collected to evaluate the relation between herbicide analytical results for unfiltered and filtered samples. The sampling method used in most herbicide studies by the USGS, particularly projects assisted by the USGS Organic Geochemistry Research Group (OGRG) (Kolpin and Burkart, 1991; Zimmerman and Thurman, 1999), requires filtering to provide samples that essentially are free of particulates. Traditionally, the IEPA collects unfiltered samples that represent total concentrations of constituents (dissolved and undissolved fractions). The IEPA sampling method is dictated by one aspect of their mission -- to evaluate and regulate the quality of drinking water (Illinois Environmental Protection Agency, 2003b). Ground water supplied to the public generally is not processed to remove particulates. Because ground water withdrawn by public-supply wells typically is essentially

Figure 4. Monitoring of field water-quality characteristics (temperature, pH, specific conductance, oxidation-reduction potential) for stabilization of values prior to collection of ground-water samples.

Figure 5. Ground-water sampling at well 47725, Oreana, Ill., for analysis of herbicide compounds: (A) routing of discharge from the well through Teflon tubing to a 0.7-micron, baked, glass-fiber filter enclosed in a stainless-steel filter plate, and (B) collection of samples in 125-milliliter, amber, baked-glass bottles (refer to figure 3, Macon Co., for well location; photographs by Steven E. Stammer, U.S. Geological Survey).

free of visible particulates, the following assumption was evaluated – herbicide-analytical results for unfiltered and filtered samples should be similar (cannot be differentiated statistically). If this assumption is valid, it would indicate that filtering is unnecessary for most samples collected from public-supply wells for herbicide analysis (assumed to be at least 95 percent of samples, as indicated by the samples collected during this study), because unfiltered-sample results can be considered as equally representative of herbicide concentrations as filtered-sample results in ground water supplied to the public. Elimination of filtering would (1) allow better comparison of herbicide-analytical results to those of other constituents from public-supply wells using IEPA methods and (2) substantially reduce the time and expense of sample collection.

Unfiltered samples were collected at 13 wells (11 percent of all samples) for comparison with the filtered samples that were collected routinely during the year-long study. Eleven of the wells were selected randomly. Two of the wells, including well 52095 and 50351 (fig. 3), were selected on the basis of field observations of clay-sized particulates on the filter media following routine sample collection (fig. 6).

Thirty-six herbicide compounds were analyzed by the USGS OGRG laboratory in Lawrence, Kans. Samples were analyzed for 16 herbicides and 3 triazine transformation products by gas chromatography/mass spectrometry (GC/MS) following solid-phase extraction (Zimmerman and Thurman, 1999; Kish and others, 2000); 14 chloroacetanilide transformation products (6 ethane sulfonic (ESA), 6 oxanilic (OA), and 2 sulfynil acetic (SAA) acids) by high-performance liquid chromatography/mass spectrometry (HPLC/MS) following solid-phase extraction (Zimmerman and others, 2000; Lee and others, 2001); and glyphosate, its transformation product amino methyl phosphonic acid (AMPA), and glufosinate by HPLC/MS following solid-phase extraction (Lee and others, 2002). The reporting limit for most herbicides and transformation products was 0.05 µg/L; the limit for glyphosate and related compounds was 0.1 µg/L.

Figure 6. Examples of clay-sized particulates on filter media for samples collected from two public-supply wells that tap source-water aquifers in Illinois, 2001-02 (filter diameter is 142 millimeters—about 5.5 inches; refer to figure 3, Montgomery Co. and Peoria Co., respectively, for well locations).

Quality Assurance

The quality-assurance program for the data collected during this study are divided into two categories: on-site (sampling) methods and laboratory methods. Discussion of those methods and results of quality-assurance evaluations follows.

Differences in paired quality-assurance samples were evaluated by the relative percent difference (RPD) method (U.S. Environmental Protection Agency, 1989). For this method,

$$RPD = \frac{(C1 - C2) \times 100 \text{ percent}}{(C1 + C2) / 2},$$

where

C1 is the concentration of the field sample,

and

C2 is the concentration of the quality-assurance sample.

The method is used to assess the relative magnitude to which the analytical results of the sample pairs differ, and possible bias in the analytical results; that is, whether concentrations are typically over- or under-estimated for a particular sample type.

Quality-assurance methods used during sample collection are described in the previous section "Sample Collection and Laboratory Analysis" and in Kolpin and Burkart (1991) and Wilde and others (1997 to present). Other on-site methods consisted of collecting equipment (or field) blanks and sample duplicates for analysis. Each equipment blank was collected after cleaning the filtering equipment following sample collection. The blanks were collected, as if routine field samples, by pumping laboratory-certified, volatile-organic-free water through the filtering equipment using a peristaltic pump. Analytical results of equipment-blank samples are used to evaluate the effectiveness of routine equipment-cleaning methods and the possibility of atmospheric contamination of the filtering equipment. Twelve equipment blanks were collected (10 percent of field samples). A pseudo-random process was used to select blank-sample sites. Sites were weighted to ensure that blanks were collected (1) during the primary period of herbicide application in Illinois (March-May) (30 percent of blanks) and (2) by each sampler (representing from 5 percent of the sites visited by a sampler to 22 percent of the sites visited by a sampler).

No herbicide compounds were detected in any of the equipment blanks. Five of the field samples collected immediately prior to collection of the blank samples had detectable concentrations of three to seven herbicide compounds, with a maximum concentration of 1.59 µg/L. Thus, equipment-cleaning methods used in the study were determined to be effective.

Each duplicate sample was collected after its associated routinely scheduled field sample was collected and the filtering equipment was cleaned. Thus, the duplicate-sample site was treated as if had not been sampled previously. Analytical results of duplicates are used to evaluate the random variation of sampling. Five duplicates were collected (4 percent of field samples). A pseudo-random process was used to select duplicate-sample sites. Sites were weighted to ensure that duplicates were collected by each sampler; duplicates were collected from 2 percent of the sites visited by a sampler to 15 percent of the sites visited by a sampler.

No herbicide compounds were detected in three of the paired field and duplicate samples. Two of the field samples had detectable concentrations of one to three herbicide compounds. RPD's for the detectable concentrations in the paired samples ranged from -15 to +8 percent. The RPD's are exaggerated by the low herbicide concentrations (0.06-0.41 µg/L). No difference in concentration between individual analyte pairs was greater than 0.02 µg/L. The measured differences in concentrations were at or near the expected variance of the analytical methods (Zimmerman and Thurman, 1999; Zimmerman and others, 2000). With minimal random variation associated with sample collection, the analytical results were determined to accurately represent ground-water quality at the sample sites.

Laboratory quality-assurance samples are used for evaluation of laboratory-analytical methods, which include guidelines for sample-holding times, quantitation-reporting limits, instrument calibration, sample preparation, and sample analysis. Laboratory quality-assurance methods consisted of collecting laboratory duplicates and spikes for analysis. Specific methods for selecting and preparing laboratory quality-assurance samples can be obtained from the OGRL laboratory, on request. Laboratory duplicates were prepared and evaluated for 15 samples analyzed primarily for parent triazine and chloroacetanilide herbicides by GC/MS, 12 samples analyzed for glyphosate and related compounds

by HPLC/MS, and 15 samples analyzed for chloroacetanilide transformation products by HPLC/MS.

No parent triazine, chloroacetanilide, or glyphosate-related herbicides were detected in any of the paired laboratory duplicates. Three of the paired field and laboratory-duplicate samples had detectable concentrations of one to three chloroacetanilide transformation products. RPD's for the detectable concentrations in the paired samples ranged from -13 to +15 percent. The RPD's are exaggerated by the low herbicide concentrations (0.06-0.18 µg/L). No difference in concentration between individual analyte pairs was greater than 0.01 µg/L. The measured differences in concentrations were within the expected variance of the analytical methods (Zimmerman and Thurman, 1999; Zimmerman and others, 2000). With minimal variability associated with sample preparation and analysis, the analytical results were determined to accurately represent ground-water quality at the sample sites.

Data Analysis

Selected factors that may relate to the occurrence of herbicide compounds in the source-water aquifers of the State or indicate their possible occurrence were analyzed graphically and statistically. Relational factors included herbicide use, hydrogeologic characteristics, well-construction aspects, land use, and occurrence of excess nitrate. Most of the factors have been identified in other studies of herbicides in ground water. Among these studies is a regional study of the Midwestern cornbelt States (fig. 1), including Illinois, conducted during 1991-94 (Kolpin and others, 1994, 1995, 1996, 1998a). The factors were reexamined in this study to determine whether the relations were consistent at a scale smaller than the Midwestern study and, in particular, whether they were consistent with hydrogeologic conditions specific to Illinois.

The correlation between frequency of detection (representing occurrence) of herbicide compounds in the State's source-water aquifers and the selected relational factors was examined using the Pearson's r method (Helsel and Hirsch, 1995). The method provides the degree of linear relation (from -1 to +1) between selected variables. A correlation of +1 indicates a perfect positive linear (but not causal) relation between two variables (for example, frequency of herbicide detection and confining-unit thickness). For the correlations, rank values were used to represent selected ranges of the measurable factors. For example, for the factor confining-unit thickness, ranks of 1 to 5 were used to represent the following intervals of increasing thickness: 0-10, 11-20, 21-50, 51-100, and greater than 100 ft. Examination of the histograms used to graphically evaluate the correlation between herbicide occurrence and the various relational factors verifies the linear relation between these variables and, thus, the use of the Pearson's r method. The Spearman's rank test was used to determine the covariance (degree of linear relation from -1 to +1) between nitrate and herbicide-compound concentrations; that is, whether, as nitrate concentrations increase, herbicide-compound concentrations tend to increase or decrease.

The difference in concentration of herbicide compounds determined from paired unfiltered and filtered samples was evaluated, in part, using the RPD method (U.S. Environmental Protection Agency, 1989). As described in the section "Quality Assurance", the method is used to assess the relative magnitude to which the analytical results of sample pairs differ. For the evaluation of unfiltered and filtered sample pairs, concentration C1 represents the unfiltered sample and C2 represents the filtered sample. It was assumed that if concentrations of the paired samples differ, concentrations of the unfiltered sample likely would be higher than those of the filtered sample.

Concentrations of herbicide compounds determined from paired unfiltered and filtered samples also were evaluated to determine if the resultant populations were statistically different. Various statistical tests were used, including the sign test, Wilcoxon signed-ranks test, and paired Student's *t*-test (Helsel and Hirsch, 1995). These tests were considered appropriate because the differences between values (concentrations) in the data pairs, although typically nonparametric (not distributed normally), can be considered symmetric. For paired data, the sign test determines whether values from one data set generally are larger, smaller, or different than values from its paired data set. This test may be used regardless of the distribution of the differences and, thus, is fully nonparametric. The Wilcoxon signed-ranks test determines whether the median difference between paired values is significantly different than zero. That is, do the paired data sets represent different populations? For this test, the distribution of the difference in paired values need not be normal but is assumed to be symmetric. The paired Student's *t*-test determines whether mean differ-

ence between paired values is significantly different than zero. For this test, the distribution of the difference in paired values is assumed to be normal.

A significance level of 0.05 was used for all statistical evaluations. This probability of error (ρ=0.05) means that there is 1 chance in 20 that the statistical test reported a significant difference between the paired data when there was not a significant difference. The smaller the ρ value, the greater is the certainty that a reported statistical difference is real.

Occurrence of Herbicides and Transformation Products

Analytical results for the study of herbicides and herbicide transformation products in source-water aquifers tapped for public supply in Illinois are presented in this section. Frequencies of detection and representative concentrations of the herbicide compounds are described and considered with respect to the results of other studies conducted in Illinois and elsewhere of herbicides in ground water. The analytical results, representing samples collected from October 2001 through September 2002, are summarized in table 1 and presented in full in appendixes 1 and 2. Location and construction information for the sampled wells and their field-measured characteristics of water quality also are presented in appendix 1.

Frequencies of Detection and Concentrations

Herbicide compounds were prevalent in Illinois' source-water aquifers, as indicated by the frequency (table 1) and spatial distribution of detected herbicides and (or) transformation products (fig. 7). Parent herbicides (atrazine and metolachlor) were detected in 4 percent of all samples, and parent herbicides and transformation products were detected in 34 percent of samples. Multiple herbicide compounds were detected in 70 percent of samples, with a median of three and a maximum of eight compounds in samples in which more than one compound was detected. Of the 11 detected compounds, 9 were transformation products (table 1; fig. 8). Six chloroacetanilide transformation products, including alachlor ESA (28 percent), metolachlor ESA (26 percent), metolachlor OA (14 percent), and acetochlor ESA (9 percent), were the most frequently detected compounds. Detection frequencies of ESA compounds consistently exceeded those of OA compounds for all chloroacetanilides (for example, alachlor ESA, 28 percent; alachlor OA, 6 percent).

Concentrations of detected herbicide compounds ranged from the reporting limit of 0.05 µg/L to 7.24 µg/L (metolachlor ESA), with concentrations of parent herbicides substantially lower than those of their transformation products (table1; fig. 9). The median concentration of detected parent herbicides was 0.07 µg/L, whereas the median concentration of all detected herbicide compounds, including transformation products, was 0.16 µg/L. The highest concentration of a parent herbicide (atrazine) was 0.22 µg/L, whereas concentrations of the chloroacetanilide transformation products in seven samples (including metolachlor ESA in four samples) exceeded 1 µg/L. Concentrations (maximum, mean, and usually median) of ESA compounds exceeded those of OSA compounds and both exceeded the concentrations of their parent compounds. As indicated by these findings, the frequency of detection and the concentration greatly increase for most herbicides when their transformation products also are considered (table 1; figs. 8, 9).

No concentrations exceeded Federal maximum contaminant levels (MCL's) or health advisory levels (U.S. Environmental Protection Agency, 2003b) (table 1) or Illinois standards (Illinois Pollution Control Board, 2003) established for the protection of public-water supplies. However, these levels have been established for only seven herbicides and have not been established for any transformation products. In addition, no regulatory levels consider the toxicity or aquatic effect of multiple compounds in water. The concentration of alachlor ESA in one sample exceeded the MCL (2 µg/L) of its parent compound. S-metolachlor was not analyzed for in this study. Recorded use of this herbicide began in 2000 with about 3 million pounds applied in that year and 4 million pounds applied in 2001 (U.S. Department of Agriculture, 2001). These herbicide-application rates rank fourth and third, respectively, in those years. S-metolachlor reportedly requires only 65 percent of the application rate of metolachlor, thus less active ingredient than that of metolachlor may be available for leaching to ground water (Ecologic-IPM, 2003).

Deethylatrazine, deisopropylatrazine, and cyanazine amide were the only transformation products of triazine herbicides analyzed for in this study. Other triazine transformation products that previously have been

Table 1. Summary statistics for herbicides and their transformation products in ground-water samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001–September 2002.

[µg/L, micrograms per liter; na, not applicable]

Analyzed for but not detected: acetochlor sulfynil acetic acid (SAA), alachlor SAA, ametryn, flufenacet, flufenacet ethanesulfonic acid (ESA), flufenacet oxanilic acid (OA), glufosinate, AMPA, pendimethalin, prometon, prometryn, propazine, and terbutryn; SAA, ESA, and OA are transformation products (TP) of the associated herbicides; AMPA is a transformation product of glyphosate. Reporting limit for most herbicide compounds was 0.05 µg/L; reporting limit for glyphosate, amino methyl phosphonic acid (AMPA), and glufosinate was 0.10 µg/L.

Maximum contaminant levels (MCL)[1] for atrazine, alachlor, simazine, and glyphosate are 3, 2, 4, and 700 µg/L, respectively; health advisory levels[1] for cyanazine, metolachlor, and metribuzin are 1, 100, and 200 µg/L, respectively.

Herbicide compound	Detection frequency, in percent	Median detected concentration, in µg/L	Maximum detected concentration, in µg/L	Herbicide-application rate in Illinois[2], in 1,000 pounds	
	2001-02	2001-02	2001-02	1991	2001
Any parent herbicide	4.3	0.07	0.22	na	na
Any herbicide or TP	34.2	.16	7.24	na	na
Acetochlor	0	na	na	0	8,059
Acetochlor ESA	9.4	.16	4.18	na	na
Acetochlor OA	5.5	.16	.25	na	na
Alachlor	0	na	na	9,400	0
Alachlor ESA	28.2	.12	2.15	na	na
Alachlor OA	6.0	.09	.41	na	na
Atrazine	3.4	.06	.22	10,615	14,143
Deethylatrazine[3]	4.3	.08	.21	na	na
Deisopropylatrazine[4]	0	na	na	na	na
Cyanazine	0	na	na	4,267	0
Cyanazine amide[5]	0	na	na	na	na
Dimethenamid	0	na	na	0	2,270
Dimethenamid ESA	2.6	.05	.16	na	na
Dimethenamid OA	0	na	na	na	na
Glyphosate	0	na	na	381	7,157
Metolachlor	.9	.16	.16	9,277	993
Metolachlor ESA	26.5	.34	7.24	na	na
Metolachlor OA	14.5	.18	2.95	na	na
Metribuzin	0	na	na	395	0
Propachlor	0	na	na	0	0
Propachlor ESA[6]	1.0	.10	.10	na	na
Propachlor OA	0	na	na	na	na
Simazine	0	na	na	0	265

[1]U.S. Environmental Protection Agency (2003b).

[2]U.S. Department of Agriculture (2003). Data are not reported for herbicides applied on less than 1 percent of corn and soybean row-crop acreage or herbicides applied on sweet or processed-corn acreage, which generally represents less than 1 percent of total application on row-crop acreage.

[3]Transformation product of atrazine.

[4]Transformation product of atrazine, cyanazine, and simazine.

[5]Transformation product of cyanazine.

[6]Ninety-nine samples were collected for analysis of propachlor ESA and OA, and all SAA compounds.

detected in Illinois ground water but were not analyzed for in this study include hydroxyatrazine and deeythyldeisopropylatrazine (Kelly L. Warner and William S. Morrow, U.S. Geological Survey, written commun., 2003). Frequency of occurrence and concentrations of these compounds in ground water are expected to be low, as indicated by field experiments and (or) sampling history. Hydroxyatrazine has a greater tendency to sorb to soils than atrazine, its primary parent compound, or other atrazine transformation products (Barbash and Resek, 1996). Thus, frequent occurrence of hydroxyatrazine in ground water, particularly at concentrations above those of other atrazine compounds, would be unexpected.

Deeythyldeisopropylatrazine is a transformation product of the first-order products deethylatrazine, deisopropylatrazine, and hydroxyatrazine. In a study of herbicides in shallow ground water (depth less than about 50 ft) in central Illinois (Kelly L. Warner and William S. Morrow, U.S. Geological Survey, written commun., 2003), hydroxyatrazine was detected in only 4 percent of 69 samples collected for the study; the maximum concentration was 1.14 µg/L. Deeythyldeisopropylatrazine was detected in only 4 percent of 28 samples (1 sample); the maximum concentration was 1.0 µg/L.

The extensive use of alachlor, acetochlor, and metolachlor during the past decade (1991-2001) (fig. 10)

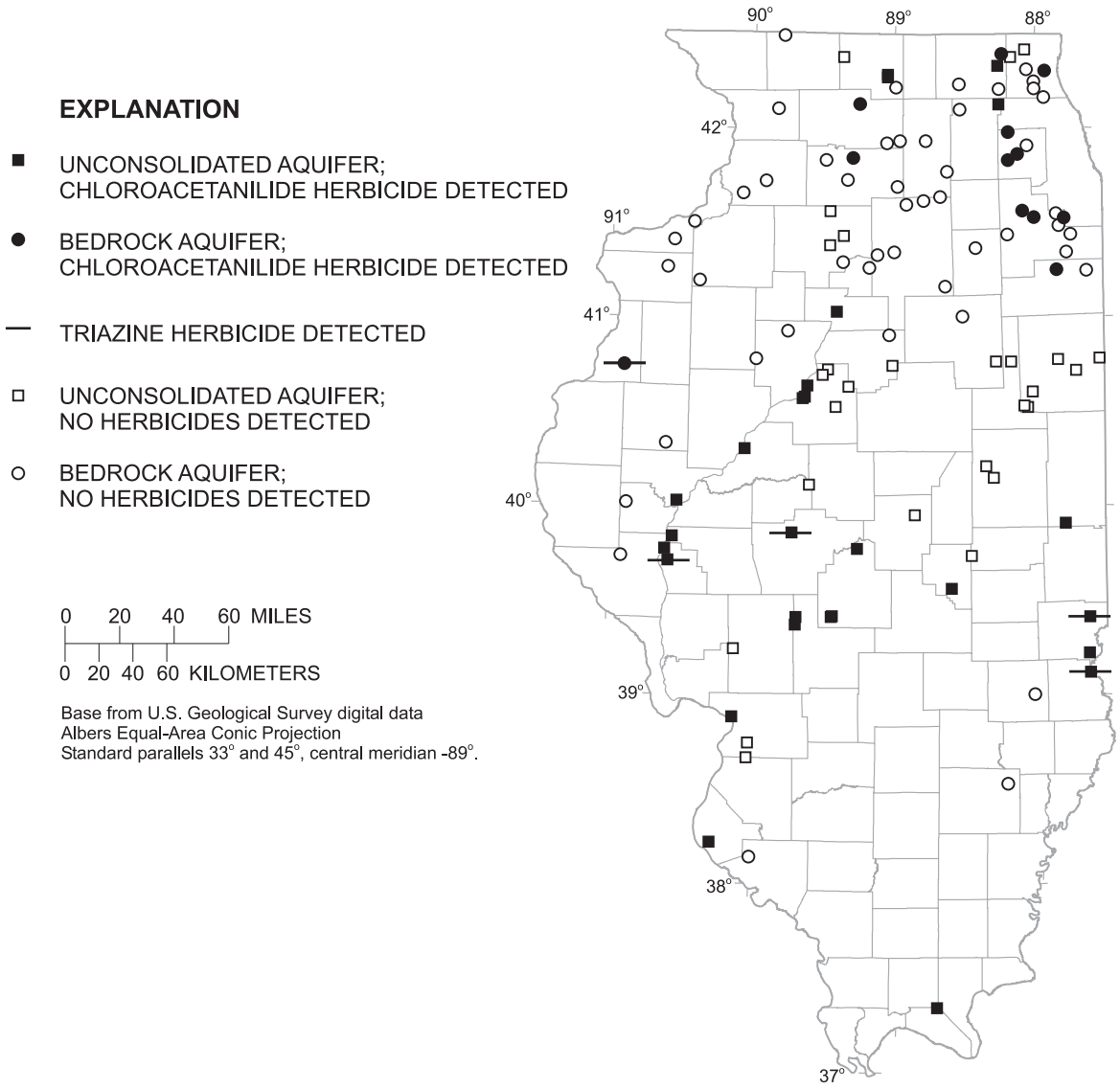

Figure 7. Locations of selected public-supply wells that tap source-water aquifers in Illinois, aquifer type (unconsolidated or bedrock) that the wells tap, and occurrence of herbicides or transformation products, 2001-02.

along with their comparatively low detection rates and concentrations in ground water as parent compounds and comparatively high detection rates and concentrations as transformation products (ESA and OA compounds) (table 1) confirms previous observations that each of these structurally related chloroacetanilide herbicides transforms similarly (Kolpin and others, 1996; Aga and others, 1995). Each of the parent herbicides degrade readily in the soil zone and persist in ground water as mobile transformed compounds. Furthermore, the frequencies of detection and concentrations in this and other studies (Kalkhoff and others, 1998, Kolpin and others, 2000b) for the parent compounds and their transformation products support conclusions that regardless of which chloroacetanilide, ESA compounds are more mobile and persistent than OA compounds (Kalkhoff and others, 1998). The finding that frequencies of detection of metolachlor compounds consistently are greater than those of the alachlor compounds and much greater than those of acetochlor compounds can be attributed, in part, to the extent to which these compounds have been used in Illinois (fig. 10) and other Midwestern States in recent years. However, the comparatively low occurrence of acetochlor relative to its comparatively heavy use since about 1995 indicates that differences in the physical properties and related rates of transformation of the various chloroacetanilide compounds are contributing factors (Kalkhoff and others, 1998) to the consistent ranking of the detection frequencies of these compounds.

The more even distribution between the detection frequencies of atrazine (detected in 3 percent of samples) and deethylatrazine (detected in 4 percent of samples) than that determined for the chloroacetanilide herbicides and their transformation products (table 1) is related to the comparatively slower transformation rate for atrazine, particularly under hypoxic conditions (Clay and others, 1995; Agertved and others, 1992; McMahon and others, 1992; Klint and others, 1993; Nair and Schnoor, 1992), and is indicative of the relative stability of atrazine and deethylatrazine (Thurman and others, 1992;

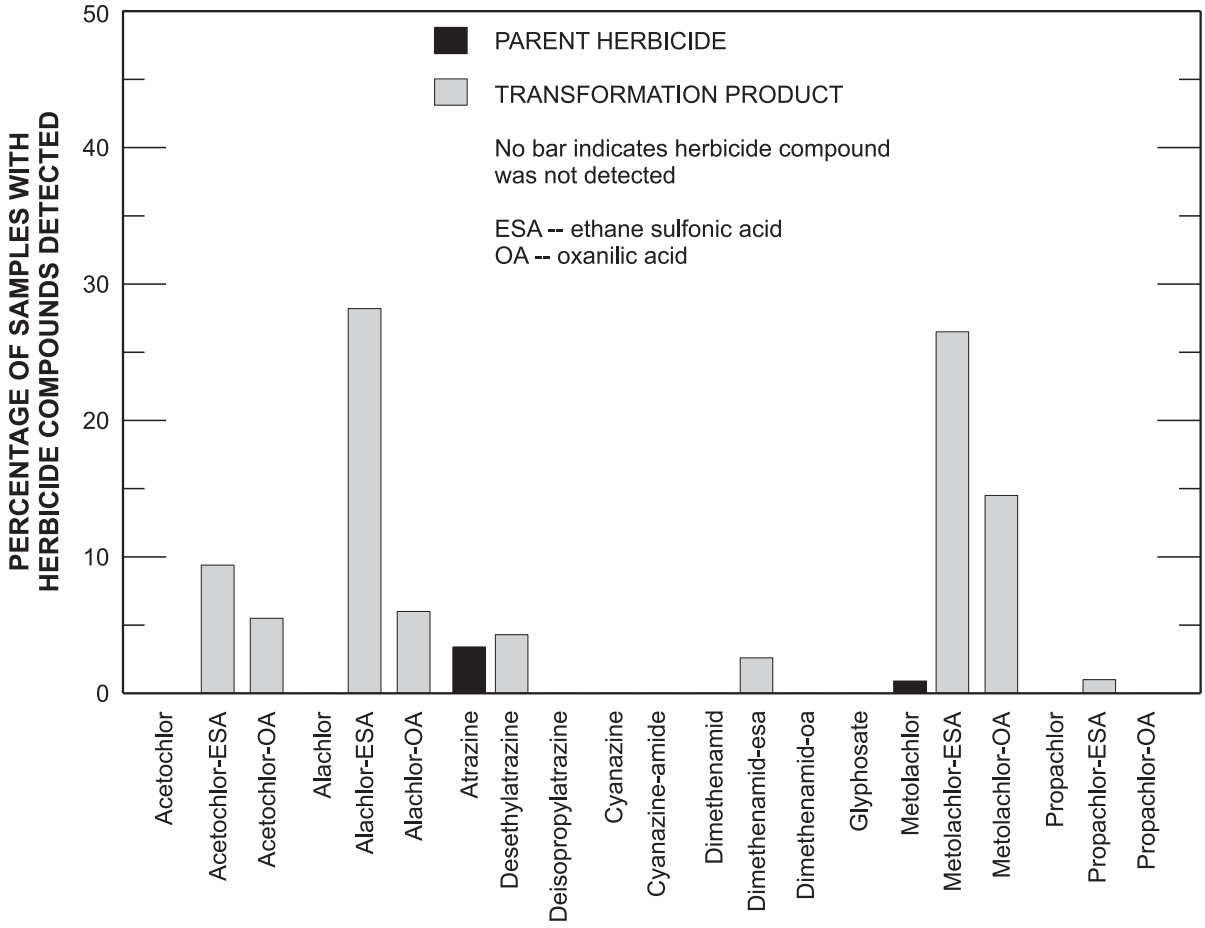

Figure 8. Frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois, 2001-02.

Roy and Krapac, 1994; Jayachandran and others, 1994). The slow transformation of atrazine allows more of it to be transported to ground water than the parent chloroacetanilides.

The concentration ratios of deethylatrazine to atrazine (DAR's) recorded in this study (from 0.95 to 4.2) indicate that the atrazine detected in the ground-water samples likely was derived from slow infiltration through well-oxygenated soil, as typically associated with nonpoint sources (Adams and Thurman, 1991; Thurman and others, 1991). Such hydrogeologic conditions allow for increased transformation of atrazine to deethylatrazine by soil microorganisms. Rapid infiltration to poorly oxygenated hydrogeologic environments below the soil zone, as typically associated with point sources and (or) preferential flow, limits transformation of atrazine and, thus, results in lower DAR's than those recorded in this study (ratios may range down to 0.1 or less).

Deisopropylatrazine, a transformation product of atrazine, cyanazine, and simazine, was not detected in samples collected for this study. Its nondetection probably relates in part to (1) its instability relative to atrazine and deethylatrazine (Geller, 1980; Adams and Thurman, 1991) and (2) the limited recent and (or) historical use of cyanazine and simazine in Illinois row-crop production (fig. 10).

The extensive use of glyphosate since 1996 (fig. 10), following introduction of Roundup Ready soybeans, along with its nondetection in Illinois' source-water aquifers as either a parent compound or transformation product (AMPA) confirms previous observations (Giesy and others, 2000) that the mobility and persistence of glyphosate are low. Field and laboratory studies indicate that glyphosate sorbs strongly to soils and degrades readily by microbial processes (Extension Toxicology Network, 2003).

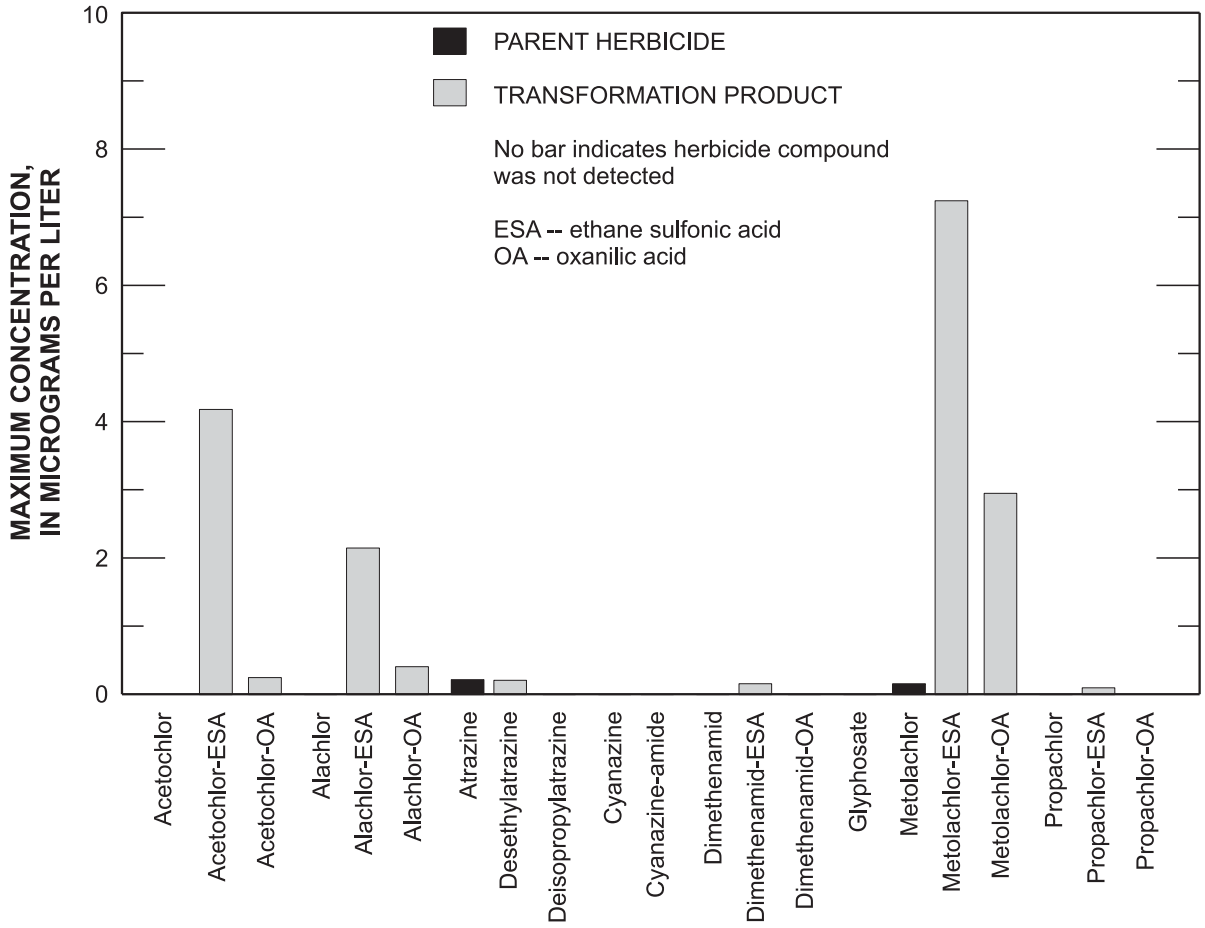

Figure 9. Maximum concentrations of herbicides and their transformation products in source-water aquifers in Illinois, 2001-02.

Comparison to Other Studies

The findings of the present study generally are similar to those of other statewide herbicide studies in Illinois (Dana W. Kolpin, U.S. Geological Survey, written commun., 2004) and Iowa (Kolpin and others, 1997, 1998b, 2000b; Kalkhoff and others, 1998), particularly for the various relations associated with frequencies of detection and concentrations between parent herbicides and their transformation products. In the previous statewide studies and the present study, transformation products were detected in groundwater samples more frequently and at higher concentrations than their parent compounds. Typically frequencies of detection and concentrations of almost all of the herbicide compounds analyzed for were lower in the present study than in the other statewide studies. For example, in the previous Illinois study (Dana W. Kolpin, U.S. Geological Survey, written commun., 2004), one or more parent herbicide or transformation products were detected in 56 percent of the ground-water samples collected from 55 wells during fall 2000; the maximum concentration detected was 40 µg/L (metalochlor ESA). In the Iowa study (Kalkhoff and others, 1998), one or more parent herbicide or transformation products were detected in 75 percent of the ground-water samples collected from 88 wells during summer 1996; the maximum concentration detected was 50 µg/L (alachlor ESA).

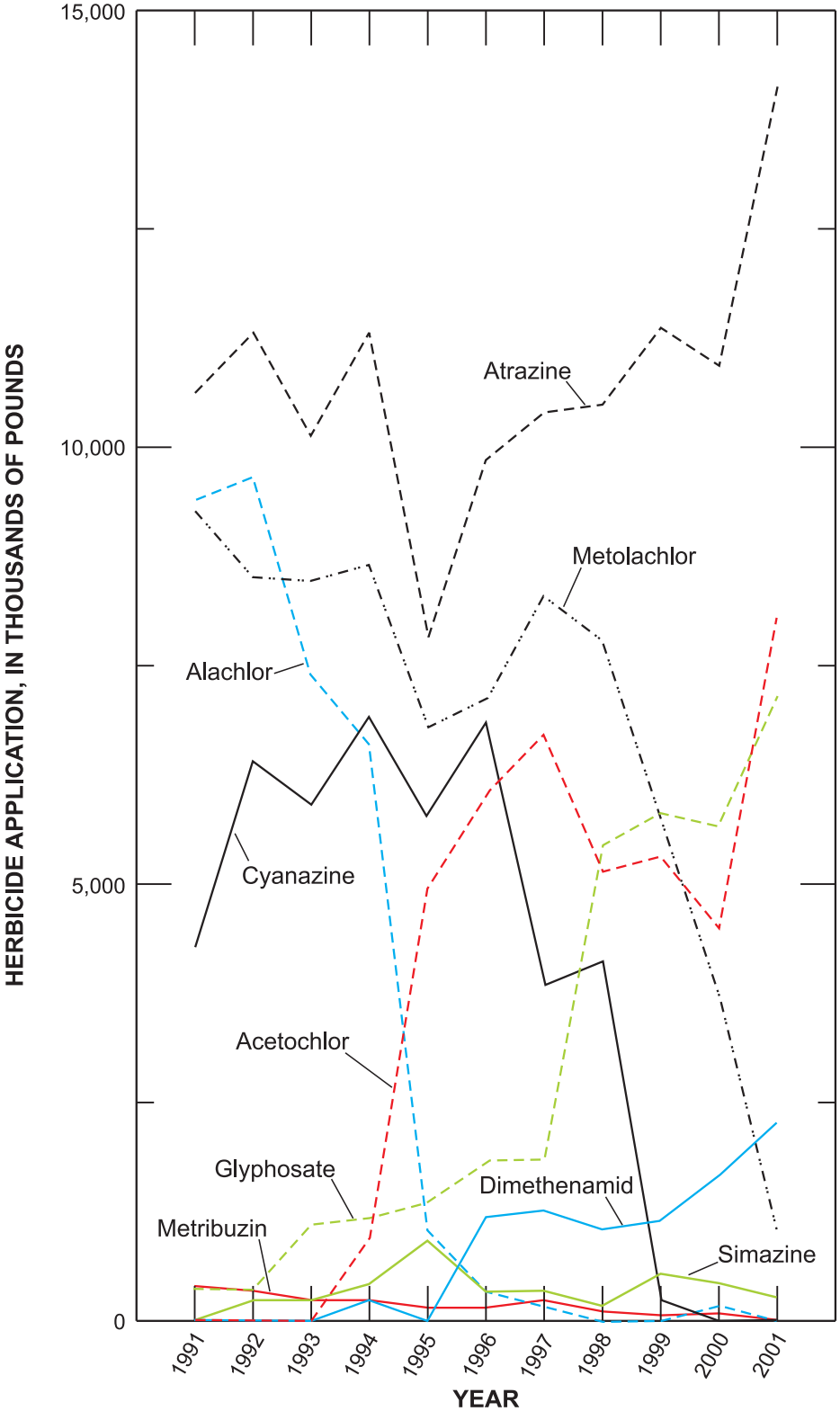

Figure 10. Annual herbicide-application rates in Illinois during 1991-2001 (herbicide-application-rate data are from U.S. Department of Agriculture, 2003; data are not reported for herbicides applied on less than 1 percent of corn and soybean acreage).

Each of the statewide studies described above used the same sample-collection methods, laboratories, analytical methods, and reporting concentrations, and analyzed for essentially the same herbicide compounds. Differences between the results of the present study and those of the previous Illinois study (Dana W. Kolpin, U.S. Geological Survey, written commun., 2004) can be attributed primarily to differences in the ground-water systems under investigation and the types of wells that were sampled. The previous study focused on aquifers expected to be vulnerable to herbicide contamination – that is, on shallow aquifers (unconsolidated and bedrock aquifers less than 50 ft below land surface) in areas where at least 25 percent of the land use was for row-crop production. Wells of various types were sampled, of which only 13 were public-supply wells. Differences between the results of the present study and those of the Iowa study cannot be attributed readily to differences in the ground-water systems under investigation and the types of wells that were sampled. The Iowa samples, like those in the present study, were collected from public-supply wells of various depths that tap Iowa's major types of source-water aquifers (alluvial, glacial drift, bedrock/karst, bedrock/nonkarst, as defined in that study). Temporal sampling bias (Detroy and others, 1988; Risch, 1994; Kolpin and others, 1994) likely accounts for much of the difference in results. In the present study, samples were collected year-round, whereas in the Iowa study, samples were collected during the summer growing season (thus, closer to the time of herbicide application).

The findings of the present study also are similar to those of the Midwestern study that included Illinois (Kolpin and others, 1994, 1995, 1996, 1998a). However, as with the statewide studies, frequencies of detection and concentrations of almost all of the herbicide compounds analyzed for were lower in the present study than in the Midwestern study. In the Midwestern study, one or more parent herbicide or atrazine-transformation products were detected in 28 percent of the ground-water samples collected from about 300 wells during summer 1991; the maximum concentration was 2.3 µg/L (deethylatrazine). Differences between the results of the present study and those of the Midwestern study can be attributed primarily to differences in the ground-water systems under investigation and the types of wells that were sampled. The focus of the Midwestern study was the same as that of the previous Illinois study (aquifers expected to be vulnerable to herbicide contamination) (Dana W. Kolpin, U.S. Geological Survey, written commun., 2004). As with the present study and the statewide studies, the same collection methods, laboratories, analytical methods, and reporting concentrations were used and essentially the same herbicide compounds were analyzed for (although analysis of transformation products in the Midwestern study was limited primarily to analysis of atrazine compounds).

Comparison of results of the present study with other large-scale studies is difficult because, typically, herbicide transformation products were not analyzed for in samples collected in other studies. In addition, higher analytical reporting limits and different well-selection and sample-collection methods usually were used in other studies. The largest comparable study of public-supply wells is the National Pesticide Survey (NPS) (U.S. Environmental Protection Agency, 1990, 1992). The frequency of detecting one or more herbicide compound was lower in the present study (4 percent, for parent herbicides) than in the NPS study (9 percent, for parent herbicides plus the atrazine transformation product deethylatrazine); concentrations were lower than MCL's or health advisory levels in both studies. Reasons for the greater frequency of detection in the NPS study than the present study are uncertain. Given the typically higher reporting limits and analysis of fewer transformation products in the NPS study, the comparatively higher detection frequencies of the NPS study could be attributed, in part, to differences in the population of wells/aquifers that were sampled and temporal sampling bias (use of herbicides may have been greater in and near the time of the NPS study than that of the present study).

Factors Related to Occurrence of Herbicide Compounds

Awareness of the types of herbicides identified in large-scale studies, such as the present study of Illinois' source-water aquifers, and the factors that may be related to their occurrence can aid in determining the vulnerability of various source-water aquifers to herbicide contamination by certain herbicides and developing more efficient strategies for sample collection. Sampling can be focused on herbicides that likely are to be detected in ground water and on locations and times where potential for contamination is greatest. Focused sampling should

reduce time and costs associated with the collection of samples and with regulatory management of these contaminants. As a result, there may be increased opportunities for addressing other water-quality issues, including further study of the toxicity of various herbicide transformation products and their fate and transport.

Various factors that may be related to the occurrence and detection of herbicides in Illinois' source-water aquifers are examined in the following section. These factors include herbicide use, various hydrogeologic characteristics, well-construction aspects, and land use. Excess nitrate in ground water also is examined as an indicator of herbicide occurrence. Specific herbicide-use factors examined include rates and timing of herbicide applications. Hydrogeologic characteristics examined include depth to the top of the aquifer, thickness of the confining unit, designation as a confined aquifer, and stream proximity. Well-construction aspects examined include well depth and depth to base of the well casing.

Herbicide Use

When only parent compounds are considered, a moderate positive relation (r = 0.72) was found between current (2001) herbicide-application rates and current (2001-02) frequency of detection of herbicides in Illinois' source-water aquifers (table 1). Atrazine, the most frequently detected parent herbicide in ground-water samples collected from these aquifers, is consistently the most heavily applied herbicide in Illinois (fig. 10). In 2001, about 14 million pounds of atrazine were applied to the State's corn crops -- almost double the amount of the next most heavily applied herbicides (acetochlor and glyphosate). Metolachlor, the only other parent herbicide that was detected during this study, was the second most heavily applied herbicide during most of 1991-98 (fig. 10).

When herbicide transformation products are considered, there is little to no relation between current herbicide-application rates and current frequency of detection of herbicide compounds in the State's source-water aquifers (r = -0.20) (fig. 11A). For example, in 2001,

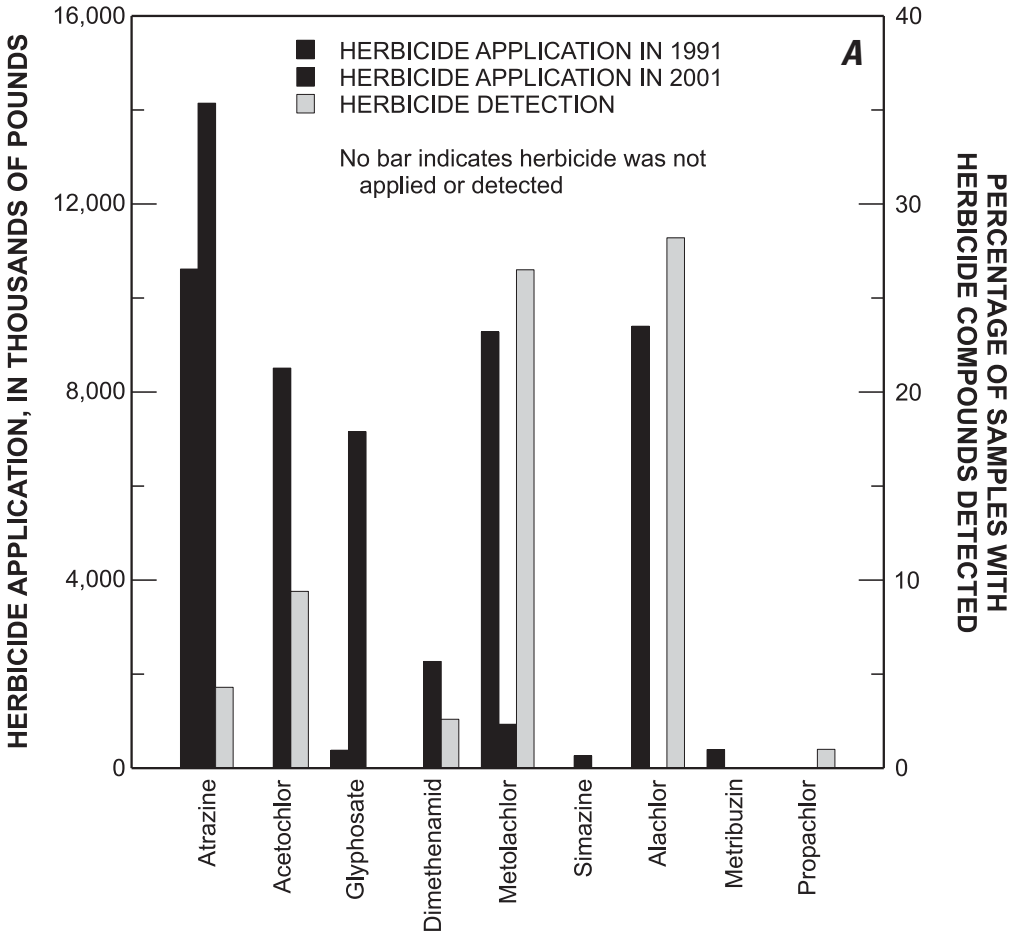

Figure 11. Relation of (A) frequency of detection of herbicides and their transformation products and (B) maximum total concentration of herbicides and their transformation products in source-water aquifers in Illinois (2001-02) to annual herbicide-application rates in Illinois (1991, 2001) (herbicide-application-rate data are from U.S. Department of Agriculture, 2003; data are not reported for herbicides applied on less than 1 percent of corn and soybean acreage).

there was no recorded use of alachlor in the State (U.S. Department of Agriculture, 2003). Yet, alachlor ESA was the most frequently detected herbicide compound (28 percent) in samples collected from these aquifers during approximately the same time period (2001-02). Similarly, there is little to no relation between current herbicide-application rates and current herbicide concentrations in the State's source-water aquifers (fig. 11B). The time lag between land application of herbicides and herbicide transport to ground water requires that the mobility and persistence of herbicide transformation products and historical herbicide-application rates be considered to better determine the relations between herbicide-application rates and the occurrence and concentrations of herbicides in source-water aquifers. Use of certain herbicides has varied substantially during the past decade (1991-2001) (figs. 10, 11). Other contributing factors related to hydrogeology, herbicide chemistry, and soil biochemistry that can affect the mobility and persistence of many herbicide transformation products also need to be considered when relating application rates of herbicides to their occurrence and concentrations in ground water. The weak relation between herbicide-application rates and detection frequency, as determined in this study, also may be attributed, in part, to the use of statewide-application-rate data. Annual statewide-application-rate data available from the U.S. Department of Agriculture (2003) were used for the examination, because location-specific data, even at the county scale, were not readily available.

When the timing of herbicide application during the cycle of crop planting to harvesting is considered, it is evident that herbicides and their transformation products are detected most frequently (50 percent of samples) in the State's source-water aquifers during the spring crop-planting and herbicide-application period (fig. 12). At least 90 percent of the annual application of the detected herbicides currently (2001) in use in Illinois (atrazine, acetochlor, dimethenamid, and metolaclor) is during March through June (most in Aril and May), primarily for pre-emergent control of competing weeds and grasses (Aaron Hager, University of Illinois Extension, oral commun., 2003; Dennis P. McKenna, Illinois Department of Agriculture, written commun., 2003).

Concentrations of herbicide compounds detected in the State's source-water aquifers also are indicated to be highest during the spring crop-planting and herbicide-application period. The highest total concentration of all wells sampled during an application period was from samples collected in the spring period (26.7 µg/L). Additionally, three of the five highest total concentrations (12.9, 2.7, and 2.1 µg/

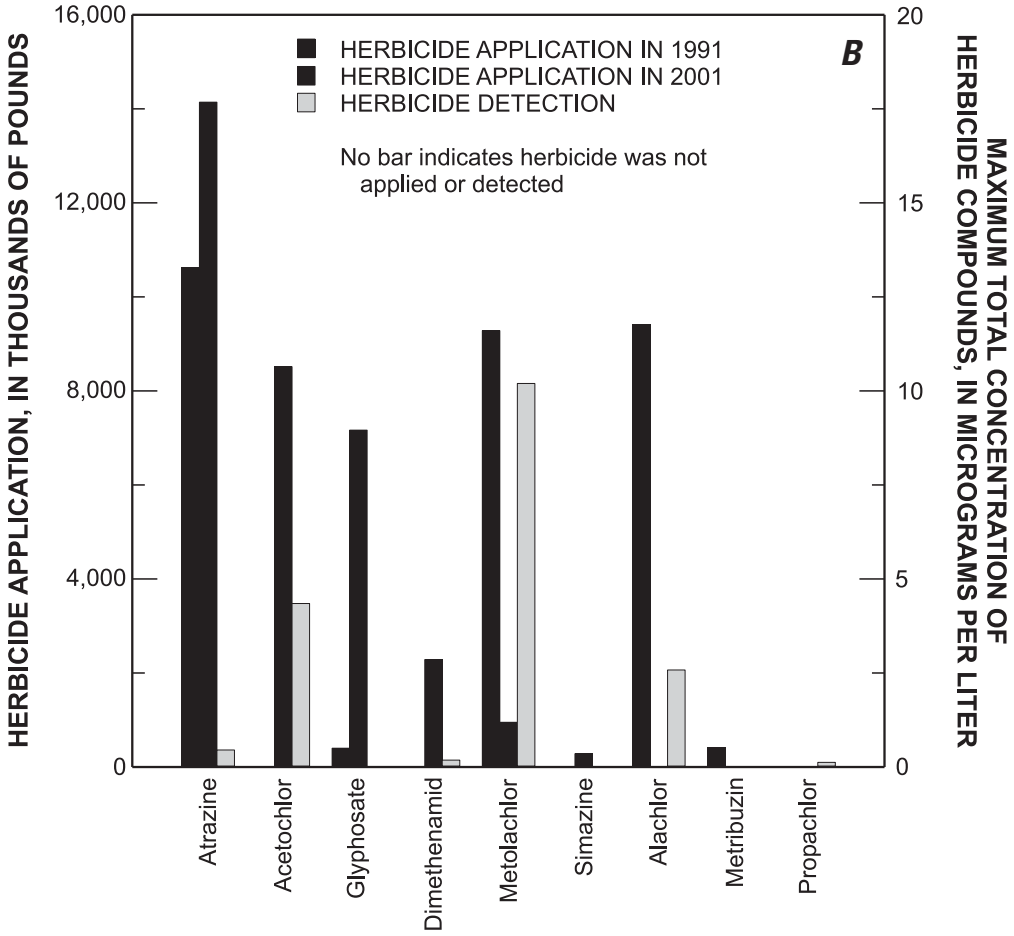

Figure 11. – Continued.

L; 1st, 4th, and 5th highest, respectively) in samples from individual wells and three of the five detections of parent compounds were from samples collected during this period. The comparatively high frequency of detection of herbicide compounds during the fall through winter crop- harvesting (and post-harvesting) period seems to represent the detection of increasing numbers of herbicide compounds with low concentrations as the parent herbicides transform and (or) the transportation of herbicide compounds in ground water to increasing distances from their application source areas. The higher percentage of sample wells open to unconsolidated aquifers in the crop-harvesting period (33 percent) than in the summer crop-growing period (17 percent) also may account for the comparatively high frequency of detection of herbicide compounds in the crop-harvesting period (fig. 12). Eighty-four percent of herbicide-compound detections were in samples from wells open to unconsolidated aquifers in which the ground water typically is recharged more recently than in bedrock aquifers.

Hydrogeology

Ground water typically is younger in unconsolidated aquifers than in bedrock aquifers. The younger age of ground water in unconsolidated aquifers can be attributed, in part, to the comparatively shallower depths and more rapid recharge rates of most unconsolidated aquifers (Kolpin and others, 2000b). Considering the factors of age and aquifer depth, unconsolidated aquifers are expected to be more vulnerable to contamination by herbicide compounds than bedrock aquifers; shallow bedrock aquifers are expected to be more vulnerable to contamination than deep bedrock aquifers.

Herbicide compounds were detected in more than twice as many samples from unconsolidated source-water aquifers in Illinois (representing 46 percent of sample locations) than from shallow bedrock aquifers (representing 46 percent of sample locations) (figs. 7, 13). No herbicide compounds were detected in samples from deep bedrock aquifers (representing 8 percent of sample locations). As indicated previously, source-water bedrock aquifers in Illinois are considered to be shallow where the depth to the top of the aquifer is less than or equal to 500 ft and are considered to be deep where the depth to the top of the aquifer is greater than 500 ft. Depth to the top of shallow bedrock aquifers with detections of herbicide compounds ranged from 71 to 246 ft, with 85 percent of the detections from aquifers with depths of 150 ft or less.

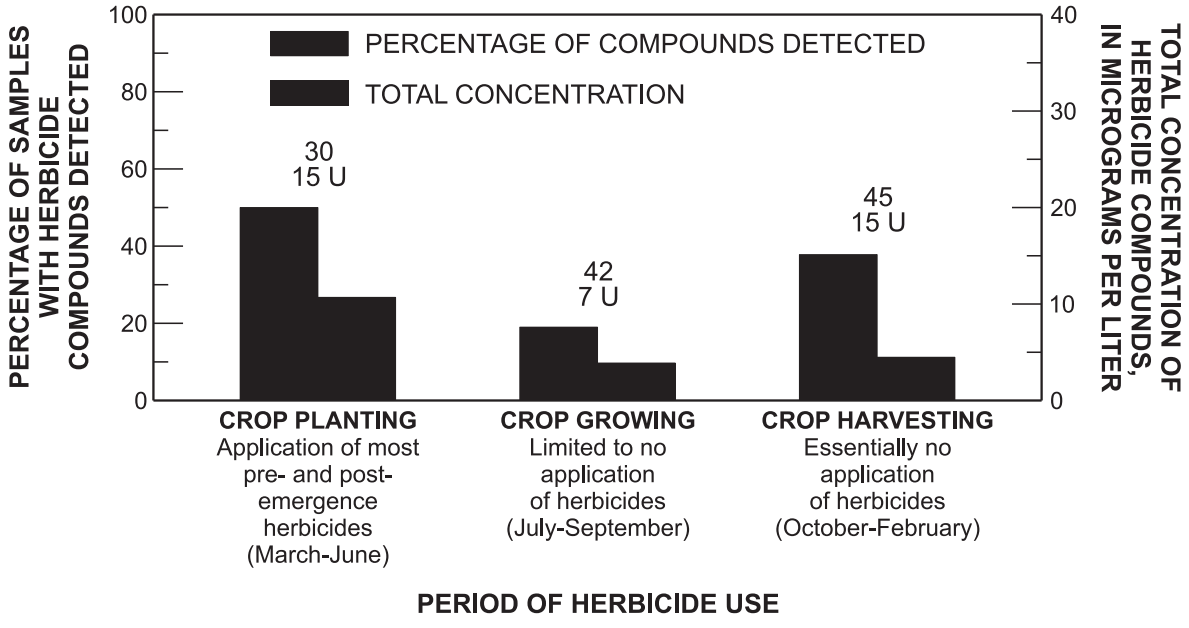

Figure 12. Relation of frequency of detection and total concentration of herbicides and their transformation products in source-water aquifers in Illinois to timing of herbicide application, 2001-02 (total number of wells and number of wells open to unconsolidated aquifers (U) in herbicide-application period are shown above bar).

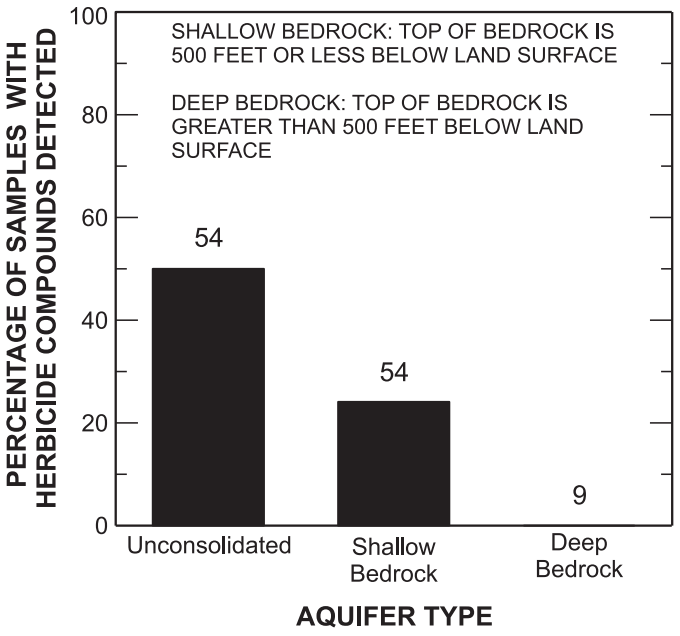

Figure 13. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to aquifer type, 2001-02 (total number of samples in aquifer type is shown above bar).

With travel times for recharge to most confined aquifers (whether bedrock or unconsolidated) used in Illinois for public-water supply expected to be greater than 50 years (assuming only advective water movement through continuous porous media), occurrence of herbicide compounds in these aquifers generally should not be expected. Most synthetic organic herbicides, including the herbicides examined in this study, have been used routinely for less than about 40 years. By standard definition, aquifers are considered confined when the potentiometric surface (water level) in the aquifer is above the top of the aquifer; confined aquifers are bounded above and below by lithologic units of distinctly lower permeability than that of the aquifer (U.S. Geological Survey, 1989). For the present study, water-level data generally were unavailable for direct designation of aquifers as confined or unconfined and, thus, the relation between aquifer confinement and herbicide occurrence in the State's source-water aquifers could not be evaluated directly. Alternatively, thickness of the confining unit, frequently used as an indirect indicator of aquifer confinement, was evaluated to determine its relation to occurrence of herbicide compounds. For the evaluation, designations of confining-unit thickness were made by IEPA on the basis of geologic information available from well-construction logs or from other sources (Illinois Environmental Protection Agency, 1995).

There was a strong inverse relation (r = -0.93) between confining-unit thickness and frequency of detection of herbicide compounds in Illinois' source-water aquifers (fig. 14). Herbicide compounds were detected in 88 percent of samples from wells that tap aquifers confined below low-permeability units 10 ft thick or less and in 47 percent or less of samples from wells that tap aquifers confined below low-permeability units greater than 10 ft thick. Aquifer depth (depth to the top of the aquifer) seemed to be a factor where herbicide compounds were detected in samples from aquifers confined by units greater than 10 ft thick. For example, for aquifers confined below units greater than 10 to 20 ft thick, herbicides were not detected where aquifer depths exceeded 163 ft. Unexpectedly, herbicide compounds were detected in 20 percent of samples (4 samples) from wells that tap aquifers presumably confined below units greater than 50 ft thick (to a maximum thickness of 153 ft). One of the wells (00572) (fig. 3) where herbicide compounds were detected tapped an unconsolidated aquifer; three of the wells (20180, 20766, 20328) tapped a shallow bedrock aquifer of fractured carbonate (Silurian). For this study, construction logs of the sampled

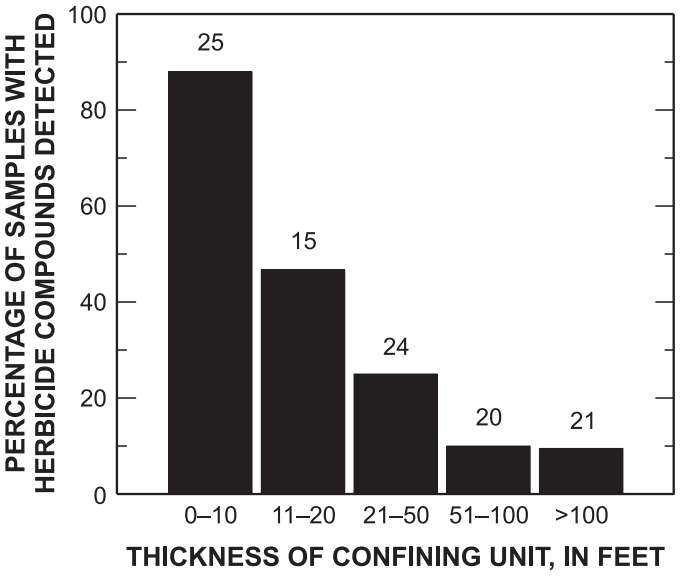

Figure 14. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to thickness of the confining unit, 2001-02 (>, greater than; total number of samples in classification is shown above bar, confining-unit thicknesses were not available for 12 sampled wells).

wells were unavailable for evaluation of the lithology and lateral extent of the confining units. However, the occurrence of herbicide compounds in these samples indicates that the confining units in the vicinity of the sampled wells may not be vertically and (or) horizontally continuous. The occurrence of herbicide compounds in samples from three wells that tapped a fractured-carbonate aquifer indicates the fractures may, in some undetermined way, contribute to preferential movement of water and herbicides through the confining unit that overlies the aquifer.

Wells included in IEPA's ambient ground-water-quality well network have been designated officially as tapping either confined or unconfined aquifers to evaluate the vulnerability of Illinois' source-water aquifers to contamination (Illinois Environmental Protection Agency, 1995). The primary approach used for the official designations was a weighted-ranking method that includes five hydrogeologic criteria determined from well-construction logs and other data sources. For example, one criteria is – Does at least one contiguous unit of impermeable geologic materials greater than 10 ft thick overlie the aquifer (excluding the top 10 ft of soil materials)? When sufficient data are unavailable for the weighted-ranking method, the designations are based on alternative hydrogeologic-related vulnerability criteria (Berg and others, 1984), tritium data (Illinois Environmental Protection Agency, 2003c; Mills and others, 2002, p. 9), evidence of human sources of contaminants (such as synthetic organic compounds), and (or) information from detailed hydrogeologic investigations of the aquifer (Wade Boring, Illinois Environmental Protection Agency, oral commun., 2003). Each of these approaches for designating aquifers as confined or unconfined for the evaluation of aquifer vulnerability to contamination is based on the assumption that ground water from confined aquifers generally represents old recharge (about 50 years or older) and ground water in unconfined aquifers generally represents comparatively young recharge (about 50 years or younger). Thus, ground water in confined aquifers generally is considered to be less vulnerable to anthropogenic contamination (contaminants from human sources) than ground water in unconfined aquifers.

Aquifer confinement, as designated by the IEPA, was related strongly to frequency of detection of herbicide compounds in Illinois' source-water aquifers (fig. 15). Herbicide compounds were detected in samples from 11 percent of wells that tap aquifers designated as confined (representing 68 percent of wells/aquifers) and 84 percent of wells that tap aquifers designated as unconfined (representing 32 percent of wells/aquifers). Most of the samples (71 percent) obtained from aquifers designated as confined were obtained from bedrock aquifers. All detections of herbicide compounds in samples from confined aquifers were from bedrock aquifers.

Ground-water age and aquifer vulnerability to contamination by herbicide compounds can be related to proximity and hydraulic connection to recharge areas (Kolpin and others, 1998b). Generally, the closer and (or) better the hydraulic connection to a recharge area, the younger the ground water and more vulnerable an aquifer is to herbicide contamination. The length of the recharge flow path, in part, determines the time available for transport, transformation, or sorption of herbicides. This is important particularly if a thick unsaturated zone overlies an aquifer and, thus, extensive aerobic and biotic transformation of herbicides can result. One indication of proximity to a recharge area is the depth from land surface of various hydrogeologic characteristics (top of aquifer, for example) and well-construction aspects (base of well casing, for example). In this study, depth to the top of an aquifer (or aquifer depth) is defined as depth to

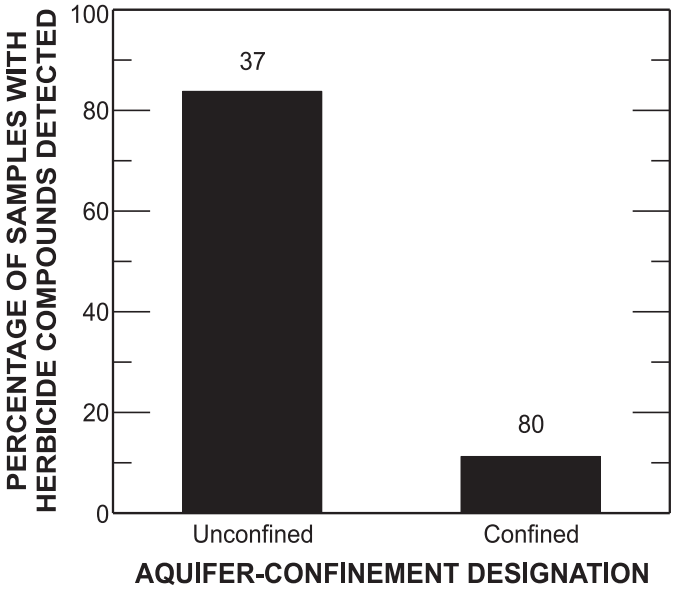

Figure 15. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to designation as a confined aquifer (policy designation of the Illinois Environmental Protection Agency), 2001-02 (total number of samples in designation is shown above bar).

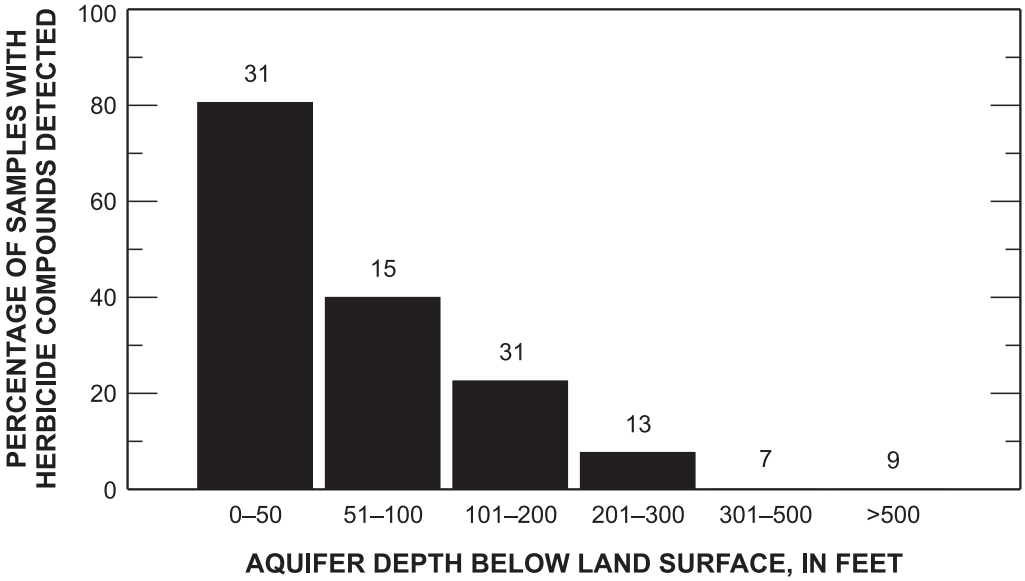

Figure 16. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to aquifer depth, 2001-02 (aquifer depth is the depth from land surface to the top of aquifer material; >, greater than; total number of samples in depth interval is shown above bar; aquifer depths were not available for 11 sampled wells).

the top of the aquifer material, regardless of whether the aquifer material is fully saturated.

There was a strong inverse relation (r = -0.92) between aquifer depth and frequency of detection of herbicide compounds in Illinois' source-water aquifers, with a monotonic decline in detections with increasing aquifer depth (fig. 16). The number of samples with detected herbicides from aquifers within 50 ft of land surface was almost twice the number from all other aquifer-depth intervals (25 and 14 samples, respectively). No herbicide compounds were detected in samples from aquifers with depths greater than 300 ft. Of the various depth-related factors examined in the study of Midwestern cornbelt States that included Illinois, aquifer depth was associated most strongly with detection of herbicide compounds (Kolpin and others,1994).

Proximity of public-supply wells to a stream was related positively to the frequency of detection of herbicide compounds in Illinois' source-water aquifers (fig. 17). Herbicide compounds were detected in 70 percent of samples from the 10 wells within 100 ft of a stream, but in only 31 percent of samples from wells that were further than 100 ft from a stream (fig. 16). Similarly, herbicides were detected in 47 percent of samples from the 34 wells within about 0.25 mi of a stream, but in only 29 percent of samples from wells that were located more remotely (greater than 0.25 mi from a stream). Additionally, herbicides were detected in 78 percent of samples from the nine wells that tap unconsolidated (alluvial) aquifers adjacent to the Illinois and Mississippi Rivers (fig. 3). Two wells (50237, 50238; fig. 3) with the highest total (12.93, 5.13 µg/L, respectively) and individual (7.24, 4.18, 2.95, and 2.15 µg/L) concentrations of four herbicide compounds (metolachlor ESA, acetochlor ESA, metolachlor OA, and alachlor ESA, respectively) were immediately adjacent to a stream.

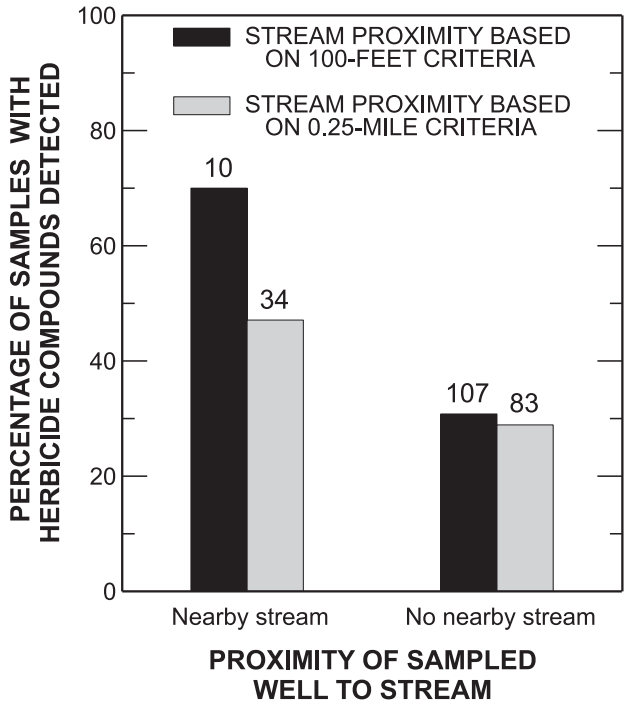

Figure 17. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to proximity of well to a stream, 2001-02 (total number of samples in stream-proximity interval is shown above bar)

Kolpin and others (1994) report similar findings in their study of Midwestern States.

There are various hydrogeologic factors that could contribute to the greater frequency of detection of herbicide compounds and, possibly, higher concentrations in samples from wells near streams. Wells near streams may draw some water from the streams, as hydraulic gradients between the aquifer and stream are reversed during periods of high stream stage and (or) groundwater withdrawal by pumping. Herbicides frequently occur in streams in the Midwestern States and typically their frequencies of occurrence and concentrations substantially exceed those in ground water (Fuhrer and others, 1999). In addition, flow paths, possibly from many areas in an aquifer, tend to converge near discharge locations, such as streams. This comparatively wide source area providing recharge to near-stream wells can increase the likelihood of herbicide contamination of the water provided by the wells. Water in near-stream alluvial aquifers also tends to be younger than water in other aquifers (Kolpin and others, 2000b). The younger the ground water, the closer the source of recharge and potentially contaminating herbicides are likely to be.

Well Construction

As with aquifer depth, there was a strong inverse relation (r = -0.95) between well depth (depth from land surface to base of the well) and frequency of detection of herbicide compounds in Illinois' source-water aquifers (fig. 18). However, the decline in detection frequency associated with increasing well depth was less monotonic (more irregular) than the decline in detection frequency associated with increasing aquifer depth or another construction-related factor (well-casing depth) that was examined. Other studies indicate a weaker relation than this study does between frequency of herbicide detection and well depth (Holden and others, 1992; Kolpin and others, 1994; Dana W. Kolpin, U.S. Geological Survey, written commun., 2004). Herbicide compounds were detected in 100 percent of samples (6 samples) from wells 50 ft deep or less and 79 percent of samples from wells greater than 50 ft deep and less than about 100 ft deep. Detection frequencies in samples from wells between 100 and 500 ft deep were less than half the detection frequency in samples from shallower wells. In addition, herbicide compounds were detected in 7 percent of samples (2 samples) from wells greater than 500 ft deep (to a maximum of 769 ft deep).

The comparatively weaker association between well depth and herbicide detection than that between aquifer depth or well-casing depth and herbicide detection likely pertains to well-construction aspects. Many of the wells included in the study (54 percent) tap bedrock aquifers and, thus, are open to the aquifer from the base of the well casing to the base of the well (lengths of open intervals were up to 1,300 ft). Water may enter these wells from various depths in the aquifer. Generally, screened intervals of wells that tap unconsolidated aquifers more closely approximate total well depths than do open intervals of wells that tap bedrock aquifers.

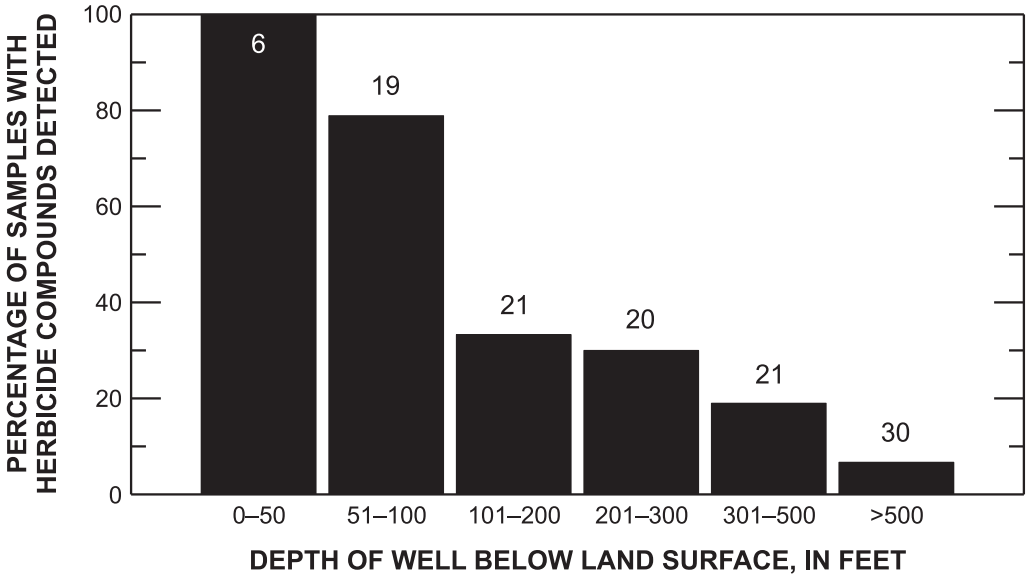

Figure 18. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to well depth, 2001-02 (>, greater than; total number of samples in depth interval is shown in or above bar).

Well-casing depth should provide a more realistic indicator of distance from recharge areas (and, thus, vulnerability to herbicide contamination) than does total well depth. Well-casing depth represents the top of the open interval of a well and, thus, the shallowest depth below land surface at which ground water may enter a well. As with well depth, there was a strong inverse relation (r = -0.93) between casing depth and frequency of detection of herbicide compounds in Illinois' source-water aquifers (fig. 19), yet a stronger monotonic relation was indicated than that for well depth. Herbicides were detected in 93 percent of samples from wells with casing depths 50 ft or less and the detections declined steadily with increasing depth. No herbicides were detected in samples from wells with casings depths greater than 300 ft.

There are various explanations other than those given previously for the unexpected detection of herbicide compounds in samples of older ground water from confined aquifers, deep wells, or below thick confining units. Three of the explanations are (1) any given sample water mass is a composite of waters of multiple ages; thus, mixing of only a small quantity of recent recharge containing herbicide compounds with older recharge is necessary to result in detection of trace concentrations, (2) water quality can be affected at recharge locations that are remote from locations of older ground water and the effects can persist over long horizontal flow paths if ground-water velocities are sufficiently rapid and herbicide compounds are sufficiently persistent, and (3) recent recharge may circumvent confining units and (or) move rapidly to depth in an aquifer by preferential flow through fractures or improperly constructed (grouted), damaged, or unused wells.

Land Use

Land use was evaluated to determine a possible relation to the frequency of detection of herbicide compounds in Illinois' source-water aquifers. Frequencies were compared with percentage of land used for corn or soybean (row-crop) production within radial distances of 1 and 2 mi from sampled wells. Because of the routine use of herbicides in row-crop production, a positive relation between frequency of herbicide detection and percentage of land used for row-crop production is expected. However, there was a strong inverse relation (r = -0.81) between frequency of detection of herbicide compounds in ground-water samples collected from the source-water aquifers and percentage of land used in row-crop production (fig. 20). There was little difference in the results for the 1- and 2-mi radial distances.

Interestingly, herbicide transformation products were detected in 71 percent of the samples (5 of 7 samples) from wells with no identified corn or soybean production within a 2-mi radius. One of the samples with detected transformation products was from a well (50383; fig. 3) located adjacent to the Illinois River, indicating that recharge to this well, in part, originates greater than 2 mi from the well and (or) all or part of the recharge is from the river. Four of the five samples with detected transformation products were collected from-

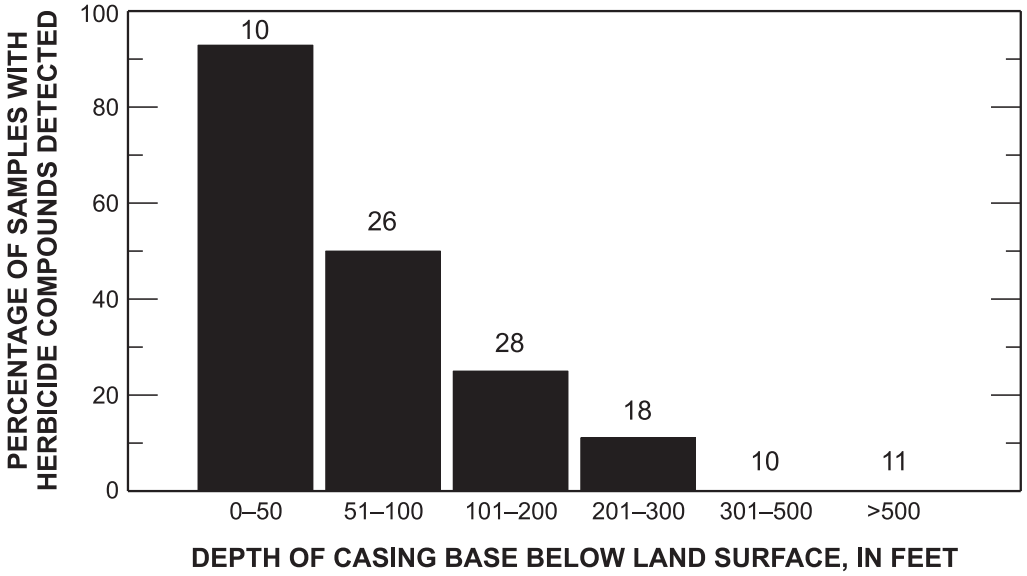

Figure 19. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to depth to base of the well casing, 2001-02 (>, greater than; total number of samples in depth interval is shown above bar).

wells in urbanized areas of Rockford (11629, 11636) and suburban Chicago (20285, 20497). Detections in urban areas have been reported by others (Barbash and others, 1999). Metolachlor, detected as an ESA compound in one sample, sometimes is used in non-agricultural settings (about 1 percent of total national use); however, alachlor, detected as an ESA compound in all four samples, reportedly is not (Barbash and others, 1999). The detections of herbicide compounds in these urban areas may be attributable to previous agricultural activity in the area of the wells, particularly near one well location in suburban Chicago. The detections also indicate the possibility that recharge to the high-capacity public-supply wells that were sampled may originate up to about 10 mi from the wells. Finally, although least likely, atmospheric deposition may account for the occurrence of these herbicide compounds in the urban ground waters (U.S. Geological Survey, 1995; Dana W. Kolpin, U.S. Geological Survey, oral commun., 2003).

Other studies that consider the relation between land use and occurrence of herbicide compounds in ground water report similar findings (Kolpin and others, 1994; Kolpin and others, 1997). Even when land-use patterns are considered in detail (for example, more land-use categories are applied, locations are mapped, and estimated areas contributing recharge to wells are removed from the statistical analysis), the relation between land use and occurrence of herbicide compounds in ground water seems only to be strengthened marginally (Kolpin and others, 1997). These findings pertaining to land use indicate the complexity of identifying factors and relations associated with the occurrence and concentrations of herbicide compounds in source-water aquifers and other ground waters. Many factors can contribute collectively to the occurrence of herbicides in ground water, including historical trends in land and herbicide use, ground-water age, local flow patterns, geology, soil microbiology, and herbicide chemistry. Furthermore, the detections of herbicide compounds in urban areas point to the necessity of evaluating ground-water-flow systems and mapping areas contributing recharge to wells in detail in development of aquifer-protection strategies by water-resources managers.

Excess Nitrate

Nitrogen fertilizers are used commonly in conjunction with herbicides to increase crop production and frequently nitrate is detected in aerobic ground water where these fertilizers are used (Nolan and others, 1997). Because the occurrence of nitrate in aerobic ground water can indicate the proximity of a source of ground-water recharge, nitrate occurrence, particularly as excess nitrate, also may indicate the co-occurrence of herbicide compounds (Kolpin and others, 1994; Kolpin and others, 2002; Dana W. Kolpin, U.S. Geological Survey, written commun., 2004). In ground water, concentrations of nitrate, as nitrogen, higher than 3.0 mg/L generally are considered to represent excess nitrate; this is considered the division between natural and human sources of nitrate (Madison and Brunett, 1985). Some research indicates concentrations as low as 2.0 mg/L (Mueller

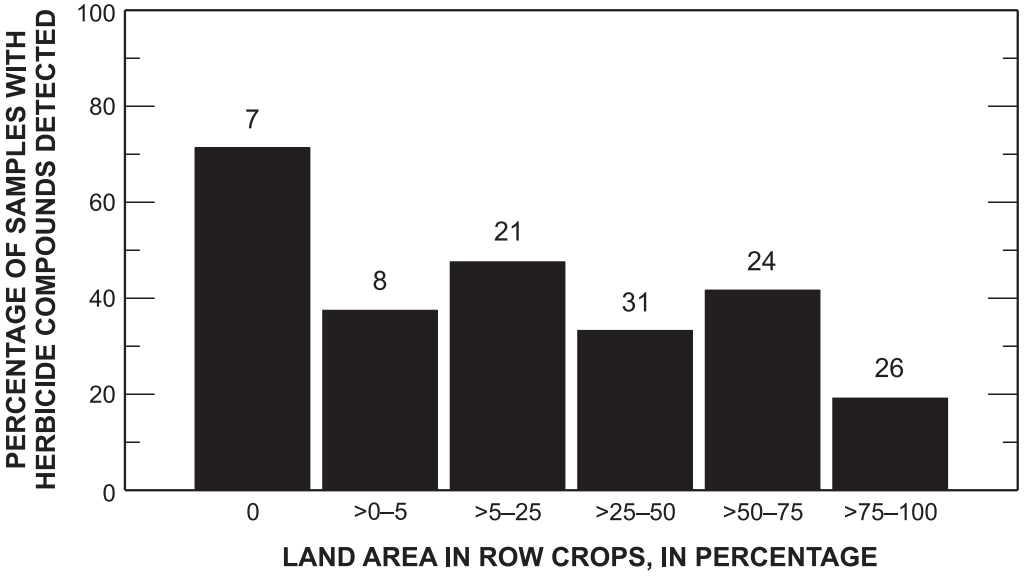

Figure 20. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to percentage of land area used for corn and soybean (row crop) production within a 2-mile radius of the sampled wells, 2001-02 (>, greater than; total number of samples in land-area interval is shown above bar).

and Helsel, 1996) or even 1 mg/L (Nolan and Hitt, 2003) represent excess nitrate.

The occurrence of excess nitrate in Illinois' source-water aquifers generally was indicative of the co-occurrence of herbicide compounds (fig. 21), particularly if nitrate concentrations greater than 1 mg/L are considered representative of excess nitrate. Herbicide compounds were detected in 100 percent of samples (8 of 8) with nitrate concentrations greater than 3.0 mg/L and less than 10 mg/L and in 89 percent of samples (8 of 9) with concentrations greater than 1 mg/L and less than 3.0 mg/L. However, herbicide compounds also were detected in numerous samples with nitrate concentrations less than 1 mg/L, which is below the lowest level considered to represent excess nitrate. Herbicide compounds were detected in 83 percent of samples (5 of 6) with nitrate concentrations greater than 0.2 mg/L and less than 1 mg/L and in 21 percent of samples (19 of 90) with nitrate concentrations less than or equal to 0.2 mg/L. No nitrate concentrations exceeded the MCL of 10 mg/L. The detection of herbicide compounds in samples with nitrate concentrations less than 1 mg/L may be explained, in part, by differences in the amounts of nitrogen fertilizer and herbicide applied near the ground-water-sample sites, and the timing of the application and subsequent transport of these compounds to ground water.

Nitrate (as nitrogen) concentrations in ground-water samples do not seem a reliable indicator of concentrations of herbicide compounds in Illinois' source-water aquifers. A weak to moderate positive relation (r = 0.52) was indicated between nitrate concentrations and total concentrations of herbicide compounds in samples from the State's public-supply wells (fig. 22). The limited strength of this correlation may be attributed, in part, to the relative stability of nitrate concentrations in ground water; concentrations of herbicide compounds generally decrease with time. Water samples collected for this study represent various ages of ground water. Additionally, nitrogen-based fertilizer applications are not restricted to the spring-planting period to the extent of herbicide applications; nitrogen-based fertilizers often are applied during the fall, after crops have been harvested.

Representativeness of Unfiltered Samples

The herbicide analytical results of paired unfiltered and filtered samples were evaluated to determine if the results for unfiltered samples significantly differ from those for filtered samples. Similarity in the results should indicate that the analytical results from unfiltered samples are as representative as those from filtered samples of herbicide concentrations in ground water supplied to the public.

For the purpose of this evaluation, equivalent concentrations were assumed when no herbicide compound(s) (concentrations were less than the reporting limit) was detected in either of the paired samples. Actual differences in concentrations that are less than

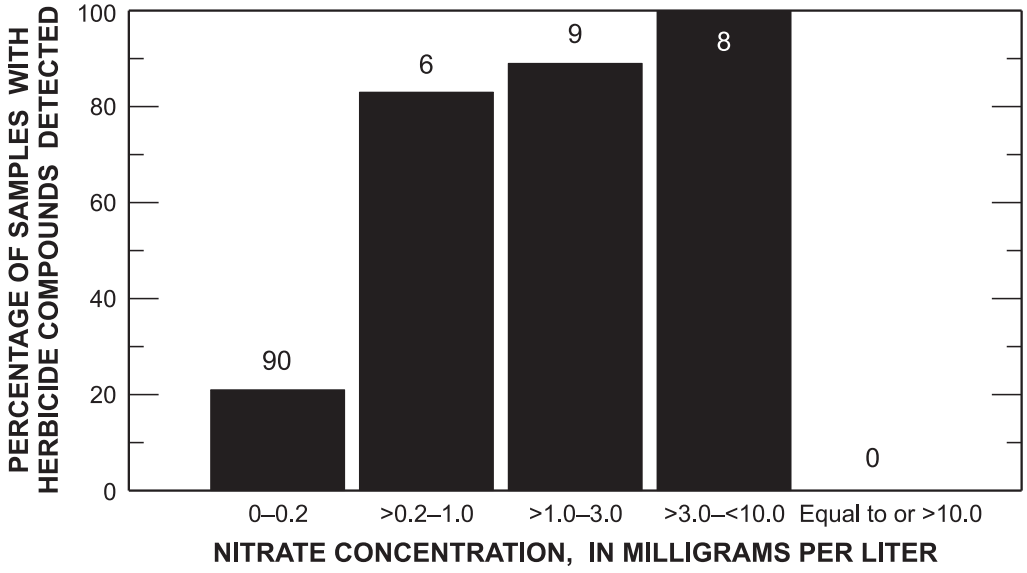

Figure 21. Relation of frequency of detection of herbicides and their transformation products in source-water aquifers in Illinois to concentrations of nitrate (as nitrogen), 2001-02 (>, greater than; <, less than; ≥, greater than or equal to; total number of samples in concentration interval is shown in and above bar).

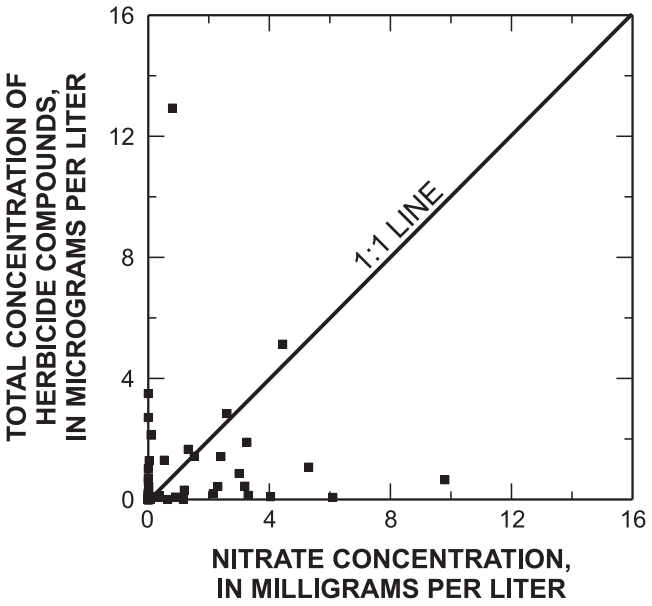

Figure 22. Relation of total concentration of herbicides and their transformation products in source-water aquifers in Illinois to concentration of nitrate (as nitrogen), 2001-02 (herbicide concentrations below the reporting limit of 0.05 microgram per liter are normalized to 0 microgram per liter; nitrate concentrations below the reporting limit of 0.10 milligram per liter are normalized to 0 milligram per liter).

the reporting limits used in this study (0.05 or 0.10 µg/L) can be ignored here because the concentrations would be substantially lower (more than an order of magnitude) than those of applicable Federal (U.S. Environmental Protection Agency, 2003b) or State (Illinois Pollution Control Board, 2003) drinking-water standards. These regulatory standards represent the benchmark for this evaluation. Concentrations that fall below the reporting limits used in this study are well below those of regulatory concern.

No particulates of any size were observed in the 117 water samples collected. However, clay-sized particulates were observed on the filter media of six samples, representing 5 percent of the samples (fig. 6; appendix 1). No difference in analytical results was recorded in 396 of 400 analyte pairs that resulted from analysis of up to 36 herbicide compounds in 13 samples (table 2; fig. 23). Glyphosate, AMPA, and glufosinate were not detected in the nine sample pairs that were analyzed; parent chloroacetanilides were not detected in the eight sample pairs. Triazine compounds were detected in only one of eight sample pairs. For the two detected compounds, the RPD was 0 in one analyte pair (atrazine) and -10 percent in the other (deethylatrazine, with a -0.02 µg/L difference in concentration between the unfiltered (0.19 µg/L) and filtered (0.21 µg/L) sample. Chloroacetanilide transfomation products were detected in only 2 of 11 sample pairs. For the two detected compounds (alachlor ESA and metolachlor ESA) in each sample pair, RPD's ranged from 0 to 18 percent, with no difference in concentration greater than 0.01 µg/L. The measured differences in concentrations were within the expected variability of the analytical methods (Zimmerman and Thurman, 1999; Zimmerman and others, 2000) and, therefore, unlikely to represent consequences of differing sample-collection methods. The statistical evaluation of the paired unfiltered and filtered samples supports this conclusion. There was no significant difference in the analytical results of the paired samples, as indicated by the results of the signed, Student's t, and Wilcoxon signed-ranks tests ($\rho = 0.62$, 0.71, and 0.88, respectively).

Although this initial evaluation of herbicide analytical results of unfiltered and filtered samples indicates that there is essentially no difference between resulting concentrations, some unresolved concerns remain regarding the comparative results. Concentrations of herbicide compounds in more than 99 percent of the

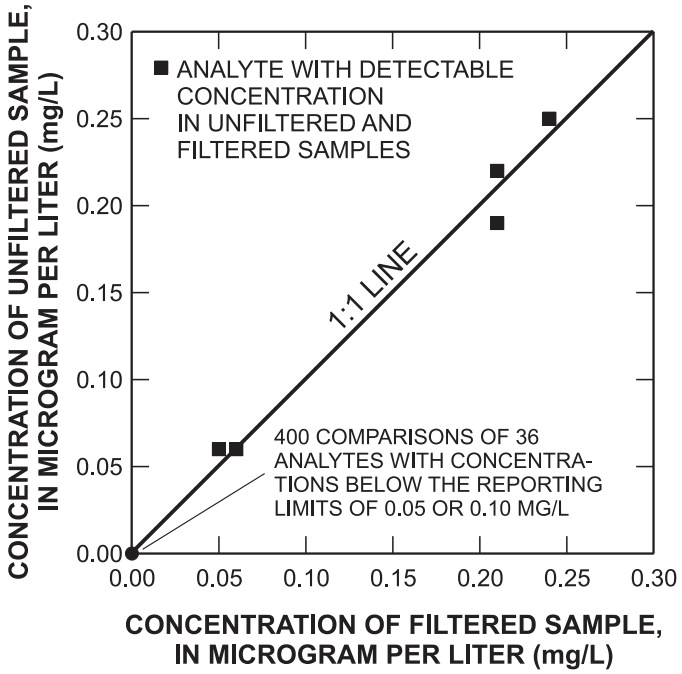

Figure 23. Comparison of herbicide-analytical results for paired unfiltered and filtered samples from selected public-water supply wells that tap source-water aquifers in Illinois, 2001-02.

Table 2. Herbicides and their transformation products detected in paired unfiltered and filtered samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001–September 2002.

[IEPA, Illinois Environmental Protection Agency; µg/L, microgram per liter; ESA, ethanesulfonic acid; OA, oxanilic acid; F, filtered with 0.7-micron baked, glass-fiber filter; U, unfiltered; <, less than; na, not applicable]

Analyzed for but not detected: acetochlor, acetochlor sulfynil acetic acid (SAA), alachlor, alachlor SAA, ametryn, cyanazine, cyanazine amide, deisopropylatrazine, dimethenamid, dimethenamid ESA, dimethenamid OA, flufenacet, flufenacet ESA, flufenacet OA, glufosinate, AMPA, metribuzin, pendimethalin, prometon, prometryn, propachlor, propachlor ESA, propachlor OA, propazine, simazine, and terbutryn. Reporting limit for most herbicide compounds was 0.05 µg/L; reporting limit for glyphosate, amino methyl phosphonic acid (AMPA), and glufosinate was 0.10 µg/L.

IEPA well number	Sample status	Sample date	Atrazine, dissolved (µg/L)	Deethylatrazine, dissolved (µg/L)	Glyphosate dis-, solved (µg/L)	Acetochlor ESA, dissolved (µg/L)	Acetochlor OA, dissolved (µg/L)	Alachlor ESA, dissolved (µg/L)	Alachlor OA, dissolved (µg/L)	Metolachlor ESA, dissolved (µg/L)	Metolachlor OA, dissolved (µg/L)
47518	F	12-04-01	<0.05	<0.05	<0.10	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
50251	F	12-12-01	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
50094	F	12-18-01	<.05	.21	<.10	<.05	<.05	.21	<.05	.24	<.05
do.	U	do.	<.05	.19	<.10	<.05	<.05	.22	<.05	.25	<.05
47532	F	01-15-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
11347	F	03-05-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
11363	F	04-09-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
50298	F	04-29-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
00276	F	05-20-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
60089	F	08-14-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	na	na	na	<.05	<.05	<.05	<.05	<.05	<.05
47586	F	08-28-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	na	na	na	<.05	<.05	<.05	<.05	<.05	<.05
[1]52095	F	09-04-02	<.05	<.05	<.10	<.05	.06	.05	<.05	<.05	<.05
do.	U	do.	<.05	<.05	<.10	<.05	.06	.06	<.05	<.05	<.05
60171	F	09-18-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	na	na	na	<.05	<.05	<.05	<.05	<.05	<.05
[2]11456	F	09-24-02	<.05	<.05	<.10	<.05	<.05	<.05	<.05	<.05	<.05
do.	U	do.	na	na	na	<.05	<.05	<.05	<.05	<.05	<.05

[1]Tan-to-orange, clay-sized particulates on filter media; well taps an unconsolidated aquifer.

[2]Light tan, clay-sized particulates on filter media; well taps a bedrock aquifer.

analyte pairs were below reporting limits, and of the few compounds that were detected, most of the measured concentrations were low (less than 0.25 µg/L, thus, an order of magnitude or more lower than typical MCL's for herbicides (table 1; appendix 1)). Additionally, comparative analyses were done on only two water samples from wells with observable particulates on the filter media. The evaluation of about 10 more unfiltered/filtered sample pairs (thus, representing about 20 percent of total field samples) could provide more conclusive results regarding the representativeness of unfiltered herbicide samples. Further evaluation of unfiltered/filtered sample pairs would benefit from pairs that represent (1) high herbicide-compound concentrations, as measured during the initial evaluation and (2) samples with visible particulates, including those from wells at which particulates previously were identified, but unfiltered samples were not collected (and, if possible, wells where particulates are identified for the first time during the additional sampling). Before the results of this evaluation are applied to uses other than regulation of herbicides in public-water supplies, the evaluation probably should be repeated using more sensitive laboratory analytical methods with lower reporting limits than those used in this evaluation.

Summary

During 2001-02, the U.S. Geological Survey, in cooperation with the Illinois Environmental Protection Agency, studied the occurrence and factors related to the distribution of herbicides and their transformation products (also referred to as degradates, metabolites, or breakdown products) in source-water aquifers tapped by the State's public-supply wells. Herbicides, used heavily in Illinois to control grass and weeds during production of corn and soybeans (row crops), have been detected in ground water in other studies in Illinois and the Midwestern States.

Ground-water samples were collected from 117 public-supply wells selected using a stratified-random method to ensure distributed representation of the major types of source-water aquifers (sand and gravel; Pennsylvanian to Cambrian bedrock) in the State. Samples were analyzed for 18 herbicides and 18 transformation products, including 3 triazine and 14 chloroacetanilide products. Eleven percent of the herbicide samples were collected unfiltered to determine if analytical results of unfiltered samples collected from public-supply wells are similar to the results of paired filtered samples and, thus, can be considered equally representative of herbicide concentrations in ground water supplied to the public.

Herbicide compounds (field-applied parent herbicides and their transformation products) were detected in 34 percent of all samples collected; only 4 percent of the samples contained residues of parent herbicides. The six most frequently detected herbicide compounds (from 6 to 28 percent of samples) were transformation products of the chloroacetanilides metolachlor, alachlor, and acetochlor. The frequent occurrence of transformation products and their higher concentration relative to those of most other parent herbicides confirm the importance of obtaining information on transformation products to understand the mobility and fate of herbicides in ground-water systems. No concentrations exceeded current (2003) Federal or State drinking-water standards; however, standards are established for only seven parent herbicides. In addition, the toxicity and aquatic effects of multiple herbicide compounds in water are not considered in the setting of standards.

Factors related to the occurrence of herbicide compounds in the State's source-water aquifers include unconsolidated and unconfined conditions, various hydrogeologic characteristics (top of aquifer, for example) and well-construction aspects (base of well casing, for example) at shallow depths, and proximity to streams. Generally, the closer an aquifer (or well location) is to a recharge area and (or) the stronger the hydraulic connection between an aquifer and a recharge area, the younger the ground water and the more vulnerable the aquifer will be to contamination by herbicide compounds. The weak relation ($r = -0.20$) between current (2001) statewide application rates of herbicides (in pounds) and current (2001-02) occurrence of herbicide compounds in source-water aquifers indicates that additional factors must be considered when relating herbicide-application rates to occurrence. These factors include historical application rates and the mobility and persistence of the various herbicide compounds in ground-water systems. Frequency of detection and concentrations of herbicides compounds in the State's source-water aquifers are indicated to be highest during the spring, when crops are planted and herbicides are primarily applied. Excess nitrate (concentrations

of nitrate, as nitrogen, higher than 3 mg/L) in ground water strongly indicates the co-occurrence of herbicide compounds. However, nitrate concentrations are not a reliable indicator of herbicide-compound concentrations. Concentrations of nitrate and total concentrations of herbicide compounds in samples were weakly to moderately related (r = 0.52). The strong inverse relation (r = -0.81) between current use of land for corn and soybean production and current occurrence of herbicide compounds in underlying aquifers indicates that various factors, along with current agricultural land use, contribute to herbicide occurrence. These factors include, among others, land-use history, ground-water age, ground-water-flow patterns, geology, soil microbiology, and chemistry and persistence of the herbicide compounds. Detection of agriculture-specific herbicide compounds in 71 percent of samples from urban areas with no current or recent agricultural land use near the sampled wells indicates that recharge to the high-capacity supply wells may originate at considerable distances (up to about 10 mi) from the wells. Essentially no difference was found between the analytical results for herbicides in paired unfiltered and filtered samples, although additional study of this issue is warranted. Further evaluation of unfiltered/filtered sample pairs would benefit from pairs that represent (1) high herbicide-compound concentrations, as measured during the initial evaluation and (2) samples with visible particulates, including those from wells at which particulates previously were identified, but unfiltered samples were not collected.

Awareness of the types of herbicides identified in large-scale studies, such as this study, and the factors that may be related to their occurrence can aid in determining the vulnerability of various source-water aquifers to herbicide contamination and result in more efficient strategies for sample collection. Sampling could be focused on herbicide compounds that likely are to be detected in ground water and on locations where potential for contamination is greatest.

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Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002.

[IEPA, Illinois Environmental Protection Agency; USGS, U.S. Geological Survey; FT, feet below land surface; US/CM, microsiemens per centimeter; DEG C, degrees Celsius; UG/L, micrograms per liter; ESA, ethanesulfonic acid; OA, oxanilic acid; SAA, sulfynil acetic acid; <, less than; --, not analyzed; geologic unit codes are listings in USGS National Water Information System (NWIS) data base; numbers listed under units in column headings are parameter codes for listings in U.S. Environmental Protection Agency STORET data base]

GEOLOGIC UNIT: 110QRNR, Quaternary; 350SLRN, Silurian; 360ODVC, Ordovician; 340DVSL, Silurian-Devonian; 367OVCB, Cambrian-Ordovician; 365ANCL, Ancell; 333VLMR, Valmeyeran; 320PSLV, Pennsylvanian; 362MQKT, Maquoketa; 355NIGR, Niagaran; 365GLPV, Galena-Platteville; 372IRGL, Ironton-Galesville; 330MSSP, Mississippian; 365STPR, St. Peter

IEPA WELL NUMBER	USGS STATION NUMBER	USGS STATION NAME	GEOLOGIC UNIT	SAMPLE DATE	DEPTH OF WELL, TOTAL (FT) (72008)	SPECIFIC CONDUCTANCE (US/CM) (00095)	PH WATER WHOLE FIELD (STANDARD UNITS) (00400)	TEMPERATURE WATER (DEG C) (00010)
47583	403143088054701	24N10E-20.8g1	110QRNR	10-09-01	226	690	7.3	12.7
00823	411447087513101	32N12E-20.1f	350SLRN	10-10-01	262	963	7.0	12.0
20458	412603087452601	34N13E-17.5e1	350SLRN	10-10-01	460	1080	7.2	11.5
50060	403432089380901	25N 5W-35.4d1	110QRNR	10-15-01	145	1280	6.8	14.2
60053	383932090012601	3N 8W-31.2a1	110QRNR	10-16-01	102	772	6.8	14.4
11782	415551088575201	40N 2E-23.2f2	360ODVC	10-23-01	723	492	6.9	11.6
31409	404418089011802	26N 2E- 5.7a1	110QRNR	10-23-01	120	709	6.4	12.9
50055	403116089251401	24N 3W-23.8e1	110QRNR	10-30-01	75	613	7.1	12.6
50057	403404089390701	24N 5W- 3.3h1	110QRNR	10-30-01	91	708	7.1	14.3
20250	421433088002701	44N10E-36.2b1	340DVSL	10-31-01	204	918	7.4	11.7
00602	414320089201301	20N10E-15.6e1	367OVCB	11-07-01	1115	607	6.2	11.7
11470	413654088475601	36N 4E- 8.4h1	350SLRN	11-07-01	230	552	6.4	11.8
11678	421256088593801	43N 2E-10.7d2	365ANCL	11-07-01	530	560	6.2	10.9
50308	401754090032001	21N 8W- 6.8e	110QRNR	11-13-01	96	346	7.8	13.9
00366	421840088033001	44N10E-10.6g	350SLRN	11-20-01	250	767	7.3	11.1
02305	422225088102501	45N 9E-15.6a1	110QRNR	11-20-01	123	1010	6.5	11.5
20357	412608088121401	34N 9E- 9.4a1	365ANCL	11-21-01	765	850	7.8	13.0
47561	404625087335301	26N11W- 2.4g1	110QRNR	11-27-01	116	465	7.6	12.7
01118	400111090312502	1N 1W-33.6e	110QRNR	12-04-01	60	655	7.0	13.1
11509	411928089073001	33N 1E-20.1h1	367OVCB	12-04-01	2591	1560	6.4	24.0
47518	410002088313401	29N 6E-10.8e1	367OVCB	12-04-01	1940	2220	6.7	20.6
20152	422231088221101	45N 7E-13.6c1	110QRNR	12-09-01	220	551	6.9	10.9
20141	421222088152701	43N 8E-14.1e1	367OVCB	12-09-01	1300	430	6.9	15.3
11891	422931089473201	29N 6E-22.2b1	367OVCB	12-11-01	355	530	6.4	11.2
20330	413137088011901	35N11E-18.5g	340DVSL	12-11-01	192	962	7.1	12.0
50251	400623089361201	19N 5W-13.2h2	110QRNR	12-12-01	145	465	6.8	13.0
50376	403737089195401	25N 2W-16.2g1	110QRNR	12-12-01	335	556	6.9	12.4
50094	404417090541502	9N 5W-25.1c1	333VLMR	12-18-01	71	664	6.6	12.4
47819	385946088020701	7N11E-31.8a2	320PSLV	12-19-01	269	784	8.2	14.2
50224	394605089155301	15N 2W-12.6g1	110QRNR	01-09-02	70	710	6.4	13.4
52120	394557090361601	15N14W-12.4g1	110QRNR	01-09-02	93	663	6.5	18.2
20285	421803087554801	44N11E-10.3b1	350SLRN	01-14-02	242	1030	7.2	7.2
47532	404532088111201	26N 9E- 4.2f1	110QRNR	01-15-02	78	709	6.5	12.4
47551	404614087512502	26N13W- 5.6h2	110QRNR	01-15-02	132	691	6.8	13.0
00757	372000088431001	14S 4E- 1.5f1	110QRNR	01-22-02	101.5	413	6.7	14.6
20700	415410088034301	39N10E- 2.4h1	362MQKT	01-23-02	350	972	7.2	11.3
31307	410134089244503	30N 3W-26.1b3	110QRNR	01-29-02	50	580	6.5	13.3
31428	405404089022301	28N 2W- 7.5c2	367OVCB	01-29-02	2005	2780	6.9	21.9

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	USGS STATION NAME	GEO-LOGIC UNIT	SAMPLE DATE	DEPTH OF WELL, TOTAL (FT) (72008)	SPE-CIFIC CON-DUCT-ANCE (US/CM) (00095)	PH WATER WHOLE FIELD (STAND-ARD UNITS) (00400)	TEMPER-ATURE WATER (DEG C) (00010)
11562	415035089175901	21N10E- 1.7g1	367OVCB	02-05-02	769	550	6.3	11.1
11636	421611089031101	44N 2E-19.7b1	110QRNR	02-05-02	237	810	6.3	11.5
11406	415552088465601	40N 4E-21.4f1	367OVCB	02-06-02	1307	473	6.6	12.4
11438	413805088405102	37N 5E-32.1c2	367OVCB	02-06-02	502	608	6.5	11.6
20497	415134088075001	39N10E-17.8a1	350SLRN	02-11-02	200	989	6.6	17.2
47692	395411087485701	17N13W-27.6e1	110QRNR	02-19-02	28	530	6.9	12.7
60127	381238090160001	4S11W- 1.5a1	110QRNR	02-25-02	59	621	6.1	14.9
00251	391251087393801	9N11W-20.6h2	110QRNR	03-04-02	115	684	6.4	14.0
47811	390644087391802	8N11W-29.4h2	110QRNR	03-04-02	32	647	6.3	12.9
11327	411722089221201	15N10E-17.7g1	355NIGR	03-05-02	334	3260	7.2	13.4
11347	412534089215601	17N10E-29.5c1	110QRNR	03-05-02	270	655	6.5	11.8
11904	414305089550901	20N 5E-15.8b1	365GLPV	03-06-02	820	571	6.4	14.0
00795	420559088322101	42N 6E-21.3b2	372IRGL	03-11-02	1195	508	6.6	13.2
01416	394953090331202	16N13W-22.5g2	110QRNR	03-12-02	90	623	6.3	14.3
47725	395640088515501	17N 3E- 9.2e1	110QRNR	03-12-02	132	893	6.1	12.6
45155	392418087392101	11N11W- 8.4a1	110QRNR	03-18-02	68	536	7.1	11.1
11892	413920090044401	19N 4E- 6.3a1	350SLRN	03-19-02	567	498	6.5	12.3
31889	412421090334501	16N 2W- 1.6d1	340DVSL	03-19-02	509	647	6.5	12.9
50101	394212090344901	15N13W-31.2d2	110QRNR	04-01-02	76	680	7.2	13.4
50227	400022090522301	1S 4W- 4.1b1	330MSSP	04-01-02	483	158	8.6	15.4
20207	421943088160501	45N 8E-35.5a1	110QRNR	04-02-02	60	1200	6.4	12.2
45081	400836088185101	20N 8E-33.8a1	110QRNR	04-08-02	338	614	8.1	12.9
47687	401217088220301	20N 7E-12.8c1	110QRNR	04-08-02	282.5	634	7.7	12.5
11363	413328089274501	18N 9E- 9.5c2	110QRNR	04-09-02	404	477	7.6	12.1
71531	383124088135401	1N 9E-17.5g1	320PSLV	04-16-02	215	1650	7.4	15.0
50395	391411090072001	9N 9W- 8.5b2	110QRNR	04-22-02	51	867	6.8	13.3
20297	420947087561801	43N11E-34.5f1	350SLRN	04-23-02	280	940	7.2	11.1
20447	413337088055501	36N10E-33.7e1	350SLRN	04-23-02	303	992	6.9	12.0
50298	401930090361701	4N 2W-15.2b1	330MSSP	04-29-02	380	1200	6.9	13.8
50383	403758089370801	25N 5W-12.5e	110QRNR	04-29-02	78	969	7.1	13.4
11367	412232089275101	16N 9E-16.6g	110QRNR	04-30-02	270	497	7.5	11.9
11495	412030089001901	33N 2E- 9.7b1	367OVCB	04-30-02	1078	1430	7.0	13.3
11600	411529089105701	32N 1W-11.1e1	365ANCL	04-30-02	1800	1580	7.4	19.6
20180	422322088140901	45N 9E- 7.8d1	350SLRN	05-14-02	294	573	6.7	11.6
22040	412143088254901	33N 7E- 4.4c1	367OVCB	05-14-02	1462	648	7.1	15.8
00276	421359088323201	43N 6E- 4.5d1	365STPR	05-20-02	760	500	7.1	12.8
11560[a]	415027089175501	21N10E- 1.6f1	350SLRN	05-20-02	298	760	6.9	11.8
00572	393324088363301	13N 5E-23.3f	110QRNR	05-21-02	115	742	7.2	12.8
50237	392439089262001	11N 3W- 8.3a2	110QRNR	05-21-02	44	830	6.9	13.4
52062	394338090542001	4S 4W- 7.5a1	330MSSP	06-05-02	429	554	8.5	15.6
20613	415839088113901	40N 9E- 3.5b1	350SLRN	06-11-02	392	716	6.6	11.5
50351[b]	404511090004001	9N 5E- 7.6d1	367OVCB	06-11-02	1572	3710	8.5	21.9
20442	412854087503501	35N12E-34.6g1	350SLRN	06-17-02	428	936	7.0	12.0

[a]Minor light tan, clay-sized particulates on filter media.

[b]Black clay-sized particulates on filter media.

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	USGS STATION NAME	GEO-LOGIC UNIT	SAMPLE DATE	DEPTH OF WELL, TOTAL (FT) (72008)	SPE-CIFIC CON-DUCT-ANCE (US/CM) (00095)	PH WATER WHOLE FIELD (STAND-ARD UNITS) (00400)	TEMPER-ATURE WATER (DEG C) (00010)
47573	403551088021302	25N10E-26.6d2	110QRNR	06-17-02	152	1200	7.3	13.4
11557	414955089291901	21N 9E- 5.5a1	367OVCB	06-18-02	1870	537	6.9	11.4
11629	421656089031301	44N 2E-18.7a2	110QRNR	06-18-02	295	651	6.9	11.4
20029	420727088153901	42N 8E-14.2h1	110QRNR	06-24-02	183	783	6.5	11.4
11570	414122088585401	37N 2E-10.1c1	367OVCB	06-25-02	1053	378	7.3	12.9
11943[c]	415509089032801	40N 1E-25.2h1	367OVCB	06-25-02	888	534	7.0	11.5
20766	414945088115101	39N 9E-34.5h1	350SLRN	07-01-02	365	1210	6.4	11.2
50003	395106089432801	16N 6W-12.5g	110QRNR	07-02-02	55	703	6.4	13.0
60058	385235090072601	5N 9W-20.4h2	110QRNR	07-02-02	92	815	9.1	15.0
50051	404126089305001	26N 4W-24.5a	110QRNR	07-17-02	260	695	7.3	13.4
11707	420608089501401	24N 6E- 5.6d1	367OVCB	07-29-02	1082	544	6.9	12.8
11514	410931088384401	31N 5E-16.1d1	320PSLV	07-30-02	280	986	7.3	12.9
20328[d]	413117087481701	35N12E-13.6e1	350SLRN	08-06-02	500	1050	6.4	12.2
20389	413234087513401	35N12E- 9.4h1	350SLRN	08-07-02	420	1400	6.5	12.0
20436	412015087472301	33N12E-24.2g1	350SLRN	08-07-02	300	1000	6.3	18.4
31701	411129090225701	14N 1E-21.1f1	367OVCB	08-13-02	1209	1560	7.5	17.5
31820	411537090363701	15N 2W-27.8c1	34ODVSL	08-13-02	604	1890	7.5	14.4
31930	413003090252401	18N 1E-31.4a1	350SLRN	08-13-02	554	577	7.2	12.5
60089	384417090005201	3N 8W- 5.6d1	110QRNR	08-14-02	106	800	6.4	15.1
11706	420614089500101	24N 6E- 5.5e1	367OVCB	08-19-02	1100	524	7.0	12.2
11778	420739089132901	25N11E-32.6g1	367OVCB	08-19-02	740	607	7.1	11.6
00546	422448088041901	45N10E- 4.4g2	110QRNR	08-27-02	150	668	7.0	12.0
47501	404533088173901	26N 8E- 3.8g1	110QRNR	08-28-02	100	1000	6.9	15.7
47578	403059088041202	24N10E-28.4h2	110QRNR	08-28-02	158	1060	6.8	14.3
47586	404247087434701	26N12W-29.2g2	110QRNR	08-28-02	124	845	6.9	13.2
40013	394343088281801	15N 7E-19.8c1	110QRNR	09-03-02	84	814	6.6	15.0
50238	392438089262101	11N 3W- 9.8c1	110QRNR	09-04-02	47	592	6.7	19.4
52095[e]	392422089411901	11N 5W-18.4h1	110QRNR	09-04-02	44	645	6.6	18.1
50064	404305089283501	26N 3W-17.5h1	110QRNR	09-09-02	333	385	7.3	12.8
01233	421234088002501	43N10E-13.2b	350SLRN	09-16-02	259	1990	7.4	11.8
60171	380805089594701	5S 8W- 4.7h1	330MSSP	09-18-02	315	560	7.4	17.6
00140	392154089413401	11N 5W-30.6b1	110QRNR	09-23-02	59	724	6.9	13.2
11419	414608088375201	38N 5E-14.4d1	367OVCB	09-24-02	573	546	7.1	11.2
11456[f]	413528088552101	36N 3E-18.4d1	367OVCB	09-24-02	150	576	7.4	13.5
22073	411436087384801	32N14E-19.1b1	350SLRN	09-24-02	330	830	6.9	12.4
50138	405525089452201	11N 6E-24.1e1	367OVCB	09-24-02	1680	2450	7.2	22.1

[c]Minor light brown, clay-sized particulates on filter media.

[d]Minor light gray-to-brown, clay-sized particulates on filter media.

[e]Tan-to-orange, clay-sized particulates on filter media.

[f]Light tan, clay-sized particulates on filter media.

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	ACETOCHLOR, DISSOLVED (UG/L) (49260)	ACETOCHLOR ESA, DISSOLVED (UG/L) (61029)	ACETOCHLOR OA, DISSOLVED (UG/L) (61030)	ACETOCHLOR, SAA, DISSOLVED (UG/L) (62847)	ALACHLOR, DISSOLVED (UG/L) (46342)	ALACHLOR ESA, DISSOLVED (UG/L) (50009)	ALACHLOR OA, DISSOLVED (UG/L) (61031)	ALACHLOR, SAA, DISSOLVED (UG/L) (62848)
47583	403143088054701	10-09-01	<0.05	<0.05	<0.05	--	<0.05	<0.05	<0.05	--
00823	411447087513101	10-10-01	<.05	.21	<.05	--	<.05	<.05	<.05	--
20458	412603087452601	10-10-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
50060	403432089380901	10-15-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
60053	383932090012601	10-16-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
11782	415551088575201	10-23-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
31409	404418089011802	10-23-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
50055	403116089251401	10-30-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
50057	403404089390701	10-30-01	<.05	.45	.18	--	<.05	<.05	<.05	--
20250	421433088002701	10-31-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
00602	414320089201301	11-07-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
11470	413654088475601	11-07-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
11678	421256088593801	11-07-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
50308	401754090032001	11-13-01	<.05	<.05	<.05	--	<.05	.06	<.05	--
00366	421840088033001	11-20-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
02305	422225088102501	11-20-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
20357	412608088121401	11-21-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
47561	404625087335301	11-27-01	<.05	<.05	<.05	--	<.05	<.05	<.05	--
01118	400111090312502	12-04-01	<.05	<.05	<.05	<0.05	<.05	.07	<.05	<0.05
11509	411928089073001	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47518	410002088313401	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20152	422231088221101	12-10-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20141	421222088152701	12-10-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11891	422931089473201	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20330	413137088011901	12-11-01	<.05	<.05	<.05	<.05	<.05	.09	<.05	<.05
50251	400623089361201	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50376	403737089195401	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50094	404417090541502	12-18-01	<.05	<.05	<.05	<.05	<.05	.21	<.05	<.05
47819	385946088020701	12-19-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50224	394605089155301	01-09-02	<.05	<.05	<.05	<.05	<.05	.07	<.05	<.05
52120	394557090361601	01-09-02	<.05	.16	.16	<.05	<.05	.34	.09	<.05
20285	421803087554801	01-14-02	<.05	<.05	<.05	<.05	<.05	.05	<.05	<.05
47532	404532088111201	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47551	404614087512502	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00757	372000088431001	01-22-02	<.05	<.05	<.05	<.05	<.05	.11	<.05	<.05
20700	415410088034301	01-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31307	410134089244503	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31428	405404089022301	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11562	415035089175901	02-05-02	<.05	<.05	<.05	<.05	<.05	.38	<.05	<.05
11636	421611089031101	02-05-02	<.05	<.05	<.05	<.05	<.05	.05	<.05	<.05
11406	415552088465601	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11438	413805088405102	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20497	415134088075001	02-11-02	<.05	<.05	<.05	<.05	<.05	.06	<.05	<.05
47692	395411087485701	02-19-02	<.05	<.05	<.05	<.05	<.05	.09	<.05	<.05
60127	381238090160001	02-25-02	<.05	.25	.25	<.05	<.05	.19	.08	<.05
00251	391251087393801	03-04-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47811	390644087391802	03-04-02	<.05	.08	<.05	<.05	<.05	.12	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	ACETOCHLOR, DISSOLVED (UG/L) (49260)	ACETOCHLOR ESA, DISSOLVED (UG/L) (61029)	ACETOCHLOR OA, DISSOLVED (UG/L) (61030)	ACETOCHLOR, SAA, DISSOLVED (UG/L) (62847)	ALACHLOR, DISSOLVED (UG/L) (46342)	ALACHLOR ESA, DISSOLVED (UG/L) (50009)	ALACHLOR OA, DISSOLVED (UG/L) (61031)	ALACHLOR, SAA, DISSOLVED (UG/L) (62848)
11327	411722089221201	03-05-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
11347	412534089215601	03-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11904	414305089550901	03-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00795	420559088322101	03-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01416	394953090331202	03-12-02	<.05	<.05	<.05	<.05	<.05	.08	<.05	<.05
47725	395640088515501	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
45155	392418087392101	03-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11892	413920090044401	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31889	412421090334501	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50101	394212090344901	04-01-02	<.05	<.05	<.05	<.05	<.05	.13	<.05	<.05
50227	400022090522301	04-01-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20207	421943088160501	04-02-02	<.05	<.05	<.05	<.05	<.05	.12	<.05	<.05
45081	400836088185101	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47687	401217088220301	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11363	413328089274501	04-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
71531	383124088135401	04-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50395	391411090072001	04-22-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20297	420947087561801	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20447	413337088055501	04-23-02	<.05	.05	<.05	<.05	<.05	.52	.06	<.05
50298	401930090361701	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50383	403758089370801	04-29-02	<.05	.07	.09	<.05	<.05	.41	.06	<.05
11367	412232089275101	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11495	412030089001901	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11600	411529089105701	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20180	422322088140901	05-14-02	<.05	<.05	<.05	<.05	<.05	.18	<.05	<.05
22040	412143088254901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00276	421359088323201	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11560	415027089175501	05-20-02	<.05	<.05	<.05	<.05	<.05	.39	<.05	<.05
00572	393324088363301	05-21-02	<.05	.11	.20	<.05	<.05	.81	.41	<.05
50237	392439089262001	05-21-02	<.05	.13	<.05	<.05	<.05	2.15	.41	<.05
52062	394338090542001	06-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20613	415839088113901	06-11-02	<.05	<.05	<.05	<.05	<.05	.13	<.05	<.05
50351	404511090004001	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20442	412854087503501	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47573	403551088021302	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11557	414955089291901	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11629	421656089031301	06-18-02	<.05	<.05	<.05	<.05	<.05	.07	<.05	<.05
20029	420727088153901	06-24-02	<.05	<.05	<.05	<.05	<.05	.17	<.05	<.05
11570	414122088585401	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11943	415509089032801	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20766	414945088115101	07-01-02	<.05	<.05	<.05	<.05	<.05	.11	<.05	<.05
50003	395106089432801	07-02-02	<.05	.28	.06	<.05	<.05	.11	<.05	<.05
60058	385235090072601	07-02-02	<.05	<.05	<.05	<.05	<.05	.37	.12	<.05
50051	404126089305001	07-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11707	420608089501401	07-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11514	410931088384401	07-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20328	413117087481701	08-06-02	<.05	<.05	<.05	<.05	<.05	.10	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	ACETOCHLOR, DISSOLVED (UG/L) (49260)	ACETOCHLOR ESA, DISSOLVED (UG/L) (61029)	ACETOCHLOR OA, DISSOLVED (UG/L) (61030)	ACETOCHLOR, SAA, DISSOLVED (UG/L) (62847)	ALACHLOR, DISSOLVED (UG/L) (46342)	ALACHLOR ESA, DISSOLVED (UG/L) (50009)	ALACHLOR OA, DISSOLVED (UG/L) (61031)	ALACHLOR, SAA, DISSOLVED (UG/L) (62848)
20389	413234087513401	08-07-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
20436	412015087472301	08-07-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31701	411129090225701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31820	411537090363701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31930	413003090252401	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60089	384417090005201	08-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11706	420614089500101	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11778	420739089132901	08-19-02	<.05	<.05	<.05	<.05	<.05	.35	<.05	<.05
00546	422448088041901	08-27-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47501	404533088173901	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47578	403059088041202	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47586	404247087434701	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
40013	394343088281801	09-03-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50238	392438089262101	09-04-02	<.05	4.18	.15	<.05	<.05	<.05	<.05	<.05
52095	392422089411901	09-04-02	<.05	<.05	<.05	<.05	<.05	.06	<.05	<.05
50064	404305089283501	09-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01233	421234088002501	09-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60171	380805089594701	09-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00140	392154089413401	09-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11419	414608088375201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11456	413528088552101	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
22073	411436087384801	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50138	405525089452201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	AMETRYN, DISSOLVED (UG/L) (38401)	ATRAZINE, DISSOLVED (UG/L) (39632)	DEETHYL ATRAZINE, DISSOLVED (UG/L) (04040)	DEISOPROPYL ATRAZINE, DISSOLVED (UG/L) (04038)	CYANAZINE, DISSOLVED (UG/L) (04041)	CYANAZINE AMIDE, DISSOLVED (UG/L) (61709)	DIMETHENAMID, DISSOLVED (UG/L) (61588)	DIMETHENAMID ESA, DISSOLVED (UG/L) (61951)	DIMETHENAMID OA, DISSOLVED (UG/L (62482)
47583	403143088054701	10-09-01	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00823	411447087513101	10-10-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20458	412603087452601	10-10-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50060	403432089380901	10-15-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60053	383932090012601	10-16-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11782	415551088575201	10-23-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31409	404418089011802	10-23-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50055	403116089251401	10-30-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50057	403404089390701	10-30-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20250	421433088002701	10-31-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00602	414320089201301	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11470	413654088475601	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11678	421256088593801	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50308	401754090032001	11-13-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00366	421840088033001	11-20-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
02305	422225088102501	11-20-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20357	412608088121401	11-21-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47561	404625087335301	11-27-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01118	400111090312502	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11509	411928089073001	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47518	410002088313401	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20152	422231088221101	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20141	421222088152701	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11891	422931089473201	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20330	413137088011901	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50251	400623089361201	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50376	403737089195401	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50094	404417090541502	12-18-01	<.05	.05	.21	<.05	<.05	<.05	<.05	<.05	<.05
47819	385946088020701	12-19-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50224	394605089155301	01-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
52120	394557090361601	01-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	.05	<.05
20285	421803087554801	01-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47532	404532088111201	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47551	404614087512502	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00757	372000088431001	01-22-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20700	415410088034301	01-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31307	410134089244503	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31428	405404089022301	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11562	415035089175901	02-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11636	421611089031101	02-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11406	415552088465601	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11438	413805088405102	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20497	415134088075001	02-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47692	395411087485701	02-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60127	381238090160001	02-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00251	391251087393801	03-04-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	AMETRYN, DISSOLVED (UG/L) (38401)	ATRAZINE, DISSOLVED (UG/L) (39632)	DEETHYL ATRAZINE, DISSOLVED (UG/L) (04040)	DEISOPROPYL ATRAZINE, DISSOLVED (UG/L) (04038)	CYANAZINE, DISSOLVED (UG/L) (04041)	CYANAZINE AMIDE, DISSOLVED (UG/L) (61709)	DIMETHENAMID, DISSOLVED (UG/L) (61588)	DIMETHENAMID ESA, DISSOLVED (UG/L) (61951)	DIMETHENAMID OA, DISSOLVED (UG/L (62482)
47811	390644087391802	03-04-02	<0.05	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
11327	411722089221201	03-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11347	412534089215601	03-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11904	414305089550901	03-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00795	420559088322101	03-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01416	394953090331202	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47725	395640088515501	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
45155	392418087392101	03-18-02	<.05	.06	.08	<.05	<.05	<.05	<.05	<.05	<.05
11892	413920090044401	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31889	412421090334501	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50101	394212090344901	04-01-02	<.05	<.05	.05	<.05	<.05	<.05	<.05	<.05	<.05
50227	400022090522301	04-01-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20207	421943088160501	04-02-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
45081	400836088185101	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47687	401217088220301	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11363	413328089274501	04-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
71531	383124088135401	04-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50395	391411090072001	04-22-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20297	420947087561801	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20447	413337088055501	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50298	401930090361701	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50383	403758089370801	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11367	412232089275101	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11495	412030089001901	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11600	411529089105701	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20180	422322088140901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
22040	412143088254901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00276	421359088323201	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11560	415027089175501	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00572	393324088363301	05-21-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50237	392439089262001	05-21-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	.05	<.05
52062	394338090542001	06-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20613	415839088113901	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50351	404511090004001	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20442	412854087503501	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47573	403551088021302	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11557	414955089291901	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11629	421656089031301	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20029	420727088153901	06-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11570	414122088585401	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11943	415509089032801	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20766	414945088115101	07-01-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50003	395106089432801	07-02-02	<.05	.22	.21	<.05	<.05	<.05	<.05	.16	<.05
60058	385235090072601	07-02-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50051	404126089305001	07-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11707	420608089501401	07-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	AMETRYN, DISSOLVED (UG/L) (38401)	ATRAZINE, DISSOLVED (UG/L) (39632)	DEETHYLATRAZINE, DISSOLVED (UG/L) (04040)	DEISOPROPYLATRAZINE, DISSOLVED (UG/L) (04038)	CYANAZINE, DISSOLVED (UG/L) (04041)	CYANAZINE AMIDE, DISSOLVED (UG/L) (61709)	DIMETHENAMID, DISSOLVED (UG/L) (61588)	DIMETHENAMID ESA, DISSOLVED (UG/L) (61951)	DIMETHENAMID OA, DISSOLVED (UG/L (62482)
11514	410931088384401	07-30-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
20328	413117087481701	08-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20389	413234087513401	08-07-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20436	412015087472301	08-07-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31701	411129090225701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31820	411537090363701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31930	413003090252401	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60089	384417090005201	08-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11706	420614089500101	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11778	420739089132901	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00546	422448088041901	08-27-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47501	404533088173901	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47578	403059088041202	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47586	404247087434701	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
40013	394343088281801	09-03-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50238	392438089262101	09-04-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
52095	392422089411901	09-04-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50064	404305089283501	09-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01233	421234088002501	09-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60171	380805089594701	09-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00140	392154089413401	09-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11419	414608088375201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11456	413528088552101	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
22073	411436087384801	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50138	405525089452201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	FLUFENACET, DISSOLVED (UG/L) (62481)	FLUFENACET ESA, DISSOLVED (UG/L) (61952)	FLUFENACET OA, DISSOLVED (UG/L) (62483)	GLYPHOSATE, DISSOLVED (UG/L) (62722)	GLUFOSINATE, DISSOLVED (UG/L) (62721)	AMINOMETHYL PHOSPHONIC ACID, DISSOLVED (UG/L) (62649)
47583	403143088054701	10-09-01	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
00823	411447087513101	10-10-01	<.05	<.05	<.05	<.10	<.10	<.10
20458	412603087452601	10-10-01	<.05	<.05	<.05	<.10	<.10	<.10
50060	403432089380901	10-15-01	<.05	<.05	<.05	<.10	<.10	<.10
60053	383932090012601	10-16-01	<.05	<.05	<.05	<.10	<.10	<.10
11782	415551088575201	10-23-01	<.05	<.05	<.05	<.10	<.10	<.10
31409	404418089011802	10-23-01	<.05	<.05	<.05	<.10	<.10	<.10
50055	403116089251401	10-30-01	<.05	<.05	<.05	<.10	<.10	<.10
50057	403404089390701	10-30-01	<.05	<.05	<.05	<.10	<.10	<.10
20250	421433088002701	10-31-01	<.05	<.05	<.05	<.10	<.10	<.10
00602	414320089201301	11-07-01	<.05	<.05	<.05	<.10	<.10	<.10
11470	413654088475601	11-07-01	<.05	<.05	<.05	<.10	<.10	<.10
11678	421256088593801	11-07-01	<.05	<.05	<.05	<.10	<.10	<.10
50308	401754090032001	11-13-01	<.05	<.05	<.05	<.10	<.10	<.10
00366	421840088033001	11-20-01	<.05	<.05	<.05	<.10	<.10	<.10
02305	422225088102501	11-20-01	<.05	<.05	<.05	<.10	<.10	<.10
20357	412608088121401	11-21-01	<.05	<.05	<.05	<.10	<.10	<.10
47561	404625087335301	11-27-01	<.05	<.05	<.05	<.10	<.10	<.10
01118	400111090312502	12-04-01	<.05	<.05	<.05	<.10	<.10	<.10
11509	411928089073001	12-04-01	<.05	<.05	<.05	<.10	<.10	<.10
47518	410002088313401	12-04-01	<.05	<.05	<.05	<.10	<.10	<.10
20152	422231088221101	12-09-01	<.05	<.05	<.05	<.10	<.10	<.10
20141	421222088152701	12-09-01	<.05	<.05	<.05	<.10	<.10	<.10
11891	422931089473201	12-11-01	<.05	<.05	<.05	<.10	<.10	<.10
20330	413137088011901	12-11-01	<.05	<.05	<.05	<.10	<.10	<.10
50251	400623089361201	12-12-01	<.05	<.05	<.05	<.10	<.10	<.10
50376	403737089195401	12-12-01	<.05	<.05	<.05	<.10	<.10	<.10
50094	404417090541502	12-18-01	<.05	<.05	<.05	<.10	<.10	<.10
47819	385946088020701	12-19-01	<.05	<.05	<.05	<.10	<.10	<.10
50224	394605089155301	01-09-02	<.05	<.05	<.05	<.10	<.10	<.10
52120	394557090361601	01-09-02	<.05	<.05	<.05	<.10	<.10	<.10
20285	421803087554801	01-14-02	<.05	<.05	<.05	<.10	<.10	<.10
47532	404532088111201	01-15-02	<.05	<.05	<.05	<.10	<.10	<.10
47551	404614087512502	01-15-02	<.05	<.05	<.05	<.10	<.10	<.10
00757	372000088431001	01-22-02	<.05	<.05	<.05	<.10	<.10	<.10
20700	415410088034301	01-23-02	<.05	<.05	<.05	<.10	<.10	<.10
31307	410134089244503	01-29-02	<.05	<.05	<.05	<.10	<.10	<.10
31428	405404089022301	01-29-02	<.05	<.05	<.05	<.10	<.10	<.10
11562	415035089175901	02-05-02	<.05	<.05	<.05	<.10	<.10	<.10
11636	421611089031101	02-05-02	<.05	<.05	<.05	<.10	<.10	<.10
11406	415552088465601	02-06-02	<.05	<.05	<.05	<.10	<.10	<.10
11438	413805088405102	02-06-02	<.05	<.05	<.05	<.10	<.10	<.10
20497	415134088075001	02-11-02	<.05	<.05	<.05	<.10	<.10	<.10
47692	395411087485701	02-19-02	<.05	<.05	<.05	<.10	<.10	<.10
60127	381238090160001	02-25-02	<.05	<.05	<.05	<.10	<.10	<.10
00251	391251087393801	03-04-02	<.05	<.05	<.05	<.10	<.10	<.10

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	FLUFENACET, DISSOLVED (UG/L) (62481)	FLUFENACET ESA, DISSOLVED (UG/L) (61952)	FLUFENACET OA, DISSOLVED (UG/L) (62483)	GLYPHOSATE, DISSOLVED (UG/L) (62722)	GLUFOSINATE, DISSOLVED (UG/L) (62721)	AMINOMETHYL PHOSPHONIC ACID, DISSOLVED (UG/L) (62649)
47811	390644087391802	03-04-02	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
11327	411722089221201	03-05-02	<.05	<.05	<.05	<.10	<.10	<.10
11347	412534089215601	03-05-02	<.05	<.05	<.05	<.10	<.10	<.10
11904	414305089550901	03-06-02	<.05	<.05	<.05	<.10	<.10	<.10
71531	383124088135401	04-16-02	<.05	<.05	<.05	<.10	<.10	<.10
50395	391411090072001	04-22-02	<.05	<.05	<.05	<.10	<.10	<.10
20297	420947087561801	04-23-02	<.05	<.05	<.05	<.10	<.10	<.10
20447	413337088055501	04-23-02	<.05	<.05	<.05	<.10	<.10	<.10
50298	401930090361701	04-29-02	<.05	<.05	<.05	<.10	<.10	<.10
50383	403758089370801	04-29-02	<.05	<.05	<.05	<.10	<.10	<.10
11367	412232089275101	04-30-02	<.05	<.05	<.05	<.10	<.10	<.10
11495	412030089001901	04-30-02	<.05	<.05	<.05	<.10	<.10	<.10
11600	411529089105701	04-30-02	<.05	<.05	<.05	<.10	<.10	<.10
20180	422322088140901	05-14-02	<.05	<.05	<.05	<.10	<.10	<.10
22040	412143088254901	05-14-02	<.05	<.05	<.05	<.10	<.10	<.10
00276	421359088323201	05-20-02	<.05	<.05	<.05	<.10	<.10	<.10
11560	415027089175501	05-20-02	<.05	<.05	<.05	<.10	<.10	<.10
00572	393324088363301	05-21-02	<.05	<.05	<.05	<.10	<.10	<.10
50237	392439089262001	05-21-02	<.05	<.05	<.05	<.10	<.10	<.10
52062	394338090542001	06-05-02	<.05	<.05	<.05	<.10	<.10	<.10
20613	415839088113901	06-11-02	<.05	<.05	<.05	<.10	<.10	<.10
50351	404511090004001	06-11-02	<.05	<.05	<.05	<.10	<.10	<.10
20442	412854087503501	06-17-02	<.05	<.05	<.05	<.10	<.10	<.10
47573	403551088021302	06-17-02	<.05	<.05	<.05	<.10	<.10	<.10
11557	414955089291901	06-18-02	<.05	<.05	<.05	<.10	<.10	<.10
11629	421656089031301	06-18-02	<.05	<.05	<.05	<.10	<.10	<.10
20029	420727088153901	06-24-02	<.05	<.05	<.05	<.10	<.10	<.10
11570	414122088585401	06-25-02	<.05	<.05	<.05	<.10	<.10	<.10
11943	415509089032801	06-25-02	<.05	<.05	<.05	<.10	<.10	<.10
20766	414945088115101	07-01-02	<.05	<.05	<.05	<.10	<.10	<.10
50003	395106089432801	07-02-02	<.05	<.05	<.05	<.10	<.10	<.10
60058	385235090072601	07-02-02	<.05	<.05	<.05	<.10	<.10	<.10
50051	404126089305001	07-17-02	<.05	<.05	<.05	<.10	<.10	<.10
11707	420608089501401	07-29-02	<.05	<.05	<.05	<.10	<.10	<.10
11514	410931088384401	07-30-02	<.05	<.05	<.05	<.10	<.10	<.10
20328	413117087481701	08-06-02	<.05	<.05	<.05	<.10	<.10	<.10
20389	413234087513401	08-07-02	<.05	<.05	<.05	<.10	<.10	<.10
20436	412015087472301	08-07-02	<.05	<.05	<.05	<.10	<.10	<.10
31701	411129090225701	08-13-02	<.05	<.05	<.05	<.10	<.10	<.10
31820	411537090363701	08-13-02	<.05	<.05	<.05	<.10	<.10	<.10
31930	413003090252401	08-13-02	<.05	<.05	<.05	<.10	<.10	<.10
60089	384417090005201	08-14-02	<.05	<.05	<.05	<.10	<.10	<.10
11706	420614089500101	08-19-02	<.05	<.05	<.05	<.10	<.10	<.10
11778	420739089132901	08-19-02	<.05	<.05	<.05	<.10	<.10	<.10
00546	422448088041901	08-27-02	<.05	<.05	<.05	<.10	<.10	<.10
47501	404533088173901	08-28-02	<.05	<.05	<.05	<.10	<.10	<.10

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	FLUFENACET, DISSOLVED (UG/L) (62481)	FLUFENACET ESA, DISSOLVED (UG/L) (61952)	FLUFENACET OA, DISSOLVED (UG/L) (62483)	GLYPHOSATE, DISSOLVED (UG/L) (62722)	GLUFOSINATE, DISSOLVED (UG/L) (62721)	AMINOMETHYL PHOSPHONIC ACID, DISSOLVED (UG/L) (62649)
01233	421234088002501	09-16-02	<0.05	<0.05	<0.05	<0.10	<0.10	<0.10
60171	380805089594701	09-18-02	<.05	<.05	<.05	<.10	<.10	<.10
00140	392154089413401	09-23-02	<.05	<.05	<.05	<.10	<.10	<.10
11419	414608088375201	09-24-02	<.05	<.05	<.05	<.10	<.10	<.10
11456	413528088552101	09-24-02	<.05	<.05	<.05	<.10	<.10	<.10
22073	411436087384801	09-24-02	<.05	<.05	<.05	<.10	<.10	<.10
50138	405525089452201	09-24-02	<.05	<.05	<.05	<.10	<.10	<.10

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	METOLACHLOR, DISSOLVED (UG/L) (39415)	METOLACHLOR ESA, DISSOLVED (UG/L) (61043)	METOLACHLOR OA, DISSOLVED (UG/L) (61044)	METRIBUZIN, DISSOLVED (UG/L) (82630)	PENDIMETHALIN, DISSOLVED (UG/L) (82683)	PROMETON, DISSOLVED (UG/L) (04037)	PROMETRYN, DISSOLVED (UG/L) (04036)
47583	403143088054701	10-09-01	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
00823	411447087513101	10-10-01	<.05	.86	.21	<.05	<.05	<.05	<.05
20458	412603087452601	10-10-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50060	403432089380901	10-15-01	<.05	.09	<.05	<.05	<.05	<.05	<.05
60053	383932090012601	10-16-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11782	415551088575201	10-23-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31409	404418089011802	10-23-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50055	403116089251401	10-30-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50057	403404089390701	10-30-01	<.05	.57	.23	<.05	<.05	<.05	<.05
20250	421433088002701	10-31-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00602	414320089201301	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11470	413654088475601	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11678	421256088593801	11-07-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50308	401754090032001	11-13-01	<.05	.13	<.05	<.05	<.05	<.05	<.05
00366	421840088033001	11-20-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
02305	422225088102501	11-20-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20357	412608088121401	11-21-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47561	404625087335301	11-27-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01118	400111090312502	12-04-01	<.05	.17	.07	<.05	<.05	<.05	<.05
11509	411928089073001	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47518	410002088313401	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20152	422231088221101	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20141	421222088152701	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11891	422931089473201	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20330	413137088011901	12-11-01	<.05	.12	<.05	<.05	<.05	<.05	<.05
50251	400623089361201	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50376	403737089195401	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50094	404417090541502	12-18-01	<.05	.24	<.05	<.05	<.05	<.05	<.05
47819	385946088020701	12-19-01	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50224	394605089155301	01-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
52120	394557090361601	01-09-02	<.05	.60	.26	<.05	<.05	<.05	<.05
20285	421803087554801	01-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47532	404532088111201	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47551	404614087512502	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00757	372000088431001	01-22-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20700	415410088034301	01-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31307	410134089244503	01-29-02	<.05	.14	<.05	<.05	<.05	<.05	<.05
31428	405404089022301	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11562	415035089175901	02-05-02	<.05	2.46	<.05	<.05	<.05	<.05	<.05
11636	421611089031101	02-05-02	<.05	.07	<.05	<.05	<.05	<.05	<.05
11406	415552088465601	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11438	413805088405102	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20497	415134088075001	02-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47692	395411087485701	02-19-02	<.05	.77	<.05	<.05	<.05	<.05	<.05
60127	381238090160001	02-25-02	<.05	.17	.13	<.05	<.05	<.05	<.05
00251	391251087393801	03-04-02	.16	.07	<.05	<.05	<.05	<.05	<.05
47811	390644087391802	03-04-02	<.05	.52	.52	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	METOLACHLOR, DISSOLVED (UG/L) (39415)	METOLACHLOR ESA, DISSOLVED (UG/L) (61043)	METOLACHLOR OA, DISSOLVED (UG/L) (61044)	METRIBUZIN, DISSOLVED (UG/L) (82630)	PENDIMETHALIN, DISSOLVED (UG/L) (82683)	PROMETON, DISSOLVED (UG/L) (04037)	PROMETRYN, DISSOLVED (UG/L) (04036)
11327	411722089221201	03-05-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
11347	412534089215601	03-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11904	414305089550901	03-06-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00795	420559088322101	03-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01416	394953090331202	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47725	395640088515501	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
45155	392418087392101	03-18-02	<.05	.29	<.05	<.05	<.05	<.05	<.05
11892	413920090044401	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31889	412421090334501	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50101	394212090344901	04-01-02	<.05	.41	.06	<.05	<.05	<.05	<.05
50227	400022090522301	04-01-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20207	421943088160501	04-02-02	<.05	1.56	.45	<.05	<.05	<.05	<.05
45081	400836088185101	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47687	401217088220301	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11363	413328089274501	04-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
71531	383124088135401	04-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50395	391411090072001	04-22-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20297	420947087561801	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20447	413337088055501	04-23-02	<.05	.21	.06	<.05	<.05	<.05	<.05
50298	401930090361701	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50383	403758089370801	04-29-02	<.05	.45	.22	<.05	<.05	<.05	<.05
11367	412232089275101	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11495	412030089001901	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11600	411529089105701	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20180	422322088140901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
22040	412143088254901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00276	421359088323201	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11560	415027089175501	05-20-02	<.05	2.32	<.05	<.05	<.05	<.05	<.05
00572	393324088363301	05-21-02	<.05	1.59	.28	<.05	<.05	<.05	<.05
50237	392439089262001	05-21-02	<.05	7.24	2.95	<.05	<.05	<.05	<.05
52062	394338090542001	06-05-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20613	415839088113901	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50351	404511090004001	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20442	412854087503501	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47573	403551088021302	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11557	414955089291901	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11629	421656089031301	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20029	420727088153901	06-24-02	<.05	.05	<.05	<.05	<.05	<.05	<.05
11570	414122088585401	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11943	415509089032801	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20766	414945088115101	07-01-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50003	395106089432801	07-02-02	<.05	.70	.15	<.05	<.05	<.05	<.05
60058	385235090072601	07-02-02	<.05	.34	.18	<.05	<.05	<.05	<.05
50051	404126089305001	07-17-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11707	420608089501401	07-29-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11514	410931088384401	07-30-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
20328	413117087481701	08-06-02	<.05	.20	.12	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	METOLACHLOR, DISSOLVED (UG/L) (39415)	METOLACHLOR ESA, DISSOLVED (UG/L) (61043)	METOLACHLOR OA, DISSOLVED (UG/L) (61044)	METRIBUZIN, DISSOLVED (UG/L) (82630)	PENDIMETHALIN, DISSOLVED (UG/L) (82683)	PROMETON, DISSOLVED (UG/L) (04037)	PROMETRYN, DISSOLVED (UG/L) (04036)
20389	413234087513401	08-07-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
20436	412015087472301	08-07-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31701	411129090225701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31820	411537090363701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
31930	413003090252401	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60089	384417090005201	08-14-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11706	420614089500101	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11778	420739089132901	08-19-02	<.05	.09	<.05	<.05	<.05	<.05	<.05
00546	422448088041901	08-27-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47501	404533088173901	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47578	403059088041202	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
47586	404247087434701	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
40013	394343088281801	09-03-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50238	392438089262101	09-04-02	<.05	.70	.10	<.05	<.05	<.05	<.05
52095	392422089411901	09-04-02	<.05	.05	<.05	<.05	<.05	<.05	<.05
50064	404305089283501	09-09-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
01233	421234088002501	09-16-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
60171	380805089594701	09-18-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
00140	392154089413401	09-23-02	<.05	.45	.07	<.05	<.05	<.05	<.05
11419	414608088375201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
11456	413528088552101	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
22073	411436087384801	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05
50138	405525089452201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	PROPACHLOR, DISSOLVED (UG/L) (04024)	PROPACHLOR, ESA, DISSOLVED (UG/L) (62766)	PROPACHLOR, OA, DISSOLVED (UG/L) (62767)	PROPAZINE, DISSOLVED (UG/L) (38535)	SIMAZINE, DISSOLVED (UG/L) (04035)	TERBUTRYN, DISSOLVED (UG/L) (38888)
47583	403143088054701	10-09-01	<0.05	--	--	<0.05	<0.05	<0.05
00823	411447087513101	10-10-01	<.05	--	--	<.05	<.05	<.05
20458	412603087452601	10-10-01	<.05	--	--	<.05	<.05	<.05
50060	403432089380901	10-15-01	<.05	--	--	<.05	<.05	<.05
60053	383932090012601	10-16-01	<.05	--	--	<.05	<.05	<.05
11782	415551088575201	10-23-01	<.05	--	--	<.05	<.05	<.05
31409	404418089011802	10-23-01	<.05	--	--	<.05	<.05	<.05
50055	403116089251401	10-30-01	<.05	--	--	<.05	<.05	<.05
50057	403404089390701	10-30-01	<.05	--	--	<.05	<.05	<.05
20250	421433088002701	10-31-01	<.05	--	--	<.05	<.05	<.05
00602	414320089201301	11-07-01	<.05	--	--	<.05	<.05	<.05
11470	413654088475601	11-07-01	<.05	--	--	<.05	<.05	<.05
11678	421256088593801	11-07-01	<.05	--	--	<.05	<.05	<.05
50308	401754090032001	11-13-01	<.05	--	--	<.05	<.05	<.05
00366	421840088033001	11-20-01	<.05	--	--	<.05	<.05	<.05
02305	422225088102501	11-20-01	<.05	--	--	<.05	<.05	<.05
20357	412608088121401	11-21-01	<.05	--	--	<.05	<.05	<.05
47561	404625087335301	11-27-01	<.05	--	--	<.05	<.05	<.05
01118	400111090312502	12-04-01	<.05	<0.05	<0.05	<.05	<.05	<.05
11509	411928089073001	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05
47518	410002088313401	12-04-01	<.05	<.05	<.05	<.05	<.05	<.05
20152	422231088221101	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05
20141	421222088152701	12-09-01	<.05	<.05	<.05	<.05	<.05	<.05
11891	422931089473201	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05
20330	413137088011901	12-11-01	<.05	<.05	<.05	<.05	<.05	<.05
50251	400623089361201	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05
50376	403737089195401	12-12-01	<.05	<.05	<.05	<.05	<.05	<.05
50094	404417090541502	12-18-01	<.05	<.05	<.05	<.05	<.05	<.05
47819	385946088020701	12-19-01	<.05	<.05	<.05	<.05	<.05	<.05
50224	394605089155301	01-09-02	<.05	<.05	<.05	<.05	<.05	<.05
52120	394557090361601	01-09-02	<.05	<.05	<.05	<.05	<.05	<.05
20285	421803087554801	01-14-02	<.05	<.05	<.05	<.05	<.05	<.05
47532	404532088111201	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05
47551	404614087512502	01-15-02	<.05	<.05	<.05	<.05	<.05	<.05
00757	372000088431001	01-22-02	<.05	<.05	<.05	<.05	<.05	<.05
20700	415410088034301	01-23-02	<.05	<.05	<.05	<.05	<.05	<.05
31307	410134089244503	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05
31428	405404089022301	01-29-02	<.05	<.05	<.05	<.05	<.05	<.05
11562	415035089175901	02-05-02	<.05	<.05	<.05	<.05	<.05	<.05
11636	421611089031101	02-05-02	<.05	<.05	<.05	<.05	<.05	<.05
11406	415552088465601	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05
11438	413805088405102	02-06-02	<.05	<.05	<.05	<.05	<.05	<.05
20497	415134088075001	02-11-02	<.05	<.05	<.05	<.05	<.05	<.05
47692	395411087485701	02-19-02	<.05	<.05	<.05	<.05	<.05	<.05
60127	381238090160001	02-25-02	<.05	<.05	<.05	<.05	<.05	<.05
00251	391251087393801	03-04-02	<.05	<.05	<.05	<.05	<.05	<.05
47811	390644087391802	03-04-02	<.05	<.05	<.05	<.05	<.05	<.05

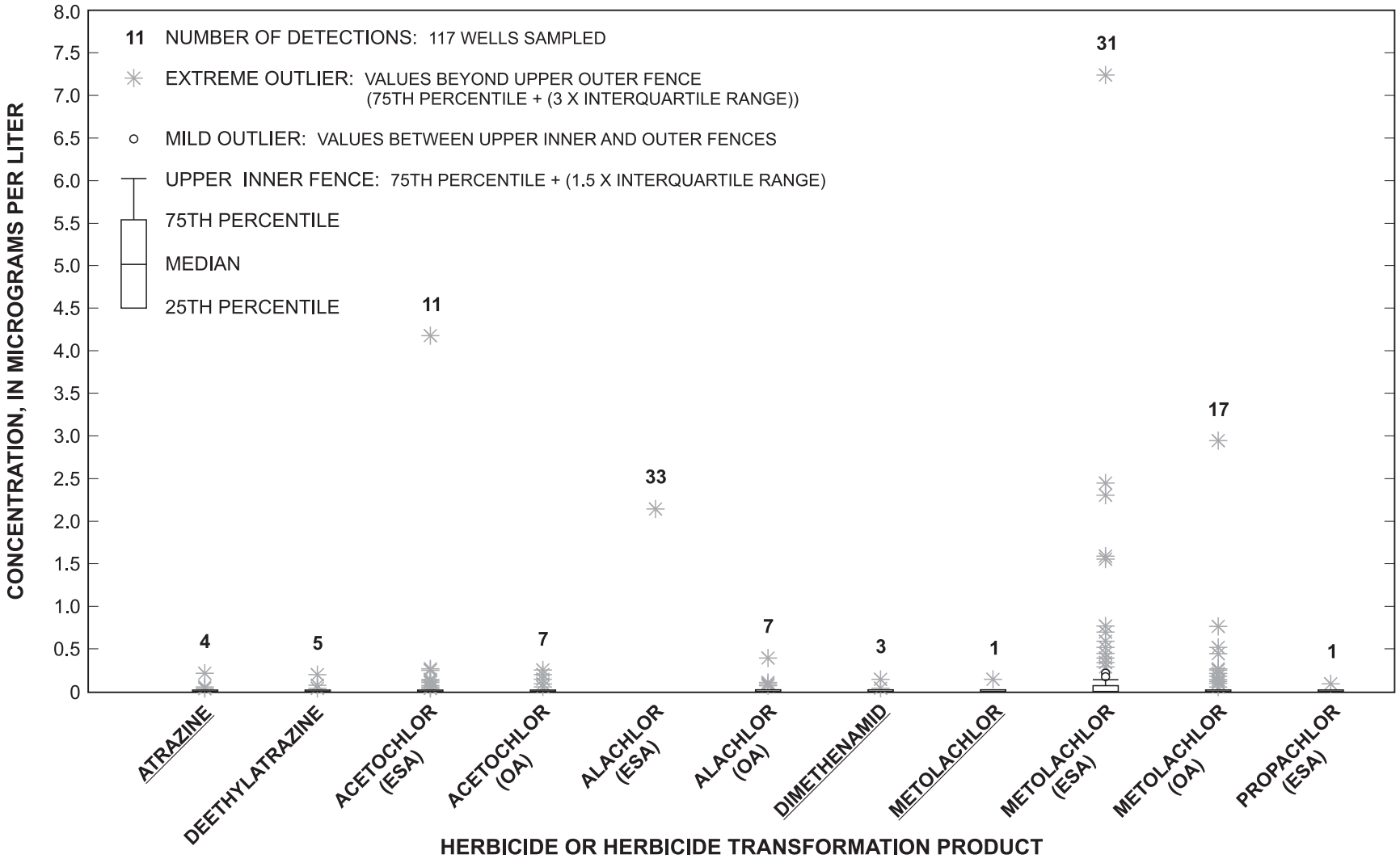
Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	PROPACHLOR, DISSOLVED (UG/L) (04024)	PROPACHLOR, ESA, DISSOLVED (UG/L) (62766)	PROPACHLOR, OA, DISSOLVED (UG/L) (62767)	PROPAZINE, DISSOLVED (UG/L) (38535)	SIMAZINE, DISSOLVED (UG/L) (04035)	TERBUTRYN, DISSOLVED (UG/L) (38888)
11327	411722089221201	03-05-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
11347	412534089215601	03-05-02	<.05	<.05	<.05	<.05	<.05	<.05
11904	414305089550901	03-06-02	<.05	<.05	<.05	<.05	<.05	<.05
00795	420559088322101	03-11-02	<.05	<.05	<.05	<.05	<.05	<.05
01416	394953090331202	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05
47725	395640088515501	03-12-02	<.05	<.05	<.05	<.05	<.05	<.05
45155	392418087392101	03-18-02	<.05	<.05	<.05	<.05	<.05	<.05
11892	413920090044401	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05
31889	412421090334501	03-19-02	<.05	<.05	<.05	<.05	<.05	<.05
50101	394212090344901	04-01-02	<.05	<.05	<.05	<.05	<.05	<.05
50227	400022090522301	04-01-02	<.05	<.05	<.05	<.05	<.05	<.05
20207	421943088160501	04-02-02	<.05	<.05	<.05	<.05	<.05	<.05
45081	400836088185101	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05
47687	401217088220301	04-08-02	<.05	<.05	<.05	<.05	<.05	<.05
11363	413328089274501	04-09-02	<.05	<.05	<.05	<.05	<.05	<.05
71531	383124088135401	04-16-02	<.05	<.05	<.05	<.05	<.05	<.05
50395	391411090072001	04-22-02	<.05	<.05	<.05	<.05	<.05	<.05
20297	420947087561801	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05
20447	413337088055501	04-23-02	<.05	<.05	<.05	<.05	<.05	<.05
50298	401930090361701	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05
50383	403758089370801	04-29-02	<.05	<.05	<.05	<.05	<.05	<.05
11367	412232089275101	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05
11495	412030089001901	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05
11600	411529089105701	04-30-02	<.05	<.05	<.05	<.05	<.05	<.05
20180	422322088140901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05
22040	412143088254901	05-14-02	<.05	<.05	<.05	<.05	<.05	<.05
00276	421359088323201	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05
11560	415027089175501	05-20-02	<.05	<.05	<.05	<.05	<.05	<.05
00572	393324088363301	05-21-02	<.05	.10	<.05	<.05	<.05	<.05
50237	392439089262001	05-21-02	<.05	<.05	<.05	<.05	<.05	<.05
52062	394338090542001	06-05-02	<.05	<.05	<.05	<.05	<.05	<.05
20613	415839088113901	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05
50351	404511090004001	06-11-02	<.05	<.05	<.05	<.05	<.05	<.05
20442	412854087503501	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05
47573	403551088021302	06-17-02	<.05	<.05	<.05	<.05	<.05	<.05
11557	414955089291901	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05
11629	421656089031301	06-18-02	<.05	<.05	<.05	<.05	<.05	<.05
20029	420727088153901	06-24-02	<.05	<.05	<.05	<.05	<.05	<.05
11570	414122088585401	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05
11943	415509089032801	06-25-02	<.05	<.05	<.05	<.05	<.05	<.05
20766	414945088115101	07-01-02	<.05	<.05	<.05	<.05	<.05	<.05
50003	395106089432801	07-02-02	<.05	<.05	<.05	<.05	<.05	<.05
60058	385235090072601	07-02-02	<.05	<.05	<.05	<.05	<.05	<.05
50051	404126089305001	07-17-02	<.05	<.05	<.05	<.05	<.05	<.05
11707	420608089501401	07-29-02	<.05	<.05	<.05	<.05	<.05	<.05
11514	410931088384401	07-30-02	<.05	<.05	<.05	<.05	<.05	<.05
20328	413117087481701	08-06-02	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 1. Well information, field characteristics of water quality, herbicides, and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002—Continued

IEPA WELL NUMBER	USGS STATION NUMBER	SAMPLE DATE	PROPACHLOR, DISSOLVED (UG/L) (04024)	PROPACHLOR, ESA, DISSOLVED (UG/L) (62766)	PROPACHLOR, OA, DISSOLVED (UG/L) (62767)	PROPAZINE, DISSOLVED (UG/L) (38535)	SIMAZINE, DISSOLVED (UG/L) (04035)	TERBUTRYN, DISSOLVED (UG/L) (38888)
20389	413234087513401	08-07-02	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
20436	412015087472301	08-07-02	<.05	<.05	<.05	<.05	<.05	<.05
31701	411129090225701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05
31820	411537090363701	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05
31930	413003090252401	08-13-02	<.05	<.05	<.05	<.05	<.05	<.05
60089	384417090005201	08-14-02	<.05	<.05	<.05	<.05	<.05	<.05
11706	420614089500101	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05
11778	420739089132901	08-19-02	<.05	<.05	<.05	<.05	<.05	<.05
00546	422448088041901	08-27-02	<.05	<.05	<.05	<.05	<.05	<.05
47501	404533088173901	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05
47578	403059088041202	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05
47586	404247087434701	08-28-02	<.05	<.05	<.05	<.05	<.05	<.05
40013	394343088281801	09-03-02	<.05	<.05	<.05	<.05	<.05	<.05
50238	392438089262101	09-04-02	<.05	<.05	<.05	<.05	<.05	<.05
52095	392422089411901	09-04-02	<.05	<.05	<.05	<.05	<.05	<.05
50064	404305089283501	09-09-02	<.05	<.05	<.05	<.05	<.05	<.05
01233	421234088002501	09-16-02	<.05	<.05	<.05	<.05	<.05	<.05
60171	380805089594701	09-18-02	<.05	<.05	<.05	<.05	<.05	<.05
00140	392154089413401	09-23-02	<.05	<.05	<.05	<.05	<.05	<.05
11419	414608088375201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05
11456	413528088552101	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05
22073	411436087384801	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05
50138	405525089452201	09-24-02	<.05	<.05	<.05	<.05	<.05	<.05

Appendix 2. Box-and-whisker plots of concentrations of detected herbicides and herbicide transformation products in samples from selected public-supply wells that tap source-water aquifers in Illinois, October 2001-September 2002.



HERBICIDES ARE UNDERLINED IN LISTING ABOVE

ETHANESULFONIC ACID (ESA); OXANILIC ACID (OA)

VALUES BELOW REPORTING LIMIT OF 0.05 MICROGRAMS PER LITER (μg/L) ARE NORMALIZED TO 0 μg/L

U.S. ENVIRONMENTAL PROTECTION AGENCY (2003b) MAXIMUM CONTAMINANT LEVELS: ATRAZINE (3 μg/L), ALACHLOR (2 μg/L), SIMAZINE (4 μg/L)

District Chief
U.S. Geological Survey
221 N. Broadway Ave.
Urbana, IL 61801

Mills and McMillan—**Herbicides and Their Transformation Products in Source-Water Aquifers Tapped by Public-Supply Wells in Illinois, 2001-02**—U.S. Geological Survey Water-Resources Investigations Report 03-4226

1879–2004

Attachment

1I

1

Class I Groundwater Quality Standard (GQS) Updates						Chemical-Specific Factors for HTTAC Calculations			Chemical-Specific Factors for HNTAC Calculations	
CASRN	Constituent	Current Class I GQS mg/L	Current Class I GQS Basis	Proposed Updated Class I GQS mg/L	Updated Class I GQS Basis	Oral Reference Dose (RfD) mg/kg-day	RfD Source	Relative Source Contribution (RSC) unitless	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	SF_o Source
Section 620.410(a) - Inorganics										
7429-90-5	Aluminum	----	----	1.9	HTTAC	1E+00	PPRTV	0.1	----	----
7440-48-4	Cobalt	1.0	Livestock	0.0012	HTTAC	3E-04	PPRTV	0.2	----	----
7440-50-8	Copper	0.65	Lead/Copper Rule	0.5	Livestock	----	----	----	----	----
7681-49-4	Fluoride (sodium fluoride)	4	MCL	2	Livestock	----	----	----	----	----
7439-93-2	Lithium	----	----	0.04	LLOQ/LCMRL	2E-03	PPRTV	0.2	----	----
7439-98-7	Molybdenum	----	----	0.019	HTTAC	5E-03	IRIS	0.2	----	----
7440-02-0	Nickel	0.1	Livestock	0.077	HTTAC	2E-02	IRIS	0.2	----	----
14797-73-0	Perchlorate	0.0049	HTTAC	0.0081	HTTAC	7E-04	IRIS	0.6	----	----
7440-14-4	Radium (combined 226+228) (pCi/L)	----	----	5	MCL	----	----	----	----	----
7782-49-2	Selenium	0.05	MCL	0.02	Irrigation	----	----	----	----	----
7440-22-4	Silver	0.05	MAC	0.058	HTTAC	5E-03	IRIS	0.2	----	----
7440-62-2	Vanadium	0.049	HTTAC	0.00027	HTTAC	7E-05	PPRTV	0.2	----	----
7440-66-6	Zinc	5	Livestock	1.2	HTTAC	3E-01	IRIS	0.2	----	----
Section 620.410(b) – Organics										
83-32-9	Acenaphthene	0.42	HTTAC	0.23	HTTAC	6E-02	IRIS	0.2	----	----
67-64-1	Acetone	6.3	HTTAC	3.5	HTTAC	9E-01	IRIS	0.2	----	----
120-12-7	Anthracene	2.1	HTTAC	1.2	HTTAC	3E-01	IRIS	0.2		
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)*	0.00011	PQL	0.000012	HNTAC	----	----	----	6.3E+00	IRIS
56-55-3	Benzo(a)anthracene**	0.00013	PQL	0.00025	HNTAC-Mutagen	----	----	----	1.0E-01	IRIS
205-99-2	Benzo(b)fluoranthene**	0.00018	PQL	0.00025	HNTAC-Mutagen	----	----	----	1.0E-01	IRIS
207-08-9	Benzo(k)fluoranthene**	0.00017	PQL	0.0025	HNTAC-Mutagen	----	----	----	1.0E-02	IRIS

Class I Groundwater Quality Standard (GQS) Updates						Chemical-Specific Factors for HTTAC Calculations			Chemical-Specific Factors for HNTAC Calculations	
CASRN	**Constituent**	**Current Class I GQS mg/L**	**Current Class I GQS Basis**	**Proposed Updated Class I GQS mg/L**	**Updated Class I GQS Basis**	**Oral Reference Dose (RfD) mg/kg-day**	**RfD Source**	**Relative Source Contribution (RSC) unitless**	**Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$**	**SF_o Source**
65-85-0	Benzoic acid	28.0	HTTAC	15	HTTAC	4E+00	IRIS	0.2		
78-93-3	2-Butanone (methyl ethyl ketone)	4.2	HTTAC	2.3	HTTAC	6E-01	IRIS	0.2		
75-15-0	Carbon disulfide	0.7	HTTAC	0.38	HTTAC	1E-01	IRIS	0.2		
218-01-9	Chrysene**	0.012	HNTAC	0.025	HNTAC-Mutagen	----	----	----	1.0E-03	IRIS
53-70-3	Dibenzo(a,h)anthracene**	0.0003	PQL	0.0001	LLOQ/LCMRL	----	----	----	1.0E+00	IRIS
1918-00-9	Dicamba	0.21	HTTAC	0.12	HTTAC	3E-02	IRIS	0.2		
75-71-8	Dichlorodifluoromethane	1.4	HTTAC	0.77	HTTAC	2E-01	IRIS	0.2		
75-34-3	1,1-Dichloroethane	1.4	HTTAC	0.77	HTTAC	2E-01	PPRTV	0.2		
84-66-2	Diethyl phthalate	5.6	HTTAC	3.1	HTTAC	8E-01	IRIS	0.2	----	----
84-74-2	Di-*n*-butyl phthalate	0.7	HTTAC	0.38	HTTAC	1E-01	IRIS	0.2	----	----
99-65-0	1,3-Dinitrobenzene	0.0007	HTTAC	0.001	LLOQ/LCMRL	1E-04	IRIS	0.2	----	----
121-14-2	2,4-Dinitrotoluene*	0.0001	HNTAC	0.001	LLOQ/LCMRL	----	----	----	3.1E-01	CalEPA
606-20-2	2,6-Dinitrotoluene*	0.00031	PQL	0.001	LLOQ/LCMRL	----	----	----	1.5E+00	PPRTV
123-91-1	1,4-Dioxane (*p*-dioxane)*	0.0077	HNTAC	0.00078	HNTAC	----	----	----	1.0E-01	IRIS
206-44-0	Fluoranthene	0.28	HTTAC	0.15	HTTAC	4E-02	IRIS	0.2	----	----
86-73-7	Fluorene	0.28	HTTAC	0.15	HTTAC	4E-02	IRIS	0.2	----	----
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	----	----	0.000012	HTTAC	3E-06	U.S. EPA OW	0.2		
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	1.4	HTTAC	0.77	HTTAC	5E-02	IRIS	0.8	----	----
193-39-5	Indeno(1,2,3-c,d)pyrene**	0.00043	PQL	0.00025	HNTAC-Mutagen	----	----	----	1.0E-01	IRIS
98-82-8	Isopropylbenzene (cumene)*	0.7	HTTAC	0.38	HTTAC	1E-01	IRIS	0.2	----	----
93-65-2	MCPP (Mecoprop)	0.007	HTTAC	0.1	LLOQ/LCMRL	1E-03	IRIS	0.2	----	----

Class I Groundwater Quality Standard (GQS) Updates						Chemical-Specific Factors for HTTAC Calculations			Chemical-Specific Factors for HNTAC Calculations	
CASRN	Constituent	Current Class I GQS mg/L	Current Class I GQS Basis	Proposed Updated Class I GQS mg/L	Updated Class I GQS Basis	Oral Reference Dose (RfD) mg/kg-day	RfD Source	Relative Source Contribution (RSC) unitless	Oral Slope Factor (SF_o) $(mg/kg\text{-}day)^{-1}$	SF_o Source
1634-04-4	MTBE (methyl tertiary-butyl ether)	0.07	HTTAC	0.038	HTTAC	1E-02	IEPA	0.2	----	----
90-12-0	1-Methylnaphthalene	----	----	0.27	HTTAC	7E-02	ATSDR	0.2	----	----
91-57-6	2-Methylnaphthalene	0.028	HTTAC	0.015	HTTAC	4E-03	IRIS	0.2	----	----
95-48-7	2-Methylphenol (*o*-cresol)	0.35	HTTAC	0.19	HTTAC	5E-02	IRIS	0.2	----	----
91-20-3	Naphthalene	0.14	HTTAC	0.077	HTTAC	2E-02	IRIS	0.2	----	----
98-95-3	Nitrobenzene	0.014	HTTAC	0.0077	HTTAC	2E-03	IRIS	0.2	----	----
375-73-5	PFBS (perfluorobutanesulfonic acid)	----	----	0.0012	HTTAC	3E-04	PPRTV	0.2	----	----
355-46-4	PFHxS (perfluorohexanesulfonic acid)	----	----	0.000077	HTTAC	2E-05	ATSDR	0.2	----	----
375-95-1	PFNA (perfluorononanoic acid)	----	----	0.000012	HTTAC	3E-06	ATSDR	0.2	----	----
335-67-1	PFOA (perfluorooctanoic acid)*	----	----	0.000002	LLOQ/LCMRL	----	----	----	1.4E+02	CalEPA
1763-23-1	PFOS (perfluorooctanesulfonic acid)	----	----	0.0000077	HTTAC	2E-06	ATSDR	0.2	----	----
129-00-0	Pyrene	0.21	HTTAC	0.12	HTTAC	3E-02	IRIS	0.2	----	----
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	0.084	HTTAC	0.062	HTTAC	4E-03	IRIS	0.8	----	----
118-96-7	TNT (2,4,6-trinitrotoluene)	0.014	HTTAC	0.0077	HTTAC	5E-04	IRIS	0.8	----	----
75-69-4	Trichlorofluoromethane	2.1	HTTAC	1.2	HTTAC	3E-01	IRIS	0.2	----	----
99-35-4	1,3,5-Trinitrobenzene	0.84	HTTAC	0.46	HTTAC	3E-02	IRIS	0.8	----	----

Class I Groundwater Quality Standard (GQS) Updates						Chemical-Specific Factors for HTTAC Calculations			Chemical-Specific Factors for HNTAC Calculations	
CASRN	**Constituent**	**Current Class I GQS mg/L**	**Current Class I GQS Basis**	**Proposed Updated Class I GQS mg/L**	**Updated Class I GQS Basis**	**Oral Reference Dose (RfD) mg/kg-day**	**RfD Source**	**Relative Source Contribution (RSC) unitless**	**Oral Slope Factor (SF_o) $(mg/kg-day)^{-1}$**	**SF_o Source**
Section 620.410(c) - Complex Organic Chemical Mixtures										
Section 620.410(c)(2)										
1912-24-9	Total Atrazine	0.003	MCL	0.003	MCL with Addition of Metabolites in Standard	----	----	----	----	----
6190-65-4	Desethyl-atrazine (DEA) – metabolite	----	----							
1007-28-9	Desisopropyl-atrazine (DIA) - metabolite	----	----							
3397-62-4	Diaminochlorotriazine (DACT) - metabolite	----	----							

* = Carcinogen

** = Mutagen

ATSDR = Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services.

CalEPA = Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

HNTAC = Human Nonthreshold Toxicant Advisory Concentration.

HNTAC - Mutagen = Human Nonthreshold Toxicant Advisory Concentration for Mutagens.

HTTAC = Human Threshold Toxicant Advisory Concentration.

IRIS = Integrated Risk Information System (National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency).

Irrigation = The standard is based on beneficial use for irrigation of crops, per “Water Quality Criteria”, by National Academy of Sciences.

LCMRL = Lowest Concentration Minimum Reporting Level, formerly termed "PQL".

LLOQ = Lower Limit of Quantitation, formerly termed "PQL".

Lead/Copper Rule = The standard is 50% of the U.S. EPA “action level” of 0.015 mg/L for lead. The U.S. EPA action level applies at the service connection. The standard is reduced by 50% as a safety margin, based on the assumption that 50% of water would be treated.

Livestock = The standard is based on beneficial use for watering livestock, per “Water Quality Criteria”, by National Academy of Sciences.

MAC = Maximum Allowable Concentration. The standard is promulgated at 35 Ill. Adm. Code 611.300.

MCL = The standard is based the Maximum Contaminant Level (“MCL”), promulgated by U.S. EPA, Office of Water, and Illinois EPA Primary Drinking Water Standards at 35 Ill. Adm. Code 611.

PPRTV = Provisional Peer-Reviewed Toxicity Value (Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency).

PQL = Practical Quantitation Limit.

U.S. EPA OW = United States Environmental Protection Agency Office of Water.

95% Confidence Value = The standard is the 95% confidence concentration stated in Illinois EPA's "Integrated Water Quality Report and Section 303(d) List".

Attachment

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EPA/635/R-17/003Fc
www.epa.gov/iris

Toxicological Review of Benzo[a]pyrene

Executive Summary

[CASRN 50-32-8]

January 2017

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Benzo[a]pyrene is a five-ring polycyclic aromatic hydrocarbon (PAH). Benzo[a]pyrene (along with other PAHs) is released into the atmosphere as a component of smoke from forest fires, industrial processes, vehicle exhaust, cigarettes, and through the burning of fuel (such as wood, coal, and petroleum products). Oral exposure to benzo[a]pyrene can occur by eating certain food products, such as charred meats, where benzo[a]pyrene is formed during the cooking process, or by eating foods grown in areas contaminated with benzo[a]pyrene (from the air and soil). Dermal exposure may occur from contact with soils or materials that contain soot, tar, or crude petroleum products or by using certain pharmaceutical products containing coal tars, such as those used to treat the skin conditions, eczema and psoriasis. The magnitude of human exposure to benzo[a]pyrene and other PAHs depends on factors such as lifestyle (e.g., diet, tobacco smoking), occupation, and living conditions (e.g., urban versus rural setting, domestic heating, and cooking methods).

Animal studies demonstrate that exposure to benzo[a]pyrene is associated with developmental (including developmental neurotoxicity), reproductive, and immunological effects. In addition, epidemiology studies involving exposure to PAH mixtures have reported associations between internal biomarkers of exposure to benzo[a]pyrene (benzo[a]pyrene diol epoxide-DNA adducts) and adverse birth outcomes (including reduced birth weight, postnatal body weight, and head circumference), neurobehavioral effects, and decreased fertility.

Studies in multiple animal species demonstrate that benzo[a]pyrene is carcinogenic at multiple tumor sites (alimentary tract, liver, kidney, respiratory tract, pharynx, and skin) by all routes of exposure. In addition, there is strong evidence of carcinogenicity in occupations involving exposure to PAH mixtures containing benzo[a]pyrene, such as aluminum production, chimney sweeping, coal gasification, coal-tar distillation, coke production, iron and steel founding, and paving and roofing with coal tar pitch. An increasing number of occupational studies demonstrate a positive exposure-response relationship with cumulative benzo[a]pyrene exposure and lung cancer.

Effects Other Than Cancer Observed Following Oral Exposure

In animals, oral exposure to benzo[a]pyrene has been shown to result in developmental toxicity (including developmental neurotoxicity), reproductive toxicity, and immunotoxicity. Developmental effects in rats and mice include neurobehavioral changes and cardiovascular effects following gestational exposures. Reproductive and immune effects include decreased sperm counts, ovary weight, and follicle numbers, and decreased immunoglobulin and B cell numbers and thymus weight following oral exposures in adult animals. In humans, benzo[a]pyrene exposure occurs in conjunction with other PAHs and, as such, attributing the observed effects to

benzo[a]pyrene is complicated. However, some human studies report associations between particular health endpoints and internal measures of exposure, such as benzo[a]pyrene-deoxyribonucleic acid (DNA) adducts, or external measures of benzo[a]pyrene exposure. Overall, the human studies report developmental, neurobehavioral, reproductive, and immune effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for hazards associated with benzo[a]pyrene exposure.

Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ- or system-specific RfDs were derived for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by neurobehavioral changes persisting into adulthood, was chosen as the basis for the overall oral RfD as the available data indicate that developmental neurotoxicity represents the most sensitive hazard of benzo[a]pyrene exposure. The neurodevelopmental study by Chen et al. (2012) was used to derive the RfD. Altered responses in three behavioral tests (i.e., Morris water maze, elevated plus maze, and open field tests) were selected to represent the critical effect of abnormal behavior, due to the consistency (i.e., each of these responses were affected in two separate cohorts of rats, including testing as juveniles and as adults; similar effects in these behavioral tests were observed across studies) and sensitivity of these responses, and the observed dose-response relationship of effects across dose groups. Benchmark dose (BMD) modeling for each of the three endpoints resulted in $BMDL_{1SD}$ values that clustered in the range $0.092–0.16$ mg/kg-day. The lower end of this range of BMDLs, 0.092 mg/kg-day, was selected to represent the point of departure (POD) from these three endpoints for RfD derivation.

The overall RfD was calculated by dividing the POD for altered behavior in three tests of nervous system function by a composite uncertainty factor (UF) of 300 to account for the extrapolation from animals to humans (10), for interindividual differences in human susceptibility (10), and for deficiencies in the toxicity database (3).

Table ES-1. Organ/system-specific RfDs and overall RfD for benzo[a]pyrene

Effect	Basis	RfD (mg/kg-d)	Confidence
Developmental	Neurobehavioral changes Gavage neurodevelopmental study in rats (postnatal days [PNDs] 5–11) Chen et al. (2012)	3×10^{-4}	Medium
Reproductive	Decreased ovarian follicles and ovary weight Gavage subchronic (60 d) reproductive toxicity study in rats Xu et al. (2010)	4×10^{-4}	Medium
Immunological	Decreased thymus weight and serum IgM Gavage subchronic (35 d) study in rats De Jong et al. (1999) and Kroese et al. (2001)	2×10^{-3}	Low
Overall RfD	**Developmental toxicity (including developmental neurotoxicity)**	3×10^{-4}	**Medium**

Confidence in the Overall Oral RfD

The overall confidence in the RfD is medium. Confidence in the principal study (Chen et al., 2012) is medium. The design, conduct, and reporting of this neurodevelopmental study was good and a wide variety of neurotoxicity endpoints were measured across 40 litters of rats. However, some uncertainty exists regarding the authors' use of dam rotation across litters (an attempt to reduce potential nurturing bias) and a within-litter dosing design, by potentially introducing maternal stress or other unanticipated consequences in the pups, and some informative experimental details were omitted, including the sensitivity of some assays at the indicated developmental ages and lack of reporting of individual animal- or gender-specific data for all outcomes. Several subchronic and developmental studies covering a wide variety of endpoints are also available; however, a multigeneration toxicity study with exposure throughout development and across generations is not available, and the available neurotoxicity studies did not comprehensively evaluate all potentially vulnerable lifestages of nervous system development. Therefore, confidence in the database is medium.

Effects Other Than Cancer Observed Following Inhalation Exposure

In animals, inhalation exposure to benzo[a]pyrene has been shown to result in developmental and reproductive toxicity. Studies in rats following inhalation exposure show decreased embryo/fetal survival and nervous system effects in offspring, and decreased testes weight and sperm counts in adult animals. Overall, the available human PAH mixtures studies report developmental and reproductive effects that are generally analogous to those observed in animals, and provide qualitative, supportive evidence for the hazards associated with benzo[a]pyrene exposure.

Inhalation Reference Concentration (RfC) for Effects Other Than Cancer

An attempt was made to derive organ- or system-specific RfCs for hazards associated with benzo[a]pyrene exposure where data were amenable (see Table ES-2). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Developmental toxicity, represented by decreased embryo/fetal survival, was chosen as the basis for the proposed inhalation RfC as the available data indicate that developmental effects represent a sensitive hazard of benzo[a]pyrene exposure. The developmental inhalation study in rats by Archibong et al. (2002) and the observed decreased embryo/fetal survival (i.e., increased resorptions) following exposure to benzo[a]pyrene on gestation days (GDs) 11–20 were used to derive the overall RfC. The lowest-observed-adverse-effect level (LOAEL) of 25 μg/m³ based on decreased embryo/fetal survival was selected as the POD. The LOAEL was adjusted to account for the discontinuous daily exposure to derive the POD_{ADJ} and the human equivalent concentration (HEC) was calculated from the POD_{ADJ} by multiplying by the regional deposited dose ratio ($RDDR_{ER}$) for extrarespiratory (i.e., systemic) effects, as described in *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b). These adjustments resulted in a POD_{HEC} of 4.6 μg/m³, which was used as the POD for RfC derivation.

The RfC was calculated by dividing the POD by a composite UF of 3,000 to account for toxicodynamic differences between animals and humans (3), interindividual differences in human susceptibility (10), LOAEL-to-no-observed-adverse-effect level (NOAEL) extrapolation (10), and deficiencies in the toxicity database (10).

Table ES-2. Organ/system-specific RfCs and overall RfC for benzo[a]pyrene

Effect	Basis	RfC (mg/m³)	Confidence
Developmental	Decreased embryo/fetal survival Developmental toxicity study in rats (GDs 11–20) Archibong et al. (2002)	2×10^{-6}	Low-medium
Reproductive	Reduced ovulation rate and ovary weight Premating study in rats (14 d) Archibong et al. (2012)	3×10^{-6}	Low-medium
Overall RfC	**Developmental toxicity**	2×10^{-6}	Low-medium

Confidence in the Overall Inhalation RfC

The overall confidence in the RfC is low-to-medium. Confidence in the principal study (Archibong et al., 2002) is medium. The conduct and reporting of this developmental inhalation study were adequate; however, a NOAEL was not identified. Confidence in the database is low due to the lack of a multigeneration toxicity study and the lack of information on varied toxicity

endpoints following subchronic and chronic inhalation exposure. However, confidence in the RfC is bolstered by consistent systemic effects observed by the oral route (including reproductive and developmental effects) and similar effects observed in human populations exposed to PAH mixtures.

Evidence for Human Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), benzo[a]pyrene is "carcinogenic to humans" based on strong and consistent evidence in animals and humans. The evidence includes an extensive number of studies demonstrating carcinogenicity in multiple animal species exposed via all routes of administration and increased cancer risks, particularly in the lung and skin, in humans exposed to different PAH mixtures containing benzo[a]pyrene. Mechanistic studies provide strong supporting evidence that links the metabolism of benzo[a]pyrene to DNA-reactive agents with key mutational events in genes that can lead to tumor development. These events include formation of specific DNA adducts and characteristic mutations in oncogenes and tumor suppressor genes that have been observed in humans exposed to PAH mixtures. This combination of human, animal, and mechanistic evidence provides the basis for characterizing benzo[a]pyrene as "carcinogenic to humans."

Quantitative Estimate of Carcinogenic Risk From Oral Exposure

Lifetime oral exposure to benzo[a]pyrene has been associated with forestomach, liver, oral cavity, jejunum or duodenum, and auditory canal tumors in male and female Wistar rats, forestomach tumors in male and female Sprague-Dawley rats, and forestomach, esophagus, tongue, and larynx tumors in female B6C3F_1 mice (male mice were not tested). Less-than-lifetime oral exposure to benzo[a]pyrene has also been associated with forestomach tumors in more than 10 additional bioassays with several strains of mice. The Kroese et al. (2001) and Beland and Culp (1998) studies were selected as the best available studies for dose-response analysis and extrapolation to lifetime cancer risk following oral exposure to benzo[a]pyrene. These studies included histological examinations for tumors in many different tissues, contained three exposure levels and controls, contained adequate numbers of animals per dose group (~50/sex/group), treated animals for up to 2 years, and included detailed reporting methods and results (including individual animal data).

Time-weighted average (TWA) daily doses were converted to human equivalent doses (HEDs) on the basis of (body weight [BW])$^{3/4}$ scaling (U.S. EPA, 1992). EPA then used the multistage-Weibull model for the derivation of the oral slope factor. This model was used because it incorporates the time at which death-with-tumor occurred and can account for differences in mortality observed between the exposure groups. Using linear extrapolation from the $BMDL_{10}$, human equivalent oral slope factors were derived for each gender/tumor site combination (slope factor = $0.1/BMDL_{10}$) reported by Kroese et al. (2001) and Beland and Culp (1998). The oral slope factor of **1 per mg/kg-day** based on the tumor response in the alimentary tract (forestomach,

esophagus, tongue, and larynx) of female B6C3F$_1$ mice (Beland and Culp, 1998) was selected as the factor with the highest value (most sensitive) among a range of slope factors derived.

Quantitative Estimate of Carcinogenic Risk From Inhalation Exposure

Inhalation exposure to benzo[a]pyrene has been associated with squamous cell neoplasia in the larynx, pharynx, trachea, nasal cavity, esophagus, and forestomach of male Syrian golden hamsters exposed for up to 130 weeks to benzo[a]pyrene condensed onto sodium chloride particles (Thyssen et al., 1981). Supportive evidence for the carcinogenicity of inhaled benzo[a]pyrene comes from additional studies with hamsters exposed to benzo[a]pyrene via intratracheal instillation. The Thyssen et al. (1981) bioassay represents the only study of lifetime exposure to inhaled benzo[a]pyrene.

A time-to-tumor dose-response model was fit to the TWA continuous exposure concentrations and the individual animal incidence data for the overall incidence of tumors in the upper respiratory tract or pharynx. The inhalation unit risk of $\mathbf{6 \times 10^{-4}\ per\ \mu g/m^3}$ was calculated by linear extrapolation (slope factor = $0.1/\text{BMCL}_{10}$) from a BMCL_{10} of $0.16\ \text{mg/m}^3$ for the occurrence of upper respiratory and upper digestive tract (forestomach) tumors in male hamsters chronically exposed by inhalation to benzo[a]pyrene (Thyssen et al., 1981).

Quantitative Estimate of Carcinogenic Risk From Dermal Exposure

Skin cancer in humans has been documented to result from occupational exposure to complex mixtures of PAHs including benzo[a]pyrene, such as coal tar, coal tar pitches, unrefined mineral oils, shale oils, and soot. In animal models, numerous dermal bioassays have demonstrated an increased incidence of skin tumors with increasing dermal exposure of benzo[a]pyrene in all species tested, although most benzo[a]pyrene bioassays have been conducted in mice.

Carcinogenicity studies in animals by the dermal route of exposure are available for benzo[a]pyrene and are supportive of the overall cancer hazard. A quantitative estimate of skin cancer risk from dermal exposure is not included in this assessment, as methodology for interspecies extrapolation of dermal toxicokinetics and carcinogenicity are still under development.

Susceptible Populations and Lifestages

Benzo[a]pyrene has been determined to be carcinogenic by a mutagenic mode of action in this assessment. According to the *Supplemental Guidance for Assessing Susceptibility from Early Life Exposure to Carcinogens* (U.S. EPA, 2005b), individuals exposed during early life to carcinogens with a mutagenic mode of action are assumed to have an increased risk for cancer. The oral slope factor of $1\ \text{per mg/kg-day}$ and inhalation unit risk of $0.0006\ \text{per } \mu\text{g/m}^3$, calculated from data applicable to adult exposures, do not reflect presumed early life susceptibility to this chemical. Although some chemical-specific data exist for benzo[a]pyrene that demonstrate increased early life susceptibility to cancer, these data were not considered sufficient to develop separate risk estimates for childhood exposure. In the absence of adequate chemical-specific data to evaluate differences in

age-specific susceptibility, the *Supplemental Guidance* (U.S. EPA, 2005b) recommends that age-dependent adjustment factors (ADAFs) be applied in estimating cancer risk. The ADAFs are 10- and 3-fold adjustments that are combined with age specific exposure estimates when estimating cancer risks from early life (<16 years of age) exposures to benzo[a]pyrene.

Regarding effects other than cancer, there are epidemiological studies that report associations between developmental effects (decreased postnatal growth, decreased head circumference, and neurodevelopmental delays), reproductive effects, and internal biomarkers of exposure to benzo[a]pyrene. Studies in animals also indicate alterations in neurological development and heightened susceptibility to reproductive effects following gestational or early postnatal exposure to benzo[a]pyrene. More preliminary data suggest that effects on cardiovascular, kidney, pulmonary, and immune system development may result from early life exposures, although few in vivo developmental studies exist to confirm these findings.

Key Issues Addressed in Assessment

The overall RfD and RfC were developed based on effects observed following exposure to benzo[a]pyrene during a critical window of development. The derivation of a general population toxicity value based on exposure during development has implications regarding the evaluation of populations exposed outside of the developmental period and the averaging of exposure to durations outside of the critical window of susceptibility. Discussion of these considerations is provided in Sections 2.1.5 and 2.2.5.

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Attachment

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United States
Environmental Protection
Agency

Office of Research and
Development
Washington, DC 20460

EPA/600/R-93/089
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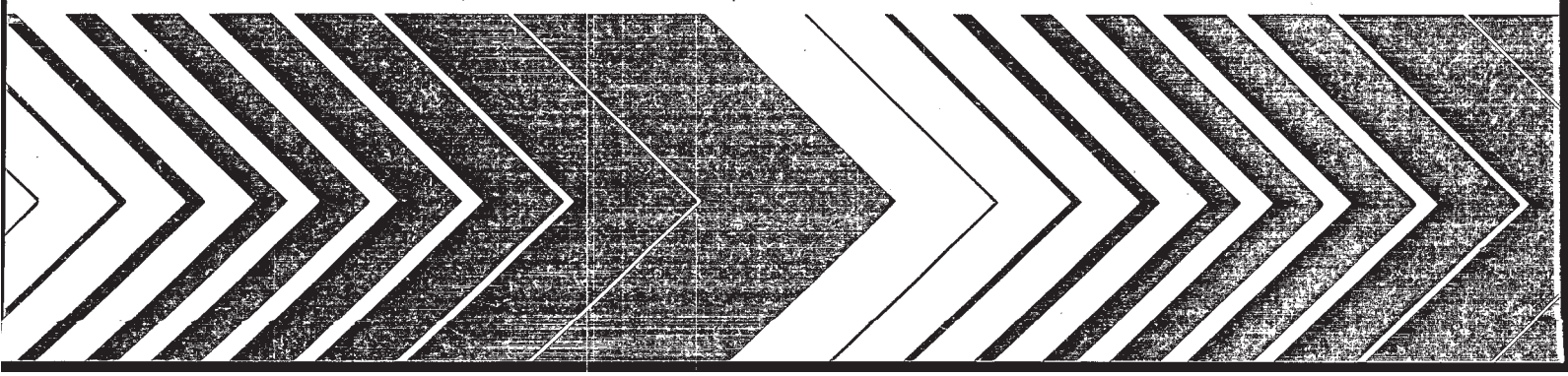
EPA

Provisional Guidance for Quantitative Risk Assessment of Polycyclic Aromatic Hydrocarbons



EPA/600/R-93/089
July 1993

PROVISIONAL GUIDANCE FOR QUANTITATIVE RISK ASSESSMENT OF POLYCYCLIC AROMATIC HYDROCARBONS

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Cincinnati, OH 45268

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PREFACE

The Office of Health and Environmental Assessment (OHEA) has prepared this Interim Guidance Document at the request of the Office of Emergency and Remedial Response. The purpose of this publication is to provide interim guidance for the quantitative risk assessment of polycyclic aromatic hydrocarbons (PAH).

For a more complete discussion of potential hazards from PAH exposure, the reader is referred to the 1992 Drinking Water Criteria Document for Polycyclic Aromatic Hydrocarbons (PAH). A literature search was not done in support of this short guidance document. A comprehensive, multimedia document for polycyclic aromatic hydrocarbons is in preparation by OHEA.

This document was prepared by Drs. Rita Schoeny and Ken Poirier, Environmental Criteria and Assessment Office, Cincinnati, OH and reviewed by Jeanette Wiltse, Office of Health and Environmental Assessment, and Drs. V. James Cogliano and Robert McGaughy, Human Health Assessment Group, OHEA, Washington, DC.

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INTRODUCTION

The Office of Health and Environmental Assessment (OHEA) recently completed an extensive document entitled "Drinking Water Criteria Document (DWCD) for Polycyclic Aromatic Hydrocarbons (PAHs)." In this document, weight-of-evidence judgments of Group B2, probable human carcinogen, are presented for seven PAHs; namely, benz[a]anthracene (BAA), benzo[b]fluoranthene (BBF), benzo[k]-fluoranthene (BKF), benzo[a]pyrene (BAP), chrysene (CHY), dibenz[a,h]anthracene (DBA), and indeno[1,2,3-cd]pyrene (IDP). All of these categorizations were found appropriate by the Carcinogen Risk Assessment Verification Endeavor (CRAVE), and files are available on the Agency's Integrated Risk Information System (IRIS) data base (U.S. EPA, 1993).

The 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986) support the calculation of quantitative risk estimates for those materials for which there is a reasonable concern for potential human health risk; for example, PAHs categorized as B2, probable human carcinogen. In the 1992 DWCD for PAHs, a quantitative risk estimate for oral exposure to BAP was given as a range of values from 4.5-9.0 per (mg/kg)/day with a geometric mean of 5.8 per (mg/kg)/day; the drinking water unit risk calculated from the mean was 1.7E-4 per (µg/L) (U.S. EPA, 1992).

NOTE: At the June 1992 meeting of the CRAVE a revised risk estimate was verified. It was noted that an error had been made in the 1991 document "Dose-Response Analysis of Ingested Benzo[a]pyrene" which is cited in the DWCD for PAHs. In the calculation of the doses in the Brune et al. (1981) study it was erroneously concluded that doses were given in units of mg/year, whereas it was in fact mg/kg/year. When the doses are corrected the slope factor is correctly calculated as 11.7 per (mg/kg)/day as opposed to 4.7 per (mg/kg)/day as reported in the DWCD. The correct range of slope factors is 4.5-11.7 per (mg/kg)/day with a geometric mean of 7.3 per (mg/kg)/day. A drinking water unit risk based on the revised slope factor is 2.1E-4 per (µg/L). These values are being changed on IRIS and an Erratum to the DWCD is being prepared.

Data were insufficient for the calculation of slope factors for any other PAHs discussed in the DWCD. While PAHs in general, and BAP in particular, are well-studied as carcinogens, data are by and large unsuitable for the calculation of quantitative risk estimates by conventional methods for one or more of the following reasons.

- Data were from exposures not typically used in deriving quantitative estimates for oral or inhalation exposure (e.g., skinpainting or subcutaneous exposure).
- Study populations were too small.
- Studies were done at only one exposure level.
- Dose-response data were not reported.

EPA quantitative risk estimates for mixtures of PAHs have often assumed that all carcinogenic PAHs are equipotent to BAP, and that the carcinogenic effect of the mixture can be estimated by the sum of the effects of each individual PAH (U.S. EPA, 1980). It has been recognized that some PAHs are less carcinogenic in animal studies than is BAP, so that application of this policy could result in an overestimation of the effect of those PAHs. On the other hand, PAH mixtures are likely to contain carcinogenic PAHs that are not considered indicator compounds and thus would not be measured. Some PAHs, moreover, have been shown to be more potent animal carcinogens than BAP.

This practice has been inconsistent; some risk assessments applied the BAP slope factor to all measured PAHs, rather than only those categorized as probable or possible human carcinogens. This would be expected to result in an overestimation of the mixture risk. Other risk assessments have used comparative potencies for PAHs published in the open literature, those cited in a contractor report to EPA (Clement Associates, 1988), or those based on ranking of PAHs presented in an Erratum to the Ambient Water Quality Criteria for PAHs (U.S. EPA, 1983).

This paper presents some comparative risk estimates for assessment of potentially carcinogenic PAHs. These are not proposed as toxicity equivalency factors

(TEF). A series of guiding criteria have been discussed for the application of a TEF to mixtures (U.S. EPA, 1991). They include the following:

1. A demonstrated need for the TEF. The PAHs meet this criterion. PAHs are found in all media; as a group they are among the most common contaminants at waste sites. PAHs are the subject of constant inquiry at the Superfund Technical Support Center. The lack of numerical estimates of risk for any PAH except BAP has had the potential for negative impacts on many risk-based regulatory decisions.
2. A well-defined group of chemicals. This criterion is also met. Any compound consisting of three or more fused aromatic rings qualifies as a PAH. At this time OHEA is limiting the definition to exclude all compounds with substituents on the ring or compounds with anything other than carbon and hydrogen in their composition. For purposes of this paper (and the Multimedia Document in preparation), only those PAHs classified as B2, probable human carcinogen, are being considered.
3. A broad base of toxicologic data. The data for PAHs are limited. Studies have, for the most part, been confined to carcinogenicity, genotoxicity and metabolism studies (generally concerned with the identification of metabolites that are genotoxic or carcinogenic). For this reason and others below, a weighting of potential potency is recommended only for carcinogenicity.
4. Consistency in the relative toxicity of congeners across toxicological endpoints, both *in vivo* and *in vitro*. As noted above there is not a broad toxicological data base. Consistency is observed among cancer bioassays in various animal models and by different routes. The point of congruency is in the generation of biologically active metabolites; if the PAH is administered to a system capable of "activating" metabolism, then tumors will be observed. If the site of administration is capable of metabolism (e.g., skin), contact point tumors will be observed. If the PAH can be absorbed and metabolized, then distant site tumors will also be observed. There are data which show that genotoxicity for individual PAHs and mixtures of PAHs are generally proportional to tumorigenicity. There are also

some limited data to indicate that immunotoxicity is roughly correlated with carcinogenic potency. Data for other noncancer effects are generally lacking but indicate that carcinogenicity is the most sensitive endpoint for PAH toxicity. The ranking of potential potency in this document is recommended only for PAH carcinogenicity.

5. Demonstrated additivity between the toxicity of individual congeners. Few studies have been reported which are an adequate test of an additivity assumption. In this regard the data bases for PAH, PCB congeners and dibenzo-p-dioxins and dibenzofurans are about of equal quality. Both additive and nonadditive effects have been observed for the carcinogenicity or genotoxicity of PAHs by various routes. Both inhibition and cocarcinogenicity have been observed for mixtures of PAHs; effects are dependent on route and proportion of materials and solvents (see U.S. EPA, 1992 for a review). It is logical to assume that in skin PAHs act as their own promoters; most B2, probable human carcinogens, in this group have been shown to be complete carcinogens in mouse skin. There have been few demonstrations that one PAH can serve as a promoter for a different PAH. According to the Guidelines for the Health Risk of Chemical Mixtures (U.S. EPA, 1986), ". . . none of the models for toxicant interactions can predict the magnitude of toxicant interactions in the absence of extensive data." The Guidelines make no recommendation as to the use of any risk model for promotion.
The guidelines further state the following:

> Based on current information, additivity assumptions are expected to yield generally neutral risk estimates (i.e., neither conservative nor lenient) and are plausible for component compounds that induce similar types of effects at the same sites of action (U.S. EPA, 1986).

A National Research Council Report (NRC, 1988) notes that a consideration of the mathematical considerations of low-dose extrapolation shows that interactions which are demonstrable at high doses will not be detectable at low doses. All of the above indicates that the use of an additivity assumption for PAHs is not

contraindicated and is consistent with the practice of the Risk Assessment Guidelines.

6. Some mechanistic rationale as to why TEFs would be applicable to a particular group of chemicals. This criterion is met for PAHs assuming that one accepts the hypothesis that mutation or some DNA change is a necessary step in carcinogenesis. All the PAHs for which ranking of potential potency is proposed can be shown both to induce tumors in animals and genetic changes (generally mutations) in some systems.
7. Some method of gaining consensus as to what TEFs ought to be. This process has not yet been undertaken for PAHs. The proposed ranking of potential potency was developed by a small group of OHEA scientists and has received only OHEA review.

In summary, not all of the guiding criteria are met for TEF. For this reason OHEA has chosen not to label the risk assessment numbers in this document a "toxicity equivalency factor" but rather an "estimated order of potential potency." It should be recognized in the application of these risk estimates that there are many limitations. First, these risk estimates are applicable only to cancer evaluation. Second, additivity of PAH response has not been proved (or refuted). Last, the estimated order of potential potency described herein is an OHEA interim recommendation and does not constitute an Agency consensus.

ESTIMATED ORDERING OF POTENTIAL POTENCIES OF PAHS

In studies of rodents, wherein BAP was assayed for carcinogenicity in conjunction with other PAHs, a range of carcinogenic potencies were observed. For example, as seen in Table 1, several PAHs were less effective in tumor induction in a mouse lung adenoma assay than was BAP at smaller or equivalent doses (LaVoie et al., 1987). Likewise, ranges of potency have been observed in many species and by different routes; for example, intrapulmonary injection in rat lungs (Table 2) and skin painting in mice (Table 3).

Inspection of these data suggest that one should be able to estimate orders of potential carcinogenic potencies for various PAHs by comparison with the activity of a standard compound. If BAP is used as the standard, then estimates of individual slope factors could be done as a percentage of the calculated slope factor for BAP. This approach could be applied to estimating the amounts of group B2 (probable human carcinogen) PAHs in a particular exposure situation and calculating their weighted contribution (by comparison to BAP) to total carcinogenic activity of the mixture.

The choice of the data set or sets to be used for estimating the potency is important, as is the modeling procedure used to provide estimates of carcinogenic activity. A discussion of various approaches is given in the DWCD (U.S. EPA, 1992).

Previous work attempted to derive relative potencies for PAHs. One derivation was done by T. Thorslund of ICF-Clement Associates on contract to U.S. EPA. An interim report (Clement Associates, 1988) is described in some detail in U.S. EPA (1992). In this report data were used from studies wherein BAP and several other PAHs were administered in the same time frame by routes including skin painting, intraperitoneal or subcutaneous injection, and lung implantation. For each study considered, a comparison was made between BAP carcinogenic activity and the activity of a particular PAH in that same report.

Two forms of dose-response models were used: either $P(d) = 1-\exp[-a(1+bd)]$; or $P(d) = 1-\exp[-a(1+bd)^2]$, where a and b are background and exposure-related parameters, respectively (Clement Associates, 1988). The first equation is simply a one-hit model, which is a special case (one-stage) of the multistage model. The second equation is a special case of the multistage model with two stages and an additional assumption that the first and second transition rates are identical relative to their respective background rates. In the application of these models it was assumed that carcinomas can develop from papillomas. For studies which reported only combined tumors or did not classify tumors, the simple form, or one-stage model was used. The two-stage model was used for data in which malignant tumors were reported separately.

In deriving the potency for each PAH relative to BAP, it was assumed that the PAHs and BAP have similar dose-response curves, but that it takes a proportionally

larger concentration of non-BAP material to induce an equivalent tumor response. The relative potency of each PAH was calculated as the ratio of the estimated transition rates with the potency of BAP indexed as 1. Point estimates (maximum likelihood estimates) were compared rather than upper bounds. An example of relative potencies from one data set is given in Table 4. In this and all subsequent tables, transition rates and relative potencies for PAHs are given as reported in Clement Associates (1988). This is to allow the reader to follow derivation of the numbers; it is acknowledged that the number of significant figures is a reflection only of the precision of numerical calculations and does not accurately transmit the degree of experimental uncertainty.

The result of all calculations based on 11 separate studies is a range of comparative potencies; the ranges reported in Clement Associates (1988) for PAHs classified as B2, probable human carcinogen, are given in Table 5.

Clement Associates (1988) selected what they considered to be the most appropriate relative potency for each PAH based on a consideration of qualitative differences in studies. Their selections are presented in Table 6. It should be noted that the application of study selection criteria other than those described in the Clement Associates (1988) report could result in the selection of different "most appropriate" relative potencies. In this context, a peer review panel convened in 1988 to review the DWCD on PAHs and felt that potencies based on the Deutsch-Wenzel et al. (1983) study would be less reliable than those based on other bioassays because of the unusual route of exposure (surgical implantation of wax pellets in the lung). Arguments for the validity of this exposure method have also been presented, however.

Other approaches for obtaining a single estimate of relative potency are feasible; for example, taking a mean, a weighted mean, or some other measure of central tendency of the individual estimates comprising the range. Calculated means are given in Table 7 as well as order of magnitude potencies based on the following rounding scheme: 0.51-5.0 = 1.0; 0.051-0.50 = 0.1; 0.0051-0.050 = 0.01.

The approach chosen here was to select a test system that provides a complete set of comparisons. Of the data sets modeled in Clement 1988, mouse skin painting

bioassays wherein PAHs were tested as complete carcinogens rather than as initiators only, meets this criterion. This data set is compiled from four reports with standard study protocols, using adequate numbers of test animals (20-36). These studies are not without deficiencies. For example, neither the Bingham and Falk (1969) paper nor Wynder and Hoffmann (1959) reported solvent control tumor incidences. Estimated orders of potential potencies based on skin painting tests as reported by Clement (1988) are given in Table 8. These are rounded to orders of magnitude using the rule presented above.

The values in Table 8 are recommended for interim use. They are based on well conducted studies using a standard, easily comparable endpoint well-known to be associated with exposure to PAHs; namely, complete carcinogenesis after repeated exposure to mouse skin. The potencies of PAH for comparison were calculated by Clement Associates (1988) using both forms of the model (one and two stages as indicated in Table 8). For this exercise no claim as to biological relevance is made for the modeling procedure; rather, it represents a convenient curve-fitting procedure, based on plausible assumptions. It is recommended that only the order of magnitude ranking be used. The quality of the data and the analysis thereof do not support any greater precision.

CONCLUSIONS

The values in Table 8 are provided for interim use. Research on relative potencies for PAHs and on the development of a TEF methodology is being undertaken by OHEA and other parts of the Agency. Areas of research include: the assumption of additivity of carcinogenic activity of PAHs; the basis for choice of studies and data sets; and the choice of modeling procedures.

In summary, a series of relative potency values (orders of magnitude) is provided as temporary guidance for the risk evaluation of PAHs. It is recognized that the list of PAHs in Table 8 is not sufficiently extensive to meet the needs of Programs and Regions; part of the continuing work on PAHs will be the consideration of the expert panel approach of ranking PAH hazards undertaken by OERR. Also in progress is

work to expand the series to include PAH for which there are animal carcinogenicity studies that did not include BAP as a positive control.

The guidance in this paper should be applied only to assessment of carcinogenic hazard from oral exposure to PAHs. There is currently no inhalation unit risk for BAP that has been found acceptable by the CRAVE. At this time, there is no basis for judgment that BAP or other PAHs will be equipotent by oral and inhalation routes. The documented effects of particulate matter and other cocarcinogens on BAP carcinogenic effects in animal lungs are confounding issues for the derivation of an inhalation unit risk for BAP and the establishment of potencies for inhalation vs. oral exposure to other PAHs.

In order to apply this guidance of relative potencies to mixtures, empirical data are needed on the additivity (or lack thereof) of carcinogenic effects of PAHs. Results of testing simple mixtures of PAHs and mixture components must be compared to assessments made from bioassays of complex environmental mixtures. Research of this nature is being undertaken by the U.S. EPA Health Effects Research Laboratory and by several research groups under contract to the Electrical Power Research Institute.

TABLE 1

Incidence of Lung Adenomas Observed in Newborn Mice for Various PAHs[a]

Treatment	Total Dose (umol)	Lung Adenomas	
		Incidence	% Response
Control[b]	0	0/35	0
Benzo[a]pyrene	1.1	23/31	74.2
Benzo[b]fluoranthene	0.5	5/32	15.6
Benzo[j]fluoranthene	1.1	15/39	38.5
Benzo[k]fluoranthene	2.1	4/34	11.8
Indeno[1,2,3-cd]pyrene	2.1	1/20	5.0

[a]Source: Adapted from LaVoie et al., 1987

[b]Dimethylsulfoxide was used as the vehicle control.

TABLE 2

Tumor Incidence in Female Osborne-Mendel Rats Administered PAHs by Intrapulmonary Injection[a]

Treatment	Total Dose (mg)	Epidermoid Carcinomas	
		Incidence	% Response
CONTROL[b]	0	0/35	0
Benzo[a]pyrene	1.0	33/35	94.3
Benzo[b]fluoranthene	1.0	9/35	25.7
Benzo[e]pyrene	5.0	1/35	2.9
Benzo[k]fluoranthene	4.15	12/27	44.4
Indeno[1,2,3-cd]pyrene	4.15	21/35	60.0
Benzo[g,h,i]perylene	4.15	4/34	11.8

[a]Source: adapted from Deutsch-Wenzel et al., 1983

[b]Neither untreated nor vehicle (beeswax and trioctanoin pellets) controls were observed to develop epidermoid carcinomas.

TABLE 3

Tumor Initiating Activity of PAHs in Female CD-1 Mouse Skin[a]

Treatment	Total Initiating Dose (µg)	Incidence	Tumor Response[b] % Response	# Tumors/ Animal
Control[c]	0	0/20	0	0
Benzo[a]pyrene	30	17/20	85.0	4.9
Benzo[b]fluoranthene	30	12/20	60.0	2.3
Benzo[j]fluoranthene	30	6/20	30.0	0.6
Benzo[k]fluoranthene	30	1/20	5.0	0.1

[a]Data from LaVoie et al., 1982. Initiating doses were applied in 10 doses, one every other day followed by applications of TPA 3 times/week for 20 weeks.

[b]Tumors were largely papillomas.

[c]Acetone was used as the vehicle control.

TABLE 4

Relative Potency Estimates for PAH Based on Skin Tumor Data[a]

Treatment	Total Dose (mg/animal)	Tumor Incidence	Estimated Transition Rate	Relative Potency[b]
DMSO	0	0/35	–	–
Acetone	0	0/36	–	–
BAP	1.7 2.8 4.6	8/43 24/35 22/36	3.92	1.0
BBF	3.4 5.6 9.2	2/38 5/34 20/37	0.656	0.167
BJF	3.4 5.6 9.2	1/38 1/35 2/38	0.241	0.241
BKF	3.4 5.6 9.2	1/39 0/38 0/38	0.078	0.020
IDP	3.4 5.6 9.2	1/35 0/37 0/37	0.081	0.021

[a]Source: Data from Habs et al. (1980); transition rates and relative potencies from Clement Assoc. (1988).

[b]Model: $P(d)=1-\exp[-a(1+bd^2)]$

TABLE 5

Summary of Relative Potency Estimates for Indicator PAHs[a]

Compound	Test System				
	Mouse Skin Carcinogenesis	Subcutaneous Injection into Mice	Intrapulmonary Administration to Rats[b]	Initiation-Promotion on Mouse Skin	Intraperitoneal Injection in Newborn Mice
Benzo[a]pyrene	1.0	1.0	1.0	1.0	1.0
Benz[a]anthracene	0.145[c]				0.057, 0.524, 0.496[d]
Benzo[b]fluoranthene	0.167[e]		0.140	0.258[f], 0.125[g]	0.232, 1.067, 0.874[h]
Benzo[k]fluoranthene	0.020[e]		0.066	0.022[f]	0.040, 0.097, 0.044[h]
Chrysene	0.0044[i]			0.040[g]	0.125, 0.33[d]
Dibenz[ah]anthracene	1.11[i]	2.82[j], 4.50[k]			
Indeno[1,2,3-cd]pyrene	0.021[e], 0.089[l]		0.232	0.074[l]	0.013[h]

[a]Where more than one potency estimate is shown, they were derived from the same study using different tumor types as endpoints. Both forms of the dose-response model in the text were used.
[b]Deutsch-Wenzel et al., 1983
[c]Bingham and Falk, 1969
[d]Wislocki et al., 1986
[e]Habs et al., 1980
[f]LaVoie et al., 1982
[g]Van Duuren et al., 1966
[h]LaVoie et al., 1987
[i]Wynder and Hoffmann, 1959
[j]Pfeiffer, 1977
[k]Bryan and Shimkin, 1943
[l]Hoffmann and Wynder, 1966

TABLE 6

Comparative Potency Estimates Based on Single Data Sets as Calculated by Clement Associates, 1988

Compound	Relative Potency	Reference
Benzo[a]pyrene	1.0	
Benzo[a]anthracene	0.145[a]	Bingham and Falk, 1969
Benzo[b]fluoranthene	0.140[a]	Deutsch-Wenzel et al., 1983
Benzo[k]fluoranthene	0.066[a]	Deutsch-Wenzel et al., 1983
Chrysene	0.0044[a]	Wynder and Hoffmann, 1959
Dibenzo[a,h]anthracene	1.11[a]	Wynder and Hoffmann, 1959
Indeno[1,2,3-cd]pyrene	0.232[b]	Deutsch-Wenzel et al., 1983

[a]Model: $P(d)=1-\exp[-a(1+bd)^2]$

[b]Model: $P(d)=1-\exp[-a(1+bd)]$

TABLE 7

Ranges and Combined Potencies for Seven PAHs*

Compound	Range	Potency Relative to BAP		
		Simple Mean	Geometric Mean	Order of Magnitude
Benzo[a]pyrene	–	–	–	1.0
Benzo[a]anthracene	0.057-0.524	0.31	0.22	0.1
Benzo[b]fluoranthene	0.125-1.067	0.41	0.29	0.1
Benzo[k]fluoranthene	0.020-0.097	0.05	0.04	0.01
Chrysene	0.0044-0.33	0.12	0.05	0.01
Dibenz[a,h]anthracene	1.11-4.5	2.81	2.42	1.0
Indeno[1,2,3-cd]pyrene	0.013-0.232	0.08	0.08	0.1

*Relative potencies given in the range are from Clement Associates, 1988. Both forms of the dose-response model in the text were used.

TABLE 8

Estimated Order of Potential Potencies of Selected PAH Based on Mouse Skin Carcinogenesis

Compound	Relative Potency[a]		Reference
Benzo[a]pyrene	1.0	1.0	
Benz[a]anthracene	0.145	0.1	Bingham and Falk, 1969
Benzo[b]fluoranthene	0.167	0.1	Habs et al., 1980
Benzo[k]fluoranthene	0.020	0.01	Habs et al., 1980
Chrysene	0.0044	0.001	Wynder and Hoffmann, 1959
Dibenz[a,h]anthracene	1.11	1.0	Wynder and Hoffmann, 1959
Indeno[1,2,3-cd]pyrene	0.055[b]	0.1	Habs et al., 1980; Hoffmann and Wynder, 1966

[a]Model was $P(d)=1-\exp[-a(1+bd)^2]$ for all but indeno[1,2,3-cd]pyrene

[b]Simple mean of relative potencies (0.021 and 0.089) the latter of which was derived using the one-hit model.

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TECHNICAL SUPPORT DOCUMENT FOR CANCER POTENCY FACTORS

APPENDIX B.

Chemical-specific summaries of the information used to derive unit risk and cancer potency values.

ACETALDEHYDE

CAS No: 75-07-0

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	44.05
Boiling point	20.5° C
Vapor pressure	740 mm
Conversion factor	1 ppm = 1.8 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor	2.7 E-6 $(\mu g/m^3)^{-1}$
Slope Factor	1.0 E-2 $(mg/kg\text{-}day)^{-1}$

[Calculated from rat nasal tumor incidence data (Wouterson *et al.*, 1986) by OEHHA (1993) using a linearized, time-dependent multistage procedure.]

III. CARCINOGENIC EFFECTS

Human Studies

Bittersohl (1974) conducted a morbidity survey to study the incidence of total cancer in an aldol and aliphatic aldehyde factory in the German Democratic Republic (GDR). The work force in this factory was potentially exposed to a product primarily consisting of acetaldol (70)% combined with smaller, but variable, amounts of acetaldehyde; butylaldehyde; crotonaldehyde; "large" condensed aldehydes such as hexatrial, hexatetral, and ethylhexal; traces of acrolein; and 20 to 22% water. The observation period extended from 1967 to 1972. The study cohort consisted of 220 people, approximately 150 were employed for more than 20 years. Acetaldehyde concentrations were found to range from 0.56 to 1 ppm.

Nine cases of cancer (five squamous cell carcinomas of the bronchi, two squamous cell carcinomas of the mouth cavity, one adenocarcinoma of the stomach, and one adenocarcinoma of the cecum) were identified in male workers during the 6-year study period. An incidence rate of 6,000 per 100,000 population (9 cases/150 individuals employed for more than 20 years) for total cancer was calculated for this study cohort, compared to 1,200 per 100,000 in the general population. All cases had a history of smoking.

This study had the following major methodological limitations: the incidence rate was not age adjusted; concurrent exposure to other chemicals and cigarette smoke occurred; duration of exposure was short; a small number of subjects was studied; and information on subject selection, age, and sex distribution was lacking. Because of the limitations, the International Agency for Research on Cancer (IARC, 1985) considered this study to be inadequate to evaluate the carcinogenicity of acetaldehyde.

Animal Studies

Rats

Woutersen *et al.* (1984, 1986) exposed groups of 105 male and female SPF-Wistar rats to atmospheres containing acetaldehyde concentrations of 0, 750, 1500, or 3000/1000 ppm (0, 1350, 2700 or 5400/1800 mg/m^3, respectively), 6 hours/day, 5 days/week for up to 28 months. The highest concentration was gradually decreased from 3000 ppm (days 0 to 141) to 1000 ppm (from day 313 forward) because of severe growth retardation, loss of body weight, and early mortality.

Treatment-related nonneoplastic histopathological lesions were observed in the nose, larynx, and lungs, with the most severe lesions seen in the nose and in the vocal cord region of the larynx. Nasal tumors observed were mainly squamous cell carcinomas and adenocarcinomas originating from the respiratory and olfactory epithelium, respectively. Tumor incidences are listed in Table 1. The incidences of adenocarcinomas were significantly ($p < 0.01$) higher in both sexes of rats at all exposure concentrations when compared to controls. Squamous cell carcinomas were significantly ($p < 0.01$) increased in males in the mid- and high-dose groups and in females in the high-dose group. No laryngeal or lung tumors were seen in male rats and tumors observed in the other organs of treated rats were comparable to those in the controls. The presence of nasal tumors at all exposure levels suggested that the latency period for nasal tumor induction was independent of the acetaldehyde concentration. The authors concluded that under the conditions of this study, acetaldehyde was carcinogenic to the nasal mucosa of rats.

Table 1: Nasal tumor incidence in male and female Wistar rats exposed to acetaldehyde by inhalation (Woutersen *et al.*, 1986)

Sex	Exposure Concentration (ppm)	Nasal Tumor Incidence
males	0	1/49
	750	17/52
	1500	41/53
females	0	0/50
	750	6/48
	1500	36/53

In an extension of the above study, Woutersen and Feron (1987) examined the process of regeneration of damaged nasal mucosa in rats exposed to acetaldehyde at concentrations as described above for 52 weeks. Animals were sacrificed after a recovery period of 26 weeks. The number of nasal tumors observed was almost the same as in the lifetime study, which indicated that proliferative epithelial lesions of the nose may develop into tumors even without continued acetaldehyde exposure.

Hamsters

Feron *et al.* (1982) exposed groups of male and female Syrian golden hamsters to room air (0 ppm) or to decreasing concentrations of acetaldehyde. The initial concentration was 2500 ppm (4500 mg/m^3), which was gradually decreased (between weeks 9 and 44) to 1650 ppm (2970 mg/m^3) 6 hours/day, 5 days/week for 52 weeks. Acetaldehyde-induced nonneoplastic lesions were seen in the nose, larynx, and trachea. Tumors were seen in both the nose (adenoma, adenocarcinoma, and anaplastic carcinoma) and the larynx (carcinoma *in situ*, squamous cell carcinoma, and adenosquamous carcinoma). The tumor incidences were 2/27 (7%) and 6/23 (26%) in males and 1/26 (4%) and 4/26 (20%) in females for the nose and larynx, respectively; no nasal or laryngeal tumors were observed in the controls. Only the increases in laryngeal tumors were statistically significant ($p < 0.05$) compared to controls. Under the conditions of this study, acetaldehyde was considered to be carcinogenic in male and female hamsters.

In a second part of the above study (Feron, 1979), groups of male Syrian golden hamsters were exposed by inhalation to 0 or 1500 ppm (2700 mg/m^3) acetaldehyde vapor 7 hours/day, 5 days/week for 52 weeks. The animals also received a concurrent, weekly intratracheal instillation of 0, 0.625, 0.125, 0.225, 0.5, or 1 mg benzo[a]pyrene (BaP) in saline for the same duration. Simultaneous exposure to acetaldehyde and BaP induced marked nonneoplastic lesions in the nasal cavity and trachea which disappeared after the 26-week recovery period. No respiratory tract tumors were seen in hamsters exposed to acetaldehyde alone. Various types of benign and malignant respiratory tract tumors were found in male hamsters treated with BaP or BaP plus acetaldehyde. The results of this study indicated no evidence for carcinogenicity of acetaldehyde and limited evidence of co-carcinogenicity. This study had a number of methodological limitations such as the exposure level exceeding the maximum tolerated dose (MTD).

Feron *et al.* (1982) repeated the above study using male and female Syrian golden hamsters. Some of the animals were also treated with subcutaneous injections of 0.0625% diethylnitrosamine (DENA) once every 3 weeks. The enhancing effect of BaP-initiated respiratory tract tumor formation observed in this study was similar to that observed in the previous study (Feron, 1979). There was no evidence that acetaldehyde exposure increased the incidence or affected the type of DENA-induced tumors in any part of the respiratory tract. Based upon these findings, the authors concluded: "acetaldehyde is an irritant as well as a carcinogen to the nose and larynx with a weak initiating and a strong 'promoting' (co-carcinogenic) activity."

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The IARC concluded that there is inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde (IARC, 1985). Therefore IARC classified acetaldehyde as class 2B, a possible human carcinogen. The U.S. EPA, using the guidelines for Carcinogen Risk Assessment, has classified acetaldehyde as a Group B2 probable human carcinogen, based on sufficient evidence of carcinogenicity in animals and inadequate evidence in humans (IRIS, 1997). OEHHA staff concurred that acetaldehyde is a potential human carcinogen.

OEHHA staff have used the rat nasal tumor data from the Woutersen *et al.* (1986) inhalation study and hamster laryngeal tumor data from the Feron *et al.* (1982) inhalation study to assess the cancer potency with the multistage model. Acetaldehyde tumors occurred in the nasal area for rats and in the larynx for hamsters. While it is assumed that the respiratory tract is the only organ affected by acetaldehyde, tumors in the nose in rats and in the upper larynx of hamsters do not directly mean that only tumors in the nose or larynx would occur in humans. Unlike the rodents, humans are not obligate nose breathers. Thus, the entire human respiratory tract, including the lung, may be at risk for cancer induction by acetaldehyde.

Methodology

Data from the Woutersen *et al.* (1986) inhalation study were used to calculate cancer risk from the male and female rat nasal carcinoma incidence. Three types of nasal tumors were observed: squamous cell carcinomas, adenocarcinomas, and carcinomas *in situ*. OEHHA staff used only 49-53 (out of 55) animals of each group that were examined for nasal changes in the quantitative risk assessment. Doses were converted to an equivalent continuous dose (US EPA, 1987; IRIS, 1997), because the animals were exposed for only 6 hours/day, 5 days/week. Because of the excessive morbidity with the animals exposed at the highest concentration (3000 ppm) and the eventual lowering of the concentration to 1000 ppm, data from this group were not used in deriving the cancer potency.

Using the computer program GLOBAL86 (Howe *et al.*, 1986), a linearized, time-independent multistage procedure was fit to the nasal carcinoma dose-response data. The male rat nasal tumor data yielded a maximum likelihood estimate (MLE) for q_1 of 1.6×10^{-8} ppb^{-1}, and an Upper 95% Confidence Limit (UCL) on q_1 (q_1*) of 3.2×10^{-6} ppb^{-1}. The female rat data yielded a somewhat lower risk with a q_1* equal to 9.3×10^{-7} ppb^{-1}. For acetaldehyde, 1 ppb = 1.8 $\mu g/m^3$. Using the latter units, the MLE for q_1 equals 8.8×10^{-9} $(\mu g/m^3)^{-1}$ and the 95% UCL for q_1 (q_1*) equals 1.8×10^{-6} $(\mu g/m^3)^{-1}$ for the male rat data.

The following scaling factors are used for scaling from animals to humans: 1.5 for the 400 g male rat and 1.6 for the 250 g female rat, assuming 70 kg body weight for both human sexes. Using these scaling factors, risk values of 1.6×10^{-6} ppb^{-1} from female rat data and 4.8×10^{-6} ppb^{-1} from male rat data were obtained.

For rat nasal carcinomas, contact scaling factors were obtained: 5.6 for a 400 g male rat and 6.5 for a 250 g female rat, again assuming both human sexes have 70 kg body mass. The resultant risks of 6.3×10^{-6} ppb^{-1} for female rats and 2.7×10^{-5} ppb^{-1} for male rats would be used only to predict nasal or respiratory system cancers.

In the case of acetaldehyde, a best value of 2.7×10^{-6} $(\mu g/m^3)^{-1}$ (4.8×10^{-6} ppb^{-1}) was chosen from the range. The value was obtained from the male rat (more sensitive to tumor induction than the female rat) data using an interspecies surface area correction factor of body weight to the 2/3 power.

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ACETAMIDE

CAS No: 60-35-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	59.07
Boiling point	222 °C
Melting point	81 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 2.416 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.0 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 7.0 E-2 $(mg/kg\text{-}day)^{-1}$
[Male Fischer 344 rat liver tumor data (Fleischman *et* al., 1980), contained in Gold *et al.* database (1990), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of acetamide on humans are known to exist.

Animal Studies

Dessau and Jackson (1955) exposed 5 Rockland albino rats to acetamide by gavage (4 g/kg body weight/day in distilled water) for 205 days. One animal developed an hepatocellular adenoma.

Male Wistar rats (25/group) were fed diet containing 0, 1.25, 2.5 or 5% acetamide for 1 year (Jackson and Dessau, 1961). One rat/group was sacrificed at monthly intervals; the remaining animals were sacrificed at 1 year. Liver tumors (described as trabecular carcinomas or adenocarcinomas) were seen in 0/25, 4/24, 6/22 and 1/18 animals from the control, low, medium and high dose groups. In the same study, a group of 50 male Wistar rats were fed a diet containing 5% acetamide for 1 year. One animal was killed weekly for 26 weeks, after which 1 animal was killed every other week. Liver tumors (trabecular carcinomas or adenocarcinomas) were observed in 4/48 animals treated for 38-42 weeks, compared to 0/43 in controls.

Male Wistar rats were fed control diet (15 animals), or diets containing 2.5% acetamide (40 animals), 2.5% acetamide + 5.6% L-arginine L-glutamate (40 animals), or 5.6% L-arginine L-glutamate (15 animals) for 1 year. Hepatomas were observed in 2/8 animals fed acetamide alone and killed after 1 year; 7/16 animals fed acetamide alone for 1 year followed by control diet for 3 months also developed liver tumors. In contrast, 1/11 animals fed diet containing acetamide + L-arginine L-glutamate for 1 year developed hepatomas, and 1/19 animals fed diet containing

acetamide + L-arginine L-glutamate for 1 year followed by control diet for 3 months developed hyperplastic liver nodules, but not tumors. No liver tumors were noted in either the control or 5.6% L-arginine L-glutamate treatment groups (Weisburger *et al.*, 1969).

Fleischman *et al.*(1980) fed male and female C57BL/6 mice (50/sex/group) and Fischer 344 rats (50/sex/group) a diet containing 1.18% (mice) or 2.36% (mice, rats) acetamide for 365 consecutive days; animals were then fed a control diet for an additional 4 months. Male mice demonstrated a treatment-related increase in hematopoietic tumors, primarily malignant lymphomas; tumor incidence was 7/50 and 7/46 for the low and high dose groups, respectively, compared to 0/95 for the pooled (male and female) control group. Neoplastic nodules and hepatocellular carcinomas were observed in both male and female rats. However, the incidence, speed of onset and frequency of metastases were greater in males (Fleischman *et al.*, 1980). No liver tumors were noted in control animals. Incidence data for hepatocellular carcinomas in F344 rats, the most sensitive species tested, are given in Table 1.

Table 1: Incidence of hepatocellular carcinomas in F344 rats treated with acetamide by dietary administration (Fleischman *et al.*, 1980).

Dietary Concentration (%)	Average Dose[1] (mg/kg-day)	Tumor Incidence[2]	
		Male	Female
0	0	0/50	0/49
2.36	710	41/47	33/48

1. Doses as reported by Gold *et al*. (1984).

2. Decreased survival of treatment group according to Gold *et al.* (1990) (56% survival at study termination compared to 86% for controls); potency may be an underestimate (Cal/EPA, 1992).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The carcinogenicity bioassay by Fleischman *et al*. (1980) indicated that acetamide causes hematopoietic tumors in male C57BL/6 mice, and hepatocellular carcinomas in male and female Fischer 344 rats. Rats were more sensitive than mice, and male rats were more sensitive than female rats in this study; therefore, the male Fischer 344 rat liver tumor data was used as the basis of a cancer potency factor.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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ACRYLAMIDE

CAS No: 79-06-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	71.08
Boiling point	125°C at 25 mm Hg
Melting point	84.5
Vapor pressure	0.007 mm Hg at 25°C
Air concentration conversion	1 ppm = 2.91 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.3 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 4.5 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated by US EPA/IRIS (1988, 1993) from female Fischer 344 rat tumor data (central nervous system, mammary and thyroid glands, uterus, oral cavity) (Johnson *et al*., 1986) using a linearized multistage procedure, extra risk; adopted by CDHS/RCHAS (1990).]

III. CARCINOGENIC EFFECTS

Human Studies

US EPA (1993) reviewed a study of cancer mortality in workers exposed to acrylamide by Collins (1984). Data from a long duration exposure group (10 individuals) and a short duration/intermittent exposure group (52 individuals) was analyzed using a standardized proportional mortality ratio (SPMR) procedure. No excess mortality for all types of cancer combined was noted in either group. Mortality from lung and central nervous system cancer appeared to be slightly elevated. However, the SPMRs were not significantly different from expected values, due to small group size. US EPA (1993) also noted additional study limitations including underrepresentation of the potential at-risk worker population, incomplete cause of death ascertainment, and incomplete exposure data.

Sobel *et al*. (1986) studied the mortality experience of 371 workers (365 white males, 6 white females) employed in acrylamide monomer production and polymerization operations at the Michigan Division of the Dow Chemical Company from 1955 through 1979. Vital status followup was performed from the date of the first potential exposure to December 31, 1982. Mortality comparisons were made between the cohort and United States white male mortality rates; comparisons were made with a subcohort of workers previously exposed to organic dyes both included and excluded. Slight excesses of mortality from all cancers (11 observed/7.9 expected), digestive tract cancer (4 observed/1.9 expected) and respiratory tract cancer (4 observed/2.9 expected) were observed in the total cohort; these excesses were not observed when the organic dye exposure subcohort was excluded. The authors concluded that the study did not support a relationship between acrylamide exposure and general or specific cancer mortality. However, US EPA (1988) considers this study insufficient to assess the carcinogenicity of acrylamide in humans

because of small cohort size, multiple chemical exposures, limited followup, and short exposure duration (167 cohort members had < 1 year of employment; 109 had 1-4 years of employment).

Animal Studies

Bull *et al*. (1984a) exposed female Sencar mice and male and female A/J mice to acrylamide. Female Sencar mice (40/treatment group) were exposed to 0, 12.5, 25.0 or 50.0 mg/kg body weight acrylamide by gavage, intraperitoneal injection or dermal application. Doses were administered 6 times over a 2 week period; total doses were 0, 75, 150 and 300 mg/kg. Acrylamide was dissolved in distilled water for gavage and intraperitoneal injection administration, and in ethanol for dermal application. Two weeks after the cessation of acrylamide exposure, 1.0 µg 12-*O*-tetradecanoyl-phenol-13-acetate (TPA) dissolved in 0.2 ml acetone was applied to the shaved back of each animal 3 times/week for 20 weeks. A promotion control group was included which received 300 mg/kg acrylamide followed by dermal applications of 0.2 ml acetone on the same treatment schedule and duration as the animals receiving TPA. All animals were sacrificed at 52 weeks, and were evaluated for the presence of skin tumors. Male and female A/J mouse (40/sex/treatment group) acrylamide exposures were conducted at laboratories of the US EPA (Cincinnati, OH) and the Medical College of Ohio (Toledo, OH) (MCO) . Animals exposed at US EPA received acrylamide dissolved in distilled water by gavage 3 times/week for 8 weeks at doses of 0, 6.25, 12.5 or 25 mg/kg. Animals exposed at MCO initially received acrylamide by intraperitoneal injection 3 times/week for 8 weeks at doses of 0, 1, 3, 10, 30 or 60 mg/kg; however, peripheral neuropathy and decreased survival forced treatment termination on the 60 mg/kg group after the 11th injection. An untreated control group was also included. Animals were sacrificed after either 7 months (US EPA) or 6 months (MCO) and examined for lung adenomas. Acrylamide induced skin tumors (squamous cell papillomas and carcinomas) in TPA-promoted female Sencar mice in a dose-dependent manner when administered by gavage, intraperitoneal injection or dermal application. Acrylamide did not induce skin tumors by any route of administration in animals not receiving TPA. Tumor incidence data from female Sencar mice exposed to acrylamide are listed in Table 1.

The incidence of lung adenomas in both male and female A/J mice exposed to acrylamide by either gavage or intraperitoneal injection was significantly increased in a dose-related manner (Bull *et al*., 1984a). Tumor incidence data for animals treated by intraperitoneal injection is listed in Table 2; numerical tumor incidence data for animals exposed to acrylamide by gavage was not listed.

Acrylamide dissolved in water was administered by gavage (0, 75, 150 or 200 mg/kg body weight, divided into 6 equal portions) to female ICR-Swiss mice (40 animals/treatment group) over a 2 week period (Bull *et al*., 1984b). Two weeks after the last acrylamide exposure, the animals were exposed 3 times/week to dermal applications of 2.5 µg TPA for 20 weeks. Another group of 20 animals were exposed to a total dose of 300 mg/kg acrylamide, but received dermal applications of acetone alone. All animals were sacrificed after 52 weeks. Acrylamide caused a significant dose-related increase in the incidence of skin tumors (papillomas and carcinomas combined). The incidence in animals also receiving TPA was 0/35, 4/34, 4/32 and 13/32 (number of animals with tumors/number of animals examined) for the control, low, mid and high dose groups, respectively; the skin tumor incidence in animals receiving 300 mg/kg acrylamide but not TPA was 10/36. Acrylamide-treated animals also demonstrated a significant dose-related increase in the incidence

of lung tumors (alveolar and bronchiolar adenomas and carcinomas). The incidence in animals also receiving TPA was 4/36, 8/34, 6/36 and 11/34 for the control, low, mid and high dose groups, respectively; the lung tumor incidence in animals receiving 300 mg/kg acrylamide but not TPA was 14/36.

Table 1. Skin tumor (squamous cell papillomas and carcinomas) incidence in female Sencar mice exposed to acrylamide (Bull *et al*., 1984a)

Total administered dose[1] (mg/kg body weight)	Route of administration	TPA[2]	Tumor incidence
0	gavage	+	2/40
75		+	12/40
100		+	23/40
300		+	30/40
300		-	0/20
0	intraperitoneal injection	+	0/40
75		+	10/40
100		+	13/40
300		+	21/40
300		-	0/20
0	dermal	+	7/40
75		+	4/40
100		+	11/40
300		+	18/40
300		-	0/20

1. The exposure duration was less than lifetime (2 weeks); the total administered dose listed was not adjusted to reflect a less-than-lifetime exposure.
2. TPA = 12-*O*-tetradecanoyl-phenol-13-acetate

Table 2. Lung adenoma incidence in male and female A/J mice exposed to acrylamide by intraperitoneal injection (Bull *et al*., 1984a)

Dose level[1] (mg/kg body weight)	Percent of animals with tumors	
	males	females
0	13	8
1	50	35
3	38	53
10	59	79
30	93	93

1. The exposure duration was less than lifetime (8 weeks); the dose level listed was not adjusted to reflect a less-than-lifetime exposure.

Robinson *et al*. (1986) exposed female SENCAR, BALB/c, A/J and ICR-Swiss mice (60 mice/strain/treatment group) to a single 50 mg/kg body weight dose of acrylamide by intraperitoneal injection; 2 days later 40 of the 60 mice in each treatment group received 1.0 µg (SENCAR), 2.5 µg (A/J and ICR-Swiss) or 5.0 µg (BALB/c) TPA in 0.2 ml acetone applied dermally 3 times/week for 20 weeks. The remaining 20 mice/strain/treatment group received acetone alone for the same treatment schedule and duration. All animals were sacrificed at 40 weeks, and were only examined for the number of skin papillomas and lung adenomas/animal. Acrylamide induced a significant increase in the number of skin papillomas and lung adenomas per animal in SENCAR mice receiving TPA treatment. The total number of animals bearing tumors was not listed. No significant increase in either tumor type was noted in the other mouse strains tested; tumor data for the animals receiving acrylamide but not TPA was not reported.

Male and female Fischer 344 rats (90/sex/treatment group) were exposed to acrylamide in drinking water for 2 years (Johnson *et al*., 1986). Acrylamide water concentrations were adjusted to provide dosages of 0, 0.01, 0.1, 0.5 or 2 mg/kg body weight/day. Interim sacrifices (10 animals/sex/treatment group) were performed at 6, 12 and 18 months. A maximum tolerated dose (MTD) was achieved based on decreased weight gain, increased mortality during the last 4 months of the study and the appearance of several toxic effects (including peripheral nerve degeneration) in the 2 mg/kg/day group. Increases in the incidences of a number of tumor types were observed in the 2.0 mg/kg/day exposure group animals. An increased incidence of thyroid gland-follicular epithelium tumors was observed in both males and females. In females, increased tumor incidences were noted in the mammary glands, central nervous system, oral tissues, uterus and clitoral gland. An increased incidence of scrotal mesothelioma was noted in males, in both the 2.0 and 0.5 mg/kg/day exposure group; additionally, although not statistically significant, the incidence of scrotal mesothelioma in the 0.1 mg/kg/day group was greater than either the control group or historical control incidences. Male rats in the 2.0 mg/kg/day exposure group also had a significant increase in adrenal pheochromocytomas, and an increased incidence of central nervous system tumors when compared to historical controls but not when compared to concurrent controls. Tumor incidence data is listed in Table 3.

Table 3. Acrylamide-induced tumor incidences in male and female Fischer 344 rats (Johnson *et al*., 1986)

Administered dose (mg/kg/day)	Human equivalent dose[1] (mg/kg/day)	Tumor type	Tumor incidence	
			males	females
0	0	combined central nervous	NA	13/60
0.01	0.001	system (CNS), mammary	NA	18/60
0.1	0.015	gland, oral cavity, thyroid	NA	14/60
0.5	0.076	gland, uterus[2]	NA	21/60
2.0	0.305		NA	46/60
0	0	adrenal pheochromacytomas[3]	3/60	NA
0.01	0.001		7/60	NA
0.1	0.015		7/60	NA
0.5	0.076		5/60	NA
2.0	0.305		10/60	NA
0	0	central nervous system[4]	5/60	1/60
0.01	0.001		2/60	2/60
0.1	0.015		0/60	1/60
0.5	0.076		3/60	1/60
2.0	0.305		8/60	9/60
0	0	oral cavity[5]	6/60	0/60
0.01	0.001		7/60	3/60
0.1	0.015		1/60	2/60
0.5	0.076		5/60	3/60
2.0	0.305		6/60	8/60
0	0	mammary gland[6]	NA	2/60
0.01	0.001		NA	2/60
0.1	0.015		NA	1/60
0.5	0.076		NA	5/58
2.0	0.305		NA	8/61
0	0	scrotal mesotheliomia	3/60	NA
0.01	0.001		0/60	NA
0.1	0.015		7/60	NA
0.5	0.076		11/60	NA
2.0	0.305		10/60	NA
0	0	thyroid[7]	1/60	1/58
0.01	0.001		0/58	0/59
0.1	0.015		2/59	1/59
0.5	0.076		1/59	1/58
2.0	0.305		7/59	5/60
0	0	uterine adenocarcinomas	NA	1/60
0.01	0.001		NA	2/60
0.1	0.015		NA	1/60
0.5	0.076		NA	0/59
2.0	0.305		NA	5/60

Table 3 (continued). Acrylamide-induced tumor incidences in male and female Fischer 344 rats (Johnson *et al*., 1986)

1, 2. As calculated by US EPA (1988).
3. Benign and malignant.
4. Tumors of glial origin or glial proliferation suggestive of early tumor.
5. Squamous cell papillomas and carcinomas.
6. Adenomas and adenocarcinomas.
7. Males: follicular adenomas; females: follicular adenomas and adenocarcinomas.
NA not available

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The studies by Bull *et al*. (1984a, 1984b), Robinson *et al*. (1986) and Johnson *et al*. (1986) indicate that acrylamide is capable of acting as both an initiator and a complete carcinogen in animals. However, only the Johnson *et al*. (1986) study contained a data set suitable for generating a cancer potency factor. Female Sencar mice developing tumors after exposure to acrylamide in the study by Bull *et al*. (1984a) were also additionally exposed to TPA; animals not exposed to TPA did not develop skin tumors. Female A/J mice exposed in that study to acrylamide by either gavage or intraperitoneal injection developed an increased incidence of lung adenomas without requiring TPA exposure. However, the animals were not evaluated for tumor types other than lung adenomas, and numerical tumor incidence data for animals exposed to acrylamide by gavage was not listed. Also, the exposure and observation durations for animals exposed by gavage (8 weeks and 7 months, respectively) and by intraperitoneal injection (8 weeks and 6 months, respectively) were short. Female ICR-Swiss mice exposed to acrylamide by gavage in the study by Bull *et al*. (1984b) were generally also exposed to TPA; only one exposure group was included which received acrylamide (300 mg/kg) but not TPA. Additionally, the exposure duration was only 2 weeks and the exposure duration was less than lifetime (52 weeks). In the study by Robinson *et al*. (1986), all animals for which tumor incidence data was reported were exposed to TPA as well as acrylamide. Animals in the Johnson *et al*. (1986) study were exposed to acrylamide alone for the lifetime of the animals, and were comprehensively examined for tumors. For these reasons, tumor incidence data from the Johnson *et al*. (1986) study was used to derive a cancer potency factor for acrylamide.

Methodology

As recommended in the US EPA Guidelines for Carcinogen Risk Assessment (1986), US EPA (1988) pooled tumor incidence data from different tumor sites, under the consideration that risk numbers derived from site-specific tumor incidence data potentially may not be predictive of, and may in fact underestimate, "whole-body" risks that are determined using the pooled individual animal data. The dose-response curves for each sex based on the pooled tumor incidence (benign and malignant) constituted the data sets of choice for risk assessment. Tumors at a particular site were added into the pool only when the tumor site had statistically significantly increased incidence at least at the high dose level (treated vs. control). The female rat was considered to be

the more sensitive sex, as there were significantly increased tumor incidences at a greater number of sites than in the males; the female rat tumor data was therefore used as the basis of a risk estimate. A linearized multistage procedure (GLOBAL 83) was used to calculate a cancer potency factor (q_1^*) from the female rat tumor incidence data. Surface area scaling was employed to transform animal cancer potency factors to human cancer potency factors, using the relationship ($q_{human} = q_{animal} * (bw_h / bw_a)^{1/3}$), where q_{human} is the human potency, q_{animal} is the animal potency, and bw_h and bw_a are the human and animal body weights, respectively. Body weight values used for humans and rats were 70 kg and 0.2 kg, respectively. No exposure route adjustment was made to the risk estimates because data exists which indicates that the pharmacokinetics and tissue distribution of acrylamide were not significantly affected by the dose administered or the route of administration (Dearfield *et al*., 1988). US EPA calculated a cancer potency value (q_{human}) of 4.5 E+0 $(mg/kg\text{-}day)^{-1}$. A unit risk factor was then calculated from the cancer potency factor by OEHHA/ATES using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day. The unit risk should not be used if the air concentration exceeds 8 $\mu g/m^3$, as above this concentration the unit risk may not be appropriate.

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ACRYLONITRILE

CAS No: 107-13-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	53.06
Boiling point	77.3°C
Melting point	-82°C
Vapor pressure	100 mm Hg at 23°C
Air concentration conversion	1 ppm = 2.2 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.9 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.0 E+0 $(mg/kg\text{-}day)^{-1}$
[Human respiratory tract cancer incidence data (O'Berg, 1980), relative risk model (US EPA, 1983), reevaluated by CDHS/RCHAS (1988).]

III. CARCINOGENIC EFFECTS

Human Studies

Cancer incidence and mortality in a cohort of 1345 male workers exposed to acrylonitrile at a E.I. du Pont de Nemours and Co., Inc. textile plant in Camden SC was studied by O'Berg (1980). The study cohort was identified as having had potential exposure to acrylonitrile in the period between plant startup in 1950 and 1966. The 1966 cutoff date allowed for a 10-year follow-up through the end of 1976. Worker exposure levels were assessed qualitatively by the author and a committee of six DuPont employees with long-term experience in the acrylonitrile exposure area; plant environmental monitoring data was not available for the 1950-1966 period. High, moderate and low exposure categories were established. The U.S. Environmental Protection Agency (US EPA) (1983) noted that DuPont representatives agreed that 5, 10 and 20 ppm might be used to represent the low medium and high exposure classification levels. Expected numbers of cancer cases and deaths were calculated from both DuPont corporate data and from the 1969-1971 National Cancer Institute survey data set. However, the author only listed the results of cancer incidence and mortality calculations using the DuPont "control" data set as the source of the expected numbers of cancer cases and mortality; US EPA (1983) stated that the presentation of results based on this control cohort only would ignore the possible effects of other chemicals on the company control cohort. Exposed workers demonstrated 25 cases of all types of cancer, with 20.5 expected. Of these cases, 8 were lung cancer versus 4.4 expected. For workers employed during plant startup (1950-1952) and exposed for at least 6 months, 8 cases of lung cancer were noted vs. 2.6 expected ($p < 0.01$). Most of this excess occurred during the latest followup period (1970-1976) (6 cases of lung cancer vs. 1.5 expected, $p < 0.01$). Total cancer cases in this period were also significantly increased (17 observed, 5.6 expected, $p < 0.01$). Also, a trend was observed correlating increased cancer risk with increased severity of exposure. Workers in the moderate exposure group with a

probable latent period of at least 15 years exhibited 13 cases of cancer of all types vs. 5.5 expected, and 5 cases of lung cancer vs. 1.4 expected ($p < 0.05$).

One potential confounding factor in this study is the lack of controls for tobacco smoking. US EPA (1983) investigated the possible impact of smoking on lung cancer incidence in the O'Berg (1980) study. DuPont provided additional data on 32 of 36 cancer cases reported on in the plant under study (some cases were not in the study cohort) and on smoking data for a matched group of non-cancer cases. Of the 32 cancer cases for which smoking data was available, 22 were cancer types other than lung, and 16 of the non-lung cancer cases (73%) were smokers. Of the matched noncancer controls, 25 (69%) were smokers. US EPA estimated that 70% of the plant population were smokers and 30% nonsmokers, and of the smoker population, 50% were "moderate" smokers and 20% were "heavy" smokers. Based on the assumption that the relative risk of lung cancer for nonsmokers, moderate, and heavy smokers is 1, 10 and 20, respectively, US EPA adjusted the number of expected lung cancer cases in the study cohort to reflect the smoking prevalence data. The number of expected lung cancer cases after adjustment for smoking is 1.61 cases. This is about 15% higher than the 1.4 cases expected without considering smoking differences; however, this adjustment did not substantially alter the significance of the increased prevalence of lung cancer in the workers exposed to acrylonitrile. US EPA (1983) concluded that the observations by O'Berg (1980) of a statistically significant excess of lung cancer in acrylonitrile-exposed workers constitutes significant evidence that acrylonitrile is likely to be a human carcinogen.

A followup to the O'Berg (1980) study was conducted by O'Berg *et al*. (1985). Observations of cancer incidence and mortality for the study cohort of 1345 DuPont workers exposed to acrylonitrile was extended through 1981 for mortality and through 1983 for cancer incidence. Exposed workers demonstrated 43 cases of all types of cancer, with 37.1 expected. Of these cases, 10 were lung cancer versus 4.4 expected. These rates were in excess but were not statistically significant. Additionally, prostate cancer rates were significantly elevated, with 6 cases observed compared to 1.8 cases expected ($p < 0.05$).

Theiss *et al.* (1980) (reviewed by US EPA, 1983) conducted a cohort mortality study of 1469 workers from 12 factories owned by the BASF company in the Federal Republic of Germany (FRG). BASF purchased acrylonitrile during the study period in order to produce styrene-acrylonitrile and acrylonitrile-butadiene-styrene polymers in addition to organic intermediate products. Processing methods differed between factories, and worker exposure data was not available. The study population was defined as all workers employed for over 6 months in acrylonitrile processing from the time of first use of acrylonitrile (approximately 1956) to the study cut-off date of May 15, 1978. The cohort included 1081 German workers and 338 workers of other nationalities. Followup was 98% complete on the German workers, but only 56% complete on the foreign workers. Expected deaths were calculated from mortality rates for the city of Ludwigshafen, the state of Rheinhessen-Pfalz, and the FRG as a whole. An elevated risk of cancer (all types) mortality was noted in the study cohort (27 observed, 20.5 expected based on FRG mortality rates). The study cohort also demonstrated a significantly elevated risk of lung cancer (11 observed, 5.65 expected based on FRG rates, $p < 0.05$; 5.92 expected based on Rheinhessen-Pfalz rates, $p < 0.05$). An excess significant risk of lung cancer remained after 78 cohort members from one factory who reported "contact with other substances since proven to be carcinogenic" were removed from the calculations (9 observed, 4.37 expected based on FRG mortality rates, $p <$

0.05); additionally, a significant excess risk of lymphatic cancer was seen (4 observed, 1.38 expected based on FRG mortality rates, $p < 0.05$).

US EPA (1983) noted that the members of the study cohort were exposed to a number of other carcinogens, including vinyl chloride, and distillation residues including polycyclic aromatic hydrocarbons, cadmium, ß-napthylamine, dimethyl sulfate and epichlorohydrin. Additionally, tobacco smoking was a potential confounding factor; all lung cancer cases were smokers. However, US EPA also noted that the lung cancer risk associated with exposure to acrylonitrile estimated from this study could actually be an underestimate of the actual risk because 1) combining workers from 12 factories between which acrylonitrile exposure levels varied could have lend to an underestimate of risk due to the inclusion of unexposed or minimally exposed workers; 2) the "healthy worker" effect could have resulted in an underestimate of risk; 3) followup on the relatively youthful cohort was insufficient, and did not allow sufficient latency in the cohort segments most at risk - only 447.1 person-years were accumulated in members over 64 years of age and 4) underascertainment of vital status (12% of the study cohort were lost to followup) may have resulted in an undercount of observed deaths. US EPA concluded that it was possible that exposure to acrylonitrile might be related to the excess risk of lung cancer demonstrated by the study cohort of Theiss *et al*. (1980).

Werner and Carter (1981) studied the mortality of 1111 men who worked in acrylonitrile polymerization and acrylic fiber production (6 plants, located in England, Northern Ireland, Scotland and Wales) from 1950 to 1968; surveillance was continued to the end of 1978. An excess of total cancer deaths was noted (21 observed, 18.6 expected) but was not statistically significant. Expected deaths were calculated from mortality rates from England and Wales combined. Only 68 deaths from all causes had occurred as of the end of 1978; 72.4 were expected. An excess of deaths from all types of cancer combined was noted (21 observed, 18.6 expected), but this excess was not statistically significant. Significant increases in deaths due to stomach cancer were noted in all age groups combined (5 observed, 1.9 expected, $p < 0.05$), with deaths in the 55-64 age group comprising the largest portion of those deaths (3 observed, 0.7 expected, $p < 0.05$). A statistically significant elevated risk of lung cancer was also noted in the 15-44 age group (3 observed, 0.7 expected, $p < 0.05$), but not in other age groups or in all age groups combined. The authors note the lack of acrylonitrile exposure data, including potential differences in exposure levels between the 6 plants surveyed. US EPA (1983) also notes the relatively short followup in the cohort subgroup which would be expected to have incurred the greatest risk, the 158 men having the earliest exposure (during the 1950-1958 period). US EPA (1983) concluded that because of the relative youth of the cohort resulting in a small number of expected deaths, the lack of followup, and the lack of control for smoking, the findings of this study are only suggestive.

A cohort mortality study of 327 white male workers employed for 2 or more years between January 1, 1940 and July 1, 1971 at a rubber manufacturing plant in Akron OH who were potentially exposed to acrylonitrile was conducted by Delzell and Monson (1982). Acrylonitrile exposure levels were not reported. Cause-specific expected deaths were calculated based on both U.S. age and calendar specific white male mortality and mortality rates for other rubber workers from the same city; however, most results reported in this study used expected deaths calculated using U.S. white male mortality rates. An excess risk of lung cancer mortality was observed when either U.S. mortality rates (9 observed, 5.9 expected) or Akron rubber industry mortality rates (9 observed,

4.7 expected) were used to calculate expected lung cancer deaths. Workers employed for 5-15 years and followed for at least 15 years demonstrated a significantly increased risk of lung cancer (4 observed, 0.8 expected, $p < 0.01$). Cohort members could potentially have been exposed to other chemicals used in the same area (butadiene, styrene, vinyl pyridine). Also, smoking controls were not included. However, US EPA (1983) commented that the possibility that the excess risk of lung cancer demonstrated in this study was due to acrylonitrile exposure could not be dismissed.

Chen *et al*. (1987) examined cancer incidence and mortality in a cohort of 1083 male employees at a E.I. du Pont de Nemours and Co., Inc. textile plant in Waynesboro, VA who were potentially exposed to acrylonitrile in the period 1944-1970. Worker exposure levels were assessed by an Exposure Classification Committee consisting of seven DuPont employees with long-term experience in the acrylonitrile exposure area; plant environmental monitoring data was not available for the 1944-1970 period. High, moderate and low exposure categories were established. Expected numbers of deaths were calculated from both U.S. and DuPont mortality rates; however, the authors only listed the results of cancer incidence and mortality calculations using the DuPont "control" data set as the source of the expected numbers of cancer cases and mortality. No significant increase in incidence was noted for either all types of cancer (37 observed, 36.5 expected) or lung cancer (5 observed, 6.9 expected). However, a significant increase in the incidence of prostate cancer (5 observed, 1.9 expected) was noted; of these, 4 occurred in the 1975-1983 period (0.9 expected).

US EPA (1983) also reviewed several unpublished studies of cancer mortality and/or morbidity potentially caused by acrylonitrile (Kiesselbach *et al*., 1980; Zack, 1980; Gaffey and Strauss, 1981; Herman, 1981; Stallard, 1982). These studies indicated no cancer increase in workers potentially exposed to acrylonitrile. However, US EPA concluded that due to design and methodological deficiencies including short followup, small cohort size and young cohort age, "none of these studies can be cited as adequate evidence that acrylonitrile is not carcinogenic".

Animal Studies

Maltoni *et al*. (1977) (reviewed by US EPA, 1983) exposed male and female Sprague-Dawley rats (30/sex/exposure group) to 0, 5, 10, 20 or 40 ppm acrylonitrile by inhalation for 4 hours/day, 5 days/week for 12 months. The animals were then maintained for the remainder of their lifetime. Slight increases in the incidence of the following tumor types were noted: mammary gland tumors in males and females, nonglandular forestomach tumors in males and skin tumors in females. Tumor incidence data are listed in Table 1.

The authors claimed that these results indicated a "border-line carcinogenic effect". US EPA (1983) noted that low sensitivity of this study due to the low concentrations of acrylonitrile used and the short duration of acrylonitrile exposure (12 months). Additionally, male and female Sprague-Dawley rats (40/sex/group) were exposed to 0 or 5 mg/kg body weight acrylonitrile by gavage 3 times/week for 52 weeks.

Table 1. Tumor incidence in male and female Sprague-Dawley rats exposed to acrylonitrile by inhalation (Maltoni *et al*., 1977)

Tumor type	Tumor incidence Acrylonitrile concentration (ppm)				
	0	5	10	20	40
mammary tumors (females)	5/30	10/30	7/30	10/30	7/30
mammary tumors (males)	1/30	0/30	1/30	4/30	4/30
nonglandular forestomach papillomas (males)	0/30	1/30	2/30	0/30	3/30
skin carcinomas (females)	0/30	4/30	1/30	1/30	1/30

On spontaneous death, a moderate increase in the incidence of female rat mammary gland tumors and nonglandular forestomach tumors was noted. US EPA (1983) commented that although the observation period was relatively short (52 weeks) and only a single dose level was used, this study provides additional evidence for the carcinogenicity of acrylonitrile.

A three-generation reproductive study on the effect of acrylonitrile exposure in male and female Charles River rats [CRL:COBS CD (SD) BR] was conducted by Litton-Bionetics, Inc. for the Chemical Manufacturers Association (Beliles *et al*., 1980; reviewed by US EPA, 1983). The rats and their offspring were exposed to drinking water containing 0, 100 or 500 ppm acrylonitrile starting 15 days post-weaning and were mated after 100 days. After delivery of two litters, the animals were exposed to acrylonitrile for approximately 45 weeks. After exposure, all animals in generations F_0, F_1b and F_2b were sacrificed and examined histologically. Second-generation rats in the 500 ppm exposure group demonstrated a significant increase in the incidence of astrocytomas and Zymbal gland tumors. Tumor incidence data are listed in Table 2.

Table 2. Tumor incidence data in Charles River rats during a three-generation reproductive study (Beliles *et al*., 1980)

Tumor type	Generation	Tumor incidence Acrylonitrile dose (ppm)		
		0	100	500
astrocytomas	F_0	0/19	1/20	2/25
	F_1b	0/20	1/19	4/17
	F_2b	0/20	1/20	1/20
Zymbal gland	F_0	0/19	0/20	1/25
	F_1b	0/20	2/19	4/17
	F_2b	0/20	0/20	3/20

Bio/Dynamics Inc. conducted a study on the toxicity and carcinogenicity of acrylonitrile in Sprague-Dawley rats (Bio/Dynamics, 1980a) for the Monsanto Company (St. Louis, MO); the results of this study were subsequently submitted to the US EPA by the Monsanto Company on June 30, 1980. Male and female Sprague-Dawley rats (100/sex/treatment group) were exposed to acrylonitrile in drinking water at concentrations of 0,1, and 100 ppm. Interim sacrifices (10/sex/treatment group) were conducted at 6, 12 and 18 months. The study was terminated at less than 2 years because of low survival rates; males were sacrificed at 22 months and females were

sacrificed at 19 months. Statistically significant increases were noted in the incidence of astrocytomas of the brain and spinal cord, adenomas and carcinomas of the Zymbal gland, and nonglandular forestomach squamous cell papillomas and carcinomas in males and females of the 100 ppm group. Tumor incidence data are listed in Table 3.

Table 3. Tumor incidences in male and female Sprague-Dawley rats exposed to acrylonitrile in drinking water (Bio/Dynamics, 1980a)

Tumor type	Dose level (ppm)	Tumor incidence	
		males	females
brain astrocytomas	0	2/98	0/99
	1	3/95	1/100
	100	23/97	32/97
spinal cord astrocytomas	0	NA	0/96
	1	NA	0/99
	100	NA	7/98
Zymbal gland carcinomas	0	1/100	0/99
	1	0/91	0/95
	100	14/93	7/98
nonglandular forestomach papillomas/carcinomas	0	3/98	1/100
	1	3/98	4/99
	100	12/97	7/99

NA - not analyzed

A similar study was conducted by Bio/Dynamics Inc. on the toxicity and carcinogenicity of acrylonitrile in Fischer 344 rats (Bio/Dynamics, 1980b) for the Monsanto Company (St. Louis, MO); the results of this study were subsequently submitted to the US EPA by the Monsanto Company on December 12, 1980. Male and female Fischer 344 rats (100/sex/acrylonitrile treatment group; 200/sex/control group) were exposed to acrylonitrile in the drinking water at concentrations of 0,1, 3, 10, 30 and 100 ppm. Interim sacrifices (10/sex/acrylonitrile treatment group; 20/sex/control group) were conducted at 6, 12 and 18 months. The study was designed to be 24 months in duration; however, because of poor survival, all females were sacrificed at 23 months. Males were continued on study until 26 months, when survival rates comparable to females were achieved. Statistically significant increases were noted in the incidence of astrocytomas of the brain and spinal cord in males (30 and 100 ppm groups) and females (10, 30 and 100 ppm group), adenomas and carcinomas of the Zymbal gland in males (30 and 100 ppm groups) and females (10, 30 and 100 ppm groups), and nonglandular forestomach squamous cell papillomas and carcinomas in males (3, 10 and 30 ppm groups) and females (30 ppm group). Tumor incidence data are listed in Table 4.

Table 4. Tumor incidences in male and female Fischer 344 rats exposed to acrylonitrile in drinking water (Bio/Dynamics, 1980b)

Tumor type	Dose level (ppm)	Tumor incidence	
		males	females
brain astrocytoma	0	2/200	1/199
	1	2/100	1/100
	3	1/100	2/101
	10	2/100	4/95*
	30	10/99*	6/100*
	100	21/99*	23/98*
spinal cord astrocytoma	0	1/196	1/197
	1	0/99	0/97
	3	0/92	0/99
	10	0/98	1/92*
	30	0/99	0/96
	100	4/93*	1/91
Zymbal gland[1]	0	2/189	0/193
	1	1/97	0/94
	3	0/93	2/92
	10	2/88	4/90*
	30	7/94*	5/94*
	100	16/93*	10/86*
nonglandular forestomach[2]	0	0/199	1/199
	1	1/100	1/100
	3	4/97*	2/100
	10	4/100*	2/97
	30	4/100*	4/100*
	100	1/100	2/97

* Statistically significant at $p < 0.05$

Male and female Sprague-Dawley rats (Spartan strain) (100/sex/group) were exposed to acrylonitrile by gavage at dose levels of 0, 0.1 and 10 mg/kg-day, 5 days/week in a study conducted by Bio/Dynamics Inc. for the Monsanto Company (St. Louis, MO) (Bio/Dynamics, 1980c). Study termination was originally planned for 24 months; however, because only 10 and 13 high dose males and females, respectively, were still alive at 20 months, all surviving animals in all groups were killed during the 20th month to ensure that at least 10 animals/sex/group were available for histopathological examination. Interim sacrifices were performed at 6, 12 and 18 months (10 animals/sex/group). Statistically significant increases in tumor incidence were noted in the following tumor types: brain astrocytomas and Zymbal gland squamous cell carcinomas (high dose males and females), stomach papillomas and carcinomas and intestinal tumors (high dose males), and mammary gland tumors (high dose females). Tumor incidence data are listed in Table 5.

Table 5. Tumor incidence in male and female Sprague-Dawley rats (Spartan strain) exposed to acrylonitrile by gavage (Bio/Dynamics, 1980c)

Tumor type	Sex	Tumor incidence Dose level (mg/kg-day)		
		0	0.10	10.0
brain astrocytoma	male female	2/100 1/99	0/97 2/100	16/98 17/100
spinal cord astrocytoma	male female	0/94 0/100	0/93 0/95	1/97 1/99
Zymbal gland squamous cell carcinomas	male female	1/96 0/85	0/93 0/94	10/96 9/94
stomach papillomas/carcinomas	male female	2/99 2/99	6/97 4/99	40/99 17/99
Intestine	male female	0/100 NA	1/100 NA	6/100 NA
mammary gland	male female	NA 7/101	NA 6/100	NA 22/101

Dow Chemical Company (Midland, MI) performed a study in which male and female Sprague-Dawley rats (48 animals/sex/acrylonitrile exposure group; 80 animals/sex/control group) were exposed to acrylonitrile in drinking water for 2 years (Quast *et al*., 1980a). For the first 21 days of the study, the concentrations used were 0, 35, 85 and 210 ppm; the two higher concentrations were subsequently raised to 100 and 300 ppm. Animals at the highest 2 concentrations demonstrated treatment-related toxicity after 9 months. The mean administered doses of acrylonitrile were calculated to be 0, 3.42, 8.53 and 21.18 mg/kg-day for males and 4.36, 10.76 and 24.97 mg/kg-day for females for the 35, 100 and 300 ppm exposure groups, respectively. All surviving animals were sacrificed at 24 months. Statistically significant increases in tumor incidence were noted for the following tumor types: central nervous system tumors (astrocytomas, gliomas) in males and females (all treatment groups), Zymbal gland adenomas and carcinomas in females (all treatment groups) and males (300 ppm group), nonglandular forestomach squamous cell papillomas and carcinomas in males (all treatment groups) and females (100, 300 ppm groups), tongue squamous cell papillomas and carcinomas in males (all treatment groups) and females (100, 300 ppm groups), mammary gland tumors (benign and malignant) in females (35, 100 ppm groups), and small intestine cystadenocarcinomas in females (100, 300 ppm). Tumor incidence data are listed in Tables 6 and 7.

Table 6. Tumor incidence in male Sprague-Dawley rats exposed to acrylonitrile in drinking water (Quast *et al.*, 1980a)

Tumor type	Tumor incidence Acrylonitrile dose level (ppm)			
	0	35	100	300
brain and/or spinal cord[1]	1/80	12/47	22/48	30/48
Nonglandular forestomach[2]	0/80	3/46	23/48	39/47
tongue[2]	1/75	2/7	4/9	5/40
Zymbal gland carcinomas	3/80	4/47	3/48	15/48

1. Benign and/or malignant
2. Squamous cell papillomas and/or carcinomas

Table 7. Tumor incidence in female Sprague-Dawley rats exposed to acrylonitrile in drinking water (Quast *et al.*, 1980a)

Tumor type	Tumor incidence Acrylonitrile dose level (ppm)			
	0	35	100	300
brain and/or spinal cord[1]	0/80	17/48	22/48	24/48
mammary gland[1]	57/80	42/48	42/48	35/48
Nonglandular forestomach[2]	1/80	1/47	12/48	30/48
small intestine[3]	0/80	1/7	4/11	4/48
tongue[2]	0/78	1/5	2/3	12/45
Zymbal gland carcinomas[4]	1/80	5/48	8/48	18/48

1. Benign and/or malignant
2. Squamous cell papillomas and/or carcinomas
3. Mucinous cystadenocarcinomas
4. Adenomas and carcinomas

Male and female Sprague-Dawley rats (Spartan substrain; 100 animals/sex/exposure group) were exposed to acrylonitrile by inhalation in a study conducted by Dow Chemical Company for the Chemical Manufacturers Association (Quast *et al.*, 1980b). Study animals were exposed to 0, 20 or 80 ppm of acrylonitrile for 6 hours/day, 5 days/week for 2 years. Statistically significant increases in tumor incidence were noted for the following tumor types: brain and spinal cord glial cell tumors (males and females), mammary gland adenocarcinomas (females), small intestine tumors (benign and malignant) (males), tongue squamous cell papillomas and carcinomas (males) and Zymbal gland tumors (males and females). All tumor incidence increases were noted at the highest concentration tested, 80 ppm, except for brain and spinal cord glial cell tumors in females, which were also noted in the 20 ppm group. Tumor incidence data are listed in Table 8.

Table 8. Tumor incidence in male and female Sprague-Dawley rats exposed to acrylonitrile by inhalation (Quast *et al.*, 1980b)

Tumor type	Sex	Tumor incidence Acrylonitrile concentration (ppm)		
		0	20	80
Brain and/or spinal cord glial cell tumors[1]	male	0/100	4/99	22/99
	female	0/100	8/100	21/100
mammary gland adenocarcinomas	female	9/100	8/100	20/100
small intestine[1]	male	2/99	2/20	15/98
Zymbal gland tumors[1]	male	2/100	4/100	11/100
	female	0/100	1/100	11/100

1. Benign and/or malignant

Bigner *et al.* (1987) exposed male and female Fischer 344 rats to acrylonitrile in drinking water. Exposure groups were as follows: 147 males and 153 females exposed to 500 ppm acrylonitrile; 50 males and 50 females exposed to 500 ppm acrylonitrile; 50 males and 50 females exposed to 100 ppm acrylonitrile and 51 males and 49 female control animals. The study was not complete at the time of the report (18 months of exposure); however, they reported 49 primary brain tumors in 215 animals examined from the high dose treatment groups.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Acrylonitrile has been demonstrated to cause cancer in humans (O'Berg, 1980; Werner and Carter, 1981; Delzell and Monson, 1982) and rats; routes of administration for rats include gavage (Bio/Dynamics, 1980c), oral exposure (Beliles *et al*., 1980; Bio/Dynamics, 1980a; Bio/Dynamics, 1980b; Quast *et al*., 1980a; Bigner *et al.* 1987) and inhalation (Maltoni *et al*., 1977; Quast *et al*., 1980b). US EPA (1991) chose to use the O'Berg acrylonitrile occupational exposure study as the basis of derivation of a cancer potency factor for acrylonitrile. This study demonstrated the carcinogenicity of acrylonitrile in a cohort which was sufficiently large and which was followed for an adequate time period. Exposure levels were estimated by representatives of the company employing the study cohort, and a dose-response relationship was observed for the increased cancer risk. This increased risk remained after adjusting for smoking. US EPA (1983) noted that the cancer potency values for acrylonitrile derived from human exposure (O'Berg, 1980) was within one order of magnitude of the cancer potencies derived from rat oral exposure (Bio/Dynamics, 1980a; Bio/Dynamics, 1980b; Quast *et al*., 1980a) and inhalation exposure (Quast *et al*., 1980b) studies.

Methodology

A unit risk (UR) for acrylonitrile was calculated by US EPA (1991) from a relative risk model adjusted for smoking and based on a continuous lifetime equivalent of occupational exposure using the relationship

$$UR = PO\ (R-1) / X = 1.5E\text{-}4/ppb * 0.45\ ppb/\mu g/m^3 = 6.8E\text{-}5\ (\mu g/m^3)^{-1}$$

where: PO = 0.036 = background lifetime probability of death from respiratory cancer
R = 5.0/1.6 = 3.1 = relative risk of respiratory cancer adjusted for smoking (O'Berg, 1980)
X = 500 ppb = continuous equivalent lifetime exposure when 9 years = estimated average exposure duration, and 60 years = estimated maximum possible age at the end of the observation period.

CDHS (1988) reestimated the unit risk factor for acrylonitrile, using the standard lifespan typically assumed by CDHS in risk assessments (70 years) and taking into account the uncertainty in the relative risk estimate. The unit risk was corrected using the following relationship:

$$B^* = B(95) * (70/60)^3$$

where B^* is the unit risk and B(95) is the upper 95% bound on B. This bound was estimated directly by substituting R(95), the upper 95% confidence bound on R, which was found to be 6.6; the second factor $[(70/60)^3]$ was used to extrapolate from a 60 year observation period to a 70 year observation period. The resulting unit risk factor derived was 2.9E-4 $(\mu g/m^3)^{-1}$.

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ALLYL CHLORIDE

CAS No: 107-05-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	76.5
Boiling point	44.96°C
Freezing point	-134.5°C
Vapor pressure	295.5 mm Hg at 20°C
Air concentration conversion	1 ppm = 3.13 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.0 E-6 $(\mu g/m^3)^{-1}$

Slope Factor: 2.1 E-2 $(mg/kg\text{-}day)^{-1}$

[Linearized multistage procedure (GLOBAL82) (US EPA, 1986) fitted to NCI (1977) female mouse forestomach tumor data, body weight scaling, adopted by RCHAS/OEHHA (1994), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

A retrospective cohort mortality study of 1,064 male workers potentially exposed to epichlorohydrin and allyl chloride was conducted by Olsen *et al*. (1994). Study subjects had a minimum of 1 month work experience between 1957-1986 in the production or use of epichlorohydrin and allyl chloride and 1 year total employment duration at Dow Chemical's Texas Operations (Freeport, TX). Job exposure categorization was used to quantify individual exposure based on an evaluation of work practices, production processes and available environmental monitoring data. Vital status follow-up occured through 1989; 66 total deaths were recorded. Standardized mortality ratios (SMR) for all malignant neoplasms or lung cancer were not significantly increased when compared to external (U.S.) or internal (Texas Operations) populations. The authors noted that the study results are limited by the cohort's size, duration of follow-up, relatively few number of observed and expected deaths, and the level of potential epichlorohydrin and allyl chloride exposure.

Animal Studies

Several studies exist on the potential carcinogenicity of allyl chloride in animals; these studies have been reviewed by IARC (1985) and U.S. EPA (1986, 1991).

Male and female $B6C3F_1$ mice and Osborne-Mendel rats (50/group) were exposed to allyl chloride (technical grade; 98% pure) by gavage daily 5 days/week for 78 weeks (NCI, 1977). Exposure groups for mice were initially 172 or 199 mg/kg body weight for males and 129 or 258 mg/kg for

females. Exposure groups for rats were initially 70 or 140 mg/kg body weight for males and 55 or 110 mg/kg for females. Due to toxicity, the initial doses were reduced as the study progressed. Final time-weighted average doses for the 78 week dosing period were 172 and 199 mg/kg/day for male mice; 129 and 258 mg/kg/day for female mice; 57 and 77 mg/kg/day for male rats and 55 and 73 mg/kg/day for female rats. Mice and rats were observed for an additional 13 and 30-33 weeks after the end of the dosing period, respectively. Excessive mortality (50% after 14-38 weeks) was noted in the high-dose rats (both sexes) and male mice. The number of surviving animals in all low-dose groups and high-dose female mice were adequate to evaluate late-developing tumor risk.

No significant increases in tumor incidence were noted in rats. Proliferative nonneoplastic lesions of the stomach were noted in mice of both sexes. In male mice, squamous cell carcinomas of the stomach were found in 0/29 controls (17 vehicle and 12 untreated), 2/36 low-dose animals, and 0/10 high-dose animals (only 10 survived past 52 weeks). In female mice, squamous cell papillomas and carcinomas of the forestomach were found in 0/39 controls (19 vehicle and 20 untreated), 3/47 low-dose animals (2 carcinomas) and 3/45 high-dose animals (no carcinomas). Tumor incidence was not significantly increased compared to controls for either dose group of either sex. However, the combined tumor incidence in females and the carcinoma incidence in low-dose males was significantly increased at both doses compared to historical vehicle controls (1/180 female mice with squamous cell papilloma or carcinoma of the forestomach; 1/180 male mice with squamous cell carcinoma of the forestomach). The authors considered the findings to be strongly suggestive of carcinogenicity in mice because of the rarity of the tumor type involved and because the proliferative lesions demonstrated could be preneoplastic.

Female Ha:ICR Swiss mice (30/group) were exposed to allyl chloride by topical application (31 or 94 mg allyl chloride in 0.2 ml acetone) 3 times/week for 63-85 weeks (Van Duuren *et al*., 1979). Skin tumors were not induced. Lung and stomach papillomas were induced in both the low dose group (3 stomach, 14 lung papillomas) and the high dose group (3 stomach, 12 lung papillomas, 1 glandular stomach adenocarcinoma). Tumor incidences were not significantly increased compared to vehicle or untreated controls (control incidence not reported).

Female Ha:ICR Swiss mice (30/group) received a single dermal application of 94 mg technical grade allyl chloride in 0.2 ml acetone followed 2 weeks later by dermal applications of 5 µg 12-*O*-tetradecanoylphorbol 13-acetate (TPA) 3 times/week for life (median survival 61-82 weeks) (Van Duuren *et al*., 1979). Skin papilloma incidence was significantly increased (7/30 treated animals compared to 6/90 TPA control animals, $p < 0.025$) and time to tumor was decreased (first tumor in treated animals at day 197 compared to day 449 in TPA controls) in allyl chloride -treated animals.

Male and female A/St mice (10/group) received intraperitoneal injections of allyl chloride in tricaprylin 3 times/week for 8 weeks; total doses were 1.2, 2.9 and 5.9 g/kg body weight (Theiss *et al*., 1979). Animals were killed 24 weeks after exposure initiation. The only pathological endpoint examined was the induction of lung tumors as determined by gross examination. The average number of adenomas/mouse (20 animals/group, both sexes combined) was 0.19 ± 0.1, 0.60 ± 0.2, 0.50 ± 0.27 and 0.60 ± 0.15 in the control, low, medium and high-dose groups,

respectively. The incidence of lung adenomas in the high-dose group was significantly increased ($p < 0.05$ by Student's T-test or chi-square test).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The NCI (1978) carcinogenicity bioassay demonstrated a statistically significant increased incidence of squamous cell papillomas and carcinomas of the forestomach in low-dose male mice (2/46, $p < 0.029$) and low-dose (3/47; $p < 0.003$) and high-dose (3/45; $p < 0.003$) female mice when compared to tumor incidences in male (1/180) and female (1/180) historical controls. The female mouse tumor incidence data from this study was chosen as the basis of a cancer potency factor because it demonstrated induction of a rare tumor type by allyl chloride in the most sensitive sex of a sensitive species.

Methodology

Transformed doses were calculated as follows:

transformed dose = experimental dose × (5 days/7 days) × (78 weeks/92 weeks)

Animals were dosed 5 days/week, and the duration of exposure and of the experiment were 78 and 92 weeks, respectively. Experimental doses were 129 and 258 mg/kg/day; transformed doses were 78 and 156 mg/kg/day. A linearized multistage procedure (GLOBAL82) was then applied to the tumor incidence data; the resulting unadjusted cancer potency factor (q_1^*) was 1.01 E-3 $(mg/kg/day)^{-1}$. A q_1^* for humans was calculated from the unadjusted q_1^* as follows:

human q_1^* = unadjusted $q_1^* \times (70\ kg/0.025\ kg)^{1/3} \times (104\ weeks/92\ weeks)^3$
= 2.1 E-2 $(mg/kg/day)^{-1}$

The reference human body weight and the average female mouse weight were 70 kg and 0.025 kg, respectively, and the experiment length and the mouse lifespan were 92 weeks and 104 weeks, respectively. A unit risk factor of 6.0 E-6 $(\mu g/m^3)^{-1}$ was derived from the human q_1^* by OEHHA/ATES using an inspiration rate of 20 m^3/day.

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2-AMINOANTHRAQUINONE

CAS No: 117-79-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1994) except where noted)

Molecular weight	223.24
Boiling point	sublimes (IARC, 1982)
Melting point	302 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 9.131 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 9.4 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 3.3 E-2 $(mg/kg\text{-}day)^{-1}$
[Male rat liver tumor data (NCI, 1978), contained in Gold *et al*. database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 2-aminoanthraquinone (2-AA) on humans are known to exist.

Animal Studies

Results from the National Cancer Institute (NCI) (1978) feeding study in male and female B6C3F$_1$ mice and Fischer 344 rats are tabulated in Gold *et al*. (1984). 2-Aminoanthraquinone (technical grade, unspecified impurities) was administered in feed to groups of 50 male and 50 female animals of each species. Matched control groups were included for each mouse dose group (50 animals/sex/species). Control groups of 50 male and 25 female rats were also included; these animals were observed for 107-109 weeks. Diet fed to mice contained 5000 or 10000 mg/kg 2-AA; diet fed to female rats contained 2000 mg/kg 2-AA. Diet fed to male rats contained 10000 or 20000 mg/kg 2-AA for the first 10 weeks; this was reduced to 2500 or 5000 mg/kg for the remaining 68 weeks. For rats, NCI reported the time-weighted average dietary concentrations to be 0.69% and 0.35% for high and low dose males, and 0.2% for treated females over a 78-week period. An additional observation period of 28-32 weeks was included after treatment ended. High and low dose mice of both sexes were administered time-weighted average dietary concentrations of 1.0% (over 80 weeks) and 0.5% (over 78 weeks) respectively, and were observed for an additional 15-16 weeks after treatment ended.

At study termination, 82, 78, 94 and 86% of male mice and 78, 76, 88 and 76% of female mice were still alive in the low-dose control, high-dose control, low-dose and high-dose groups, respectively. In male rats, 54% of the controls, 64% of low-dose and 70% of high-dose animals

were alive at the end of the study. Insufficient numbers of female rats survived to the latter portion of the experimental period to permit analysis of late-developing tumors.

High-dose male and female mice demonstrated a significantly increased incidence of hepatocellular carcinomas. Tumor incidence in male mice was 12/46 in low-dose controls, 6/48 in high-dose controls, 20/47 in low-dose animals, and 36/49 ($p < 0.001$) in high-dose animals; in female mice, the frequencies were 4/46, 1/50, 5/47 and 12/47 ($p < 0.001$) (NCI, 1978; Murthy *et* al., 1979). A dose-dependent increase in hepatic neoplastic nodules and hepatocellular carcinomas ($p < 0.001$) was noted in 18/41 low-dose and 18/45 high dose males; tumors were observed in 0/36 control male rats.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The NCI carcinogenicity bioassay of 2-AA indicated that 2-AA induced tumor formation in both rats and mice. The cancer potency value derived is based on the dose-response data for hepatic tumors in the more sensitive sex and species, the male rat (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. The average dose administered to the male rat high-dose group as calculated by Gold *et al*. (1984) was 102 mg/kg/day. Analysis of the data set using the computer program TOX_RISK (Crump *et al*., 1991) indicated that inclusion of the high dose group resulted in a p-value of = 0.05 based on the chi-square goodness-of-fit test, indicating non-linearity. Following procedures described by US EPA (Anderson *et al*., 1983), the high dose group was excluded from the analysis to correct for the poor fit (Cal/EPA, 1992). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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ANILINE

CAS No: 62-53-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	93.12
Boiling point	184-186°C
Melting point	-6.3°C
Vapor pressure	0.67 mm Hg at 25°C
Air concentration conversion	1 ppm = 3.82 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.6 E-6 ($\mu g/m^3$)$^{-1}$
Slope Factor: 5.7 E-3 (mg/kg-day)$^{-1}$
[Derived from a cancer potency factor calculated by US EPA/IRIS (1990, 1994) from male rat primary splenic sarcoma incidence data (CIIT, 1982) using a linearized multistage procedure, extra risk; adopted by CDHS/RCHAS (1990)]

III. CARCINOGENIC EFFECTS

Human Studies

US EPA (1994) reviewed a study that examined the occurrence of bladder tumors in British workers in the chemical dye industry (Case *et al.*, 1954). A group of 4622 men employed for more than 6 months in the United Kingdom chemical industry during the period 1910-1952 were studied. In a subgroup of 1233 men exposed solely to aniline, one death from bladder cancer was observed compared to 0.83 expected from English/Welsh male mortality data. Among the entire group (who had generally been exposed to a number of aromatic amines including napthylamine, benzidine, auramine and aniline; no detailed exposure information was available), 127 deaths from bladder cancer were observed compared to 4.1 expected. The authors concluded that the data provided insufficient evidence to suggest that aniline itself causes bladder tumors.

Animal Studies

Forty-three male and female Osborne-Mendel rats were fed diets containing 330 mg/kg aniline hydrochloride for up to 1032 days (White *et al.*, 1948). Hepatomas and splenic sarcomas were noted in 4 and 3 animals, respectively. No control group was included in the study; however, the authors claimed that liver and spleen tumors were rare in the rat strain used in the study.

IARC (1982) reviewed a study by Druckrey (1950) in which rats (random bred, sex unspecified) were exposed to aniline hydrochloride in drinking water (22 mg/rat/day) over their lifetime. Mortality was quite high; 50% mortality occurred at day 450 and 100% mortality at day 750. No tumors were observed in the treated animals; however, only the bladder, liver, spleen and kidney were evaluated for tumors.

Male and female Fischer 344 (F344) rats and B6C3F$_1$ mice were fed diets containing aniline hydrochloride for 103 weeks (NCI, 1978). Rats were fed diets containing 0, 3000 or 6000 mg/kg diet aniline hydrochloride; mice were fed diets containing 0, 6000 or 12000 mg/kg diet aniline hydrochloride. Group sizes were 50/sex/group, except for high dose female mice (n = 49), and control rats (25/sex). Surviving rats and mice were sacrificed at 107-108 and 107 weeks, respectively. No significantly increased treatment-related tumor incidences were noted in treated mice. Male rats demonstrated significantly elevated incidences of hemangiosarcomas in the spleen, as well as fibrosarcomas and sarcomas (not otherwise specified) in multiple organs of the body cavity and spleen. There were also significant dose-related trends in the incidence of hemangiosarcomas, sarcomas or fibrosarcomas and malignant pheochromocytomas. For female rats, a dose-related trend was observed in the incidence of fibrosarcomas and sarcomas in the spleen and in multiple organs of the body cavity. No fibrosarcomas, or sarcomas of the spleen or multiple organs of the body cavity were observed in pooled (249 female and 250 male) control animals. Tumor incidence data is listed in Table 1.

Table 1. Aniline hydrochloride-induced tumor incidence data in male and female Fischer 344 rats (NCI, 1978)

Tumor type	Sex	Tumor incidence aniline hydrochloride dietary concentration (mg/kg diet)		
		0	3000	6000
spleen hemangiosarcoma	male	0/25	19/50	20/46
spleen fibrosarcoma/sarcoma NOS	male	0/25	7/50	9/46
multiple organ fibrosarcoma/sarcoma NOS	male	0/25	2/50	9/48
	female	0/24	1/50	7/50
adrenal pheochromocytomas	male	2/24	6/50	12/44

NOS = not otherwise specified

Hagiwara *et al.* (1980) administered aniline in the diet at a concentration of 300 mg/kg diet to 28 male Wistar rats over a period of 80 weeks. An untreated control group of 28 rats was also included. No significant increase in tumor incidences were observed as a result of aniline exposure. However, the treatment group sizes used were relatively small, and the exposure was relatively low and less than lifetime.

Male and female CD-F rats (130/sex/exposure group) were exposed to aniline hydrochloride in the diet for 2 years at exposure levels of 0, 10, 30 and 100 mg/kg body weight/day (CIIT, 1982). An increased incidence of primary splenic sarcomas was noted in the male 100 mg/kg exposure group (high dose group); stromal hyperplasia and fibrosis of the splenic red pulp, a potential sarcoma precursor lesion, was also observed in high dose males, and to a lesser degree, in high dose females. No fibrosarcomas, stromal sarcomas, capsular sarcomas or hemangiosarcomas were noted in female rats. Tumor incidence data is listed in Table 2.

Table 2: Incidence of splenic tumors in male CD-F rats fed diets containing aniline hydrochloride (CIIT, 1982)

Dietary aniline hydrochloride concentration (approximate) (ppm)	Aniline hydrochloride exposure level (mg/kg body weight/day)	Human equivalent dose[1] $(mg/kg/day)^{-1}$	Tumor incidence[2]
0	0	0	0/64
200	10	1.23	0/90
600	30	3.69	1/90
2000	100	12.29	31/90

1. Calculation of the doses included a correction for the difference in molecular weight of aniline and aniline hydrochloride (compound administered) (US EPA, 1992).
2. Tumor incidence includes fibrosarcomas, stromal sarcomas, capsular sarcomas, and hemangiosarcomas as reported by US EPA (1992).

Syrian golden hamsters (15 male and 15 female) received subcutaneous injections of aniline (521 mg/kg body weight; total dose 9219 mg/kg) for 52 weeks (Hecht *et al*., 1983). Although mean survival was reduced in the aniline-treated groups, no increase in tumor incidence was observed. However, the experimental exposure was less than lifetime, and the number of exposed animals was small.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Cancer potency values are based on the most sensitive site, species and study demonstrating carcinogenicity of a particular chemical, unless other evidence indicates that the value derived from that data set is not appropriate (CDHS, 1985). Male rat spleen tumor data (CIIT, 1982) was used to generate a cancer potency factor for aniline. Male rats in the high-dose group showed a marked increase in the incidence of splenic tumors (see Table 2). US EPA (1994) also noted the presence of stromal hyperplasia and fibrosis of the splenic red pulp in high-dose males and, to a lesser degree, in females; this may represent a precursor lesion of sarcoma.

Methodology

A linearized multistage procedure (US EPA,1980) was used to calculate a slope factor of 5.7 E-3 $(mg/kg\text{-}day)^{-1}$ from the CIIT (1982) male splenic tumor incidence data. Calculation of the transformed doses for aniline included a correction for the difference in molecular weights of aniline and aniline hydrochloride, the form in which the compound was administered in the NCI and CIIT bioassays. Calculation of the unit risk by OEHHA/ATES from the US EPA slope factor assumed a body weight of 70 kg and an inspiration rate of 20 m^3/day.

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ARSENIC (INORGANIC)

CAS No: 7440-38-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	74.92
Boiling point	613 °C (sublimes)
Melting point	817 °C @ 28 atm
Vapor pressure	1 mm Hg at 372 °C
Air concentration conversion	1 ppm = 2.21 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.3 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 1.2 E+1 $(mg/kg\text{-}day)^{-1}$
[Human occupational exposure lung tumor incidence (Enterline *et al*., 1987a);. relative risk model, adjusted for interaction with tobacco smoking (CDHS, 1990).]

Oral slope factor: 1.5 E+0 $(mg/kg\text{-}day)^{-1}$
[Human skin cancer incidence (Tseng *et al*., 1968, 1977), time- and dose-related formulation of the multistage procedure (U.S. EPA, 1988).]

III. CARCINOGENIC EFFECTS

Human Studies

Inhalation

Cancer mortality has been studied among workers employed in three major smelters in the U.S., in (1) Tacoma, Washington, (2) Anaconda, Montana, and (3) Garfield, Utah. Smelter workers in Sweden (Ronnskarverken) and in Japan (Sagnoseki-Machi) and cohorts of both miners and smelter workers in China have also been studied.

Enterline and Marsh (1982) and Enterline *et al*. (1987a) examined the longest follow-up period for the Tacoma, Washington cohort. The 1982 report used cumulative doses based on urinary arsenic measurements. Standardized mortality ratios (SMRs) for respiratory cancer ranged from 170 for those receiving the lowest intensity and shortest duration of exposure, to 578 for those with the highest intensity and with 20-29 years duration of exposure. A strong dose-response relationship was evident only when the analysis was limited to the 582 retired workers in the cohort. In the 1987 reanalysis, Enterline and colleagues incorporated newly available historical air sampling data (Enterline *et al*., 1987a); in this study, the dose-response relationship appears more clearly.
The Anaconda, Montana cohort was also the subject of numerous publications. Lee-Feldstein (1983, 1986) divided the 8,045 men of the full study group into nine subcohorts based on arsenic exposure and year of first employment. When considering only those men who had been in their maximum exposure category for at least 12 months, each of the nine subcohorts, except for one

subgroup which had a very small sample size, showed significantly elevated respiratory cancer rates relative to the combined male population of Idaho, Montana and Wyoming (Lee-Feldstein, 1983). Lee-Feldstein (1983) also investigated the association of sulfur dioxide (SO_2) with respiratory cancer in this cohort. Findings from this study could not conclude that arsenic trioxide was the primary environmental agent causing the excessive respiratory cancer seen in the study group.

In another study, Lee-Feldstein (1986) incorporated quantitative exposure estimates based on industrial hygiene data collected between 1943 and 1958. In all but the latest-employed cohort, a statistically significant linear dose-response relationship was observed between arsenic exposure and the directly standardized death rate (DSDR) for respiratory cancer. Lee-Feldstein (1986) reiterated that the latest-employed cohort may not have been followed long enough to display a dose-related mortality pattern, and the men in this cohort may have experienced prior exposures which confound the relationship between arsenic exposure and respiratory cancer mortality. Also, men who were younger at the start of employment were at greater risk for lung cancer than those who began employment later in life. Further analysis of the same cohort by Welch *et al.* (1982) and Higgins *et al*. (1985) confirmed the linear dose-response relationship between exposure and respiratory cancer.

The Garfield, Utah smelter was studied by Rencher *et al*. (1977), who found a three- to five-fold increase in the proportion of deaths due to lung cancer among smelter workers when compared to workers in the mine or concentrator. Similar results were reported by Tokudome and Kuratsune (1976) who studied 839 copper smelter workers in Japan. A dose-response effect on respiratory cancer was observed using either duration of employment or intensity of exposure. For the same duration of employment, risks were greater among those employed in earlier periods.

Workers at the Ronnskarverken smelter in Sweden experienced lung cancer mortality at about five times the rate of residents of the county (Wall, 1980). Pershagen *et al*. (1981) conducted a nested case-control study within this cohort, focusing on the interrelationship of smoking, arsenic and lung cancer. For smokers and nonsmokers, the age-standardized rate ratios (SRRs) were 3.0 and 2.9. Among roaster workers, the most heavily exposed in this plant, the SRRs were 4.4 and 4.5. Exposures were not quantified in this analysis.

Other studies of cancer incidence and mortality among workers at the Ronnskar smelter in Sweden confirmed the excess lung cancer risk (Sandstrom *et al*., 1988; Jarup *et al*., 1989a,b). An analysis of age-adjusted rates by calendar year showed a decline in lung cancer starting in the mid-1970s, possibly due to lower exposures, earlier notification of health problems, and/or changing smoking habits (Sandstrom *et al*., 1988). However, among the most recently hired cohort, lung cancer incidence was greater than expected.

Taylor *et al*. (1989) conducted a case-control study among tin miners in China to examine the relationship between arsenic exposure and lung cancer. After adjusting for tobacco use and radon exposure, the risk of lung cancer for subjects in the highest quartile of arsenic exposure was 22.6-fold higher than for those in the lowest quartile. Duration but not intensity of exposure appeared to be a predictor of lung cancer risk.

Another report from China covers a cohort consisting of workers employed at two copper smelters, one arsenic smelter and a mine (Wu, 1988). Wu reported that nearly 19,000 person-years were followed, resulting in 40 lung cancer deaths.

Enterline *et al*. (1987b) analyzed data from eight smelters with fairly low levels (relative to the Anaconda, Tacoma, and Ronnskar smelters) of arsenic. When data from the six smelters were combined and examined, the results suggested an increasing trend in risk with increasing exposure ($p = 0.06$). A significant effect was observed for cumulative exposure to arsenic and for smoking.

Reports on cancer and insecticide manufacturing exposures to arsenic were by Ott *et al*. (1974), Baetjer *et al*. (1975b; as cited in Mabuchi *et al*., 1979), Mabuchi *et al*. (1979), and Sobel *et al*. (1988). A study of orchardists who potentially sprayed arsenic-containing pesticides is reviewed by Wicklund *et al*. (1988). The report by Mabuchi *et al*. (a more extensive follow-up of the same cohort Baetjer analyzed) found a sharp increase in the lung cancer SMR with increasing duration of employment among those predominantly exposed to arsenic, although no increase was observed for those with exposure to arsenic only. However, more than 99 percent of this latter group were employed for five years or less.

Ott *et al*. (1974) observed a marked increase in respiratory cancer mortality with increasing cumulative dose in workers previously employed in an insecticide manufacturing plant. Sobel *et al*. (1988) updated the study by Ott *et al.* by 9 additional years of follow-up and by tracing more than 99% of those who had been lost to follow-up in the study by Ott *et al*. The 9 follow-up years yielded a non-statistically significant respiratory cancer SMR of 116.

A case-control study of deaths among orchardists (Wicklund *et al*., 1988) found no association between exposures to arsenic-containing pesticides and respiratory cancer, after controlling for smoking.

In summary, for smelter workers, the association between respiratory cancer mortality and arsenic exposure is a consistent, replicable finding of substantial magnitude with a clear dose-response relationship, and high statistical significance. The mortality data on workers employed in the manufacturing of insecticides provide further evidence that arsenic acts as a respiratory tract carcinogen.

Oral

Chronic exposure to high levels of arsenic in drinking water has been identified as increasing skin cancer incidence in humans (US EPA, 1988, 1995).

In a region on the southwest coast of Taiwan, artesian well water with high arsenic concentrations ranging from 0.01-1.82 ppm had been in use for more than 45 years (Tseng *et al*., 1968, 1977). 40,421 inhabitants of 37 villages of the regions were examined for skin lesions, peripheral vascular disorders and cancers. The study identified 7,418 cases of hyperpigmentation, 2,868 of keratosis (Type A/benign), 428 of skin cancer (squamous cell carcinoma, basal cell carcinoma, *in situ* squamous cell carcinoma, and Type B keratoses/intraepidermal carcinomas) and 360 cases of

Blackfoot disease. The incidence rates for keratosis and skin cancer were 183.5 and 10.6/1000, respectively. A control population of 7,500 people did not exhibit any of the above disorders.

The above exposed population was divided into "low", "mid" and "high" exposure groups based upon the well-water arsenic concentration in each village (<0.3, 0.3-0.6, and >0.6 ppm, respectively). A dose-response relationship was identified for the prevalence of skin cancer and Blackfoot disease (no dose-response data was presented for hyperpigmentation and keratosis). The prevalence of both disease was also found to increase with age. Males were found to have higher prevalence rates than females (male to female ratios for skin cancer and Blackfoot disease were 2.9 and 1.3, respectively).

Additional studies of chronic human arsenic exposure resulting in increased skin cancer or internal organ cancer incidence have been identified and reviewed (Fierz, 1965; Borgono and Greiber, 1972; Cebrian *et al*., 1983; Yue-Zhen *et al*., 1985; Chen *et al*., 1985, 1986; reviewed by US EPA, 1988).

Animal Studies

There were two animal inhalation studies on the carcinogenicity of arsenic available at the time the document *Report to the Air Resources Board on Inorganic Arsenic. Part B. Health Effects of Inorganic Arsenic Compounds* was written (CDHS, 1990). Berteau *et al.* (1977, 1978) exposed mice to a respirable aerosol of arsenic(III) (containing approximately 27 mg arsenic(III)/m^3) for 40 minutes/day for 26 days and 20 minutes/day thereafter. Inhaled doses were approximately 1.3 mg arsenic/kg/day and 0.69 mg arsenic/kg/day. No evidence of neoplasia was observed grossly in exposed animals.

In an inhalation study of arsenic trioxide, Glaser *et al*. (1986) exposed 20 rats for 18 months, at approximately 60 µg arsenic/kg/day and 40 rats at approximately 20 µg arsenic/kg/day. No tumors were observed in exposed animals. The report lacked important methodological details, including sampling to verify exposure levels. Also, the study tested fewer animals than required by standard cancer bioassay protocols.

In an arsenic (III) trioxide-treated group of 47 male hamsters, Pershagen *et al*. (1984) found three carcinomas: two of bronchi or lungs (an adenocarcinoma, and an anaplastic carcinoma) and one of larynx or trachea (a squamous cell carcinoma). These carcinomas were not statistically significant when considered in relation to the concurrently treated controls but were statistically significant when considering additional controls from the same colony ($p = 0.01$, one-tailed test). In female hamsters, benign lung tumors (adenomas) were induced by intratracheal instillation of a suspension of solid arsenic trioxide in a phosphate buffer (Ishinishi *et al*., 1983; Ishinishi and Yamamoto, 1983), but Ohyama *et al*. (1988) did not induce lung tumors in male hamsters similarly treated with arsenic trioxide or gallium arsenide.

Arsenic (V) has also induced tumors in animals. Calcium arsenate injected intratracheally induced lung adenomas in male hamsters (Pershagen and Bjorklund, 1985) and leukemia and lymphoma were produced by sodium arsenate by subcutaneous injection in mice (Osswald and Goerttler, 1971).

Among oral studies, only one study reported positive findings. Tumors, including adenocarcinomas of the skin, lung, and lymph nodes, were noted in mice given Fowler's solution (potassium arsenite), but the report lacks experimental details necessary for critical assessment (Knoth, 1966; as reviewed in U.S. EPA, 1984).

Other oral studies reported that arsenite (3 μg arsenic/l in drinking water) reduced the total tumor incidence in male and female white Charles River CD mice (Kanisawa and Schroeder, 1967), and enhanced the growth rate of "spontaneous" (common) mammary tumors in female inbred C3H mice (Schrauzer and Ishmael, 1974; Schrauzer *et al*., 1978).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Inhalation

The International Agency for Research on Cancer (IARC) evaluated arsenic in 1980 and classified "arsenic and arsenic compounds" in Group 1, which includes the "chemicals and groups of chemicals (which) are causally associated with cancer in humans." Ingestion of arsenic is associated with cancer at sites different from those associated with arsenic inhalation: ingestion is associated with skin cancer, while inhalation results in lung neoplasms.

In contrast, arsenic has not conclusively produced carcinogenesis in animals. Arsenic produces tumors in animals, but these tumors are rarely malignant. The few reports of carcinogenic effects of arsenic compounds in animals are seriously flawed. The hypothesis that arsenic may act as a tumor promoter has been tested but not proven in animals.

CDHS (1990) used human data for its cancer risk assessment of arsenic because (1) these data showed a strong, consistent association with increased respiratory cancer in epidemiologic studies, (2) quantitative exposure measurements were made in several of these studies, and (3) clear dose-response relationships were observed. No risk assessment has been conducted using animal data because the cancer bioassays using relevant routes of exposure have been negative and because no adequate inhalation bioassay has been published.

The quantitative cancer risk assessment for arsenic considered data from the occupational mortality studies of smelter workers in Anaconda, Montana by Welch *et al*. (1982), Higgins *et al*. (1985) and Lee-Feldstein (1986), and in Tacoma, Washington by Enterline *et al*. (1987a).

Oral

US EPA (1995) conducted a review of the available literature and identified the studies by Tseng *et al*. (1968, 1977) as the key references for quantifying ingested arsenic cancer potency. US EPA stated that these studies demonstrate a causal association between arsenic ingestion and an elevated risk of skin cancer. These data were considered reliable for the following reasons: 1) the study and control populations (40,421 and 7,500, respectively) were large enough to provide reliable estimates of the skin cancer incidence rates; 2) a statistically significant elevation in skin cancer incidence in the exposed population compared to the control population was observed many years after first exposure; 3) a pronounced skin cancer dose-response by exposure level was demonstrated; 4) the exposed and control populations were similar in occupational and socioeconomic status, with ingestion of arsenic-contaminated drinking water the only apparent difference between the two groups, and 5) over 70% of the observed skin cancer cases were pathologically confirmed.

Methodology

Inhalation

Data from the Anaconda and Tacoma smelters show nonlinear relationships between cumulative dose and the relative risk (or SMR) for death from lung cancer. These dose-response curves are concave downward (their slopes remain positive but decrease as exposure increases). Notwithstanding this observation, the staff of DHS used linear models for this risk assessment. In these models, the dose of arsenic was measured as cumulative $\mu g/m^3$-years; the response was measured as the relative increase in risk over the background (risk ratio). In addition, the models assume that the mechanism of carcinogenesis is a nonthreshold process.

The data from Enterline *et al*. (1987a), Higgins *et al*. (1985), and Lee-Feldstein (1986) were fitted to the model. The regression model used to achieve a linear extrapolation is described by the equation:

$$E[obs_i] = [\alpha + \beta(d_i)] \times Exp_i$$

where $E[\cdot]$ represents the expectation of a random variable, d_i represents the average cumulative dose of arsenic (in $\mu g/m^3$-years) for exposure group i, obs_i represents the observed number of deaths in exposure group i, Exp_i represents the expected number of deaths in group i based on the standard population, α represents the risk ratio predicted for a cumulative dose (d) of zero, and β is the slope parameter (in $[\mu g/m^3\text{-years}]^{-1}$).

To calculate unit risk, the staff of DHS selected the MLE (maximum likelihood estimate) slope and upper 95% confidence limit (UCL) based on use of the four lowest exposure groups from the Enterline *et al*. (1987a) analysis.
A risk assessment was also conducted using an adjustment for the strong interaction between arsenic and smoking observed in several occupational cohorts. The prevalence of smoking was independent of the level of arsenic exposure in the Anaconda cohort (Welch *et al*., 1982), but may have been higher than in the general population. Also, there appeared to be no reason for smokers

to be distributed differently among the exposure levels in the Tacoma cohort, hence smoking was assumed to be independent of arsenic exposure in this cohort as well.

Each dose-specific crude SMR was adjusted taking the low-dose SMR in each study as the baseline. Next, a nonsmokers' SMR and a smokers' SMR were derived. From the nonsmokers' SMR, observed and expected deaths among nonsmokers were inferred. Finally, a regression model was fitted to the inferred nonsmokers' data to find the slope of the line relating cumulative arsenic dose to excess relative risk. This procedure was applied to the data of Enterline *et al.* (1987a) under the assumption that the interaction between smoking and arsenic varies as a function of dose, and that the joint effects at low doses are multiplicative.

The MLE for β was 2.30×10^{-4} using the data on nonsmokers from the study by Enterline *et al.* (1987a). An 95% UCL was estimated and used in evaluating unit risks.

Risks were evaluated separately by sex and for four smoking categories: never, former, light (< 1 pack/day) and heavy smokers. Unit risks for these categories range from 400 to 8,400 per million persons, with upper bounds ranging from 630 to 13,000 per million.

The staff of DHS recommended that the range of risk for ambient exposures to arsenic be based on the 95% UCL predicted from fitting a linear model to the human data adjusted for interaction with smoking. The staff of DHS further recommended that the overall unit risk, 3.3×10^{-3} per $\mu g/m^3$, be considered the best estimate of the upper bound of risk.

Oral

A generalized multistage procedure with both linear and quadratic dose assumptions was used to predict the prevalence of skin cancer as a function of arsenic concentration in drinking water (d) and age (t), assuming exposure to a constant dose rate since birth. F(t,d) represents the probability of developing skin cancer by age t after lifetime exposure to arsenic concentration d. The procedure used is expressed as follows: $F(t,d) = 1 - \exp[-g(d)\, H(t)]$, where g(d) is a polynomial in dose with non-negative coefficients, and H(t) is $(t-w)^k$, where k is any positive real number, and $t > w$ for induction time w. The cancer potency calculation was based on skin cancer incidence data for Taiwanese males (Tseng *et al.*, 1968) because their skin cancer prevalence rates were higher than the females studied. The calculation was also based on several assumptions listed below.

1. The mortality rate was equal for both diseased (skin cancer) and nondiseased persons.

2. The population composition (with respect to skin cancer risk factors) remained constant over time, implying that there was no cohort effect.
3. Skin cancers were not surgically removed from diseased persons.

The population at risk was classified into 4 age groups (0-19, 20-39, 40-59 and ≥ 60 years of age) and three dose groups (0 - 0.3, 0.3 - 0.6 and > 0.6 ppm drinking water arsenic concentration) for males and females separately from the reported prevalence rates (Tseng *et al.*, 1968, 1977) as percentages. The assumption was made that the Taiwanese persons had a constant arsenic exposure from birth, and that males and females consumed 3.5 L and 2 L drinking water/day,

respectively. The multistage procedure was used to predict dose-specific and age-specific skin cancer prevalence rates associated with ingestion of inorganic arsenic. Both linear and quadratic model fitting of the data were conducted. The maximum likelihood estimate (MLE) of skin cancer risk for a 70 kg person drinking 2 L of water/day, adjusted for U.S. population survivorship by life-table analysis, ranged from 1 E-3 to 2 E-3 for an arsenic intake of 1 µg/kg/day. Expressed as a single value, the cancer unit risk for drinking water is 5 E-5 $(\mu g/L)^{-1}$; the corresponding cancer potency value is 1.5 E-0 $(mg/kg/day)^{-1}$.

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ASBESTOS

CAS No: 1332-21-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1998) except as noted)

Molecular weight	not applicable
Boiling point	decomposes
Melting point	decomposes at 600ºC (NIOSH, 1994)
Vapor pressure	not applicable
Air concentration conversion	not applicable

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.3 E-2 $(\mu g/m^3)^{-1}$ [1.9 E-4 (100 PCM fibers/m^3)$^{-1}$; see Appendix D]
Slope Factor: 2.2 E+2 $(mg/kg\text{-}day)^{-1}$
[Human occupational asbestos lung tumor and mesothelioma incidence data, excess relative risk model (CDHS, 1986).]

III. CARCINOGENIC EFFECTS

Human Studies

Asbestos has been consistently demonstrated to be carcinogenic in humans and is recognized as a human carcinogen by the International Agency for Research on Cancer (IARC, 1977; NRC, 1984; Ontario Royal Commission, 1984). In occupational cohort mortality studies, exposure to the three principal commercial forms of asbestos – chrysotile, amosite, and crocidolite – has been repeatedly linked with increased risks for lung cancer, mesothelioma and, to a lesser extent, other neoplasm, particularly gastrointestinal and laryngeal cancer (IARC, 1977; NRC, 1984). Occupational exposure to anthophyllite has been associated with an increased risk for lung cancer. Cigarette smoking acts synergistically with occupational exposure to asbestos in increasing the risk of lung cancer, but not mesothelioma (Hammond *et al.*, 1979; NRC, 1974).

Tremolite and actinolite are often contaminants of other ores and have not been extensively studied with respect to their biological effects in humans.

The epidemiologic studies on asbestos are extensive. These have been reviewed by the Consumer Product Safety Commission (1983), the National Academy of Sciences (NRC, 1984), Nicholson (1985), and the Ontario Royal Commission (1984). The relevant studies used in deriving the cancer risk for asbestos are summarized in the tables below.

Table 1: Summary of epidemiologic studies used in quantitative risk assessment for lung cancer.

Study	Cohort Occupation	Fiber type	Sex	Cohort Number	F/U	Lung Cancer Mortality		
						Exp	Obs	SMR
Finkelstein, (1983)	asbestos cement manufacturing	chrysotile, crocidolite	M	241	1963-80	3.3	20	606
Selikoff *et al*., (1979)	insulation	chrysotile, amosite	M	17,800	1967-76	93.7	390	416
Seidman *et al*., (1979)	insulation manufacturing	amosite	M	820	1961-76	21.9	83	380
Dement *et al*. (1982; 1983a-b)	textile products manufacturing	chrysotile	M	1,261	1940-75	9.8	33	336
Henderson and Enterline (1979)	asbestos manufacturing	chrysotile, amosite, crocidolite	M	1,075	1941-73	23.3	63	270
Newhouse and Berry (1979)	asbestos products manufacturing	chrysotile, amosite, crocidolite	M	4,600	1936-75	43.2	103	238
Newhouse and Berry (1979)	asbestos products manufacturing	chrysotile, amosite, crocidolite	F	922	1936-75	3.2	27	843
Nicholson *et al*. (1979)	mining	chrysotile	M	544	1961-77	11.1	25	225
Peto (1977; 1980)	textile products manufacturing	chrysotile	M	822	1933-74	22.9	49	214
McDonald *et al*. (1984)	friction products	chrysotile	M	3,177	1938-77	49.1	73	1149
Weill *et al*. (1979)	asbestos cement manufacturing	chrysotile, crocidolite	M	5,645	1940-74	49.2	51	104
Berry and Newhouse (1983)	friction products	chrysotile, crocidolite	M	7,474	1942-80	139.5	143	103
Berry and Newhouse (1983)	friction products	chrysotile, crocidolite	F	3,708	1942-80	11.3	6	50
Rubino *et al*. (1980)	mining	chrysotile	M	952	1946-75	8.7	9	103
McDonald *et al*. (1980)	mining	chrysotile	M	9,767	1926-75	184	230	125

F/U = Follow-up, Exp = Expected, Obs = Observed, SMR = Standard Mortality Ratio

Table 2. Summary of epidemiologic studies used in quantitative risk assessment for mesothelioma

Study	Cohort Occupation	Fiber type	Cohort Number	Sex	F/U	No. Mesotheliomas	
						Pleural	Peritoneal
Selikoff *et al*. (1979)	Insulation	Chrysotile, Amosite	17,800	M	1967-76	63	112
Peto (1980)	Textile manufacturing.	Chrysotile	822	M	1933-74	9	0
Seidman *et al*. (1979)	Insulation manufacturing.	Amosite	820	M	1961-76	7	7
Finkelstein (1983)	Asbestos Cement manufacturing.	Chrysotile, Crocidolite	241	M	1963-80	6	5

F/U = Follow-up

Animal Studies

Many studies using laboratory animals have been conducted to investigate the carcinogenic potential of various forms of asbestos administered by inhalation, by ingestion (in food or drinking water), and via intraperitoneal and intrapleural injection or deposition. The animal studies have been reviewed by Condie (1983), NRC (1984), and Nicholson (1985).

Gross *et al*. (1967) exposed male rats to airborne chrysotile to a mean concentration of 86 mg/m^3, 30 hours/week for their lifetime and found that a large number of treated animals developed malignant lung tumors (24/72; adenocarcinomas, squamous cell carcinomas and fibrosarcomas, compared to 0/39 for controls) and one developed mesothelioma. Reeves *et al*. (1971) exposed rats, rabbits, mice and hamsters to amosite, crocidolite or chrysotile at a concentration of approximately 48 mg/m^3, 16 hours/week for up to two years. No lung tumors were reported in control or treated hamsters, mice or rabbits. Lung tumors were found in 2/31 rats exposed to crocidolite; no lung tumors were reported for the controls or other fiber exposure groups. In a similar study, Reeves *et al*. (1974) found that gerbils, guinea pigs, hamsters and rabbits exposed to amosite, crocidolite or chrysotile at a concentration of approximately 49 mg/m^3, 16 hours/week for up to two years did not develop lung tumors. Lung tumor incidences in rats exposed to chrysotile, amosite or crocidolite were 3/43, 4/46 and 3/36, respectively; no lung tumors were noted in the 12 controls. Lung tumors were noted in the chrysotile-exposed mice (2/18), but this incidence was not significantly increased compared to controls (1/6).

Wagner *et al*. (1974) compared the carcinogenic effect of five different Union Internationale contre le Cancer (UICC) asbestos samples, amosite, anthophyllite, crocidolite, chrysotile (Canadian), and chrysotile (Rhodesian). Exposure varied from 9.7 to 14.7 mg/m^3 from one day to 24 months, although all animals were followed for their lifetimes. Malignant lung tumors (adenocarcinoma and squamous cell carcinoma) were found in rats from all five asbestos exposure groups (11/146, 16/145, 16/141, 17/137, and 30/144, for the respective fiber types). All but the group exposed to Rhodesian chrysotile had at least one animal demonstrating a mesothelioma. Davis *et al*. (1978) exposed rats to chrysotile (2 or 10 mg/m^3), crocidolite (5 or 10 mg/m^3), or amosite (10 mg/m^3).

Twenty percent (8/40) of the animals exposed to 10 mg/m^3 chrysotile developed malignant lung tumors. One out of 40 animals exposed to the low concentration of chrysotile (2 mg/m^3) developed a peritoneal mesothelioma. Neither amosite nor crocidolite induced malignant lung tumors in the rats.

Several long-term ingestion studies have been conducted on asbestos. Cunningham *et al*. (1977) and Gross *et al*. (1974) fed diets containing chrysotile to rats. Neither study indicated that ingested chrysotile induced an increased incidence of intestinal tumors. Smith *et al*. (1980) reported that amosite given to male and female hamsters via their drinking water did not significantly increase the incidence of cancer. In a lifetime rat feeding study using a diet containing 10% chrysotile, there was some evidence of penetration of asbestos into the colonic mucosa and possible cytotoxicity to the colonic tissues (Donham *et al*., 1980). McConnell *et al*. (1983a, b) reported on a number of studies conducted by the National Toxicology Program (NTP) in which hamsters and rats were fed diets containing different types of asbestos. These studies were generally negative; however, NTP (1985) stated that there was some evidence of carcinogenicity in male rats exposed to intermediate-range (size) chrysotile asbestos as indicated by an increased incidence of adenomatous polyps in the large intestine.

A number of studies have shown that intrapleural administration of asbestos results in the development of mesothelioma (Donna, 1970; Reeves *et al*., 1971; Pylev and Shabad, 1973; Shabad *et al*., 1974; Smith and Hubert, 1974). Chrysotile, amosite, anthophyllite, and crocidolite have all induced mesothelioma when administered intrapleurally to rats, rabbits, and/or hamsters.
Wagner *et al*. (1973) demonstrated a dose-response relationship between the amount of asbestos (superfine chrysotile or crocidolite) administered intraperitoneally and incidence of mesothelioma in treated rats. Stanton and Wrench (1972) showed that commercial asbestos fibers as well as glass and other mineral fibers implanted onto the pleural surface of rats were able to induce formation of mesotheliomas.

Maltoni and Annoseia (1974) found that intraperitoneal injection of crocidolite into Sprague-Dawley rats resulted in over 60% developing mesothelial tumors. Pott and Friedrichs (1972) and Pott *et al*. (1976) reported that several commercial varieties of asbestos, as well as other fibrous materials, induced peritoneal mesotheliomas in mice and rats injected intraperitoneally.

In summary, the animal studies clearly indicated that asbestos is carcinogenic in a variety of species when administered by inhalation or directly into the peritoneum or pleural space. Results of bioassays where asbestos was ingested are inconclusive.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The IARC and US EPA carcinogen classifications for asbestos are 1 and A, respectively – that is, a known human carcinogen. Ample data exists indicating that asbestos induces lung tumors and mesotheliomas in both humans and animals. In cases where both human and animal cancer data exist for a substance and both are suitable for quantitative risk assessment, use of the human data

is preferred by OEHHA. The cancer quantitative risk assessment of asbestos was therefore based on human occupational lung cancer and mesothelioma incidence data, since both posed potential population risks at ambient concentrations of asbestos.

Methodology

The CDHS (1986) risk assessment relies extensively on work done by the Consumer Product Safety Commission (1983), the National Academy of Sciences (NRC, 1984), Nicholson (1985), and the Ontario Royal Commission (1984). As with these risk assessments, the DHS risk assessment on asbestos was based exclusively on the results of occupational epidemiologic studies.

Animal bioassay data were excluded from this analysis as there are numerous epidemiologic studies of populations occupationally exposed to asbestos, which contain or have been supplemented with exposure data adequate for purposes of quantitative risk assessment.

DHS adapted linear models developed and/or used in the work cited above to estimate risks of mesothelioma and lung cancer to the general population. The models extrapolate risks observed in numerous occupationally exposed cohorts to lower levels of asbestos found in the general environment. In this case the range of extrapolation was four to five orders of magnitude. Results are presented below.

Table 3: Estimated lifetime risks of lung cancer and mesothelioma due to continuous exposure to 0.0001 fibers/cm^3 of asbestos (expressed as cases per million population)

Exposure Group	Lung Cancer	Mesothelioma
Male Smokers	11 (110)	24 (120)
Female Smokers	5 (50)	32 (160)
Male Nonsmokers	2 (15)	32 (160)
Female Nonsmokers	1 (6)	38 (190)

Numbers in parentheses represent approximate upper 95% confidence limits.

The use of excess lung cancer lifetime risk values between 11 and 110 per million for 0.0001 fibers/cm^3***** of asbestos exposure were recommended. (*****Fiber/cm^3 = asbestos fibers ≥ 5μm in length, ≥ 0.3 μm in width, with a length/width ratios of ≥ 3:1. Such fiber counts can be converted to total fibers measurable by transmission electron microscopy (TEM) by multiplying by 100 to 1,000. Therefore, 0.0001 (PCM) fibers/cm^3 = 0.01 to 0.1 TEM fibers/cm^3 = 10,000 to 100,000 TEM fibers/m^3.) For mesothelioma, recommended lifetime risk values are between 18 and 190 per million for each 0.0001 fibers/cm^3***** of asbestos exposure. These recommendations are based on best estimates and approximate upper confidence limits for the groups theoretically at highest risk for lung cancer and mesothelioma: male smokers and female nonsmokers, respectively. The above values represent theoretical lifetime risk of cancer assuming continuous average daily exposure to 0.0001 fibers/cm^3***** throughout life.

These fibers can be measured by phase contrast microscopy (PCM) and for historical reasons represent the basis for all recent asbestos risk assessments. The unit risk factor selected for asbestos is 1.9 E-4 (100 PCM fibers/m^3)$^{-1}$ and in units of μg/m^3, 6.3 E-2(μg/m^3)$^{-1}$. This was based on mesothelioma in female nonsmokers. The original TAC unit risk value has been converted

using a factor of 0.003 µg asbestos = 100 asbestos fibers which has been derived from information published by U.S. EPA (1985) (see Appendix D). The number of asbestos fibers associated with a given mass of asbestos can vary appreciably. Also, U.S. EPA (1985) has stated that this conversion factor is the geometric mean of measured relationships between optical fiber counts and mass airborne chrysotile in several published studies. The range of the conversion factor between the different studies is large (0.0005 - 0.015 µg asbestos/100 asbestos fibers) and carries with it an appreciable uncertainty. Use of the unit risk factor listed in the asbestos TAC document [1.9 E-4(100PCM fibers/m^3)$^{-1}$], wherever possible, will result in a more precise risk estimation.

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BENZENE

CAS No: 71-43-2

I. PHYSICAL AND CHEMICAL PROPERTIES (from HSDB, 1998)

Molecular weight	78.1
Boiling point	80.1° C
Melting point	5.5° C
Vapor pressure	100 mm Hg @ 26.1° C
Air concentration conversion	1 ppm = 3.2 mg/m^3 @ 25° C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor	2.9 E-5 $(\mu g/m^3)^{-1}$
Slope Factor	1.0 E-1 $(mg/kg\text{-}day)^{-1}$

[Human occupational exposure leukemia incidence (Rinsky *et al.*, 1981); excess risk calculated using a Weighted Cumulative Exposure/relative risk procedure (CDHS, 1984).]

III. CARCINOGENIC EFFECTS

Human Studies

Case studies of workers exposed to benzene were responsible for generating the hypothesis that benzene causes leukemia in humans. Epidemiological studies performed to test the hypothesis supported the causal nature of the benzene-leukemia association. A summary of some of the more salient features from 23 major epidemiologic studies are shown in Table 1.

Table 1: Epidemiologic studies of carcinogenicity in humans.

Study	Population Studied	Duration	Results[1]
Tabershaw Cooper Assoc. (1974)	petroleum industry workers		Increase in rate of lymphomas (NS)
Thorpe (1974)	petroleum industry workers	1962-1972	Leukemia SMR=121 (NS) (SMR in worker controls = 60)
Aksoy *et al*. (1974, 1976)	Shoemakers exposed to 210-650 ppm benzene	1967-1975	Annualized crude rate of acute leukemia 2-fold greater than expected
McMichael *et al*. (1976)	rubber industry workers		Excess in mortality from: chronic lymphatic leukemia, myelogenous leukemia, lymphosarcoma
Vigliani (1976)	patients with benzene hemopathy; exposures estimated at 200-500 ppm	1942-1975 1959-1974	Leukemia incidence: 11/66 13/135 Estimated relative risk (RR)=20

Table 1 (continued): Epidemiologic studies of carcinogenicity in humans.

Study	Population Studied	Duration	Results[1]
Infante *et al*. (1977)	748 rubber industry workers followed to 7/75		1940-1949: 7 cases of myeloid/monocytic leukemia SMR=506 (U.S. white males) SMR=474 (worker controls)
Ott *et al*. (1978)	benzene workers (Dow); 594 workers followed to 1973	1938-1970	2 deaths from anemia (one pernicious, one aplastic); 3 deaths from leukemia. Mortality results NS but leukemia rates exceed expectation ($p < 0.05$)
Fishbeck *et al*. (1978)	10 chemical workers exposed to benzene	1953-1963	Changes in blood but '… no persisting significant adverse health effects.'
Brandt *et al*. (1978)	case-control study of 50 acute non-lymphocytic leukemia	1969-1977	History of exposure to petroleum products among cases.
Vianna and Polan (1979)	workers in NY State exposed to benzene		RR=2.1 lymphosarcoma RR=1.6 reticulum cell sarcoma RR=1.6 Hodgkin's Disease For workers > 45 years old, the observed number of cases was SS greater than the expected number.
Greene *et al*. (1979)	U.S. Gov't Printing Office workers		Higher proportion of deaths from multiple myeloma, leukemia, and Hodgkin's disease related to exposure to benzene (SS).
Linos *et al*. (1980)	case-control study of 138 leukemics		4 cases found, 3 were chronic lymphocytic leukemia. RR = 3.3 (NS)
Schottenfeld *et al*. (1981)	worker cancer registry compiled by API		Incidence of lymphocytic leukemia & multiple myeloma increased (SS).
Rushton and Alderson (1981)	petroleum refinery workers		Risk of leukemia increased 2-fold in high and medium benzene exposed vs. low exposed ($p = 0.05$).
Rinsky *et al*. (1981)	continuation of follow-up of Infante study		SMR=560 leukemia SMR=2100 leukemia in workers with 5 or more years exposure.
Thomas *et al*. (1982)	refinery workers		SMRs for multiple myeloma and other lymphomas elevated (SS)
Hanis *et al*. (1982)	refinery and chemical workers		SMR for cancer of the lymphopoietic tissues elevated but NS.
Decoufle *et al*. (1983)	chemical plant workers; 259 men followed through 1977	1960-1974	SMR=377 (SS) L & H SMR=682 (SS) leukemia
Tsai *et al*. (1983)	454 refinery workers		No deaths observed from L & H cancer; 0.42 expected (NS).
Arp *et al*. (1983)	rubber industry workers		For lymphocytic leukemia: RR=4.5 (NS) benzene exposure RR=4.5 (NS) other solvent exposure
Environmental Health Associates (Wong *et al*., 1983)	chemical workers	1946-1975	SMRs elevated (NS) for L & H cancer, leukemia, non-Hodgkin's lymphoma; RR=3.2 (SS) L & H cancer (vs. worker control). Dose-response trend found, SMRs for lung cancer and several other cancers elevated (NS)

[1] NS = not statistically significant; SS = statistically significant ($p < 0.05$)
SMR = standardized mortality ratio; L & H = lymphocytic and hematopoietic cancer

Animal Studies

Available experimental data prior to 1976 has been summarized by Maltoni *et al.* (1983). These earlier studies did not provide evidence for carcinogenicity in animals. Since then two significant series of bioassay studies have been reported, those of Maltoni *et al.* (1983) and the National Toxicology Program (NTP, 1983).

In a series of oral studies, reported by Maltoni *et al.* (1983), rats were administered benzene via gavage tube. Male and female Sprague-Dawley rats were administered benzene at 0, 50 or 250 mg/kg benzene in olive oil, 4 to 5 times per week, for 52 weeks. Dose-related increases were observed for Zymbal gland carcinoma in the female rats only. In Sprague-Dawley rats administered 0 and 500 mg/kg benzene in olive oil, 4 to 5 times per week for 104 weeks significant increases relative to controls were reported for Zymbal gland carcinoma (males and females), and oral cavity carcinoma (males and females).

Maltoni *et al.* (1983) also chronically exposed pregnant Sprague-Dawley rats (breeders) and their offspring to high concentrations of benzene using a complex dosing regimen. Exposure concentrations ranged from 200 to 300 ppm. Among the breeders, a slight increase (not significant) in the incidences of Zymbal gland carcinoma and mammary tumors were reported. Among offspring, significant increased incidences in Zymbal gland tumors and non-significant increases in cancers of oral and nasal cavity, mammary gland and liver were reported. Selected study data are listed in Table 2.

Table 2: Maltoni *et al.* (1983) benzene rat bioassay summary

Organ (Site)	Tumor Type	Route of Exposure	Sex	Dose (mg/kg-day)	Cochran-Armitage Linear Trend Test	Fisher Exact Test	Difference in Cancer Attack Rate per 100 (Dose-Control)
Zymbal gland	carcinomas	gavage[1]	F		$p < 0.001$		
				13.9	----	$p = 0.25$	6.7
				66.7	----	$p = 0.003$	25.0
Hemolympho -reticular	'leukemias'	gavage[1]	M		$p = 0.005$		
				13.9	----	$p = 1.0$	0.0
				66.7	----	$p = 0.078$	12.1
Mammary	carcinomas	gavage[1]	F		$p = 0.091$		
				13.9	----	$p = 0.5$	3.3
				66.7	----	$p = 0.178$	11.9
Zymbal gland	carcinomas	gavage[2]	F	321.4	N/A (single dose level)	$p = 0.007$	15.0
	carcinomas	gavage[2]	M, F	321.4		$p < 0.001$	14.0
Oral Cavity	carcinomas	gavage[2]	M	321.4	N/A (single dose level)	$p = 0.003$	17.5
	carcinomas	gavage[2]	M, F	321.4		$p < 0.001$	13.7
Zymbal gland	carcinomas	inhalation[3]	F	17	N/A (single dose level)	$p = 0.27$	3.9

Table 2 (continued): Maltoni *et al.* (1983) benzene rat bioassay summary.

Organ (Site)	Tumor Type	Route of Exposure	Sex	Dose (mg/kg-day)	Cochran-Armitage Linear Trend Test	Fisher Exact Test	Difference in Cancer Attack Rate per 100 (Dose-Control)
Zymbal gland	carcinomas	inhalation[4]	M, F	16.4	N/A (single dose level)	$p = 0.002$	5.1
Liver	hepatomas	inhalation[5]	F	1.42	N/A (single dose level)	$p = 0.022$	5.1

Source: CDHS (1984) TAC document. Results of Maltoni *et al.* (1983) studies.

[1]Experiment 1: (#BT901), animals dosed for 52 weeks.
[2]Experiment 2: (#BT902), 92-week interim results, dosing to be carried out for 104 weeks, 118-week interim results.
[3]Experiment 3: (#BT4004), inhalation exposure of 13-week old breeder rats for 104 weeks, 118-week interim results.
[4]Experiment 4: (#BT4004), inhalation exposure of 12-day old embryos for 104 weeks. Dose *in utero* not considered, 118-week interim results.
[5]Experiment 5: (#BT4006), inhalation exposure of 12-day old embryos for 15-weeks. Dose *in utero* not considered, 118 week interim results.

The National Toxicology Program (NTP, 1983) conducted a 2-year bioassay on the carcinogenic effects of oral (gavage) exposure to benzene in F344 rats and $B6C3F_1$ mice. Female rats and mice were administered benzene in corn oil at doses of 0, 25, 50, and 100 mg/kg, 5 days/week, for 103 weeks. Male rats and mice were administered benzene at doses of 0, 50, 100, and 200 mg/kg, 5 days/week for 103 weeks. In F344 rats, statistically significant dose-related increases in the incidences of neoplasms were reported for the oral cavity (males and females), Zymbal gland (males and females), uterus (females) and skin (males). In $B6C3F_1$ mice, statistically significant dose-related increases in the incidences of tumors were reported for the Zymbal gland (males and females), ovary (females), mammary gland (females), Harderian gland (males and females), lung (males and females), preputial gland (males) and for lymphoma/leukemia combined (males and females). Selected study data are listed in Table 3.

Table 3: Summary of NTP bioassay results for significant neoplasms[1,2].

Organ (site)	Tumor Type	Species	Sex	Cochran-Armitage Trend Test	Fisher Exact Test	Difference in Cancer Attack Rate per 100 (High Dose - Control)
Zymbal gland	squamous cell carcinoma	rat	M	$p < 0.001$	$p < 0.001$	30
		rat	F	$p < 0.001$	$p < 0.001$	28
		mouse	M	$p < 0.001$	$p < 0.001$	43
		mouse	F	$p = 0.022$	$p = 0.121$	6
Skin	squamous cell carcinoma	rat	M	$p = 0.007$	$p = 0.003$	16
		mouse	M	$p = 0.028$	$p = 0.121$	6
Lip	squamous cell carcinoma	rat	M	$p = 0.012$	$p = 0.003$	16
Tongue	squamous cell carcinoma	rat	M	$p = 0.078$	$p = 0.059$	8
		rat	F	$p = 0.078$	$p = 0.059$	8
Oral cavity	squamous cell carcinoma	rat	M	$p = 0.006$	$p = 0.006$	14
		rat	F	$p = 0.011$	$p = 0.028$	10
Hematopoietic system	malignant lymphomas or leukemia	mouse	M	$p = 0.006$	$p = 0.005$	23
Lung	alveolar/bronchiolar carcinoma	mouse	M	$p = 0.028$	$p = 0.020$	19
		mouse	F	$p = 0.021$	$p = 0.013$	12
Preputial gland	all carcinomas	mouse	M	$p < 0.001$	$p < 0.001$	63
Mammary gland	carcinomas	mouse	F	$p < 0.001$	$p < 0.001$	20
	carcinosarcoma	mouse	F	$p = 0.006$	$p = 0.059$	8
Harderian gland	adenoma or carcinoma	mouse	M	$p = 0.001$	$p < 0.001$	27
	carcinoma	mouse	F	$p = 0.004$	$p = 0.059$	8
Ovary	granulosa cell tumor or carcinoma	mouse	F	$p = 0.003$	$p = 0.017$	15

[1]Source: CDHS (1984) TAC document. Results of NTP gavage study (NTP, 1983).
[2]Comparison of highest dose group with control. Mice of both sexes and female rats were administered 71.4 mg/kg-day by gavage, male rats were administered 143 mg/kg-day.

In summary, benzene has been shown to be carcinogenic in animal studies either by inhalation or oral routes of administration. Cancer was observed at multiple sites in these studies.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

CDHS (1984) used both animal and human data for this quantitative risk assessment. The cancer potency estimates based on animal data were obtained from data on Zymbal gland carcinomas in rats exposed via inhalation or gavage by Maltoni *et al.* (1983); and Zymbal gland carcinomas, preputial gland carcinomas, and lymphoma or leukemia in male mice or mammary carcinomas in female mice exposed by gavage by the National Toxicology Program (NTP, 1983). The epidemiological studies analyzed were those of leukemia in workers exposed to benzene via inhalation reported by Infante *et al.* (1984), Rinsky *et al.* (1981), Aksoy *et al.* (1974; 1976), Aksoy (1977), and Ott *et al.* (1978).

Methodology

A summary of the calculated low-dose risk assessments from a number of animal and human studies is shown in Table 4. The epidemiological data were analyzed using a linear nonthreshold model to estimate risk. The animal data were analyzed by fitting a linearized multistage procedure to dose-response data from the animal cancer bioassays. The results of the U.S. EPA's Carcinogen Assessment Group (CAG) epidemiologic-based assessments are also included in the table for comparative purposes (U.S. EPA, 1979).

CDHS (1984) recommended that cancer potency values in the range of 24 to 170 × 10^{-6} per ppb (i.e., 0.024 to 0.17/ppm) be used in estimating risks from low level exposure to benzene. Slightly higher upper bound values were obtained when shortened survival times in the studies analyzed were taken into account. Assuming that humans breathe 20 m^3 per day and weigh 70 kg and that an air concentration of 1 ppm benzene is equivalent to 3.25 mg/m^3, the CDHS range of potency values is equivalent to 0.03 to 0.2 $(mg/kg\text{-}day)^{-1}$.

In 1988, CDHS (under Proposition 65) recommended that the potency value of 0.1 $(mg/kg\text{-}day)^{-1}$ [unit risk = 2.9 × 10^{-5} $(\mu g/m^3)^{-1}$] be used to estimate risk specific intake levels from exposure to benzene. This value falls within the range of estimates derived by CDHS (1984) and the U.S. EPA (1979, 1985), and is the upper 95% confidence bound estimate from the analysis of human data considered most credible by the U.S. EPA.

Subsequent to the development of the benzene TAC cancer unit risk value, OEHHA described new benzene occupational exposure cancer epidemiology data in the 2001 Public Health Goal (PHG) for Benzene in Drinking Water document (OEHHA, 2001)

Yin *et al.* (1987) reported on a large retrospective cohort study of benzene-exposed workers in China. The study examined 28,460 exposed workers from 233 factories and 28,257 control workers from different industries. Thirty leukemia cases were identified (23 acute, 7 chronic) in the exposed workers compared with four cases in the unexposed controls (SMR 574, $p < 0.01$). Exposure estimates from grab-samples taken at the time of the survey ranged from 3 to 313 ppm with the majority of exposures in the range of 16 to 157 ppm.

A number of detailed reports describing further study and analysis of the Chinese Worker Cohort have been published. Yin *et al.* (1994) reported that the cohort had been expanded to include 74,828 benzene-exposed workers (since 1949) and 35,805 controls from 712 factories located in 12 Chinese cities. Dosemeci *et al.* (1994) described the exposure assessment methods. Quantitative estimates of benzene exposure took into account job title and assignment to individual work units, and reflected exposures of individual workers. Li *et al.* (1994) investigated gender differences in hematopoietic and lymphoproliferative disorders and other cancers among the benzene-exposed cohort. No statistically significant differences in cancer mortality were observed for males versus females, although the number of cases for most endpoints was small.

Travis *et al.* (1994) reported on the hematopoietic malignancies and other blood disorders in the benzene-exposure workers in China. Eighty-two hematopoietic neoplasms and related disorders were observed, including 32 cases of acute leukemia, seven cases of myelodysplastic syndromes,

nine cases of chronic granulocytic leukemia, 20 cases of malignant lymphoma, and nine cases of aplastic anemia. In the control workers, 13 hematological malignancies were observed, including six leukemias. Yin *et al*. (1996) reported the overall cancer findings among the expanded benzene-exposed and control worker cohorts. An increased incidence in the benzene-exposed group compared to controls was observed for leukemia (RR 2.6, 95 percent CI = 1.3 to 5.0), malignant lymphoma (RR 3.5, 95 percent CI = 1.2 to 14.9), and lung cancer deaths (RR 1.4, 95 percent CI = 1.0 to 2.0). Among leukemia cases, incidence of acute myelogenous leukemia was increased in the benzene-exposed group (RR 3.1, 95 percent CI = 1.2 to 10.7). Significant increases were also reported for aplastic anemia and myelodysplastic syndromes.

The best upper-bound estimates of leukemia risk resulting from continuous lifetime air exposures of the general population to benzene in the benzene PHG document (OEHHA, 2001) were similar for the U.S. rubber workers (0.044 ppm^{-1}), the Chinese workers (0.056 ppm^{-1}), and for the mean of the two studies combined (0.050 ppm^{-1}). The three lifetime risk estimates all convert to a population-based cancer potency of 0.1 $(mg/kg\text{-}d)^{-1}$ for oral exposures after rounding, which can be scaled to 0.05 $(mg/kg\text{-}d)^{-1}$ for inhalation exposures. These values are similar to the TAC benzene cancer potency value of 0.1 $(mg/kg\text{-}d)^{-1}$.

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Table 4: Summary of benzene low-dose risk assessments.

Study	Route of Exposure	Lifetime[a] TWA Dosage	Tumor Type	Species	Sex	Type of Analysis	Multi-Stage Model for Human Equivalent Cancer Risk/ppb Benzene
NTP (1983)	Gavage	17.9 mg/kg-day[b]	Zymbal gland carcinomas	Mouse	M	Crude Attack Rate	MLE: 7.4×10^{-6} 95% UCL: 34×10^{-6}
						Lifetable Adj. Rate	MLE: 6.9×10^{-6} 95% UCL: 47×10^{-6}
NTP (1983)	Gavage	17.9 mg/kg-day[b]	Preputial gland carcinomas	Mouse	M	Crude Attack Rate	MLE: 78×10^{-6} 95% UCL: 170×10^{-6}
						Lifetable Adj. Rate	MLE: 140×10^{-6} 95% UCL: 340×10^{-6}
NTP (1983)	Gavage	17.9 mg/kg-day[b]	Lymphoma or Leukemia	Mouse	M	Crude Attack Rate	MLE: 49×10^{-6} 95% UCL: 81×10^{-6}
						Lifetable Adj. Rate	MLE: 170×10^{-6} 95% UCL: 230×10^{-6}
NTP (1983)	Gavage	17.9 mg/kg-day[b]	Mammary carcinomas	Mouse	F	Crude Attack Rate	MLE: 32×10^{-6} 95% UCL: 57×10^{-6}
						Lifetable Adj. Rate	MLE: 61×10^{-6} 95% UCL: 92×10^{-6}
Maltoni *et al*. (1983)	Gavage	13.9 mg/kg-day[c]	Zymbal gland carcinomas	Rat	F	Crude Attack Rate	MLE: 26×10^{-6} 95% UCL: 42×10^{-6}
Maltoni *et al*. (1983)	Inhalation[d]	16.45 mg/kg-day[e]	Zymbal gland carcinomas	Rat	M, F	Crude Attack Rate	MLE: 6.4×10^{-6} 95% UCL: 12×10^{-6}
Infante *et al*. (1977)	Inhalation	2.81 ppm[f] (2.99 mg/kg-day)	Leukemia (Myelocytic or Monocytic)	Human	M	Fatal Tumor Life Table	15×10^{-6}
Rinsky *et al*. (1981)	Inhalation	2.81 ppm[f] (2.99 mg/kg-day)	Leukemia (Myelocytic or Monocytic)	Human	M	Fatal Tumor Life Table	48×10^{-6}
Askoy *et al*. (1974, 1976,1977)	Inhalation	4.22 ppm[f] (4.49 mg/kg-day)	Leukemia	Human	M	Fatal Tumor	20×10^{-6}
Ott *et al*. (1978)	Inhalation	0.171 ppm[f] (0.182 mg/kg-day)	Leukemia	Human	M	Fatal Tumor	46×10^{-6}
CAG (US EPA, 1979)	Inhalation		Leukemia	Human	M	Fatal Tumor	24×10^{-6}

Assumptions: 60 kg person, human inhalation at 20 m^3/day. MLE = Maximum likelihood estimate. 95% UCL = 95% upper confidence limit on risk for provided dose.

[a] Dosages provided without scaling factors.

[b] Lowest dose used in three dose risk assessment, Cochran-Armitage linear trend test for these tumors: preputial gland, $p < 0.001$; Zymbal gland, $p < 0.001$; lymphoma or leukemia, $p = 0.035$; mammary carcinoma, $p < 0.001$.

[c] Lowest dose used in two point risk assessment, Cochran-Armitage linear trend test for these tumors, $p < 0.001$.

Table 4 (continued): Summary of benzene low-dose risk assessments.

[d] Pregnant Sprague-Dawley rats from the twelfth day of pregnancy at a concentration of 200 ppm, 4 hours/day, 5 days/week to delivery, then offspring assumed to be exposed to 200-300 ppm 4-7 hours/day, 5 days/week for 104 weeks. Exposure *in utero* not calculated for total lifetime dosage.
[e] Provided for comparative purposes.
[f] Estimated lifetime dosage by the U.S. EPA-CAG (US EPA, 1979)

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BENZIDINE

CAS No: 92-87-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	184.2
Boiling point	402°C
Melting point	115-120°C
Vapor pressure	0.0005 mm Hg
Air concentration conversion	1 ppm = 7.53 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.4 E-1 $(\mu g/m^3)^{-1}$
Slope Factor: 5.0 E+2 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

Case *et al.* (1954) examined mortality among British chemical workers exposed to benzidine. Among the population examined (total number not specified), ten deaths were certified as due to bladder cancer. The death rate from bladder cancer in the male Welsh and English population predicts 0.72 deaths from this cause, giving a standard mortality ratio of 13.9 (10/0.72, $p < 0.001$). Non-fatal bladder cancers were also noted among the exposed population.

Mancuso and El-Atar (1967) examined the incidence of urinary tract cancer among 639 male employees of an Ohio plant where benzidine and β-naphthylamine were made, with potential exposure occurring between 1938 and 1939. Six cases of bladder cancer occurred among white workers, giving a population incidence of 204 per 10^5. The expected incidence of bladder cancer among Ohio white males is 4.4 per 10^5. The authors do not present the significance of the change in incidence because of exposure of workers to other compounds.

Zavon *et al.* (1973) conducted a prospective study of workers exposed to benzidine during its manufacture. The authors report on the health surveillance of all 25 employees of a chemical plant who were exposed to benzidine from 3 to 28 years (1930-1958). During the 13 year follow-up period (1957-1970), 11 cases of transitional cell bladder cancer and 2 cases of benign bladder tumors developed. Among the workers with bladder cancers,

two also developed kidney carcinomas and one a benign tumor of the kidney. No background tumor incidences were reported. The average exposure duration for workers with tumors was 13.6 years, whereas the average exposure duration for those without tumors was 8.9 years. Levels of urinary benzidine were measured at the beginning of the follow-up period of the study, with samples taken at the beginning and end of the work day and at the before the work week began. Mean levels were reported to be ~0.01 mg/l before shift, ~0.04 mg/l after shift, and ~0.004 mg/l Monday morning. Quantitative estimates of exposure have been based on these levels. Exposure conditions at the time of the sampling were reported to be representative of conditions in the previous years of plant operation. Air sampling at different locations in the chemical plant showed benzidine concentrations ranged from <0.007 to 17.6 mg/m^3. Potential confounding variables in the study include smoking and exposure to other carcinogens in the work environment such as β-naphthylamine, o-toluidine, and dichlorobenzidine.

Tsuchiya *et al.*(1975) report on the incidence of bladder cancer among 1303 Japanese workers employed in benzidine production or use. Among workers involved in the manufacture of benzidine 61/542 developed bladder cancer. Among workers involved in benzidine use, 11/761 developed bladder cancer. Exposure levels to benzidine and population background incidence of bladder cancer in the Japanese population were not provided in the study. A statistically significant difference in bladder cancer incidence was observed between workers involved in benzidine production versus those involved in benzidine use ($p < 10^{-10}$, Fisher's exact test).

Meigs *et al.* (1986) conducted a 30-year follow-up study of 597 male workers at a benzidine manufacturing plant in Connecticut. Workers were categorized based upon time of employment, but benzidine levels were not quantitated. Among workers in the high exposure category (> 2 years of employment) 6/105 developed bladder cancer. Among workers in the medium (6 mo.-2 yrs.) and low (1 day-6 mo.) exposure categories, 1/147 and 0/345 developed bladder cancer, respectively. Connecticut cancer statistics predict 1.77 cases of bladder cancer in an unexposed population of 597 people. A significant difference in incidence between high exposure workers and unexposed populations was found ($p < 0.003$).

Animal Studies

Saffioti *et al.*(1967) exposed Syrian Golden hamsters (30/sex/group) for life to 0 or 1000 ppm benzidine or benzidine dihydrochloride in feed, and examined them for evidence of liver tumors. Among animals treated with benzidine, 19/22 males and 6/26 females developed cholangiomatous liver tumors (none in controls). Among animals treated with benzidine dihydrochloride, 10/20 males and 12/27 females developed cholangiomatous liver tumors (none in controls). Liver tumor incidence was found to be significantly elevated in exposed animals in each group ($p < 0.001$, Fisher's exact test).

Griswold *et al* .(1968) treated female Sprague-Dawley rats with benzidine by oral gavage. Animals received 1.2 or 2.5 mg/dose (10 animals/group) or 3.5 or 5 mg/dose (20 animals/group) every 3 days, with a total of 10 administrations. After 9 months, surviving

animals were examined for tumors. Increased incidence of mammary carcinoma was found in benzidine treated groups, with 5/10, 7/9, and 4/5 showing tumors in the 1.2, 2.5, and 5 mg dose groups, respectively, versus 3/127 in control animals. There were no survivors among the animals receiving 3.5 mg benzidine. Miakawa and Yoshida (1975) fed female dd strain mice (50/group) diet containing either 0 or 0.2% benzidine for 280 days. Hepatocellular carcinomas were identified in 11 of 32 mice surviving 140 days or more, whereas no hepatocellular carcinomas were reported among control mice. The significance level of the difference in incidence was $p < 0.001$ by Fisher's exact test.

Littlefield *et al.*(1984) exposed male and female F_1 generation mice (BALB/c males × C57BL females) to benzidine in drinking water for life (~33 months). Mice from a cross of F_1 generation males and females were also exposed as above . Exposure levels and incidence of hepatocellular carcinomas are presented in Table 1. Significant differences in the incidence of hepatocellular carcinoma were observed in all exposed groups ($p < 0.05$, Fisher's exact test). Frith *et al.* (1980) also exposed F_1 and F_2 generation mice (BALB/c males × C57BL/6 females) to 30-400 ppm benzidine dihydrochloride in drinking water for 40, 60, or 80 weeks at which time animals were sacrificed. As in the Littlefield *et al.* (1984) study, animals showed a dose-dependent increase in hepatocellular carcinoma incidence. This effect was also shown to be dependent upon duration of exposure.

Table 1. Incidence of hepatocellular carcinoma in F_1 and F_2 generation mice (BALB/c × C57BL) exposed to benzidine in drinking water (Littlefield *et al.*, 1984).

hepatocellular carinoma incidence					
males			Females		
exposure level (ppm)	F_1	F_2	exposure level (ppm)	F_1	F_2
0	14/125	17/123	0	3/124	10/125
30	24/119	20/118	20	51/120	54/119
40	30/96	20/95	30	52/95	43/95
60	32/71	23/72	40	45/72	31/71
80	35/71	24/71	60	55/71	37/72
120	61/71	37/71	80	60/69	51/69
160	49/71	32/71	120	64/72	56/72

Vesselinovitch *et al.* (1975) treated male $B6C3F_1$ mice with feed containing 150 ppm benzidine dihydrochloride from weeks 6 to 45 of life. Groups of 50 mice thus treated were sacrificed at 45, 60, 75, or 90 weeks and examined for liver tumors. Hepatoma incidence was reported to be 8/50, 20/50, 31/50, and 35/50, respectively, at successive sacrifice times, while only one hepatoma was found among 98 control animals sacrificed at 90 weeks ($p < 0.001$; Fisher's exact test). Among the animals with hepatomas, the incidence of hepatocellular carcinoma was 2/50, 5/50, 14/50, and 24/50, respectively, at the successive sacrifice times.

Two other feeding studies have been conducted. Boyland *et al.* (1954) found cases of hepatocellular carcinoma in rats fed diet containing 0.017% benzidine or benzidine plus

tryptophan for life. Inadequate study size, data on controls and poor survival, however, limit the usefulness of this study. Marhold *et al.* (1968) found no tumors in lifetime benzidine feeding study, but poor survival also limits the study's value.

Zabezhinski (1970) exposed 48 albino rats (male and female numbers not specified) to 10-20 mg/m^3 benzidine aerosol for 4 hours/day, 5 days/week for 20 months. Among animals surviving at 13 months, 5/28 developed leukemia (0/21 untreated; p =0.052)
Tumors have also been observed in animals injected subcutaneously with benzidine. They include hepatocellular carcinomas, Zymbal gland tumors and injection-site tumors (Spitz *et al*., 1950; Bonser *et al*, 1956; Pliss, 1964; Prokofjeva, 1971). Intraperitoneal injection of benzidine resulted in the induction of mammary tumors in a single study (Morton *et al*, 1981).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The data presented by Zavon *et al*. (1973) are the only human cancer data appropriate for the development of a cancer potency value for benzidine. The US EPA (1986, 1987, 1988), Allen *et al.* (1987), and CDHS (1988) have each provided estimates of cancer potency based on human data. However, different assumptions made in the calculation of exposure levels has resulted in different estimates of the cancer potency. Potencies derived in these studies assume that cancer risk is proportional to cumulative exposure. Previously, IARC (1982) had suggested that benzidine cancer risk be based on the assumption that the empirical distribution of cumulative incidence rate is a function of the duration of continual exposure. Derivations of cancer potencies from the human data using these different methodologies and exposure assessments are described in the *Methodology* section below.

Cancer potency values have also been derived from animal studies, in particular those of Griswold *et al*. (1968), Miakawa and Yoshida (1975), Saffioti *et al* (1967), and Littlefield *et al*. (1984). Resulting potency estimates were well below those derived from the human data, suggesting humans may be more sensitive to the carcinogenic effects of benzidine, and therefore animal data are not appropriate for use in the establishing a cancer potency value. CDHS has based its benzidine cancer potency value on the human data of Zavon *et al*. (1973) using exposure level assessment modifications of Allen *et al.* (1987) and the methodology of US EPA (1986, 1987, 1988).

Methodology

US EPA (1986, 1987, 1988) made exposure estimates from the Zavon *et al.* study (1973) based upon reported mean urinary concentrations of 0.04 mg/l. Assuming that average body weight is 70 kg, average urinary output is 1.2 l/day, and 1.45% of absorbed benzidine is excreted in the urine, US EPA calculated the average daily dose to be 0.047 mg/kg-day. Adjusting this value for work time exposure, with 11.46 years the average time exposed,

56.5 years the average age of the cohort, and 240 work days per year, final lifetime exposure levels were calculated to be 0.0063 mg/kg-day.

US EPA (1986,1987, 1988) based estimates of cancer potency on the following relationship where p(t) is the probability of developing a tumor in a study of cohort of average age t at the end of the follow-up period (56.5 years) and an average lifespan t_L (71.3 years), exposed to dose level d (0.0063 mg/kg-day), and assuming that background tumor incidence is negligible:

$$\text{cancer potency} = \frac{-\ln(1 - p(t))}{(d)(\frac{t}{t_L})^3}$$

With this model, US EPA used total tumor incidence (13/25, both benign and malignant) in its calculation, giving a final potency value of 234 $(mg/kg\text{-}day)^{-1}$ (See Table 2). Using the upper 95% confidence bound on the tumor incidence (0.68 vs. 0.52) resulted in a cancer potency value of 363 $(mg/kg\text{-}day)^{-1}$.

Using mean urine benzidine concentrations reported at the beginning (0.01 mg/l) and end (0.04 mg/l) of the work day, Allen *et al.* (1987) adjusted benzidine exposure estimates on the assumption of linear increases in urine concentration during the workday and first-order decay during non-work hours. The resulting average urine concentration during workdays was 0.023 mg/l. Based on assumptions that 1.5% of the inhaled benzidine is present in the urine (100% absorption), urinary output is 1.5 l/day, breathing rate is 10 m^3/8-hour work day, and average exposure time of the cohort is 11.24 years, Allen *et al.* (1987) estimated average cumulative dose to be 2.59 mg-yrs/m^3.

Allen *et al.* (1987) calculated potency using this dose value and the malignant tumor incidence only [p(t) = 11/25 = 0.44] with a background tumor incidence factor (α = 0.002; NIH, 1981) and without using a time adjustment factor. That is:

$$\text{cancer potency} = \frac{-\ln [1 - p(t)] + \alpha}{d}$$

The resulting estimate of cancer potency from work place exposure was 0.22 $(mg{\cdot}yrs/m^3)^{-1}$. with upper and lower 90% confidence bounds of 0.81 and 0.045 $(mg{\cdot}yrs/m^3)^{-1}$. Cancer potency from continuous lifetime exposure was calculated by assuming 240 workdays of a 365 day year and 10 of 20 m^3/day total air breathed during the workday. Potencies thus expressed are 0.67 $(mg\text{-}yr/m^3)^{-1}$ with upper and lower confidence bounds of 2.5 and 0.14 $(mg\text{-}yr/m^3)^{-1}$. Assumptions of 70 kg body weight, 70 yr lifespan, and 20 m^3/day breathing rate (CDHS, 1988) result in a cancer potency of 160 $(mg/kg\text{-}day)^{-1}$ with upper and lower 90% confidence bounds of 600 and 30 $(mg/kg\text{-}day)^{-1}$.

An estimate of cancer potency was also made based on a description of cumulative risk described by IARC (1982) (CDHS, 1988). IARC (1982) describe risk based on the assumption that the risk varies linearly with the duration of exposure. IARC (1982) report

the data from Zavon *et al.* (1973) show a cumulative bladder tumor incidence of 25% among workers exposed to benzidine for 15 years. Under such an assumption, cancer potency (B) from lifetime (t_L = 70 yrs) exposure can be based on the following relationship, where $C(t_1)$ is the experimentally derived cumulative tumor incidence (25%) and $d(t_1)$ is the average daily dose at time t_1 (15 years):

$$B = [C(t_1)/d(t_1)] \times [(t_L/t_1)^k]$$

The factor k describes the relationship of time of exposure to potency, in this case k=1 because of the assumption of linear proportionality. From this relationship, CDHS (1988) derived a cancer potency value. Using the average daily dosing derived by Allen *et al.* (0.023 mg/kg-day) and the cumulative incidence data described by IARC (1982), the calculated cancer potency was 50 $(mg/kg\text{-}day)^{-1}$ with upper and lower 95% confidence bounds of 130 and 16 $(mg/kg\text{-}day)^{-1}$ derived from the confidence bounds of the dose.

CDHS (1988) considers the Allen *et al* .(1987) estimation of daily urine concentration of 0.023 mg/l to be the most useful for establishing exposure levels. Assuming 1.5 ℓ/day urinary output, 70 kg body weight, 240 work days per year, and average duration of exposure 11.46 yrs in a cohort of 56.5 yrs average age, lifetime average exposure is 0.0044 mg/kg-day. Using the US EPA (1986, 1987, 1988) methodology and the upper 95% confidence bound on incidence [p(t) = 0.68], the cancer potency is 5.0 E+2 $(mg/kg\text{-}day)^{-1}$.

A unit risk factor of 0.14 $(\mu g/m^3)^{-1}$ was derived by OEHHA/ATES assuming a breathing rate of 20 m^3/day, 70 kg body weight, and 100% fractional absorption of inhaled benzidine.

Table 2. Benzidine cancer potencies derived from Zavon *et al.* (1973).

Source of methodology	Potency $[(mg/kg\text{-}day)^{-1}]$	Upper 95% confidence bound $[(mg/kg\text{-}day)^{-1}]$
US EPA (1986, 1987, 1988)	234	363
Allen *et al.*(1987)	160	600
IARC (1982)*	50	130
CDHS (1988)		500

*Estimate based on IARC methodology using the dosage estimation of Allen *et al.* (1987).

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BENZO[*a*]PYRENE

CAS No: 50-32-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	252.3
Boiling point	360° C
Melting point	179° C
Vapor pressure	1 mm Hg at 20° C
Air concentration conversion	1 ppm = 10.3 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor:		1.1 E-3 $(ug/m^3)^{-1}$
Slope Factor:	(inhalation)	3.9 E+0 $(mg/kg\text{-}day)^{-1}$
	(oral)	1.2 E+1 $(mg/kg\text{-}day)^{-1}$

[Inhalation: male hamster respiratory tract tumor incidence (Thyssen *et al.*, 1981), unit risk calculated using a linearized multistage procedure (OEHHA, 1993). Oral: male and female gastric tumor (papillomas and squamous cell carcinomas) incidence (Neal and Rigdon, 1967), cancer potency factor calculated using a linearized multistage procedure (OEHHA, 1993).]

III. CARCINOGENIC EFFECTS

Human Studies

The predominant sources of airborne benzo[*a*]pyrene (BaP) are combustion processes. Thus, this compound rarely enters the environment alone but rather is associated with additional PAHs and other components frequently present in both vapor phase and particulate form. Available epidemiological information, therefore, is from persons exposed to mixtures such as tobacco smoke, diesel exhaust, air pollutants, synthetic fuels, or other similar materials. Several IARC publications have been dedicated to the analysis of cancer in processes which involve exposure to polynuclear aromatic compounds (PAHs) (IARC, 1983; 1984a; 1984b; 1985; 1987). The types of cancer reported are often consistent with the exposure pathway: scrotal cancer and lung cancer in chimney sweeps exposed to soot; skin cancer (including scrotal cancer) where shale oils are used; and lung cancer where airborne exposure of PAHs occurs, such as in iron and steel foundries.

Shamsuddin and Gan (1988) examined several human tissues collected at surgery or autopsy using rabbit high-specificity antibody to benzo[*a*]pyrene diol epoxide (BPDE)-DNA adducts and light immunocytochemistry. Antigenicity was detected in the lung, ovary, placenta, uterine cervix, and white blood cells. Their results indicated that the tissue concentration of adducts varies substantially in the human population and that BPDE-DNA adducts can be detected in human tissues by immunochemical techniques.

Five of twelve human lung samples obtained at surgery, from smokers or former smokers, showed positive antigenicity for BPDE-DNA adducts (Garner *et al.*, 1988). Higher DNA-adduct levels were detected in the white blood cells of Finnish iron workers with jobs in high PAH exposure areas than in the white blood cells of workers with jobs in low PAH exposure areas (Perera *et al.*, 1988; Hemminki *et al.*, 1990). Workers were classified as high, medium, or low BaP exposure and there was a highly significant correlation between BaP exposure and DNA-adduct levels (Reddy *et al.*, 1991). A similar observation was noted by Ovrebo *et al.* (1992) in a study of workers exposed around coke ovens. Perera *et al.* (1993) extended the technique and found that PAH adducts were higher in an industrialized area in winter than both in a more rural area in winter and in the same urban area in summer (when less burning of fuel would occur).

In studies looking at PAH-derived adducts bound to serum protein, higher levels of PAH-albumin adducts were found in foundry workers and in roofers than in their respective reference groups (Lee *et al.*, 1991). Smokers had higher levels of BaP-derived adducts bound to serum protein than non-smokers, and workers in high BaP exposure areas (foundry) had two to three times the levels of workers in low exposure areas (Sherson *et al.*, 1990).

Studies with human placental tissues have shown that aryl hydrocarbon hydroxylase (AHH) activity is several times higher in smokers than non-smokers and that this activity increases in a sigmoidal manner with increased numbers of cigarettes smoked (Gurtoo *et al.*, 1983). Genetic factors probably contribute to this variability and, ultimately, to susceptibility of individuals to tumor development (Manchester and Jacoby, 1984).

Animal Studies

BaP is carcinogenic by intratracheal, inhalation, and dermal exposure, by intraperitoneal injection, and when given in the diet.

(a) Inhalation and Intratracheal Exposures

Early experiments by Saffiotti *et al.* (1968) indicated that PAHs are at least weakly carcinogenic to the respiratory tract. A mixture of BaP (3 mg) and Fe_2O_3 (hematite, 0.25 μm) (3 mg) in a saline suspension was administered to Syrian golden hamsters by intratracheal instillation, once per week for 15 weeks. Most surviving animals receiving BaP plus Fe_2O_3 developed tumors of the respiratory tract (mostly bronchogenic carcinoma) whereas control animals receiving Fe_2O_3 only or those receiving no treatment did not develop tumors.

Subsequently, Saffiotti *et al.* (1972) determined the carcinogenic dose-response relationship after intratracheal instillation of a suspension of BaP and Fe_2O_3 in male and female Syrian golden hamsters. Test materials were administered once weekly for 30 weeks at 2.0, 1.0, 0.5, and 0.25 mg BaP/animal and an equivalent weight of Fe_2O_3 (hematite) as particulate carrier. Tumors were not present in animals receiving ferric oxide or in untreated controls. Respiratory tract tumors (including squamous cell carcinomas of

the larynx, of the trachea, and of the bronchi, adenocarcinomas of the bronchi, and adenomas of the bronchi and of the bronchioles and alveoli) developed in all groups of BaP/Fe_2O_3 treated animals. The response was dose related.

In another experiment, Feron *et al.* (1973) gave male Syrian golden hamsters intratracheal doses of 0, 0.0625, 0.125, 0.5, or 1 mg BaP weekly for 52 weeks. A variety of tumors were produced throughout the respiratory tract, including bronchoalveolar adenomas and carcinomas.

Thyssen *et al.* (1980) conducted an inhalation study in which male Syrian golden hamsters were exposed to BaP condensation aerosol (in 0.1% saline; particle size ranging from 0.2 to 1.5 μm) for 10 to 16 weeks at a concentration of 9.8 to 44.8 mg BaP/m^3. Neoplastic changes in the respiratory tract were not seen.

In a subsequent experiment, Thyssen *et al.* (1981) exposed male Syrian golden hamsters to BaP condensed onto sodium chloride particles at BaP concentrations of 2.2, 9.5, and 46.5 mg BaP/m^3. Tumors were not observed in the respiratory tract of the unexposed control group or the group that received 2.2 mg/m^3. The incidence of tumors in this organ system increased in a dose dependent manner for the 9.5 and 46.5 mg/m^3 exposure groups. Papillomas, papillary polyps, and squamous cell carcinomas were seen in the nasal cavity, larynx, trachea, pharynx, esophagus, and forestomach. Lung tumors were absent.

(b) Feeding Studies

Feeding of pelletized chow containing BaP (50 to 250 ppm BaP for 4 to 6 months) to male and female CFW mice caused gastric tumors (papillomas and squamous cell carcinomas), pulmonary adenomas, and leukemia (Rigdon and Neal, 1966; 1969; Neal and Rigdon, 1967). The pulmonary adenomas, gastric tumors, and leukemia occurred independently of each other (Rigdon and Neal, 1969). The overall data strongly suggest a positive carcinogenic effect since there were no gastric tumors in 289 control mice while 178 out of 454 mice fed various levels of BaP had gastric tumors (Neal and Rigdon, 1967).

(c) Dermal Application

BaP has been shown to be carcinogenic by dermal application (ATSDR, 1990). Wynder and associates demonstrated a positive dose-response relationship for BaP-induction of skin tumors in Swiss and in C57BL mice and showed a tumor response at doses as low as 0.001% BaP applied topically in acetone every 2 weeks for up to 2 years (Wynder and Hoffmann, 1959; Wynder *et al.*, 1957; 1960). In addition, incidences of 95% for papillomas and carcinomas of the skin were obtained by chronic administration (3 times weekly for 1 year) of 0.001% BaP to the skin of Swiss mice (Wynder and Hoffman, 1959). Extensive experiments conducted by Conney and associates demonstrated the tumor initiating activity of BaP and several of its epoxide and hydroxy derivatives (summarized by US EPA, 1979 and by Conney, 1982).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

A very large number of experiments have demonstrated that BaP causes tumors at several sites, by several routes of administration, in both sexes, and in several animal species. Many studies, however, are very limited in scope or in data reported and are not suitable for risk assessment (Zeise and Crouch, 1984).

OEHHA guidelines prescribe that risk assessment use the most sensitive sex, site, and species where a significant increase in cancer incidence is observed (CDHS, 1985). Since there is no adequate information regarding the carcinogenicity of BaP to humans from epidemiological studies, data from animal bioassays were extrapolated to estimate human cancer risk. Potency estimates were derived by OEHHA (1993) from gastric tumors (papillomas and squamous cell carcinomas) observed in male and female mice due to feeding of BaP (Neal and Rigdon, 1967), respiratory tract tumors in hamsters from the inhalation bioassay of Thyssen *et al.* (1981), and from data obtained after intratracheal administration of BaP (Saffiotti *et al.*, 1972; Feron *et al.*, 1973). The dose-response data from these studies are presented in Tables 1-4 below.

Table 1: Gastric tumors in mice from feeding benzo[*a*]pyrene[a].

Exposure (ppm)	Calculated daily dose (mg/kg-day) (animal)	Incidence of gastric tumors
0	0	0/289
1	0.078	0/25
10	0.781	0/24
20	1.563	1/23
30	2.344	0/37
40	3.126	1/40
45	3.516	4/40
50	3.908	24/34
100	7.815	19/23
250	19.538	66/73

[a]Source: OEHHA (1993). Adapted from Neal and Rigdon (1967) and US EPA (1984).

Table 2: Respiratory tract tumors in hamsters from benzo[*a*]pyrene inhalation[a]

Exposure (mg/m^3)	Hamster dose (mg/kg-day)		Tumor incidence
	based on U.S EPA (1994)	based on US EPA (1988)	
0	0	0	0/27
2.2	0.089	0.152	0/27
9.5	0.385	0.655	9/26
46.5			13/25[b]

[a]Source: OEHHA (1993). Adapted from Thyssen *et al*. (1981) and US EPA (1984)
[b]These data were not used due to shortened lifespan of the hamsters in the exposure group. The carcinogenic response, however, is apparent.

Table 3: Respiratory tract tumors from intratracheal instillation of benzo[*a*]pyrene in hamsters – 30 week exposure[a].

Weekly Dose (mg)	Average Daily Dose (mg)	Lifetime Adjusted Daily Dose (mg/kg-day)	Human Equivalent Dose (mg/kg-day)	Tumor Incidence (Males)	Tumor Incidence (Females)
0	0	0	0	0/47	0/45
0.25	0.036	0.119	0.013	6/47	4/41
0.5	0.071	0.239	0.027	10/33	9/30
1.0	0.143	0.477	0.054	22/33	20/34
2.0	0.286	0.953	0.107	17/28	17/29[b]

[a]Source: OEHHA (1993). Adapted from Saffiotti *et al.*, 1972.
[b]Data group was not used since exposure started 7 weeks after other groups.

Table 4: Bronchoalveolar tumors from intratracheal instillation of benzo[*a*]pyrene in hamsters – 52 week exposure[a].

Weekly dose (mg)	Average daily dose (mg)	Lifetime adjusted daily dose (mg/kg-day)	Human equivalent dose (mg/kg-day)	Tumor incidence
0	0	0	0	0/29
0.0625	0.009	0.0495	0.0059	1/30
0.125	0.018	0.0989	0.0118	4/30
0.25	0.036	0.198	0.0237	6/30
0.5	0.071	0.395	0.0473	17/30
1.0	0.143	0.791	0.0947	19/30

[a]Source: OEHHA (1993). Adapted from Feron *et al.*, 1973.

Methodology

Cancer risk associated with exposure to ambient levels of BaP was estimated by extrapolating from the experimental data to ambient levels by means of the best fitting linearized multistage procedure GLOBAL86 (Howe *et al.*, 1986). In addition, other models were fit to the data for comparison. In its risk assessment, the US EPA used the data for stomach tumors from oral exposure to BaP in mice and the data for respiratory tract tumors from inhalation exposure in hamsters to estimate cancer potency and unit risks associated with exposure to BaP (US EPA, 1984).

For BaP there is compelling evidence that it is genotoxic and an initiator of tumorigenesis. Therefore, OEHHA staff treated BaP-induced carcinogenesis as a nonthreshold phenomenon and, as such, applied a nonthreshold, linear extrapolation model for cancer potency estimation.

The linearized multistage model was fit to the respiratory tract tumor data resulting from inhalation exposure of hamsters to BaP (OEHHA, 1993; Thyssen *et al.*, 1981). The data from the highest dose group were not used since these animals had an appreciably shortened lifespan (59 weeks versus 96 weeks in other groups) (Thyssen *et al.*, 1981; US EPA, 1984). By considering the conditions of exposure given in the report and using an inhalation rate of 0.063 m^3/day and a "standard" body weight of 0.12 kg for hamsters (US EPA, 1988), a dose of BaP in mg/kg-day was estimated. A q_1^* (animal) equal to 0.43 $(mg/kg\text{-}day)^{-1}$ is obtained. Multiplying by the interspecies surface area correction factor of $(70/0.1)^{1/3}$ yields a human equivalent $q_1^* = 1.1 \times 10^{-3}$ $(\mu g/m^3)^{-1}$ for inhalation.

Because of the limited amount of data currently available for risk assessment of BaP, the inhalation unit risk of 1.1×10^{-3} $(\mu g/m^3)^{-1}$ based on respiratory tract tumors in hamsters is used as a best value for inhalation exposures. For exposures to BaP by other routes, the potency of 11.5 $(mg/kg\text{-}day)^{-1}$ based on gastric tract tumors in mice can be used (Neal and Rigdon, 1967).

Cancer Potency for Other PAHs

IARC (1987; 1989) has classified a number of PAHs, their mixtures and derivatives, as carcinogens (Group 1, Groups 2A and Group 2B) and a large number of PAHs into Group 3, a class of chemicals for which there are no human data but limited or inadequate data in animals (Tables 5 and 6). The US EPA has classified several PAHs in Group B2, possibly carcinogenic to humans and Group D, unclassifiable as to carcinogenicity (Table 7).

In their risk assessment, OEHHA staff concluded that while the studies available for carcinogenic risk assessment of BaP are not ideal for risk assessment, those for practically all other individual PAHs are less complete for risk assessment (OEHHA, 1993). However, there are extensive data establishing the genotoxicity, and in some cases the carcinogenicity, of many PAHs or their genotoxic metabolites. In other cases, some PAHs are not considered carcinogens. Several authors have used mutagenicity and various tests of carcinogenicity to rank several PAHs for their relative carcinogenicity (e.g., Deutsch-

Wenzel *et al.*, 1983; Bingham and Falk, 1969; Habs *et al.*, 1980; Wynder and Hoffman, 1959; Wislocki *et al.*, 1986) and their relative genotoxicity (Brown, 1989). Many of these comparisons were summarized by Clement Associates (1988) and Krewski *et al.* (1989). In these analyses dibenz(*a,h*)anthracene was shown to be more potent than BaP, while other PAHs tested were less or much less potent. These comparisons indicated that considering all PAHs to be equivalent in potency to BaP would overestimate the cancer potency of a PAH mixture, but such an assumption would be health protective and is likely to be helpful in a screening estimate of PAH risks (OEHHA, 1993).

If one assumes that PAHs are as carcinogenic as they are genotoxic, then their hazard relative to BaP would be dependent on their concentration in the environment. In light of the limited information available on other PAHs, BaP remains an important representative or surrogate for this important group of chemically diverse air pollutants.

Selection of Risk Values for Other PAHs

BaP was chosen as the primary representative of the class because of the large amount of toxicological data available on BaP (versus the relatively incomplete database for other PAHs), the availability of monitoring techniques for BaP, and the significant exposure expected (and found). Nisbet and LaGoy (1992) presented a Toxic Equivalency Factor (TEF) scheme for 17 PAHs. The paper was an extension of earlier work by other investigators (Clement Associates, 1987; 1988; Krewski *et al.*, 1989). Along similar lines, OEHHA has developed a Potency Equivalency Factor (PEF) procedure to assess the relative potencies of PAHs and PAH derivatives as a group. This would address the impact of carcinogenic PAHs in ambient air since they are usually present together.

Due to the variety of data available on the carcinogenicity and mutagenicity of PAHs, an order of preference for the use of available data in assessing relative potency was developed. If a health effects evaluation and quantitative risk assessment leading to a cancer potency value had been conducted on a specific PAH, then those values were given the highest preference.

Table 5: IARC groupings of PAHs, mixtures with PAHs, and derivatives.

Group 1	Group 2A	Group 2B
Coal-tar pitches Coal-tar Coke production Mineral oils Shale-oils Soots Tobacco smoke	Benz[*a*]anthracene Benz[*a*]pyrene Creosotes Dibenzo[*a,h*]anthracene	Benzo[*b*]fluoranthene Benzo[*j*]fluoranthene Benzo[*k*]fluoranthene Carbon black extracts Dibenz[*a,h*]acridine Dibenz[*a,j*]acridine 7H-Dibenzo[*c,g*]carbazole Dibenzo[*a,e*]pyrene Dibenzo[*a,h*]pyrene Dibenzo[*a,i*]pyrene Dibenzo[*a,l*]pyrene Indeno[1,2,3-*cd*]pyrene 5-Methylchrysene 5-Nitroacenaphthene 1-Nitropyrene 4-Nitropyrene 1,6-Dinitropyrene 1,8-Dinitropyrene 6-Nitrochrysene 2-Nitrofluorene

Source: OEHHA (1993)
Abstracted from IARC Supplement 7 (1987) and IARC Volume 46 (1989).
Group1: carcinogenic to humans.
Group 2A: probably carcinogenic to humans.
Group 2B: possibly carcinogenic to humans.

Table 6: IARC Group 3 PAHs and PAH derivatives[1]

Chemical	Animal Evidence
Acridine orange	inadequate
5-Aminoacenaphthene	inadequate
2-Aminoanthraquinone	limited
Anthanthrene	limited
Anthracene	inadequate
Benz[*a*]acridine	inadequate
Benz[*c*]acridine	limited
Benzo[*g*,*h*,*i*]fluoranthene	inadequate
Benzo[*g*,*h*,*i*]perylene	inadequate
Benzo[*c*]phenanthrene	inadequate
Benzo[*e*]pyrene	inadequate
Carbazole	limited
Chrysene	limited
Cyclopenta[*c*,*d*]pyrene	limited
Dibenz[*a*,*c*]anthracene	limited
Dibenz[*a*,*j*]anthracene	limited
Dibenz[*a*,*e*]fluoranthene	limited
Dibenzo[*h*,*rst*]pentaphene	limited
3,7-Dinitrofluoroanthene	limited
3,9-Dinitrofluoroanthene	limited
1,3-Dinitropyrene	limited
Fluoranthene	inadequate
Fluorene	inadequate
1-Methylchrysene	inadequate
2-Methylchrysene	limited
3-Methylchrysene	limited
4-Methylchrysene	limited
6-Methylchrysene	limited
2-Methylfluoranthene	limited
1-Methylphenanthrene	inadequate
1,5-Naphthalenediamine	limited
9-Nitroacenaphthene	limited
9-Nitroanthracene	no adequate data
7-Nitrobenz[*a*]anthracene	limited
6-Nitrobenzo[*a*]pyrene	limited
3-Nitrofluoranthene	inadequate
1-Nitronaphthalene	inadequate
2-Nitronaphthalene	inadequate
3-Nitroperylene	inadequate
2-Nitropyrene	inadequate
Perylene	inadequate
Phenanthrene	inadequate
N-Phenyl-2-naphthylamine	limited
Pyrene	inadequate
Triphenylene	inadequate

Table 6 (continued): IARC Group 3 PAHs and PAH derivatives[1].

[1]Source: OEHHA (1993). Abstracted from IARC Supplement 7 (1987) and IARC Volume 46. (1989). Group 3 have either limited or inadequate evidence in animals and are not classifiable as to their carcinogenicity in humans due to no adequate data.

Table 7: US EPA groupings of PAHs[1]

Group B2	Group D
Benz[*a*]anthracene	Acenaphthylene
Benzo[*a*]pyrene	Anthracene
Benzo[*b*]fluoranthene	Benzo[*e*]pyrene
Benzo[*j*]fluoranthene	Benzo[*g,h,i*]perylene
Benzo[*k*]fluoranthene	Fluorene
Chrysene	Naphthalene
Dibenz[*a,h*]anthracene	Phenanthrene
Indeno[1,2,3-*cd*]pyrene	

[1]Source: OEHHA (1993). Abstracted from US EPA (1993a). Group B2: possibly carcinogenic to humans. Group D is unclassifiable as to carcinogenicity.

If potency values have not been developed for specific compounds, a carcinogenic activity relative to BaP, rather than a true potency, can be developed. These relative activity values are referred to by OEHHA as PEFs. For air contaminants, relative potency to BaP based on data from inhalation studies would be optimal. Otherwise, intrapulmonary or intratracheal administration, such as those published by Deutsch-Wenzel *et al.* (1983), would be most relevant, since such studies are in the target organ of interest. Next in order of preference is information on activity by the oral route and skin painting. Intraperitoneal and subcutaneous administration rank at the bottom of the *in vivo* tests considered useful for PEF development because of their lack of relevance to environmental exposures. Next in decreasing order of preference are genotoxicity data which exist for a large number of compounds. In many cases genotoxicity information is restricted to mutagenicity data. Finally, there are data on structure-activity relationships among PAH compounds. Structure-activity considerations may help identify a PAH as carcinogenic, but at this time have not been established as predictors of carcinogenic potency.

Using this order of preference, PEFs were derived for 21 PAHs and are presented in Table 8. The cancer potencies of four other PAH compounds are given in Table 9. Explanation of the derivation of each PEF, type of data used in the derivation, and the relevant references are given below.

Table 8: OEHHA PEF weighting scheme for PAHs[1]

PAH or derivative	PEF
benzo[*a*]pyrene	1.0 (index compound)
benz[*a*]anthracene	0.1
benzo[b]fluoranthene	0.1
benzo[*j*]fluoranthene	0.1
benzo[k]fluoranthene	0.1
dibenz[*a*,*j*]acridine	0.1
dibenz[*a*,*h*]acridine	0.1
7H-dibenzo[c,*g*]carbazole	1.0
dibenzo[*a*,*e*]pyrene	1.0
dibenzo[*a*,*h*]pyrene	10
dibenzo[*a*,*i*]pyrene	10
dibenzo[*a*,*l*]pyrene	10
indeno[1,2,3-*cd*]pyrene	0.1
5-methylchrysene	1.0
1-nitropyrene	0.1
4-nitropyrene	0.1
1,6-dinitropyrene	10
1,8-dinitropyrene	1.0
6-nitrochrysene	10
2-nitrofluorene	0.01
chrysene	0.01

[1]Source: OEHHA (1993)

Table 9: Potencies of PAHs and derivatives

Chemicals	Cancer potency factors $(mg/kg\text{-}day)^{-1}$	Unit risks $(\mu g/m^3)^{-1}$
benzo[*a*]pyrene[1]	11.5	1.1×10^{-3}
dibenz[*a*,*h*]anthracene[1]	4.1	1.2×10^{-3}
7,12-dimethylbenzanthracene[1]	250	7.1×10^{-2}
3-methylcholanthrene[1]	22	6.3×10^{-3}
Naphthalene[2]	0.12	3.4×10^{-5}
5-nitroacenaphthene[1]	0.13	3.7×10^{-5}

Source: [1]OEHHA (1993), [2]OEHHA (2004): It is assumed that unit risks for inhalation have the same relative activities as cancer potencies for oral intake.

Potency and Potency Equivalency Factors (PEFs) for Selected PAHs

1. Benzo[*a*]pyrene. Benzo[*a*]pyrene (BaP) was the index compound for relative potency and for Potency Equivalency Factors (PEF) for PAHs and derivatives. It has a cancer potency of 11.5 $(mg/kg\text{-}day)^{-1}$ and inhalation unit risk of 1.1×10^3 $(\mu g/m^3)^{-1}$. For the potency equivalency scheme, it was assigned a PEF of 1.

2. Dibenz[*a*,*h*,]anthracene. An expedited potency of 4.1 $(mg/kg\text{-}day)^{-1}$ was derived using the linearized multistage model with the only dose-response data set available - a drinking water study (Snell and Stewart, 1962) which reported alveolar carcinomas of the lung in male DBA/2 mice due to dibenz[*a*,*h*]anthracene (incidence = 14/21 at 28.3 mg/kg-day versus 0/25 in controls). An inhalation unit risk can be obtained from a potency under the assumption that the chemicals are equally absorbed and are equally potent by oral and inhalation routes and that a 70 kg person inhales 20 cubic meters of air per day. When the potency in units of $(mg/kg\text{-}day)^{-1}$ is divided by 3500 (70 kg * 1000 µg/mg/20 m^3), an inhalation unit risk is obtained in units of $(\mu g/m^3)^{-1}$.

3. 7,12-Dimethylbenzanthracene. An expedited potency of 250 $(mg/kg\text{-}day)^{-1}$ was derived. The only study listed in the Gold *et al.* cancer potency (TD50) database (Gold *et al.*, 1984; 1986; 1987; 1989; 1990) is the feeding study by Chourolinkov *et al.* (1967) in female albino mice. Significant increases in malignant angioendotheliomas of the mesenteric intestine and papillomas of the forestomach were observed in animals treated with 0.39 mg/kg-day of 7,12-dimethylbenzanthracene. Cancer potency is based on mesenteric intestine angioendothelioma incidence (incidence = 49/75 versus 0/40 in controls).

4. 3-Methylcholanthrene. An expedited potency of 22 $(mg/kg\text{-}day)^{-1}$ was derived. Results of 3 studies in male Long Evans rats, one study in an unspecified strain of female rats, and 10 studies in female Wistar rats were included in the Gold *et al.* database. All studies in female rats found highly significant increases in tumors of the mammary gland. The cancer potency for 3-methylcholanthrene was taken as the geometric mean of cancer potencies estimated from 9 of the 10 studies in female rats (Shay *et al.*, 1962; Gruenstein *et al.*, 1964; Shay *et al.*, 1961). The upper bound on potency could not be estimated from one of the studies by Shay *et al.* (1961), because 100% of the treated animals developed mammary gland tumors.

5. 5-Nitroacenaphthene. An expedited potency of 0.13 $(mg/kg\text{-}day)^{-1}$ was derived based on the combined incidence of benign and malignant tumors of the ear canal in female rats. Usable studies were feeding studies by Takemura *et al.* (1974) in female Syrian golden hamsters and by the National Cancer Institute (1978) in male and female $B6C3F_1$ mice and F344 rats. The compound 5-nitroacenaphthene induced increases in tumor incidences at multiple sites in rats and female mice. Rats were the most sensitive species; the sensitivity of males were similar to that of females.

6. Benzo[*b*]fluoranthene. Benzo[*b*]fluoranthene was assigned a PEF of 0.1. Clement Associates (1988) applied both a two stage model and the multistage model to various data sets for several PAHs. The two models generally gave similar results for relative potency. In order to verify the results, OEHHA staff (OEHHA, 1993) used GLOBAL86 to fit the multistage model to the tumor data used by Clement Associates and obtained relative cancer potencies similar to those obtained by Clement Associates. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs *et al.* (1980) and the intrapulmonary administration to rats by Deutsch-Wenzel *et al.* (1983) to estimate a cancer potency for benzo[*b*]fluoranthene relative to BaP. As an example of the type of data used,

Deutsch-Wenzel *et al.* obtained pulmonary tumor incidences of 0, 2.9, and 25.7% after intrapulmonary administration of 0.1, 0.3, and 1 mg benzo[*b*]fluoranthene, respectively, whereas they obtained 11.8, 60.0, and 94.3% tumor incidence after the same doses of benzo[*a*]pyrene. Clement Associates estimated a relative cancer potency for benzo[*b*]fluoranthene of 0.140 after fitting the two stage model to the data and 0.105 after fitting the multistage model. Using the data of Habs *et al.* a relative cancer potency of 0.167 was obtained with the two stage model and 0.201 with the multistage model. The results from the multistage model were averaged, then rounded (down) to 0.1 to obtain the PEF. OEHHA obtained a relative potency of 0.208 for benzo[*b*]fluoranthene fitting the multistage model to the data from Habs *et al.* OEHHA staff were also able to reproduce the calculations for the two stage model in the accepted model for cancer risk assessment in California; results from the multistage model have been used to obtain PEFs although the two models usually gave the same PEF.

7. Benzo[*j*]fluoranthene. Benzo[*j*]fluoranthene was assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs *et al.* (1980) to estimate a cancer potency relative to BaP of 0.0648. OEHHA staff estimated 0.065 using the same data. This was rounded to 0.1 to obtain the PEF. Clement Associates did not use the data of Deutsch-Wenzel *et al.* (1983) on benzo[*j*]fluoranthene to calculate a relative potency but Deutsch-Wenzel *et al.* found that it was very similar in tumorigenic activity to benzo[*k*]fluoranthene.

8. Benzo[*k*]fluoranthene. Benzo[*k*]fluoranthene was assigned a PEF of 0.1. Clement Associates (1988) used mouse skin carcinogenesis data obtained by Habs *et al.* (1980) to obtain a cancer potency relative to BaP of 0.0235 and the intrapulmonary administration to rats by Deutsch-Wenzel *et al.* (1983) to estimate a PEF of 0.085. Because the latter was obtained by the pulmonary route it was chosen to be the basis of the PEF. The value was rounded to 0.1 to obtain the PEF.

9. Benz[*a*]anthracene. Benz[*a*]anthracene was assigned a PEF of 0.1. In the case of benz[*a*]anthracene, mouse skin carcinogenesis data obtained by Bingham and Falk (1969) were used by Clement Associates (1988) to calculate potencies for benz[*a*]anthracene. For this chemical the multistage model gave a relative potency of 0.0137. Using the two stage model a higher cancer potency of 0.145 relative to BaP was obtained. In the Wislocki *et al.* (1986) report, in which lung adenomas were induced in newborn mice, benz[*a*]anthracene (2.8 micromoles) was less carcinogenic (12/71 or 17% versus 7/138 or 5% in controls) relative to 0.56 micromoles BaP (24/64 or 38% versus 7/138 in controls). The relative potency was 0.08, which rounds to 0.1. Since the US EPA was using a PEF of 0.1 for this PAH (US EPA, 1993b) and the data from the Wislocki study were consistent with a PEF of 0.1, a value of 0.1 was selected by OEHHA.

10. Dibenz[*a,j*]acridine. Dibenz[*a,j*]acridine was assigned a PEF of 0.1. Warshawsky *et al.* (1992) compared the tumor-initiating ability of dibenz[*a,j*]acridine to BaP in mouse skin. Two hundred nanomoles of each compound were applied to groups of 30 mice, then the skin lesion was promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP mice (90%) had skin papillomas, while 17 of 30 (57%) of the dibenz[*a,j*]acridine mice

had skin papillomas. The multistage model was fit to both sets of data and the ratio of upper 95% confidence limits on the linear coefficient was 0.36. This was rounded to a PEF of 0.1.

11. Dibenz[*a*,*h*]acridine. Dibenz[*a*,*h*]acridine was also assigned a PEF of 0.1. Its carcinogenic classification by IARC was based on studies published in 1940 and earlier and the studies did not appear appropriate for estimation of a PEF. Since its structure is similar to dibenz[*a*,*j*]acridine, it was assigned the same PEF as dibenz[*a*,*j*]acridine until usable compound-specific bioassay data becomes available.

12. 7H-Dibenzo[*c*,*g*]carbazole. 7H-dibenzo[*c*,*g*]carbazole was assigned a PEF of 1.0. Warshawsky *et al.* (1992) compared the tumor-initiating ability of 7H-dibenzo[*c*,*g*]carbazole to BaP in mouse skin. Two hundred nanomoles of each compound were applied to 30 mice, then promoted with a phorbol ester for 24 weeks. Twenty-seven out of 30 BaP-treated mice (90%) had skin papillomas, while 26 of 30 (87%) of the dibenzo[*a*,*j*]acridine-treated mice had skin papillomas for a relative tumorigenic activity of 0.97. This was rounded to a PEF of 1.

13. Dibenzo[*a*,*l*]pyrene. Dibenzo[*a*,*l*]pyrene was assigned a PEF of 10. Cavalieri *et al.* (1989; 1991) studied the tumor-initiating and dose-response tumorigenicity of dibenzo[*a*,*l*]pyrene in mouse skin and rat mammary gland. BaP was used as a reference compound in some experiments. Dibenzo[*a*,*l*]pyrene was the most potent member of the group. Several levels of PAHs were tested. When results from 33.3 nanomoles of dibenzo[*a*,*l*]pyrene as a skin tumor initiator (with promotion by a phorbol ester) were compared to results using the same amount of BaP, dibenzo[*a*,*l*]pyrene induced skin tumors in 23/24 (96%) of the animals while BaP induced tumors in 10/23 (43%) which resulted in a relative potency of 5.8. Dibenzo[*a*,*l*]pyrene induced approximately 5 times as many tumors per tumor-bearing animal. In a second experiment 4 nanomoles of each chemical were compared. Ninety-two percent (22/24) of the dibenzo[*a*,*l*]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals, which yielded a relative potency of 25.1. In a third experiment 100 nM were compared without promotion. Twenty-nine percent (7/24) of the dibenzo[*a*,*l*]pyrene-treated mice had tumors but only 4% (1/24) of the BaP animals, for a relative potency of 4. Finally, with direct application to the mammary gland, 0.25 and 1.0 nanomoles dibenzo[*a*,*l*]pyrene led to tumors in all the rats treated (19 and 20 per group, respectively) whereas only one animal in the 0.25 micromoles BaP group showed a tumor for a relative potency greater than 100. Based on its much greater tumorigenic activity than BaP in the above tests, dibenzo[*a*,*l*]pyrene was assigned a PEF of 10.

14. Dibenzo[*a*,*h*]pyrene. Dibenzo[*a*,*h*]pyrene was assigned a PEF of 10 since, in the experiments by Cavalieri *et al.* (1989) in which all four dibenzo[a]pyrenes were studied, its tumor causing activity was similar to dibenzo[*a*,*l*]pyrene. For example, when used to initiate tumors in mouse skin, 18 of 24 (75%) of mice treated with dibenzo[*a*,*h*]pyrene had tumors compared to 22 of 24 (92%) with dibenzo [*a*,*l*]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

15. Dibenzo[*a*,*i*]pyrene. Dibenzo[*a*,*i*] pyrene was assigned a PEF of 10 since, in the experiments by Cavalieri *et al.* (1989) in which all four dibenzo[*a*]pyrenes were studied, its tumor-causing activity was similar to dibenzo[a,l]pyrene. For example, when used to initiate tumors in mouse skin, 15 of 24 (63%) of mice treated with dibenzo[a,i]pyrene had tumors compared to 22 of 24 (92%) with dibenzo-[*a*,*l*]pyrene. Controls showed skin tumors in 2 of 23 mice (9%).

16. Dibenzo[*a*,*e*]pyrene. Dibenzo[*a*,*e*]pyrene was assigned a PEF of 1.0. Dibenzo[*a*,*e*]pyrene was the least potent of the four dibenzo[*a*]pyrenes studied by Cavalieri *et al.* (1989; 1991). In the experiments in which all four dibenzo[*a*]pyrenes were compared (Cavalieri *et al.*, 1989), its tumor-causing activity was approximately one-tenth to one-twentieth that of dibenzo[*a*,*l*]pyrene.

17. Indeno[1,2,3-*cd*]pyrene. Indeno[1,2,3-*cd*]pyrene was assigned a PEF of 0.1. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Habs *et al.* (1980) and by Hoffman and Wynder (1966) and the lung tumor data obtained by Deutsch-Wenzel *et al.* (1983) after intrapulmonary administration to estimate cancer potencies relative to BaP of 0.0302, 0.0292, and 0.246, respectively. These were averaged and rounded to obtain a PEF of 0.1.

18. 5-Methylchrysene. 5-Methylchrysene was assigned a PEF of 1.0. The activity of 5-methylchrysene relative to BaP has been studied by Hecht *et al.* (1976) using skin tumor initiation with phorbol ester (tetradecanoy1 phorbol acetate) promotion as well as skin tumor induction in mice. In the skin tumor induction test the tumorigenic activities of 5-methylchrysene and BaP were comparable enough so that a PEF of 1.0 was selected for 5 methylchrysene. Weekly application of 0.01% 5-methylchrysene led to skin carcinomas in 10 of 15 mice treated for up to 62 weeks, while 0.01% BaP led to skin carcinomas in 14 of 18 mice. The results for 0.005% of the 2 chemicals were 6 of 9 and 7 of 10, respectively.

19. 1-Nitropyrene. 1 Nitropyrene has been assigned a PEF of 0.1. In the Wislocki *et al.* (1986) report, in which lung tumors were induced in newborn mice, 1-nitropyrene (0.7 micromoles) was weakly carcinogenic in males (6/34 or 18% versus 4/45 or 9% in controls) and not carcinogenic in females (3/50 or 6% versus 2/34 or 6% in controls) relative to 0.56 micromoles BaP (13/37 or 35% in males versus 1/28 or 4% in control males and 13/27 or 48% in females versus 0/31 in control females). The relative potency was 0.348 in males and 0.076 in females. A PEF of 0.1 was assigned based on the experiment.

20. 4-Nitropyrene. 4-Nitropyrene was assigned a PEF of 0.1. Wislocki *et al.* (1986) compared the lung tumorigenicity of nitrated derivatives of pyrene to BaP in a newborn mouse assay. The background incidences were 4% in males and 0% in females. The administration of 2.8 micromoles of 4-nitropyrene gave a net incidence of 34% tumors in males and 31% in females, while 0.56 micromoles BaP gave 31% tumors in males and 48% in females. The potency of 4-nitropyrene relative to BaP was 0.23 in males and 0.12 in females. These were averaged and rounded to a PEF of 0.1.

21. 1,6-Dinitropyrene. 1,6-Dinitropyrene was assigned a PEF of 10. In the Wislocki *et al.* (1986) report, 1,6-dinitropyrene (0.2 micromoles) was weakly carcinogenic in inducing lung tumors in females (2/29 versus 0/31 in controls) and essentially not carcinogenic in males (1/25 versus 1/28 in controls) relative to 0.56 micromoles BaP (see 1-nitropyrene above for BaP data). The weak response combined with the low dose of 1,6-dinitropyrene (0.2 micromoles) relative to BaP (0.56 micromoles) resulted in a relative potency of 0.52 in females and 0.54 in males. In an intratracheal injection experiment (Takayama *et al.*, 1985) hamsters were given 26 weekly instillations of 0.5 mg BaP. All 10 males and 9 of 10 females developed respiratory tract tumors. A unit risk of 2.9×10^{-2} $(\mu g/m^3)^{-1}$ obtained from the female data which is 6.4 times the unit risks obtained from intratracheal studies using BaP and 26 times that using inhalation data. In a study by Iwagawa *et al.* (1989) using several doses of 1,6-dinitropyrene or BaP implanted directly into the lungs, a relative potency of 5.1 was obtained from the resulting lung cancer data. In light of the two experiments showing high relative potency and of 1,6-dinitropyrene's strong mutagenicity, a PEF of 10 appeared to be more appropriate than 1.0.

22. 1,8-Dinitropyrene. 1,8-Dinitropyrene was assigned a PEF of 1.0. In the Wislocki *et al.* (1986) report, 1,8-dinitropyrene (0.2 micromoles) was weakly carcinogenic in females (2/29 versus 0/31 in controls) and not carcinogenic in males (1/31 versus 1/28 in controls) relative to 0.56 micromoles BaP. However, due again to the low dose of 1,8-dinitropyrene chosen, the relative potency was 0.46 in females and 0.41 in males. In view of the high PEF of 1,6-dinitropyrene derived above and the very high mutagenicity of 1,8-dinitropyrene, the default PEF of 1.0 was assigned to 1,8-dinitropyrene until better *in vivo* data becomes available to derive a PEF.

23. 6-Nitrochrysene. 6-Nitrochrysene was assigned a PEF of 10. In the Wislocki *et al.* (1986) report, 0.7 micromoles of 6-nitrochrysene gave a net incidence of 76% lung tumors in males (28/33 versus 4/45 in controls) and 84% in females (36/40 versus 2/34 in controls). The potency of 6-nitrochrysene relative to BaP was 3.27 in males and 2.50 in females. In the newborn mouse assay of Busby *et al.* (1988), "(t)he ED50 for total lung tumors was 0.02 μmol for 6-NC and 0.2 μmol for BaP, thus showing a 10-fold higher potency for 6-NC compared with the 25-fold difference noted with tumor multiplicity." In a subsequent report (Busby *et al.*, 1989), 0.03 micromoles of 6-nitrochrysene caused lung adenomas and adenocarcinomas in 19/26 males and 13/22 females (versus controls of 13/91 in males and 7/101 in females) while 0.24 micromoles BaP caused lung adenomas and adenocarcinomas in 13/28 males and 19/27 females (against the same controls). The relative potencies were 17.51 for males and 6.17 for females. Based on the several experiments a PEF of 10 was selected.

24. 2-Nitrofluorene. 2-Nitrofluorene was assigned a PEF of 0.01. Miller *et al.* (1955) fed 2-nitrofluorene at a level of 1.62 mmol(215 mg)/kg diet to rats. This is estimated to give an animal dose of 33.1 mg/kg-day and a human equivalent dose of 4.7 mg/kg-day. In one experiment 17 of 20 male rats (85%) developed forestomach tumors by 12 months. In another experiment 4 of 9 female rats (44%) developed mammary tumors by 10 months. These experiments yielded cancer potencies of 0.25 and 0.62 $(mg/kg\text{-}day)^{-1}$, approximately

0.02 and 0.05 that of BaP obtained in this risk assessment. The values of 0.02 and 0.05 were averaged and rounded down to obtain a PEF of 0.01.

25. Chrysene. Chrysene was assigned a PEF of 0.01. Clement Associates (1988) used the mouse skin carcinogenesis data obtained by Wynder and Hoffman (1959) to estimate a cancer potency relative to BaP of 0.0132. This was rounded to obtain a PEF of 0.01.

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BENZYL CHLORIDE

CAS No: 100-44-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	126.58
Boiling point	179°C
Melting point	-43 to -48°C
Vapor pressure	1 mm Hg at 22°C
Air concentration conversion	1 ppm = 5.26 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.9 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 1.7 E-1 $(mg/kg\text{-}day)^{-1}$
[Cancer potency factor derived by US EPA/IRIS (1989) from female rat C-cell thyroid tumor incidence data (Lijinsky, 1986) using a linearized multistage procedure, extra risk; adopted by RCHAS/CDHS (1991).]

III. CARCINOGENIC EFFECTS

Human Studies

Several studies report on cancer mortality in workers occupationally exposed to benzyl chloride.

Sakabe *et al*. (1976) studied cancer incidences among 41 workers exposed to chemicals including benzyl chloride over 18 years (ending in 1972) in a plant producing benzoyl chloride in Japan. Four cases of cancer were reported among the workers: two fatal cases of lung cancer, one fatal maxillary malignant lymphoma, and one squamous cell carcinoma of the lung (still surviving in 1973). The range of employment duration among the workers with cancer was 6 to 14 years. Both cases of lung cancer were in smokers. The expected number of lung cancer deaths among 41 Japanese males was 0.06. In addition to smoking, another potential confounding factor is the reporting of exposure to other compounds in the work environment including benzotrichloride, benzoyl chloride, toluene, chlorine gas, hydrogen chloride, benzal chloride, and other chlorinated toluenes and polymers. Exposure levels were not quantitated.

Sakabe and Fukuda (1977) also reported on cancer deaths among workers exposed to chemicals including benzyl chloride in another plant involved with the production of benzoyl peroxide and benzoyl chloride between 1952 and 1963. Two lung cancer deaths (one a smoker) were reported. Expected number of deaths and exposure levels were not reported, and workers were also exposed to other chemicals as listed in the description of the study by Sakabe *et al*. (1976).

Sorahan *et al*. (1983) studied cancer mortality among British workers occupationally exposed to a number of compounds including toluene, benzotrichloride, benzoyl chloride, benzyl chloride, and benzal chloride during the course of producing chlorinated toluenes. Five digestive system cancers and 5 respiratory system cancers were reported among 163 male workers employed more than 6 months between 1961 and 1970. Expected mortality rates from these tumors in England and Wales were 1.24 and 1.78, respectively, and the mortality ratio was significantly elevated. Smoking rates were not reported among the workers. Cumulative exposure and death from any cancer among workers employed before 1951 was shown to be significantly correlated by survival analysis using the Cox Proportional Hazard Model, although this was not the case when entry cohorts were combined.

Wong and Morgan (1984) studied cancer mortality among a cohort of 697 workers employed from 1 to more than 35 years in a chlorination plant in Tennessee. Workers were exposed to benzyl chloride, benzoyl chloride, and benzotrichloride. Deaths from respiratory cancers were reported for 7 workers, 5 of whom were exposed for more than 15 years. Expected mortality for U.S. males in a group of this size was 2.84 deaths (1.32 deaths for the subgroup exposed > 15 years). No data on smoking was reported.

Animal Studies

Lijinsky (1986) treated F344 rats (52/sex/dose) and B6C3F$_1$ mice (52/sex/dose) with benzyl chloride in corn oil by gavage. The rats were dosed with 0, 15, and 30 mg/kg/day benzyl chloride and mice with 0, 50 and 100 mg/kg/day benzyl chloride, with treatments 3 days/week for 2 years. Animals were histopathologically examined 3-4 weeks after the end of the treatment using the NCI bioassay protocol. Survival in both species was not significantly affected by treatment. The incidence of C-cell adenoma/carcinoma of the thyroid was significantly increased in female rats in the high-dose group compared to control animals (14/52 treated vs. 4/52 control; $p < 0.01$ by Fisher's exact test). In male mice in the high-dose group, significantly increased incidences were found for hemangioma/hemangiocarcinoma (5/52 treated vs. 0/52 control), forestomach carcinoma (8/52 treated vs. 0/51 control) and forestomach carcinoma/papilloma (32/52 treated vs. 0/51 control). In male mice in the low-dose group only, an increased incidence of hepatic carcinoma/adenoma (28/52 treated vs. 17/52 control) was reported. In female mice in the low- and high-dose groups, an increased incidence of forestomach carcinoma/papilloma (5/50 low-dose, 19/51 high-dose vs. 0/52 control) was reported.

Injection-site sarcomas developed in 3 of 14 BD-strain rats administered benzyl chloride in peanut oil subcutaneously weekly for 51 weeks at 40 mg/kg-week and 6 of 8 rats administered 80 mg/kg-week (Druckrey *et al*., 1970). Mean induction time was 500 days.

Poirier *et al*. (1975) treated A/H mice (20/dose) 3 times weekly over 24 weeks intraperitoneally with a total dose of 0.6, 1.5 or 2 g benzyl chloride/kg body weight in tricaprylin. Surviving animals were sacrificed at 24 weeks. Among the survivors, lung tumors were found in 4/15, 7/16, and 2/8 animals, respectively. Animals treated with tricaprylin alone had an average of 0.22 lung tumors/mouse and animals receiving no

treatment had 0.21 lung tumors/mouse. The incidence of lung tumors in treated animals was not found to be statistically significant from control animals.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Human studies do not provide adequate data for the development of a cancer potency value because of the presence of confounding factors in the studies (multiple compound exposures, no data on smoking status) and no reporting of exposure levels. The animal study by Lijinsky (1986) showing development of thyroid tumors in female rats, and forestomach papillomas and carcinomas in male and female mice provides data from which cancer potency values can be derived.

Methodology

Cancer potency values were derived by US EPA (1989) from the tumor incidence data presented in the study by Lijinsky (1986). The experimentally administered doses (15 and 30 mg/kg) were converted to time-weighted dosage based on the dosing schedule (3 times/week) and the experimental duration (107.5 weeks). The human equivalent dose (HED) was calculated based on an assumed experimental animal body weight (bw_a) of 0.35 kg and human body weight (bw_h) of 70 kg using the following relationship:

$$HED = \text{time-weighted dose} \times (bw_a/bw_h)^{1/3}$$

The calculated human equivalent doses in the Lijinsky (1986) study were 1.06 and 2.12 mg/kg-day. A linearized multistage procedure (CDHS, 1985) was applied to the tumor incidence data for thyroid tumors in female rats (4/52 controls, 8/51 low-dose, 14/52 high-dose), forestomach tumors in male mice (0/51 controls, 5/50 low-dose, 32/52 high-dose) and forestomach tumors in female mice (0/52 controls, 5/50 low-dose, 19/51 high-dose). This resulted in estimations of the upper 95% confidence bound of cancer potency (q_1^*) of 0.17, 0.056, and 0.12 $(mg/kg\text{-}day)^{-1}$, respectively. Selection of the cancer potency value is made in the most sensitive species and site; therefore, the cancer potency value [0.17 $(mg/kg\text{-}day)^{-1}$] derived from the female rat C-cell thyroid tumor data was chosen.

A unit risk value of 4.9 E-5 $(\mu g/m^3)^{-1}$ was derived by ATES/OEHHA assuming a human breathing rate of 20 m^3/day, a human body weight of 70 kg, and 100% fractional absorption after inhalation exposure.

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BERYLLIUM

CAS No: 7440-41-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	9.012
Boiling point	2970°C
Melting point	1287°C
Vapor pressure	10 mm Hg @ 1860°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.4 E-3 $(\mu g/m^3)^{-1}$
[Calculated by US EPA (1992) from the human inhalation exposure data of Wagoner *et al.* (1980).]

III. CARCINOGENIC EFFECTS

Human Studies

US EPA (1992) reviewed several studies that found increased incidences of lung cancer in beryllium processing workers. A cohort mortality study of 3055 white males employed at a single beryllium processing plant in Pennsylvania with a median duration of employment of 7.2 months demonstrated a statistically significant increased incidence of mortality due to lung cancer in the entire cohort, as well as in the 2068 cohort members followed for 25 years or more since initial employment (Wagoner *et al.,* 1980). Recalculation of the number of expected deaths using 1968-1975 lung cancer mortality data indicated that the increased incidence was significant only among workers followed for 25 years or more (Bayliss, 1980; MacMahon, 1977, 1978), and was not significant when the number of expected deaths was adjusted for smoking (US EPA, 1986).

Earlier studies of workers from the same beryllium processing plant alone or combined with workers from other beryllium plants reported a statistically significant increase in lung cancer mortality (Bayliss and Wagoner, 1977; Mancuso, 1970, 1979, 1980). These studies made no adjustment for smoking and had methodological constraints and deficiencies that precluded their use to establish a causal relationship between beryllium exposure and lung cancer.

Animal Studies

Slight, non-statistically significant increases in cancer incidence (all tumor types) were observed in male Long-Evans rats (52/sex/group) following lifetime exposure to 5 ppm beryllium sulfate administered in the drinking water (Schroeder and Mitchener, 1975a).

Tumors were observed in 9/33 treated and 4/26 control rats. High mortality during the study resulting from a pneumonia epidemic at 20 months greatly reduced the power of this study to detect any potential carcinogenic effect of beryllium exposure. A non-statistically significant increase in combined lymphoma and leukemia incidence was observed in female Swiss mice administered 5 ppm beryllium sulfate in drinking water for life (9/52 exposed, 3/47 controls) (Schroeder and Mitchener 1975b).

Male and female Wistar-derived rats were exposed to diet containing beryllium sulfate at concentrations of 0, 5, 50, or 500 ppm for life (Morgareidge *et al*., 1977). Reticulum cell carcinomas of the lung were observed in 10/49 male control animals, 17/35 low dose animals, 16/40 intermediate dose animals, and 12/39 high dose animals, respectively. Since the results were published only as an abstract, and since no response was seen at the highest dose, these results are considered to be only suggestive for the induction of cancer via this route.

Beryllium and beryllium compounds have been shown to cause statistically significant tumor increases in male and female rhesus monkeys and several strains of rats via inhalation and intratracheal installation, and the induction of osteosarcomas in rabbits by intravenous or intramedullary injection. Studies describing the induction of lung tumors (adenomas, adenocarcinomas) by beryllium via inhalation during exposure periods of up to 72 weeks are listed in Table 1. Intratracheal instillation of beryllium also resulted in the induction of lung tumors and extrapulmonary lymphosarcomas and fibrosarcomas in rats (Groth *et al*., 1980; Ishinishi *et al*., 1980).

Table 1. Induction of lung tumors in animals exposed to beryllium via inhalation

Study	Species/strain	Compound
Reeves *et al*., 1967	male, female Sprague-Dawley rats	beryllium sulfate
Schepers, 1961	male, female Sherman and Wistar rats	beryllium phosphate, beryllium fluoride, zinc beryllium silicate
Wagner *et al*., 1969	male Charles River CR-CD rats	beryl ore
Vorwald, 1968	male, female rhesus monkeys	beryllium sulfate

Beryllium compounds were shown to induce osteogenic sarcomas in rabbits by intravenous injection in 12 studies and by intramedullary injection in 4 studies (US EPA, 1991)

V. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Wagoner *et al*. (1980) studied a cohort of 3055 white males employed at a single beryllium processing plant in Pennsylvania (exposed to beryllium metal, oxide or hydroxide) sometime between January 1, 1942 and December 31, 1967 with a median duration of employment of 7.2 months. A significantly increased incidence of mortality due to lung

cancer was observed in the entire cohort (47 observed versus 34.29 expected, $p < 0.05$), as well as in the 2068 cohort members followed for 25 years or more since initial employment (20 observed versus 10.79 expected, $p < 0.01$). When the number of expected deaths was recalculated using 1968-1975 lung cancer mortality data, significance was lost for the cohort overall (38.2 expected), but not for the subgroup followed for 25 years or more (13.36 expected, $p \approx 0.05$) (Bayliss, 1980; MacMahon, 1977, 1978). However, significance was lost for the subgroup when the number of expected deaths was adjusted for smoking (14.67 expected) (US EPA, 1986).

The data of Wagoner *et al*. (1980) was used for the quantitation of cancer potency due to inhalation exposure despite study limitations. Human inhalation exposure is usually to beryllium oxide rather than other beryllium salts. Animal studies utilizing beryllium oxide have used intratracheal instillation instead of inhalation exposure. The use of the available human data therefore avoids uncertainties due to cross-species extrapolation, and uses the most relevant route of administration and beryllium species.

Methodology

A risk assessment was performed based on the occupational exposure study of Wagoner *et al*. (1980). The narrowest range for median exposure that could be obtained on the basis of available information was 100 to 1000 $\mu g/m^3$. Effective dose was calculated by adjusting for the duration of daily (8 of 24 hours) and annual (240 of 365 days) exposure, and the fraction of the lifetime at risk (time from start of employment to study termination). Smoking-adjusted expected lung cancer deaths were found to range from 13.91 to 14.67 (based on exposure range) compared to 20 observed. Relative risk estimates of 1.36 and 1.44 were calculated and the 95% confidence limits of these estimates used to calculate the lifetime cancer risk (Table 2). These estimates were based on one data set and a range of estimated exposure levels and times. To account for estimation uncertainties, unit risks were derived using two estimates each of concentration, fraction of lifetime exposed and relative risk. The listed unit risk factor [2.4 E-3 $(\mu g/m^3)^{-1}$] is the arithmetic mean of the 8 derived unit risks. US EPA has stated that this unit risk may not be appropriate if the air concentration exceeds 4 $\mu g/m^3$ and should not be used under those circumstances.

Table II. Effective dose, upper-bound estimate of relative risk and unit risk of carcinogenicity due to human beryllium exposure via inhalation (US EPA, 1992).

Beryllium concentration in workplace ($\mu g/m^3$)	Fraction of lifetime	Effective dose ($\mu g/m^3$)	95% upper-bound estimate of relative risk	Unit risk/ ($\mu g/m^3$)
100	1.00	21.92	1.98	1.61E-3
			2.09	1.79E-3
	0.25	5.48	1.98	6.44E-3
			2.09	7.16E-3
1000	1.00	219.18	1.98	1.61E-4
			2.09	1.79E-4
	0.25	54.79	1.98	6.44E-4
			2.09	7.16E-4

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BIS(2-CHLOROETHYL)ETHER

CAS No: 111-44-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	143.02
Boiling point	176-178.5°C
Melting point	-24.5°C
Vapor pressure	0.7 mm Hg @ 20°C
Air concentration conversion	1 ppm = 5.8 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 7.1 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 2.5 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There are no human carcinogenicity studies available for bis(2-chloroethyl)ether (BCEE).

Animal Studies

Two studies address the carcinogenicity of bis(2-chloroethyl)ether by the oral route of exposure. Innes *et al.* (1969) administered 100 mg/kg body weight bis(2-chloroethyl)ether by oral gavage to two F_1 generation strains of mice termed X (C57BL/6 × C3H/Anf) and Y (C57BL/6 × AKH) (18/sex/strain) from day 7 to 28 of life, without adjusting the initial dose to account for weight gain. After 28 days, BCEE was added to feed at a concentration of 300 ppm for 76 weeks. Surviving animals were sacrificed at 80 weeks. Ninety animals of each strain were included as controls. Tumor incidence in surviving animals is summarized in Table 1. A statistically significant increase in hepatomas ($p < 0.05$) was noted in both males and females of strain X and in males of strain Y. No other tumor type showed significant increases in incidence.

Charles River CD rats (26/sex/group) were treated with 0, 25 or 50 mg/kg-day bis(2-chloroethyl)ether by gavage for 18 months by Weisburger *et al.* (1981). Animals were observed for 2 years. No carcinogenic effects were observed, although the authors report increased mortality among the high-dose females and reduction in mean weight among high-dose males and females.

Van Duuren *et al.* (1972) report on two experimental approaches to evaluate the carcinogenicity of bis(2-chloroethyl)ether; a subcutaneous injection study and an initiation study by dermal application. ICR/Ha Swiss mice (30 females) were injected weekly with 1 mg BCEE for 68 days. No tumors remote from the injection site were observed. Two sarcomas were noted at the injection site (2/30) but were not found to be statistically different from controls (0/30) ($p > 0.05$ by Fisher's Exact Test).

Van Duuren *et al.* (1972) also treated ICR/Ha Swiss mice (20 females/group) with a single dose of 1 mg BCEE applied to the skin followed by three applications/week of the tumor promoter phorbol myristate acetate (PMA) in acetone for life. Control groups included animals not treated with BCEE and animals treated with BCEE but without the promoter. Skin papillomas were noted among animals treated with both BCEE and PMA, but the incidence was not significantly higher than among control animals (3/20 treated, 2/20 controls; $p>0.05$ by Fisher's Exact Test). No tumors were observed among animals treated only with BCEE.

Theiss *et al.* (1977) injected A/St mice (20 males) intraperitoneally with 8, 20, or 40 mg/kg BCEE 3 times/week for 8 weeks. After 24 weeks all surviving mice were sacrificed and examined only for lung tumors. The incidence of tumors among treated animals was not found to be significantly higher than among controls.

Table 1. Incidence of tumors in rats treated with bis(2-chloroethyl)ether* (Innes *et al.*,1969).

		Tumor Incidence			
		Strain X		Strain Y	
Tumor Type/Treatment		female	male	female	male
hepatomas	treated	4/18**	14/16**	0/18	9/17**
	control	0/87	8/79	1/82	5/90
pulmonary tumors	treated	0/18	0/16	0/18	2/16
	control	3/83	5/79	3/92	10/90
lymphomas	treated	0/18	2/16	0/18	0/17
	control	4/87	5/79	4/82	1/90

* F_1 generation mice were administered 100 mg/kg body weight bis(2-chloroethyl)ether by oral gavage from day 7 to 28 of life and subsequently in feed at a concentration of 300 ppm for 76 weeks (calculated dose is 39 mg/kg-day). Surviving mice were sacrificed at 80 weeks.

** statistically significant increase in incidence ($p < 0.001$ by Fisher's exact test)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

In the absence of studies in humans useful in evaluating the carcinogenicity of bis(2-chloroethyl)ether, a single animal study (Innes *et al.*, 1969) has been identified as appropriate for the development of a cancer potency value. The most sensitive endpoint from this study is the development of hepatomas in treated male strain X rats. Other studies either do not show development of tumors (Weisburger, 1981) or experimental duration/dosing limited the interpretation of negative data (Van Duuren, 1972; Theiss, 1977).

Methodology

Lifetime average dose estimates from the Innes *et al.* study (1969) have been calculated to be 39 mg/kg-day bis(2-chloroethyl)ether (US EPA, 1980). A linearized multistage procedure polynomial was applied to the tumor incidence data (CDHS, 1985; Anderson, 1983). The upper 95% confidence bound on the cancer potency estimate is termed q_1^*. Using the data presented in Table 1, the following cancer potencies were derived from groups showing significant increases in hepatoma incidence:

animal group	q_1^* (mg/kg-day)$^{-1}$
Strain X - males	0.086
Strain X - females	0.013
Strain Y - males	0.031

The selection of the cancer potency value has been based on the q_1^* value from the most sensitive sex and strain in this case, 0.086 (mg/kg-day)$^{-1}$ in Strain X males derived from Innes *et al.*(1969). Calculation of the cancer potency in animals (q_{animal}) can be made from the following relationship, where T is the natural lifespan of the animal (104 weeks) and T_e is the experimental duration (80 weeks):

$$q_{animal} = q_1^* \times (T/T_e)^3$$

The resulting q_{animal} is 0.19 (mg/kg-day)$^{-1}$. Conversion to human cancer potency (q_{human}) is based on the following relationship, where bw_{animal} is the assumed body weight for the test species (0.03 kg - mice; US EPA, 1980) and bw_{human} is the assumed human body weight (70 kg):

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

The estimate of q_{human} based on this relationship is 2.5 (mg/kg-day)$^{-1}$. A unit risk value based upon air concentrations was derived by OEHHA/ATES assuming a human breathing rate of 20 m^3/day, 100% fractional absorption, and average human body weight of 70 kg. The calculated unit risk value is 7.1 E-4 ($\mu g/m^3$)$^{-1}$.

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BIS(CHLOROMETHYL)ETHER

CAS No: 542-88-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	114.96
Boiling point	106°C
Melting point	-41.5°C
Vapor pressure	30 mm Hg at 22°C
Air concentration conversion	1 ppm = 4.75 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.3 E-2 $(\mu g/m^3)^{-1}$
Slope Factor: 4.6 E+1 $(mg/kg\text{-}day)^{-1}$
[Calculated from potency value derived by RCHAS, cross-route extrapolation (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

Increases in the incidence of lung cancer have been reported in a number of studies of workers exposed to both bis(chloromethyl)ether (BCME) and chloromethyl methyl ether (CMME). Some of these studies involve workers primarily exposed to CMME contaminated with 1-8 % BCME. Exposure to CMME, a known human carcinogen, is a confounding variable in these studies. However, there are several studies in which individuals were known to have been exposed to BCME, but exposure to CMME was not known to have occurred and appears unlikely.

Theiss *et al*. (1973) (reviewed by IARC, 1973) reported a retrospective study of a small group of BCME workers exposed between 1956 and 1962. Six cases of lung cancer were found in 18 men employed in a testing facility; 5 of the 6 men were smokers. Two additional lung cancer cases were found in a group of 50 production workers. Five of the 8 total cases were oat-cell carcinomas. Exposure periods were 6-9 years, and tumor latency was 8-16 years.

Sakabe (1973) reported on lung cancer cases occurring in 32 workers exposed to BCME in a Japanese dyestuff factory in the period 1955-1970. Five cases of lung cancer were reported compared to 0.024 expected cases ($p < 0.001$). One case was reported to be oat-cell carcinoma; the others were of mixed histological types. Duration of exposure to BCME ranged from 4 to 7 years; cancer mortality latency ranged from 8 to 14 years after initial exposure. It was noted that all the workers that developed lung cancer were also smokers, and that 4 of the 5 cases were also exposed to other industrial chemicals.

Lemen *et al*. (1976) conducted a retrospective cohort study of cancer incidence in a group of 115 white male anion-exchange resin manufacturing workers in San Mateo County, California. Worker tobacco smoking status was evaluated and used to adjust expected tumor incidence rates. Five cases of lung cancer were observed compared to 0.54 cases expected ($p < 0.01$), representing a nine-fold increased lung cancer risk. The histological type of lung cancer primarily observed was small cell-undifferentiated; exposure ranged from 7.6 to 14 years (mean of 10 years). The mean induction-latency period was 15 years. No quantitative worker exposure evaluation was performed.

The studies described above demonstrated a significant increase in lung cancer incidence, predominantly small-cell-undifferentiated carcinoma. This histologic type is not the one generally associated with smoking (squamous cell carcinoma).

Animal Studies

Male Sprague-Dawley rats and golden Syrian hamsters (50/exposure group) received 1, 3, 10 or 30 exposures (6 hours/exposure) to 1 ppm BCME by inhalation (Drew *et al*., 1975). After exposure, the animals were exposed for the remainder of their lifetime. Median survival time for hamsters receiving 0, 1, 3, 10 or 30 exposures was 675, 620, 471, 137 and 42 days, respectively. Median survival time for exposed rats was 467, 457, 168, 21 and 23 days, respectively. One rat in the 3 exposure group developed a squamous-cell carcinoma of the skin; additionally, one hamster in the 1 exposure group developed an undifferentiated nasal tumor. These tumor incidences were not statistically significant. However, the study treatment durations were short, and survival of the treated animals was poor.

Kuschner *et al*. (1975) exposed male Sprague-Dawley rats and golden Syrian hamsters to BCME by inhalation. Groups of 100 hamsters and 70 rats were exposed to 0.1 ppm BCME 6 hours/day, 5 days/week. Control group sizes were not stated. Exposure was generally for the life of the animals. After 80 exposures, 57/70 rats were still alive; 20 rats were then removed from the exposure schedule and observed for the remaining life of the animals. Mortality at 60 weeks was approximately 90% for rats (both animals exposed for their entire lifetime and animals receiving 80 exposures) and hamsters; corresponding control mortality at 60 weeks was approximately 40% and 15% for rats and hamsters, respectively. Two rats in the group receiving 80 exposures developed tumors; the tumor types were a nasal esthesioneuroepitheloma and a keratinizing squamous cell carcinoma of the lung. Additionally, one hamster developed an undifferentiated carcinoma of the lung. No corresponding tumors were reported in control rats or hamsters. Additional groups of rats were given 0, 10, 20, 40, 60, 80 or 100 6-hour exposures to 0.1 ppm BCME (group sizes 240, 50, 50, 20, 20, 30, and 30, respectively), then observed for the life of the animals. Mortality of the exposed animals in all exposure groups was equivalent to that of controls. Nasal and lung tumors were noted in the exposed animals. Nasal tumor types included esthesioneuroepithelomas, unclassified malignant olfactory tumors, squamous cell carcinomas involving the turbinates and gingiva, poorly differentiated epithelial tumors and adeno-carcinomas of the nasal cavity. Lung tumors included squamous cell carcinomas and adeno-carcinomas. Tumor incidence data for combined respiratory tract tumors is listed in Table 1.

Table 1. Bis(chloromethyl)ether-induced respiratory tract tumors in male Sprague-Dawley rats (Kuschner *et al*., 1975)

Number of exposures (6 hours, 0.1 ppm)	Human equivalent[1] $(mg/kg/day)^{-1}$	Tumor incidence[2]
0	0	0/240
10	0.00027	11/41
20	0.000541	3/46
40	0.00105	4/18
60	0.00184	4/18
80	0.00347	15/34
100	0.00373	12/20

1. Calculated by US EPA (1991)
2. Incidence of respiratory tract cancers in animals surviving beyond 210 days.

Male Sprague-Dawley rats (Spartan substrain) (120/group) and Ha/ICR mice (144-157/group) were exposed to 0, 1, 10 or 100 ppb BCME by inhalation for 6 hours/day, 5 days/week for 6 months (Leong *et al*., 1981). The animals were then observed for the duration of their lifespan. No significant increases in mortality were associated with BCME exposure, except for the 100 ppb exposure group; all animals in this group were dead by 19 months. Significant treatment-related increases in the incidence of respiratory tract tumors were noted. Tumor types included nasal esthesioneuroepithelomas and carcinomas, and pulmonary adenomas. Tumor incidence data is listed in Table 2.

Table 2. Bis(chloromethyl)ether-induced nasal tumors in male Sprague-Dawley rats (Leong *et al*., 1981)

Concentration[1] (ppb)	Tumor Incidence[2]
0	0/112
1	0/113
10	0/111
100	97/112

1. Animals were exposed to 0, 1, 10, 100 ppb BCME for 6 hours/day, 5 days/week for 6 months.
2. Incidence of nasal tumors as reported by CDHS (1988).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Cancer potency factors for BCME were derived from male Sprague-Dawley rat respiratory tract tumor data (Kuschner *et al*., 1975; Leong *et al*., 1981). Cancer potency values are based on the most sensitive site, species and study demonstrating carcinogenicity of a particular chemical, unless other evidence indicates that the value derived from that data set is not appropriate (CDHS, 1985). The Kuschner *et al.* (1975) study used relatively high exposure levels of BCME. The exposure levels used in the Leong *et al*. (1981) study were lower, and the dose-response exhibited is highly non-linear. Therefore, a cancer potency estimated from the Leong *et al*. (1981) data set may be more representative of low-dose rate potency. For low dose exposures to BCME (below 1 ppb), the potency value was calculated from dose-response data published by Leong *et al*. (1981); for periodic high dose exposures (at or above 1 ppb BCME), the potency was derived from the study by Kuschner *et al*. (1975) (CDHS, 1988).

Methodology

Cancer potency factors (q_1^*) were derived using a linearized multistage procedure (CDHS, 1985) with the dose-response data for male Sprague-Dawley rat respiratory tract tumors (Kuschner *et al.*, 1975) and nasal tumors (Leong *et al*., 1981). Absorbed doses were calculated assuming complete absorption of inhaled BCME, using an inspiration rate of 0.29 m^3/day for Sprague-Dawley rats. The dose from a continuous exposure to 1 ppb BCME (4.7 µg/m^3) would therefore be 1.36 µg/day, or 2.6 µg/kg-day. The cancer potency factors (q_1^*) derived from the Leong *et al*. (1981) and Kuschner (1975) data sets were 8.9 $(mg/kg/day)^{-1}$ and 47 $(mg/kg/day)^{-1}$, respectively. Surface area scaling was employed to transform animal cancer potency factors to human cancer potency factors, using the relationship ($q_{human} = q_{animal} * (bw_h / bw_a)^{1/3}$), where q_{human} is the human potency, q_{animal} is the animal potency, and bw_h and bw_a are the human and animal body weights, respectively. Body weight values used for humans and Sprague-Dawley rats were 70 kg and 0.52 kg, respectively. The human cancer potency factors (q_1^*) derived from the Leong *et al*. (1981) and Kuschner (1975) data sets were 45.6 $(mg/kg/day)^{-1}$ and 240 $(mg/kg/day)^{-1}$, respectively. The unit risk factor was derived by OEHHA/ATES from the low dose exposure cancer potency value using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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1,3-BUTADIENE

CAS No.: 106-99-0

I. PHYSICAL AND CHEMICAL PROPERTIES (from HSDB, 1998)

Molecular weight	54.09
Boiling point	-4.4° C
Melting point	-108.9° C
Vapor pressure	910 mm Hg at 20° C
Air concentration conversion	1 ppm = 2.21 mg/m^3 at 25° C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor:	1.7 E-4 $(\mu g/m^3)^{-1}$
Slope Factor:	6.0 E-1 $(mg/kg\text{-}day)^{-1}$

[Calculated from lung alveolar and bronchiolar neoplasms in female mice (Melnick *et al.*, 1990) using a linearized multistage procedure (OEHHA, 1992).]

III. CARCINOGENIC EFFECTS

Human Studies

Several studies have examined cancer mortality rates among industrial workers who were likely to have been exposed to butadiene. However, these studies generally considered workers likely to have had contemporaneous exposure to other potential carcinogens (most notable styrene). Nevertheless, studies of two work environments are sufficiently specific to butadiene exposure to provide limited supporting evidence for the carcinogenic effects observed in animal bioassays. These studies are a case-control study of rubber workers by Matanoski *et al.* (1989) and cohort studies of a butadiene manufacturing plant by Downs *et al.* (1987) and Divine (1990). In addition, Checkoway and Williams (1982) observed statistical associations of blood abnormalities with butadiene exposure at a facility where excess leukemia and lymphoma had been reported.

The first epidemiological study evaluating the possibility of an increased risk of carcinogenicity following occupational exposure to butadiene and other compounds was conducted by the National Institute of Occupational Safety and Health (NIOSH). A study conducted at the University of North Carolina (Spirtas, 1976) prompted NIOSH to examine the issue of styrene-butadiene exposures and a possible link to leukemia (NIOSH, 1976). Leukemia rates in the area surrounding the Port Neches plants, Texas, were found to be above national rates.

McMichael *et al.* (1976) examined deaths occurring from 1964 through 1973 in a male population which had been employed at a large tire manufacturing plant in Akron, Ohio. Standardized Mortality Ratios (SMRs) for the study population indicated that deaths due to several types of cancer exceed rates for the 1968 U.S. male population. Statistically

significantly elevated SMRs were found for stomach cancer (171) and lymphatic and hematopoietic cancers (136). Other solvents and monomers to which these workers were exposed included styrene, benzene, and toluene. Work for five years or more in the synthetic plant was associated with significantly elevated risk ratios for lymphatic and hematopoietic neoplasms (6.2), lymphatic leukemia (3.9), and stomach cancer (2.2).

Andjelkovich *et al.* (1976, 1977) reported the mortality experience of 8,418 white male workers in a large rubber manufacturing plant, also in Akron, Ohio. The cohort was initially divided into two age groups, those under 65 and those 65 or older. SMRs were elevated (but not necessarily significantly) in both age groups in both cohorts for cancers of the stomach, large intestine, and prostate as well as for lymphosarcoma. The SMR for monocytic leukemia (311) was significantly elevated for this entire cohort.

Another cohort of 13,570 white males who had worked for ≥ 5 years in a Goodrich plant in Akron, Ohio was examined for mortality outcome from 1940-1976 (Monson and Fine, 1978). External comparisons (SMR) of mortality, based on U.S. white males, and internal comparisons of incidence were performed in this study. Leukemia and lymphatic cancers were elevated in a number of job categories as was the incidence of gastrointestinal cancer. Solvents were suggested by the authors to have been responsible for the increased incidences; the elevation cannot be specifically attributed to butadiene.

In an investigation of the health effects of styrene exposure, Ott *et al.* (1980) studied 2,904 employees of Dow Chemical plants who had worked for at least one year over the years from 1937 to 1970. SMRs for leukemia (176) and for lymphatic and hematopoietic neoplasms (132) were elevated but not significantly. Therefore, as with the previous studies (McMichael *et al.*, 1976; Andjelkovich *et al.*, 1976, 1977; Monson and Fine, 1978), this report is suggestive of an increase in incidence of lymphatic and hematopoietic cancers in a cohort associated with multiple types of chemical exposures. The increase cannot be definitely attributed to butadiene exposure.

White male workers who had been employed for at least six months in two SBR plants in eastern Texas were studied for an excess of leukemia (Meinhardt *et al.*, 1982) and an attempt was made to correlate the results to occupational chemical exposures. There were 1,662 study subjects in plant A and 1,094 in plant B. Workers were followed from 1943-1976 at plant A from 1950-1976 at plant B. There were no significantly elevated SMRs observed from workers in plant B. In plant A, SMRs were elevated but not significantly for lymphatic and hematopoietic neoplasms (155) and for several subcategories within that classification including leukemia (203).

Divine (1990) found a significantly elevated SMR for lymphosarcoma among 2,582 workers in a butadiene manufacturing facility. The SMR for lymphosarcoma was even higher in those with routine exposure to butadiene. Matanoski *et al.* (1990) observed an excess of leukemia and lymphatic and hematopoietic cancers in black production workers and an elevated SMR for residual cancers of the lymphohematopoietic system for all production workers in several styrene-butadiene rubber (SBR) plants in the U.S. and Canada. Presumably, in SBR plants, production workers had the highest likelihood of

exposure to butadiene, although they may have been exposed to other substances that are, or may be, linked to some of these cancers. While it is difficult to establish a causal relation with butadiene exposure, the fact that cancers of the lymphohematopoietic system were reported in mice suggests that the association deserves close attention in future studies.

Animal Studies

Inhalation of butadiene has been shown to induce tumors in mice and rats at multiple sites. These sites include the heart, lung, mammary gland, ovaries, forestomach, liver, pancreas, Zymbal gland, thyroid, testes, and hematopoietic system. Butadiene is only one of two chemicals known to induce cancer of the heart in laboratory animals.

Mice

The most detailed evaluations of the carcinogenicity of butadiene are the mouse inhalation studies sponsored by the National Toxicology Program (NTP), mouse I (NTP, 1984), and mouse II (Melnick *et al.*, 1990). The nominal doses of study I were 0, 652 or 1,250 ppm administered 6 hours/day, 5 days/week for either 60 weeks (males) or 61 weeks (females). Fifty animals per sex/dose were used. Although the study was designed for 103 weeks, early deaths resulted largely from malignant neoplasms involving multiple organs (heart, hematopoietic lymphomas, lung, mammary gland, ovaries, forestomach, and liver). The incidences of total significant tumor bearing animals (i.e., the number of animals bearing one or more significant tumors) at control, middle, and high doses were 2/50, 43/49, and 40/45 in the males and 4/48, 31/48, and 45/49 in the females. Tumor incidence data are provided in Table 1.

In study II, lower exposure concentrations of butadiene (i.e., 0, 6.25, 20, 62.5, 200, and 625 ppm) were used than had been employed in the first study. Interim sacrifices at 40 and 65 weeks of exposure were also added to the original study design in order to follow progression of lesions. As in the previous study, hemangiosarcomas of the heart, hematopoietic lymphomas, squamous cell neoplasms of the forestomach, alveolar-bronchiolar neoplasms, and/or adenocarcinomas of the mammary gland were frequently observed in mice which died between weeks 40 and 65 of the study. Tumor incidence data used in the quantitative risk assessment are given in Table 2.

Table 1: Incidence of primary tumors in mice exposed to butadiene in the "Mouse I" study[a] (OEHHA, 1992).

Site /Lesion	Sex	Nominal Dose (ppm) in Air		
		0	625	1250
Heart / Hemangiosarcoma	M	0/50[d]	16/49[c]	7/49[c]
	F	0/49[b]	11/48[c]	18/49[c]
Hematopoietic System /	M	0/50[b]	23/50[c]	29/50[c]
Malignant Lymphoma	F	1/50[b]	10/49[b]	10/49[c]
Lung / Alveolar and Bronchiolar Adenoma	M	2/50	12/49	11/49
	F	3/49	9/48	20/49
Lung / Alveolar and Bronchial Neoplasm	M	2/50[b]	14/49[c]	15/49[c]
	F	3/49[b]	12/48[c]	23/49[c]
Mammary / Acinar Cell Carcinoma	F	0/50[b]	2/49	6/49[e]
Ovary / Granulosa Cell Neoplasm	F	0/49[b]	6/45[c]	13/48[c]
Forestomach / Papilloma and Carcinoma	M	0/49	7/40[c]	1/44
	F	0/49[b]	5/42[e]	10/49[c]
Liver / Adenoma	F	0/50	1/47	4/49
Liver / Adenoma and Carcinoma	F	0/50[d]	2/47	5/49[e]

[a] Tumor incidences based on U.S. EPA evaluation of NTP (1984) study.
[b] Increasing trend ($p < 0.01$); [c] Increase compared to control ($p < 0.01$).
[d] Increasing trend ($p < 0.05$); [e] Increase compared to control ($p < 0.05$).

Table 2: Incidence of Primary Tumors in Mice Exposed to Butadiene in the "Mouse II" Study[1] (OEHHA, 1992).

Site / Lesion	Sex	Nominal Dose (ppm) in Air					
		0	6.25	20	62.5	200	625
Heart / Hemangiosarcoma	M	0/70	1/49	1/50	5/38	20/35	6/11
	F	0/70	0/50	0/50	1/33	20/31	26/31
Hematopoietic system /	M	4/50	3/50	8/42	11/44	9/33	69/71
All malignant lymphomas	F	10/50	14/47	18/44	10/38	19/33	43/48
Lymphocytic lymphomas	M	2/50	1/50	2/40	4/40	2/29	62/65
	F	2/50	4/44	6/43	3/38	11/27	36/42
Lung / Alveolar and	M	22/48	23/48	20/44	33/46	42/48	12/16
bronchiolar neoplasm	F	4/50	15/44	19/43	27/44	32/40	25/30
Forestomach / Papilloma and carcinoma	M	1/70	0/50	1/60	5/38	12/33	13/17
	F	2/70	2/50	3/38	4/33	7/23	28/33
Ovary / Granulosa cell Neoplasm	F	1/69	0/59	0/59	9/38	11/25	6/14

[1] Source: Data of Melnick *et al.*, 1990. Figures adjusted for intercurrent mortality.

Rats

A two-year rat inhalation toxicity/carcinogenicity study (Hazleton Europe, 1981) was also evaluated. Groups of 100 per sex/dose were exposed to 0, 1,000, or 8,000 ppm butadiene for 6 hours/day, 5 days/week for 105-111 weeks. Unlike the mouse I study, survival of treated animals was not adversely affected in the first year of the study, but during the second year there was a statistically significant relationship between mortality and air concentration of butadiene. The published incidences (Owen, 1987) are slightly different form those given by U.S. EPA (1985). The total significant tumor incidences (number of animals bearing one or more significant tumors) in males based on U.S. EPA criteria and 1987 published incidences are 4/100, 5/100 and 20/100 for the control, low, and high dose groups (Leydig cell tumors, pancreatic exocrine tumors, and Zymbal gland tumors). Total female significant tumor incidences in the control, low and high dose groups were: 18/100, 19/100, and 41/100 (mammary carcinoma, thyroid follicular cell tumors, and Zymbal gland tumors). Tumor incidence data are shown in Table 3.

Table 3: Incidence of Primary Tumors in Rats Exposed to Butadiene[a] (OEHHA, 1992)

Site / Lesion	Sex	Nominal Dose (ppm) in Air		
		0	1000	8000
Mammary / Fibroadenoma	F	32	64	55
Mammary / Carcinoma	F	18	15	26
TOTAL		50[b]	79[c]	81[c]
Thyroid / Follicular cell adenoma	F	0	2	10
Thyroid / Follicular cell carcinoma	F	0	2	1
TOTAL		0[b]	4	11[c]
Uterus / Cervical stromal sarcoma	F	1	4	5
Testis / Leydig cell adenoma or carcinoma	M	0[d]	3	8[c]
Pancreas / Exocrine adenoma	M	3[d]	1	10[e]
Zymbal gland adenoma	M	1	1	1
	F	0	0	0
Zymbal gland carcinoma	M	0	0	1
	F	0	0	4
TOTAL	M	1	1	2
	F	0[d]	0	4

[a] Data of Hazleton Europe (1981) as published by Owen *et al.*, 1987. Number of rats examined: 100 males and 100 females.
[b] Increasing trend ($p < 0.01$); [c] Increase compared to control ($p < 0.01$)
[d] Increasing trend ($p < 0.05$); [e] Increase compared to control ($p < 0.05$)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

OSHA (1990) has classified butadiene as a "potential occupational carcinogen". U.S. EPA (1985) and IARC (1987) have concluded that the evidence for carcinogenicity of butadiene in animals is sufficient. These organizations have classified the chemical as Group B2 and 2B respectively in their schemes of ranking potential human carcinogens.

With respect to quantitative risk assessment, the epidemiological data base is still considered inadequate for predicting risks of community exposure to butadiene. Thus, the quantitative risk assessment presented in this document relies on data from animal bioassays rather than epidemiologic studies. Cancer potencies were calculated using tumor incidence data from NTP (1984), Melnick *et al.* (1990), and Hazelton Europe (1981).

Methodology

Cancer potency estimates were made for mice and rats using total significant tumor incidences and individual site incidences, three measures of dose, and the linearized multistage procedure of low dose extrapolation. The most sensitive tumor site was the lung alveolar and bronchiolar neoplasms in female mice (mouse II bioassay data of Melnick *et al.*, 1990). The continuous internal dose was considered to be the best measure of dose available. When interspecies equivalent units of mg/m^2 surface area were used, the resulting upper range of human cancer potency based on all rodent assays was 4.4×10^{-6} to 3.6×10^{-4} $(\mu g/m^3)^{-1}$. The range of upper bound risk is based on the two orders of magnitude difference between potency figures for the mouse and the rat. This difference has been the subject of much additional metabolic and kinetic investigation. In addition to a higher metabolic rate for butadiene in the mouse, limited detoxification and accumulation of the primary reactive genotoxic metabolite (BMO) may be a significant factor in the increased susceptibility of mice to butadiene-induced carcinogenesis. The most detailed evaluation of the carcinogenicity of butadiene has been conducted in the mouse.

The staff of the Office of Environmental Health Hazard Assessment concluded that, for use in risk assessment, the quality of the mouse II bioassay data is superior to that of the rat data. The primary reasons for this conclusion are: 1) the use of lower, more relevant dose levels in the mouse II study; 2) the use of five dose levels in the mouse II study, compared to two in the rat study; 3) the presence of two mouse studies; 4) the fact that the rat study has not been replicated; 5) the consistency in sites of carcinogenicity between the two mouse studies; 6) the greater detail in the available mouse data which allows in-depth analysis; and 7) suggestions from limited epidemiological observations that butadiene exposure may be associated in humans with lymphatic and hematopoietic cancers, effects that were seen in mice. The analysis above using lung alveolar and bronchiolar neoplasm incidences in female mice (mouse II bioassay data of Melnick *et al.*, 1990) resulted in a cancer potency of 6.0 $(mg/kg\text{-}day)^{-1}$, and a cancer unit risk of 1.7×10^{-4} $(\mu g/m^3)^{-1}$.

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CADMIUM

CAS No: 7440-43-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1998) except as noted)

Molecular weight	112.41
Boiling point	765 ºC
Melting point	321 ºC
Vapor pressure	1 mm Hg at 394 ºC
Air concentration conversion	1 ppm = 1.8 mg/m^3 (from NIOSH, 1994)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.2 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 1.5 E+1 $(mg/kg\text{-}day)^{-1}$
[Human occupational exposure lung cancer data (Thun *et al*., 1985), Poisson regression model fitted by CDHS (1986), resulting model parameters applied to California life table to calculate cancer risk, reevaluated by CDHS (1990).]

III. CARCINOGENIC EFFECTS

Human Studies

US EPA has reviewed the epidemiologic evidence on health effects due to cadmium exposure (US EPA, 1985). Most of the studies were occupational mortality studies in which cause-specific death rates were compared to expected rates based on a standard population, with the ratio of observed to expected deaths yielding a standardized mortality ratio (SMR). Outcomes examined in these investigations included cancer of the respiratory tract, prostate, bladder, kidney, and gastrointestinal tract. The results were not entirely consistent, but the evidence for an effect of cadmium exposure was strongest for lung cancer, prostate cancer and renal cancer.

Lemen *et al*. (1976) found an excess of prostate cancer deaths among 292 workers employed for greater than two years in a job with potential cadmium exposure. The excess was significant if the analysis assumed a 20-year latency period. However, a follow-up study of this cohort by Thun *et al*. (1985) uncovered no new deaths due to prostatic cancer. The authors suggested that given the generally nonfatal nature of the disease, mortality studies frequently may not be sensitive enough to detect a potentially real association with incidence of prostate cancer. Sorahan and Waterhouse (1983, 1985) followed up a 1967 report by Kipling and Waterhouse which had found a highly significant excess incidence of prostatic cancer. Both the incidence report (Sorahan and Waterhouse, 1985) and the mortality study (Sorahan and Waterhouse, 1983) found no significantly elevated risk if the original four index cases were excluded. However, inclusion of these cases in the analysis yielded a highly significant association. For mortality, using cumulative years of high

exposure to cadmium, the *p*-value was less than 0.05 when controlling for sex, year of study, employment, age at starting employment, and duration of employment. For morbidity, using more than one year of high exposure, the value of *p* was less than 0.001 (1.99 expected, 8 observed, *p*-value not given by authors but calculated by DHS staff based on a Poisson distribution). Tumor incidence was determined using the Birmingham Regional Cancer Registry. The authors do not provide information on completeness of ascertainment by this registry.

The SMR study by Andersson *et al*. (1984) and the matched case-control study by Ross *et al*. (1983) both failed to reject the null hypothesis of no effect on risk of prostate cancer. However, the SMR was considered "possibly" increased and the lack of statistical significance could have been due to deficiencies in the measure of exposure. Similarly in the study by Ross *et al*., the odds ratio (OR) for cadmium exposure among prostatic cancer cases as compared to controls was 2. The smallest OR which would have an 80% chance of being detected as statistically significant in a study this size is 4; therefore, the lack of significance should be interpreted cautiously. In general, the power of these studies was not sufficient to detect the small relative risks for prostate cancer deaths expected from cadmium exposure.

The evidence available at that time appeared to be inconclusive regarding the effect of cadmium exposure on prostatic cancer. Given the highly significant early reports, it may be that cadmium acts as a promoting agent, inducing earlier tumors in those already susceptible. This effect may have been reduced markedly in recent years due to the lowering of exposure levels, sometimes by an order of magnitude or more (Thun *et al*. 1985, Andersson *et al*. 1984, Sorahan and Waterhouse 1985). Therefore, those with earlier exposure may have been at highest risk, and the cohorts most recently studied, being heterogeneous with respect to their exposures, show only nonsignificant increases in prostate cancer incidence, e.g., a doubling or less, and no increase in mortality. Because the human studies repeatedly found some elevation in risk, albeit a nonsignificant one, the staff of DHS decided that the evidence does not permit a conclusive rejection of a possible effect of cadmium on prostate cancer.

Table 1 summarizes the epidemiologic evidence relating respiratory cancer SMR's to cadmium exposure. A significantly increased risk of respiratory cancer deaths was seen by Lemen *et al*. (1976), Thun *et al*. (1985), Sorahan and Waterhouse (1983), Varner (1983), and Armstrong and Kazantzis (1983), but not by Inskip *et al*. (1982) nor by Andersson *et al*. (1984). However, the assessment of exposure in the two towns investigated by Inskip *et al*. relied only on 1979 soil samples for exposure from 1939 to 1979. Even with a questionable exposure assessment, males in the exposed town had a lung cancer SMR which, while not statistically significant, was nearly double that of males in the unexposed town (101 vs. 55). The other negative study (Andersson *et al*., 1984) had low statistical power to detect a SMR of less than 200. In other studies, the range of SMR's for respiratory cancer was 120-230 (see Table 1). The staff of DHS concluded that the two negative studies for respiratory cancer are not convincing evidence of no effect, due to low statistical power in one study and poor exposure data in the other.

The study by Thun *et al*. (1985) showed a positive dose-response relationship where dose was expressed as cumulative mg-days/m^3. Varner (1983) reported the lung cancer PMR (proportional mortality ratio) to be elevated. Sorahan and Waterhouse (1983), in two separate analyses, found an elevated risk of respiratory cancer. The first analysis was based on the SMR and included all potentially exposed workers. The second analysis used the regression method of life tables (RMLT) and assessed exposure by cumulative years employed in a (1) high exposure job or (2) high or moderate exposure job or (3) high or moderate exposure job excluding welding. Measures (2) and (3) resu1ted in a significant effect of exposure on respiratory cancer, particularly for those with more than 30 years of follow-up.

Table 1: Association Between Cadmium Exposure and Respiratory Cancer Mortality

Authors	SMR	Significant at $p < 0.05$
Sorahan and Waterhouse 1983	127	Y
Lemen *et al*., 1976	235	Y
Thun *et al*., 1985	229	Y
Armstrong and Kazantzis 1983	126[a]	Y
Inskip *et al*., 1982	101[b]	N
Andersson *et al*., 1984	120	N

[a] For workers with >10 years exposure in the "always low" category (the number of workers with "ever medium" and "ever high" exposures was small).
[b] vs. SMR-55 for the unexposed town.

Heavier smoking among cadmium workers as compared to the general population could account for the small but statistically significant SMR for lung cancer (126) observed by Armstrong and Kazantzis (1983) for those exposed >10 years at the "always low" category. No smoking histories were available. Sorahan and Waterhouse (1983) also lacked data on smoking, but they argue that smoking was unlikely to have been a confounder for two reasons. First, their analysis showed an increasing association with duration of employment, while smoking habits are unlikely to be well-correlated with duration of employment. Secondly, deaths from other diseases of the respiratory system were not elevated, as they would have been if the cohort had included a disproportionate number of smokers. However, the effect of nickel hydroxide could not be disentangled from that of cadmium oxide in this cohort.

The strongest evidence for cadmium-induced carcinogenicity in humans is the study conducted by Thun *et al*. (1985). The characteristics of this study which make it particularly convincing are the quality of the exposure data and the analysis of potential confounding. Since the quantitative results of this study constituted the basis for the DHS risk assessment of cadmium, a full description of this study is presented below.

Thun *et al*. (1985) conducted a follow-up of the report by Lemen *et al*. (1976), who had found an increase in mortality from respiratory and prostate cancer and from nonmalignant lung disease in a cohort of cadmium smelter workers. Thun *et al*. (1985) expanded the cohort and extended the follow-up period. The final cohort included those hired after 1925

and employed 6 months or longer in production areas of the plant during the period 1940-1969. The cause-specific death rates were adjusted by the indirect method to yield standardized mortality ratios (SMRs) and by the direct method to yield standardized rate ratios (SRRs). The SMR for lung cancer in the overall cohort was 147, while for those with 2 or more years of employment it was 229, with a 95% confidence interval of (131,371).

Exposure data that had been collected since the 1940's allowed evaluation of the lung cancer SMR by dose. Industrial hygiene measurements for departments and job sites with potential cadmium exposure were available (Smith *et al*. 1980). These were combined with individual work histories for each member of the cohort in order to assign an exposure level to each work day. Interruptions of employment were taken into account and exposure levels were adjusted to reflect respirator usage in departments where these were worn. A cumulative exposure in mg-years/m^3 was then assigned to each person-year of follow-up for each worker. The range of cumulative exposures was divided into three categories and both SMRs and SRRs were calculated for each category. The results are shown in Table 2 using US white males as the comparison population, and in Table 3 using Colorado white males as the comparison population. (Thun presented the analysis using Colorado white males as the control group at the Fifth International Cadmium Conference, February 1986, in San Francisco. This analysis assumes that pre-1950 lung cancer rates equaled those in 1950, since cause-specific rates were not tabulated in that state before 1950.)

Table 2: Lung Cancer (ICD 162-163) Mortality By Cumulative Exposure White Male Cadmium Workers Hired on or After 1/1/26 Compared to U.S. Death Rates (Adapted from Thun *et al*. (1986), Table 7)

Exposure (cumulative mg/m^3)					
Range	Median	Person years at risk	Deaths	SMR	SRR
≤384	184.1	7005	2	53	0.48
385-1920	795.6	5825	7 (6)*	152 (130)*	1.55 (l.33)*
≥1921	2761.6	2214	7	280	3.45
U.S. WHITE MALES				100	1.00

* Numbers in parentheses exclude one lung cancer death which was originally miscoded as being due to another cause.

The data indicate a clear dose-response relationship between cumulative cadmium exposure and the risk of death due to lung cancer. Using the US population as the comparison group, both the SMR and the SRR rise from about 1/2 the expected at "low" cumulative exposure to about 3 times the expected at high cumulative exposure. Both of

these measures of risk are larger when the Colorado population is used as a standard, with the SRR rising from 0.7 to over 5.0.

Table 3: Lung Cancer (ICD 162-163) Mortality by Cumulative Exposure; White Male Cadmium Workers Hired on or After 1/1/26 Compared to Colorado Death Rates, 1950-79 (Adapted from Thun *et al.* 1985, Table 8).

Cumulative exposure (mg-days/m^3)	Person-years at risk	Deaths	SMR	SRR
≤384 385-1920 ≥1921	7005 5825 2214	2 7(6)* 7	76 212(182)* 387	.70 2.29(l.96)* 5.09
Colorado white males			100	1.00

* Numbers in parentheses exclude one lung cancer death which was originally miscoded as being due to another cause.

Thun *et al*. (1985) calculated the standardized rate ratio (SRR) for each of 3 exposure groups. (The person-years at risk, rather than individual workers, were classified by cumulative exposure to that point in time.) The SRR is suitable for subgroup comparisons, but not for external comparisons. A regression of the SRRs yielded a slope of 7.33×10^{-7} which differed from zero with a probability of 0.0001.

Selection criteria described by Thun *et al*. (1985) appear to have been unbiased: all retired, deceased, and active employees who had worked a minimum of 6 months in production areas of the plant were included in the cohort. In calculating cumulative exposure, dates of interruption of employment were accounted for. Since more than 80% of the workers were followed for 20 or more years it is likely that the follow-up was sufficient for many latent cadmium-induced cancers to become manifest and lead to death. Trained nosologists evaluated the death certificates. As indicated by Thun *et al*., one lung cancer death was originally miscoded as being due to another cause. Removal of this death from the lung cancer deaths (i.e. restoring it to the original, but incorrect coding) is necessary in order that the comparison with general population rates be unbiased (since miscodings also occur in the general population). However, the findings are not altered in any substantial way by this reclassification.

Exposure categories were chosen prior to the analysis. The cumulative exposure for all person-years was miscalculated by Thun *et al*. (1985) because they included non-workdays. This does not cause bias for purposes of. inference since the misclassification was equivalent for all exposure categories. It would, however, alter the dose-response relationship, and therefore DHS staff adjusted for this error in conducting their risk assessment, since an overestimate of exposure would result in an underestimation of potency. The corrected exposures are shown in Tables 2 and 3.

If the cadmium-exposed workers included a disproportionate number of individuals with exposures to other agents responsible for lung cancer, then the observed association might be spurious. The potential confounders with regard to lung cancer mortality in this cohort were smoking and arsenic exposure.

(a) Smoking:

Indirect evidence that smoking was not a confounder in this cohort is provided by the cardiovascular death rate in this cohort, which was 35% lower than expected based on U.S. white male death rates. If this cohort included a higher proportion of total smokers or heavy smokers as compared to the general population of white males in the same age categories, then one would expect an increase (or at least not a deficit) in the cardiovascular death rate as well.

Data on the smoking habits of these workers were provided to Thun *et al*. (1985) by the company. The data came from company medical records and from a questionnaire survey mailed to surviving workers or the next-of-kin in 1982. The results of this survey have elicited differing interpretations depending on the choice of measure of smoking and on the choice of the comparison group. The 1985 paper by Thun *et al*. reported data on 70% of the workers. For these workers, the data indicated that as of 1982, 77.5% were current or former smokers compared to 72.9% current or former smokers among U.S. white males 20 years or older reported in the 1965 Health Interview Survey (HIS) conducted by the National Center for Health Statistics. It is clear that these 2 figures are not comparable since data from 1982 for the exposed group were compared with data from 1965 for the control group.

In the updated report by Thun *et al*. (1986), presented at the Fifth International Cadmium Conference in San Francisco, February 6, 1986, the authors provided a more meaningful comparison by limiting the smoking analysis to the 49% of the cohort for whom lifetime smoking histories were available. These data indicated that as of 1965 a larger percentage of the cadmium-exposed cohort were nonsmokers and a smaller percentage were heavy smokers compared to general population rates available from the HIS. The year 1965 was chosen since this was the midpoint of the study.

The percent who "ever smoked" was 77.5% in the cadmium-exposed cohort, and 76% in the total HIS sample. The data on the cadmium workers represented information from only 36% of the cohort. Given that the cohort under study was considerably older than the HIS sample, that the HIS survey was done about 10 years earlier than the survey of the cadmium cohort, and that different information was reported from these two surveys, the differences between the smoking habits of the total HIS sample and those of the cadmium-exposed workers do not appear to be very large.

The magnitude of confounding from differential smoking habits can be assessed. A method to estimate the contribution of smoking to lung cancer mortality in the cohort is described by Axelson (1978). The method is applied to the lifetime smoking histories summarized by Thun *et al*. (1985). The calculations (summarized in Table 4) are based on information

regarding smoking habits in the exposed group, smoking habits in the comparison group, and the relative risk for lung cancer at each level of smoking. In view of the data indicating a deficit of smokers in this cohort compared to the general population, the baseline SMR for lung cancer would have been reduced 30%.

It is unknown, however, whether the smoking histories of the 49% sample were representative of the cohort as a whole, and whether the histories themselves were biased, since they were collected retrospectively. While smoking may have confounded the relationship between cadmium and lung cancer, it is unlikely that smoking was responsible for all of the excess. Furthermore, if the smoking habits in this cohort were correctly reported, i.e., if the observed deficit of smokers was real, then the excess of lung cancer deaths is larger than originally calculated. In other words, confounding due to smoking did not create the appearance of a nonexistent carcinogenic effect from cadmium; rather, the confounding reduced the apparent magnitude of cadmium's carcinogenicity.

Table 4: Technique Used to Adjust for Cigarette Smoking (Thun *et al*., 1986)

Population	Percent of Population, 1965 Nonsmokers[3] (1X)	Moderate[1] Smokers (10X)	Heavy [2] Smokers (20X)	Rate Ratio of Overall Population Relative to Nonsmokers	Rate Ratio Relative to U.S.
Exposed	48.4%	40.8%	10.8%	6.724	0.70
U.S.	27.1%	53%	20%	9.571	1.0

1. 1-24 cigarettes/day
2. 25+ cigarettes/day
3. The numbers in parentheses refer to the relative risk for lung cancer associated with each level of smoking.
4. Usable information available on 250 persons hired after 1926.

(b) Arsenic

The plant employing the workers in this cohort refined cadmium metals and compounds from 1926 onwards. Between 1918 and 1925 it had functioned as an arsenic smelter. Therefore, the analysis by Thun *et al*. excluded workers employed prior to January 1, 1926. (For those employed prior to 1926 the lung cancer SMR was 714). Nevertheless, it is possible that residues of arsenic contributed to the lung cancer excess for those first employed in 1926 or later.

To estimate the possible contribution of arsenic to lung cancer in this cohort, Thun *et al.*:

(1) identified the departments and job categories which were likely to have involved continued exposure to arsenic;

(2) calculated the proportion of person-years spent in areas with probable arsenic exposure based on personnel records (20%);

(3) evaluated industrial hygiene measurements to estimate air concentrations (range - 300 to 700 $\mu g/m^3$; Thun used midpoint - 500 $\mu g/m^3$);

(4) estimated the total years of employment for workers in the cohort (1728 years);

(5) based on (2), (3), and (4), estimated that total arsenic exposure amounted to 345.6 person-years of exposure to air levels of 500 $\mu g/m^3$;

(6) assumed a 75% respirator protection factor (i.e. inhaled exposures were 25% of air concentrations or 125 $\mu g/m^3$. This yielded a total exposure of 43,200 μg-years/m^3.

Using a risk assessment model developed by OSHA for arsenic carcinogenicity, Thun calculated that 43,200 $\mu g/m^3$ years of exposure to arsenic would contribute no more than 0.768 lung cancer deaths. This may represent an overestimate of the contribution of the arsenic exposure to the lung cancer excess. The reasons submitted by Thun are as follows:

1) Only a fraction of jobs in the "arsenic areas" had exposures as high as the furnace area (500 $\mu g/m^3$);

2) The high exposure jobs were frequently staffed with brief employment-entry (sic) level workers who are not in the study cohort;

3) Urinary arsenic levels on workers in the "high arsenic" areas from 1960-80 averaged only 46 $\mu g/l$ (equaling an inhaled arsenic of 14 $\mu g/m^3$);

4) Thus, assuming an average inhaled arsenic concentration of 125 $\mu g/m^3$ for these years overestimates the dose by 9-fold;

5) ASARCO has previously argued that the OSHA risk assessment overestimates "by a factor of three or more" the expected increase in mortality from respiratory cancer. (Thun, personal communication; cited in CDHS, 1986)

The last issue with respect to confounding concerns the combined effects of arsenic and smoking on lung cancer, which are more than additive, though probably less than multiplicative. Therefore, if any of the workers who were exposed to arsenic were smokers, there could also be confounding from the interactive effect of these two exposures. However, when relative risks are small (e.g., less than 1.3), there is very little difference between additive and multiplicative effects. Since it is unlikely that in this cohort the relative risk associated with either arsenic or smoking is larger than 1.3, the effect of any interaction is likely to be negligible. (If both relative risks are 1.3, multiplying yields 1.69, adding yields 1.6, difference = .09.)

In conclusion, given the low level of arsenic exposure and the evidence indicating a deficit of smokers in this cohort, DHS staff decided that the apparent association between cadmium exposure and lung cancer were not likely to be explained by confounding from smoking and/or arsenic exposure.

To summarize the DHS staff's findings with regard to the study by Thun *et al*. (1985) - the SMR of 2.3 in those with more than 2 years of cadmium exposure and the dose-response relationship are unlikely to be explained by chance, by bias, or by confounding from smoking and/or arsenic exposure. The staff of DHS concluded that the excess of lung cancer deaths in the study by Thun *et al*. (1985) is best explained by exposure to high levels of cadmium. The DHS staff further concluded that this study constitutes strong evidence of human carcinogenicity.

Animal Studies

Cadmium has been the subject of numerous studies in experimental animals to determine its carcinogenic potential. These studies have been extensively reviewed elsewhere (IARC 1973, 1976; EPA 1981, 1985); only the inhalation and intratracheal administration studies will be discussed here.

Sanders and Mahaffey (1984) examined the carcinogenic potential of cadmium oxide in male rats by intratracheal instillation. The rats were treated one, two or three times with 25 μg of cadmium oxide. The first administration was given at 70 days of age and then at 100 and 130 days of age depending on the total dose to be given (25, 50, or 75 μg). The animals were then followed for their lifetime. No differences were found in survival times or organ weights between treated and control groups. Using life-table and contingency table statistical analyses a significant increase in benign mammary fibroadenomas was observed in the high dose group. Additionally, there was a significant increase in the number of rats in the high dose group that had three or more tumor types.

Hadley *et al*. (1979) exposed a group of 61 male Wistar strain rats one time to an airborne cadmium oxide aerosol concentration of 60 mg/m^3 for 30 minutes. The mass median diameter of the particles was 1.4 μm with a geometric standard deviation of 1.9 μm. Seventeen animals were used as controls. Twenty-seven exposed animals died within three days from acute pulmonary edema. The remaining animals were then observed for one year. No morphological changes were noted in the lungs of exposed animals, although one animal did have a well-differentiated pulmonary adenocarcinoma. The authors observed that this tumor's relatively short latency period and the low spontaneous incidence (0.1%) of such tumors suggested that it resulted from cadmium exposure.

Both the Sander and Mahaffey (1984) study and the Hadley *et al*.(1979) study were not adequate to assess carcinogenic potency, since the animals were only exposed for short periods and, in the Hadley *et al*. (1979) study, were not followed for sufficient time. Without continuous exposure, effects in the lungs may not occur or the study may not be sensitive enough to detect adverse effects.

In the only long-term inhalation study available to the cadmium TAC document, Takenaka *et al*. (1983) exposed rats to several concentration of a cadmium chloride aerosol. Groups of 40 male Wistar rats were exposed to a continuous (23.5 hours/day) airborne concentration of 13.4, 25.7, or 50.8 µg of cadmium/m^3 of air for 18 months. A control group of 41 rats was exposed to filtered room air. The aerodynamic mass median diameter of the aerosol particles was 0.55 µm with a arithmetic standard deviation of 0.48 µm and a geometric standard deviation of 1.8 µm. The rats were followed for an additional 13 months before surviving rats were sacrificed.

There were no statistically significant differences seen in body weight or survival between exposed and control groups. The incidence of lung carcinomas was significantly increased ($p > 0.014$, Fisher's exact test) in all exposure groups. Three lung tumor types were identified, adenocarcinoma, epidermoid carcinoma, and mucoepidermoid carcinoma. The numbers of animals in each group that had these tumor types are given in Table 5. The first lung tumor was observed at 20 months. In the high-dose group, the first tumors were observed at 23 months and 23 out of 25 animals in this group dying or sacrificed after 27 months had lung tumors. Therefore, these appear to be late-developing tumors.

Table 5: Lung tumors in rats exposed to cadmium chloride aerosols (Takenaka *et al*., 1983)

Exposure Group	# rats with tumors				
	# rats examined histologically	Adenocarcinoma	Epidermoid Carcinoma	Mucoepidermoid Carcinoma	Total Carcinomas
Control	38	0	0	0	0
13.4 µg/m^3	39	4	2	0	6
25.7 µg/m^3	38	16	5	0	20[b]
50.8 µg/m^3	35	15	8	3	25[b]

a Airborne exposure concentrations are based on the cadmium, not cadmium chloride, concentration.

b One rat had both an adenocarcinoma and an epidermoid carcinoma.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

A quantitative cancer risk assessment for cadmium using the data from the occupational mortality study by Thun et al. (1985) and extrapolating to ambient levels in California was done by DHS staff. The exposure data in this study were based on industrial hygiene measurements and individual work histories. These measurements consisted of historical area monitoring samples and, when appropriate, were adjusted to reflect respirator protection in departments where respirators had been worn. For workers employed 6 months or longer in production areas of the plant the person-years of follow-up were

divided into 3 categories according to cumulative exposure in mg-days/m^3 (see Table 6). The risk of death from lung cancer for each exposure group was measured by the standardized mortality ratio (SMR). The data indicated a clear dose-response, with SMRs of 53, 152 and 280 for the low, moderate and high exposure groups. Because the study related quantified exposure levels to quantified measures of lung cancer risk, the data were suitable for a risk assessment.

Table 6: Cadmium Exposure Levels of Workers in Thun *et al*. (1985) Occupational Exposure Study

	Cumulative exposure in mg-days/m^3			Equivalent lifetime dose rate* in μg/m^3
	Range reported by Thun *et al*.	Median	Median adjusted for 240 workdays/year	Median
Low	≤584	280	184.1	2
Middle	585-2920	1210	795.6	11.8
High	≥2921	4200	2761.6	41.0

* Assumes 24 hour/day exposure and an estimated average lifetime of 61.5 years.

Methodology

A Poisson regression model was fitted to the data. In this model the observed deaths are a function of two variables: the dose and the expected deaths. The function has two parameters: one for the carcinogenic potency of cadmium, the other to account for the healthy worker effect.

Obs_i = observed deaths in exposure group i

Exp_I = expected deaths in exposure group i based on the indirect method of age adjustment

d_i = median dose received by group I

The model is then expressed as:

$E[\ Obs_i\ ] = (1 + \beta d_i) \times \alpha \times Exp_i$ where E[ ] represents the expectation of a random variable, α = healthy worker effect, and β = potency of cadmium per unit dose.

This model predicts that in the absence of cadmium exposure ($d_i = 0$), the observed deaths will equal the expected deaths times some factor which distinguishes the workers from the general population, a factor which can be termed the "healthy worker effect". The appropriateness of this model is indicated by the mortality experience of the low exposure group, which had an SMR for lung cancer of 53. (The cohort also had a low SMR for cardiovascular deaths.) This model therefore separates the carcinogenic effect of cadmium

from the opposing, healthy worker effect. Using a nonlinear regression procedure (NLIN), the parameters were estimated at:

$\hat{\alpha}$ = 0.500 (unitless parameter) and
$\hat{\beta}$ = 0.0017 (cumulative mg-days/m^3)$^{-1}$

the 95% (two-tailed) upper confidence limit for β was 0.0079, and the χ^2 goodness-of-fit statistic was 0.15 (1 df, p = 0.70). Lung cancer deaths predicted by the model were compared with the observed lung cancer deaths for the three exposure groups (Table 7).

Table 7: Lung Cancer Deaths Among Cadmium-Exposed Workers: Observed And Predicted (Data of Thun *et al*., 1985)

	Cumulative Exposure Groups		
	Low	Middle	High
Observed	2	6	7
Predicted*	2.94	5.99	7.44

* Linear relative risk model with healthy worker effect, U.S. controls.

With these estimates of the parameters, the model was then applied to the California population to predict the excess number of lung cancer deaths induced by cadmium exposure. First, a current life table was produced for California males and females separately, using five-year age intervals. The background hazard of lung cancer death for each five-year age interval was calculated using 1980 census data for California (Bureau of the Census, 1982) and age-specific death rates for California from 1979-80 vital statistics data (California Department of Health Services, 1982) by standard statistical techniques (Chiang, 1984). These were then summed over a lifetime.

Next, using the estimated value for β and setting α = 1 for the general population (i.e. no healthy worker effect), the hazard of lung cancer death given a continuous lifetime exposure to 1 μg/m^3 cadmium was calculated from the model. Using these hazard rates, a new life table was constructed. Subtracting the background probability of a lung cancer death from that obtained for an exposed population resulted in a range of risk for excess lifetime cancer from 2×10^{-3} to 1.2×10^{-2} (μg/m^3)$^{-1}$. CDHS also suggested that a "best" cancer unit risk value for regulatory purposes was 1.6×10^{-3} (μg/m^3)$^{-1}$ for cadmium in air.

CDHS (1990) reviewed the previous CDHS (1986) estimate of parameters generated from the fit of the Poisson regression model to the Thun *et al.* (1985) lung tumor data. The 97.5% upper confidence limit for β was calculated for a number of values of α that were in the range $0.5 < \alpha < 1.0$. All of the calculated 97.5% upper confidence limit for β were less than the corresponding values calculated from the statistical analysis program used previously by CDHS (1986).

CDHS (1990) decided that it is reasonable to restrict α to values between 0.5 and 1.0. If α were less than 0.5, the study cohort background incidence of respiratory cancer would be less than 50% of the incidence among males in the Colorado population. On the other hand, CDHS (1990) concluded that it was not appropriate to assume that the background incidence of respiratory cancer is higher ($\alpha > 1$) in the study cohort than it is in the control population. Because the lung cancer incidence for Colorado males is 72% of the rate for U.S. males, CDHS selected the value $\alpha = 0.7$ as a reasonable midrange estimate. With this choice, the values of the MLE and 95% UCL for 0 are 0.0017 and 0.0028 $(\text{mg-days/m}^3)^{-1}$, respectively. When the upper confidence limit estimate for β is used to estimate lifetime cancer risk from the data and life-table methodology used by CDHS (1986), the resulting cancer unit risk is 4.1×10^{-3} $(\mu\text{g/m}^3)^{-1}$. This value is within the range of unit risks (2×10^{-3} to 1.2×10^{-2} $(\mu\text{g/m}^3)^{-1}$) contained in the cadmium TAC document and approved by the Scientific Review Panel.

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CARBON TETRACHLORIDE

CAS No: 56-23-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1998) except as noted)

Molecular weight	153.8
Boiling point	76.7°C
Melting point	-23°C
Vapor pressure	91.3 mm Hg @ 20°C
Air concentration conversion	1 ppm = 6.3 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.2 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 1.5 E-1 $(mg/kg\text{-}day)^{-1}$
[Calculated from mouse liver tumor incidence data (Edwards *et al*., 1942) using a linearized multistage procedure, extra risk (US EPA, 1984); revised by CDHS (1987).]

III. CARCINOGENIC EFFECTS

Human Studies

Capurro (1979) reported a study on the residents in a rural valley polluted by vapors from a solvent recovery plant for at least 10 years. Chloroform, benzene, methyl isobutyl ketone, trichloroethylene and 26 other organic agents were detected in the air in addition to carbon tetrachloride (Capurro, 1973). The author reported four excess cases of lymphoma. Attributing these cancer cases to carbon tetrachloride alone would be inappropriate due to exposure to the other contaminants.

In a preliminary study of 330 laundry and dry cleaning workers, Blair *et al*. (1979) examined occupational exposure to carbon tetrachloride and other dry cleaning agents. Information from death certificates indicated an excess of deaths from lung, cervical and liver cancer, and leukemia. Katz and Jowett (1981) studied female laundry and dry cleaning workers in Wisconsin. Their results failed to show an overall increase in malignant neoplasms, but they did report an elevated risk for cancers of the kidney and genitals (unspecified), along with smaller excesses of bladder and skin cancer and lymphosarcoma. However, the use of carbon tetrachloride has been of only minor importance in dry cleaning since the 1950's and quantitative data on exposure to carbon tetrachloride were not presented in these studies.

Hernberg *et al*. (1984) reported a case-control study on primary liver cancer and exposure to solvents. Of 126 cases, two had a history of exposure to carbon tetrachloride, among

other solvents. They concluded that there was an association between primary liver cancer and exposure to "solvent" among women, but not for men.

Two reports were published on cancer mortality in a population of rubber workers (Checkoway *et al*., 1984; Wilcosky *et al*., 1984). Information on cause of death was reported earlier by McMichael *et al*. (1974). They reported a significantly elevated odds ratio relating carbon tetrachloride with lymphatic leukemia (OR = 15.3, $p < 0.0001$) and lymphosarcoma and reticulum cell sarcoma (OR = 4.2, $p < 0.05$). Attributing these outcomes to carbon tetrachloride alone is inappropriate since different solvents were used simultaneously in a given process area. A high degree of correlation also existed between exposure to several other solvents and the incidence of lymphatic leukemia (carbon disulfide, ethyl acetate, acetone, and hexane) and lymphosarcoma (xylenes, carbon disulfide and hexane). Although some of these solvents are not recognized carcinogens, these potentially confounding exposures, the lack of association of carbon tetrachloride exposures with these cancers in other studies, and the small number of cases (19/6678), preclude any causal inference from this study.

In summary, the epidemiological studies and human case reports are inadequate for use in a quantitative risk assessment.

Animal Studies

Mice

Edwards (1941) and Edwards and Dalton (1942) administered carbon tetrachloride by gavage to different strains of male and female mice (Strains A, C, CH3 and Y) two to three times a week for 8 to 23 weeks. To assess the tumor-producing ability of carbon tetrachloride, animals were necropsied 12 to 21 weeks after the last treatment. For those animals exposed to approximately 2100 mg/kg of carbon tetrachloride the incidence of hepatoma was 88.2 percent (strain CH3). Whether the carbon tetrachloride-induced hepatomas were malignant was not established histologically in the study. The animals were dosed on a non-daily schedule for a maximum of 16 weeks and sacrificed starting at 4 months of age. Since tumor expression is a function of both dosage and the latency period, any risk assessment based on these studies, with their short observational periods, will underestimate the true carcinogenic risk. In another experiment Edwards and Dalton (1942) administered 1, 2, or 3 doses of carbon tetrachloride (≈260 to 2100 mg/kg) followed by long-term observation in Strain A mice. The doses were hepatotoxic, but when the animals were examined 12 months later no tumors were observed.

Edwards *et al.* (1942) treated 56 male and 19 female L mice with 0.1 ml of 40% carbon tetrachloride 2 or 3 times/week over 4 months, for a total of 46 treatments. Animals were killed 3 to 3.5 months after the last treatment. The combined hepatoma incidence of treated male and female mice was 47% (34/73 vs. 2/152 in the untreated controls) (Table 1).

Eschenbrenner and Miller (1943; 1946) extensively examined carbon tetrachloride-induced tumor production in Strain A mice. In the first study they administered 30 doses

of carbon tetrachloride at intervals of one to five days (0, ≈160, 315, 625, 1250 and 2500 mg/kg). All animals were examined for tumors at 150 days following the first dose. Centrilobular liver necrosis was observed at all exposure levels. They reported that the incidence of hepatomas was increased as the time interval between doses increased.

In a second study Eschenbrenner and Miller (1946) administered the same total quantity of carbon tetrachloride, either in 30 doses at four-day intervals or in 120 doses on consecutive days. This study was conducted to determine the effect of liver necrosis on tumor development. They found that mice receiving the smaller dose over 120 days (a "non-necrotizing" dose) developed tumors at roughly the same or greater rate as those animals that received necrotizing doses (30 large doses at four-day intervals). The tumor incidence was not statistically significant. It appears that liver necrosis was not a required precondition for the production of tumors with carbon tetrachloride. This study showed that the total length of the exposure period (i.e., 120 versus 30 days), not the time between doses, may have been the major determining factor in the production of tumors.

Three NCI mouse bioassays used carbon tetrachloride as a positive control (NCI, 1976a,b; 1977; Weisburger, 1977) and excess mortality was a severe problem in the studies. Mice ($B6C3F_1$, males and females) were dosed by gavage (0, 1250 and 2500 mg/kg body weight) for 5 days/week for up to 78 weeks and they were to be sacrificed at 92 weeks. However, only 14% of the animals survived to 78 weeks and less than 1% survived to 92 weeks. This compares with 66% of the controls surviving the 92-week experiment. Hepatocellular carcinoma was found in almost every treated animal (Table 1). Carcinomas were observed as early as 16 weeks for the low-dose female group. The high mortality and virtual 100% tumor response are the more serious limitations of this study for use in quantitative risk assessment.

Table 1: Carbon tetrachloride-induced liver tumor incidence in mice.

Study	Strain	Dose (mg/kg-day)	Tumor incidence
Edwards et al., 1941	strain Y (male, female)	0	2/152 (2%)
		≈2100	34/73 (47%)
NCI 1976a,b; 1977	$B6C3F_1$ (male, female)	0	6/157 (4%)
		1250	89/89 (100%)
		2500	90/93 (97%)

Rats

Reuber and Glover (1970) compared the carcinogenicity of carbon tetrachloride in 12-week-old male rats (Japanese, Osborne-Mendel, Wistar, Black and Sprague-Dawley strains). The animals were subcutaneously injected (0, 2080 mg/kg body weight) twice a week for up to 105 weeks. Corn oil was administered to controls. All the Black and Sprague-Dawley strains died within 18 weeks. No carcinomas were observed. Hyperplastic nodules and hepatic carcinoma were reported in the other three strains (80%, 20%; 63%, 31%; 14%, 14% for Japanese, Osborne-Mendel and Wistar rats, respectively).

Other lesions reported were hemangiomas (13% and 8% for Japanese and Osborne-Mendel rats, respectively), carcinomas of the thyroid gland (20% and 23% for Japanese and Osborne-Mendel rats, respectively), and subcutaneous leiomyosarcoma (7% in Japanese rats). Cirrhosis was reported in all animals. Due to the small group size, poor survival of several strains and the incomplete reporting of the total dosage, and most importantly, the inappropriate route of exposure (subcutaneous injections), this study was not used in a quantitative risk assessment. As in the mouse studies, NCI used carbon tetrachloride as a positive control in rat bioassays for chloroform, 1,1,1-trichloroethane and trichloroethylene (NCI, 1976a,b; 1977; Weisburger, 1977). The Osborne-Mendel rats were administered a time-weighted average dose of carbon tetrachloride by gavage for 78 weeks (47, 97 and 80, 159 mg/kg body weight, respectively for males and females). Hepatic carcinomas were found at both doses in both sexes (4%, 4% and 8%, 2% in low and high dosage, males and females, respectively). A lower incidence was reported in the high-dose females, but this may have been a result of that dose group's high mortality rate prior to tumor expression. Tumors in other tissues were not discussed.

Male and female Syrian golden hamsters were administered carbon tetrachloride in corn oil weekly by gavage (190 and 380 mg/kg of body weight, respectively) for a total of 30 weeks (Della Porta *et al*., 1961). Following treatment, the animals were kept 25 weeks, sacrificed and examined. Only eight of the original 20 animals survived the full 55 weeks. Carcinomas were not observed in the animals that died prior to the 43rd week, but one or more liver-cell carcinomas were reported in all the surviving animals, indicating that tumors may be produced at lower levels in this species. Liver tumor incidence in carbon tetrachloride-treated animals (males and females combined) was 10/19 (53%) compared to 0/80 (0%) for controls.

In summary, carbon tetrachloride has been shown to produce liver tumors in mice, rats and hamsters by the oral and subcutaneous routes. No inhalation cancer bioassays have been conducted.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Carbon tetrachloride has been observed to induce liver tumors in male and female hamsters, mice and rats, as described above. CDHS (1987) decided that the tumor incidence data from studies by Della Porta *et al.* (1961) (hamster), Edwards *et al.* (1942) (mouse) and NCI (1977a, b) (mouse) were suitable for use in developing a quantitative risk assessment.

Methodology

A health assessment document for carbon tetrachloride was prepared by US EPA (1984). This document contained a quantitative cancer risk assessment for carbon tetrachloride. A linearized multistage procedure was applied to liver tumor incidence data (Della Porta *et*

al., 1961; Edwards *et al.*, 1942; NCI 1976a, b; 1977 [rat, mouse]) to estimate a cancer unit risk.

The quantitative risk assessment of carbon tetrachloride conducted by US EPA (1984) using the linearized multistage procedure was modified by DHS (1987) by: 1) applying an absorption fraction of 50% instead of 40%; 2) omitting one rat bioassay US EPA used (NCI, 1976a, b; 1977); 3) assuming an average inhalation intake of 18 µg/day instead of 20 µg/day; and 4) presenting the range of resulting unit risks instead of the geometric mean. DHS chose not to include the NCI rat bioassay data in the unit risk estimation because when the data are adjusted for excess mortality there is no statistically significant association between dose and tumor response. Cross-route extrapolation was used to calculate inhalation unit risk values from oral exposure data. Using an absorption fraction of 50%, an estimated human weight of 60 kg and an estimated respiration rate of 18 m^3/day, the unit inhalation intake is 4.5 times the unit oral intake. Therefore, the US EPA 95% upper confidence limit oral cancer risk values calculated were multiplied by a factor of 4.5 to obtain the values of 1.5×10^{-4} $(\mu g/m^3)^{-1}$ (Della Porta *et al.*, 1961), 4.2×10^{-5} $(\mu g/m^3)^{-1}$ (Edwards *et al.*, 1942) and 9.9×10^{-6} $(\mu g/m^3)^{-1}$ (NCI, 1976a, b; 1977 [mouse]). The cancer unit risk of 4.2×10^{-5} $(\mu g/m^3)^{-1}$ was recommended by CDHS for continuous air exposures.

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CHLORINATED DIBENZO-*p*-DIOXINS
CAS No: 1746-01-6

CHLORINATED DIBENZOFURANS
CAS No: 5120-73-19

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1998) except as noted)

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

Molecular weight	322
Boiling point	decomposes (NIOSH, 1994)
Melting point	305-306 ºC
Vapor pressure	7.4×10^{-10} mm Hg at 25 ºC
Air concentration conversion	not available

2,3,7,8-Tetrachlorodibenzofuran

Molecular weight	305.99
Boiling point	not available
Melting point	not available
Vapor pressure	not available
Air concentration conversion	not available

II. HEALTH ASSESSMENT VALUES

Congener	Unit Risk $(\mu g/m^3)^{-1}$	Slope Factor $(mg/kg/day)^{-1}$
PCDDs		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	3.8 E+1	1.3 E+5
1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin	3.8 E+1	1.3 E+5
1,2,3,4,7,8-Hexachlorodibenzo-*p*-dioxin	3.8 E+0	1.3 E+4
1,2,3,6,7,8-Hexachlorodibenzo-*p*-dioxin	3.8 E+0	1.3 E+4
1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin	3.8 E+0	1.3 E+4
1,2,3,4,6,7,8-Heptachlorodibenzo-*p*-dioxin	3.8 E-1	1.3 E+3
1,2,3,4,5,6,7,8-Octachlorodibenzo-*p*-dioxin	3.8 E-3	1.3 E+1
PCDFs		
2,3,7,8-Tetrachlorodibenzofuran	3.8 E+0	1.3 E+4
1,2,3,7,8-Pentachlorodibenzofuran	1.9 E+0	6.5 E+3
2,3,4,7,8-Pentachlorodibenzofuran	1.9 E+1	6.5 E+4
1,2,3,4,7,8-Hexachlorodibenzofuran	3.8 E+0	1.3 E+4
1,2,3,6,7,8-Hexachlorodibenzofuran	3.8 E+0	1.3 E+4
1,2,3,7,8,9-Hexachlorodibenzofuran	3.8 E+0	1.3 E+4
2,3,4,6,7,8-Hexachlorodibenzofuran	3.8 E+0	1.3 E+4
1,2,3,4,6,7,8-Heptachlorodibenzofuran	3.8 E-1	1.3 E+3
1,2,3,4,7,8,9-Heptachlorodibenzofuran	3.8 E-1	1.3 E+3
1,2,3,4,5,6,7,8-Octachlorodibenzofuran	3.8 E-3	1.3 E+1

Congener		Unit Risk $(\mu g/m^3)^{-1}$	Slope Factor $(mg/kg/day)^{-1}$
PCBs (IUPAC #, structure)			
77	3,3',4,4'-Tetrachlorobiphenyl	3.8 E-3	1.3 E+1
81	3,4,4',5- Tetrachlorobiphenyl	3.8 E-3	1.3 E+1
105	2,3,3',4,4'- Pentachlorobiphenyl	3.8 E-3	1.3 E+1
114	2,3,4,4',5- Pentachlorobiphenyl	1.9 E-2	6.5 E+1
118	2,3',4,4',5- Pentachlorobiphenyl	3.8 E-3	1.3 E+1
123	2',3,4,4',5- Pentachlorobiphenyl	3.8 E-3	1.3 E+1
126	3,3',4,4',5- Pentachlorobiphenyl	3.8 E+0	1.3 E+4
156	2,3,3',4,4',5- Hexachlorobiphenyl	1.9 E-2	6.5 E+1
157	2,3,3',4,4',5'- Hexachlorobiphenyl	1.9 E-2	6.5 E+1
167	2,3',4,4',5,5'- Hexachlorobiphenyl	3.8 E-4	1.3 E+0
169	3,3',4,4',5,5'- Hexachlorobiphenyl	3.8 E-1	1.3 E+3
189	2,3,3',4,4',5,5'- Heptachlorobiphenyl	3.8 E-3	1.3 E+1

PCDDs = polychlorinated dibenzo-*p*-dioxins. PCDFs = polychlorinated dibenzofurans. PCBs = polychlorinated biphenyls. IUPAC = International Union for Pure and Applied Chemistry.

[Linearized multistage procedure (GLOBAL79), fitted to male mouse hepatic adenoma and carcinoma data (NTP, 1982), body weight scaling, cross-route extrapolation (CDHS, 1986).]

III. CARCINOGENIC EFFECTS

Human Studies

Comprehensive reviews of the human studies of dioxin exposure and cancer risk available at the time the document entitled *Health Effects of Chlorinated Dioxins and Dibenzofurans* was written for the Toxic Air Contaminant (TAC) program (CDHS, 1986) are found in US EPA (1984) and Veterans Administration (VA) (1981, 1984). A more recent review of human dioxin exposure and cancer risk studies can be found in ATSDR (1999).

Dioxins have never been intentional products. In human exposure studies, PCDDs (polychlorinated dibenzo-*p*-dioxins) and PCDFs (polychlorinated dibenzofurans) have only been present as contaminants of other toxic chemicals, such as herbicides. Hence all studies of human PCDD/PCDF exposures have been studies of exposure to chemical mixtures that may have contained PCDD and PCDF.

VA (1981, 1984) summarized what is known about the presence of PCDD and PCDF in commercially-used chemicals. In general, PCDDs and PCDFs may be present as contaminants in the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5T). Levels of 2,3,7,8-TCDD in 2,4,5-T have been found as high as six parts per million (Rappe *et al.* 1982). Another widely used herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D) is generally regarded as uncontaminated with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD). Cochrane

et al. (1982) did detect traces of di-, tri-, and TetraCDD as high as one part per billion in technical grade 2,4-D from Canada. However, the TetraCDD isomer found in these samples was the 1,3,6,8-TCDD isomer, not the more toxic 2,3,7,8-TCDD.

Agent Orange, which was a mixture of 2,4,5-T and 2,4-D, has been shown to contain 2,3,7,8-TCDD concentrations as high as 15-47 parts per million with an average of about 2 ppm (VA 1981). PCDDs and/or PCDFs have also been found in the parts per million range in commercially used polychlorinated biphenyls (PCB), trichlorophenol (TCP), tetrachlorophenol, and pentachlorophenol (PCP) (Rappe *et al*. 1982, Hardell 1983).

Several case/control studies have been conducted in Sweden and in New Zealand. In these countries, phenoxyacetic acids and chlorophenols were used extensively for agriculture and forestry. After clinical observations of several patients with soft-tissue sarcomas (STS) and a history of heavy exposure to phenoxyacetic acids, Hardell and Sandstrom (1979) conducted a case/control study of STS and herbicide exposure. Cases were drawn from a university hospital in Northern Sweden, and consisted of 52 adult males with STS diagnosed between 1970 and 1977. Controls were drawn from general population registries, at a 4:1.matching ratio, and matched to cases on sex, age, place of residence, and vital status (whether alive or deceased). The investigators considered only non-malignant deaths for deceased controls. Study subjects (or their next of kin) provided exposure histories by a mailed questionnaire with a telephone follow-up. The odds ratio (OR) for exposure to phenoxyacetic acids only (excluding subjects exposed to chlorophenols) was 5.3 (95% confidence interval (95% CI) 2.4-11.5). For exposure to chlorophenols only (excluding those exposed to phenoxyacetic acids) the OR was 6.6 (95% CI 2.1-20.9).

To confirm these findings, Ericksson *et al*. (1981) replicated this study in Southern Sweden, using cases from a cancer registry. Similar study methods were used, including matching controls from a population registry (at a 2:1 ratio), and determining exposure by mail and telephone questionnaires. The investigators calculated separate odds ratios for exposure to phenoxy acids known to be contaminated with PCDD and PCDF (OR-17.0; 95% CI 2.1-140.0) and for exposure to phenoxy acids thought to be free of PCDD and PCDF (OR-4.2; 95% CI 1.2-14.9). When exposure was dichotomized into categories of 30 days or less, or more than 30 days, the ORs were 5.7 and 8.5, respectively, possibly indicating a dose-response trend.

One of the drawbacks of this study is that, exposure histories were provided by the study subjects; therefore, the results may be influenced by recall bias. Cases (or their next of kin) may be more likely to recall an exposure than a healthy person. In order to investigate this possible bias, Hardell (1981) duplicated the study methods using cases of colon cancer. Here there was no significant association with exposure to herbicides. Therefore, Hardell concluded that the association with STS was not due to reporting differences between diseased cases and healthy controls.

Smith *et al*. (1984) reported a similar case/control study in New Zealand. Here, male cases of STS were gathered from a national cancer registry, with controls also being selected from the same registry. This method of control selection was designed to avoid differential recall. Unlike the Swedish studies, however, the New Zealand study showed no significant

associations with reported phenoxy herbicide spraying. The authors suggested that if dioxin were the necessary agent, that Swedish herbicides may have been more contaminated than New Zealand herbicides. However, Smith *et al*. (1984) note that the Swedish investigators also found a significant association between STS and non-dioxin-contaminated herbicides, indicating that if the association were true, dioxin would not be the sole agent.

Another case/control study reported in brief by Olsen and Jensen (1984) of cases from the Danish Cancer Registry failed to show an association between nasal cancer and chlorophenol exposure, although nasal cancer was associated with occupational exposure to wood dust.

In a letter to Lancet, Milham (1982) reported proportionate mortality data from Washington state indicating that farmers suffered a significantly larger proportion of deaths due to STS. No other group occupationally exposed (foresters, orchardists, tree farmers) showed an excess of STS; however, the exposure assessment was based on occupations taken from death certificates. Furthermore, Milham indicated that 2,4-D was the predominant herbicide used, and 2,4-D is not generally contaminated with 2,3,7,8-TCDD.

A cohort study of phenoxy acid herbicide applicators in Finland was reported by Riihimaki *et al*. (1983). A historical cohort of 1926 herbicide applicators was assembled from the records of four large employers, including the Finnish Highway Authority and State Railways. These male workers had used chlorinated phenoxyacids for at least two weeks between 1955 and 1971. Their mortality between 1972 and 1980 was studied by comparing their names against population registers. National mortality figures provided expected age-standardized numbers of deaths. Deaths from all causes, and for all cancers, were less than expected. The power of this study to detect an increase in STS was poor, however, as only 0.1 case of STS was expected based on general population rates. Furthermore, as deaths in the cohort were studied only after 1972, 45 deaths that occurred in this group before 1972 were not tallied. (Even for post-1971 deaths, however, the follow-up period may also have been too short for a sufficient tumor latency period to have elapsed.)

There have been four potentially exposed occupational cohorts studied in the United States. Zack and Suskind (1980) reported the follow-up of Monsanto employees in Nitro, West Virginia, who were involved in a 1949 accident during the processing of trichlorophenol. A sudden violent reaction released fumes and residues into a building interior. Apparently, the released chemical mixture was not analyzed, but the authors assumed that it contained TCDD, as exposed workers developed chloracne. A historical cohort of 121 white male employees was assembled from company records on the basis of their having exhibited skin disorders "attributed to the 1949 TCP process accident." Their vital status was traced through 1978, providing a maximum of 29 years of follow-up per person. The standardized mortality ratio (SMR) for all causes of death in this cohort (relative to US white males) was significantly decreased (32 observed deaths vs. 46.4 expected). One cancer site showed an excess: lung cancer (5 observed vs. 2.85 expected), although this SMR of 1.75 was not statistically significant. Interestingly, there occurred one STS, a fibrous histiocytoma. However, the authors calculated SMRs (and expected numbers of deaths) only for causes with five or more observed deaths.

Zack and Gaffey (1983) described another cohort from this plant, composed of 884 male workers employed for at least one year between 1955 and 1977. It is not clear whether workers exposed in the 1949 accident were included. The same methods were used to calculate SMRs. Only 25 malignancies occurred, compared to 30.9 expected. However, two specific sites were notably elevated: lung cancer, with 14 observed vs. 9.9 expected (SMR 1.4; 95% CI 0.8-2.4), and bladder cancer, with 9 observed vs. 0.9 expected (SMR 9.9; 95% CI 4.5-18.8). One STS occurred in a worker judged to have been exposed to TCDD. One drawback to this study is that exposure histories were only constructed for the 163 decedents - and only 36% of these were judged to have had potential exposure to 2,4,5-T (and therefore TCDD). Therefore, the true exposed cohort may only have been one-third the size of the entire study group.

Cook *et al*. (1980) presented a similar historical cohort study of Dow chemical employees. In 1964, chloracne occurred in workers in a trichlorophenol manufacturing area. Industrial hygiene investigations concluded that TCDD was responsible and changes were made in the operations to decrease exposure. Levels of TCDD during this period were unknown because concentrations fell below the limit of detection at that time, 0.02 µg/ml of air (Cook 1981a); however, wipe samples were positive for TCDD. Cook *et al.* (1980) assembled a cohort of 39 workers thought to have high exposure potential, and 22 workers thought to have lower exposure. Among the high-exposure group, 87% had a history of chloracne, compared to 68% of the low-exposure group. Their vital status was determined through 1978. There were only four deaths (vs 7.8 expected based on US white males), although three of these deaths were due to neoplasms (vs 1.6 expected). One neoplasm was a fibrosarcoma.

Another Dow cohort was investigated by Ott *et al*. (1980). This cohort contained 204 white males involved in 2,4,5-T production between 1951 and 1971. The authors determined each worker's vital status through 1976, resulting in a median length of time since first exposure of about 20 years. Only one malignancy (a respiratory cancer) was recorded vs. 3.6 expected from US population rates. This cancer death occurred among the employees with 20 or more years of latency; in this group 0.9 deaths were expected.

Besides the small sample size, there are other problems with using this study for risk assessment. The exposure to TCDD may have been minimal. Environmental sampling of the breathing zone in 1969 revealed 2,4,5-T concentrations between 0.2 and 0.8 mg/m^3. Product specifications at that time called for a maximum TCDD concentration of 1 ppm. Assuming the maximum level of both 2,4,5-T in the breathing zone, and TCDD in the 2,4,5-T, the concentration of TCDD in the breathing zone would have been 10^{-6} of the concentration of 2,4,5-T, or 0.8 ng/m^3. Ott *et al*. also noted that 157 of the 204 workers (77%) were exposed for less than one year. Furthermore, a review of medical records of the cohort uncovered no cases of chloracne.

A further analysis of Dow employees was presented by Bond *et al*. (1983), who reported a morbidity survey on the combined cohorts previously described by Cook *et al.* (1980) and Ott *et al*. (1980). Bond *et al.* found few differences between the morbidity of these workers and a matched control group of workers from other locations in the plant. There were,

however, more ulcers and diseases of the digestive system (excluding liver) in the 2,4,5-T cohort, at roughly twice the prevalence in the controls. However, because the investigators only studied cohort members who participated in company medical programs between 1976 and 1978, only 69% of the original cohort was included. The study did not include workers who had died, retired, or left the company, raising the possibility that the most affected workers might have been missed.

Following the publication of the four US mortality studies, reports began to appear in Lancet of four additional cases of STS among these cohorts, bringing the apparent total to seven (Honchar and Halperin 1981, Cook 1981b, Moses and Selikoff 1981, Johnson *et al*. 1981). The proportion of deaths in these merged cohorts due to STS appeared to be far greater than would be expected (Fingerhut and Halperin 1983), although there is great difficulty in estimating expected rates of STS using general population statistics (Cook and Cartmill 1984). Fingerhut (cited in VA 1984) had the diagnoses of the seven cases reviewed by two pathologists. The pathologists could only agree on a diagnosis of STS for three of the seven, another three being reclassified, and the last diagnosis being disputed. Of the three definite cases, only two had frank chloracne to corroborate exposure. The VA review (1984) concluded that the occurrence of even two cases of STS among these relatively small cohorts warranted continued surveillance.

Other cohort studies of occupational exposures have come from Great Britain, West Germany, and the Netherlands. May (1973 and 1982) only briefly described the aftermath of a 1968 accidental release of TCP with a "higher than normal" concentration of TCDD. A total of 79 cases of chloracne were recorded, but May did not specify how many workers were exposed, so that an attack rate cannot be calculated. A survey of 46 of these workers, who were still with the company 10 years later, revealed that roughly half still had some chloracne (May, 1982). There were no other clinical problems reported, and no cases of cancer (although clearly few if any would be expected in a group this small).

Thiess *et al*. (1982) published a carefully-reported study of 74 workers exposed to dioxins during a 1953 reactor accident in a German 2,4,5-T plant. After a 23-year follow-up, this cohort exhibited seven deaths due to malignancies (vs. 4.09 expected from West German population rates), including three deaths due to stomach cancer (vs. 0.7 expected). The latter was statistically significant at a one-sided 95% level. No cases of STS occurred, although less than 0.1 would have been expected.

A mortality study of workers present at an explosion in an herbicide factory in Amsterdam was summarized by Dalderup and Zellenrath (1983). Between 200 and 500 g of TCDD were thought to have been liberated. The investigation traced 141 of 145 workers potentially exposed, and 69 (49%) had developed chloracne. After 20 years of follow-up, 8 of the workers had died with cancer (vs. 6.9 expected), yielding an SMR of 1.2 (95% CI 0.5-2.3). No STS deaths were seen. Unfortunately, the authors did not calculate SMRs separately for the group with frank chloracne (an indicator of stronger exposure), as the crude mortality for this chloracne group was 20%, and for the non-chloracne group 15%.

At the time the dioxin TAC document was prepared (CDHS, 1986), reports were starting to appear in the literature on the effects of Agent Orange herbicide exposure in Vietnam. However, most of those reports were at the time primarily anecdotal, or interim results. Agent Orange was composed of equal parts 2,4-D and 2,4,5-T, and about 90,000 tons of herbicides were sprayed in Vietnam between 1962 and 1971. Hay (1983) mentioned evidence from Vietnamese studies that "suggests a link" between herbicide exposure and liver cancer, but provided no details. Sarma and Jacobs (1982) reported three patients with STS who claimed Agent Orange exposure while serving in Vietnam.

The US Air Force's Ranch Hands study (summarized by VA, 1984) had released some initial results at the time the dioxin TAC document was prepared. This was a cohort study of some 1200 military personnel who worked on Operation Ranch Hand, the herbicide spraying operation. These subjects were matched (in a 5:1 ratio) with personnel who flew only cargo missions in Vietnam. As of 1983, the total mortality rates were nearly identical between the two groups. Only four cases of cancer had occurred among the exposed, and none were STS. The investigators stressed the preliminary nature of the data, the relatively low power of a study of this size to detect rare tumors such as STS, and the relatively short latency period up to that time (12-21 years).

A report by Greenwald *et al*. (1984) gave the results of a case/control study of STS in New York State. Cases of STS (n = 281) diagnosed between 1962 and 1980, who were between the ages of 18 to 29 during the war in Vietnam, were selected from the state cancer registry. Cases were individually age matched to living controls drawn from drivers' license files. The investigators gathered exposure information from subjects or next of kin by a telephone questionnaire. The questions focused on Vietnam service (and Agent Orange exposure in particular), but included other exposures such as chemical manufacturing and herbicide spraying in general. Only 3% of the cases and 4% of the controls had a history of Agent Orange, dioxin, or 2,4,5-T exposure. None of the various exposures proved statistically significant.

The power of this study can be criticized, with exposures as rare as they were. Also, the inclusion of cancer cases from the early 1960s can be questioned. These cases would not have had sufficient latency to have been caused by an exposure in Vietnam.

In 1983, an Australian Royal Commission began investigating the effects of Agent Orange exposure to Australian Vietnam veterans. However, their report, released in 1985, does not supply much information on the effects of PCDDs. The executive summary concluded that "only a very limited number of Australian servicemen were ever directly exposed," and further, that the dose received by the majority of Australian veterans was "so minute that it may, without doubt, be ignored," (e.g., it noted that no Australians developed chloracne). Not surprisingly, the Commission found no evidence of any cancer excess among the "exposed" servicemen (Royal Commission, 1985).

There are only a few cases where dioxin exposure of the general population has been documented; the Seveso incident in Italy, is one of them. In 1976, a chemical plant producing 2,4,5-trichlorophenol, exploded and released into the air several chemicals including TCDD in the vicinity of Seveso. The Seveso incident represents a unique event

in the sense that exposure to the toxic chemical was not limited to occupational exposure by workers but the whole population was affected by the TCDD release in the area surrounding a pentachlorophenol manufacturing facility that experienced an explosion and fire releasing dioxins into the atmosphere. Children, woman and men of various age were exposed to different degrees depending on the distance and direction from the origin of the plume.

Abate *et al*. (1982) summarized the series of studies following the 1976 accidental release of TCDD from a TCP-producing plant in Seveso, Italy. The investigators looked at mortality rates for 11 municipalities for four years after the accident and reported no increase in cancer mortality. These studies served mainly to provide baseline rates for future studies, because clearly not enough time had elapsed to provide the minimum 10 to 20 years required for an increased cancer risk to become manifest (Bruzzi, 1983).
Fifteen years after the industrial accident, Bertazzi et al. (1997) examined the cancer mortality among residents (20 to 74 years old) of Seveso by comparing populations living in dioxin contaminated areas (divided into three zones: highest, lower and lowest zone of exposure to dioxin, zone A, B, and R, respectively) with population from neighboring noncontaminated areas (zone nonABR). No increase for all-cancer mortality, or major specific sites like respiratory cancer among males and breast cancer among females, was found. However, other specific cancer mortality was observed and could be associated with dioxin exposure. Table 1 represents cancer mortality for men and women living in zone B.

Increased mortality from stomach cancer (RR = 2.4; 95% CI = 0.8-5.7) was reported 10 years after the accident in women living in zone B. In men, increased mortality from rectal cancer (RR = 6.2; 95% CI = 1.7-15.9) was observed. Leukemia in men represented one of the highest risks seen in zone B for hematologic neoplasms and was statistically significant (RR = 3.1; 95% CI = 1.3-6.4). Multiple myeloma in women (RR = 6.6; 95% CI = 1.8-16.8), and Hodgkin's disease in both genders (RR = 3.3; 95% CI = 0.4-11.9 in men; and RR = 6.5; 95% CI = 0.7-23.5 in women) were also noted in that zone. In the young population (20,000 subjects aged 0 to 19 years old), some cases of cancer were also found (Pesatori et al., 1993). Cancer cases noted included two ovarian cancers and Hodgkin’s lymphoma; myeloid leukemia represented the most evident increase although not statistically significant (RR = 2.7; 95% CI = 0.7-11.4). Two cases of thyroid cancer were also reported (RR = 4.6; 95% CI = 0.6-32.7). This observation represents an important result because of its magnitude and its correlation with experimental observations. None of the elevated cancer incidences in zone A, the area with the highest exposure, were statistically significant; however, this area also had the smallest population. Additionally, it should be noted that the Seveso population was exposed to 2–3 orders of magnitude times the level of dioxin normally experienced by the general population of industrialized countries. In 1997, individuals living in the contaminated area at the time of the accident still experienced high level of plasma TCDD 20 years after the industrial accident in Seveso. Geometric means for plasma TCDD concentration for individuals who lived in zone A, B and nonABR (control zone) in 1976 were 53.2, 11.0 and 4.9 ppt, respectively. Women in these three groups represented the gender with the highest plasma TCDD contamination (Landi et al., 1997). The authors concluded that the results indicate a

positive association between dioxin exposure and certain cancers, but further study is needed to clarify this association.

Table 1. Female and male deaths in zone B for selected causes, 1976-1991, ten years or more since first exposure (latency) and duration of exposure (length of stay in contaminated area) (Adapted from Bertazzi et al., 1997).

		Latency > 10 years		Length of stay > 10 years	
		Female	Male	Female	Male
All cancers	OBS	23	31	20	29
	RR	1.4	1.0	1.4	1.1
	(95% CI)	(0.9 –2.1)	(0.7 –1.4)	(0.8 –2.1)	(0.7 – 1.6)
Digestive cancer	OBS	10	12	9	12
	RR	1.5	1.0	1.6	1.2
	(95% CI)	(0.7 – 2.7)	(0.5 – 1.8)	(0.7 – 2.9)	(0.6 – 2.1)
Stomach cancer	OBS	5	X	4	
	RR	2.4	X	2.3	
	(95% CI)	(0.8 – 5.7)		(0.6 – 6.0)	
Lymphatic and hemopoietic	OBS	4	4	3	4
	RR	2.8	2.5	2.4	2.5
	(95% CI)	(0.7 – 7.1)	(0.7 –6.4)	(0.5 – 7.1)	(0.7 –6.4)
Multiple myeloma	OBS	3		2	
	RR	15.9		11.0	
	(95% CI)	(3.2 – 46.5)		(1.2 – 39.6)	
Rectal cancer	OBS		4		4
	RR		6.2		7.2
	(95% CI)		(1.7 – 15.9)		(1.9 - 18.4)
Leukemia	OBS		2		2
	RR		3.4		3.9
	(95% CI)		(0.4 – 12.3)		(0.4 – 14.1)

OBS = observed deaths RR = relative risk CI = confidence interval

Animal Studies

Van Miller *et al.* (1977a,b) reported the results of a study in which rats were fed diets containing from 1 ppt to 1 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) for 78 weeks. Surviving rats were killed after 95 weeks. Laparotomies were performed on all surviving rats at 65 weeks and all tumors were biopsied. Rats in the three highest dose groups, receiving 50 ppb or more, died early. A variety of tumors were found in rats receiving 5 ppt to 5 ppb while no-neoplasms were found in the control or low-dose groups. The absence of tumors in these two groups is unusual in this strain of rats. In addition, because of the small number of animals in each group (10) the study was inadequate to determine the carcinogenic potential of TCDD.

Toth *et al*. (1979) administered TCDD to male Swiss/H/Riop strain mice by gavage once a week for a year, then followed them for their lifetime. The weekly doses were 0.007,

0.7, and 7.0 µg/kg. Analysis of the results from this study focused on the incidence of liver tumors. A significant increase in the incidence of liver tumors was observed in the intermediate-dose group compared to the four separate control groups. The high-dose group, however, had an incidence of liver tumors that was similar to the control group. This finding may be explained by the early mortality in the high-dose group. The average life span was 424 days for this group, compared to average life spans of between 577 and 651 days for the control groups. If the treated animals had lived it is possible that more tumors may have formed.

Kociba *et al*. (1978) conducted a two-year feeding study in male and female Sprague-Dawley rats given diets containing 2200, 210, or 22 parts per trillion (w/w) TCDD for two years. Consumption of these diets resulted in daily doses of 0.1, 0.01, and 0.001 µg/kg body weight, respectively. There were 50 male and 50 female rats in each treatment group and 86 animals of each sex in the control group. There was a statistically significant ($p < 0.05$) increase in cumulative mortality for the high-dose female group in the latter half of the study. Body weights of the male and female high-dose groups were significantly ($p < 0.05$) reduced for the last three quarters of the study; however, food intake was not altered. The combined incidence of hepatocellular carcinomas and hepatocellular neoplastic nodules in the intermediate and high-dose groups of female rats was increased above the control group. Statistically significant increased incidences of stratified squamous cell carcinomas of the hard palate and/or nasal
turbinates were observed in both male and female high-dose groups. The male group also had an increased incidence of squamous cell carcinoma of the tongue, while the female group had an increased incidence of keratinizing squamous cell carcinoma of the lung.

US EPA (1981) reviewed this study and had an independent pathologist, Robert Squire, review the tissue pathology. The incidences of significant tumors reported by Kociba *et al*. (1978) and by Squire (US EPA, 1981) are given in Table 2 for male and female rats. The results of Squire's review did not differ greatly from those reported by Kociba *et al*. (1978).

CDHS staff members concurred with earlier reviewers (IARC 1982, EPA 1984) that the study reported by Kociba *et al*. (1978) was an adequately conducted chronic carcinogenicity bioassay of TCDD, with significant effects observed at the two higher dose levels.

Table 2: Tumor incidences in Osborne-Mendel rats receiving 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in the diet for two years (US EPA, 1984)

Tumor type, sex	Dose level (µg/kg-day)			
	0	0.001	0.01	0.1
	Tumor incidence[a]			
Tongue, stratified squamous cell carcinoma				
male	0/76 (0/77)	1/49 (1/44)	1/49 (1/49)	4/42 ($p = 0.015$) (3/44) ($p = 0.046$)
Nasal turbinates/hard palate, squamous cell carcinoma				
male	0/51 (0/55)	1/34 (1/34)	0/27 (0/26)	4/30 ($p = 0.017$) (6/30) ($p = 0.002$)
female	1/54 (0/54)	0/30 (0/30)	1/27 (1/27)	5/24 ($p = 0.009$) (5/22) ($p = 0.001$)
lung, keratinizing squamous cell carcinoma				
female	0/86 (0/86)	0/50 (0/50)	0/49 (0/49)	7/49 ($p < 0.001$) (8/47) ($p < 0.001$)
Liver, hepatocellular hyperplastic nodules, carcinomas				
female	9/86 (16/86)	3/50 (8/50)	18/50 ($p < 0.001$) (27/50) ($p < 0.001$)	34/48 ($p < 0.001$) (33/47) ($p < 0.001$)

P values determined using Fisher's exact test.

[a] Number of animals with tumor over number of animals examined (incidence reported by Kociba *et al.*, 1978). Numbers in parentheses give the incidence reported by Squire (US EPA, 1984).

The National Toxicology Program (NTP 1982a) conducted an oncogenicity bioassay of TCDD in male and female Osborne-Mendel rats. They were administered TCDD in a 9:1 corn oil:acetone vehicle by gavage at dose levels of 0.005, 0.025, or 0.25 µg/kg twice a week for 104 weeks. The treatment groups consisted of 50 rats of each sex and a vehicle control group that was made up of three subgroups of 25 rats of each sex. An untreated control group, also made up of three subgroups of 25 rats of each sex, was included in the study, but not in the statistical analysis of the results by NTP. At the dose levels used, TCDD did not have a significant effect on survival of any treatment group. The high-dose group of male rats did have a statistically-significant increased incidence of subcutaneous tissue fibromas, but it was not considered biologically significant because of the variability found. All male treatment groups had significantly ($p < 0.05$) increased incidences of thyroid follicular cell adenomas or adenomas and carcinomas, although the low- and intermediate-dose level group incidences were not significant when compared to the untreated control group by CDHS staff. The female high-dose group had significantly ($p < 0.05$) increased incidences of several tumor types, including subcutaneous tissue fibrosarcomas, liver neoplastic nodules or hepatocellular carcinomas, and adrenal cortical adenomas. Of these 3 tumors, NTP considered only the liver tumors to be related to TCDD

administration. The incidences of these tumors are given in Table 3. Toxic hepatitis was found in 14 male and 32 female high-dose level rats.

Table 3: Tumor incidences in male and female Osborne-Mendel rats given 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) by gavage for two years (NTP, 1982a)

Sex, tumor type	Dose level (µg/kg-week)			
	0	0.01	0.05	0.5
	Tumor incidence[a]			
Males				
Thyroid				
Follicular cell adenoma	1/69	5/48 ($p = 0.042$)	6/50 ($p = 0.021$)	10/50 ($p = 0.001$)
Follicular cell adenoma/carcinoma	1/69	5/48 ($p = 0.042$)	8/50 ($p = 0.004$)	11/50 ($p < 0.001$)
Females				
Subcutaneous tissue, fibrosarcoma	0/75	2/50	3/50	4/49 ($p = 0.023$) [3][b]
Liver				
Neoplastic nodules/ hepatocellular carcinoma	5/75	1/49	3/50	14/49 ($p = 0.001$)
Adrenal				
Cortical adenoma or adenoma NOS	11/73	8/49	4/49	14/46 ($p = 0.039$)

[a] Number of animals with tumor over number of animals examined.
[b] Number of animals with hepatocellular carcinoma.
NOS = Not otherwise specified. *P* values determined using Fisher's exact test.

NTP (1982a) also conducted a carcinogenicity bioassay with TCDD in male and female $B6C3F_1$ hybrid strain mice. The protocol was similar to that used in the rat study with male mice receiving the same doses of TCDD. Female rats, however, received larger doses of 0.02, 0.1 or 1.0 µg/kg twice a week. These dose levels did not have a statistically significant effect on survival of any treatment group. Male mice in the highest dose group had a significantly increased incidence of hepatocellular carcinomas. The high-dose female group had significantly increased incidences of subcutaneous tissue fibrosarcomas, hepatocellular adenomas or carcinomas, and thyroid follicular-cell adenomas. NTP considered only liver tumors and thyroid tumors to be related to TCDD administration. NTP also considered histiocytic lymphomas to have been increased in the high-dose female group; however, the staff of DHS did not consider that these lymphomas were increased when the incidences in all control subgroups were considered. The observed tumor incidences in both male and female mice are given in Table 4. Toxic hepatitis was observed in 44 male and 34 female high-dose group animals. It was also observed in several animals of the other treatment groups.

Table 4: Tumor incidences in male and female B6C3F$_1$ mice given 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TCDD) by gavage for two years (NTP, 1982a).

Sex, tumor type	Dose level (µg/kg-week)[a]			
	0	0.01 (0.04)	0.05 (0.2)	0.5 (2.0)
	Tumor incidence[b]			
males				
liver (hepatocellular carcinoma)	8/73	9/49	8/49	17/50 (p = 0.002)
Hepatocellular adenoma or carcinoma	15/73	12/49	13/49	27/50 (p < 0.001)
females				
Subcutaneous tissue, fibrosarcoma	1/74	1/50	1/48	5/47 (p = 0.032)
liver, hepatocellular carcinoma	1/73	2/50	2/48	6/47 (p = 0.014)
hepatocellular adenoma or carcinoma	3/73	6/50	6/48	11/47 (p = 0.002)
thyroid, follicular cell adenoma	0/69	3/50	1/47	5/46 (p - 0.009)

P values determined using Fisher's exact test.
[a] Dose administered to male mice; dose administered to female mice in parentheses.
[b] Number of animals with tumor over number of animals examined.

Both rat and mouse carcinogenicity bioassays conducted by NTP appear to have been done in an adequate manner. The number of treatment groups and the large dose range used in the studies are not typical of NTP bioassays, although it was similar to that used by Kociba *et al*. (1978). However, it may not have been large enough to include a dose level which produced no effect. Most significantly increased tumor incidences only occurred in the high-dose level groups, but a statistically significant dose-related trend was found in all groups.

NTP (1982b) also conducted a dermal oncogenicity bioassay on TCDD in male and female Swiss-Webster mice. TCDD in an acetone suspension was applied to the skin three days per week for 104 weeks. The male rats received 0.001 µg per application and the females received 0.005 µg per application. Separate groups of male and female mice were treated with one application of 50 µg 7,12-dimethylbenz(*a*)anthracene (DMBA) one week prior to the start of TCDD treatments. The only significantly (p = 0.01) increased incidences of tumors observed were among female mice. Both the TCDD- and DMBA/TCDD-treated groups had a similar incidences of fibrosarcoma in the integumentary system (8/27 and 8/29, respectively), compared to the vehicle control of 2/41. In NTP's judgment, the results of this experiment indicated that TCDD was carcinogenic.

HexaCDDs have been tested for carcinogenicity by NTP (1980a) in both Osborne-Mendel rats and B6C3F$_1$ mice. The bioassay tested a mixture of HexaCDDs containing 31 percent 1,2,3,6,7,8-HexaCDD and 67 percent l,2,3,7,8,9-HexaCDD. Lower chlorinated PCDDs made up the remaining 2% of the mixture, including 0.04 percent TetraCDDs. Male and female rats and male mice received weekly doses of 1.25, 2.5 or 5 µg/kg, administered by

gavage twice a week. The female mice were administered doses of 2.5, 5.0, or 10 μg/kg/week.

A dose-related "toxic hepatitis", which was noninflammatory and consisted of degenerative changes in the liver, was observed in treated rats. The treated groups of female rats had significantly increased incidences of liver neoplastic nodules. Four high-dose animals were diagnosed as having hepatocellular carcinoma. The mice also had a dose-related incidence of "toxic hepatitis" and the high-dose male and female mouse groups had statistically significant increased incidences of hepatocellular adenomas and combined incidences of hepatocellular adenomas and carcinomas. The incidences of these tumors are given in Table 5.

Several pathologists have independently evaluated the slides made from the female rat livers in this bioassay. The re-evaluations found fewer neoplastic nodules and carcinomas than did the original evaluation. Although the incidences of neoplastic nodules and carcinomas are probably lower than originally reported, the incidence is still significant in the high-dose group. The results of four separate evaluations of the liver pathology of the female rats are given in Table 6.

A dermal application carcinogenicity bioassay of the same mixture of HexaCDD in male and female Swiss-Webster mice was also conducted by NTP (1980b). This study was similar to the TCDD dermal oncogenicity bioassay in its protocol. Thirty mice of each sex were treated with 0.005 μg of the dioxin mixture three times per week for the first 16 weeks, which was increased to 0.01 μg thereafter. A similar group was initially treated once with 50 μg DMBA before being treated with the HexaCDD mixture. Thirty untreated and 45 vehicle-treated mice of each sex were used as controls. Although there was a slight increase in fibrosarcomas of the integumentary system, this was not considered by NTP to be a significant carcinogenic response. DMBA pretreatment had no additional effect.

DHS staff members agreed with IARC (1982) that there is adequate evidence to support a conclusion that TCDD is carcinogenic to rats and mice and that TCDD should be considered a potential carcinogen to humans. The NTP bioassays (NTP 1980a) of HexaCDDs also indicated that the mixture used was tumorigenic.

Table 5: Tumor incidences in female Osborne-Mendel rats and male and female B6C3F$_1$ mice given HexaCDD by gavage for two years (NTP, 1980a)

Sex, species, tumor type	Dose level (µg/kg-week)			
	0	1.25 (2.5)	2.5 (5.0)	5.0 (10)
	Tumor incidence			
female rat				
liver, neoplastic nodule or hepatocellular carcinoma	5/75	10/50 ($p = 0.026$)	12/50 ($p = 0.007$)	30/50 ($p < 0.001$)
male mice				
liver, hepatocellular adenoma	7/73	5/50	9/49	15/4 ($p = 0.003$)
liver, hepatocellular adenoma or carcinoma	15/73	14/50	14/49	24/48 ($p = 0.001$)
female mice				
liver, hepatocellular adenoma	2/73	4/48	4/47	9/47 ($p = 0.003$)
liver, hepatocellular adenoma or carcinoma	3/73	4/48	6/47	10/47 ($p = 0.004$)

P values determined using Fisher's exact test.
[a] Dose administered to male mice; dose administered to female mice in parentheses.
[b] Number of animals with tumor over number of animals examined.

Table 6: Incidence of liver tumors based on four separate pathological evaluations of female rats given HexaCDD by gavage for two years[a] (CDHS, 1986)

Pathologist and Diagnosis	dose level (µg/kg-week)			
	0	1.25	2.5	5
	Tumor incidence[b]			
NTP (1980) Neoplastic nodules or hepatocellular carcinoma	5/75	10/50 $p = 0.026$ p - 0.026	12/50 $p = 0.007$	30/50 (4)[c] $p < 0.001$
Squire (1983) Neoplastic nodules	1/75	4/50	7/50 $p = 0.007$	7/50 $p = 0.007$
Haberman and Schueler (Schueler 1983) Neoplastic nodules or hepatocellular carcinoma	NA	NA	NA	17/50 (3)[d]
Hildebrandt (1983) Neoplastic nodules or hepatocellular carcinoma	1/75	5/50 $p = 0.037$	7/50 $p = 0.007$	18/50(2) $p < 0.001$

[a] Chi-square test for trend in proportions for NTP, Squire, and Hildebrandt studies significant at $\alpha = 0.05$ level.
[b] Number of animals with tumor over number of animals examined.
[c] Number of animals diagnosed with hepatocellular carcinoma is shown in parentheses.
[d] The diagnosis for nine of the animals with neoplastic nodules was considered a matter of judgment by the pathologist.
NA = Not available.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Several human epidemiological studies of PCDD exposure reviewed in the dioxin TAC document (CDHS, 1986) reported results which suggested an increase in cancer incidence or mortality associated with PCDD exposure (Hardell and Sandstrom, 1979; Ericksson *et al*., 1981; Zack and Gaffey, 1983). However, these and the other studies described in the dioxin TAC document suffer from a number of limitations. The characterization of exposure to PCDD/PCDF were at best, uncertain. Usually the exposure occurred at a time when there were no sensitive measures of exposure levels. Exposure was often based on job title, self-reported use of substances which may have had PCDD contamination, or exposure to an event thought to have liberated PCDDs. Additionally, none of the human exposures described have been solely to PCDDs or PCDFs, but rather to a mixture of chemicals. PCDDs were only trace contaminants of other toxic chemicals. Many of the occupationally exposed subjects were exposed only briefly (e.g., during an accidental release), or worked in a possibly contaminated environment for a short time. For example, more than 75% of the workers studied by Ott *et al*. (1980) had been exposed for less than one year. Finally, many of the discussed studies, including the four US cohorts, have been hampered by small samples. Studies of only a few hundred subjects lack sufficient power to detect small increases in the risk of rare tumors. For these reasons, DHS staff members concluded that the epidemiologic data available at the time the dioxin TAC document was written provided insufficient information to conclude whether or not PCDDs or PCDFs are human carcinogens.

CDHS (1986) found that the most sensitive species, sex, and site for the induction of cancer by TCDD is the male mouse with hepatocellular adenomas or carcinomas (NTP, 1982a). This response is an order of magnitude greater than the least sensitive species, sex, and site examined, the female mouse subcutaneous fibromas. It is interesting to note that there is less than a four-fold difference in the unit risk between animal species for liver tumors. CDHS therefore developed an inhalation cancer unit risk value for TCDD based on the NTP (1982a) male mouse hepatocellular adenoma/carcinoma tumor data. CDHS also developed an inhalation cancer unit risk value for HexaCDD based on the most sensitive species, sex, and site for the induction of cancer. The data set chosen was the NTP (1980b) female rat liver neoplastic nodule or hepatocellular carcinoma incidence data as evaluated by Hildebrandt (1983).

Methodology

GLOBAL79 was used to fit a linearized multistage procedure to the NTP (1982a) male mouse hepatocellular adenoma/carcinoma tumor data for TCDD, and the NTP (1980b) female rat neoplastic nodule/hepatocellular carcinoma data for HexaCDD as evaluated by Hildebrandt (1983). This procedure provided point estimates of the extra risk for both the maximum likelihood estimate (MLE) and the linearized 95% upper confidence value (UCL). The UCL is calculated by maximizing the linear term of the procedure, or forcing a best fitting linear term if one is not present. This method of calculating the UCL is

consistent both with the expected low-dose linearity and the linear nonthreshold theory of carcinogenesis. The slope of the 95% UCL, q_1*, is taken as a plausible upper bound of cancer potency of TCDD at low doses.

The animal exposure data (NTP 1980a, 1982a) was converted into equivalent human exposures by applying appropriate scaling factors. The following assumptions were made: Oral and inhalation routes are equivalent, the concentration of TCDD in the air was assumed to be the daily oral dose, the route of exposure does not affect absorption, and there is no difference in metabolism and pharmacokinetics between animals and humans. The total weekly dose levels were averaged over the entire week to get the daily dose level. This procedure assumes that daily dosing of the animals in the NTP studies would have given the same results as did the actual twice weekly dosing schedule. Since the half-life of TCDD is relatively long, both dosing schedules should produce similar concentrations of TCDD in the animal tissues, and therefore would be expected to give similar results. The calculated daily doses are given in Table 7. Human equivalent exposures are listed in Table 8.

Because the animal dose levels for TCDD were converted to human equivalent exposure from inhalation, the 95% UCL, q_1*, is a measure of the greatest potential excess cancer risk for humans. If the lifetime daily exposure is expressed in $\mu g/m^3$, then q_1* is the excess risk associated with this exposure. Since q_1* for humans is a unit measure of excess lifetime cancer risk associated with exposure to TCDD, it is termed the unit risk. With the unit risk, the 95% UCL of excess risk may be calculated for any low-level exposure to TCDD by the equation R = unit risk × dose, where R is the 95% UCL of excess lifetime cancer risk. The cancer unit risks calculated by CDHS using the above procedure for TCDD and HexaCDD were 38 $(\mu g/m^3)^{-1}$ and 1 $(\mu g/m^3)^{-1}$, respectively.

Table 7: Calculated daily dose levels for NTP (1980a, 1982a) TCDD and HexaCDD chronic studies in rats and mice (CDHS, 1986)

Chemical	Animal	Reported Dose Level (µg/kg-week)	Calculated Dose Level (µg/kg-day)
TCDD	male and female rats, male mice	0.01 0.05 0.5	0.0014 0.0071 0.071
	female mice	0.04 0.2 2.0	0.0057 0.029 0.29
HexaCDD	female rats	1.25 2.5 5.0	0.18 0.36 0.71
	female mice	2.5 5.0 10	0.36 0.71 1.40

Table 8: Calculated equivalent human exposure to TCDD and HexaCDD based on daily animal dose levels from NTP (1980a, 1984a) carcinogenicity studies (CDHS, 1986)

Chemical	Animal	Daily Dose Level (µg/kg-day)	Airborne Concentration for Equivalent Human Exposure (ng/m^3)
TCDD	female rat (0.45)[a]	0.0014	0.93
		0.0071	4.6
		0.071	46
	male mice (0.048)	0.0014	0.44
		0.0071	2.2
		0.071	22
	female mice (0.04)	0.0057	1.7
		0.029	8.4
		0.29	84
HexaCDD	female rats (0.45) a	0.18	120
		0.36	230
		0.71	460
	female mice (0.04)	0.36	100
		0.71	210
		1.43	420

[a] Number in parentheses is animal body weight in kilograms.

CDHS recognized that total PCDD/PCDF in the air is composed of dozens of PCDD and PCDF homologues and isomers. The chemicals in such a mixture are difficult to quantitate analytically. As a result, usually only total PCDD and total PCDF are measured. In the Air Toxics Hot Spots program, certain dioxin sources are required to perform stack testing and speciate the 2,3,7,8-congeners. Thus, more data are becoming available to adequately characterize the risk from dioxin sources in California.

To estimate cancer risks from such mixtures requires information about: (1) the proportion of each PCDD and PCDF in the mixture, and (2) the carcinogenic potency of each. However, these data are not generally available. The proportion of isomers differs depending on the emission source, and only three isomers had been tested for carcinogenic potency (2,3,7,8-TCDD and a mixture of 1,2,3,6,7,8- and l,2,3,7,8,9-HexaCDD). It was also recognized that not all 2,3,7,8-isomer PCDDs and PCDFs are equally carcinogenic. The results of the bioassays on TCDD and HexaCDD suggested that carcinogenic potency may decline in homologues more chlorinated than TCDD. It was therefore assumed that PCDDs and PCDFs that are not chlorinated on the 2,3,7,8 positions or do not have at least one ring position open are noncarcinogenic. Additionally, it was also considered that the 2,3,7,8-isomer PentaCDD has a carcinogenic potency equivalent to TCDD, and that 2,3,7,8-isomer HeptaCDD is equivalent in carcinogenic potency to 2,3,7,8-isomer HexaCDD. The potencies for the homologous PCDDs were also used for the PCDFs. Using this approach, the potency of a given concentration of PCDDs would be 2% of the potency of TCDD. The potency of a mixture of PCDFs would be 3% of the potency of TCDD.

Another toxicity equivalency factor (TEF) scheme was developed after 1986 during an international symposium (NATO/CCMS, 1988a,b), and it was adopted by US EPA (US EPA, 1989) and the Department of Toxic Substances Control (DTSC) (DTSC, 1992). The international scheme, referred to as ITEFs, is based on experimental cancer and noncancer data for many 2,3,7,8-PCDDs and 2,3,7,8-PCDFs and on the assumption that the mechanism of all PCDD/PCDF-related biologic effects are based on initial binding to a specific protein, the *Ah* receptor. Because the ITEF scheme incorporated more experimental data from cancer and noncancer studies for more PCDDs/PCDFs than does the CTEF scheme, the replacement of the CTEFs by the ITEFs was considered appropriate for use in risk assessment. This approach also increases uniformity among Cal/EPA guidelines. The TEFs contained in the dioxin TAC (CDHS, 1986) document and the ITEFs are listed in Table 8. The cancer unit risks and potency factors for chlorinated dibenzo-*p*-dioxins and dibenzofurans listed in the 1999 chemical summary and Hot Spots Unit Risk and Cancer Potency Values table (OEHHA, 1999) were generated by applying the appropriate ITEFs to the cancer unit risk and potency factor for 2,3,7,8-TCDD calculated in the dioxin TAC document.

As TEFs for PCDDs and PCDFs were developed, considerable efforts went into the study of quantitative structure activity relationships (QSAR) for polychlorinated biphenyls (PCBs). PCB congeners substituted in the para and at least 2 of the meta positions but not at any of the ortho positions can adopt structural conformations most resembling that of 2,3,7,8-TCDD, therefore have the greatest potency and exert their toxicity through the *Ah* receptor pathway. These coplanar PCB congeners are structurally similar to 2,3,7,8-tetrachorodibenzo-*p*-dioxin and therefore are termed dioxin-like PCBs. Introduction of one chlorine in the ortho position results in a decrease in toxic potency and PCBs with more than one chlorine in the ortho positions lack some effects exerted by non- and mono-ortho PCBs. These PCB congeners show a different spectrum of toxic effects (Safe, 1994).

In 1991, U.S. EPA considered using the TEF methodology for PCBs. They noted that only a small subset of the 209 PCB congeners elicits dioxin-like activity and meet the criteria for inclusion in the TEF methodology. In an attempt to harmonize TEF schemes for dioxin-like compounds, the World Health Organization - European Center for Environmental Health (WHO-ECEH) and the International Program on Chemical Safety (IPCS) generated a database consisting of almost 1,200 peer-reviewed publications, representing all the available toxicological data for PCBs up to the end of 1993. From a selected number of these publications and based on four inclusion criteria, the WHO-ECEH and the IPCS proposed TEF values for 13 dioxin-like PCBs (Ahlborg *et al.*, 1994). The inclusion criteria are:

1. The compound should show structural similarity to PCDDs and PCDFs.
2. It should bind to the *Ah* receptor.
3. It should induce dioxin-specific biochemical and toxic responses.
4. It should be persistent and accumulate in the food chain.

In addition, the first WHO PCB TEF consultation (Ahlborg *et al.*, 1994) recommended expanding the current database to include all relevant information on PCDDs, PCDFs and other dioxin-like compounds that satisfied the four inclusion criteria.

Some terminologies and definitions applicable to TEFs were reviewed prior to the second WHO-ECEH consultation (van Leeuwen, 1997). The term TEF, used in the past to describe any experimental end point to be compared with TCDD was reconsidered since not all end points are "toxic" end points. For example, end points such as binding to the *Ah* receptor and induction of ethoxyresorufin-O-deethylase (EROD) are mostly considered biological/biochemical responses. Therefore, experimental end points, for which numerical values are compared to the response to TCDD, should be termed "Relative Potency" values (REPs). These REPs could be the result of a single laboratory experiment looking at a single end point. REPs are derived from the available data either used as reported in each publication, or calculated by comparing dose-response curves or ratios of medium effective doses (ED_{50}), median lethal dose (LD_{50}), median effective concentration (EC_{50}) etc. A chemical's TEF is then derived from all available REPs examined for that compound. Thus, the term TEF is be restricted to describe an overall estimate of the order-of-magnitude of the toxicity of a compound relative to the toxicity of TCDD. This estimate is derived by consensus, using careful scientific judgment of all available data (van Leeuwen, 1997; van den Berg *et al.*, 1998). The derivation of TEF consensus using *Ah* receptor-specific end points gives more weight to toxic responses than to biochemical (e.g., enzyme induction) responses and it puts more weight on *in vivo* data than on *in vitro* results. In fact, the weighting order of contributing *in vivo* data was: chronic > subchronic > subacute > acute.

In its most recent consultation in 1997, the WHO-ECEH proposed amendments to the previous NATO/WHO I-TEF scheme (NATO/CCMS, 1989). For revision of the existing mammalian TEFs, the WHO-ECEH committee agreed that if the available information was considered insufficient to warrant a change, the existing value would remain. The suggested WHO_{97} TEFs for humans and mammals along with the CTEFs and ITEFs are presented in Table 9. Taking advantage of new data and understanding of the underlying mechanisms of toxicity of dioxin-like compounds, the WHO-ECEH's re-evaluation and extension of the TEF concept lead to the following amendments:

1) For 1,2,3,7,8-PeCDD, an increase in TEF value from 0.5 to 1.0 was recommended, based on new *in vivo* tumor promotion data and CYP 1A1/A2 induction potencies from subchronic studies.

2) For OCDD, the TEF value was reduced from 0.001 to 0.0001 based on a recalculation of the old data in which exposure versus tissue concentrations were compared (administered dose); originally the TEF was based on body burdens of the chemical following subchronic exposures.

3) For OCDF, the TEF value was changed from 0.001 to 0.0001 based on new *in vivo* EROD induction potency values (81) and an expected structural similarity with OCDD; thus, for the *in vivo* situation, a change in analogy with OCDD is recommended.

The Scientific Review Panel on Toxic Air Contaminants (SRP) reviewed and endorsed the use of the WHO_{97} TEFs in Hot Spots risk assessments at its June 20, 2003 meeting. The cancer unit risks and potency factors for chlorinated dibenzo-*p*-dioxins and dibenzofurans and polychlorinated biphenyls listed in this chemical summary and the Hot Spots Unit Risk and Cancer Potency Values table were generated by applying the appropriate WHO_{97} TEFs to the cancer unit risk and potency factor for 2,3,7,8-TCDD calculated in the dioxin TAC document.

Table 9: Toxicity equivalency factors for chlorinated dibenzo-*p*-dioxins and dibenzofurans (relative to 2,3,7,8-TCDD)

Congener	**California TEF [a]**	**I-TEF [b]**	**TEF $_{WHO/97}$[c]**
PCDDs			
2,3,7,8-TCDD	1	1	1
1,2,3,7,8-PeCDD	1	0.5	1
1,2,3,4,7,8-HxCDD	0.03	0.1	0.1
1,2,3,6,7,8-HxCDD	0.03	0.1	0.1
1,2,3,7,8,9-HxCDD	0.03	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.03	0.01	0.01
1,2,3,4,6,7,8,9-OCDD		0.001	0.0001
PCDFs			
2,3,7,8-TCDF	1	0.1	0.1
1,2,3,7,8-PeCDF	1	0.05	0.05
2,3,4,7,8-PeCDF	1	0.5	0.5
1,2,3,4,7,8-HxCDF	0.03	0.1	0.1
1,2,3,6,7,8-HxCDF	0.03	0.1	0.1
1,2,3,7,8,9-HxCDF	0.03	0.1	0.1
2,3,4,6,7,8-HxCDF	0.03	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.03	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.03	0.01	0.01
1,2,3,4,6,7,8,9-OCDF		0.001	0.0001
PCBs (IUPAC #, Structure)			
77 3,3',4,4'-TCB			0.0001
81 3,4,4',5-TCB			0.0001
105 2,3,3',4,4'-PeCB			0.0001
114 2,3,4,4',5-PeCB			0.0005
118 2,3',4,4',5-PeCB			0.0001
123 2',3,4,4',5-PeCB			0.0001
126 3,3',4,4',5-PeCB			0.1
156 2,3,3',4,4',5-HxCB			0.0005
157 2,3,3',4,4',5'-HxCB			0.0005
167 2,3',4,4',5,5'-HxCB			0.00001
169 3,3',4,4',5,5'-HxCB			0.01
189 2,3,3',4,4',5,5'-HpCB			0.0001

Value introduced or changed

[a] CDHS, 1986 [b] NATO/CCMS, 1989. [c] van Leeuwen, 1997.

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CHLORINATED PARAFFINS (average chain length C12, 60% chlorine by weight)

CAS No: 108171-26-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1994) except where noted)

Molecular weight	411 (average) [NTP, 1986]
Boiling point	not available
Melting point	not available
Vapor pressure	not available
Air concentration conversion	1 ppm = 17 mg/m^3 (approximate)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.5 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 8.9 E-2 $(mg/kg\text{-}day)^{-1}$
[NTP (1986) female mouse liver tumor data, contained in Gold *et* al. database (1990), expedited Proposition 65 methodology (OEHHA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of chlorinated paraffins are known to exist.

Animal Studies

Male and female $B6C3F_1$ mice and Fischer 344N rats (50/sex/group) were treated by gavage with a commercial-grade chlorinated paraffin product dissolved in corn oil 5 days/week; treatment duration was 103 and 104 weeks for mice and rats, respectively (NTP, 1986). Exposure levels were 0, 125 and 250 mg/kg body weight for mice, and 0, 312 and 625 mg/kg for rats. Significant increases in the incidence of liver tumors were noted in male and female mice; the incidence of alveolar and bronchiolar carcinoma was significantly increased in male mice, as was the combined incidence of thyroid follicular-cell adenomas and carcinomas in female mice. Tumor incidences in mice are listed in Table 1.

Significant increases in liver tumor incidence were noted in male and female rats. Significant increases were also noted in the incidence of leukemia in male rats, and in the incidence of thyroid follicular-cell tumors in female rats. Tumor incidences in rats are listed in Table 2.

Table 1. Tumors induced in $B6C3F_1$ mice by gavage administration of chlorinated paraffins (NTP, 1986)

Dose (mg/kg bw)	Hepatocellular adenomas	Hepatocellular adenomas and carcinomas	Alveolar/bronchiolar carcinomas	Thyroid follicular-cell tumors
Males				
0	11/50	20/50	0/50	
125	20/50	34/50	3/50	
250	29/50	38/50	6/50	
Females				
0	0/50	3/50		8/50
125	18/50	22/50		12/49
250	22/50	28/50		13/49

Table 2. Tumors induced in Fischer 344 rats by gavage administration of chlorinated paraffins (NTP, 1986)

Dose (mg/kg bw)	Hepatocellular carcinomas	Hepatocellular adenomas and carcinomas	Mononuclear cell leukemia	Thyroid follicular-cell tumors
Males				
0	0/50	0/50	7/50	
312	10/50	13/50	12/50	
625	16/48	16/50	14/50	
Females				
0	0/50	0/50		0/50
312	4/50	5/50		6/50
625	7/50	7/50		6/50

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results of the NTP (1986) gavage study of chlorinated paraffins in male and female $B6C3F_1$ mice and F344 rats are listed in Gold *et al*. (1990). Benign and malignant liver tumors were observed in both sexes and species; significant elevations in tumor incidences at other sites were also observed. Estimates of cancer potency are similar for male and female mice and male rats. Cancer potency is based on dose-response data for benign and malignant liver tumors in female mice (see Table 1) (OEHHA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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CHLOROFORM

CAS No.: 67-66-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	119.49
Boiling point	61° C
Melting point	-63.5° C
Vapor pressure	200 mm Hg 25º C
Air concentration conversion	1 ppm = 4.9 mg/m^3 at 25º C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 5.3 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 1.9 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated by CDHS (1990) using a nonthreshold linear procedure. This unit risk is the arithmetic average of unit risks generated by CDHS and Bogen *et al.* (1989) for renal tumors observed in rats and mice reported by Jorgenson *et al.* (1985) and NCI (1976), and the geometric mean for supporting data sets (Roe *et al.*, 1979; Tumasonis *et al.*, 1985).]

III. CARCINOGENIC EFFECTS

Human Studies

There is no information currently available in the open literature which examines the potential relationship between exposure to chloroform in an occupational setting and human cancer. However, several studies are available which examine the relationship between trihalomethanes (THM) in drinking water and human cancer.

Many studies have concentrated on chlorination of water and concomitant production of halogenated carcinogens as a causative factor in human cancers. Cantor *et al.* (1978) compared age-adjusted cancer mortality rates by site and sex for whites in the years 1968-71 to measures of THM and the drinking water. A weighed linear regression model was used to predict cancer rates in 923 U.S. counties which were over 50% urban in 1970. Reasonably strong associations between bladder cancer and THM levels in drinking water were found after controlling for confounding by urbanization, ethnicity, social class, and county industrialization. The association was not changed by controlling for occupation in certain high-risk (for bladder cancer) industries nor by lung cancer rates used as a surrogate measure for cigarette smoking. The measure of THM most associated with bladder cancer in both white males and females was that of bromine-containing trihalomethanes (BTHM). Chloroform and total trihalomethanes (TTHM) were not as well associated. There were inconsistent associations between other cancer sites and THM levels. However, there was some evidence of and association of chloroform in drinking water with kidney cancer in males, which Cantor *et al.* believed warrants further study.

Hogan *et al.* (1979) examined the potential association between chloroform levels in finished drinking water supplies and various site-specific cancer mortality rates. The most consistent associations were between chloroform "exposure" and cancers of the bladder, rectum and large intestine. Hogan *et al.* stated that the results of this ecological study must be interpreted with caution and the association between chloroform levels in drinking water and certain types of cancer (e.g., bladder, large intestine and rectum) warrant further study.

Carlo and Mettlin (1980) analyzed 4,255 cases of cancer reported in Erie County, NY, between 1973 and 1976 for any relationship between cancer and type of water source, THM levels, and a variety of socioeconomic variables. No significant association between THM and cancers were noted in the regression analyses for the total population. When regression analyses were conducted for population stratified by race-sex, a significant association was found between THM levels in drinking water and pancreatic cancer in white males ($p <$ 0.05). The investigators caution that the lack of association between THM and pancreatic cancer in other sex-race groups and absence of association between THM and other cancer raises doubts as to the validity of this finding.

Brenniman *et al.* (1980) conducted a case-control study in Illinois to determine whether an association exists between chlorination of drinking water and gastrointestinal and urinary tract cancers. Cases (3,208) and controls (43,666) were classified according to residence in chlorinated and unchlorinated groundwater communities. Elevated risk was found for cancers of the gallbladder, large intestine, total gastrointestinal, and urinary tract for women. However, the investigators considered the results tenuous because, when the data were subclassified according to several control variables, the associations were not strengthened. Many confounding factors were not controlled including smoking, diet, ethnicity, and occupation.

Alavanja *et al.* (1980) conducted a case-control study on all gastrointestinal and urinary tract cancer deaths occurring from January 1, 1968 through December 31, 1970 in seven counties in New York. There was a statistically significant excess risk of stomach cancer in females, and of stomach, esophagus, large intestine, rectum, liver and kidney, pancreas, and urinary bladder in males residing in chlorinated water areas in the seven counties studied. The investigators concluded that the excess risk was associated with living in chlorinated areas of certain counties and was not due to a disparity in the age, race, or ethnic distribution, or to urban/rural classification, hazardous occupation, or a surface vs. ground water difference. Several confounding factors were not controlled including cigarette smoking and diet.

The association between site-specific cancer mortality and THM exposure, as estimated by chlorine dose, was investigated by Young *et al.* (1981). Cases were obtained from death certificates provided by the Wisconsin Bureau of Health Statistics and consisted of all white female deaths that occurred 1972-77 within 28 counties due to malignant neoplasms of esophagus, stomach, colon, rectum, liver, bile ducts, pancreas, urinary bladder, kidney, lung, breast, and brain. Only death from colon cancer was associated with chlorine dose (- < 0.05). The risk of colon cancer, calculated as odds ratios, was over twice as great when

the water source was affected by rural runoff. This variable was tested because of the assumption that rural runoff increased the organic precursors to THMs. While the association of colon cancer with chlorination and rural runoff factors is provocative, the findings of this study must be considered inconclusive due to the possible underestimation of risk associated with misclassification error and spurious contribution from unknown colon cancer risk factors (Young *et al.*, 1981).

Wilkins and Comstock (1981) conducted a nonconcurrent prospective study to investigate possible relationships between products of water chlorination and human cancer. Site and sex-specific incidence rates for malignant neoplasm of liver, biliary passages, kidney, and bladder were constructed from hospital records, a cancer registry, and death certificates. Incidence rates for cancer of the bladder among men and cancer of the liver among women were not significant relative to the other exposure groups among persons using water from the chlorinated surface supply. While the results were only weakly suggestive, Wilkins and Comstock noted that bladder cancer has been suggestively linked with chloroform and other indices of THM in drinking water in other studies.

Gottlieb and Carr (1982) studied the potential relationship between chlorination of drinking water and cancer in 20 south Louisiana parishes. Chlorinated surface water was associated with a significant risk for rectal cancer ($p = 0.012$). The odds ratio for rectal cancer in groups receiving high chlorination level (> 1.09 ppm chlorine) to groups with no chlorinations is 1.53 (95% CI=1.15-2.04) in surface water supplied areas. Gottlieb and Carr concluded that there appears to be some cancer risk associated with water chlorination, but definitive studies are needed with respect to the role of industrial confounders and the importance of co-contaminants.

Lawrence *et al.* (1984) used a case-control approach to study the association of chloroform exposure via drinking water to colorectal cancer in white female teachers in upstate New York. Analysis was based on 395 cases of colon and rectal cancer and 395 control noncancer deaths matched with respect to age and year of death. No effect of cumulative chloroform exposure on incidence of colorectal cancer deaths was observed.

Cantor *et al.* (1987) examined the association between use of chlorinated drinking water and bladder cancer by a case-control study design. The investigators interviewed 2,982 cases and 5,782 controls in 10 geographic areas of the U.S. Risk of bladder cancer was primarily associated with use of tap water rather than nontap beverages. Among white males, the coefficients for tap and nontap beverages were 0.176 ($p < 0.001$) and 0.037 ($p = 0.42$), and among white females, the coefficients were 0.123 ($p = 0.09$) and 0.089 ($p = 0.39$), respectively. It was suggested that nonvolatile components of tap water may be associated with risk of bladder cancer since both heated and nonheated tap water beverages were significantly associated with bladder cancer risk among males. The relative risk increased with increasing tap water intake. While this investigation was quite thorough in many respects, there is a need for confirmation of these findings. The contribution of chloroform in the etiology of human bladder cancer in men may be overshadowed by other nonvolatile chemicals present in the drinking water.

Overall, the present epidemiological evidence suggests an association between chlorinated drinking water consumption and human cancer, particularly bladder and gastrointestinal cancers. However, these relationships cannot be directly correlated to chloroform exposure because many other carcinogens are found in drinking water including other chlorinated halomethanes, brominated halomethanes, industrial pollutants, and nonvolatile halogenated compounds.

Animal Studies

The National Cancer Institute conducted carcinogenesis bioassays of chloroform in both sexes of Osborne-Mendel rats and $B6C3F_1$ mice (NCI, 1976). Mice and rats were given either corn oil or chloroform in corn oil by gavage, 5 days/week for 78 weeks. Time-weighted average doses for female rats were 100 and 200 mg/kg, and for male and female mice were 138 and 277 mg/kg, and 238 and 477 mg/kg, respectively. Tumor incidences are listed in Table 1.

A statistically significant increase ($p < 0.05$) in epithelial tumors of renal tubular origin was noted in the treated males. Ten carcinomas, two of which had metastasized, and three adenomas of renal tubular origin were found in 12 high dose male rats. In the low dose males, two carcinomas and two adenomas of tubular origin were observed in four out of 50 animals. Among the 48 high dose female rats, one tubular epithelial carcinoma and one renal squamous cell carcinoma were observed. No renal epithelial tumors were noted in matched or colony controls. The NCI reported that these type of tumors rarely occur spontaneously in Osborne-Mendel rats.

The incidence of thyroid tumors in female rats was statistically higher than controls in both treated groups ($p = 0.05$, Fisher exact test) but not in treated male rats. The incidence of hepatocellular carcinoma or neoplastic nodules was not increased in the chloroform-treated rats. Although inflammatory pulmonary lesions occurred in all test groups, the lesions were more severe and occurred more frequently in the chloroform-treated rats.

The incidence of hepatocellular carcinomas in mice was significantly elevated in all treatment groups ($p < 0.001$, Fisher exact test). The NCI reported that in their experience the spontaneous incidence of hepatocellular carcinomas in $B6C3F_1$ mice is about 5-10% in males and 1% in females. The NCI concluded that chloroform treatment was associated with increased incidences of hepatocellular carcinomas in male and female mice and renal epithelial tumors in male rats.

In addition, Reuber (1979), based on his examination of the histological sections from the NCI study, concluded that chloroform treatment was also associated with cancer of the liver in rats and an increased incidence of malignant lymphomas in mice. However, the NCI did not agree with his findings.

The carcinogenicity of chloroform given in drinking water was evaluated in male Osborne-Mendel rats and female $B6C3F_1$ mice (Jorgenson *et al.*, 1985). The chloroform used (technical grade), was found to contain 100 ppb diethylcarbonate, and trace amounts of

1,1-dichloroethane, dichloroethylene, carbon tetrachloride, and an unidentified C_5H_{10} hydrocarbon. Time-weighted average doses of chloroform calculated based on water consumption rates and body weight, ranged up to 160 and 263 mg/kg-day for rats and mice, respectively. Two control groups were used, an untreated control, and a control group of animals with restricted access to water.

Jorgenson *et al.* observed a dose-related significant increase in renal tubular cell adenomas and adenocarcinomas in male rats, but found no treatment-related increases in tumor incidence in the female mice (Table 1). The lack of liver tumors in female $B6C3F_1$ mice is in sharp contrast to the results of the NCI study. A major difference between the NCI study and the Jorgenson study is the mode of administration. Administration of chloroform to rats in a corn oil vehicle slowed the gastrointestinal absorption of chloroform relative to the absorption rate observed after administration as a bolus in water (Withey *et al*., 1983). In the Jorgenson *et al*. study, the rats received small doses of chloroform each time they drank water. The corn oil vehicle effect (Withey *et al.*, 1983) may have diminished the differences in absorption kinetics expected with the two different methods of administration. Therefore, any differences in peak blood concentrations between the NCI study and the Jorgenson study may not have been sufficient to account for the difference in liver tumor incidence. Physiologic or metabolic changes produced by corn oil consumption might interact with chemical carcinogens altering the production of liver tumors (Bull *et al.*, 1986; Newberne *et al.*, 1979).

A series of experiments was conducted by the Huntingdon Research Center to determine the effects of chronic ingestion of chloroform in a toothpaste base in mice, rats, and beagle dogs. In the first set of experiments (Roe *et al.*, 1979), doses of 17 and 60 mg chloroform/kg were administered by gavage in toothpaste to male and female ICI mice, 6 days/week for 80 weeks followed by a 16 week observation period (Experiment I). Controls (N=104) were treated with 1 ml chloroform-free toothpaste/kg-day. Aside from increased nonneoplastic liver lesions (moderate fatty degeneration), the only significant difference in pathology reported was an increase in the incidence of kidney tumors in high dose male mice, three were hypernephromas (tubular adenocarcinoma) and the remainder were adenomas (tumor incidences listed in Table 1). The incidence of renal tumors in high-dose male ICI mice was significantly greater than control mice ($p = 0.00012$, Fisher exact test). None of the female ICI mice examined developed renal tumors (Roe *et al.*, 1979). Roe *et al.* (1979) also investigated other components of the toothpaste base for carcinogenicity using male ICI mice. No lesion in this part of the study could be correlated with treatment.

In a third mouse experiment (Experiment III), Roe *et al.* (1979) compared the effects of toothpaste containing 3.5% chloroform on male mice of four different strains (C57BL, CBA, CF/1, and ICI). Treatment with chloroform was not associated with any increase in liver or lung neoplasms relative to vehicle-treated controls in any of the four strains tested but was associated with significantly higher incidences of moderate to severe kidney pathology in CBA and CF/1 mice relative to the controls ($p < 0.0001$, chi-square test).

Palmer *et al.* (1979) gave groups of 50 Sprague-Dawley rats (both sexes) 0 or 60 mg chloroform/kg-day, 6 days/week by gavage in a toothpaste base for 80 weeks, followed by a 15 week observation period. There were no differences in the incidences of tumors of any site examined, including brain, lung, liver, kidney, and mammary gland, between treated and control animals. Heywood *et al.* (1979) investigated the carcinogenicity of chloroform in a toothpaste base in beagle dogs. Groups of male and female dogs received toothpaste base with 0, 15 or 30 mg chloroform/kg-day, 6 days/week for 7.5 years (8-16 dogs/sex), followed by a 20-24 week recovery period. Treatment with chloroform at the high dose was associated with significant elevations in SGPT levels but no treatment-related tumors were observed.

Chloroform treatment of rats via drinking water was associated with hepatic neoplastic nodules and hepatic adenofibrosis (Tumasonis *et al.*, 1985). Chloroform was administered to male and female Wistar rats in the drinking water at about 220 mg/kg/day and 160 mg/kg/day for the female and male rats, respectively. The incidence of hepatic neoplastic nodules was significantly elevated in treated females compared to controls ($p < 0.03$, Fisher exact test). In males, the incidence of hepatic neoplastic nodules did not differ in control and chloroform-treated groups. Increased incidences of hepatic adenofibrosis were observed in chloroform-treated males and females relative to controls. In contrast to the NCI and the Jorgenson *et al.* studies, renal tumors were not associated with chloroform treatment. However, Tumasonis *et al.* indicated that kidneys were only examined when grossly observable lesions were evident. Hence, kidney tumors may have been missed by this protocol. Tumasonis *et al.* concluded that chloroform is a hepatocarcinogen in Wistar rats.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Chloroform is carcinogenic to rats and mice (NCI, 1976; Roe *et al.*, 1979; Jorgenson *et al.*, 1985). The International Agency for Research on Cancer (IARC) has classified chloroform as a possible human carcinogen (Group 2B). Similarly, the U.S. EPA has placed chloroform in Group B2 in their classification scheme, based on sufficient evidence of carcinogenicity in animals, but inadequate epidemiologic evidence. Current evidence and understanding of the carcinogenic process is insufficient to classify chloroform as either a genotoxic or epigenetic carcinogen, and it is possible that both types of effects are involved.

Table 1: Chloroform carcinogenicity bioassay tumor incidence data used to estimate cancer potency (CDHS, 1990)

Study	Strain/Species	Sex	Tumor Site	Lifetime Daily Dose (mg/kg-day)	Tumor Incidence
NCI (1976)	$B6C3F_1$ mouse	M	hepatocellular carcinoma	control 83 167	1/18 18/50 44/45
	$B6C3F_1$ mouse	F	hepatocellular carcinoma	control 143 287	0/20 36/45 39/41
	Osborne-Mendel rat	M	renal tubular adenoma or adenocarcinoma	control 45 90	0/19 4/38 12/27
Jorgenson *et al.* (1985)	Osborne-Mendel rat	M	renal tubular adenoma or adenocarcinoma	control 18 38 79 155	4/301 4/313 4/148 3/48 7/50
Roe *et al.* (1979)	ICI mouse (Experiment I)	M	renal tubular adenoma or adenocarcinoma	control 12 43	0/72 0/37 8/37
	ICI mouse (Experiment II)	M	renal tubular adenoma or adenocarcinoma	control 40	6/237 9/49
	ICI mouse (Experiment III)[a]	M	renal tubular adenoma or adenocarcinoma	control 42	1/49 5/47
	ICI mouse (Experiment III)[b]	M	renal tubular adenoma or adenocarcinoma	control 42	1/50 12/48
Tumasonis *et al.* (1985)	Wistar rat	F	cholangiocarcinoma	control 220	0/18 34/40
	Wistar rat	M	cholangiocarcinoma	control 160	0/22 17/28
Reuber *et al.* (1979) using NCI (1976)	Osborne-Mendel rat	F	cholangiocarcinoma and cholangiofibroma	control 50 100	0/20 3/39 11/39

[a] toothpaste base was used as the vehicle; [b] arachis oil was used as the vehicle

The estimation of cancer risk to humans from exposure to chloroform by CDHS (1990) is based on animal studies. Data were chosen based primarily on statistical significance, as discussed below.

Methodology

The following data sets were evaluated to estimate chloroform cancer potency: 1) Liver tumor data in male and female $B6C3F_1$ mice, and renal tubular cell tumors in male Osborne-Mendel rats from the NCI (1976) study were chosen because statistically significant increases in these tumor types were observed in chloroform treated animals relative to controls; 2) Renal tubular cell tumor data in male Osborne-Mendel rats from the Jorgenson *et al.* (1985) study and in male ICI mice in the Roe *et al.* (1979) study were used for risk estimation based on a statistically significant increase in kidney tumors in

chloroform treated animals relative to controls; 3) Liver cholangiocarcinoma ("adenofibrosis") data in female rats from Tumasonis *et al.* (1985), and from Reuber's reanalysis of the NCI (1976) slides (Reuber, 1979) were also analyzed with the linearized multistage model (GLOBAL86). Administered doses were transformed to lifetime doses by adjusting for the number of days exposed per week and the ratio of the length of exposure to the length of the experiment (exposure plus observation period).

Calculated q_1* values from the above studies ranged from 8.1×10^{-4} to 1.9×10^{-2} $(mg/kg\text{-}day)^{-1}$. These represent cancer potency estimates for rats and mice and must be converted to theoretical equivalent potency values for humans. This conversion is based on equivalency of dose per unit surface area according to Anderson *et al.* (1983). These "human" cancer potencies range from 4.2×10^{-3} to $2.6 \times l0^{-1}$ $(mg/kg\text{-}day)^{-1}$. Scaling factors ranged from 5.19 to 13.57.

The NCI (1976) and Jorgenson *et al.* (1985) studies were the most thorough studies in terms of the number of doses tested, sample size, histological examination of the animals, and other procedural and statistical methods presented. As such, CDHS placed more confidence in the potency slopes from these studies than in the other studies. The potency slopes derived from Roe *et al.* (1979) and Tumasonis *et al.* (1985) fall within the range of those from the NCI and Jorgenson studies.

Bogen *et al.* (1989) used a physiologically based pharmacokinetic (PBPK) model to estimate metabolized dose for chloroform to use in the analysis of cancer potency with the linearized multistage model to carcinogenicity bioassay data from NCI (1976), Jorgenson *et al.* (1985), Roe *et al.* (1979), and Tumasonis *et al.* (1985). In the application of the model, the liver was considered to metabolize chloroform through a saturable enzyme system following Michaelis-Menten kinetics. This approach is consistent with the evidence that chloroform metabolites are responsible for toxicity and probably for the carcinogenicity of chloroform. The potency estimates made from these studies ranged from 4.8×10^{-3} to 5.0×10^{-1} $(mg/kg\text{-}day)^{-1}$. These corresponded to unit risks of 4.5×10^{-6} to 4.7×10^{-4} $(ppb)^{-1}$. These potency estimates are incorporated into DHS staff's best estimate of cancer potency for chloroform.

There are no studies on the carcinogenicity of chloroform by the inhalation route. Therefore, estimation of the cancer risk from exposure to chloroform in the ambient air required extrapolation from the oral route. In so doing, it is assumed that chloroform is also carcinogenic by the inhalation route, and that the risk posed by an absorbed inhaled dose of chloroform is equivalent to that posed by the same dose absorbed after oral administration. In the final risk range, the DHS staff included tumor sites that did not appear to be vehicle-dependent. Therefore, the liver tumors were not included in the range of risks or the best estimate of risk, due to the possible potentiation of liver tumors by the corn oil vehicle.

The best estimate of unit risk was considered by CDHS (1990) to be the arithmetic average of unit risks generated by CDHS (1990) and Bogen *et al.* (1989) for rat renal tumors in Jorgenson *et al.* (1985) and NCI (1976) and of the geometric mean for supporting data sets

(Roe *et al.*, 1979; Tumasonis *et al.*, 1985). This unit risk, 5.3 E-6 $(\mu g/m^3)^{-1}$, represents the best estimate using a nonthreshold linear procedure and using most of the data on the carcinogenicity of chloroform. It included analysis by PBPK modeling of metabolized dose, as well as analysis of potency based on applied dose.

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4-CHLORO-*o*-PHENYLENEDIAMINE

CAS No: 95-83-0

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	142.60
Boiling point	not available
Melting point	76 °C (NCI, 1978)
Vapor pressure	not available
Air concentration conversion	1 ppm = 5.83 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.6 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 1.6 E-2 $(mg/kg\text{-}day)^{-1}$
[Male rat urinary bladder tumor data (NCI, 1978), contained in Gold *et al.* (1990), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 4-chloro*o*-o-phenylenediamine in humans are known to exist.

Animal Studies

Male and female Fischer 344 (F344) rats and B6C3F$_1$ mice were fed diets containing 4-chloro-*o*-phenylenediamine (NCI, 1978). Dietary 4-chloro-*o*-phenylenediamine concentrations and treatment durations for rats and mice are listed in Table 1. Treatment group sizes were 50 animals/sex/species/group, except for low-dose male rats, where a group size of 49 was used.

At study termination, 84, 84 and 70% of male mice, and 72, 88 and 78% of female mice in the control, low-dose and high-dose groups, respectively, were still alive. Survival in treated rats was somewhat lower; 64, 80 and 56% of male rats and 72, 84 and 54% of female rats in the control, low-dose and high-dose groups, respectively, were still alive at study termination. Significantly increased treatment-related liver tumor (hepatocellular adenomas, carcinomas) incidences were noted in both male and female mice. These data are listed in Table 2. A significant dose-related trend was also noted in the increased incidence of urinary bladder carcinomas in male (transitional cell papillomas, carcinomas) and female (papillary or transitional cell carcinomas) rats (Table 2). Increases in the incidence of forestomach tumors also occurred in both male and female rats. The

forestomach tumor increases were not statistically significant; however, these tumors are rare in F344 rats.

Table 1. Study design summary for NCI (1978) carcinogenicity bioassay of 4-chloro-*o*-phenylenediamine in Fischer 344 rats and B6C3F$_1$ mice.

Sex/species	Treatment group	4-chloro-*o*-phenylenediamine concentration (mg/kg diet)	Observation period		Time-weighted average concentration (mg/kg diet)
			Treated (weeks)	Untreated (weeks)	
Male rats	control	0		105	
	low-dose	5000	78	27	
	high-dose	10000	78	28	
Female rats	control	0		106	
	low-dose	5000	78	28	
	high-dose	10000	78	28	
Male mice	control	0		97	0
	low-dose	10000 5000 0	33 45	 17	7000
	high-dose	20000 10000 0	33 45	 18	14000
Female mice	control	0		97	0
	low-dose	10000 5000 0	33 45	 18	7000
	high-dose	20000 10000 0	33 45	 18	14000

Table 2. Tumor induction in F344 rats and B6C3F$_1$ mice fed diet containing 4-chloro-*o*-phenylenediamine (NCI, 1978)

Sex/species	Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
Male rats	control	0	urinary bladder tumors	0/50
	low-dose	149		15/49
	high-dose	294		25/50
Female rats	control	0	urinary bladder tumors	0/50
	low-dose	184		5/50
	high-dose	368		22/50
Male mice	control	0	liver tumors	15/50
	low-dose	701		28/50
	high-dose	1390		34/50
Female mice	control	0	liver tumors	0/50
	low-dose	752		11/50
	high-dose	1500		10/50

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al.* (1984) list results of the NCI (1978) feeding study in male and female B6C3F$_1$ mice and F344 rats. Benign and malignant neoplasms of the liver were elevated in treated male and female mice. Forestomach tumors were also observed in treated rats; these tumors are relatively uncommon in this strain. In addition, substantial increases in the incidences of urinary bladder cancers were seen in rats of both sexes. Dose-response data are given in Table 2. Rats appear to be more sensitive than mice. Quantitative analysis of dose-response data for urinary bladder tumors indicate that male and female rats have nearly the same sensitivity. The upper confidence bound on potency for data on male rats is slightly higher, and this is the value used as a cancer potency for 4-chloro-*o*-phenylenediamine (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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p-CHLORO-*o*-*T*OLUIDINE

CAS No: 95-69-2

p-CHLORO-*o*-TOLUIDINE HYDROCHLORIDE

CAS No: 3165-93-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

***p*-Chloro-*o*-toluidine**

Molecular weight	141.61
Boiling point	241 °C
Melting point	30 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 5.79 mg/m^3

***p*-Chloro-*o*-toluidine hydrochloride**

Molecular weight	178.07
Boiling point	not available
Melting point	not available
Vapor pressure	not available
Air concentration conversion	1 ppm = 7.3 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 7.7 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 2.7 E-1 $(mg/kg\text{-}day)^{-1}$
[Male and female mouse hemangioma and hemangiosarcoma tumor data (Weisburger *et* al., 1978; NCI, 1979), contained in Gold *et* al. database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

IARC (1990) reviewed studies by Currie (1933) and Uebelin and Pletscher (1954) which investigated bladder tumor incidence in small groups of male workers exposed to *p*-chloro-*o*-toluidine; one case of bladder carcinoma was discovered (Currie, 1933).

A cohort study by Ott and Langner (1983) investigated 342 male workers involved in the manufacture of organic dyes in the US between 1914 and 1958. One plant area involved 117 workers with potential exposure to *p*-chloro-*o*-toluidine and other raw materials and intermediates, including *ortho*-toluidine. Followup of this subcohort from 1940 to 1975

indicated that a nonsignificant excess of total cancer deaths occurred (12 observed, 8 expected) and no bladder cancer was observed.
Two studies were conducted on a cohort of 355 male workers in *p*-chloro-*o*-toluidine manufacturing plants in the Federal Republic of Germany (FRG) who had been followed up for mortality from 1929 to 1982. No deaths due to bladder cancer were found in the first study (Stasik *et al*., 1985). The second study examined a subcohort of 116 workers exposed prior to 1970 (the implementation date of improved exposure controls) with presumed high *p*-chloro-*o*-toluidine exposure levels. Excluding 2 cases of urinary bladder carcinomas in the current work force, 6 cases of bladder carcinoma were found between January 1983 and June 1986 in hospital and other institution records. No cancer registry data was available for the area of the FRG where the plant was located; cancer registration rates for a different area of the FRG was therefore used as a basis of comparison. The expected number of tumors was 0.11 based on sex- and age-specific cancer rates. Two patients had hemorrhagic cystitis thought to be due to massive exposure to *p*-chloro-*o*-toluidine. Cigarette smoking was discounted as a confounding variable after reviewing patient smoking histories (3 patients were nonsmokers). Quantitative exposure data was unavailable, but the predominant chemical exposure was to *p*-chloro-*o*-toluidine; however, exposure to other amines could have occurred.

Animal Studies

Male and female random-bred CD-1 albino mice (derived from HaM/ICR mice) and male Charles River CD Sprague-Dawley-derived rats (25/sex/group) were fed diets containing *p*-chloro-*o*-toluidine hydrochloride (97-99% pure) as part of a larger carcinogenicity study of several compounds (Weisburger *et al*., 1978). Mouse exposure levels were 0, 750 or 1500 mg/kg diet and 0, 2000 or 4000 mg/kg diet for males and females, respectively; the mice were fed treated diet for 18 months, followed by an additional 3 month observation period. Rats were fed diet containing 2000 or 4000 mg/kg diet *p*-chloro-*o*-toluidine for 3 months; the doses were then reduced to 500 and 1000 mg/kg diet for 15 months. An untreated control group (25 males) was included. All rats were killed after 24 months. Tumor incidence differences between control and exposed rat groups were not statistically significant. Hemangiomas and hemangiosarcomas were observed in male and female mice; tumor incidence data is listed in Table 1. These tumor types were found in 5/99 male and 9/102 female pooled controls from the larger carcinogenicity study, but were not present in the simultaneous controls.

Diets containing *p*-chloro-*o*-toluidine hydrochloride (99% pure) were fed to groups of male and female B6C3F$_1$ mice and Fischer 344 (F344) rats (50 animals/sex/species/treatment group) (NCI, 1979). Exposure levels for mice were 3750 or 15000 and 1250 or 5000 mg/kg diet for males and females, respectively. Exposure levels for rats were 1250 or 5000 mg/kg diet. Untreated control groups (20 animals/sex/species) were included. Exposed animals were fed treated diet for the duration of the study. All surviving mice were killed at 99 weeks; however, all high-dose females had died by 92 weeks. All surviving rats were killed at 107 weeks.

Exposure to *p*-chloro-*o*-toluidine did not affect mortality of either male or female rats; a dose-related increase in mortality was noted for both male and female mice (NCI, 1979). However, sufficient numbers of mice of each sex were at risk for tumor development for determination of tumor incidence significance. The percentage of mice surviving to study week 52 was at least 95% for all sexes and treatment groups. No significant tumor incidence increase was observed in male or female F344 rats as a result of *p*-chloro-*o*-toluidine exposure. Significant treatment-related increases ($p < 0.001$) were observed in the incidence of both hemangiosarcomas and hemangiomas and hemangiosarcomas combined in both male and female mice. Tumor incidence data is listed in Table 1.

Table 1: Incidence of vascular tumors (hemangiomas and hemangiosarcomas) in male and female mice treated with *p*-chloro*-o*-toluidine hydrochloride by dietary administration

Study	Sex/Strain	Dietary concentration (mg/kg diet)	Average Dose[1] (mg/kg-day)	Tumor Incidence
Weisburger *et al*. (1978)[2]	male CD-1	0 750 1500	0 90 180	0/14 12/20 13/20
Weisburger *et al*. (1978)[3]	female CD-1	0 2000	0 260	0/15 18/19
NCI (1979)[2]	male $B6C3F_1$	0 3750 15000	0 450 1800	0/20 6/50 41/50
NCI (1979)[3]	female $B6C3F_1$	0 1250	0 162	1/20 43/50

1. Doses as reported by Gold *et al*. (1984).
2. Decreased survival according to Gold *et al*.; a time-to-tumor analysis was performed using TOX_RISK (Crump *et al*., 1991; Cal/EPA, 1992).
3. Analysis of the data set using the computer program TOX_RISK (Crump *et al*., 1991) indicated that inclusion of the high dose group resulted in a *p*-value of = 0.05 based on the chi-square goodness-of-fit test, indicating non-linearity. Following procedures described by US EPA (Anderson *et al*., 1983; US EPA, 1986), the high dose group was excluded from the analysis to correct for the poor fit (Cal/EPA, 1992).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

On the basis of positive bioassay results, the hydrochloride salt of *p*-chloro*-o*-toluidine was classified as a compound with sufficient evidence of carcinogenicity in animals by IARC

(1987). The results of feeding studies on by NCI (1979) using male and female $B6C3F_1$ mice and Fischer 344 rats and by Weisburger *et al.* (1978) using male and female CD-1 HaM/ICR mice and male Charles River CD rats are reported in Gold *et al.* (1984). In contrast to rats, mice are sensitive to *p*-chlor*o*-*o*-toluidine hydrochloride-induced carcinogenicity. Vascular tumors (hemangiomas and hemangiosarcomas) were induced in treated mice of both strains and sexes tested.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. The cancer potency for *p*-chlor*o*-*o*-toluidine is based on the bioassay results for the hydrochloride, adjusted for differences in molecular weight. An overall cancer potency was estimated by taking the geometric mean of the 4 values derived from dose-response data for vascular tumors from each of the mouse sex and strains tested (male and female CD-1 and $B6C3F_1$ mice) (Weisburger *et* al., 1978; NCI, 1979; see Table 1). Male $B6C3F_1$ mouse survival in the NCI (1979) study was poor; a cancer potency for that sex and strain was therefore derived using a time-to-tumor analysis (Crump *et al.*, 1991; Cal/EPA, 1992). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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CHROMIUM (HEXAVALENT)

CAS No: 18540-29-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	51.966
Boiling point	2642 °C
Melting point	1900 °C
Vapor pressure	1 mm Hg at 1616 °C
Air concentration conversion	not available

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.5 E-1 $(\mu g/m^3)^{-1}$
Slope Factor: 5.1 E+2 $(mg/kg\text{-}day)^{-1}$
[Human lung cancer mortality data (Mancuso, 1975), linearized multistage procedure, extra risk (US EPA, 1984), reevaluated by CDHS (1985).]

Oral Slope Factor: 4.2 E-1$(mg/kg\text{-}day)^{-1}$
[Calculated by CDHS (1991) from female mouse benign and malignant stomach tumor data (Borneff *et al.*, 1968), using a linearized multistage procedure.]

III. CARCINOGENIC EFFECTS

Human Studies

Several reviewers have summarized the epidemiologic studies examining the effects of chromium exposure on cancer morbidity and/or mortality (IARC, 1980; Hayes, 1982; US EPA, 1984). The chromium Toxic Air Contaminant (TAC) document (CDHS, 1985) included a summary table listing 14 occupational studies reporting an increase in cancer morbidity and/or mortality (primarily but not exclusively lung cancer). At the time the chromium TAC document was released, the relevant studies with exposure data were those by Pokrovskaya and Shabynina (1973, as cited in US EPA, 1984), Mancuso (1975) and Langard *et al*. (1980).

Pokrovskaya and Shabynina (1973) compared the cancer mortality of a group of ferroalloy workers in the Soviet Union to the local population for the time period 1955-1969. No specific cohort was defined nor were the number of cancer cases, individuals in the comparison groups, or person-years at risk given. Workers in the plant were reported to be exposed to low-solubility chromium compounds with concentrations of hexavalent chromium exceeding the allowable level of $0.01 mg/m^3$ by 2 to 7 times. In addition, some workers were exposed to smelting process fumes for the chromium ore, which included benzo[*a*]pyrene.

Age-specific cancer mortality ratios (SMR) were reported. The ratios for cancers in males aged 50-59 were significantly increased ($p < 0.001$) for all sites (SMR = 3.3), lung (SMR = 6.67), and esophagus (SMR = 2.0). Esophageal cancer mortality was also elevated among 60-69 year old males (SMR = 11.3, $p < 0.001$). However, the lack of methodological detail reporting as well as the absence of a defined worker cohort leave the results of this study open to question.

Mancuso and Hueper (1951) studied the lung cancer-chromium association in employees of the Painesville, Ohio chromate plant. A cohort of workers was defined as consisting of employees who had worked for at least one year during the period 1931-1949. The male population of the county in which the plant was located served as the comparison group. Denominator data were not reported; rather, the results were presented as proportionate mortality ratios (PMR). The PMR for cancer of the respiratory system was 18.2% (6/33) among chromate workers and 1.2% among the general male population. This difference is significant at $p < 0.01$. The authors also stated that about 96% of the workers were exposed predominantly to insoluble chromium (chromite ore; Cr(III)), suggesting that insoluble chromium, because of its relatively long pulmonary retention time may have played a causal role in carcinogenesis. However, since all work environments were contaminated with both trivalent and hexavalent chromium, (i.e., both insoluble and soluble chromium) the data are too limited to ascribe the carcinogenic form.

Mancuso (1975) followed up a segment of this population (new employees for the years 1931-37). A major concern of the author was to determine whether an association existed between lung cancer deaths and exposure to chromium of different oxidation states and solubilities. Data from a 1949 industrial hygiene study of the plant were used to derive weighted average exposures to insoluble, soluble and total chromium which were then applied to the worker cohort. Water-soluble chromium was considered to be hexavalent while insoluble chromium was assumed to be trivalent. The author noted that since the plant's inception in 1931, production had dramatically increased, possibly increasing chromium dust concentrations. This was likely to have continued until 1949, when the company instituted control measures, which markedly reduced the exposure. Thus, the 1949 exposure data probably represent an average exposure for the cohort; that is, the data underestimate exposure from 1931 to 1949 and overestimate it subsequently.

Of the 332 cohort employees, 173 (52%) had died by 1974, including 41 from lung cancer. No comparison to a reference group was made. The age-adjusted data showed an increase in lung cancer rates with increasing exposure to chromium, regardless of solubility (and hence oxidation state). No statistical evaluation of those trends was reported, but the staff of DHS tested the data and found a statistically significant positive trend ($p < 0.001$).

Langard *et al*. (1980) studied the incidence of cancer in male workers at a Norwegian ferroalloy plant (chromium and silicon alloys were produced). The cohort studied included all men who had worked at least one year in the period 1928-77, but the analysis focused on 976 workers who started before January 1, 1960. Both overall cancer mortality and incidence were lower than would have been expected based on national data. Lung cancer incidence was elevated; however, 7 cases were found among ferrochromium workers while

3.1 were expected ($p > 0.05$). The authors note that the expected rate may be inflated because the age-corrected lung cancer rate in the population of the county in which the plant is located is only 58% of the incidence in the whole country. Applying 58% to the expected rate results in a significant increase in the incidence ratio ($p < 0.01$). Furthermore, using non-ferrochromium workers as an internal referent population resulted in an 8.5-fold increase in lung cancer incidence ($p = 0.026$). Exposure data were based on a 1975 industrial hygiene survey of the plant. The total chromium content of dust was "with few exceptions" below 1 mg/m^3. This level probably underestimates past exposures. Water-soluble chromium (assumed to be hexavalent) ranged from 11-33% of the total. The presence of high levels of Cr(VI) in previous years was also confirmed by the finding of' 2 workers with nasal septum perforations. Exposure to asbestos and low levels of polycyclic aromatic hydrocarbons also occurred, but concentrations were not reported. However, since the 243 ferrosilicon workers studied were similarly exposed yet experienced no lung cancers, the
effect of these exposures may be minimal.

Table 1 Age, chromium exposure concentrations, lung cancer mortality and person-years of exposure for male chromate workers (Mancuso, 1975; as cited in US EPA, 1998).

Age (years)	Midrange (μg/m^3)	Deaths from Lung Cancer	Person Years
50	5.66	3	1345
	25.27	6	931
	46.83	6	299
60	4.68	4	1063
	20.79	5	712
	39.08	5	211
70	4.41	2	401
	21.29	4	345

Animal Studies

There have been at least eighty reported attempts to induce cancer in rodents by administration of chromium compounds by various routes. These have been reviewed by IARC (1980, 1982) and US EPA (1984). US EPA (1998) has stated that hexavalent chromium compounds were carcinogenic in animal assays producing the following tumor types: intramuscular injection site tumors in Fischer 344 and Bethesda Black rats and in C57BL mice; intraplural implant site tumors for various chromium VI compounds in Sprague-Dawley and Bethesda Black rats; intrabronchial implantation site tumors for various Cr VI compounds in Wistar rats; and subcutaneous injection site sarcomas in Sprague-Dawley rats.

It was noted in the chromium TAC document that at the time of document preparation, no chromium compound had been unequivocally shown to cause a significantly increased number of neoplasms in experimental animals after exposure by inhalation. At least 7 experiments involving dusts containing Cr(VI) and/or Cr(III) compounds had been conducted. Although Nettesheim et al. (1971) reported a significantly increased incidence of alveologenic (not bronchogenic) adenomas and adenocarcinomas in mice exposed to calcium chromate dust (13 mg/m3) over their lifetimes for 5 hr/day, 5 days/wk, this conclusion could not be confirmed on the basis of the data reported. The authors' statistical methodology was not reported. Fourteen treated animals (6 males and 8 females) developed tumors, whereas only 5 control animals (3 males and 2 females) did. However, the numbers of exposed and control animals were not reported, nor was the distribution of tumor types, so that the claim of a significant increase of treatment-related tumor incidence could not be validated. CDHS (1990) found that a later study (Glaser *et al*., 1986) reported increases in tumors of the lung, pharynx, pituitary, pancreas, and liver of male rats exposed for 18 months to aerosols of $Na_2Cr_2O_7$ at chromium concentrations of 100 mg/m^3. Statistical criteria used for the evaluation of tumor increases were not discussed by the authors. When analyzed by staff of CDHS, the incidence of respiratory tumors in the high-dose group, 4/19, was found to be significantly increased above the incidence, 0/37, in controls.

CDHS (1990) also described a oral chromium carcinogenicity bioassay done by Borneff *et al.* (1968). Male and female NMRI mice were exposed to 1 mg K_2CrO_4 per day (added to the regular diet); a control group of 79 females and 47 males was maintained on regular diet for three generations. During 880 days of observation, each generation was dosed for two years. Approximately two-thirds of the animals in both the treatment and the control group died during months 8-11. Necropsies were performed on 66 dosed females and 35 dosed males and on 79 female and 47 male controls. Two carcinomas of the stomach were found in dosed females, but no stomach carcinomas were found in dosed males or controls. In addition, benign stomach tumors (papillomas and hyperkeratomas) were seen in 9 females and 2 males given chromium and in 2 females and 3 males in the control group. The increased incidence of malignant stomach tumors was not significant when compared with controls, but the incidence of malignant or benign stomach tumors in dosed females, 11/66, was significantly increased above 2/79, the incidence in controls ($p = 0.003$).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Inhalation

A number of human occupational studies (relevant reviews listed above) have demonstrated that inhalation exposure to chromium results in an increased risk of lung cancer mortality in humans. An occupational exposure study by Mancuso (1975) was used by US EPA (1984) as the basis for an inhalation cancer unit risk for chromium. This study demonstrated the carcinogenicity of chromium in a cohort which was sufficiently large and

which was followed for an adequate time period. Data from an industrial hygiene study were used to derive weighted average exposures to insoluble, soluble and total chromium which were then applied to the worker cohort. Table 1 lists the age, exposure and lung cancer mortality study data.

Oral

Borneff *et al.* (1968) exposed male and female NMRI mice to 1 mg K_2CrO_4 per day for two years. A significantly increased incidence of stomach carcinomas were noted in female mice; a significantly increased incidence of benign stomach tumors (papillomas and hyperkeratomas) were noted in both male and female mice.

Methodology

Inhalation

US EPA (1984) used both multistage linearized "competing risks" and "crude" procedures to estimate human cancer risks associated with chromium inhalation exposure from the data set of Mancuso (1975). The resulting cancer unit risk (1.2 E-2 $(\mu g/m^3)^{-1}$) (US EPA, 1998) was a maximum likelihood estimate (MLE) derived from the "competing risks" procedure, calculated on the basis of total chromium exposure. It was also assumed that the smoking habits of chromate workers were similar to those of the U.S. white male population. CDHS (1985) adopted the US EPA linear nonthreshold procedure to estimate low-dose chromium inhalation cancer risk. A multistage linearized "crude" procedure was used to derive a cancer unit risk of 1.5 E-1 $(\mu g/m^3)^{-1}$, which was the 95% upper confidence limit for the estimate of the relative risk in the Mancuso (1975) study. The cancer mortality in Mancuso (1975) was assumed to be due to Cr(VI), which was further assumed to be no less than one-seventh of total chromium. This contrasts with the unit risk developed by US EPA (1984), which was calculated on the basis of total chromium exposure.

Oral

An oral cancer potency factor for chromium(VI) was derived from the benign and malignant mouse stomach tumor incidence data reported by Borneff *et al.* (1968). From the average weight of the treated mice, 31 grams, the dose of 1 mg/day of K_2CrO_4 (0.26 mg/day Cr) was calculated to be 8.39 mg/kg-day, and from the incidence of benign and malignant stomach tumors combined, the oral cancer potency of Cr(VI) was calculated using a linearized multistage procedure to be 3.17×10^{-2} $(mg/kg\text{-}day)^{-1}$ (CDHS, 1991). Surface-area scaling was used to extrapolate the animal cancer potency to human cancer potency. The scaling factor was calculated by taking the ratio, human body weight divided by animal body weight, raised to the one-third power. This extrapolation factor, $(70\ kg/0.031\ kg)^{1/3}$, was calculated to be 13.1. Multiplying the above potency estimate, 3.17×10^{-2} $(mg/kg\text{-}day)^{-1}$, made from the mouse study, by the extrapolation factor gives the estimate, 0.42 $(mg/kg\text{-}day)^{-1}$, for the carcinogenic potency of Cr(VI) ingested by humans.

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CREOSOTE (COAL TAR-DERIVED)

CAS No: 8001-58-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	complex mixture
Boiling point	194 - 400ºC
Melting point	not available
Vapor pressure	not available
Air concentration conversion	not available

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: Can be calculated using PEF factors contained in the benzo[*a*]pyrene Toxic Air Contaminant (TAC) document (OEHHA, 1993).

Slope Factor: Can be calculated using PEF factors contained in the benzo[*a*]pyrene TAC document (OEHHA, 1993).

III. CARCINOGENIC EFFECTS

Human Studies

Henry (1947) reviewed 3753 cases of cutaneous epitheliomata (epitheliomatous ulceration or cancer of the skin) reported to the British Medical Inspector of Factories from 1920 to 1945. Thirty five cases (12 of the scrotum) had creosote exposure. Henry (1946) also reported that the crude mortality rate for scrotal cancer during 1911-1938 for British brickmakers exposed to creosote oil was 29 per million men based on 9 verified cases as compared to a national average of 4.2 per million and rates of 1 per million or less for groups not exposed to suspected skin carcinogens.

A cohort study reported on 123 Swedish workers who treated wood with creosote and were exposed to both creosote and arsenic between 1950 and 1980 (Axelson and Kling, 1983; reviewed in IARC, 1985). Eight workers died of cancer compared to 6 expected. Three cancer deaths (leukemia, pancreas and stomach) were observed compared to 0.8 expected in a subgroup of 21 workers exposed only to creosote for five or more years.

A case-referent study of Swedish workers examined potential relationships between past occupational and radiation exposure and multiple myeloma (Flodin *et al*., 1987). Exposure assessment employed a mailed questionnaire that asked questions about occupational (including coal tar creosote) and radiation exposure. Data analysis using the Miettinen confounder score technique indicated that an increased prevalence of multiple myeloma was associated with occupational exposure to coal tar creosote (crude rate ratio = 6.0, p = 0.001). The rate ratio point estimate for creosote exposure increased to 9.0 after age

stratification. The power of this study was limited by differences between the case group and the referent group in the number of smokers and gender composition.

Creosote contains many of the same compounds present in other polycyclic aromatic hydrocarbon (PAH) mixtures (roofing tar pitch, coke oven emissions) known to be human carcinogens (US EPA, 1986; ATSDR, 1990).

Animal Studies

Female C57BL mice (10 animals/group) were exposed to blended creosote oil (a mixture of creosote, anthracene oils and naphthalene recovery residue oil) in toluene. One drop (8.7 - 9 µl) of a 20% or 80% solution was applied to the skin three times/week for the animals' lifetimes or until tumors developed (21 - 44 weeks and 18 - 35 weeks for the 20% and 80% solution exposure groups, respectively). All treated mice developed skin papillomas and 7 mice in each group developed epidermoid carcinomas, some of which metastasized to pulmonary or regional lymph nodes. None of the vehicle control animals developed skin tumors (Poel and Kammer, 1957).

Female Swiss mice (30/group) were treated twice weekly with one drop of a 2% solution of creosote in acetone applied dermally for 70 weeks. Skin tumors (including 16 carcinomas) were reported in 23 of 26 surviving mice. The average tumor latency period was 50 weeks. No vehicle control group was included; however, no animals in a control group of 50 mice receiving a single application of 1% 7,12-dimethylbenz[*a*]anthracene in mineral oil developed tumors after 80 weeks (Lijinsky *et al*., 1957).

Undiluted creosote applied topically twice weekly (25 µl) to 30 random-bred female mice for 28 weeks induced an average of 5.4 papillomas per animal; 82% of the mice had carcinomas. The average time to tumor for papillomas and carcinomas was 20 and 26 weeks, respectively. No vehicle control group was included in the study (Boutwell and Bosch, 1958). In a similar study, a group of 24 albino mice treated dermally with 25 µl creosote twice weekly for 5 months and housed in stainless steel cages exhibited 139 lung adenomas (5.8 tumors/mouse) after 8 months. A group of 29 mice born and housed in creosote-treated wood cages treated dermally with 25 µl creosote for 5 months demonstrated 315 lung adenomas (10.8 tumors/mouse) after 8 months. A control group (19 mice) housed in stainless steel cages demonstrated 9 lung adenomas (0.5 tumors/mouse) after 8 months (Roe *et al.*, 1958).

V. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Creosote has been demonstrated to cause skin and lung tumors in mice after dermal exposure, and is predominantly composed of PAH; similar PAH-containing coal tar products (roofing tar pitch, coke oven emissions) have been shown to be human carcinogens (US EPA, 1986; ATSDR, 1990). Creosote has been given B1 and 2A classifications (probable human carcinogen) by US EPA (1987) and IARC (1985),

respectively. No creosote carcinogenicity bioassay study suitable for quantitative risk assessment exists. However, a cancer unit risk factor for the PAH benzo[*a*]pyrene (BaP) derived from an inhalation exposure study (Thyssen *et al*., 1981) has been developed, along with Potency Equivalency Factors (PEFs) for several related PAHs. (OEHHA, 1993).

Thyssen *et al*. (1981) exposed male Syrian golden hamsters (24/group) by inhalation to 0. 2.2, 9.5 or 46.5 mg BaP/m^3 in a sodium chloride aerosol (greater than 99% of the particle diameters were between 0.2 and 0.5 μm). The hamsters were exposed to BaP daily for 4.5 hours/day for the first 10 weeks of exposure; subsequent exposure was daily for 3 hours/day. Total treatment duration was 95 weeks. Animals dying in the first year of the study were replaced. The effective number of animals in the control, 2.2, 9.5 and 46.5 mg/m^3 exposure groups were 27, 27, 26 and 25, respectively. Survival time for the 46.5 mg/m^3 exposure group was significantly reduced (60 weeks) when compared to controls (96 weeks). Survival times for the other exposure groups were similar to controls. Respiratory tract (including nasal cavity, larynx and trachea) tumor incidence was significantly increased in a dose-dependent manner in the 9.5 and 46.5 mg/m^3 exposure groups (34.6% and 52%, respectively, compared to controls); those exposure groups also demonstrated an increase in upper digestive tract (including pharynx, esophagus and forestomach) tumor incidence (27% and 56%, respectively). This study was selected as the basis of a cancer potency factor for exposure to BaP by inhalation because it used the most sensitive species and sex demonstrating a dose response and using the most relevant exposure route.

Methodology

A linearized multistage procedure (GLOBAL86) (Howe and van Landingham, 1986) was used with the Syrian golden hamster respiratory tract tumor incidence data of Thyssen *et al*. (1981) to calculate a cancer potency factor. Data from the highest exposure group (46.5 mg/m^3) was not used due to the shortened lifespan of the hamsters in this group. Administered dose for the 2.2 and 9.5 mg/m^3 exposure groups based on an inspiration rate of 0.063 m^3/day and a body weight of 0.1 kg was 0.152 and 0.655 mg/kg/day, respectively. Surface area scaling was then used to extrapolate a human cancer potency factor and an inhalation unit risk factor (using assumptions of 70 kg body weight and 20 m^3/day inspiration rate). Creosote is approximately 91% PAH, nitro-PAH or hydroxy-PAH (Wright *et al*., 1985); a unit risk for creosote can be calculated using the unit risk value for BaP and the PEFs for related PAHs (OEHHA, 1993).

V. REFERENCES

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p-CRESIDINE

CAS No: 120-71-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	137.20
Boiling point	235 °C
Melting point	51.5 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 5.611 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.3 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 1.5 E-1 $(mg/kg\text{-}day)^{-1}$
[Female mouse urinary bladder tumor data (NCI, 1979), contained in Gold *et* al. (1984), expedited Proposition 65 methodology, with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of *p*-cresidine in humans are known to exist.

Animal Studies

Male and female Fischer 344 (F344) rats and B6C3F$_1$ mice (50 animals/sex/species/group) were fed diets containing *p*-cresidine (NCI, 1979). The study design is outlined in Table 1. Dose levels for male and female mice were reduced after 21 weeks; the study report did not describe the rationale for the dose reduction.

Mortality in mice was dose-related and associated with the development of bladder tumors; mortality in rats was also dose-related and was related to development of urinary bladder and nasal cavity tumors. Survival percentages after 75 weeks of treatment are listed in Table 2. Significant incidence increases were seen for urinary bladder tumors in male and female rats (squamous-cell or transitional-cell carcinomas) and mice (transitional-cell carcinomas), for liver tumors in female mice (hepatocellular adenomas or carcinomas) and male rats (neoplastic liver nodules, hepatocellular carcinomas or cholangiocarcinomas), and for nasal cavity tumors (primarily nasal cavity tumors) in male and female rats. These data are listed in Table 2. Nonsignificant increases in nasal cavity tumors were also observed in male and female mice.

Table 1. Study design summary for NCI (1979) carcinogenicity bioassay of *p*-cresidine in Fischer 344 rats and B6C3F$_1$ mice.

Sex/species	Treatment group	*p*-cresidine dietary concentration (mg/kg diet)	Observation period	
			Treated (weeks)	Untreated (weeks)
Male rats	control	0		106
	low-dose	5000	104	1
	high-dose	10000	104	1
Female rats	control	0		106
	low-dose	5000	104	2
	high-dose	10000	104	2
Male mice	control	0		97
	low-dose	5000	21	
		1500	83	
		0		2
	high-dose	10000	21	
		3000	71[a]	
Female mice	control	0		97
	low-dose	5000	21	
		1500	83	
		0		2
	high-dose	10000	21	
		3000	83	
		0		2

a. All animals in this group were dead by the end of week 92.

Table 2. Mortality and tumor incidences associated with dietary exposure of Fischer 344 rats and B6C3F$_1$ mice to *p*-cresidine (NCI, 1979)

Sex/species	Treatment group	Average Dose[1] (mg/kg-day)	Survival after 75 weeks (%)	Tumor type	Tumor incidence[2]
male mice	controls	0	98	urinary bladder tumors	0/50
	low-dose	260	50		40/50
	high-dose	552	10		31/50
female mice	controls	0	90	urinary bladder tumors	0/50
	low-dose	281	78		42/50
	high-dose	563	28		45/50
	controls			liver tumors	0/50
	low-dose				14/50
	high-dose				6/50
male rats	controls	0	94	urinary bladder tumors	0/50
	low-dose	198	96		30/50
	high-dose	396	62		44/50
	controls			liver tumors	0/50
	low-dose				13/50
	high-dose				2/50
	controls			nasal cavity tumors	0/50
	low-dose				2/50
	high-dose				23/50
female rats	controls	0	96	urinary bladder tumors	0/50
	low-dose	245	98		31/50
	high-dose	491	76		43/50
	controls			nasal cavity tumors	0/50
	low-dose				0/50
	high-dose				11/50

1. Doses as reported by Gold *et al*. (1984).
2. Tumor incidences as reported by Gold *et al*. (1984)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results of the NCI (1979) feeding study in male and female B6C3F$_1$ mice and Fischer 344 rats are listed in Gold *et al*. (1984). Urinary bladder tumors as well as tumors at other sites were observed in both sexes of mice and rats. The most sensitive site appears to be the urinary bladder. Both sexes of both species show similar sensitivities at this site. The potency derived from dose-response data on female mice (benign and malignant urinary bladder tumors) is slightly greater than those for the other groups and is taken as the best estimate here (see Table 2).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. Because female mouse survival was poor, the potency was derived using a time-to-tumor analysis (Crump *et al*., 1991; Cal/EPA, 1992). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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CUPFERRON (N-hydroxy-N-nitroso-benzenamine, ammonium salt)

CAS No: 135-20-6

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	155.16
Boiling point	not available
Melting point	163-164 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 6.346 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.3 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 2.2 E-1 $(mg/kg\text{-}day)^{-1}$
[Male rat hemangiosarcoma data (NCI, 1978), contained in Gold *et* al. database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of cupferron in humans are known to exist.

Animal Studies

Male and female Fischer 344 (F344) rats and $B6C3F_1$ mice were exposed to diets containing cupferron (NCI, 1978). A summary of the experimental design is outlined in Table 1. Treatment periods were followed by an observation period during which animals were fed control diet. Male and female mouse dose levels were reduced after 35 weeks. Group sizes were 50 animals/sex/species/group except for the high-dose male mouse group, which consisted of 49 animals.

Cupferron induced significantly increased incidences of forestomach squamous-cell carcinomas, hepatocellular neoplastic nodules and carcinomas in male and female rats, hemangiosarcomas in male and female rats and mice, auditory sebaceous gland tumors in female mice and rats, hepatocellular carcinomas in female mice and Harderian gland adenomas in male and female mice. Hemangiosarcoma incidence data is listed in Table 2.

Table 1. Experimental design for carcinogenicity bioassay of cupferron using male and female Fischer 344 (F344) rats and B6C3F$_1$ mice (NCI, 1978)

Sex/species	Group	Cupferron concentration (%)	Experiment duration (weeks)		Time-weighted average concentration[1]
			treatment period	observation period	
male rats	control	0	0	110	
	low dose	0.15	78	26	
	high dose	0.3	78	19	
female rats	control	0	0	110	
	low dose	0.15	78	28	
	high dose	0.3	78	28	
male, female mice	control	0		98	0
	low dose	0.3	35		0.2
		0.1	43	18	
	high dose	0.6	35		0.4
		0.2	43	17	

1. Time-weighted concentration $= \dfrac{\sum (\text{concentration} \times \text{weeks received})}{\sum (\text{weeks receiving chemical})}$

Table 2. Cupferron-induced hemangiosarcoma incidence in male and female F344 rats and B6C3F$_1$ mice (NCI, 1978)

Sex/species	Dose group	Average dose[1] (mg/kg-day)	Tumor incidence[2]
Male rat	control	0	0/50
	low dose	45	38/50
	high dose	96.5	35/49
Female rat	control	0	0/50
	low dose	55.2	28/50
	high dose	110	37/50
Male mouse	control	0	1/50
	low dose	185	3/50
	high dose	374	7/50
Female mouse	control	0	1/50
	low dose	200	5/50
	high dose	405	6/50

[1]Doses as reported by Gold *et al.* (1984).
[2]Tumor incidences as reported by Gold *et al.* (1984)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results of the NCI (1978) feeding study in male and female B6C3F_1 mice and Fischer 344 rats are listed in Gold *et al*. (1984). Benign and malignant vascular tumors as well as tumors at other sites were observed in mice and rats of both sexes treated with cupferron. Cancer potency is based on the data for vascular tumors in the male rat (see Table 2) because the rat is the more sensitive of the species tested, and the male appears to be slightly more sensitive than the female (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. Analysis of the data set using the computer program TOX_RISK (Crump *et al*., 1991) indicated that inclusion of the high dose group resulted in a p-value of = 0.05 based on the chi-square goodness-of-fit test, indicating non-linearity. Following procedures described by US EPA (Anderson *et al*., 1983), the high dose group was excluded from the analysis to correct for the poor fit (Cal/EPA, 1992). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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2, 4-DIAMINOANISOLE

CAS No: 615-05-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	138.17
Boiling point	not available
Melting point	67-68 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 5.651 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.6 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 2.3 E-2 $(mg/kg\text{-}day)^{-1}$
[Male rat thyroid tumors (NCI, 1978), contained in Gold *et al*. (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 2,4-diaminoanisole in humans are known to exist.

Animal Studies

Male and female Fischer 344 (F344) rats and B6C3F_1 mice were fed diets containing 2,4-diaminoanisole (DAA) sulfate (NCI, 1978). Mice were fed diets containing 1200 or 2400 mg/kg DAA sulfate for 78 weeks and were observed for an additional 18-19 weeks. Rats were fed diets containing 5000 mg/kg DAA sulfate for 78 weeks, or diet containing 1250 mg/kg DAA sulfate for 10 weeks, then 1200 mg/kg diet for 68 weeks, followed by a 29 week observation period. Matched control groups were provided for each dose group. Group sizes were 50 animals/sex/species/group with the exception of the male rat high-dose control group (49 animals). Mortality of control and treated rats and mice were similar by the end of the study. Significantly increased incidences of thyroid tumors were seen in both mice (males - follicular cell adenomas; females - follicular cell adenomas, carcinomas) and rats (follicular cell adenocarcinomas, carcinomas, papillary adenocarcinomas and cystadenocarcinomas). Increased skin tumor incidences (squamous-cell carcinomas, basal-cell carcinomas, sebaceous adenocarcinomas) were observed in male rats. Male and female rats both had increased incidences of preputial or clitoral gland adenomas, papillomas or carcinomas and Zymbal gland tumors (males - squamous cell carcinomas, sebaceous adenocarcinomas; females - sebaceous adenocarcinomas). Tumor incidence data is listed in Table 1.

Table 1: Tumor induction in Fischer 344 rats and B6C3F$_1$ mice by dietary administration of 2,4-diaminoanisole (NCI, 1978)

Sex/species	Dose group	Average Dose[1] (mg/kg-day)	Tumor type	Tumor Incidence[2]
Male mouse	control	0	thyroid	1/100
	low dose	116		0/50
	high dose	234		11/50
Female mice	control	0	thyroid	0/100
	low dose	126		0/50
	high dose	253		8/50
Male rats	control	0	thyroid	2/99
	low dose	35.2		2/50
	high dose	146		17/50
	control	0	preputial gland	0/99
	low dose	35.2		2/50
	high dose	146		8/50
	control	0	skin	0/99
	low dose	35.2		2/50
	high dose	146		9/50
	control	0	Zymbal gland	0/99
	low dose	35.2		1/50
	high dose	146		6/50
Female rats	control	0	thyroid	3/100
	low dose	44		1/50
	high dose	182		10/50
	control	0	clitoral gland	3/100
	low dose	44		5/50
	high dose	182		8/50
	control	0	Zymbal gland	0/100
	low dose	44		0/50
	high dose	182		4/50

1. Doses reported by Gold *et al*., 1984.
2. Tumor incidences reported by Gold *et al*., 1984.

Diets containing 2,4-diaminoanisole at concentrations of 0, 1200, 2400 or 5000 mg/kg diet were fed to female F344 rats (40 - 60/group) for up to 82-86 weeks (Evarts and Brown, 1980). An additional 15 rats were fed diet containing 5000 mg/kg diet for 10 weeks, then fed control diet and observed for up to 87 weeks. Thyroid tumor incidences (follicular-cell adenomas or carcinomas or C cell carcinomas) were 1/37 in controls, 2/47 in the low-dose group, 3/33 in the mid-dose group and 31/40 in the high-dose group; in addition, 3/12 animals exposed to the 5000 mg/kg diet for 10 weeks had thyroid tumors. Clitoral gland tumors (squamous-cell, sebaceous-cell or squamous-sebaceous-cell carcinomas) were

noted in 0/37 controls, 8/47 of the low-dose group, 15/33 of the mid-dose group and 9/40 of the high dose-group, as well as in 1/12 of the animals in the 10 week high-dose group.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Cancer potency for 2, 4-diaminoanisole was derived from that for the sulfate using a molecular weight conversion (Cal/EPA, 1992):

$$q_h\,(\text{base}) = q_h\,(\text{sulfate}) \times \frac{\text{MW(sulfate)}}{\text{MW(base)}}$$

where q_h is the human potency and MW is the molecular weight. This conversion assumes that the intake of equivalent moles of the two forms of the chemical results in equivalent concentrations of the active species *in vivo*. Gold *et al.* (1984) list the results of the NCI (1978) feeding studies in male and female F344 rats and $B6C3F_1$ mice, and the feeding study by Evarts and Brown (1980) in female F344 rats. Cancer potency is based on dose-response data for benign and malignant thyroid tumors in male rats, the most sensitive sex and species (see Table 1) (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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Gold L, Sawyer C, Magaw R, Backman G, de Veciana M, Levinson R, Hooper N, Havender W, Bernstein L, Peto R, Pike M and Ames B. 1984. A Carcinogenic Potency Database of the standardized results of animal bioassays. Environ Health Perspect 58:9-319.

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2, 4-DIAMINOTOLUENE

CAS No: 95-80-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	122.17
Boiling point	292 °C
Melting point	99 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 4.997 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.1 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 4.0 E+0 $(mg/kg\text{-}day)^{-1}$
[Female rat mammary gland tumors (NCI, 1978), contained in Gold *et* al. database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the carcinogenic potential of 2,4-diaminotoluene in humans are known to exist.

Animal Studies

IARC (1978) reviewed a study by Umeda (1955) in which 20 rats of mixed strain and sex were injected subcutaneously with 0.5 ml of a 0.4% solution of 2,4-diaminotoluene at weekly intervals. No tumor induction was noted in 11 rats that died in the first 8 months of the study. All 9 surviving rats, which received 29-44 weekly injections, developed subcutaneous sarcomas. No concurrent control group was included in this study; however, another group of 12 rats exposed to 11 subcutaneous injections of xanthene in propylene glycol over 10 months did not develop local sarcomas.

Male Wistar rats were fed diets containing 0, 0.06% or 0.1% 2,4-diaminotoluene for 30-36 weeks (12 animals/treatment group, 6 animals/control group) (Ito *et al.*, 1969). Exposure to 2,4-diaminotoluene caused an increased incidence of hepatocellular carcinomas in the treated animals (0/6, 7/11, and 9/9 in the control, low-dose and high-dose groups, respectively).

Male and female Fischer 344 (F344) and $B6C3F_1$ mice were fed diets containing 2,4-diaminotoluene (NCI, 1979). Treatment group sizes were 50 animals/sex/species/group; matched control group sizes were 20 animals/sex/species/group. The study design is

outlined in Table 1. Male and female rat low- and high-dose levels were reduced after 40 weeks.

Table 1. Experimental design of 2,4-diaminotoluene carcinogenicity bioassay using male and female F344 rats and B6C3F$_1$ mice (NCI, 1979)

Sex/species	Study group	dietary 2,4-diaminotoluene (ppm)	Study time (weeks)	Time-weighted average dose[c] (ppm)
Male rat	matched control	0	103	0
	low dose	125	40	
		50	63	79
	high dose	250	40	
		100	39[a]	176
Female rat	matched control	0	103	0
	low dose	125	40	
		50	63	79
	high dose	250	40	
		100	44[b]	171
Male,female mouse	matched control	0	101	
	low dose	100	101	
	high dose	200	101	

a. Test diet administration was terminated at the time indicated and all high-dose males were killed because of morbidity.

b. Test diet administration was terminated at the time indicated and all high-dose females except four were killed because of morbidity.

c. Time-weighted average dose $= \dfrac{\sum (\text{dose in ppm} \times \text{weeks at that dose})}{\sum (\text{weeks receiving each dose})}$

Significantly increased tumor incidences were observed in treated rats; hepatocellular adenomas, carcinomas and neoplastic nodules in male and female rats, mammary gland adenomas and carcinomas in female rats, and subcutaneous fibromas in male rats. Significant increases in tumor incidence were also noted in female mice; hepatocellular carcinomas in the low- and high-dose groups, and lymphomas in the low-dose group. No significant tumor induction was noted in male mice. Tumor incidence data is listed in Table 2.

Table 2. 2,4-Diaminotoluene-induced tumor incidence in F344 rats and B6C3F$_1$ mice (NCI, 1978)

Sex/species	Study group	Average dose[a] (mg/kg-day)	Tumor type	Tumor incidence[b]
Male rats	matched controls	0	liver tumors[c]	0/20
			subcutaneous fibromas	0/20
	low dose	3.2	liver tumors[c]	5/50
			subcutaneous fibromas	15/50
	high dose	7.0	liver tumors[c]	10/50
			subcutaneous fibromas	19/50
Female rats	matched controls	0	liver tumors[c]	0/20
			mammary gland tumors	0/20
	low dose	3.95	liver tumors[c]	0/50
			mammary gland tumors	11/50
	high dose	8.55	liver tumors[c]	6/50
			mammary gland tumors	14/50
Female mice	matched controls	0	liver tumors	0/20
			lymphomas	2/20
	low dose	13.0	liver tumors	13/50
			lymphomas	29/50
	high dose	26.0	liver tumors	18/50
			lymphomas	11/50

a. Doses as reported by Gold *et al*. (1984).
b. Tumor incidences as reported by Gold *et al*. (1984)
c. Includes hepatocellular neoplastic nodules, adenomas and carcinomas

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results of the NCI (1978) feeding studies of 2, 4-diaminotoluene in male and female B6C3F$_1$ mice and F344 rats are listed by Gold *et al*. (1984). Significant increases in tumors were seen in rats of both sexes and in female mice. The study results indicated that rats are more sensitive than mice. The female rat appears to be slightly more sensitive than the male, although the study is not sensitive enough to definitively distinguish between the two. Cancer potency is based on mammary gland tumors in the female rat (see Table 2).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. Because female rat survival was poor in this study, the potency was derived using a time-to-tumor analysis (Crump *et al*., 1991; Cal/EPA, 1992). The individual animal data for the time-to-tumor analysis were obtained from TOX_RISK (Crump *et al*., 1991). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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1,2-DIBROMO-3-CHLOROPROPANE

CAS No: 96-12-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	236.36
Boiling point	195.5°C
Melting point	5°C
Vapor pressure	0.8 mm Hg @ 21°C
Air concentration conversion	1 ppm = 9.67 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.9 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 7.0 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

Two occupational epidemiological studies, Hearn *et al.* (1984) and Wong *et al.* (1984), were conducted using data from workers exposed during the formulation or manufacture of 1,2-dibromo-3-chloropropane (DBCP). The study by Hearn *et al.* (1984) examined a cohort of 550 chemical workers exposed to a variety of compounds, including DBCP. Twelve of the subjects in this cohort died from cancer (7.7 expected). In the study by Wong *et al.* (1984) 9 cases of respiratory cancer were reported in a cohort of 1034 workers exposed to DBCP (5.0 expected). Neither of these studies produced statistically significant ($p < 0.05$) associations between DBCP exposure and expected cancer incidence. In addition, it was not possible to account for all confounding chemical exposures in these studies. Therefore these studies were considered by IARC (1987) to be inadequate and were not used for the derivation of the cancer potency of DBCP.

An epidemiological study conducted by Jackson *et al.* (1982) found an association between DBCP in drinking water and increased incidence of stomach cancer and leukemia. In this study, patterns of DBCP contamination of well water in Fresno County, California were compared with deaths from selected cancers in the same area from 1970 to 1979. The cancers studied included stomach, esophageal, liver, kidney, and breast cancers in addition to lymphoid leukemia. Significant relationships between DBCP exposure level and cancer deaths were tested by Bartholomew's trend test. Exposed individuals were grouped into those with less than 0.05 ppb, those with 0.05 up to 1.0 ppb, and those with greater than 1.0 ppb DBCP in the drinking water. Mortality was age-adjusted using 20-year age groups. Significant trends for incidence of stomach cancer and lymphoid leukemia were found.

Results may have been confounded by smoking habits, ethnicity, and exposure to other carcinogens.

Additional analysis of these data by Environmental Health Associates (EHA, 1986) and sponsored by the Shell Oil Company did not show the above association. These data included corrections for ethnicity. This study used a different method for estimating DBCP concentrations. Although the associations between cancer incidence and DBCP exposure failed to reach significance at the $p < 0.05$ level in the EHA study, the magnitude of the associations in the high and low exposure groups were approximately the same as described in the Jackson *et al*. (1982) study. The trend for cancer risk and DBCP exposure is most closely related to the time of residence of the test subjects (Table 1).

Table 1. Relative risk of human gastric cancer in areas with high[1] concentrations of DBCP in the drinking water compared with areas of low[2] DBCP (Jackson *et al*., 1982).

Time of Residence	Relative Risk for Gastric Cancer
1 year at death	1.29
1 year prior to death	1.55
10 years prior to death	3.05

[1] DBCP concentrations greater than 1.0 ppb.
[2] DBCP concentrations less than 0.05 ppb.

Animal Studies

DBCP is a carcinogen in at least two laboratory rodent species by inhalation, ingestion, or dermal exposure. Tumors following DBCP exposure can arise not only at the site of application, but also at distal sites. Because of its carcinogenicity to multiple species, DBCP is assumed to represent a carcinogenic threat to humans (CDHS, 1985).

Three sets of long-term bioassays using mice and rats were conducted respectively by the National Cancer Institute (NCI, 1978), the National Toxicology Program (NTP, 1982) and Hazelton Laboratories (1977, 1978). In the NCI (1978) study, DBCP was administered by oral gavage to both sexes of rats and mice. Two major problems with this study, early mortality and nearly 100% forestomach carcinoma rate, precluded its usefulness in determining a cancer potency value. The inhalation study by NTP (1982) had much better survival rates than those observed in the gavage study, and carcinogenicity was observed for tissues at or near the site of the initial chemical contact.

In the NTP study, groups of 50 B6C3F1 mice or 50 F344 rats of either sex were exposed by inhalation to 0, 0.6 or 3.0 ppm DBCP for 6 hours/day, 5 days/week, for 76-103 weeks. Surviving high-dose rats were killed at week 84. High-dose female mice and low- and high-dose male mice were killed at week 76. Low-dose rats and female mice were killed at week 104. A significant increase in the combined incidence of nasal tumors was found in male and female rats at both concentrations (Table 2a, 2b). In mice, the combined

incidence of nasal tumors was significantly increased in females at both concentrations, and in males exposed to the high concentration. Proliferative lesions were observed at sites distal to the lung in the mice, including the kidney, forestomach, and spleen.

Table 2a. Incidence of combined nasal cancers from DBCP Inhalation exposure in rats and mice (NTP, 1982)

	Tumor Incidence DBCP Concentration (ppm)		
Species	0	0.6	3.0
F-344 Rats (males)	0/50	32/50	39/49
F-344 Rats (females)	1/50	21/50	32/50
B6C3F1 Mice (males)	0/45	1/42	21/48
B6C3F1 Mice (females)	0/50	11/50	38/50

Table 2b. Incidence of combined lung cancers from DBCP inhalation exposure in mice (NTP, 1982)

	Tumor Incidence DBCP Concentration (ppm)		
Species	0	0.6	3.0
B6C3F1 Mice (males)	0/41	3/40	11/45
B6C3F1 Mice (females)	4/50	12/50	18/50

In the studies conducted by the Hazelton Laboratories (1977, 1978), mice (50 males or females per group) or rats (60 males or females per group) were exposed to DBCP in the diet for 78 weeks. The intended daily doses were 0, 0.3, 1.0 and 3.0 mg/kg per day. Both species exhibited dose-dependent increases in forestomach squamous cell papillomas and carcinomas. The mouse study contained experimental errors in the diet preparation and food consumption measurements. Spillage of the food and evaporation in the mouse study may have resulted in an overestimate of the actual average daily exposure. For the rats, diets were prepared every two weeks, therefore loss of DBCP in the food due to evaporation was less significant than in the mouse study. The average amount of DBCP in the diet was estimated assuming first-order evaporation loss (Shell Oil Company, 1986). Using this model, the average daily doses of DBCP in the mouse study were 0, 0.3, 1.6 and 4.8 mg/kg per day. The study conducted by Hazelton Laboratories used lower doses than those in the NCI study, providing better information on the lower end of the dose-response curve. However, the times of death or times of tumor appearance were not reported. In addition, the study conducted by Hazelton Laboratories was terminated at 78 weeks in the case of the mice but lasted for 104 weeks in the case of the rats.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The Hazelton Laboratories (1977) study in female CD-1 mice was chosen as the critical study for the derivation of the cancer potency factor. In this study, female mice had a tumor incidence of 19/50 in the high dose group. No tumors were observed in the controls, and no histopathological examination was determined in the low and mid dose groups. The problem of food spillage and evaporation of the DBCP from the food bias the data toward an underestimation of the true cancer potency. Despite this fact, the data from this study gives a higher potency than that calculated from the rat data. The cancer potency for DBCP is based on the incidence of forestomach squamous cell carcinomas in the Hazelton Laboratory study, and is consistent with the incidence of stomach carcinomas in female mice in the NCI gavage study. In addition, the inhalation study conducted by NTP produced tumors at sites distal to the lung, including the forestomach. The cancer potency based on the NTP inhalation study is close to, but slightly lower than the potency derived from the Hazelton Laboratories study. The Hazelton Laboratory study was therefore taken to be the most appropriate animal data for the derivation of the cancer potency value.

Based on the calculated cancer potency derived from the animal studies, significant increases in cancer incidence would not be expected in the human occupational studies. The duration of exposure was too brief, the exposure too recent, and the number of subjects too small. In the ecological and case-control environmental studies by Jackson *et al*. (1982) and EHA (1986), a significant increase in the number of cancers would indicate that the true human cancer potency is an order of magnitude higher than that calculated from the animal studies.

Methodology

A linearized multistage procedure was used to estimate the cancer potency of DBCP from the Hazelton Laboratories (1977) data in female CD-1 mice (Crump *et al*., 1982). The actual daily doses received by the mice were estimated to be 0, 0.3, 1.6 and 4.8 mg/kg/day (Shell Oil Company, 1986). The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value for DBCP.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 6.6 $(mg/kg \times day)^{-1}$ was derived (CDHS, 1988). An airborne unit risk factor was calculated by OEHHA/ATES from the q_{human} value using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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1,4-DICHLOROBENZENE

CAS No: 106-46-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	147.01
Boiling point	174°C
Melting point	53.1°C
Vapor pressure	10 mm Hg @ 25°C
Air concentration conversion	1 ppm = 6 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.1 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 4.0 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by CDHS (1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There are several case reports of human leukemia associated with occupational exposure to chlorinated benzenes, including 1,4 - dichlorobenzene (1,4-DCB) (Girard *et al.*, 1969). One case of chronic lymphocytic leukemia involved exposure to a solvent mixture of 80% ortho-, 2% meta-, and 15% para-dichlorobenzene. The association between leukemia and 1,4 - dichlorobenzene exposure was confounded by multiple chemical exposure.

Animal Studies

Loeser and Litchfield (1983) conducted a chronic inhalation carcinogenicity bioassay in male and female Alderly Park rats. In this study, groups of 76-79 rats were exposed to 0, 75, or 500 ppm p-DCB 5 hours/day, 5 days/week for 76 weeks. Control rats exhibited a high mortality rate and did not differ significantly from treated rats in overall tumor incidence (Table 1) or in the incidence of animals with multiple tumors and malignant tumors.

Table 1. Tumor incidence in rats exposed to 1,4-dichlorobenzene (DCB) in air for 76 weeks (Loeser and Litchfield, 1983)

Concentration of 1,4-DCB	Combined Tumors (males)	Combined Tumors (females)
0 ppm	39/60	55/61
75 ppm	31/60	54/61
500 ppm	35/60	53/58

A parallel experiment was conducted using groups of 75 Swiss mice of either sex (Loeser and Litchfield, 1983). In this experiment, female mice were exposed to 0, 75, or 500 ppm 1,4 – DCB for 5 hours/day, 5 days/week, for 57 weeks. A similar experiment in male mice was terminated due to high mortality due to fighting and respiratory infections. As with the rats, no significant increase in any tumor type was detected.

The National Toxicology Program (NTP, 1987) studied the carcinogenicity of 1,4 - DCB in male and female F344 rats and B6C3F1 mice via chronic (103 week) oral intubation. Male rats were given 0, 150, or 300 mg/kg 1,4 - DCB for 5 days/week for 103 weeks. Male and female mice, and female rats were given 0, 300, or 600 mg/kg for the same duration. Sentinel animals were killed periodically to test for infectious pathogenic agents. The survival of male rats given 300 mg/kg was significantly lower than controls after 97 weeks, but the survival of treated female rats was unchanged from controls. The time-weighted average doses in the study were 0, 214, and 428 mg/kg/day for the mice, and 0, 107, and 214 mg/kg/day for the rats. Male rats treated with 1,4-DCB displayed nephropathy and mineralization and hyperplasia of renal tubules. The incidence of renal tubular adenocarcinomas was also dose-dependently increased in the male rats (1/50, 3/50, or 7/50 for the 0, 107, or 214 mg/kg groups, respectively). A significant dose-dependent increase in the incidence of mononuclear cell leukemia (5/50, 7/50, or 11/50 for the 0, 107, or 214 mg/kg groups, respectively) was observed in the male rats. Additionally, an increasing trend in the incidence of mesothelioma was observed in the male rats (1/50, 0/50, 4/50, for the 0, 107, or 214 mg/kg groups, respectively).

Mice of both sexes exposed to 1,4 - DCB had significantly increased incidence of hepatocellular adenomas and carcinomas (NTP, 1987). In addition, four male mice exposed to 428 mg/kg were found to have hepatoblastomas, a rare hepatocellular carcinoma. The incidence of follicular thyroid cell adenomas was increased in female mice exposed to 428 mg/kg ($p < 0.038$). As with the male rats, male mice showed evidence of kidney tubule damage when treated with 1,4 - DCB. Females were not similarly affected.

NTP concluded from these data that 1,4 - DCB was carcinogenic to male rats, but not female rats. In addition, NTP concluded that the increased incidence of hepatocellular adenomas and carcinomas was evidence of carcinogenicity in male and female mice.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The study by NTP (1987) was chosen by CDHS (1988) as the key study for the development of a cancer potency value for 1,4-DCB. In the NTP (1987) study, mice and rats exhibited significant increases in several types of tumors. The mice were exposed for 5 days/week, resulting in average daily doses of 0, 214, and 428 mg/kg/day 1,4-DCB. Mice of either sex exhibited a significant increase in hepatocellular carcinomas or adenomas. The incidence of hepatocarcinomas or adenomas was 17/50, 22/49, and 40/50 in the control, 214, and 428 mg/kg/day groups, respectively. In addition, male rats showed a significant increase in kidney adenomas and mononuclear cell leukemia. The cancer

potency for 1,4-DCB was calculated from the male mouse hepatocarcinoma and adenoma data.

Methodology

A linearized multistage procedure was used to estimate the cancer potency of 1,4-DCB from the NTP (1987) data in male B6C3F1 mice (Crump *et al*., 1982). The concentrations of 1,4-DCB given in the feed were 0, 214, or 428 mg/kg/day. The premature mortality of animals without tumors was subtracted from the sample groups. The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. In this case, the scaling factor was equal to 1. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 0.04 $[mg/kg\text{-}day]^{-1}$ was calculated (CDHS, 1988). An airborne unit risk factor of 1.1E-5 $(\mu g/m^3)^{-1}$ was calculated by OEHHA/ATES from the q_{human} value using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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3,3-DICHLOROBENZIDINE

CAS No: 91-94-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	253.1
Boiling point	402°C
Melting point	132-133°C
Vapor pressure	unknown
Air concentration conversion	1 ppm = 10.4 mg/m^3 (IARC, 1982)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.4 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.2 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

The body of literature addressing the carcinogenicity of 3,3'-dichlorobenzidine in humans is scant. Three retrospective epidemiological studies of occupational exposure have been conducted, focusing on the possibility that 3,3'-dichlorobenzidine is a bladder carcinogen like its parent compound, benzidine. None of the studies approximates exposure levels.

Gerarde and Gerarde (1974) conducted a study of 175 workers involved in the manufacture and use of dichlorobenzidine in a chemical manufacturing plant in the United States between 1938 and 1957. Workers were segregated from benzidine exposure. No bladder tumors were found among the exposed workers. General population incidence of bladder tumors predicts 0-2 cases in a cohort of this size.

Gadian (1975) conducted a study of 35 British workers exposed to dichlorobenzidine who had been segregated from exposure to benzidine in a chemical plant from 1953 to 1973. Cumulative hours of exposure were tabulated for all workers. No tumors were reported among the exposed workers at the end of the study (through 1973).

MacIntyre (1975) reports on bladder tumor incidence among 225 Scottish production and service workers, 119 of which had more than 5 years of exposure to dichlorobenzidine and 36 of which were exposed more than 16 years before the time of the study. No bladder tumors were reported among the study subjects.

Animal Studies

Stula *et al.* (1975) conducted a study on ChR-CD rats (50/sex/group), exposing animals to 1000 ppm 3,3'-dichlorobenzidine in feed for life (mean survival 51 weeks), with an interim exposed group of 6 rats/group sacrificed after 12 months. Control animals receiving no added compound were observed for up to two years (mean survival 81 weeks (males) and 90 weeks (females)). Male animals showed statistically significant increases in incidences of granulocytic leukemias (9/44 treated vs. 2/44 control; $p < 0.05$ by Fisher's exact test), mammary adenocarcinomas (7/44 treated vs. 0/44 control; $p < 0.01$), and Zymbal gland carcinomas (8/44 treated vs. 0/44 control; $p < 0.01$). Female animals only showed increased incidence of mammary adenocarcinomas (26/44 treated vs. 3/44 control; $p < 0.01$).

Stula *et al.* (1978) later conducted a study on six female beagle dogs, administering 100 mg 3,3'-dichlorobenzidine in gelatin capsules 3 times/week for 6 weeks followed by 100 mg, 5 times/week for up to 7.1 years, plus 6 untreated control animals sacrificed at 8-9 years of age. One animal which died during the course of the study (3.5 years) showed no sign of tumors, whereas another animal which died at 6.6 years showed both undifferentiated liver carcinoma and papillary transitional cell carcinoma of the bladder. Among the animals surviving to the end of the study there was an increased incidence of hepatocellular carcinoma (3/4 treated vs. 0/6 control; $p < 0.05$) and papillary transitional cell carcinoma of the bladder (4/4 treated vs. 0/6 control; $p < 0.01$). Control animals showed a high incidence of adenocarcinoma and carcinoma of the mammary gland (4/6).

Pliss (1959) reports on carcinogenesis in Rappolovskii white rats exposed to 3,3'-dichlorobenzidine in feed for 12 months. The addition of 10-20 mg added to feed in the form of a paste (50% with water) 6 days/week resulted in an estimated total dose of 4.5 g/animal. A group of 130 animals receiving injections of octadecylamine and methylstearylamine were termed a "historical control". Twenty-two of 29 animals were examined for tumors at the time of the first tumor's appearance. Findings included tumors of Zymbal gland (7/29), mammary gland (7/29), skin (3/29), bladder (3/29), hematopoietic system (3/29), adenocarcinoma of the ileum (2/29), connective tissue (2/29), salivary gland (2/29), liver (1/29), and thyroid (1/29). No tumors were reported among "control" animals. Pliss (1959, 1963) also conducted studies exposing rats to 3,3'-dichlorobenzidine by the subcutaneous route. In the first study (Pliss, 1959), animals (25 female, 36 male) received 120 mg 3,3'-dichlorobenzidine weekly for 10-11 months. The dose was reduced to 20 mg/rat after the sixth month due to toxicity. The same "control"animals were used as with the feeding study. The author notes the appearance of tumors of the Zymbal gland (10/35), mammary gland (6/35), skin (5/35), hematopoietic system (2/35), connective tissue (2/35), salivary gland (1/35), and local subcutaneous sarcomas (7/35) among animals surviving to the time of the appearance of the first tumors.

Griswold *et al.* (1968) dosed 20 female Sprague-Dawley rats with 300 mg 3,3'-dichlorobenzidine in sesame oil by gavage (10 doses at three day intervals) and observed the animals after 9 months for incidence of mammary tumors. Control groups included a

negative control (sesame oil only) and a positive control (7,12-dimethylbenz[a]anthracene). No tumors were observed in treated animals, but a 3% incidence was observed in the negative controls and 100% incidence in positive control animals. Osanai *et al.* (1976) treated 26 male ICR/JCL mice (plus 39 untreated control mice) with feed containing 0.1% 3,3'-dichlorobenzidine for 12 months with an interim sacrifice group at 6 months. Hepatomas were observed in all treated animals at 6 and 12 months ($p < 0.01$) and among control animals at an incidence of 0%, 9.5% (2/21) and 38.55% (5/13) at 6,12, and 18 months, respectively.

Tatematse *et al.* (1977) fed 22 male Wistar rats a diet containing 0.3% 3,3'-dichlorobenzidine alone or in sequence with o-N-butyl-N-(4-hydroxybutyl)nitrosamine (0.1% in drinking water), N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (0.15% in the diet) and N-fluorenylacetamide (0.025% in the drinking water) over a four week period. Twelve untreated animals served as controls. Animals were observed for a 40 week period. Only animals receiving combined exposures showed effects which included some bladder tumors and histological changes of the liver.

Saffiotti *et al.* (1967) and Sellakumar *et al.* (1969) report on a feeding study in which Syrian golden hamsters (30/sex/group) were exposed to 0.1% or 0.3% 3,3'-dichlorobenzidine in feed; an untreated control group (30/sex) was included. No significant carcinogenic effects were observed in the 0.1% 3,3'-dichlorobenzidine group. The 0.3% 3,3'-dichlorobenzidine group, however, showed increased incidence of transitional cell carcinomas of the bladder (4/30 treated, 0/30 control; $p = 0.056$ by Fisher's exact test). Other observations included some liver-cell and cholangiomatous tumors.

A single study suggests that 3,3'-dichlorobenzidine may act as a transplacental carcinogen (Golub *et al.*, 1974). Pregnant female BALB/c mice given 2 mg 3,3'-dichlorobenzidine (in 0.1 ml sesame oil) five times during the last week of pregnancy, showed increased incidence of lymphoid leukemia among the offspring of exposed animals (7/24 treated, 0/30 control; $p < 0.01$). This effect, however, could also have occurred by exposure via lactation.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

An IARC (1982) review of the human epidemiological studies deemed them inadequate for evaluating carcinogenicity due to the relatively small size of the cohorts, inadequate time since first exposure, and/or incomplete follow-up of exposed workers.

The only carcinogenesis studies amenable to the development of cancer potency values are those conducted by Stula *et al.* (1975, 1978) showing the induction of granulocytic leukemia, mammary adenocarcinoma, and Zymbal gland carcinoma in rats and papillary transitional cell carcinomas of the bladder and hepatocellular carcinomas in beagle dogs exposed to 3,3'-dichlorobenzidine. Limitations of the other available studies including

poor study design, inadequate scope of endpoints, and unclear interpretation of dose extrapolation, preclude the development of cancer potency values from these studies.

Methodology

The most sensitive experimentally determined endpoint for tumor development is mammary adenocarcinoma induction in female rats exposed to 3,3'-dichlorobenzidine (26/44 treated, 3/44 control) (Stula *et al*., 1975). A linearized multistage procedure (CDHS, 1985) applied to these data resulted in an estimation of the upper 95% confidence bound of cancer potency (q_1^*) of 0.023 $(mg/kg\text{-}day)^{-1}$. With a study duration of 49.9 weeks for females (T_e) and a natural lifespan assumption of 104 weeks (T), the cancer potency for animals (q_{animal}) was derived to be 0.21 $(mg/kg\text{-}day)^{-1}$ from the following relationship:

$$q_{animal} = q_1^* \times (T/T_e)^3$$

Human cancer potency (q_{human}) of 1.2 $(mg/kg\text{-}day)^{-1}$ based upon body weight assumptions of 0.35 kg for female rats (bw_a) and 70 kg for humans (bw_h) and the following relationship:

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

A unit risk value of 3.4 E-4 $(\mu g/m^3)^{-1}$ based upon air concentrations was derived by OEHHA/ATES assuming a human breathing rate of 20 m^3/day, a human body weight of 70 kg, and 100% fractional absorption after inhalation exposure.

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1, 1-DICHLOROETHANE

CAS No: 75-34-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	98.97
Boiling point	57.3°C (ATSDR, 1990)
Melting point	-96.7°C (ATSDR, 1990)
Vapor pressure	230 mm Hg at 25°C (ATSDR, 1990)
Air concentration conversion	1 ppm = 4.05 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.6 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 5.7 E-3 $(mg/kg\text{-}day)^{-1}$
[Female rat mammary gland adenocarcinoma tumor data (NCI, 1977), contained in Gold *et* al. database (1990), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 1,1-dichloroethane in humans are known to exist.

Animal Studies

Male and female Osborne-Mendel rats and $B6C3F_1$ mice were exposed to 1,1-dichloroethane dissolved in corn oil by gavage (NCI, 1977). The study design is summarized in Tables 1a and 1b. Dosing was performed once/day, 5 days/week. Dosing of the low- and high-dose mouse treatment groups was performed cyclically in the latter part of the experimental period; one exposure-free week was followed by 4 weeks of exposure.

Dose-related increases were noted in mammary adenocarcinomas and hemangiosarcomas in female rats. Statistically significant increases were observed in endometrial stromal polyps in high-dose female mice (4/46 compared to 0/79 for controls, $p = 0.017$) and in hepatocellular carcinomas in high-dose male mice (8/32 compared to 6/72 in pooled vehicle controls, $p = 0.027$). Female rat tumor incidence data is listed in Table 2.

Klaunig *et al*. (1986) exposed male $B6C3F_1$ mice to 1,1-dichloroethane in drinking water for 52 weeks. Exposure levels were 0, 835 and 2500 mg/l. Group sizes were 35 mice/group; 10 mice/group were killed after 24 weeks. Histology was only performed on kidney, liver and lung samples. No treatment-related increase in tumor incidence was

noted. However, histological examination was only performed on a limited number of tissues, and only male mice were used.

Table 1a. Study design for carcinogenicity bioassay of 1,1-dichloroethane (1,1-DCE): Osborne-Mendel rats (NCI, 1977)

Sex	Group	Group Size	1,1-DCE dose (mg/kg bw)	Observation period (weeks)		Time-weighted average dosage[1] (78 weeks)
				Treated	Untreated	
Male	Untreated control	20	0		109	0
	Vehicle control	20	0	78	33	0
	Low dose	50	350	8		382
			450	23		
			450[2]	37	10	
			0		33	
	High dose	50	700	8		764
			900	23		
			900[2]	37	10	
			0		33	
Female	Untreated control	20	0		105	0
	Vehicle control	20	0	78	33	0
	Low dose	50	750	8		475
			900	9		
			450	14		
			450[2]	37	10	
			0		33	
	High dose	50	1500	8		950
			1800	9		
			900	14		
			900[2]	37	10	
			0		33	

1. Time weighted average dosage = $\sum$ [(dosage × number of weeks) / 78 weeks]
2. Gavage doses were cyclically administered; one exposure-free week was followed by 4 weeks (5 days/week) of exposure at the exposure level indicated.

Table 1b. Study design for carcinogenicity bioassay of 1,1-dichloroethane (1,1-DCE): B6C3F$_1$ mice (NCI, 1977)

Sex	Group	Group Size	1,1-DCE dose (mg/kg bw)	Observation period (weeks)		Time-weighted average dosage[1] (78 weeks)
				Treated	Untreated	
Male	Untreated control	20	0		90	0
	Vehicle control	20	0	78	12	0
	Low dose	50	900	6		1442
			1200	3		
			1500	69		
			0		13	
	High dose	50	1800	6		2885
			2400	3		
			3000	69		
			0		13	
Female	Untreated control	20	0		91	0
	Vehicle control	20	0	78	12	0
	Low dose	50	900	6		1665
			1200	3		
			1500	11		
			1800	58		
			0		13	
	High dose	50	1800	6		3331
			2400	3		
			3000	11		
			3600	58		
			0		13	

1. Time weighted average dosage = $\sum$ [(dosage × number of weeks) / 78 weeks]

Table 2. Tumor induction in female Osborne-Mendel rats after gavage exposure to 1,2-dichloroethane (NCI, 1977)

Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
vehicle control	0	mammary adenocarcinomas hemangiosarcomas	0/20 0/40
low dose	238	mammary adenocarcinomas hemangiosarcomas	1/50 0/50
high dose	477	mammary adenocarcinomas hemangiosarcomas	5/50 4/50

1. Dose as reported by Gold *et al.*, 1984. 2. Tumor incidence as reported by Gold *et al.*, 1984

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al.* (1984) list the results of the NCI (1977) gavage studies in male and female $B6C3F_1$ mice and Osborne Mendel rats. Cancer potency for 1, 1-dichloroethane is based on mammary gland adenocarcinomas observed in female rats, the most sensitive of the species/sex combinations tested (see Table 2). Because female rat survival was poor in this study, the potency was derived using a time-to-tumor analysis (Crump *et al.*, 1991; Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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DI-(2-ETHYLHEXYL)PHTHALATE

CAS No: 117-81-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	390.54
Boiling point	230°C @ 5 mm Hg
Melting point	-50°C
Vapor pressure	1.32 mm Hg @ 200°C
Air concentration conversion	1 ppm = 16.0 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.4 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 8.4 E-3 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency value derived for a Proposed Maximum Contaminant Level (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

Thiess *et al.* (1978; reviewed by US EPA, 1994) report on a study of mortality among 221 workers involved in di-(2-ethylhexyl)phthalate (DEHP) production. Potential exposure periods range from 3 months to 24 years and the mean follow-up period was 11.5 years. Among workers exposed for more than 15 years, incidences of pancreatic carcinoma (1 case) and uremia (1 case with urethral and bladder papillomas) were elevated over incidence in the corresponding age group of the general population. No quantitation of exposure levels was reported.

Animal Studies

The National Toxicology Program (NTP, 1982; Kluwe *et al.,*1982) assayed the carcinogenic effects of di-(2-ethylhexyl)phthalate on rats and mice. Fischer F344 rats (50/sex/group) were treated with diet containing 0, 6000, or 12000 ppm DEHP for 103 weeks. $B6C3F_1$ mice (50/sex/group) were treated with diet containing 0, 3000, or 6000 ppm DEHP for 103 weeks. Survivors were sacrificed and examined histologically at 105 weeks. Survival of rats was not found to be significantly influenced by DEHP treatment. Increased incidence of hepatocellular carcinoma or hepatic neoplastic nodules was reported in male and female high-dose treated rats (see Table 1). The increase in incidence was found to be dose-related ($p < 0.01$). Among treated high-dose male and female mice, and

low-dose female mice, hepatocellular carcinoma incidence was increased. The increase in incidence was found to be dose-related ($p < 0.05$).

Table 1. Incidence of hepatocellular carcinoma in male and female $B6C3F_1$ mice and F344 rats fed diet containing DEHP (NTP, 1982).

species	treatment[1] (ppm in diet)	hepatocellular carcinoma incidence male	female
F344 rats	0	3/50	0/50
	6,000	6/49	2/49
	12,000	12/49[2]	8/50[3]
$B6C3F_1$ mice	0	9/50	0/50
	3,000	14/48	7/50[3]
	6,000	19/50[2]	17/50[4]

[1] Fischer F344 rats were treated with 0, 6000, or 12000 ppm DEHP in their diet for 103 weeks. $B6C3F_1$ mice were treated with 0, 3000, or 6000 ppm DEHP in their diet for 103 weeks. Survivors were sacrificed after 105 weeks.
[2] $p < 0.05$. [3] $p < 0.01$. [4] $p < 0.001$.

Carpenter *et al.* (1953) maintained 2 month old Sherman rats (32/sex/group) on a diet containing 0, 400, 1300, or 4000 ppm DEHP up to two years. Animals were sacrificed at one year with the exception of a subgroup of a maximum of 8 rats/sex/dose which were exposed for an additional year. A group of 80 F_1 generation rats, the progeny of females in the highest dose group exposed for more than 120 days, were exposed for one year to diet containing 4000 ppm DEHP. Survivors were sacrificed after one year. No malignant tumors were observed among treated animals. Three rats in the 4000 ppm DEHP dose group, four in the 1300 dose group, two in the 400 ppm dose group, and five in the control group were reported to have benign tumors. Two benign tumors in the treated F_1 rats (vs. one in the control group) had benign tumors. Mortality at two years was reported to be 70.3% among control animals and between 60 and 70% among the treated groups. Poor survival of animals precluded evaluation of carcinogenicity from this study.

Carpenter *et al.* (1953) also treated hybrid guinea pigs (~23/sex/dose) with diet containing 0, 1300, or 4000 ppm DEHP for 1 year, at which time animals were sacrificed. Survival among exposed animals was decreased. No carcinogenic effects were observed.

Carpenter *et al.* (1953) also treated 4 dogs with gelatin capsules containing a volume of 0.03 ml/kg body weight DEHP five days per week for 19 doses, then with 0.06 ml/kg body weight DEHP for 240 doses. Four control animals were also included in the study. No tumors were observed in treated or control animals.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The only data appropriate for the development of a cancer potency value come from the NTP (1982) study which showed a dose-related effect of DEHP on the incidence of hepatocellular carcinoma in Fischer 344 rats and $B6C3F_1$ mice. This study was conducted by standard protocols with an adequate number of animals and thorough reporting of results.

Methodology

For the purpose of developing a cancer potency in humans, US EPA (1986, 1987) converted the exposure levels of rats and mice in the NTP (1982) study to human equivalent doses (HEDs). Dosage levels were first converted from parts per million in the diet to mg/kg-day based upon reported food disappearance rates. The resulting daily low and high dose estimates were 322 and 674 mg/kg-day for male rats, 394 and 774 mg/kg-day for female rats, 672 and 1325 mg/kg-day for male mice, and 799 and 1821 mg/kg-day for female mice. HEDs were based on the following relationship, with D the applied dose level, bw_a the experimental animal body weight, bw_h the assumed human body weight, l_e the length of exposure, L_e the length of the study, and L the lifespan of the animal:

$$HED = D \times \frac{l_e}{L_e} \times \left(\frac{bw_a}{bw_h}\right)^{\frac{1}{3}} \times \left(\frac{L_e}{L}\right)^3$$

Using the derived HED values, US EPA (1987) applied the multistage procedure of Howe and Crump (Global 82; 1982) to the combined incidence data of hepatocellular carcinomas and neoplastic nodules reported by NTP (1982). The resulting upper 95% confidence bounds on the cancer potency (q_{human}) are presented in Table 2. The highest, and thus most sensitive, cancer potency value is derived from the incidence of hepatocellular carcinomas in male $B6C3F_1$ mice, with a q_{human} value of 8.4 E-3 $(mg/kg\text{-}day)^{-1}$. Selection of the cancer potency value for DEHP comes from the most sensitive site and species of tumor induction in experimental animals in the absence of human data appropriate for developing a potency value.

Table 2. Human cancer potency values derived by US EPA (1986, 1987) from the NTP (1982) study.

species	sex	q_{human} $[(mg/kg\text{-}day)^{-1}]$
F344 rats	male	2.95 E-3
	female	3.52 E-3
$B6C3F_1$ mice	male	8.36 E-3
	female	4.73 E-3

A unit risk value of 2.4 E-6 $(\mu g/m^3)^{-1}$ was derived by OEHHA/ATES assuming a 70 kg average human body weight, 20 m^3/day human breathing rate, and 100% fractional absorption.

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P-DIMETHYLAMINOAZOBENZENE

CAS No: 60-11-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	225.28
Boiling point	not available
Melting point	114-117 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 9.214 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.3 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 4.6 E+0 $(mg/kg\text{-}day)^{-1}$
[Female rat liver tumor data (Kirby and Peacock, 1947), contained in Gold *et al.* database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992), cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of *p*-dimethylaminoazobenzene (DAB) in humans are known to exist.

Animal Studies

IARC (1974) reviewed a number of studies on the carcinogenic potential of DAB in animals. DAB was initially reported by Kinosita (1937) to induce liver tumors in rats after dietary exposure; tumors were produced after 50 or more days of treatment (smallest total dose, 176 mg DAB). Sherman, Wistar and Evans rats were found to be equally susceptible to the induction of liver tumors after exposure to diets containing 600 mg/kg DAB (Sugiura and Rhoads, 1941).

Kirby and Peacock (1947) exposed male and female Wistar-derived rats to a low-protein diet (12% casein) containing 0 or 600 mg/kg diet DAB. Group sizes were 8 animals/sex/group except for treated females, where the group consisted of 7 animals. Four male rats received treated diet for 28 weeks, followed by control diet; the other animals received treated diet for 33 weeks. At the end of the treatment period, all animals received control diet until sacrifice at 52 weeks. Both male and female rats developed hepatomas; Gold *et al.* (1984) list a tumor incidence of 0/8 and 5/7 for control and treated (average dose, 20.9 mg/kg-day) females, respectively.

Druckrey and Küpfmüller (1948; reviewed by IARC, 1975) exposed rats to 1, 3, 10, 20 or 30 mg DAB/day by gavage for the life of the animals. All doses induced the formation of liver tumors; the induction time was inversely proportional to the dose, ranging from 34 days (30 mg/day) to 700 days (1 mg/day). For exposure groups in the 3-30 mg/day range, the total carcinogenic dose was about 1000 mg. Daily exposures of 0.1 or 0.3 mg/rat did not induce tumors.

Druckrey (1967) exposed rats to 5 mg DAB/rat by gavage for 40, 60, 100, 140 or 200 days, then observed the animals for the remainder of their lifespan. Percent incidences of liver carcinomas were 20, 26, 49, 80 and 81, respectively.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The feeding study by Kirby and Peacock (1947) conducted in Wistar-derived albino rats is listed in Gold *et al*. (1984). Cancer potency is based on liver tumors in the female rats.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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2,4-DINITROTOLUENE

CAS No: 121-14-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	182.14
Boiling point	300°C
Melting point	71°C
Vapor pressure	0.00014 mm Hg @ 25°C
Air concentration conversion	1 ppm = 7.4 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 8.9 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 3.1 E-1 $(mg/kg\text{-}day)^{-1}$
[calculated from a potency factor derived by US EPA (1987) and adopted by CDHS (see Final Statement of Reasons)]

III. CARCINOGENIC EFFECTS

Human Studies

No data are available addressing the carcinogenicity of 2,4-dinitrotoluene (2,4-DNT) in humans.

Animal Studies

Lee *et al.* (1978) exposed male and female CD rats to practical grade 2,4-DNT in feed at concentrations of 0, 15, 100, and 700 ppm for 720 days. Animals were sacrificed at 750 days. Decreased weight gain and lifespan were observed among animals in the highest treatment group. Noncancer toxic effects observed included toxic anemia and aspermatogenesis. Tumors observed included fibromas of the connective tissue of male rats and fibroadenoma of the mammary gland of female rats. The incidence data for liver and mammary tumors in female rats are presented in Table 1. Significantly increased incidence was found in the highest dose group for liver tumors ($p = 4 \times 10^{-4}$; Fisher's exact test), mammary gland tumors ($p = 1.75 \times 10^{-4}$), and combined mammary gland and liver tumors ($p = 7 \times 10^{-5}$). Mammary tumor incidence among male rats was 1/37, 0/37, 0/29, and 17/23 in the 0, 15, 100, and 700 ppm 2,4-DNT dose groups, respectively. Only the highest dose group showed significantly increased tumor incidence ($p = 4 \times 10^{-9}$).

Table 1. Incidence of liver and mammary gland tumors among female CD rats exposed to practical grade 2,4-dinitrotoluene (2,4-DNT) in feed for 24 months (Lee *et al.*, 1978).

treatment level (ppm)	tumor incidence liver[1]	mammary gland[2]	combined
0	0/31	11/31	11/31
15	3/43	12/43	13/43
100	3/35	18/35	18/35
700	30/42	34/43	35/43

[1] Tumor incidence includes neoplastic nodules and hepatocellular carcinoma of the liver.
[2] Tumor incidence includes adenoma, fibroadenoma, fibroma, or adenocarcinoma of the mammary gland.

The National Cancer Institute (NCI, 1978) conducted a study exposing Fischer rats and $B6C3F_1$ mice to practical-grade 2,4-DNT (>95% pure). Male and female Fischer rats (50/sex/group) were exposed to feed containing 0.02% or 0.008% 2,4-DNT (time-weighted concentrations). Control groups consisted of 25 rats/sex for the high-dose group and 50 rats/sex for the low-dose group. The treatment period was 78 weeks long and the observation period continued for 26 weeks. Among treated male rats in both high- and low-dose groups, the incidence of benign fibroma of the skin and subcutaneous tissue was increased over controls (high-dose: 13/49 treated vs. 0/25 control, $p = 0.003$; low-dose: 7/49 treated vs. 0/46 control, $p = 0.008$ by Fisher's exact test). Among treated female rats in the high-dose group, the incidence of fibroadenoma of the mammary gland was increased over control animals (23/50 treated vs. 4/23 control, $p = 0.016$).

In the same study (NCI, 1978), male and female $B6C3F_1$ mice (50/sex/group) were treated with diet containing 0.04% or 0.008% 2,4-DNT (time-weighted concentrations). Groups of 50 mice/sex served as controls for the high- and low-dose groups. The treatment period lasted 78 weeks and the animals were observed for an additional 13 weeks. No significant increase in tumor incidence was observed among treated animals.

Ellis *et al.* (1979) (also reported by Lee *et al.,*1985) exposed male and female CD (Sprague-Dawley) rats (38/sex/group) to 0, 15, 100, or 700 ppm 2,4-DNT in feed. At 12 months, 8 rats/group were sacrificed for necropsy; the remainder were sacrificed at 24 months. Cumulative deaths during the course of the study ranged from 55 to 100% in male rats and 60 to 97% in female rats, including control animals. Histopathological outcomes of animals that died during the course of the experiment (but after 52 weeks) were included in the final incidence data along with the incidence data among survivors. Tumors showing statistically significant increases ($p < 0.05$ by Fisher's exact test) were hepatocellular carcinomas and mammary gland tumors among female rats in the highest dose group. Hepatocellular carcinomas were reported in 18/34 treated high-dose female rats vs. 0/23 control rats ($p = 4.3 \times 10^{-6}$). Mammary gland tumors, including both benign and malignant tumors of epithelial or mesenchymal origin, were reported in 33/35 treated high-dose female rats vs. 11/23 control rats ($p < 0.0001$).

Ellis *et al.*(1979) (also reported by Hong *et al.,* 1985) exposed male and female CD-1 mice (38/sex/group) to 0, 100, 700, or 5000 ppm 2,4-DNT in feed as described in the rat study above. Since over 70% of the mice in the highest dose group died before 12 months, these animals were not included in the analysis. Mortality among the remaining dose groups and controls ranged from 70 to 85%. Tumor incidence data were drawn from animals surviving at least 12 months. A significantly increased incidence of renal tumors was found among male mice in the 700 ppm 2,4-DNT dose group (19/28 treated vs. 0/33 control; $p = 1.32 \times 10^{-9}$, Fisher's exact test). Renal tumor types included cystic papillary adenomas, solid renal cell carcinomas, and cystic papillary carcinomas. No significantly increased tumor incidence was reported among female mice.

Ellis *et al.* (1979, 1985) treated beagle dogs (6/sex/group) with 2,4-DNT in gelatin capsules daily for 2 years at dose rates of 0, 0.2, 1.5, or 10 mg/kg body weight. The highest dose was lethal to five of the 12 treated animals. Throrough examination of all animals upon sacrifice showed no evidence of carcinogenicity of 2,4-DNT.

The Chemical Industry Institute of Toxicology (CIIT, 1982) exposed male and female F344 rats (130/sex/dose) to technical grade DNT (76% 2,4-DNT and 19% 2,6-DNT) feed such that daily dosing was 0, 3.5, 10.0, and 35.0 mg/kg-day. The entire high-dose group was sacrificed at 55 weeks due to significantly reduced survival. Twenty rats (/sex) were examined histopathologically at this time. The animals in the remaining dose groups were sacrificed at the scheduled time of 104 weeks. The incidences of hepatocellular carcinoma and neoplastic nodules of the liver are reported in Table 1. Cholangiocarcinomas were also reported in 3/20 high-dose male rats (at 55 weeks) and 2/23 mid-dose male rats (at 104 weeks).

Table 1. Tumor incidence in male and female F344 rats exposed to technical grade dinitrotoluene (DNT) in feed (CIIT, 1982).

tumor type	males (mg/kg-day)				females (mg/kg-day)			
	0	3.5	10.0	35.0*	0	3.5	10.0	35.0*
hepatocellular carcinoma	1/61	9/70	22/23	20/20	0/57	0/61	40/68	11/20
neoplastic nodules	9/61	11/70	16/23	5/20	5/57	12/61	53/68	12/20

*All the high-dose group animals were sacrificed at 55 weeks due to significantly reduced survival. Histopathological examinations were performed on 20 rats/sex.

Leonard *et al.* (1987) exposed 20 male CDF(F344)/CrlBR rats to 2,4-DNT in the diet for 12 months such that daily dose rate was 27 mg/kg-day. No evidence of carcinogenicity was found, however, the study was short in duration and the number of animals was small.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The US EPA (1980) derived a cancer potency value based on the tumor incidence data in the study by Lee *et al.* (1978) showing the induction of liver and mammary tumors in female CD rats. This study was selected over the NCI (1978) study because of published reservations by NCI concerning the adequacy of the study for estimating cancer potency in humans.

Methodology

The US EPA (1980) calculated a "transformed" dose rate of 0, 0.71, 3.9, and 34.0 mg/kg-day for the animals in the study by Lee *et al.* (1978) exposed to 0, 15 100 and 700 ppm 2,4-DNT in their diet, respectively. A linearized multistage procedure was applied to the combined mammary gland and liver tumor incidence data presented in Table 1 in order to calculate an animal cancer potency value (q_{animal}). The calculated q_{animal} was 0.058 $(mg/kg\text{-}day)^{-1}$. The q_{animal} was converted to a human cancer potency (q_{humna}) based on the following relationship, where bw_{animal} is the assumed body weight for the test species (Lee *et al.*, 1978; bw_{animal} = 0.464 kg) and bw_{human} is the assumed human body weight (70 kg):

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

The resulting q_{human} is 0.31 $(mg/kg\text{-}day)^{-1}$.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 8.9 E-5 $(\mu g/m^3)^{-1}$.

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1,4-DIOXANE

CAS No: 123-91-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From ACGIH, 1994)

Molecular weight	88.1
Boiling point	101.1°C
Melting point	11.8°C
Vapor pressure	29 mm Hg @ 20°C
Air concentration conversion	1 ppm = 3.6 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: $(7.7\ E\text{-}6\ \mu g/m^3)^{-1}$
Slope Factor: $2.7\ E\text{-}2\ (mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1989)]

III. CARCINOGENIC EFFECTS

Human Studies

The two human epidemiological studies of the potential carcinogenicity of 1,4-dioxane (Thiess *et al*. (1976); Buffler *et al*. (1978)) did not show significant changes in the incidence of carcinogenicity. However, neither study had sufficient statistical power to detect moderate changes in cancer incidence due to the small size of the sample groups or the short duration of the studies.

In the study by Thiess *et al*. (1976), 74 German workers were exposed to various concentrations of 1,4-dioxane for an average of 24.9 years. Of the 74 workers, 24 were working at the end of the follow-up period (1964 - 1974), 23 were no longer working, 15 had retired, and 12 had died. Of the 12 deaths, two were attributed to neoplastic diseases (1 lamellar epithelial carcinoma and 1 myelofibrotic leukemia). The expected number of deaths during this period in the cohort was 14.5, based on Federal Republic of Germany mortality statistics. The overall death rate and the cancer death rate were not significantly increased over controls.

Buffler *et al*. (1978) studied the mortality of 165 workers exposed to 1,4-dioxane in a dioxane-manufacturing and processing facility in Texas. The employees were exposed to dioxane for at least 1-month up to 21 years (April, 1954 to June, 1975), and were divided into two cohorts. The cohort of manufacturing workers was composed of 100 individuals, and the processing workers numbered 65. The concentrations of dioxane in the workplaces were less than 25 ppm. Seven deaths occurred in the manufacturing cohort (4.9 expected), two from neoplasms (0.9 expected). Five deaths occurred in the processing cohort (4.9 expected), one from cancer (0.8 expected). These mortality and cancer rates were not

higher than the expected from Texas age- and sex-specific death rates for 1960-1969. Due to the small sample size and short exposure period of the study, the authors concluded that the negative results were not conclusive.

Animal Studies

Male Wistar rats (n = 26) were given drinking water containing 1% dioxane for 63 weeks (Argus *et al.*, 1965). A group of 9 rats given untreated water were used as controls. Six hepatomas, one kidney tumor, and one case of leukemia were found in the treated animals. One lymphosarcoma was found in the control group. The increased incidence of cancer in the treated animals was not statistically significant ($p < 0.05$). However, due to the small size of the control group, this study was of very limited sensitivity, and therefore inconclusive.

Another drinking water study in male rats was conducted by Hoch-Ligeti *et al*. (1969) and further reported on by Argus *et al*. (1973). In this study, male Charles River rats (30 per group) were given 0, 0.75, 1.0, 1.4, or 1.8% dioxane in their drinking water from 2-3 months of age for 13 months. Animals were killed 16 months following treatment or earlier if tumors in the nasal cavity were observed. Survival data was not reported. Nasal histological examinations were only performed on animals with grossly visible tumors. The incidence of tumors found in this study are presented in Table 1.

Table 1. Tumor incidence in male Charles River rats exposed to 1,4-dioxane in drinking water (Hoch-Ligeti *et al*., 1969; Argus *et al*., 1973).

	Tumor Incidence				
1,4-Dioxane Concentration (%)	0	0.75	1.0	1.4	1.8
Hepatocarcinomas	0/30	0/30	0/30	2/30	2/30
Nasal tumors	0/30	1/30	1/30	2/30	2/30
Hepatic tumors (total)	NR	4/30	8/30	16/30	25/30

NR = Not reported

Kociba *et al*. (1974) studied the effects of dioxane in the drinking water of male and female Sherman rats. Rats (60/sex/group) were exposed to 0, 0.01, 0.1, or 1.0% dioxane for 2 years. Actual dosages of dioxane were estimated using drinking water consumption and body weight data. The dosages were 0, 9, 94, or 1015 mg/kg/day for the males, and 0, 14, 148, or 1599 mg/kg/day in females. Because the tumor incidence data are averaged for the combined male and female responses, the doses were also averaged. Mortality in the combined high dose group was 45% after 1 year of exposure, compared with 12% in the control group. The tumor incidence data is summarized in Table 2.

King *et al*. (1975) exposed male and female B6C3F1 mice (50/sex/group) to dioxane in the drinking water for 40-43 weeks. Concentrations of dioxane used were 0, 0.5, or 1.0 %. No tumors were observed in any group at the end of the treatment period. According to IARC

(1976), the duration of this study was insufficient to detect hepatocarcinoma, the tumor most commonly found in the National Cancer Institute (NCI, 1978) study.

Table 2. Tumor incidence in male and female Sherman rats exposed to dioxane in drinking water (Kociba *et al*., 1974).

	Tumor Incidence			
1,4-Dioxane Concentration (%)	0	0.01	0.1	1.0
Hepatocarcinomas	1/120	0/120	1/120	10/120
Nasal tumors	0/120	NR	NR	3/120

NR = Not reported

The NCI conducted a bioassay on male and female $B6C3F_1$ mice (50/sex/group) given 0, 0.5, or 1.0% dioxane in the drinking water from 5 weeks to 90 weeks (NCI, 1978). Mortality of the male rats was only 10% in the male mice after 91 weeks. Mortality in the female mice increased with increasing dose, up to 44% in the high dose group. Tumor incidence data from this study is presented in Table 3. The incidence of hepatocarcinomas was significantly increased in both the male and female mice exposed to the low and high concentrations of dioxane, compared with controls.

Table 3. Tumor incidence in male and female $B6C3F_1$ mice exposed to 1,4-dioxane in the drinking water (NCI, 1978).

	Tumor Incidence		
1,4-dioxane Concentration	0	0.5	1.0
Hepatocarcinomas (males)	2/49	18/50	24/47
Hepatocarcinomas (females)	0/50	12/48	29/37

In addition to the mouse study. the National Cancer Institute (1978) also exposed male and female Osborne-Mendel rats to 0, 0.1, or 1.0 % dioxane in their drinking water for 110 or 90 weeks, respectively. The incidences of nasal tumors in these groups were 0/33, 12/33, and 16/33 in the males, and 0/34, 10/35, and 8/35 in the females.

Male guinea pigs (20/group) were exposed to 0 or 0.5-2.0% dioxane in the drinking water for 23 months. After 28 months, the animals were killed and tumor incidence was recorded. Tumor incidences in the treated animals included 3 animals with hepatomas, 2 with gall bladder carcinomas, and one with adenoma of the kidney. Tumors were not found in the controls (n = 10). Although the cancer incidences were not significantly different from the controls, IARC (1976) concluded that dioxane caused liver and gall bladder tumors.

In an inhalation study, Torkelson *et al*. (1974) exposed groups of 288 male or female Wistar rats to 111 ppm 1,4-dioxane for 7 hours/day, 5 days/week, for 2 years. Control rats (192

male or female rats) were exposed to filtered room air. Weight gain among males and females was not affected by dioxane treatment compared with controls. Survival rates were not significantly different between control and treated rats. Similarly, the tumor incidence was not significantly different with dioxane treatment. The estimated equivalent dose rate from the inhalation study was 100 mg/kg/day, based on default values for rat body weight and breathing rate. This estimated dose is much lower than that used in the drinking water studies described above.

Male and female Swiss-Webster mice (30/sex/group) were exposed dermally to an unspecified concentration of dioxane in acetone 3 times/week (King *et al*., 1975). Dioxane was tested either as a complete carcinogen for 60 weeks, or as a promoter, following a single exposure to DMBA followed by 59-week exposure to dioxane. Control mice were treated with the acetone vehicle alone or with DMBA. Dioxane was a significant promoter of skin carcinomas compared to controls treated with DMBA only. However, no significant increase in skin papillomas or carcinomas was observed in the test for complete carcinogenicity.

The tumor-intitiating properties of dioxane were investigated using the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) in Sencar mice (40 females/group) (Bull *et al*., 1986). Mice were exposed to a single oral, topical, or subcutaneous dose of 1,000 mg/kg dioxane, followed by TPA in acetone 3 times per week for up to 52 weeks. No significant increase in skin tumor incidence was reported in the mice, however, tumor incidence was not reported and length of observation was not specified.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Five studies (Argus *et al*., 1965; Hoch-Ligeti *et al*., 1969; Argus *et al*., 1973; Kociba *et al*., 1974; and NCI, 1978) allow the estimation of cancer potency values for dioxane. Of these studies, only the Kociba *et al*. (1974) and NCI (1978) studies were considered for the determination of the cancer potency factor for dioxane.

In the Argus *et al*. (1965) study, 26 adult male Wistar rats were given drinking water containing 1% dioxane for 63 weeks. The estimated dose of dioxane by the authors was 300 mg/day. The incidence of liver hepatomas (6/26) in the treated animals was significantly higher than in untreated control animals (0/9) but not significantly different from historical control animals. The cancer incidence in the treated animals is considered biologically significant, but is not quantitatively suitable for use as the basis of a cancer potency factor for dioxane.

The Hoch-Ligeti *et al*. (1969) and Argus *et al*. (1973) study failed to demonstrate significant differences in tumor incidences between treated and control rats. The trend for increasing tumors was marginally signficant ($p < 0.07$). When analyzed, these data yield a human cancer potency factor of 2.0×10^3 to 5.8×10^3 $(mg/kg\text{-}day)^{-1}$.

In the Kociba *et al.* (1974) study, 60 male and female Sherman rats were exposed to concentrations of dioxane of 0, 0.01, 0.1, and 1.0 % for 716 days. The tumor incidence for the combined male and female data set was 1/120, 0/120, 1/120, and 10/120. Using the multistage procedure and an interspecies scaling factor, an estimate for human cancer potency of dioxane was 5.7×10^{-4} $(mg/kg/day)^{-1}$. This dataset was not used since tumor incidences for males and females were averaged.

The National Cancer Institute (1978) exposed male and female Osborne-Mendel rats to 0, 0.1, or 1.0 % dioxane in their drinking water for 110 or 90 weeks, respectively. The incidence of nasal tumors were 0/33, 12/33, and 16/33 in the males, and 0/34, 10/35, and 8/35 in the females. From measured water consumption and body weight data, the human cancer potency from a multistage polynomial fit of these data was 9.5×10^{-3} $(mg/kg/day)^{-1}$ from male rat data, and 4.9×10^{-3} $(mg/kg/day)^{-1}$ from female rat data. An adjustment for early mortality following the procedure of EPA (1988) yielded cancer potencies of 1.1×10^{-2} $(mg/kg/day)^{-1}$ and 6.0×10^{-3} $(mg/kg/day)^{-1}$ from male and female rat data, respectively. The NCI (1978) study using B6C3F1 mice was used as the basis for the cancer potency for dioxane. This study contained the best data on the most sensitive species and sex, and the most sensitive target tissue. In this study, 50 male or female mice were exposed to 0, 0.5, or 1.0% dioxane for 90 weeks. Average doses were determined from weekly measurements of water consumption. The estimated doses were 0, 720, and 830 mg/kg/day for the males and 0, 380, and 860 mg/kg/day for the females. The incidence of hepatocarcinomas were 2/49, 18/50, and 24/47 for males, and 0/50, 12/48, and 29/37 for the females. The incidence of hepatocarcinomas or adenomas were 8/49, 19/50, and 28/47 in males, and 0/50, 21/48, and 35/37 in females.

Methodology

A linearized multistage procedure (CDHS, 1985) was applied to the female mouse combined hepatocellular carcinoma and adenoma incidence from the NCI (1978) study. The animal cancer potencies were 8.3×10^{-4} and 1.4×10^{-3} $(mg/kg/day)^{-1}$, for the males and females, respectively. The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. The animal cancer potencies were therefore adjusted for the short duration of the experiment, using the factor $(104/90)^3$. A value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse). Body weights for interspecies scaling were assumed to be 0.04 and 0.035 kg for males and females, respectively. The combined incidence of hepatocarcinomas and adenomas in males and females gave human cancer potencies of 1.5×10^{-2}, and 2.7×10^{-2} $(mg/kg/day)^{-1}$, respectively. The combined incidence of hepatocarcinomas and adenomas in females was used to derive the human cancer potency for dioxane of 2.7×10^{-2} $(mg/kg/day)^{-1}$. The airborne unit risk factor for dioxane of 7.7 E-6 $(\mu g/m^3)^{-1}$ was calculated by OEHHA/ATES assuming a human body weight of 70 kg and an inhalation rate of 20 m^3/day.

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EPICHLOROHYDRIN

CAS No: 106-89-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	92.5
Boiling point	116.5°C
Melting point	-48°C
Vapor pressure	10 mm Hg at 16.6°C
Air concentration conversion	1 ppm = 3.79 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.3 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 8.0 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

A retrospective cohort mortality study of 533 white male Dow Chemical Company employees with potential epichlorohydrin (ECH) exposure for at least 1 month between October 1957 and November 1976 was performed by Shellenberger *et al*. (1979; reviewed by US EPA, 1984). Two cancer deaths were observed; this was less than the number expected (3.5) for the entire group. However, in a review of this study, US EPA (1984) pointed out that this study is inadequate for ECH carcinogenicity evaluation because of low exposures, short exposure duration, a short study period and the very young age of the cohort.

Enterline (1978, 1981; reviewed by US EPA, 1984) conducted a retrospective cohort mortality study of epichlorohydrin workers for Shell Oil Company. The cohort consisted of 864 workers at Shell plants in Louisiana and Texas; deaths were compared by cause with expected deaths in Louisiana and Texas, respectively. Study data were analyzed by vital status as of December 31, 1977 and as of December 31, 1979 (reported by Enterline in 1978 and 1981, respectively) for the cohort exposed to ECH for at least 3 months before January 1, 1966. Overall mortality in the ECH-exposed group was not increased compared to controls; a non-statistically significant increase in respiratory cancer and leukemias was reported (standardized mortality ratios (SMRs) of 146.2 and 224.7, respectively). Additionally, the data reported in 1978 indicated an apparent increase with increasing latent period since 11 of 12 of the respiratory cancer or leukemia deaths occurred in workers 15 or more years after first exposure. The possibility existed that increasing observation time would reveal more respiratory cancer/leukemia deaths. However, the 1981 report (Enterline, 1981) including the most recent data indicated that the SMRs for both

respiratory cancer and leukemia in the ECH-exposed group decreased, especially for those with greater than 15 years since first exposure. US EPA (1984) also noted that smoking was a potential confounder, exposure analysis failed to show a dose-response trend, and exposure to multiple chemicals was also a potential confounder. The SMR for respiratory cancer was much higher in workers exposed in the isopropyl alcohol manufacturing unit to other chemicals in addition to ECH than in the group exposed to ECH alone (SMRs = 214.8 and 63.3, respectively). US EPA (1984) concluded that the studies by Enterline (1978, 1981) provide only limited evidence for the human carcinogenicity of ECH.

Tassignon *et al*. (1983) studied the mortality of workers exposed to ECH in four European manufacturing plants which produced ECH, epoxy resins, glycerin and other ECH-derived specialty chemicals. Data was collected on 606 male workers with at least one year of exposure to ECH starting at least 10 years before December 31, 1978. No excess cancer mortality due to ECH exposure was observed; however, the authors noted that the small cohort size, short duration of the observation period and the limited number of deaths due to low average age (42 years) limited the power of the study.

Animal Studies

Female ICR/Ha Swiss mice were treated with ECH by dermal application, subcutaneous injection or intraperitoneal injection (Van Duuren *et al*., 1974). Dermal applications were performed 3 times/week; 2 mg ECH in 0.1 ml acetone was applied to 50 animals for 83 weeks. Untreated and vehicle control groups of 100 and 50 animals, respectively, were included. No increased incidence in skin tumors were noted in the treated animals. Intraperitoneal injections were performed weekly for 64 weeks; 1 mg ECH in 0.05 ml tricaprylin was injected into 30 animals. Untreated and vehicle control groups of 100 and 30 animals, respectively, were included. No treatment-related tumor induction was noted in the exposed animals. Subcutaneous injections were performed weekly for 83 weeks; 1 mg ECH dissolved in 0.05 ml tricaprylin was injected into 50 animals. Untreated and vehicle control groups of 100 and 50 animals, respectively, were included. An increased incidence of injection site tumors was noted (6/50 sarcomas, 1/50 adenocarcinomas) in the treated animals as compared to controls (no tumors in untreated controls, 1/50 sarcomas in vehicle controls).

Laskin *et al.* (1980) exposed male non-inbred Sprague-Dawley rats to 10, 30 or 100 ppm ECH by inhalation for the lifetime of the animals. Exposure durations were 6 hours/day, 5 days/week. Control groups consisted of 50 untreated animals and 100 air-treated animals. All groups demonstrated a high degree of early mortality, primarily due to respiratory disease (50% mortality by 64 weeks). Of a group of animals exposed to 100 ppm ECH for 6 weeks, 18/140 developed nasal cavity tumors, which were primarily squamous cell carcinomas. A group of 100 animals exposed for life (approximately 144 weeks) to 30 ppm developed 2 respiratory tract tumors; 1 larynx squamous papilloma and 1 nasal squamous cell carcinoma. The laryngeal tumor was misidentified in the original manuscript as a nasal tumor (US EPA, 1984). These tumor incidences were not significant when compared to those of the control group; however, they were significant when compared to the 1920 historical control animals from that laboratory, none of which had

developed nasal squamous carcinoma. No nasal or respiratory tract tumors were noted in a group of 100 animals exposed for life to 10 ppm. No equivalent nasal or respiratory tumors were noted in the control groups. CDHS (1988) noted that only 18%, 26% and approximately 50% of the animals in the 30 ppm, 10 ppm and 100 ppm dose groups, respectively, survived to mean time-to-tumor observed in the 100 ppm group (86 weeks).

Konishi *et al*. (1980) exposed male Wistar rats (18/group) to epichlorohydrin in drinking water at concentrations of 0, 375, 750 or 1500 ppm for up to 81 weeks; treatment was intermittently suspended between 60 and 81 weeks for all three epichlorohydrin treatment groups due to toxicity. Total dose for the 375, 750 and 1500 ppm treatment groups was 5.0, 8.9 and 15.1 g/animal, respectively. A dose-related increase in forestomach tumor (papillomas and squamous cell carcinomas) incidence was observed. Tumor incidence data is listed in Table 1.

Table 1: Incidence of forestomach tumors in male Wistar rats exposed to epichlorohydrin in drinking water (Konishi *et al*., 1980)

Concentration (ppm)	Calculated dose[1] (mg/kg-day)	Tumor incidence	
		papillomas	squamous cell carcinomas
0	0	0/10	0/10
375	15.1	0/9	0/9
750	31.9	1/10	1/10
1500	76.1	7/12	2/10

1. As listed in CDHS (1988).

Male and female ICR/HA Swiss mice (50/sex/group) were exposed to pure (99.9%) trichlorethylene (TCE), industrial grade (99.4%) TCE, or TCE containing 0.8% ECH, 0.8% 1,2-epoxybutane, or 0.8% ECH and 0.8% 1,2-epoxybutane by gavage for 104 weeks (Henschler *et al*., 1984). Corn oil vehicle control groups were included. Initial dosing provided TCE exposures of 2400 mg/kg/day^{-1} and 1800 mg/kg/day^{-1} for male and female mice, respectively. Because of toxicity, dosing was halted for all groups during weeks 35-40, 65 and 69-78. All doses were reduced by a factor of 2 at week 40. Mortality was significantly increased compared to controls in all male treatment groups, and in female treatment groups receiving pure TCE and TCE/ECH. Significant increases in the incidence of squamous cell carcinomas of the forestomach were observed in both male and female animals exposed to TCE/ECH. The tumor incidence in animals exposed to pure TCE was comparable to control values. Tumor incidence data is listed in Table 2.

Male and female Wistar rats (50/sex/group) were exposed to 0, 2 or 10 mg/kg body weight epichlorohydrin by gavage 5 times/week for 2 years (Wester *et al*., 1985). Intestinal obstruction by trichobezoars (hairballs) resulted in intercurrent mortality after 4 months. Cumulative incidences for control, low-dose and high-dose animals, respectively, were 8, 16 and 19 for females, and 1, 0 and 5 for males. The study diet formulation was changed at 4 months; this resulted in decreased mortality from this cause for the remainder of the

study. The percentage of surviving animals after 1 and 2 years of treatment is listed in Table 3.

Table 2. Epichlorohydrin-induced forestomach tumor incidence in male and female Swiss mice (Henschler *et al*., 1984)

Treatment group	Tumor incidence[1]	
	males	females
vehicle controls	1/50	1/50
pure trichloroethylene	1/50	0/50
pure trichloroethylene + 0.8% epichlorohydrin	8/50	12/50

1. Papilloma and squamous cell carcinoma incidences combined.

Table 3. Survival of male and female Wistar rats exposed to epichlorohydrin by gavage (Wester *et al*., 1985)

Dose level (mg/kg body weight)	% survival after 1 year of treatment		% survival after 2 years of treatment	
	males	females	males	females
0	98	80	76	62
2	94	62	62	40
10	90	62	58	44

Treatment-related increases in the incidence of forestomach tumors (papillomas and squamous cell carcinomas) were observed in both male and female animals. Tumor incidence data is listed in Table 4.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Cancer potency values are based on the most sensitive site, species and study demonstrating carcinogenicity of a particular chemical, unless other evidence indicates that a value derived from that data set would not be appropriate (CDHS, 1985). Several studies describe ECH-induced tumor incidence data which can be used to generate a cancer potency factor (male Wistar rat forestomach papilloma and squamous cell carcinoma data, Konishi *et al*. (1980); male Sprague-Dawley rat nasal tumor data, Laskin *et al.* (1980); male and female ICR/HA Swiss mouse forestomach squamous cell carcinoma data, Henschler *et al.* (1984); male and female Wistar rat papilloma and squamous cell carcinoma data, Wester *et al.* (1985). The data from the study by Konishi *et al*. (1980) was chosen by CDHS (1988) as the basis for a cancer potency factor for ECH. Data from the Laskin *et al*. (1980) study was considered to be less suitable for generating a cancer potency factor than data from the Konishi *et al.* (1980) study because of the poor survival of the study animals. The studies by Henschler *et al.* (1984) and Wester *et al.*, (1985) contained potential confounding factors. The ECH-exposed animals in the Henschler *et al.* (1984)

study were also exposed to trichloroethylene. The animals used in the Wester *et al.* (1985) study exhibited trichobezoar-induced intestinal obstructions early in the study due to the diet composition; CDHS (1988) noted that those obstructions could have been a contributing factor to the observed forestomach carcinogenesis.

Table 4. Epichlorohydrin-induced forestomach tumor incidence in male and female Wistar rats (Wester *et al.*, 1985)

Sex	Dose level (mg/kg body weight)	Tumor type	Tumor incidence
male	0 2 10	papilloma	1/50 6/49 4/49
	0 2 10	squamous cell carcinoma	0/50 6/49 35/49
female	0 2 10	papilloma	2/47 3/44 0/39
	0 2 10	squamous cell carcinoma	0/47 2/44 24/39

Methodology

A linearized multistage procedure (CDHS, 1985) was applied to male Wistar rat forestomach papilloma and carcinoma incidence data (Konishi *et al.*, 1980). US EPA (1984) lists the half-life of ECH in water as 0.69 days. Assuming first order decay due to hydrolysis, this corresponds to an average concentration throughout the day of 63% of the concentration of the freshly prepared solution. The control, low, mid and high dose groups were reported to have received cumulative doses of 0, 5.0, 8.9 and 15.1 grams (Konishi *et al.*, 1980); however, these data do not compensate for hydrolysis loss of ECH. CDHS (1988) estimated the actual daily exposures after hydrolysis compensation for the low, mid and high dose groups to be 15.1, 31.9 and 76.1 mg/kg-day, respectively. This assumes a body weight of 400 grams for a control Wistar rat, and utilizes the body weight data supplied by Konishi *et al*. (1980) which indicated that the low, mid and high dose animals weighed 7.7, 22.4 and 44.9% less than the controls, respectively. Upper 95% confidence bounds on carcinogenic potency (q_1^*) were estimated using the incidences of forestomach tumors in animals surviving to the end of the study (81 weeks) and the above dose estimates. Estimates of lifetime potency values (q_{animal}) were calculated from the q_1^* derived from the 81 week study using the relationship $q_{animal} = q_1^* * (104/81)^3$. Estimates for q_{animal} of 0.015 and 0.011 $(mg/kg\text{-}day)^{-1}$ were obtained for the benign squamous cell papillomas and malignant squamous cell carcinomas, respectively. The fitted dose response functions associated with these potency estimates exhibited significant upward curvature (p = 0.03). Surface area scaling was employed to transform animal cancer potency factors to human cancer potency factors, using the relationship ($q_{human} = q_{animal}$ *

$(bw_h / bw_a)^{1/3}$), where q_{human} is the human potency, q_{animal} is the animal potency, and bw_h and bw_a are the human and animal body weights, respectively. Human carcinogenic potency values (q_{human}) of 0.08 and 0.06 mg/kg-day^{-1} were derived from the q_{animal} values for benign squamous cell papillomas and malignant squamous cell carcinomas, respectively. A unit risk factor was calculated by OEHHA/ATES from the benign squamous cell papilloma data-derived q_{human} value using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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ETHYLBENZENE

CAS No: 100-41-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 2003)

Molecular weight	106.2
Boiling point	136.2°C
Melting point	-94.9°C
Vapor pressure	9.6 mm Hg @ 25°C
Air concentration conversion	1 ppm = 4.35 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk: 2.5×10^{-6} $(\mu g/m^3)^{-1}$
Inhalation Cancer Potency: 0.0087 $(mg/kg\text{-}day)^{-1}$
Oral Cancer Potency: 0.011 $(mg/kg\text{-}day)^{-1}$
[Calculated from male rat renal tumor data (NTP, 1999), using the linearized multistage (LMS) methodology with lifetime weighted average (LTWA) doses (OEHHA, 2007).

III. METABOLISM and CARCINOGENIC EFFECTS

Metabolism

Ethylbenzene is rapidly and efficiently absorbed in humans via the inhalation route (ATSDR, 1999). Human volunteers exposed for 8 hours to 23-85 ppm retained 64% of inspired ethylbenzene vapor (Bardodej and Bardodejova, 1970). Gromiec and Piotrowski (1984) observed a lower mean uptake value of 49% with similar ethylbenzene exposures. There are no quantitative oral absorption data for ethylbenzene or benzene in humans but studies with [^{14}C]-benzene in rats and mice indicate gastrointestinal absorption in these species was greater than 97% over a wide range of doses (Sabourin *et al*., 1987).
Most of the metabolism of ethylbenzene is governed by the oxidation of the side chain (Fishbein, 1985). Engstrom (1984) studied the fate of ethylbenzene in rats exposed to 300 or 600 ppm (1305 or 2610 mg/m^3) ethylbenzene for six hours. Engstrom assumed 60 percent absorption of inhaled ethylbenzene and calculated that 83% of the 300 ppm dose was excreted in the urine within four hours of exposure. At the higher exposure of 600 ppm only 59 percent of the dose was recovered in the urine within 48 hr of exposure. Fourteen putative ethylbenzene metabolites were identified in the urine of exposed rats. The principal metabolites were 1-phenylethanol, mandelic acid, and benzoic acid. Metabolism proceeded mainly through oxidation of the ethyl moiety with ring oxidation appearing to play a minor role. Other metabolites included acetophenone, ω-hydroxyacetophenone, phenylglyoxal, and 1-phenyl-1, 2-ethandiol. Ring oxidation products include p-hydroxy- and m-hydroxyacetophenone, 2-ethyl- and 4-ethylphenol. With the exception of 4-hydroxyacetophenone all these other metabolites were seen only in trace amounts.

The metabolism of ethylbenzene was studied in humans (number unstated) exposed at 23 to 85 ppm (100 to 370 mg/m^3) in inhalation chambers for eight hours (Bardodej and Bardodejova, 1970). About 64 percent of the vapor was retained in the respiratory tract and only traces of ethylbenzene were found in expired air after termination of exposure. In 18 experiments with ethylbenzene, the principal metabolites observed in the urine were: mandelic acid, 64%; phenylglyoxylic acid, 25%; and 1-phenylethanol, 5%.

Engstrom *et al.* (1984) exposed four human male volunteers to 150 ppm ethylbenzene (653 mg/m^3) for four hours. Urine samples were obtained at two-hr intervals during exposure and periodically during the next day. For the 24-hr urine the metabolites were: mandelic acid, 71.5 ± 1.5%; phenylglyoxylic acid, 19.1 ± 2.0%; 1-phenylethanol, 4.0 ± 0.5%; 1-phenyl-1, 2-ethanediol, 0.53 ± 0.09%; acetophenone, 0.14 ± 0.04%; ω-hydroxyacetophenone, 0.15 ± 0.05%; m-hydroxyacetophenone, 1.6 ± 0.3%; and 4-ethylphenol, 0.28 ± 0.06%. A number of the hydroxy and keto metabolites were subject to conjugation. Differences were observed between the concentrations obtained with enzymatic and acid hydrolysis. For example, 50% of maximal yield of 4-ethylphenol was obtained with glucuronidase or acid hydrolysis and 100% with sulfatase indicating the presence of glucuronide and sulfate conjugates of this metabolite. Alternatively, acetophenone gave only 30-36% yield with enzymatic treatment but 100% with acid hydrolysis indicating the presence of other conjugates not susceptible to glucuronidase or sulfatase. The metabolic scheme proposed by Engstrom *et al.* (1984) is shown with modifications in Figure 1. The metabolism of ethylbenzene is similar in several respects to benzene in that benzene produces phenol, catechols and hydroquinone metabolites. As noted below these metabolites and their ethyl analogs participate in redox cycles generating the reactive oxygen species hydrogen peroxide, superoxide, and hydroxyl radical.

Gromiec and Piotrowski (1984) measured ethylbenzene uptake and excretion in six human volunteers exposed at concentrations of 18 to 200 mg/m^3 for eight hours. Average retention of ethylbenzene in the lungs was 49 ± 5% and total excreted mandelic acid accounted for 55 ± 2% of retained ethylbenzene.

Tardif *et al.* (1997) studied physiologically-based pharmacokinetic (PBPK) modeling of ternary mixtures of alkyl benzenes including ethylbenzene in rats and humans. As part of this investigation they determined Vmax and Km kinetic parameters for the rat by best fit of model simulations to the time-course data on the venous blood concentrations of ethylbenzene following single exposures. The maximal velocity (Vmax) was 7.3 mg/hr-kg body weight and the Michaelis-Menten affinity constant (Km) was 1.39 mg/L. For the human PBPK model the Vmax value from the rat was scaled on the basis of $(\text{body weight})^{0.75}$. All other chemical and metabolic parameters were unchanged.

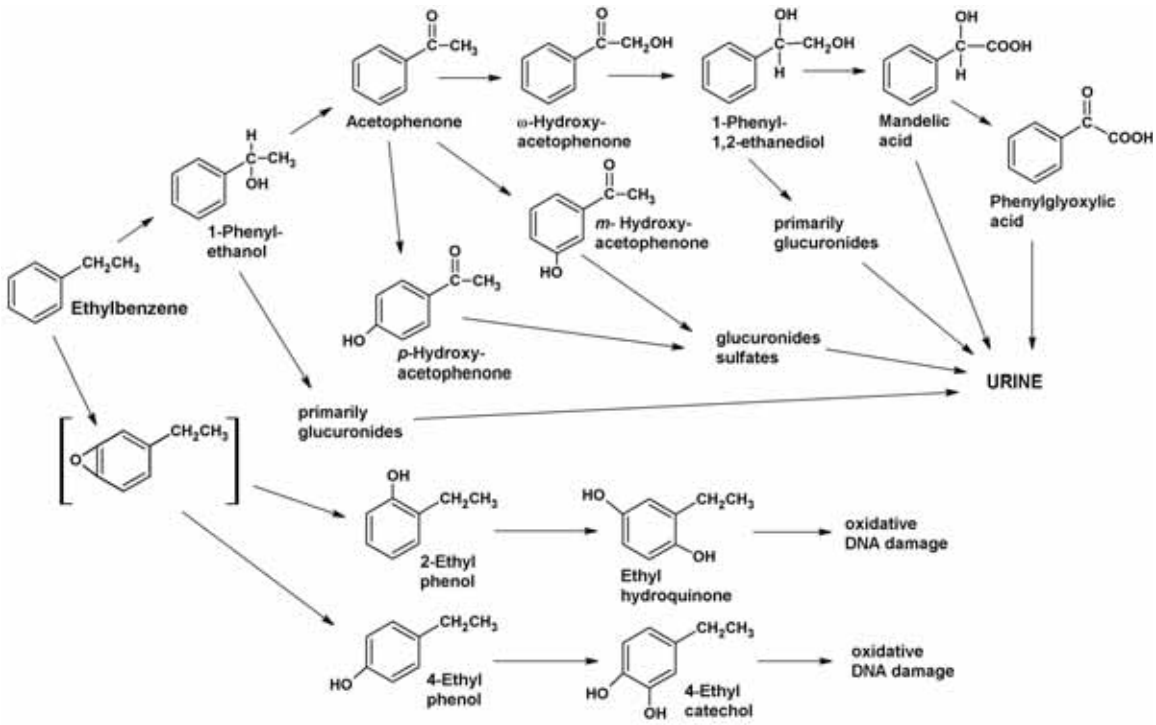

Figure 1. Ethylbenzene Metabolism (modified from Engstrom *et al.*, 1984).

The scaling of rodent metabolism of alkylbenzenes to humans was evaluated using kinetic data in an exposure study with human volunteers. Four adult male subjects (age, 22-47; body weight, 79-90 kg) were exposed to 33 ppm ethylbenzene for 7 hr/d in an exposure chamber. Urine samples were collected during (0-3 hr) and at the end (3-7 hr) of exposure and following exposure (7-24 hr). For the 0-24 hr collections mandelic acid amounted to 927 ± 281 μmol and phenylglyoxylic acid 472 ± 169 μmol. Venous blood (5.5 to 8 hr) and expired air (0.5 to 8 hr) were also measured in the subjects and exhibited good correspondence with PBPK model predictions. It is interesting that the metabolism of ethylbenzene in these human subjects was not significantly affected by simultaneous exposure to the other alkyl benzenes (toluene and xylene) studied. The metabolic parameters for ethylbenzene used by Haddad *et al.* (2001) and in the internal dosimetry modeling presented below were based on this study.

The oxidation of ethylbenzene to 1-phenylethanol by human liver microsomes and recombinant human cytochrome P450s was investigated by Sams *et al.* (2004). Human liver microsomes from seven subjects (four male, three female, age 37-74) and microsomes expressing recombinant human CYP1A2, 2A6, 2B6, 2C9*1(Arg144), 2C19, 2D6, 2E1, and 3A4 co-expressed with cytochrome P450 reductase/cytochrome b5 were both obtained from commercial sources. Kinetic experiments were conducted with microsomes and ethylbenzene over a 10-5000 μ*M* substrate concentration range. For chemical inhibition experiments, selective inhibitors of specific CYP isoforms were used to obtain maximum inhibition of the target CYP with minimum effect on other CYPs. Eadie-Hofstee plots (V vs. V/S) indicated that the reaction of ethylbenzene to 1-phenylethanol with human liver microsomes was biphasic with low and high affinity components. The Michaelis-Menten

equation was fit to the data and kinetic constants obtained by regression analysis. One microsome preparation was found to give a noticeably less curved Eadie-Hofstee plot and metabolized ethylbenzene at a much higher rate than the other preparations (Vmax = 2922 pmol/min/mg). It was excluded from the statistical analysis. For the high affinity reaction the mean Vmax was 689 ± 278 pmol/min/mg microsomal protein and the Km = 8.0 ± 2.9 μ*M* (n = 6). For the low affinity reaction the Vmax was 3039 ± 825 pmol/min/mg and Km = 391 ± 117 μ*M* (n = 6). The intrinsic clearance values of Vmax/Km were 85.4 ± 15.1 and 8.3 ± 3.0 for the high and low affinity reactions, respectively. The high affinity component of pooled human liver microsomes was inhibited 79%-95% by diethyldithiocarbamate, and recombinant CYP2E1 metabolized ethylbenzene with a low Km of 35 μ*M* and low Vmax of 7 pmol/min/pmol P450 indicating that the CYP2E1 isoform catalyzed this component. Recombinant CYP1A2 and CYP2B6 exhibited high Vmaxs (88 and 71 pmol/min/pmol P450, respectively) and Km's (502 and 219 μ*M*, respectively), indicating their role in the low affinity component. The mean Vmax and Km values above were used by OEHHA in addition to those from Haddad *et al.* (2001) in our human PBPK modeling of ethylbenzene.

Charest-Tardif *et al*. (2006) characterized the inhalation pharmacokinetics of ethylbenzene in male and female B6C3F1 mice. Initially groups of animals were exposed for four hr to 75, 200, 500 or 1000 ppm ethylbenzene. Subsequently groups of animals were exposed for six hr to 75 and 750 ppm for one or seven consecutive days. The maximum blood concentration (Cmax, mean (± SD), n = 4) observed after four hr exposure to 75, 200, 500 and 1000 ppm was 0.53 (0.18), 2.26 (0.38), 19.17 (2.74), and 82.36 (16.66) mg/L, respectively. The blood AUCs were 88.5, 414.0, 3612.2, and 19,104.1 (mg/L)-min, respectively, in female mice, and 116.7, 425.7, 3148.3, 16039.3 (mg/L)-min, respectively in male mice. The comparison of Cmax and kinetics of ethylbenzene in mice exposed to 75 ppm indicated similarity between 1 and 7-day exposures. However, at 750 ppm elimination of ethylbenzene appeared to be greater after repeated exposures. Overall, the single and repeated exposure PK data indicate that ethylbenzene kinetics is saturable at exposure concentrations above 500 ppm but is linear at lower concentrations.

Backes *et al*. (1993) demonstrated that alkylbenzenes with larger substituents (e.g., ethylbenzene, m-, p-xylene, n-propylbenzene) were effective inducers of microsomal enzymes compared to those with no or smaller substituents (benzene, toluene). Cytochrome P450 2B1 and 2B2 levels were induced with the magnitude of induction increasing with hydrocarbon size. P450 1A1 was also induced but less than 2B. A single intraperitoneal (i.p.) dose of 10 mmol/kg in rats was selected for optimum induction response with no overt toxic effects.

Bergeron *et al*. (1999) using the same daily dose of ethylbenzene for up to ten days observed changes in expression of CYP 2B1, 2B2, 2E1, and 2C11. While CYP 2C11 and 2E1 were attenuated by repeated dosing of ethylbenzene, CYP 2Bs were elevated after initial dosing despite the absence of detectable 2B1 or 2B2 mRNA. The authors interpreted this observation as the initial ethylbenzene dose leading to an increase in ethylbenzene clearance and an overall decrease in tissue ethylbenzene levels with repeated dosing and decreased induction effectiveness.

Serron *et al*. (2000) observed that treatment of rats with ethylbenzene (i.p., 10 mmol/kg) led to increased free radical production by liver microsomes compared to corn oil controls. Oxygen free radical generation was measured *in vitro* by conversion of 2', 7'-dichlorofluorescein diacetate (DCFH-DA) to its fluorescent product 2', 7'-dichlorofluorescein (DCF). A significant elevation (40%) of DCF was seen despite lack of effect on overall P450 levels. The DCF product formation was inhibited by catalase but not by superoxide dismutase suggesting a H_2O_2 intermediate. Anti-CYP2B antibodies inhibited DCF production indicating involvement of CYP2B. As noted above ethylbenzene treatment induces increased production of CYP2B.

While the doses in these studies were quite high at over 1000 mg/kg-d by the intraperitoneal route, earlier studies by Elovaara *et al*. (1985) showed P450 induction in livers of rats exposed to 50, 300 and 600 ppm (218, 1305 and 2610 mg/m^3) for 6 hours/day, 5 days/week for up to 16 weeks. So it is possible that the types of effects discussed above, notably the production of reactive oxygen species via induced CYP 2B, may have occurred during the cancer bioassays.

Genotoxicity

In vitro and *in vivo* animal studies

Ethylbenzene has been tested for genotoxicity in a variety of *in vitro* and *in vivo* genotoxicity assays. Those studies have been reviewed by ATSDR (1999). Ethylbenzene has not demonstrated genotoxicity in *Salmonella* reverse mutation assays. Those studies are listed in Table 1. All studies were performed in the presence and absence of metabolic activation (rat liver S9), and were negative. It has not been tested in strains sensitive to oxidative DNA damage.

Table 1. Ethylbenzene *Salmonella* reverse mutation studies

Test strains	**Reference**
TA98, TA100, TA1535, TA1537	Florin *et al*., 1980
TA98, TA100, TA1535, TA1537, TA1538	Nestmann *et al*., 1980
TA98, TA100, TA1535, TA1537, TA1538	Dean *et al*., 1985
TA97, TA98, TA100, TA1535	NTP, 1986
TA97, TA98, TA100, TA1535	NTP, 1999
TA98, TA100	Kubo *et al*., 2002

Ethylbenzene also did not induce mutations in the WP2 and WP2uvrA strains of *Escherichia coli* in the presence and absence of metabolic activation (Dean *et al*., 1985), or in *Saccharomyces cerevisiae* strains JDl (Dean *et al*., 1985), XVl85-14C, and D7 as measured by gene conversion assays (Nestmann and Lee, 1983).

Ethylbenzene has been observed to induce mutations in L5178Y mouse lymphoma cells at the highest nonlethal dose tested (80 µg/mL) (McGregor *et al*., 1988; NTP, 1999).

However, NTP noted significant cytotoxicity at this dose level (relative total growth was reduced to 34% and 13% of the control level in each of two trials).

Data on the ability of ethylbenzene to induce chromosomal damage in non-human mammalian cells are negative. Ethylbenzene did not cause chromosomal damage in rat liver epithelial-like (RL4) cells (Dean *et al*., 1985). Additionally, ethylbenzene did not induce an increase in either sister chromatid exchanges (SCE) or chromosomal aberrations in Chinese hamster ovary (CHO) cells in the presence or absence of metabolic activation (NTP 1986, 1999).

The frequency of micronucleated erythrocytes in bone marrow from male NMRI mice exposed to ethylbenzene by intraperitoneal injection was not significantly increased compared to controls (Mohtashamipur *et al*., 1985). Additionally, ethylbenzene did not increase the frequency of micronucleated erythrocytes in peripheral blood from male and female $B6C3F_1$ mice treated for 13 weeks with ethylbenzene (NTP, 1999).

Midorikawa *et al* (2004) reported oxidative DNA damage induced by the metabolites of ethylbenzene, namely ethylhydroquinone and 4-ethylcatechol. Ethylbenzene was metabolized to 1-phenylethanol, acetophenone, 2-ethylphenol, and 4-ethylphenol by rat liver microsomes *in vitro*. 2-Ethylphenol and 4-ethylphenol were ring-dihydroxylated to ethylhydroquinone (EHQ) and 4-ethylcatechol (EC). These dihydroxylated metabolites induced DNA damage in ^{32}P-labeled DNA fragments from the human p53 tumor suppressor gene and induced the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine in calf thymus DNA in the presence of Cu2+. Addition of exogenous NADH enhanced EC-induced oxidative DNA damage but had little effect on EHQ action. The authors suggest that Cu+ and H_2O_2 produced via oxidation of EHQ and EC were involved in oxidative DNA damage. NADH enhancement was attributed to reactive species generated from the redox cycle of EC → 4-ethyl-1, 2-benzoquinone → EC. The NADH-mediated conversion of 4-Ethyl-1, 2-benzoquinone appears to be the result of a two electron reduction which accelerates the redox reaction, resulting in enhanced DNA damage (Figure 2).

Similar effects of NADH were observed with benzene metabolites benzoquinone (BQ) and catechol (Hirakawa *et al*. 2002). In the presence of Cu^{2+} and endogenous NADH, catechol (1,2-BQH_2) induced more DNA damage than 1,4-BQH_2. In the absence of NADH the DNA damaging activities were reversed. In both cases, DNA damage resulted from base modification at guanine and thymine residues in addition to DNA strand breaks by Cu^+ and H_2O_2 generated during the oxidation of 1,2-BQH_2 and 1,4- BQH_2 to 1,2_BQ and 1,4-BQ, respectively (Hirakawa *et al*., 2002). The authors noted that NADH consumption in the presence of 1,2-BQH_2/1,2-BQ was faster than that in the 1,4-BQH_2/1,4-BQ system. The results suggest that the structure of 1,2-BQ may facilitate the two-electron reduction by NADH better than 1,4-BQ. Thus, the reduction of 1,2-BQ accelerates the turnover rate of the redox cycle in 1,2-BQH_2/1,2-BQ greater than in 1,4-BQH_2/1,4-BQ. The authors conclude that "…the NADH-dependent redox cycle may

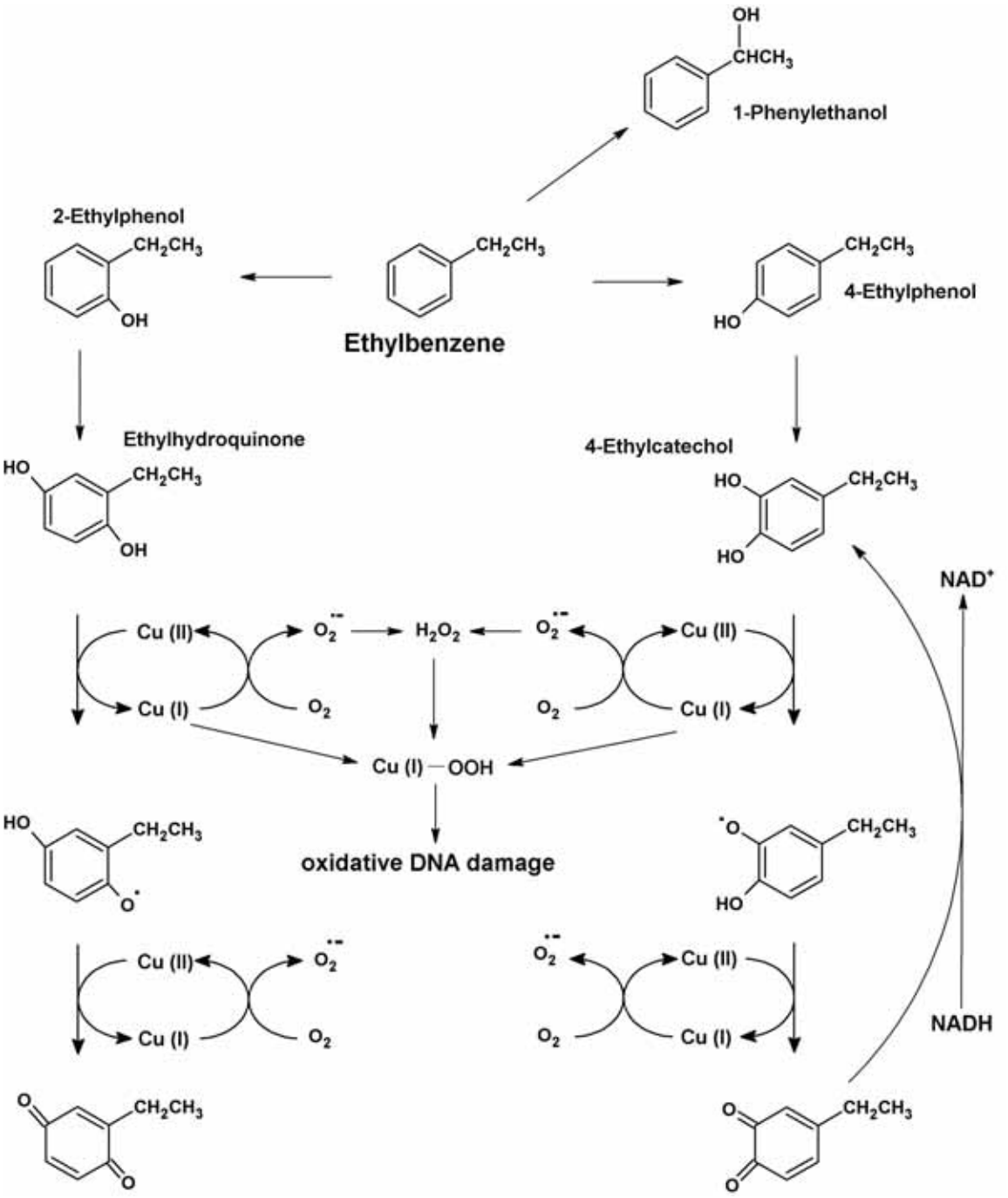

Figure 2. Possible mechanism of oxidative DNA damage induced by EHQ and EC. (Adapted from Midorikawa *et al*., 2004)

continuously generate reactive oxygen species, resulting in the enhancement of oxidative DNA damage. NADH, a reductant existing at high concentrations (100- 200 μM) in certain tissues, could facilitate the NADH-mediated DNA damage observed in this study under physiological conditions."(Hirakawa *et al*., 2002).

Similar reactions were also observed with methylcatechols, toluene metabolites that participated in Cu^{2+}-mediated DNA damage, which was enhanced by NADH compared with methylhydroquinone (Nakai *et al*., 2003; Murata *et al*., 1999).

In vitro and *in vivo* human studies

Norppa and Vainio (1983) exposed human peripheral blood lymphocytes to ethylbenzene in the absence of metabolic activation. The authors reported that ethylbenzene induced a marginal increase in SCEs at the highest dose tested, and that the increase demonstrated a dose-response.

Holz *et al*. (1995) studied genotoxic effects in workers exposed to volatile aromatic hydrocarbons (styrene, benzene, ethylbenzene, toluene and xylenes) in a styrene production plant. Peripheral blood monocytes were assayed for DNA adducts using a nuclease P1-enhanced ^{32}P-postlabeling assay, and DNA single strand breaks, SCEs and micronuclei frequencies in peripheral blood lymphocytes were determined in workers and controls. No significant increases in DNA adducts, DNA single strand breaks, SCEs or total micronuclei were noted in exposed workers. Significantly increased kinetochore-positive micronuclei (suggestive of aneuploidy-induction) were noted in total exposed workers, exposed smokers, and exposed non-smokers. However, the mixed exposures made it impossible to ascribe the kinetochore-positive micronuclei increase in exposed workers solely to ethylbenzene exposure.

The effects of benzene and ethylbenzene exposure on chromosomal damage in exposed workers were examined by Sram *et al*. (2004). Peripheral blood lymphocytes from exposed workers and controls were analyzed for chromosomal aberrations. Exposure to ethylbenzene resulted in a significant increase in chromosomal aberrations. A reduction in ethylbenzene concentration due to improved workplace emissions controls resulted in a reduction in chromosomal damage in exposed workers. However, these workers were also exposed to benzene, making it impossible to determine if the chromosomal damage was due to ethylbenzene alone.

Ethylbenzene sunlight-irradiation products

Toda *et al*. (2003) found that sunlight irradiation of ethylbenzene resulted in the formation of ethylbenzene hydroperoxide (EBH). EBH induced oxidative DNA damage in the presence of Cu^{2+} as measured by the formation of 8-hydroxy-deoxyguanosine (8-OH-dG) adducts in calf thymus DNA. The Cu^{2+}-specific chelator bathocuproine strongly inhibited EBH-induced oxidative DNA damage. Superoxide dismutase (catalyzes superoxide decomposition) partly inhibited 8-OH-dG adduct formation, and catalase (catalyzes hydrogen peroxide decomposition) slightly inhibited 8-OH-dG adduct formation.

Summary of ethylbenzene genotoxicity

The above data indicate that ethylbenzene generally has not been demonstrated to induce gene mutations or chromosomal damage in bacteria, yeast or non-human mammalian cells, with the exception of positive results in the L5178Y mouse lymphoma cell mutation assay at concentrations producing significant cytotoxicity (McGregor *et al*., 1988; NTP, 1999). Data on the genotoxicity of ethylbenzene in humans is mixed (Norppa and Vainio, 1983; Holz *et al*., 1995; Sram *et al*., 2004), and interpretation of the epidemiological studies is made difficult because of confounding due to coexposures to other chemicals, including benzene. Ethylbenzene has been demonstrated to generate reactive oxygen species in liver microsomes from exposed rats (Serron *et al*., 2000), and ethylbenzene hydroperoxide (a sunlight-irradiation product) has been demonstrated to induce oxidative DNA damage in calf thymus DNA *in vitro* (Toda *et al*., 2003). The ethylbenzene metabolites EHQ and EC have demonstrated the ability to induce oxidative DNA damage in human DNA *in vitro* (Midorikawa *et al*., 2004).

Animal Cancer Bioassays

Maltoni *et al.* (originally reported in 1985; additional information published in 1997) studied the carcinogenicity of ethylbenzene in male and female Sprague-Dawley rats exposed via gavage. The authors reported an increase in the percentage of animals with malignant tumors associated with exposure to ethylbenzene. In animals exposed to 800 mg/kg bw ethylbenzene, Maltoni *et al.* (1997) reported an increase in nasal cavity tumors, type not specified (2% in exposed females versus 0% in controls), neuroesthesioepitheliomas (2% in exposed females versus 0% in controls; 6% in exposed males versus 0% in controls), and oral cavity tumors (6% in exposed females versus 2% in controls; 2% in exposed males versus 0% in controls). These studies were limited by inadequate reporting and were considered inconclusive by NTP (1999) and IARC (2000).

The National Toxicology Program (NTP, 1999; Chan *et al*., 1998) conducted inhalation cancer studies of ethylbenzene using male and female F344/N rats and $B6C3F_1$ mice. Groups of 50 animals were exposed via inhalation to 0, 75, 250 or 750 ppm ethylbenzene for 6.25 hours per day, 5 days per week for 104 (rats) or 103 (mice) weeks.

Survival probabilities were calculated by NTP (1999) using the Kaplan-Meier product-limit procedure. For male rats in the 75 ppm and 250 ppm exposure groups, survival probabilities at the end of the study were comparable to that of controls but significantly less for male rats in the 750 ppm exposure group (30% for controls and 28%, 26% and 4% for the 75 ppm, 250 ppm and 750 ppm exposure groups, respectively). NTP (1999) stated that the mean body weights of the two highest exposure groups (250 and 750 ppm) were "generally less than those of the chamber controls from week 20 until the end of the study." Expressed as percent of controls, the mean body weights for male rats ranged from 97 to 101% for the 75 ppm group, 90 to 98% for the 250 ppm group, and 81 to 98% for the 750 ppm group.

In female rats, survival probabilities were comparable in all groups (62% for controls and 62%, 68% and 72% for the 75 ppm, 250 ppm and 750 ppm exposure groups, respectively). NTP (1999) reported that the mean body weights of exposed female rats were "generally less than those of chamber controls during the second year of the study." Expressed as percent of controls, the mean body weights for female rats ranged from 92 to 99% for the 75 ppm group, 93 to 100% for the 250 ppm group, and 92 to 99% for the 750 ppm group.

The incidences of renal tumors (adenoma and carcinoma in males; adenoma only in females) were significantly increased among rats of both sexes in the high-dose group (males: 3/50, 5/50, 8/50, 21/50; females: 0/50, 0/50, 1/50, 8/49 in control, 75 ppm, 250 ppm and 750 ppm groups respectively [standard and extended evaluations of kidneys combined]). The incidence of testicular adenomas (interstitial and bilateral) was significantly elevated among high-dose male rats (36/50, 33/50, 40/50, 44/50 in control, 75 ppm, 250 ppm and 750 ppm groups respectively). NTP noted that this is a common neoplasm, which is likely to develop in all male F344/N rats that complete a natural life span; exposure to ethylbenzene "appeared to enhance its development." NTP concluded that there was clear evidence of carcinogenicity in male rats and some evidence in female rats, based on the renal tumorigenicity findings.

The survival probabilities at the end of the study for exposed male mice were comparable to that of controls (57% for controls and 72%, 64% and 61% for the 75 ppm, 250 ppm and 750 ppm exposure groups, respectively). The same was true for exposed female mice (survival probabilities at end of study: 71% for controls and 76%, 82% and 74% for the 75 ppm, 250 ppm and 750 ppm exposure groups, respectively). Mean body weights in exposed male mice were comparable to those of controls. NTP (1999) reported that the mean body weights in exposed female mice were greater in the 75 ppm group compared to controls after week 72, and generally lower in the 750 ppm group compared to controls from week 24 through week 68. Expressed as percent of controls, the ranges of mean body weights in exposed female mice were 96 to 110% in the 75 ppm group, 93 to 108% in the 250 ppm group, and 92 to 101% in the 750 ppm group.

Increased incidences of alveolar/bronchiolar adenoma and adenoma or carcinoma (combined) were observed in male mice in the high-dose group (7/50, 10/50, 15/50, 19/50 in control, 75 ppm, 250 ppm and 750 ppm groups respectively). Among female mice in the high-dose group, the incidences of combined hepatocellular adenoma or carcinoma and hepatocellular adenoma alone were significantly increased over control animals (for adenomas and carcinomas the tumor incidences were 13/50, 12/50, 15/50, 25/50 in control, 75 ppm, 250 ppm and 750 ppm groups, respectively). NTP (1999) concluded that these findings provided some evidence of carcinogenicity in male and female mice.

Human Studies of Carcinogenic Effects

Studies on the effects of workplace exposures to ethylbenzene have been complicated by concurrent exposures to other chemicals, such as xylenes and benzene. IARC (2000) concluded that there was inadequate evidence in humans for the carcinogenicity of ethylbenzene.

Mode of Action for Ethylbenzene carcinogenesis

A mode of action (MOA) is a clear explanation of the critical events in an agent's influence on the development of tumors. An MOA analysis includes physical, chemical, and biological information and the entire range of information developed in the assessment contributes to a reasoned judgement concerning the plausibility of potential MOAs (U.S.EPA, 1996). An agent may work by more than one MOA at different sites and at the same tumor site. Inputs into an MOA analysis include tumor data in humans, animals, and in structural analogs, genetic toxicity and other key data e.g. on metabolites, DNA or protein adducts, oncogene activation and shape of the dose response. In any event conflicting data and data gaps often require careful evaluation before reaching any conclusions with respect to a prospective MOA (U.S.EPA, 1996).

OEHHA has not determined a convincing mode of action (MOA) for any of the tumor sites evaluated in this report. Various MOAs have been suggested for the tumors induced by ethylbenzene in rodent species. For instance it has been hypothesized that rat kidney tumor incidence increases are the result of ethylbenzene or its metabolites increasing the incidence and/or severity of chronic progressive nephropathy (CPN), a common process in aged control rats (Hard, 2002). However, OEHHA and others (Seely *et al.*, 2002) have found no basis to support a conclusion that the sole or primary cause of the kidney tumors is exacerbation of CPN. Similarly, it has been suggested that an increase in eosinophilic foci in the liver, possibly associated with induction of cytochrome P450 enzymes, is involved in the mechanism of production of the liver tumors. In fact, the data from which a correlation between liver eosinophilic foci and liver tumors was inferred are not consistent or convincing in this respect. Moreover, such MOAs have not been adequately elucidated with respect to their quantitative dose-response relations, or how significant they are with respect to other MOAs, possibly involving genotoxicity, which may also be operating.

A proposed MOA for ethylbenzene-induced tumors, especially those in the mouse lung, involves the generation of quinone metabolites. This is analogous to the actions of styrene and naphthalene, which are also carcinogenic. OEHHA recognizes the plausibility of quinone metabolites participating in a potential MOA for ethylbenzene-induced lung cancer in mice (see *Genotoxicity* above). However, a suggestion that the role of these metabolites is confined to cytotoxicity (resulting in promotion of spontaneous tumors) is not convincing. The observation of oxidative DNA damage *in vitro* (Midorikawa *et al.*, 2004) supports a role for quinone metabolites in carcinogenic initiation, following the analogy with benzene (a well-known genotoxic carcinogen targeting multiple sites in various species including humans). The observation of chromosomal damage in peripheral blood lymphocytes of workers exposed to ethylbenzene and benzene (Sram *et al.*, 2004) may be indicative of quinone metabolite induced DNA damage. Thus, the involvement of quinone metabolites is plausible and supported by at least some data. Although this does not of itself establish the quantitative nature of the dose-response relationship, a mechanism involving oxidative DNA damage might display low-dose linearity. Since ring oxidation may produce a genotoxic epoxide metabolite it is possible that more than one metabolic

process which generates genotoxic intermediates may be operating. In our view the genotoxicity of ethylbenzene, particularly with respect to oxidative DNA effects, merits further investigation.

OEHHA therefore concludes that the limited data do not conclusively establish any particular MOA for ethylbenzene carcinogenesis. However, one or more genotoxic processes appear at least plausible and may well contribute to the overall process of tumor induction. Because of this, the default linear approach has been used for extrapolating the dose-response curve to low doses.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Unit risk values for ethylbenzene were calculated based on data in male and female rats and mice from the studies of NTP (1999) utilizing both linearized multistage and benchmark dose methods. The incidence data used to calculate unit risk values are listed below in Tables 2 thru 6. The methodologies for calculating average concentration, lifetime weighted average (LTWA) dose and PBPK adjusted internal dose are discussed below. An internal dose metric representing the amount of ethylbenzene metabolized per kg body weight per day (metabolized dose) was used in the dose response analysis with published PBPK modeling parameters. In addition, for the mouse, recent pharmacokinetic data simulating mouse bioassay conditions were used to improve PBPK model predictions (Tables 5 and 6).

The metabolized dose metric is considered the most appropriate metric for assessment of carcinogenic risks when the parent compound undergoes systemic metabolism to a variety of oxidative metabolites which may participate in one or more mechanisms of carcinogenic action, and the parent compound is considered unlikely to be active. In this case the dose response relation is likely to be more closely related to the internal dose of metabolites than of the parent compound. Other metrics commonly investigated using PBPK methods are the area under the concentration-time curve (AUC), and the maximum concentration (Cmax) for parent or metabolites in blood and target tissues. The PBPK metabolized dose metric was used in the ethylbenzene dose-response analysis.

Table 2. Incidence of renal tubule adenoma or carcinoma in male rats exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	Tumor incidence[d]		Statistical significance[e]	
				Quantal Response	%	Fisher Exact Test	TrendTest
0	0	0	0	3/42	7.1		
75	60.7	35.6	19.09	5/42	11.9	$p = 0.356$	$p < 0.001$
250	202	119	58.78	8/42	19.0	$p = 0.0972$	
750	607	356	124.26	21/36	58.3	$p < 0.001$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 $mg/m^3/ppm$.
b. Lifetime weighted average doses determined by multiplying the lifetime average concentrations during the dosing period by the male rat breathing rate (0.264 m^3/day) divided by the male rat body weight (0.450 kg). The duration of exposure was 104 weeks, so no correction for less than lifetime exposure was required.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. Effective rate. Animals that died before the first occurrence of tumor (day 572) were removed from the denominator. Total number of tumors/number of survivors.
e. The *p*-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. The *p*-value listed for the trend test is the result obtained by the National Toxicology Program (NTP, 1999) using the life table, logistic regression and Cochran-Armitage methods, with all methods producing the same result.

Table 3. Incidence of testicular adenoma in male rats exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	Tumor incidence[d]		Statistical significance	
				Quantal Response	%	Fisher Exact Test[e]	Trend Test
0	0	0	0	36/48	75.0		
75	60.7	35.6	19.09	33/46	71.7	$p = 0.450$N	$p < 0.001$[f]
250	202	119	58.78	40/49	81.6	$p = 0.293$	$p = 0.010$[g]
750	607	356	124.26	44/47	93.6	$p < 0.05$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 mg/m^3/ppm.
b. Lifetime weighted average doses determined by multiplying the lifetime average concentrations during the dosing period by the male rat breathing rate (0.264 m^3/day) divided by the male rat body weight (0.450 kg). The duration of exposure was 104 weeks, so no correction for less than lifetime exposure was required.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. Effective rate. Animals that died before the first occurrence of tumor (day 420) were removed from the denominator. Total number of tumors/number of survivors
e. The *p*-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. An "N" after the *p*-value signifies that the incidence in the dose group is lower than that in the control group.
f. Results of trend tests conducted by NTP (1999) using the life table and logistic regression tests.
g. Result of Cochran-Armitage trend test conducted by NTP (1999).

Table 4. Incidence of renal tubule adenoma in female rats exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	Tumor incidence[d]		Statistical significance[e]	
				Quantal Response	%	Fisher Exact Test	Trend Test
0	0	0	0	0/32	0		
75	60.7	41.6	21.60	0/35	0	--	$p < 0.001$
250	202	139	67.04	1/34	2.9	$p = 0.515$	
750	607	416	144.62	8/37	21.6	$p < 0.01$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 mg/m3/ppm.
b. Lifetime weighted average doses were determined by multiplying the lifetime average concentrations during the dosing period by the female rat-breathing rate (0.193 m3/day) divided by the female rat body weight (0.282 kg). The duration of exposure was 104 weeks, so no correction for less than lifetime exposure was required.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. Effective rate. Animals that died before the first occurrence of tumor (day 722) were removed from the denominator. Total number of tumors/number of survivors
e. The p-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. The *p*-value listed for the trend test is the result obtained by the National Toxicology Program (NTP, 1999) using the life table, logistic regression and Cochran-Armitage methods, with all methods producing the same result.

Table 5. Incidence of lung alveolar/bronchiolar carcinoma or adenoma in male mice exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	PBPK metabolized dose: Charest-Tardif[d] (mg/kg-d)	Tumor incidence[e]		Statistical significance[f]	
					Quantal Response	%	Fisher Exact Test	Trend Test
0	0	0	0	0	7/46	15.2		
75	60.7	69.3	40.40	46.60	10/48	20.8	$p = 0.331$	$p = 0.004$
250	202	231	89.38	152.8	15/50	30.0	$p = 0.0688$	
750	607	693	134.77	340.2	19/48	40.0	$p < 0.01$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 mg/m^3/ppm.
b. Lifetime weighted average doses were determined by multiplying the average concentrations during the dosing period by the male mouse breathing rate (0.0494 m^3/day) divided by the male mouse body weight (0.0429 kg) and by 103 weeks/104 weeks to correct for less than lifetime exposure.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. PBPK metabolized dose based on published parameters from Charest-Tardif *et al.* (2006).
e. Effective rate. Animals that died before the first occurrence of tumor (day 418) were removed from the denominator. Total number of tumors/number of survivors.
f. The *p*-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. The *p*-value listed for the trend test is the result obtained by the National Toxicology Program (NTP, 1999) using the life table, logistic regression and Cochran-Armitage methods, with all methods producing the same result.

Table 6. Incidence of liver hepatocellular carcinoma or adenoma in female mice exposed to ethylbenzene via inhalation and relevant dose metrics (from NTP, 1999).

Chamber concentration (ppm)	Average concentration[a] (mg/m^3)	LTWA dose[b] (mg/kg-day)	PBPK metabolized dose[c] (mg/kg-d)	PBPK metabolized dose: Charest-Tardif[d] (mg/kg-d)	Tumor incidence[e]		Statistical significance	
					Quantal Response	%	Fisher Exact Test[f]	Trend Test
0	0	0	0	0	13/47	27.7		
75	60.7	71.6	41.53	47.98	12/48	25.0	$p = 0.479$N	$p = 0$[g]
250	202	239	91.22	157.3	15/47	31.9	$p = 0.411$	$p = 0.002$[h]
750	607	716	136.68	348.1	25/48	52.1	$p < 0.05$	

a. Average concentration during exposure period calculated by multiplying chamber concentration by 6.25 hours/24 hours, 5 days/7 days, and 4.35 mg/m^3/ppm.
b. Lifetime weighted average doses were determined by multiplying the average concentrations during the dosing period by the female mouse breathing rate (0.0463 m^3/day) divided by the female mouse body weight (0.0389 kg) and by 103 weeks/104 weeks to correct for less than lifetime exposure.
c. Rodent PBPK models were used to estimate internal doses under bioassay conditions; methods are described in detail below.
d. PBPK metabolized dose based on published parameters from Charest-Tardif *et al.* (2006).
e. Effective rate. Animals that died before the first occurrence of tumor (day 562) were removed from the denominator. Total number of tumors/number of survivors.
f. The *p*-value listed next to each dose group is the result of pair wise comparison with controls using the Fisher exact test. An "N" after the *p*-value signifies that the incidence in the dose group is lower than that in the control group.
g. Result of trend test conducted by NTP (1999) using the life table method.
h. Results of trend tests conducted by NTP (1999) using the logistic regression and Cochran-Armitage trend tests.

Methodology

Linearized Multistage Approach

The default approach, as originally delineated by CDHS (1985), is based on a linearized form of the multistage model of carcinogenesis (Armitage and Doll, 1954). Cancer potency is estimated from the upper 95% confidence limit, q_1^*, on the linear coefficient q_1 in a model relating lifetime probability of cancer (*p*) to dose (d):

$$p = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \cdots + q_j d^j)] \qquad (1)$$

with constraints, $q_i \geq 0$ for all i. The default number of parameters used in the model is n, where n is the number of dose groups in the experiment, with a corresponding polynomial degree of n-1.

The parameter q_1^* is estimated by fitting the above model to dose response data using MSTAGE (Crouch, 1992). For a given chemical, the model is fit to one or more data sets. The default approach is to select the data for the most sensitive species and sex.

To estimate animal potency, q_{animal}, when the experimental exposure is less than lifetime the parameter q_1* is adjusted by assuming that the lifetime incidence of cancer increases with the third power of age. The durations of the NTP experiments were at least as long as the standard assumed lifetime for rodents of 104 weeks, so no correction for short duration was required.

Benchmark Dose Methodology

U.S. EPA (2005) and others (*e.g.* Gaylor *et al.*, 1994) have more recently advocated a benchmark dose method for estimating cancer risk. This involves fitting a mathematical model to the dose-response data. A linear or multistage procedure is often used, although others may be chosen in particular cases, especially where mechanistic information is available which indicates that some other type of dose-response relationship is expected, or where another mathematical model form provides a better fit to the data. A point of departure on the fitted curve is defined: for animal carcinogenesis bioassays this is usually chosen as the lower 95% confidence limit on the dose predicted to cause a 10% increase in tumor incidence (LED_{10}). Linear extrapolation from the point of departure to zero dose is used to estimate risk at low doses either when mutagenicity or other data imply that this is appropriate, or in the default case where no data on mechanism are available. The slope factor thus determined from the experimental data is corrected for experimental duration in the same way as the q_1^* adjustments described for the linearized multistage procedure. In the exceptional cases where data suggesting that some other form of low-dose extrapolation is appropriate, a reference dose method with uncertainty factors as required may be used instead.

The quantal tumor incidence data sets were analyzed using the BMDS software (version 1.3.2) of U.S.EPA (2000). In general the program models were fit to the data with the X^2 fit criterion ≥ 0.1. In those cases when more than one model gave adequate fit the model that gave the best fit in the low dose region (visually and by X^2 residual) was chosen for the LED_{10} estimation.

Implementation of LMS and BMD Methodology

The linearized multistage approach and the benchmark dose methodology were both applied to the tumor incidence data for ethylbenzene in the NTP (1999) studies. No nonlinear mode of carcinogenic action has been established for ethylbenzene. Hard (2002) suggested that "chemically induced exacerbation of CPN [chronic progressive nephropathy] was the mode of action underlying the development of renal neoplasia" in the NTP ethylbenzene studies. In a retrospective evaluation of NTP chronic studies, Seely *et al.* (2002) found that renal tubule cell neoplasms (RTCNs) "tend to occur in animals with a slightly higher severity of CPN than animals without RTCNs. However, the differential is minimal and clearly there are many male F344 rats with severe CPN without RTCNs." Seely *et al.* (2002) go on to say that "the data from these retrospective reviews suggest that an increased severity of CPN may contribute to the overall tumor response. However, any contribution appears to be marginal, and additional factors are likely involved."

Stott *et al.* (2003) reported accumulation of the male rat specific protein α2u-globulin in 1-week and 4-week inhalation studies of ethylbenzene in groups of six (1-week study) or eight (4-week study) male rats; the accumulation measured as an increase in hyaline droplets in proximal convoluted tubules was statistically significant only in the 1-week study. In the 13-week and 2-year inhalation studies of ethylbenzene, NTP (1992; 1999) found no evidence of an increase in hyaline droplets in treated rats. NTP (1999) therefore dismissed any involvement of α2u-globulin accumulation in renal tumor development in rats. The fact that the lesion appears in both male and female rats further argues against the involvement of α2u-globulin in the development of kidney toxicity. This mechanism was discounted by Hard (2002) as well. Stott *et al.* (2003) also postulated mechanisms of tumorigenic action involving cell proliferation and/or altered cell population dynamics in female mouse liver and male mouse lung. Stott et al. (2003) propose various hypothetical mechanisms which might involve nonlinear dose responses but the metabolism data clearly show the formation of epoxides and related oxidative metabolites, which could potentially be involved in a genotoxic mechanism of carcinogenic action possibly similar to benzene. Midorikawa *et al*. (2004) reported that the oxidative metabolism of ethylbenzene metabolites ethylhydroquinone and 4-ethylcatechol resulted in oxidative DNA damage *in vitro*. In view of the variety of metabolites and possible modes of action a low-dose linearity assumption is considered appropriate when extrapolating from the point of departure to obtain an estimate of the cancer risk at low doses with the BMD methodology as is use of the LMS approach.

Calculation of Lifetime Weighted Average Dose

Male and female rats (NTP, 1999) were exposed to ethylbenzene for 6.25 hours/day, five days/week for 104 weeks. Male and female mice (NTP, 1999) were exposed to ethylbenzene for 6.25 hours/day, five days/week for 103 weeks. Average concentrations, expressed in mg/m^3, during the exposure period were calculated by multiplying the reported chamber concentrations by 6.25 hours/24 hours, five days/seven days and 4.35 mg/m^3/ppm.

The average body weights of male and female rats were calculated to be 0.450 kg and 0.282 kg, respectively, based on data for controls reported by NTP (1999). The average body weights of male and female mice were estimated to be approximately 0.0429 kg and 0.0389 kg, respectively,

based on data for controls reported by NTP (1999). Inhalation rates (I) in m^3/day for rats and mice were calculated based on Anderson *et al.* (1983):

$$I_{rats} = 0.105 \times (bw_{rats}/0.113)^{2/3} \quad (3)$$

$$I_{mice} = 0.0345 \times (bw_{mice}/0.025)^{2/3} \quad (4)$$

Breathing rates were calculated to be 0.264 m^3/day for male rats, 0.193 m^3/day for female rats, 0.0494 m^3/day for male mice, and 0.0463 m^3/day for female mice. Lifetime weighted average (LTWA) doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight. For mice, the exposure period (103 weeks) was less than the standard rodent lifespan (104 weeks), so an additional factor of 103 weeks/104 weeks was applied to determine lifetime average doses.

Physiologically Based Pharmacokinetic (PBPK) Modeling

The carcinogenic potency of ethylbenzene was calculated using rodent PBPK models to estimate internal doses under bioassay conditions. Extrapolations to human potencies were done using interspecies scaling. For comparison, a human PBPK model was used to estimate risk-specific doses for occupational and ambient environmental exposure scenarios. The PBPK models were comprised of compartments for liver, fat, vessel poor tissues (e.g., muscle), vessel rich tissues, and lung. Typical model parameters are given in Table 7 for flow-limited PBPK models and a model diagram is shown in Figure 2. Chemical and metabolic parameters for mouse and human models were taken from Haddad *et al.* (2001) and additionally from Sams *et al.* (2004) for human metabolism. The rat PBPK model was based on Dennison *et al.* (2003). Simulations were conducted using Berkeley Madonna (v.8.3.9) software (e.g., 6.25 hr exposure/day x 5 days/wk for one week simulations of bioassay exposure levels, see sample model equations in Appendix A). The chemical partition coefficients used in the Haddad et al. model were: blood:air, 28.0; fat:blood, 55.57; liver:blood, 2.99; muscle:blood, 0.93; and vessel rich:blood, 2.15 (Haddad *et al.*, 2001). For the Dennison et al. rat model the chemical partition coefficients were: blood:air, 42.7; fat:blood, 36.4; liver:blood, 1.96; muscle:blood, 0.609; and vessel rich:blood, 1.96. The metabolic parameters from Haddad *et al.* (2001) were: VmaxC = 6.39 mg/hr/kg body weight scaled to the ¾ power of body weight; Km = 1.04 mg/L. For the rat model the metabolic parameters were: VmaxC = 7.60 mg/kg-d scaled to the 0.74 power of body weight and Km = 0.1 mg/L. A second set of human metabolic parameters from Sams *et al.* (2004) was also used. In this case constants for low and high affinity saturable pathways were incorporated into the models: high affinity Vmax = 689 pmol/min/mg microsomal protein, Km = 8.0 μM; low affinity Vmax = 3039 pmol/min/mg protein, Km = 391 μM. A value of 28 mg/mL liver for microsomal protein concentration was assumed. Published values we reviewed ranged from 11 to 35 mg/g tissue. The value we used was similar to that of Kohn and Melnick (2000) (30 mg/g liver) and Medinsky *et al.* (1994) (35 mg/g liver). All model units were converted to moles, liters, or hours for simulation. A molecular weight of 106.16 g/mol for ethylbenzene was used throughout. In addition to PBPK modeling based on published parameters the recent pharmacokinetic data of Charest-Tardif *et al.* (2006) was used in the mouse PBPK modeling for comparison purposes. During the final revisions of this document we obtained the recently published paper of Nong *et al.* (2007), which describes a mouse PBPK model for ethylbenzene inhalation based on the pharmacokinetic data of Charest-Tardif *et al.*

(2006) and other parameter measurements. This model differs from that of Haddad *et al*. (2001) in having gender- and dose-specific chemical and metabolic parameters. The model also includes metabolism by lung and vessel-rich tissues in addition to liver. We employed the Nong *et al*. model in simulations of bioassay conditions identical to the Haddad *et al.* (2001) and Charest-Tardif *et al*. (2006) based models run previously, except that only the BMD dose response analysis was performed with the resulting total metabolized dose.

Although no systematic evaluation of PBPK model parameter uncertainty was conducted, the fact that we essentially used two rat models (Haddad *et al*., 2001 in the first draft and Dennison *et al*., 2004 in the revised draft) and two mouse models (Haddad *et al*. 2001, and Nong *et al*. 2007) and three key metabolic parameters (Charest-Tardif *et al*., 2006) for the mouse addresses this concern to some extent. The potency estimates in all cases were similar indicating a relative insensitivity to the PBPK parameters varied.

Johansen and Filser (1992) studied a series of volatile organic chemicals including ethylbenzene and developed theoretical values for clearance of uptake (CLupt) defined as the product of the rate constant for transfer of chemical from air to body and the volume of air in a closed chamber. The CLupt values were based on alveolar ventilation (Qalv), cardiac output (Qtot), and blood:air partition coefficients (Pbi). For most chemicals the experimentally determined values for inhalation uptake in rats and mice were about 60% of the theoretical values. The values determined for ethylbenzene in the rat of 70 mL/min for CLupt and 73 mL/min for alveolar ventilation are about 50% the value given in Table 7 (i.e., 4.38 L/hr vs. 8.58 L/hr). In the work described below selected simulations were run with lower alveolar ventilation rates for comparison with the main analysis.

The primary model prediction was the amount of ethylbenzene metabolized over the course of the simulation. The AUCs, the areas under the concentration x time curves for mixed venous concentration and liver concentration of ethylbenzene, were also recorded. The values for one week simulations of the amount metabolized (mmoles) were divided by 7d/week and body weight in kg to give daily values and multiplied by the molecular weight to give the PBPK metabolized dose in mg/kg-d. These values were then used in the dose response assessment of individual tumor site incidences using the benchmark dose software of U.S. EPA (BMDS v. 1.3.2) to obtain ED_{10}s, LED_{10}s and curve fit statistics.

Table 7. Parameters for Ethylbenzene PBPK Models.

Parameter	**Mouse**	**Rat**	**Human**
Alveolar ventilation rate Qalv, L/hr	$15*BW^{0.7}$	$12*BW^{0.74}$	$36*BW^{0.7}$ occ $15*BW^{0.7}$ env
Cardiac output Qtot, L/hr	$15*BW^{0.7}$	$15*BW^{0.74}$	$18*BW^{0.7}$ occ $15*BW^{0.7}$ env
Blood flows (fraction of cardiac output)			
Fat, Qf	0.09	0.07	0.05
Liver, Ql	0.25	0.183	0.26
Muscle, Qm	0.15	0.237	0.25
Vessel Rich Group, Qvrg	0.51	0.51	0.44
Tissue volumes, L (fraction of body weight unless otherwise indicated)			
Fat, Vf	0.06	0.035*BW + 0.0209	0.20, 0.40
Liver, Vl	0.04	0.037	0.026
Muscle, Vm	0.76	0.91*BW - (Vf + Vl + Vvrg + Vlu)	0.61, 0.41
Vessel Rich Group, Vvrg	0.05	0.054	0.036
Lung, Vlu	0.014	0.002	0.014
Body weight, BW kg	0.043 male 0.039 female	0.45 male 0.28 female	70
Metabolism VmaxC	6.39[a] 25.56[b]*	7.60 [c]	6.39[a]
Km mg/L	1.04[a]	0.10[c]	1.04[a]
Metabolism High/Low Affinity Vmax mg/hr/L_{liver} High/Low Affinity Km mg/L			 122.8/542.0 [d] 0.85/41.5[d]

Note: occ = occupational scenario values; env = environmental exposure scenario; [a]Haddad *et al*. (2001) mg/hr-$kg^{3/4}$; [b]this value provided better fit to the kinetic data of Charest-Tardif *et al*. (2006); [c]Dennison *et al*. (2003) mg/hr-$kg^{0.74}$; [d]Sams *et al*. (2004).

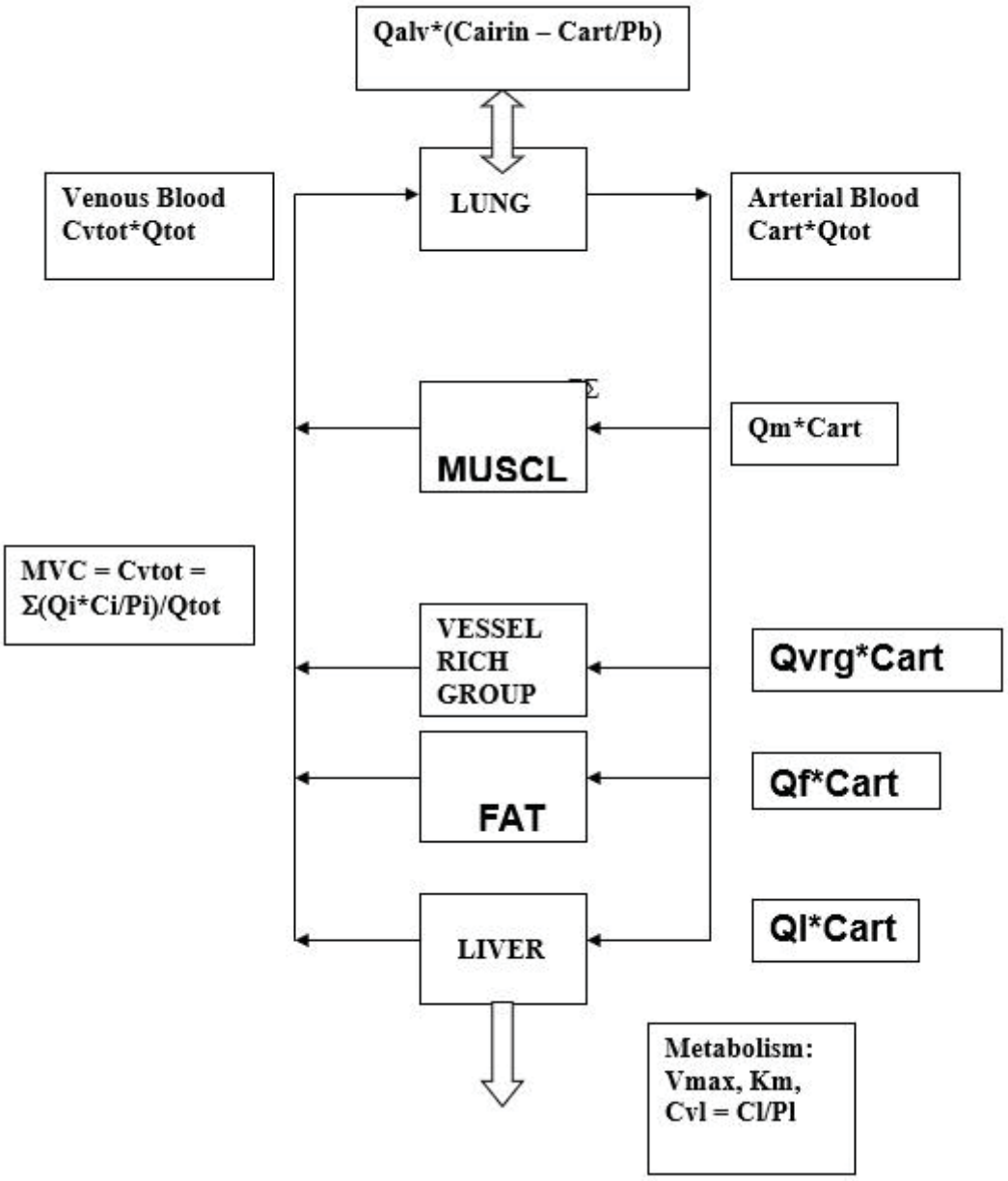

Figure 2. General Scheme for Ethylbenzene PBPK Model:

Qtot = Cardiac Output; Qalv = Alveolar Ventilation Rate ; Pb = Blood/Air Partition Coefficient; Pi = Tissue/Blood Partition Coefficients; Qi = Tissue Fractional Blood Flows ;Cart = Arterial Blood Concentration; Cvtot = Mixed Venous Blood Concentration; Cairin = Inhaled Concentration (e.g. ppm Ethylbenzene); Cexhaled = Cart/Pb(Concentration of Ethylbenzene Exhaled); Ci = Ai/Vi = Mass/Volume.

Internal to External Dose Conversion

In order to estimate external equivalent air concentrations associated with internal doses, the PBPK models were used. Simulation of 10 ppb ethylbenzene for 8 hours in the human PBPK model with the Haddad *et al*. (2001) parameters resulted in the predicted uptake of 3.04 µmoles in tissues and blood compared to 3.96 µmoles inhaled, or an uptake of 77%. Practically all of the 3.04 µmoles represents metabolized ethylbenzene. Based on these results, OEHHA assumed that all absorbed ethylbenzene is metabolized at low dose. Thus, for the inhalation route, the internal metabolized dose is converted to an external dose by applying an uptake factor of 77%. As noted above, uptake values of 49 to 65% have been observed in studies with human subjects exposed via inhalation to ethylbenzene. OEHHA has occasionally used a default value of 50% for inhalation uptake of similar volatile organic compounds.

For the oral route at low dose, OEHHA assumed that ethylbenzene is 100% metabolized and that uptake of ethylbenzene is also 100%. Thus, at low dose, the internal metabolized dose of ethylbenzene would be equivalent to an external applied dose by the oral route. No conversion factor for internal to external dose is necessary in this case.

Interspecies Extrapolation

Interspecies extrapolation from experimental animals to humans is normally based on the following relationship, where bw_h and bw_a are human and animal body weights, respectively, and potency (*e.g.*, q_{animal}) is expressed on a per dose per body weight basis (e.g., $(mg/kg\text{-}d)^{-1}$ see Watanabe *et al*. (1992):

$$q_{human} = q_{animal} \times \left(\frac{bw_h}{bw_a} \right)^{1/4} \qquad (2)$$

This is equivalent to an adjustment based on $(\text{human body weight})^{3/4}$ relative to the animal body weight or $BWh^{3/4}/BWa^{4/4} = (BWh/BWa)^{4/4-3/4} = (BWh/BWa)^{1/4}$. This is the default relationship currently recommended by OEHHA and by U.S. EPA (2005)

Alternatively, when performing calculations based on applied dose in terms of air concentrations, the assumption has sometimes been made that air concentration values are equivalent between species (CDHS, 1985). However, using the interspecies scaling factor shown above is preferred because it is assumed to account not only for pharmacokinetic differences (*e.g.*, breathing rate, metabolism), but also for pharmacodynamic considerations i.e. tissue responses to chemical exposure.

When extrapolating from an animal potency in terms of PBPK adjusted internal dose, only a pharmacodynamic scaling factor is required. Since an equal contribution of pharmacokinetic and pharmacodynamic considerations is assumed, animal potency values already adjusted for pharmacokinetic considerations require a scaling factor of only $(bw_h/bw_a)^{1/8}$:

$$q_{human} = q_{animal} \times \left(\frac{bw_h}{bw_a} \right)^{1/8} \qquad (3)$$

Derivation of the Human Inhalation Unit Risk Value

To derive the human inhalation unit risk value, the human internal potency value based on PBPK metabolized dose is multiplied by the human breathing rate (assumed to be 20 m^3/day), divided by the human body weight (assumed to be 70 kg) and multiplied by the estimated inhalation uptake factor in humans (0.77 for ethylbenzene). This yields a human inhalation unit risk value in terms of external air concentration.

For the case of LTWA doses, the human inhalation unit risk value is derived by multiplying the human inhalation cancer potency value by the human breathing rate (assumed to be 20 m^3/day), divided by the human body weight (assumed to be 70 kg). Because the LTWA doses represent external applied dose from an inhalation study, no uptake factor is necessary in deriving the unit risk value.

Inhalation and Oral Cancer Potency Values

The cancer potency derived based on internal doses (i.e., PBPK metabolized dose) is equivalent to the oral cancer potency, because of the assumption of 100% oral uptake and 100% metabolism of ethylbenzene at low doses. To derive the inhalation cancer potency, the human inhalation unit risk value is multiplied by the human body weight (assumed to be 70 kg) and divided by the human breathing rate (assumed to be 20 m^3/day).

For the case of LTWA doses, the human cancer potency derived based on these external applied doses from the inhalation study is equivalent to the inhalation cancer potency. To determine the oral cancer potency, the inhalation cancer potency is multiplied by the ratio of the oral to inhalation uptake factors (i.e., 1/0.77).

Example Calculations – BMD Approach

In this section, example calculations of the human cancer potency values (oral and inhalation) and the human unit risk value based on the LED_{10} for the male rat kidney tumor data and either the PBPK metabolized doses or the LTWA doses are provided. The same logic would apply to the derivation using the LMS methodology, with the only difference being that the animal potency is taken directly from the MSTAGE program under the LMS approach instead of being calculated from the LED_{10} in the BMD approach. To distinguish the results obtained under the two approaches, the terms P_{animal}, P_{human}, and U_{human} were used for the values derived using the BMD methodology.

Calculations based on BMD methodology and PBPK metabolized doses

Under the BMD methodology, the ED_{10}s and LED_{10}s are obtained from the BMDS program, with the animal potency value being simply $0.1/LED_{10}$ (i.e., 10% risk (0.1) divided by the 95% lower confidence limit on the dose that induced 10% risk or LED_{10}; this is the definition of a slope). To obtain the animal potency based on internal dose ($P_{animal\_internal}$), 0.1 is divided by the LED_{10} derived for the male rat kidney tumor data and the PBPK metabolized doses:

$$P_{animal\_internal} = 0.1/LED_{10} = 0.1/25.38 = 0.00394\ (mg/kg\text{-}d)^{-1}$$

The human potency value based on internal dose ($P_{human\_internal}$) is calculated from the animal potency as follows:

$$P_{human\_internal} = 0.00394\ (mg/kg\text{-}day)^{-1} \times (70\ kg/0.450\ kg)^{1/8}$$
$$= 0.0074\ (mg/kg\text{-}day)^{-1}$$

$P_{human\_internal}$ is equivalent to the oral human potency, because of the assumptions of 100% oral uptake and 100% metabolism of ethylbenzene at low dose.

The human unit risk value (U_{human}) is derived from the internal human cancer potency as follows:

$$U_{human} = 0.0074\ (mg/kg\text{-}day)^{-1} \times (20\ m^3/day/70\ kg) \times 0.77$$
$$= 1.64 \times 10^{-3}\ (mg/m^3)^{-1}$$
$$= 1.64 \times 10^{-6}\ (\mu g/m^3)^{-1}$$

As noted above the value of 0.77 was based on the prediction of the human ethylbenzene PBPK model, assuming exposure to low levels of ethylbenzene, and is similar to values obtained in studies with human subjects. By applying this uptake factor and assuming that the metabolism of ethylbenzene is 100% at low dose, the resulting unit risk value is expressed in terms of external concentration.

The inhalation cancer potency is derived from the unit risk value as follows:

$$P_{human\_inhalation} = 1.64 \times 10^{-3}\ (mg/m^3)^{-1} \times (70\ kg/20\ m^3/day)$$
$$= 0.0057\ (mg/kg\text{-}day)^{-1}$$

<u>Calculations based on BMD methodology and LTWA doses</u>

The LED_{10} based on the male rat kidney data and the LTWA doses is determined using the BMDS software. The animal potency, which in this case is the inhalation animal potency (P_{animal_inh}), is determined by dividing the LED_{10} into 0.1:

$$P_{animal\_inh} = 0.1/LED_{10} = 0.1/42.62 = 0.002346\ (mg/kg\text{-}d)^{-1}$$

The human inhalation cancer potency (P_{human_inh}) is derived from the animal potency using the interspecies scaling factor:

$$P_{human\_inh} = 0.002346\ (mg/kg\text{-}day)^{-1} \times (70\ kg/0.450\ kg)^{1/4}$$
$$= 0.0083\ (mg/kg\text{-}day)^{-1}$$

The unit risk factor is derived from the human inhalation cancer potency as follows:

$$U_{human} = 0.0083\ (mg/kg\text{-}day)^{-1} \times (20\ m^3/day/70\ kg)$$

$= 2.4 \times 10^{-3} (mg/m^3)^{-1}$
$= 2.4 \times 10^{-6} (\mu g/m^3)^{-1}$

For the calculation based on LTWA doses, the oral cancer potency is derived from the inhalation cancer potency by multiplying by the ratio of uptake factors (1/0.77):

$$P_{human\_oral} = 0.0083 (mg/kg\text{-}day)^{-1} \times (1/0.77)$$
$$= 0.011 (mg/kg\text{-}day)^{-1}$$

Results and Discussion

Linearized multistage approach

Tables 8a and 8c list the q_{animal}, q_{human} and unit risk values based on the linearized multistage approach. The cancer potencies and unit risk values were derived using the applied LTWA doses and PBPK adjusted internal doses, as described above. The most sensitive tumor sites are the male rat testicular interstitial cell adenoma and the male rat kidney adenoma and carcinoma, when the LTWA doses are used. If PBPK doses are used, the most sensitive sites are the male rat testicular interstitial cell adenoma and the male mouse lung. Regardless of whether LTWA or PBPK doses are used, the results based on the male mouse lung tumor data, the female mouse liver tumor data, and the male rat renal tumor data are comparable, producing unit risk values of approximately 0.002 $(mg/m^3)^{-1}$. Further, the results using either the LTWA doses or the PBPK metabolized doses are quite similar indicating that the PBPK modeling does not markedly improve the estimates. Some of the inherent uncertainty associated with PBPK modeling is demonstrated by the fact that the results based on the PBPK modeling using the Charest-Tardif parameters differ by roughly a factor of two for the mice compared to the results derived based on the other equally valid PBPK modeling approach.

The testicular interstitial cell adenoma site gives the highest values. However, the very high background incidences of this tumor make it less reliable and suitable for dose-response analysis than the male rat kidney site.

Thus, the unit risk value of 0.0025 $(mg/m^3)^{-1}$ derived based on the LMS approach from the male rat kidney tumor data using the LTWA doses is selected as the representative value for ethylbenzene. It is very similar to the estimate derived using the PBPK approach (0.0026 $(mg/m^3)^{-1}$), and does not require the many assumptions made in applying the more complex PBPK approach.

Table 8a. Cancer potency and unit risk values for ethylbenzene derived using the linearized multistage procedure (LMS) with applied LTWA doses based on data from NTP (1999).

Sex, species	**Site, tumor type**	**q_{animal_inh}** **$(mg/kg\text{-}day)^{-1}$**	**q_{human_inh}[a]** **$(mg/kg\text{-}day)^{-1}$**	**Human unit risk value[b]** **$(mg/m^3)^{-1}$**	**Goodness-of-fit test[c]**
Male rats	Renal tubule carcinoma or adenoma	0.002472	0.0087	**0.0025**	$p = 0.81$
	Testicular interstitial cell adenoma	0.006547	0.023	0.0066	$p = 0.52$
Female rats	Renal tubule adenoma	0.0005528	0.0022	0.00063	$p = 0.95$
Male mice	Lung alveolar/ bronchiolar carcinoma or adenoma	0.0008494	0.0054	0.0015	$p = 0.75$
Female mice	Liver hepatocellular carcinoma or adenoma	0.0009421	0.0061	0.0017	$p = 0.68$

a. The interspecies extrapolation was applied to q_{animal_inh} in $(mg/kg\text{-}d)^{-1}$ to determine q_{human_inh} $(mg/kg\text{-}day)^{-1}$, as described above.

b Unit risk was determined by multiplying the human cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate (20 m^3/day) divided by human body weight (70 kg), as described above.

c. A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit with the LMS procedure.

Table 8b. Cancer potency and unit risk values for ethylbenzene derived using the BMD procedure with applied LTWA doses based on data from NTP (1999).

Sex, species	**Site, tumor type**	**P_{animal_inh} (mg/kg-day)$^{-1}$**	**P_{human_inh}[a] (mg/kg-day)$^{-1}$**	**Human unit risk value[b] (mg/m^3)$^{-1}$**	**Model Goodness-of-fit test[c]**
Male rats	Renal tubule carcinoma or adenoma	0.002589	0.0091	0.0026	Quantal Linear $p = 0.49$
	Testicular interstitial cell adenoma	0.006333	0.022	0.0063	Quantal Linear $p = 0.73$
Female rats	Renal tubule adenoma	0.0004704	0.0019	0.00054	Quantal Quadratic $p = 0.99$
Male mice	Lung alveolar/ bronchiolar carcinoma or adenoma	0.0008062	0.0051	0.0015	Quantal Linear $p = 0.75$
Female mice	Liver hepatocellular carcinoma or adenoma	0.0009256	0.0060	0.0017	Quantal Linear $p = 0.74$

a. The interspecies extrapolation of $(BW_h/BW_a)^{1/4}$ was applied to P_{animal_inh} in $(mg/kg\text{-}d)^{-1}$ to determine P_{human_inh} $(mg/kg\text{-}day)^{-1}$, as described above.

b Unit risk was determined by multiplying the human cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate (20 m^3/day) divided by human body weight (70 kg).

c. A p-value ≥ 0.1 for the chi-square goodness-of-fit test indicates an adequate fit with the BMD procedure.

Table 8c. Cancer potency and unit risk values for ethylbenzene derived using the linearized multistage procedure with PBPK metabolized doses and bioassay data from NTP (1999).

Sex, species	**Site, tumor type**	**$q_{animal\_internal}$ $(mg/kg\text{-}day)^{-1}$**	**$q_{human\_internal}$[a] $(mg/kg\text{-}day)^{-1}$**	**Human unit risk value[b] $(mg/m^3)^{-1}$**	**Goodness-of-fit test[c]**
Male rats	Renal tubule carcinoma or adenoma	0.00473	0.0089	0.0020	$p = 0.68$
	Testicular interstitial cell adenoma	0.0154	0.029	0.0064	$p = 0.89$
Female rats	Renal tubule adenoma	0.00101	0.0020	0.00044	$p = 0.97$
Male mice	Lung alveolar/ bronchiolar carcinoma or adenoma	0.003747 0.001680[d]	0.0094 0.0042[d]	0.0021 0.00092[d]	$p = 0.99$ $p = 0.93$[d]
Female mice	Liver hepatocellular carcinoma or adenoma	0.002702 0.001705[d]	0.0069 0.0044[d]	0.0015 0.00097[d]	$p = 0.86$ $p = 0.73$[d]

a. The interspecies extrapolation of $(bw_h/bw_a)^{1/8}$ was applied to $q_{animal\_internal}$ in $(mg/kg\text{-}d)^{-1}$ to determine $q_{human\_internal}$ in $(mg/kg\text{-}day)^{-1}$, as described above.

b. Unit risk was determined by multiplying the human internal cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate (20 m^3/day) divided by human body weight (70 kg) and by an uptake factor of 0.77, as described above.

c. A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit with the LMS procedure.

d. These values obtained with PBPK model adjusted to approximate the PK data of Charest-Tardif *et al.* (2006).

Table 8d. Cancer potency and unit risk values for ethylbenzene derived using the BMD procedure with PBPK metabolized doses and bioassay data from NTP (1999).

Sex, species	Site, tumor type	$P_{animal\_internal}$ (mg/kg-day)$^{-1}$	$P_{human\_internal}$[a] (mg/kg-day)$^{-1}$	Human unit risk value[b] (mg/m^3)$^{-1}$	Model Goodness-of-fit test[c]
Male rats	Renal tubule carcinoma or adenoma	0.00394	0.0089	0.00164	Multistage (order = 3) p = 0.57
	Testicular interstitial cell adenoma	0.01460	0.027	0.00594	Quantal Quadratic p = 0.87
Female rats	Renal tubule adenoma	0.00126	0.0025	0.00055	Multistage (order = 3) p = 0.98
Male mice	Lung alveolar/ bronchiolar carcinoma or adenoma	0.003557	0.0090	0.0020	Multistage (order = 3) p = 0.99
		0.001595[d]	0.0040[d]	0.00088[d]	Quantal Linear p = 0.93
		0.000908[e]	0.00229[e]	0.00050[e]	p = 0.74
Female mice	Liver hepatocellular carcinoma or adenoma	0.002604	0.0066	0.0015	Multistage (order = 3) p = 0.86
		0.0007523[d]	0.0019[d]	0.00042[d]	Quantal Quadratic p = 0.94[d]
		0.00104[e]	0.00265[e]	0.00058[e]	Multistage (order = 3) p = 0.67

a. The interspecies extrapolation of $(BWh/BWa)^{1/8}$ was applied to $P_{animal\_intneral}$ in $(mg/kg\text{-}d)^{-1}$ to determine $P_{human\_internal}$ $(mg/kg\text{-}day)^{-1}$, as described above.

b. Unit risk was determined by multiplying the human internal cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate (20 m^3/day) divided by human body weight (70 kg) and by an uptake factor of 0.77, as described above.

c. A p-value of 0.1 or greater for the chi-square goodness-of-fit test indicates an adequate fit with the BMD procedure.

d. These values obtained with PBPK model adjusted to approximate the mouse pharmacokinetic data of Charest-Tardif *et al.* (2006).

e. These values obtained with the PBPK model of Nong *et al.* (2007). Cardiac output = 24BW^0.75; Alveolar ventilation = 0.68*Cardiac output.

Benchmark Dose Approach

Tables 8b and 8d list the P_{animal}, P_{human}, and human unit risk values based on the BMD approach. The cancer potencies and unit risk values were derived using the applied LTWA doses and PBPK adjusted internal doses, as described above. As expected the results from the BMD approach are quite similar to those just described using the LMS approach. Unit risk values ranged from 0.00054 to 0.0063 $(mg/m^3)^{-1}$. When LTWA doses are used, the most sensitive sites are the male rat testicular interstitial cell adenoma and the male rat kidney adenoma and carcinoma. When PBPK doses are used, the most sensitive sites are the male rat testicular interstitial cell adenomas and the male mice lung tumors. Regardless of whether LTWA or PBPK doses are used, the unit risk values based on male rat kidney, male mouse lung, and female mouse liver are comparable at approximately 0.002 $(mg/m^3)^{-1}$. The results based on the Charest-Tardif PBPK parameters are about a factor of two to four less than those based on the PBPK parameters from Haddad. The results obtained with the Nong *et al.* (2007) PBPK model were similar to the Charest-Tardif *et al.* (2006) adjusted mouse model. This is not surprising since they are largely based on the same kinetic data (Table 8d). The various estimates indicate some of the uncertainty in the PBPK approach.

As discussed above, the male rat testicular tumors are not considered appropriate for unit risk and potency estimation because of the high background rate. The preferred unit risk value of 0.0025 $(mg/m^3)^{-1}$, is derived from the male rat kidney data based on LTWA doses with the LMS method. The value derived using the BMD approach based on LTWA doses is not significantly different (0.0026 $(mg/m^3)^{-1}$).

Human PBPK Models

Initial predictions of risk-specific exposure concentrations from a human PBPK model used metabolic parameters from Haddad *et al.* (2001), two exposure scenarios, and two methods of risk estimation. The exposure scenarios utilized were an occupational-like time of exposure (8.0 hr exposure/day x 5 d/week; 7 days simulation) and a continuous environmental time of exposure (24 hr/d x 7d/week; 10 days simulation). Two methods of risk estimation were used. In method I a human potency value, P_{human}, was used to estimate an internal dose equivalent to 1 x 10^{-6} lifetime theoretical risk (e.g., 10^{-6} risk/0.0087 $(mg/kg`-d)^{-1}$ = 1.15 x 10^{-4} mg/kg-d.) The human PBPK model with differing exposure scenarios was then used to estimate the external ethylbenzene concentrations resulting in that internal dose. In method II the animal LED_{10} was divided by 10^5 to obtain the 10^{-6} risk specific dose and the equivalent external concentration was adjusted for possible pharmacodynamic (PD) differences between rats and humans (i.e., $(70/0.45)^{1/8}$). For the tumor site of male rat kidney the 1 x 10^{-6} values from the human models vary by 2-fold (0.48 to 0.79 ppb; Table 9). The same analysis was repeated with the human metabolic parameters from Sams *et al.* (2004) and the range was similar (0.33 to 0.74 ppb). PBPK models with higher body weight of 90 kg and 40% body fat gave only slightly higher ppb predictions. According to the discussion above, the preferred value for the unit risk of ethylbenzene is 2.5 x 10^{-6} $(\mu g/m^3)^{-1}$, based on the data for male rat kidney tumors. With the human model unit risk estimates ranged from 1.27 x 10^{-6} to 3.06 x 10^{-6} ppb^{-1} (2.9 x 10^{-7} to 7.0 x 10^{-7} $[\mu g/m^3]^{-1}$ at 4.35 $\mu g/m^3$/ppb) or somewhat lower than the animal PBPK based values. These unit risk estimates from the human PBPK models were not used as final values due to issues of tumor site concordance and human variability and

parameter uncertainty. The information is provided here for comparative purposes and methodology development.

Table 9. Estimates of Virtually Safe Exposure Levels (ppb) based on Human PBPK Modeling[a]

Method/Model	Occupational Scenario	Environmental Scenario
I . Human Potency based		
70 kg human 20% fat Haddad	0.70	0.50
20% fat Sams	0.66	0.33
90 kg human 40% fat Haddad	0.79	0.56
40% fat Sams	0.74	0.34
II. Animal LED_{10} based		
70 kg human 20% fat Haddad	0.68	0.48
20% fat Sams	0.64	0.32
90 kg human 40% fat Haddad	0.74	0.53
40% fat Sams	0.69	0.34

[a] Note: Values are calculated for 1 x 10^{-6} theoretical lifetime cancer risk. Occupational scenario was 8.0 hr/d x 5 days/week, for one-week simulations; environmental scenario was continuous exposure for one week. Method I used the human potency (Ph) in $(mg/kg\text{-}d)^{-1}$ to calculate a 10^{-6} risk internal dose in metrics of ethylbenzene metabolized by the liver (AMET, μmol/d). Method II uses the animal LED_{10} to calculate a 10^{-6} risk dose. The human models were the 70 kg default with 20% fat and a 90 kg variant with 40% fat (and comparatively less muscle). The Ph was based on the male rat kidney tumors of 0.0087 $(mg/kg\text{-}d)^{-1}$. Inhalation was 20 m^3/d. The models were run with metabolic parameters from Haddad *et al*. (2001) and Sams *et al*. (2004).

Conclusion

The male rat was the most sensitive sex and species tested by NTP (1999) in the inhalation carcinogenesis studies of ethylbenzene. While the highest potency and unit risk values were obtained for rat testicular adenomas, the high background rate of this common tumor made interpretation difficult. NTP considered the increased incidences of renal tubule carcinoma or adenoma to provide clear evidence of the carcinogenic activity of ethylbenzene, and this site was considered to be the more reliable basis for estimating human cancer potency.

OEHHA has examined various proposals for the mode of action of ethylbenzene in causing the observed increases in tumor incidence in rodent lung, kidney and liver. Some of these involve cytotoxicity or exacerbation of existing degenerative processes, which might be considered capable of increasing tumor incidence by a non-genotoxic mechanism, although the precise implications of these proposals for dose-response relationships have not been fully explored.

Moreover, it appears likely that metabolism of ethylbenzene involves generation of reactive metabolites. These metabolites include quinone/hydroquinone species capable of causing oxidative DNA damage and carcinogenesis, analogous to the processes established for benzene and some similar carcinogens. OEHHA concludes that overall, the limited data do not conclusively establish any particular mode of action for ethylbenzene carcinogenesis, and indeed several of the proposed processes may be influential. However, one or more genotoxic processes appear at least plausible and may well contribute to the overall process of tumor induction. Because of this, the default linear approach has been used for extrapolating the dose-response curve to low doses.

Using either the LMS or BMD methodology with different dose metrics, the 95% upper confidence bound on the unit risk value for purposes of calculating cancer risks associated with exposure to ethylbenzene is in the range 5.5 x 10^{-4} to 6.6 x 10^{-3} $(mg/m^3)^{-1}$, based on the incidence data from the NTP (1999) studies (Table 10). The unit risk value of 2.5 x10^{-3} $(mg/m^3)^{-1}$, or 2.5 x10^{-6} $(\mu g/m^3)^{-1}$, based on the renal tubule carcinoma or adenoma incidence data in male rats and using the LMS methodology applied to LTWA doses, is considered the most appropriate for purposes of calculating cancer risks associated with exposure to low levels of ethylbenzene. As noted above and summarized in Table 10 below, unit risks based on the PBPK internal doses were not markedly different than those based on the LTWA doses, and involved a number of assumptions. Because the PBPK modeling is uncertain and the results were relatively insensitive to the approach used, the LMS results based on the LTWA doses were selected as most appropriate. The inhalation cancer potency, from which the unit risk value was derived, is 0.0087 $(mg/kg\text{-}d)^{-1}$. The oral cancer potency value of 0.011 $(mg/kg\text{-}d)^{-1}$ is derived from the inhalation potency value by multiplying by the ratio of the uptake values (i.e., 1/0.77). The inhalation and oral cancer potency values are considered applicable to low dose ethylbenzene exposures.

Table 10. Comparison of unit risk values for ethylbenzene

Species/sex/tumor site	**Unit Risk value $(mg/m^3)^{-1}$**			
	LTWA doses, LMS approach	**LTWA doses, BMD approach**	**PBPK doses, LMS approach**	**PBPK doses, BMD approach**
Male rat kidney	0.0025	0.0026	0.0020	0.0016
Male rat testicular	0.0066	0.0063	0.0064	0.0059
Female rat kidney	0.00063	0.00054	0.00044	0.00055
Male mouse lung	0.0015	0.0015	0.0021	0.0020
Female mouse liver	0.0017	0.0017	0.0015	0.0015

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Appendix A

Berkeley Madonna Model Code Example (Male Rat 75 ppm x 6.25 hr/d x 5days/week, 1 week simulation. If cut and pasted into BM demo program available online this model will run)

```
METHOD Stiff

STARTTIME = 0
STOPTIME= 168
DT = 0.001

{ethylbenzene moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Alu = 0
Limit Alu >= 0

{moles, metabolized}
init Ametl = 0
init Ametlg = 0

{tissue flows L/hr}
Qtot = 15*BW^0.74
Qalv = 12*BW^0.74
Qf = 0.07*Qtot
Qvrg = 0.51*Qtot
Ql = 0.183*Qtot
Qm = Qtot - (Ql + Qf + Qvrg)
Qlu = Qtot

{tissue volumes L}
Vf = 0.035*BW + 0.0205
Vl = 0.037*BW
Vm = 0.91*BW - (Vf + Vl + Vvrg + Vlu)
Vvrg = 0.054*BW
Vlu = 0.014*BW
BW = 0.45

{blood/air and tissue/blood partition coefficients}
Pb = 42.7
Pl = 1.96
Pf = 36.4
Pm = 0.609
Pvrg = 1.96
Plu = 1.96

{ethylbenzene metabolic parameters, CLh, Vmax mol/hr, Km, M}
VmaxC = 7.6
Vmax = VmaxC*BW^0.74/(1000*106.16)
Km = 0.1/(1000*106.16)
```

```
{exposure in ppm converted to moles/L}
Cair = IF TIME <= 6.25 THEN 75*(1E-6/25.45) ELSE IF (24<TIME) AND (TIME <=
30.25) THEN 75*(1E-6/25.45) ELSE IF (48<TIME) AND (TIME <= 54.25) THEN 75*(1E-
6/25.45) ELSE IF (72<TIME) AND (TIME <= 78.25) THEN 75*(1E-6/25.45) ELSE IF
(96<TIME) AND (TIME <= 102.25) THEN 75*(1E-6/25.45) ELSE 0

{calculated concentrations of ethylbenzene}
Cart = Pb*(Qalv*Cair + Qtot*Cvtot)/(Pb*Qtot + Qalv)
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvm = Am/(Vm*Pm)
Cvlu = Alu/(Vlu*Plu)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg)/Qtot
Cexh = Cart/Pb

{differential equations for ethylbenzene uptake and metabolism}
d/dt(Alu) = Qtot*(Cvtot - Cvlu)
d/dt(Al) = Ql*(Cart - Cvl)  - Vmax*Cvl/(Km + Cvl)
d/dt(Af) = Qf*(Cart - Cvf)
d/dt(Avrg) = Qvrg*(Cart - Cvvrg)
d/dt(Am) = Qm*(Cart - Cvm)

{amount of ethylbenzene metabolized}
d/dt(Ametl)  = Vmax*Cvl/(Km + Cvl)
d/dt(Ametlg) = (Vmax*Cvl/(Km + Cvl))/BW
init AUCvtot = 0
init AUCvl = 0
d/dt(AUCvtot) = Cvtot
d/dt(AUCvl) =  Cvl
```

ETHYLENE DIBROMIDE

CAS No.: 106-93-4

I. PHYSICAL AND CHEMICAL PROPERTIES SUMMARY (HSDB, 1998)

Molecular weight	187.88
Boiling Point	131-132° C
Melting Point	9.8° C
Vapor pressure	0.11 mm Hg at 20° C
Air concentration conversion	1 ppm = 7.81 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 7.1 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 2.5 E-1 $(mg/kg\text{-}day)^{-1}$
[Male rat nasal tumor incidence (NTP, 1982), cancer risk range calculated using the Weibull-multistage procedure CDHS (1985); unit risk "best value" selected by CDHS (1988) for Proposition 65 purposes.]

III. CARCINOGENIC EFFECTS

Human Studies

Quantitative and qualitative information on the carcinogenicity of ethylene dibromide (EDB) in humans is limited. The epidemiological studies to date have either been suggestive but inconclusive or have lacked the statistical power to detect an effect.

Carcinogenic effects were not observed in men exposed to EDB for four years or more at two Associated Octel manufacturing facilities in the United Kingdom (Turner and Barry, 1979). Excess cancers would not have been expected, however, given the duration and magnitude of exposure, the carcinogenic potency of EDB in laboratory animals, the number of workers studied and the length of time elapsing since first exposure.

Ott *et al.* (1980) reported on the cancer mortality among workers exposed while employed at EDB manufacturing facilities in Texas and Michigan. Among workers at the Texas facility, there were no statistically significant increases in cancer above those expected. Among workers at either facility with more than six years of exposure, four died from malignant cancers, compared to an expected 1.6 deaths from cancer (p = 0.08). At the Michigan facility, five of the EDB-exposed workers died from malignant tumors, whereas 2.2 deaths were expected (p = 0.07). Excluded from this analysis were five individuals from the Michigan facility who had worked with arsenicals in addition to EDB. Two of the five died of respiratory cancer at ages 46 and 58, respectively. One had been exposed to arsenical compounds for only 1 1/2 months, in contrast to 102 months of exposure to EDB; the other had been exposed to arsenicals for 20 months and to EDB for 111 months. Had these been included in either the analysis of those exposed for more than six years

to EDB or the analysis of the Michigan facility mortalities, the results would have been found to be statistically significant ($p < 0.05$).

Animal Studies

The carcinogenic activity of EDB has been demonstrated in a number of studies using mice and rats by dermal, oral, inhalation and intraperitoneal routes of administration. Tumors occurred in both test species at sites local to, and distant from, the site of first chemical contact.

The National Toxicology Program (NTP) conducted an inhalation carcinogenesis bioassay for EDB in male and female Fischer 344 rats and $B6C3F_1$ mice (50 animals/sex/group) (NTP, 1982). The durations of exposure were 90 weeks at 40 ppm, 103 weeks at 10 ppm and 104 weeks for controls. NTP concluded that EDB was carcinogenic to rats, causing increased incidences of carcinomas, adenocarcinomas, and adenomas of the nasal cavity, hemangiosarcomas of the circulatory system (spleen), mesotheliomas of the tunica vaginalis (males only), adenomatous polyps of the nasal cavity (males only), fibroadenomas of the mammary gland (females only) and alveolar/bronchiolar adenomas and carcinomas (females only). EDB was also carcinogenic for mice, causing increased incidences of alveolar/bronchiolar adenomas and carcinomas, hemangiosarcomas of the circulatory system (females only), fibrosarcomas in the subcutaneous tissue (females only), carcinomas of the nasal cavity (females only), and adenocarcinomas of the mammary gland (females only). The tumor incidence by site for rats and mice are shown in Tables 1 and 2, respectively.

Table 1: Results of the NTP (1982) inhalation bioassay of EDB in Fischer-344 rats.

Tumor		Sex	Control	10 ppm	40 ppm
Nasal cavity:	Adenocarcinoma	M	0/50	20/50 [a]	28/50 [a]
		F	0/50	20/50 [a]	29/50 [a]
	Carcinoma	M	0/50	0/50	21/50 [a]
		F	0/50	0/50	25/50 [a]
	Adenomatous polyps	M	0/50	18/50 [a]	5/50 [e]
		F	0/50	5/50 [e]	5/50 [e]
	Adenomas	M	0/50	11/50 [a]	0/50
		F	0/50	11/50 [a]	3/50
	Squamous cell carcinoma	M	0/50	3/50	3/50
		F	1/50	10/50	5/50
Lung alveolar/bronchiolar carcinomas or adenomas		F	0/50	0/48	5/47
Tunica vaginalis mesotheliomas		M	1/50	8/50 [c]	25/50 [a]
Mammary fibroadenomas		F	4/50	29/50 [a]	24/50 [a]
Circulatory system hemangiosarcomas		M	0/50	1/50	15/50 [a]
		F	0/50	0/50	5/50 [e]
Pituitary adenomas		M	0/45	7/48 [b]	2/47
		F	1/50	18/49 [a]	4/45

[a] $p = 0.001$; [b] $p = 0.008$; [c] $p = 0.015$; [e] $p = 0.028$

Table 2: Results of the NTP (1982) inhalation bioassay of EDB in $B6C3F_1$ mice.

Tumor	Sex	Control	10 ppm	40 ppm
Lung:				
Alveolar/bronchiolar carcinomas or adenomas	M	0/41	3/48	23/46 [a]
Alveolar/bronchiolar carcinomas or adenomas	F	4/49	11/49	41/50 [a]
Circulatory system:				
Hemangioma or hemangiosarcoma	M	0/45	0/50	4/50
	F	0/50	12/50 [a]	27/50 [a]
Subcutaneous tissue fibrosarcoma	F	0/50	5/50 [c]	11/50 [a]
Mammary gland adenocarcinoma	F	2/50	14/50 [a]	8/50 [e]
Nasal cavity carcinoma or adenoma	F	0/50	0/50	8/50 [b]

[a] $p = 0.001$; [b] $p = 0.003$; [c] $p = 0.028$; [e] $p = 0.046$

A chronic inhalation bioassay was conducted by NIOSH to evaluate the effect of disulfiram on carcinogenic and other toxic effects of EDB. The findings have been reported in several publications (Plotnick, 1978; Wong *et al.*, 1982). The study exposed four groups of 48 male and four groups of 48 female Sprague-Dawley rats to room air or 20 ppm EDB for 7 hours/day for 5 days/week over an 18-month period. Diets which contained 0.05% disulfiram were given to one set of controls and EDB exposed rats.

Male rats receiving EDB exposure had significantly higher tumor incidences in spleen, adrenals, and subcutaneous tissues than either the control or disulfiram tested rats. Also a significant finding was the high incidence of hemangiosarcoma in the spleen of male rats exposed to EDB. Tumors were also found in the liver, kidneys, and lungs in these animals. Female rats exposed to EDB also showed significantly high tumor incidences in the spleen (hemangiosarcoma), adrenals, and mammary glands. Tumors were also found in the liver. The number of rats with tumors were 15/96, controls on normal diet; 13/96 controls, on disulfiram diet; 54/96, EDB-exposed on normal diet; and 90/96, EDB-exposed on disulfiram diet. The tumor incidence by site was not available.

Osborne-Mendel rats and $B6C3F_1$ mice were administered various levels of EDB in corn oil by stomach tube 5 days/week (NCI, 1978). Time-weighted averages for the high and the low dose groups were 41 and 38 mg/kg/day for male rats, 39 and 37 mg/kg/day for female rats, and 107 and 62 mg/kg/day for male and female mice, respectively (50 animals/sex/group). The responses were compared with two control groups (20 animals/group), of which one received corn oil and the other had no treatment. In rats, squamous cell carcinomas of the forestomach were observed in 45/50, 33/50, 40/50, and 29/50 of the low-dose males, high-dose males, low-dose females, and high-dose females, respectively (all statistically significant). None were observed in controls. The female rats also had statistically significant increases in hepatocellular carcinomas (5/25, time-adjusted, high dose). Hemangiosarcomas were found in male and female rats and the incidences were statistically significant in the males (11/50, low dose). Squamous cell carcinomas of the stomach were found in 45/50, 29/49, 46/49, and 28/50 of the low-dose males, high-dose males, low-dose females, and high-dose females, respectively (all statistically significant, $p < 0.001$). None were observed in controls. Male and female mice had statistically significant incidences of alveolar/bronchiolar adenomas (10/47, high-dose males; 10/43, low-dose females).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Epidemiologic studies of EDB carcinogenicity in humans have been suggestive but inconclusive, or of insufficient power to detect an effect. In animals, however, EDB is a potent carcinogen causing tumors in rats and mice of both sexes at multiple sites via various routes of exposure. CDHS (1985) based their risk assessment on the inhalation bioassays in rats and mice reported by NTP (1982). CDHS (1988) also based an oral EDB cancer potency on the NCI (1978) gavage male and female rat and mouse forestomach squamous cell carcinoma incidence data.

Methodology

CDHS (1985) fitted the Weibull-multistage, multistage, and probit models to data from the NTP inhalation study (NTP, 1982) which describes tumor incidence at two sites: tumors at the site of first chemical contact (nasal malignancies in male rats), and tumors at a remote site (hemangiosarcomas in female mice). For continuous lifetime exposures to 10 parts per trillion (ppt) in air, CDHS estimated essentially zero risk under the probit model, and for the Weibull-multistage and multistage models, risks of 1.0 to 5.5 excess cancers per million exposed. These estimates correspond to potencies of 0.05 - 0.25 $(mg/kg\text{-}day)^{-1}$ [1.4 - 7×10^{-5} $(\mu g/m^3)^{-1}$]. Particular estimates depended upon the model used, the tumor selected (rat nasal malignancies or mice hemangiosarcomas), and whether the upper 95% confidence limit or maximum likelihood estimate of potency was used. Potency estimates derived from distant site (hemangiosarcomas) and local site (nasal malignancies) did not differ substantially.

CDHS (1988), under Proposition 65, recommended that a "best value" cancer potency factor of 0.25 $(mg/kg\text{-}day)^{-1}$ be used for EDB inhalation. This potency was obtained using the NTP (1982) male rat nasal tumor data (Weibull-multistage, 95% upper confidence limit). This potency corresponds to a unit risk of 7.1×10^{-5} $(\mu g/m^3)^{-1}$.

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ETHYLENE DICHLORIDE

CAS No: 107-06-2

I. PHYSICAL AND CHEMICAL PROPERTIES (from HSDB, 1998)

Molecular weight	98.97
Boiling point	83.5º C
Melting point	-35.3º C
Vapor pressure	64 mm Hg @ 20º C
Air concentration conversion	1 ppm = 4.05 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor	2.1 E-5 $(\mu g/m^3)^{-1}$
Slope Factor	7.2 E-2 $(mg/kg\text{-}day)^{-1}$

[Calculated from the incidence of hemangiosarcomas in male rats (NCI, 1978) using a time-corrected (Weibull) multistage procedure (CDHS, 1985).]

III. CARCINOGENC EFFECTS

Human Studies

CDHS (1985), U.S. EPA (1985), and IARC (1985) reported that there was no data on the carcinogenicity of ethylene dichloride (EDC) in humans.

Animal Studies

A number of studies have investigated the carcinogenicity of EDC. The National Cancer Institute (NCI, 1978) conducted a carcinogenesis bioassay of EDC in corn oil by oral gavage in male and female Osborne-Mendel rats and $B6C3F_1$ mice. There were four groups for each sex of both species, including an untreated control, a vehicle (corn oil) control, a low dose group and a high dose (the maximum tolerated dose) group. Time-weighted average low and high dose levels were 47 and 95 mg/kg for both male and female rats, 97 and 195 mg/kg for male mice, and 149 and 299 mg/kg for female mice, respectively. The animals were dosed five days/week for 78 weeks and observed for an additional 12-32 weeks. Mortality was early and severe in dosed animals, especially for high dose rats. A statistically significant ($p \leq 0.025$) increase in the incidence of squamous cell carcinomas of the forestomach, hemangiosarcomas of the circulatory system, and fibromas of the subcutaneous tissue occurred in male rats. Female rats exhibited a statistically significant increase in the incidence of adenocarcinomas of the mammary gland and hemangiosarcomas of the circulatory systems. Male $B6C3F_1$ mice demonstrated a statistically significant increase in incidences of hepatocellular carcinomas and alveolar/bronchiolar adenomas, while female mice exhibited an increased incidence for alveolar/bronchiolar adenomas, mammary gland adenocarcinomas and endometrial stromal sarcomas (NCI, 1978).

Table 1: Tumor incidence data in rats and mice exposed to ethylene dichloride (NCI, 1978).

Tumor Type	Exposure Concentration (mg/kg-day)		
	0	47	95
Male rats			
Squamous cell carcinoma (forestomach)	0/60	3/50	9/50*
Hemangiosarcoma	1/60	9/50*	7/50*
Female rats			
Hemangiosarcoma	0/59	4/50*	4/50*
Mammary adenomas	1/59	1/50	18/50*
	Exposure Concentration (mg/kg-day)		
	0	97	195
Male mice			
Hepatocellular carcinomas	4/59	6/47	12/48*
Alveolar/bronchiolar adenomas	0/59	1/47	15/48*
	Exposure Concentration (mg/kg-day)		
	0	149	299
Female mice			
Alveolar/bronchiolar adenomas	2/60	7/50*	15/48*
Mammary adenocarcinoma	0/60	9/50*	7/48*
Endometrial polyp or stromal sarcomas	0/60	5/49*	5/47*

*Significantly increased incidence in treated animals compared with pooled vehicle controls; significance calculated using Fisher Exact Test (one-tailed); $p \leq 0.05$.

Maltoni *et al.* (1980) conducted extensive inhalation carcinogenicity studies in Sprague-Dawley rats and Swiss mice. Four groups, each consisting of 180 rats or mice of both sexes, were exposed to EDC concentrations of 5, 10, 50, or 150-250 ppm, respectively, seven hours/day, five days/week, for 78 weeks. Two groups of 180 rats per sex, or one group of 249 mice, served as controls. Although all animals received a lifetime exposure and extensive histopathology was performed on each animal, no significant increased in tumor incidences were seen (Maltoni *et al.*, 1980).

Several factors have been suggested to account for the difference in carcinogenicity between the gavage and inhalation studies, including the strains of animal used, the differences in the actual dose received by the animal, and the pharmacokinetic differences in rates of formation and/or retention of reactive metabolites in target organs for different routes of administration (US EPA, 1985; CDHS, 1985; Hooper *et al.*, 1980).

Theiss *et al.* (1977) conducted a pulmonary tumor bioassay in mice with EDC. Groups of twenty mice received intraperitoneal (ip) injections of either 0, 20, 40 or 100 mg/kg EDC three times weekly for a total of 24 injections per mouse. The mice were sacrificed 24 weeks after the first injection. The lungs were subsequently examined for surface adenomas. Although the incidence of lung tumors increased with dose, none of the groups had a significantly greater number of pulmonary adenomas than did vehicle-treated control mice (Theiss *et al.*, 1977).

Van Duuren *et al.* (1979) conducted a bioassay of EDC and one of its suspected metabolite, chloroacetaldehyde, using the two-stage mouse skin test on female Swiss mice. The results of this study indicated that neither EDC nor chloroacetaldehyde induced a statistically significant increase of papillomas or carcinomas of the skin, although dermal application of EDC was associated with a significant increase in the number of mice with benign lung papillomas (Van Duuren *et al.*, 1979).

In summary, EDC has been demonstrated to increase the incidence of tumors in rats and mice, both local to, and distant from, the initial site of chemical contact.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

EDC has caused statistically significant increases in tumor incidences in both rats and mice in several different laboratories by different routes of exposure. The State of California Scientific Advisory Panel for Proposition 65 has identified EDC as a compound known to the State to cause cancer. EDC has been classified by the US EPA and IARC as a B2 and 2B carcinogen, respectively. CDHS (1985) used the tumor incidence data from the NCI (1978) carcinogenesis bioassay for developing a quantitative risk assessment.

Methodology

DHS staff performed several different analyses to generate estimates of cancer potency of EDC in humans using the NCI tumor data. The Crump polynomial model was applied to data which summarizes the tumor incidence observed, time-dependent analyses were performed to take into account early death of treated animals, crude adjustments were made to take into account the saturable pharmacokinetics of EDC, and a physiologically-based pharmacokinetics model (PBPK) was applied to correct for the differing effects of species, routes, and dose levels on the pharmacokinetics of EDC. Due to the lack of data on the metabolism and disposition of EDC in humans and the inherent assumptions required for estimation of metabolic dose in the absence of such data, CDHS decided that animal/human scaling was insufficiently accurate to permit

reliance on PBPK-derived estimated doses to calculate "risk-specific intake levels" (CDHS, 1985).

CDHS (1985) fit the multistage Weibull-in-time model to time to tumor data. The data set for the most sensitive species and tumor type, male rat hemangiosarcomas, was used to calculate a cancer potency factor. The cancer potency values which are normalized for surface area were approximated by assuming that the ratio of animal to human surface areas is equivalent to the ratio of body weights (BW) taken to the two-thirds power. CDHS (1985) estimated the cancer potency from the hemangiosarcoma data to be 0.072 $(mg/kg\text{-}day)^{-1}$. CDHS recommended that a cancer potency of 0.072 $(mg/kg\text{-}day)^{-1}$ be used for estimating risks from exposure to EDC. A unit risk value of 2.1×10^{-5} $(\mu g/m^3)^{-1}$ was derived assuming a human breathing rate of 20 m^3/day, a human body weight of 70 kg, and 100% fractional absorption after inhalation exposure.

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ETHYLENE OXIDE

CAS No: 75-21-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1998) except as noted.)

Molecular weight	44.06
Boiling point	10.7 ºC at 760 mm Hg
Melting point	-111 ºC
Vapor pressure	1314 mm Hg at 25 ºC
Air concentration conversion	1 ppm = 1.83 mg/m^3 (NIOSH, 1994)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 8.8 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 3.1 E-1 $(mg/kg\text{-}day)^{-1}$
[Female rat mononuclear cell leukemia data (Snellings et al., 1984), analyzed by US EPA (1985) using a linearized multistage procedure (GLOBAL82), reevaluated by CDHS (1987).]

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiologic evidence for the carcinogenic effects of ethylene oxide at the time the Toxic Air Contaminant (TAC) document (CDHS, 1987) was written was based on five longitudinal studies of occupational cohorts in Sweden, the United States and West Germany. Together the studies demonstrate an association between exposure to ethylene oxide and cancer. Two additional studies, which were cross-sectional in design, evaluated leukemia incidence as part of a health evaluation of two separate occupational cohorts (Joyner 1964; Ehrenberg and Hallstrom, 1967). Neither of these studies was adequate to evaluate the carcinogenic effect of ethylene oxide since they were not designed to study this outcome.

The five longitudinal studies examined cancer outcomes for all sites and site-specific cancers, with leukemia as a focus for all studies. Four studies reported excesses in leukemia, while one study found no cases of leukemia. A discussion of these studies follows.

Hogstedt *et al*. (1979a) reported that three cases of leukemia had occurred between 1972 and 1977 among 230 Swedish workers exposed to 50% ethylene and 50% methyl formate at a factory that sterilized hospital equipment. Exposure at the plant began in 1968 and measurements taken in 1977 indicated concentrations of ethylene oxide of approximately 20 ± 10 ppm (time-weighted average); exposure levels prior to 1977 were not known. The expected number of leukemia cases at this factory for 1968-1977 was 0.2 cases, based on national rates. Three cases were observed, including two myelogenous leukemias (4 and 8 years exposure) and one primary macroglobulinemia (6 years exposure).

In order to replicate these findings, Hogstedt *et al*. (1979b) conducted a cancer mortality study of the cohort of ethylene oxide production workers originally studied by Ehrenberg and Hallstrom (1967). The findings were similar, demonstrating elevated rates of leukemia and other cancers (Table 1). The cohort was, however, exposed to other carcinogens, such as ethylene dichloride and bis(2-chloroethyl)ether. Exposure to ethylene oxide was between 5.5 and 27.5 ppm (10 to 50 mg/m^3) in the 1960s and 0.55 to 5.5 ppm (2 to 10 mg/m^3) in the 1970s. Exposed workers developed 9 tumors (including leukemias) where 3.4 would have been expected (SMR = 265)($p < 0.01$).

Table 1: Leukemia cases observed and expected in the studies of Hogstedt *et al*. (adapted from Table 5 of Hogstedt *et al*., 1986)

		Time interval					
		1960's*-1977		1978-1981		1960's*-1981	
Plant	Workers Studied	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
1	203	2**	0.09	1	0.05	3	0.14
2	175	3	0.38	1	0.14	4	0.52
3	355	1	0.09	0	0.07	1	0.16
Total	733	6	0.56	2	0.27	8	0.83

* The initial year was 1968 for plant 1, 1961 for plant 2, and 1964 for plant 3.

** One of the original cases (macroglobulinemia) was later reclassified as a non-Hodgkin's lymphoma.

Hogstedt *et al*. (1986) published findings from follow-up studies of the two cohorts described above and a group of production workers from a third plant that had not been studied previously. This last group included workers exposed only to ethylene oxide, exposed to ethylene oxide and propylene oxide, or exposed to a mixture of chemicals. All workers from this third plant had been exposed for at least one year. One leukemia was detected where 0.16 was expected. Leukemia cases from the three plants are shown in Table 4.7. An excess of leukemia cases was reported in Plants 1 and 2 during the 1978-1981 follow-up study, but not in Plant 3. Eight cases of leukemia were observed among 733 workers in the three plants where 0.83 were expected (SMR = 964)(Table 1).

In addition to excess leukemia among the three groups of workers, six stomach cancers were reported among Plant 2 workers where 0.65 were expected. Neither the leukemia nor stomach cancer cases follow a dose-response pattern when analyzed by years of employment. However, years of employment can be a poor surrogate for exposure if, for example, highly exposed workers tended to shift to jobs with lower exposure or terminate their employment early.

It should be noted that although these three studies (Hogstedt 1979a, 1979b, 1986) include many of the ethylene oxide-exposed workers in Sweden, the number of workers involved in the above calculations are relatively small, resulting in large variability in the estimates of the ratios of observed to expected cases. On the other hand, the SMRs obtained are quite large. A second consideration in the finding of an association between ethylene oxide exposure and leukemia is that the leukemias were not limited to any one particular type. However, a single agent can induce

a spectrum of cytogenetic aberrations in hematopoietic tissues resulting in the pattern of leukemias reported by Hogstedt *et al.* In addition, ethylene oxide induces cellular proliferation, including tumors in several rat tissues. Third, in these studies all production workers experienced exposure to other chemicals, including carcinogens, in addition to ethylene oxide; however, the chemical common to all was ethylene oxide.

Two other epidemiologic studies have been conducted. Morgan *et al*. (1981) conducted a cohort study of ethylene oxide production workers at a Texas plant which had been in continuous operation since 1948. To be included in the study cohort, workers had to have been employed for at least five years. Unlike the previous studies, most of the employees worked outdoors. Although Morgan *et al*. did not report specific levels, ethylene oxide exposure levels were generally below 0.2 ppm, the limit of detection of the analytical instrument used, since the authors state that in most areas sampled in a 1977 industrial hygiene survey, virtually no ethylene oxide was detected. A modified lifetable program was used to compare the mortality experience of 767 workers with the pattern expected on the basis of U.S. vital statistics. A reduced overall mortality (SMR = 58) was reported. Morgan and his colleagues observed an excess of pancreas, bladder, and brain cancers and of Hodgkin's disease, but these excesses were not statistically significant. No leukemia cases were seen; however, the cohort size was sufficient to detect only very large increases, i.e., a 10.5-fold increase in leukemia, with 80% power. A 10.5-fold excess would not be expected, given the low levels of ethylene oxide exposure, when compared to Hogstedt's series. Also, this study, by excluding workers employed at the plant for less than five years, could have excluded a significant fraction of exposed workers, since entry level jobs are often associated with higher exposure to chemicals. In a published letter (Divine and Amanollahi, 1986), one of the authors of this study has attempted to use the above data to refute the studies of Hogstedt. The reasons that the study is inadequate for such a refutation are pointed out above.

Thiess *et al.* (1982) reported on the mortality experience of 602 production workers exposed to alkylene (ethylene plus propylene) oxide and other chemicals in nine West German plants. Ninety-two percent of workers employed between 1928 and 1980 were followed. Overall observed mortality and cancer deaths for the total cohort and for those with a minimum of 10 years of observation were lower than expected, based on mortality for either the local area or for West Germany. This indicates a strong healthy worker effect. A second comparison was made using a cohort of 1662 styrene workers at the same plant in order to eliminate the healthy worker effect. However, the choice of styrene-exposed workers as a comparison group may have been inappropriate, since styrene monomer has been shown to be carcinogenic in animals (IARC 1979) and has been associated with an excess in lymphocytic leukemia among styrene-exposed workers (Ott *et al*., 1980). Nevertheless, in older workers, age 65 to 75, the relative risk of malignant tumors was 2.78 in ethylene oxide workers compared to styrene workers ($p < 0.05$). The increased relative risks in younger age groups in the cohort were not statistically significant.

CDHS noted that the evidence supporting an association between working with ethylene oxide and leukemia came from 4 out of 5 occupationally exposed cohorts. The overall evidence could not be considered conclusive due to the small numbers of workers involved and to the possibility that other workplace carcinogens may have been confounders. Nevertheless, the high estimates of risk were striking; standardized incidence and mortality ratios for leukemia ranged from 6 to 21. Furthermore, the replication of the early findings in other plants and in the follow-up of those

same cohorts reduces the probability that the observed excesses of leukemia were chance findings. Since worker recall was not used to determine exposure, bias about exposure from that source is not present. The magnitude of the effect argues against the findings being due to confounding, particularly since the other carcinogens were different for the different plants. Though not conclusive, these studies provide substantial evidence of ethylene oxide's carcinogenicity in humans.

Animal Studies

Rats

A dose-related increase in local tumors, mainly squamous cell carcinomas of the forestomach, was observed in female Sprague-Dawley rats given doses of 7.5 or 30 mg/kg body weight of 99.7% pure ethylene oxide twice a week by gastric intubation for 107 weeks (average total dose of 1186 and 5112 mg/kg body weight, respectively)(Dunkelberg 1982, US EPA 1985). Rats treated with the high dose of ethylene oxide showed increased tumor-related mortality as well as decreased tumor latency compared to the low dose or the control groups. The incidence of local tumors was 0/50 in both control groups, 8/50 in the low-dose group, and 31/50 in the high-dose group. The frequency of tumors at other sites was not increased by ethylene oxide treatment (Dunkelberg, 1982; US EPA 1985).

In a two-year inhalation study in rats, Snellings *et al*. (1984) found that ethylene oxide increased the incidence of mononuclear cell leukemia in animals of both sexes, and peritoneal mesotheliomas in males. Tumor frequency among female rats was greater in all exposed groups than in controls. In addition, brain gliomas were observed in male and female exposed rats. Since this tumor has an historically rare background occurrence in Fischer 344 (F344) rats, it was considered to be a tumor type appropriate for use in risk evaluation.

Eight-week-old F344 rats were exposed in inhalation chambers to 10, 33, or 100 ppm of 99.9% pure ethylene oxide 6 hours/day, 5 days/week, for two years. Initially, 120 males and 120 females were exposed per dose. Two control groups (C1 and C2) of 120 rats per sex were exposed in inhalation chambers to room air.

Planned terminations of 10 rats per sex per dose were performed at 6 and 12 months of exposure and of 20 rats per sex per dose at 18 months. The remainder of the females were sacrificed at 24 months and the males at 25 months. Postmortem examinations were performed on all rats. Histopathologic examinations of about 50 tissues were performed on rats from the 100 ppm and two control groups that were killed at the 6 month and final intervals and on rats in any group that died or were killed in a moribund state. About 15 major organs and tissues from rats in the 100 ppm and both control groups were examined microscopically at 12 and 18 months. At 6, 12, and 18 months, only tissues with gross lesions were examined from the 10 and 33 ppm groups. At the end of the study about 20 major organs and tissues from the rats in the 10 and 33 ppm groups were examined. During the 15th month of exposure, rats in all groups became infected with sialodacryoadenitis virus. This resulted in a loss in body weight in all groups and increased mortality in the females exposed to 100 ppm compared with the other groups. Exposure to ethylene oxide was stopped for 2 weeks after which time body weights, clinical signs, and

mortality rates returned to preinfection status. The authors concluded that this outbreak was unlikely to have affected the results of the study. Cumulative percentage mortality did not increase significantly in the 10 or 33 ppm dose groups, but did increase in the 100 ppm dose groups, after 22 months exposure for males and after 21 months for females.

Table 2: Tumor incidence in F344 rats exposed to ethylene oxide by inhalation for 24 months (adapted from Snellings *et al*., 1984).

		ppm ethylene oxide				
		C1	C2	10	33	100
Sex		# animals examined grossly				
male		48	49	51	39	30
female		60	56	54	48	26
Sex	Tumor type	# animals with tumors				
male	spleen mononuclear cell leukemia	5	8	9	12	9
female		5	6	11	14	15*
male	peritoneal mesothelioma	1	1	2	4	4
male	pituitary adenoma	16	13	15	13	12

C1, C2 control groups

* significantly greater than appropriate control incidence ($p < 0.001$)

Tumor incidence was not significantly increased at 18 months of exposure; however, increased incidences of several types of tumors were observed in groups sacrificed at 24 and 25 months, the end of the study for female and male rats, respectively (Table 2).

The incidence of mononuclear cell leukemia (MNCL) was increased for both sexes in all dose groups, but was statistically significant only for females treated with 100 ppm ethylene oxide. A positive dose-related increase in MNCL incidence in females was observed ($p < 0.01$). A statistically significant trend was not observed for males. When the incidence of MNCL in rats killed at the end of the study is combined with the incidence in the rats dying spontaneously or euthanized when moribund, a statistically significant increase also occurs in females exposed to 33 ppm ($p < 0.01$) and 100 ppm ($p < 0.001$) (Table 3). A significant increase was not observed in males. A mortality-adjusted trend analysis revealed a significant positive trend for females ($p < 0.005$) and males ($p < 0.05$). The time to first tumor was not significantly decreased for MNCL in the exposed rats, but trend analysis indicated earlier tumor development.

Table 3: Tumor incidences in F344 rats exposed to ethylene oxide which died spontaneously, were killed when moribund, or were killed after 24 months of exposure (adapted from Snellings *et al*., 1981 and cited in US EPA, 1985).

Sex, Tumor type	ppm ethylene oxide				
	C1	C2	10	33	100
spleen mononuclear cell leukemia					
males	20/80 (25%)	18/80 (23%)	21/80 (26%)	23/80 (29%)	25/80 (31%)
females	9/80 (11%)	13/76 (17%)	14/80 (18%)	24/80[a] (30%)	27/80[b] (34%)
peritoneal mesothelioma					
males	2/80 (3%)	1/80 (1%)	3/80 (4%)	6/80[c] (8%)	21/80[d] (26%)

C1, C2 control groups
a $p < 0.01$ compared to C1 and combined controls, $p < 0.05$ compared to C2
b $p < 0.001$ compared to C1 and combined controls, $p < 0.05$ compared to C2
c $p < 0.001$ compared to C1, C2 and combined controls
d not significant compared to C1 or C2; $p < 0.05$ compared to combined controls

Note: These data are from US EPA (1985); US EPA questioned whether microscopic examination of all tissues or only tissues with gross lesions was performed on animals that died spontaneously or were killed when moribund. Information from Snelling *et al*. (1984) indicates that histopathology was performed on all tissues from these animals.

The increased incidence of peritoneal mesotheliomas observed in males treated with ethylene oxide for 24 months (Table 2) was not statistically significant; however, when the rats that died spontaneously or were euthanized when moribund are included, a statistically significant increase ($p < 0.001$) for the high-dose group compared with controls was observed (Table 3, data from US EPA, 1985). A mortality-adjusted trend analysis showed a highly significant relationship ($p < 0.005$) between ethylene oxide exposure and induction of peritoneal mesotheliomas. Snellings *et al*. state that this observation indicates that exposure to ethylene oxide was associated with this earlier occurrence of mesotheliomas. Although the incidence of pituitary adenomas was not significantly increased in either sex, exposure to ethylene oxide significantly decreased the time to tumor in males ($p < 0.01$) and females ($p < 0.0001$).

From the time of the 18-month sacrifice until the end of the study, the incidence of brain tumors, including gliomas (twelve astrocytomas, one oligodendroglioma, two mixed gliomas), granular cell tumors, and malignant reticulosis was increased in both sexes. The classification of brain tumors was based on light microscopic cytomorphologic features and on patterns of growth and infiltration. Immunohistochemical staining was not done; thus, the cellular origin of these tumors remained unresolved.

Data on tumors for rats killed at 18 or 24 months and those who died spontaneously or were sacrificed due to morbidity were further evaluated by Garman *et al*. (1985, 1986). The first brain tumors were noted in animals killed at 18 months. The combined incidence of all three tumor types is shown in Table 4. The incidence in the 100 ppm and 33 ppm groups was significantly increased (p = 0.004 and p = 0.027, respectively) compared with the controls. In females a statistically non-significant, dose-related increase was noted in the combined incidence of these three tumor types (Table 4). The IARC working group (1985) noted that combining the three different histological types of tumors precluded a proper evaluation of the effects of ethylene oxide on the brain. (The IARC working group did not have the 1985 paper by Garman *et al.* available and based their results on Snellings *et al*. 1984a). However, even when only the gliomas are considered, a dose-related increase in tumor frequency is also observed (Table 4).

Table 4: Statistical analyses on adjusted ratios of primary brain tumor frequencies in F344 rats exposed to ethylene oxide for two years (Adapted from Garman *et al*., 1985)

Tumor type	Sex	ppm ethylene oxide			
		0*	10	33	100
Gliomas	males	1/181 (0.6%)	0/92 (0%)	3/85 (3.5%)	6/87 (6.9%)
	females	0/187 (0%)	1/94 (1.1%)	2/90 (2.2%)	2/78 (2.6%)
Gliomas,malignant reticulosis and granular cell tumors					
	males	1/181 (0.6%)	1/92 (1.1%)	5/85 (5.9%)	7/87 (8.0%)
	females	1/188 (0.5%)	1/94 (1.1%)	3/92 (3.3%)	4/80 (5.0%)

(a) p = 0.011; (b) p = 0.195; (c) p = 0.172; (d) p = 0.004; (e) p = 0.027; (f) p = 0.058
* control groups C1 and C2

When the data are adjusted for early deaths, the Cox test statistic for adjusted trends in males is significant ($p < 0.001$) for gliomas or the combination of the three tumor types. In females, p = 0.023 for gliomas only and p = 0.001 for the three tumor types combined. Comparison of the controls with historical controls indicates that the concurrent group and the 10 ppm groups had the expected incidence of primary brain tumors.

The frequency of multiple primary (benign plus malignant) neoplasms was significantly greater than the controls in the 100 ppm-exposed male rats. For females all three exposed groups had significantly more multiple primary neoplasms than controls ($p < 0.05$).

Lynch *et al*. (1984) reported the results of a study in male Fischer 344 rats which confirmed the findings of Snellings *et al*. (1984a). Groups of 80 weanling rats were exposed to 0 (filtered air), 50 or 100 ppm 99.7% pure ethylene oxide, 7 hours/day, 5 days/week, for two years. Histopathology was performed on standard sets of 34 tissues plus all gross lesions for all rats that died or were sacrificed. At approximately 8, 16, and 20 months into the study, rats were treated for 2 to 3 weeks with tetracycline for pulmonary infections. *Mycoplasma pulmonis* was confirmed

by serology during the 16th month outbreak. Exposure to ethylene oxide was stopped only for 14 days during the 16th month.

The median survival time and body weight gain were decreased in animals exposed to both concentrations of ethylene oxide compared with controls, and survival time in the high-dose group was significantly decreased ($p < 0.01$). The authors concluded that mortality was affected by ethylene oxide treatment as well as by the *M. pulmonis* infection. Rats exposed to 50 or 100 ppm had a higher incidence than controls of inflammatory lesions of the lungs, nasal cavities, trachea and internal ear as well as an increased incidence of bronchiectasis and bronchial epithelial hyperplasia. These findings are consistent with the manifestations seen in chronic respiratory disease complex in rodents.

The incidence of MNCL in animals dying during the study plus the terminal sacrifices was significantly greater ($p = 0.03$) in the 50 ppm group, but not the 100 ppm group, than in the controls (Table 5). Survival in the 100 ppm group was 19% compared to 49% in controls. If the incidence of MNCL of only the terminally sacrificed rats is compared, a statistically significant increased incidence of MNCL ($p < 0.01$) is observed for the 100 ppm group.

Peritoneal mesotheliomas were significantly increased in the 100 ppm group ($p = 0.002$), but not the 50 ppm group, compared with controls, even in the presence of excess mortality. Use of the Armitage test for trend suggested a proportional increase in the incidence of mesotheliomas with increased exposure.

The incidence of brain gliomas was increased in the 100 ppm dose group ($p < 0.05$) compared with controls (Table 5). Trend analysis suggested a significant increase in gliomas with increased exposure to ethylene oxide. Two additional rats exposed to 50 ppm and four additional rats exposed to 100 ppm had increased numbers of glial cells, termed "gliosis." The authors suggested that these cases of gliosis represent incipient gliomas.

Table 5: Selected tumor incidence in male F344 rats exposed to ethylene oxide for 2 years (adapted from Lynch *et al.*, 1984)

Organ	ppm ethylene oxide		
	100	50	Control
Spleen Mononuclear Cell Leukemia	30/76[a] (39%)	38/79[b] (48%)	24/77 (31%)
Peritoneal Mesothelioma	21/79[c] (27%)	9/79 (11%)	3/78 (4%)
Brain			
Glioma (Mixed cell)	5/79[b] (6%)	2/77 (3%)	0/76 (0%)
Astrocytoma	0/79	0/77	0/76

[a] Groups consisted of 80 male rats at beginning of study. Denominators less than 80 reflect tissues accidentally lost on that could not be examined histologically due to autolysis.
[b,c] Statistically significant difference versus controls: $p < 0.05$, $p < 0.01$, respectively.

Mice

The National Toxicology Program performed a two-year inhalation study of ethylene oxide at concentrations of 0, 50, and 100 ppm in male and female B6C3F$_1$ mice (NTP 1986). Statistically significant, increased incidences of both benign and malignant lung tumors and of Harderian gland tumors in both sexes and of uterine, mammary gland, and hematopoietic system (e.g., malignant lymphoma) tumors in females were observed. The incidence data for several tumors are shown in Table 6. Calculations using the several data sites from this study with the multistage model gave values for carcinogenic potency comparable to those calculated using the published data for inhalation by rats in the Bushy Run Research Center study (Snellings *et al*., 1984).

Table 6: Selected tumor incidences in NTP study of mice exposed to ethylene oxide for 2 years (adapted from NTP, 1986).

Organ/Sex		ppm ethylene oxide		
		100	50	Control
Alveolar/Bronchiolar Adenoma or Carcinoma				
Male	Overall	26/50[c] (52%)	19/50 (38%)	11/50 (22%)
	K-M Adjusted	68.3%	55.4%	33.2%
Female	Overall	22/49[c] (45%)	5/48(10%)	2/49 (4%)
	K-M Adjusted	58.6%	20.8%	7.7%
Malignant Lymphoma				
Female	Overall	22/49[c] (45%)	6/48 (12%)	9/49 (18%)
	K-M Adjusted	48.3%	19.0%	26.4%
Uterine Adenoma or Adenosarcoma				
Female	Overall	5/49[b] (10%)	2/47 (4%)	0/49 (0%)
	K-M Adjusted	14.3%	7.6%	0%
Mammary Gland Adenosarcoma or Adenosquamous Carcinoma				
Female	Overall	6/49 (12%)	8/48[b] (17%)	1/49 (2%)
	K-M Adjusted	17.1%	24.8%	2.9%

a Exposure groups consisted of 50 male and 50 female mice at the beginning of the study. Denominators less than 50 in the overall incidence category reflect tissues accidentally lost or that could not be examined histologically due to autolysis. K-M Adjusted incidences are Kaplan-Meier tumor incidences at the end of the study after adjusting for intercurrent mortality.

b, c Statistically significant difference versus controls: $p < 0.05$, $p < 0.01$, respectively (Fisher exact test).

In a more limited study in mice (Adkins *et al*., 1986), strain A/J female mice (6- to 8-weeks old) were exposed to 0, 70, and 200 ppm ethylene oxide for 6 hours/day, 5 days/week for only 6 months in one study and to 0 and 200 ppm in the same protocol in a second study. There were 30 animals

in each exposure group and at least 28 animals in each group survived. In each study 28% of the control animals developed pulmonary adenomas. At 70 ppm 56% developed adenomas. At 200 ppm 87% had adenomas in the first study; in the second study only 42% of the animals exposed to 200 ppm ethylene oxide developed pulmonary adenomas.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Both human (Hogstedt *et al*., 1979a, 1979b, 1986; Thiess *et al*.,1982) and animal (Dunkelberg, 1982; Snellings *et al*., 1981, 1984; Lynch *et al*., 1984; US EPA, 1985; Garman *et al*., 1985, 1986; Adkins *et al*., 1986; NTP, 1986) cancer data exist for ethylene oxide. CDHS decided that the overall evidence for human carcinogenicity due to ethylene oxide exposure could not be considered conclusive due to the small numbers of workers involved in the human studies and because of the possibility that other workplace carcinogens may have been confounders. However, CDHS also noted that though not conclusive, these studies provide substantial suggestive evidence of ethylene oxide's carcinogenicity in humans.

The animal studies listed above demonstrate the ability of ethylene oxide to induce tumors in multiple species (rats and mice) at multiple sites (brain gliomas, mononuclear cell leukemias, peritoneal mesotheliomas in rats; alveolar/bronchiolar adenomas/carcinomas, malignant lymphomas, mammary gland adenosarcomas or adenosquamous carcinomas and uterine adenomas/adenosarcomas in mice. CDHS developed a cancer unit risk for ethylene oxide based on data from the most sensitive sex, site and species, the female rat mononuclear cell leukemia data from Snellings *et al.* (1984).

Methodology

The data used to calculate cancer risk from the female rat mononuclear cell leukemia observed in the Bushy Run Research Center study (Snellings *et al*., 1984a) are given in Table 7 (US EPA, 1985). The table differs from Table 2 presented above. The denominators in Table 7.1 include only those animals whose tissues were examined, that were alive at the time the first leukemia was detected and were thus at risk for the tumor. There is an extra tumor in the 100 ppm group numerator because it was found in one of the rats removed for quality control at 18 months and was therefore excluded from Table 2. CDHS confirmed the EPA numbers by analysis of Table A-73 in the Appendices of the Bushy Run study (Snellings *et al*., 1981). Denominators obtained by approaches other than that used by the EPA do not differ significantly from their approach.

Table 7: Incidence of mononuclear cell leukemia in female rats among survivors to first tumor (Adapted from US EPA, 1985 (Table 9-33, p. 9-150)).

Ethylene oxide exposure (ppm)	Number with leukemia/corrected number exposed	Equivalent human lifetime dose (mg/kg/day)
0	22/186 (1-1.8%)	0
10	14/71 (19.7%)	0.28
33	24/72 (33.3%)	0.75
100	28/73 (38.4%)	2.11

Using the computer software Global 82 (Crump and Howe, 1982), a linearized multistage procedure was fit to the female leukemia dose-response data. Doses were first converted to human equivalents (Tyler and McKelvey, 1980; US EPA, 1985). The female rat leukemia data yielded a maximum likelihood estimate (MLE) for q_1, (the linear or slope term, which relates the probability of cancer to the dose of carcinogen administered in the equation for the multistage procedure) of 0.20 $(mg/kg\text{-}day)^{-1}$. An Upper 95% Confidence Limit (UCL) on q_1 of 0.29 $(mg/kg/day)^{-1}$ was also obtained from the data. (The values for q presented here are the same values EPA obtained using the same data).

Assuming that the percentage of ethylene oxide absorbed by inhalation is similar for rats and humans and using an average human body weight of 60 kg and an average air intake of 18 m^3 per day (California Department of Health Services 1985), a dose of 1 mg/kg/day ethylene oxide is equivalent to 3300 $\mu g/m^3$. Applying those units, the MLE and 95% UCL for the cancer unit risk are 6.1×10^{-5} and 8.8×10^{-5} $(\mu g/m^3)^{-1}$, respectively.

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ETHYLENE THIOUREA (ETU)

CAS No: 96-45-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	102.17
Boiling point	not available
Melting point	200-203 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 4.179 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.3 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 4.5 E-2 $(mg/kg\text{-}day)^{-1}$
[Rat thyroid tumors (Graham *et al*., 1975), contained in Gold *et al*. (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of ethylene thiourea (ETU) in humans are known to exist.

Animal Studies

Male and female 7-day old $B6C3F_1$ and $B6AKRF_1$ mice (18/sex/group) were exposed to 215 mg/kg body weight ETU by gavage from 1 week of age until 4 weeks of age (Biogenics Research Labs, Inc., 1968; Innes *et al*., 1969). The animals were then fed diets containing 646 mg/kg diet ETU until the end of the experiment (82-83 weeks). Increases in liver tumors (hepatomas) were seen in males and females of both mouse strains used; an increased incidence of lymphomas was also seen in male and female $B6AKRF_1$ mice. Tumor incidence data is listed in Table 1.

Male and female Charles River CD rats (26 animals/sex/group) were fed diets containing 0, 175 or 330 ppm technical grade ETU (97% pure) for 18 months; 5 animals of each sex were then sacrificed (Ulland *et al*.,1972). The remaining animals were followed for 6 months. Increased incidences of thyroid carcinomas were noted in both males and females; thyroid carcinoma incidences were 0/26, 3/26 and 17/26 for control, low-dose and high-dose males, respectively, and 0/26, 3/26 and 8/26 for control, low-dose and high-dose females, respectively.

Table 1. Ethylene thiourea (ETU)-induced tumor incidence in (C57BL/6×C3H/Anf)F_1 and (C57BL/6×AKR)F_1 mice (Innes *et al.*, 1969)

Sex/strain	Dose group	Tumor type	Tumor incidence
Male (C57BL/6×C3H/Anf)F_1	control	liver tumors	8/79
	treated		14/16
Female (C57BL/6×C3H/Anf)F_1	control	liver tumors	0/87
	treated		18/18
Male (C57BL/6×AKR)F_1	control	liver tumors	5/90
		lymphomas	1/90
	treated	liver tumors	18/18
		lymphomas	3/18
Female (C57BL/6×AKR)F_1	control	liver tumors	1/82
		lymphomas	4/82
	treated	liver tumors	9/16
		lymphomas	4/16

Graham *et al.* (1973, 1975) exposed male and female Charles River CD rats (initial group sizes 68 animals/sex/group) to diets containing ETU at levels of 5, 25, 125, 250 or 500 mg/kg diet. An untreated control group was included. Interim sacrifices were conducted at 2, 6, 12 and 18 months; the study was terminated at 24 months. An increased incidence of thyroid tumors (adenomas and carcinomas) was noted in males and females (combined). Tumor incidence data is listed in Table 2.

Table 2. Ethylene thiourea-induced thyroid tumor incidence in male and female (combined) Charles River CD rats (Graham *et al.*, 1973, 1975)

Ethylene thiourea dietary level (mg/kg diet)	Average dose[1] (mg/kg-day)	Tumor incidence[2]
0	0	2/72
5	0.225	2/75
25	1.13	1/73
125	5.63	2/73
250	11.3	16/69
500	22.5	62/70

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984)

Male and female Charles River CD rats were exposed to diet containing 0, 175 or 350 mg/kg ETU for 78 weeks, then switched to control diet for an additional observation period of 26 weeks (Weisburger *et al.*, 1981). A significantly increased incidence of thyroid follicular-cell carcinomas was noted in both male and female rats. Tumor incidences were 0/10, 2/26 and 15/26 in pooled

control, low-dose and high-dose male rats, respectively, and 0/10, 2/26 and 6/26 in pooled control, low-dose and high-dose female rats, respectively.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The results of several studies are listed in Gold *et al.* (1984). Innes *et al*. (1969) administered ethylene thiourea (ETU) to small groups of both sexes of $B6C3F_1$ and $B6AKF_1$ mice; Graham *et al*. (1973, 1975) performed relatively large multiple dose studies in Charles River CD rats of both sexes; Weisburger *et al.* (1981) and Ulland *et al.* (1972) conducted moderately sized studies in male and female Charles River CD rats. Because all male $B6C3F_1$ and female $B6AKF_1$ mice treated with ETU developed liver tumors, an upper bound estimate on potency could not be determined for these studies. The lower bound estimates of cancer potency derived from the mouse data are consistent with potencies derived from the studies in rats. Further, cancer potencies derived from the rat studies are consistent with one another. The value selected is derived from the highest quality study, Graham (1973, 1975), which had a large sample size and used multiple dose groups. The target site chosen for the analysis was the thyroid in the Charles River CD rats, the most sensitive site (see Table 2) (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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FORMALDEHYDE

CAS No: 50-00-0

I. PHYSICAL AND CHEMICAL PROPERTIES (HSDB, 1998)

Molecular weight	30.03
Boiling point	-19.5°C
Melting point	-92°C
Vapor pressure	1.08 torr @ 26.1°C
Air concentration conversion	1 ppm = 1.24 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.0 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 2.1 E-2 $(mg/kg\text{-}day)^{-1}$
[Rat nasal squamous carcinoma incidence data (Kerns *et al.*, 1983; U.S. EPA 1987), linearized multistage procedure (OEHHA, 1992), with pharmacokinetic interpolation of molecular dosimetry data to the tumor incidence data.]

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiological studies have shown formaldehyde exposure to be significantly associated with cancer at sites in the respiratory tract in workers and in the general population. Studies of embalmers, who have used formaldehyde, have shown increased rates of brain cancer and of leukemia.

Many studies in the epidemiological literature support a link between formaldehyde and elevated risk of cancers of the upper respiratory tract. Among the industrial cohort studies, Stayner (1988) reported a relative risk of 3.4 (90% CI: 1.2-7.9) for buccal cancer, and Blair *et al.* (1986) reported a relative risk of 3.00 (90% CI: 1.30-5.92) for nasopharyngeal cancer. Among industrial proportional mortality studies, Liebling *et al*. (1984) reported a relative risk of 8.70 (90% CI: 1.50-27.33) for buccal/pharyngeal cancer and Stayner *et al*. (1985) reported a relative risk of 7.5 (90% CI: 2.0-19) for buccal cancer. In all of these studies the elevated risk was statistically significant. The population-based case control studies reported statistically significant relationships between formaldehyde exposure and upper respiratory cancers in three studies (Vaughan *et al*., 1986a, b; Hayes *et al*., 1986; Olsen *et al*., 1984), although these cancers can appear in any of several sites.

In a subsequent report Blair *et al.* (1987) presented a summary of a further analysis resulting in a significant association between nasopharyngeal cancer and simultaneous exposure to formaldehyde and to particulate, indicating that such exposure may be a risk factor. Collins *et al.* (1988) have critiqued this finding and have added data.

The three largest - and therefore potentially most sensitive - industrial cohort studies reported elevated rates of lung cancer. The largest, Blair *et al.* (1986) with 26,561 U.S. workers, reported a statistically elevated death rate due to lung cancer, equivalent to 35% above the national average. The other two studies reporting elevated death rates due to lung cancer were Acheson *et al.* (1984a, b) with 7,680 British male workers, mostly young, and Stayner *et al.* (1988) with 11,030 U.S. workers, predominantly female. Some of the categories in the Acheson study showed statistically significant increases of lung cancer. The Stayner study found lung cancer to be elevated 14% overall, which was not statistically significant, but the exposures were well below those of the other two studies.

In the Blair *et al.* (1986) study the investigators concluded that a causal relationship between formaldehyde exposure and lung cancer was unlikely because of a lack of dose gradient for those tumors. Sterling and Weinkam (1988, 1989a, b) performed a reanalysis on the basis that Blair *et al.* (1986) failed to account for a "healthy-worker" effect in the original report. These corrected results showed that lung cancer was related to formaldehyde exposure in a dose-dependent manner, which was statistically significant. In a subsequent analysis of the same workers Blair *et al.* (1990) concluded that exposure to phenol, melamine, urea, and wood dust and other substances might account for some or all of the excess lung cancer observed.

Table 1: Cohort study on industrial exposure to formaldehyde (Blair *et al.*, 1986).

Exposure	Cancer Site	Number Observed	Number Exposed	SMR	90% Confidence Interval	
					Lower	*Upper*
0.1 - > 2.0 ppm time weighted average	brain	17	21	0.81	0.52	1.21
	leukemia	19	24	0.80	0.52	1.16
	buccal/pharynx	18	19	0.96	0.61	1.41
	lung	201	182	1.11	0.98	1.24
	larynx	12	8	1.42	0.87	2.43
	nasal	2	2.2	0.91	0.16	2.86
> 0 - 5.5 ppm-yr	lung, 20 yr latency	146	108	1.35	1.17	1.55
	hypopharynx	1	1.7	0.59	0.02	2.78
	nasopharynx	6	2.0	3.00	1.30	5.92
	oropharynx	5	2.6	1.92	0.76	4.04

Source: OEHHA (1992)

Recent epidemiological studies contribute to the conclusions only marginally. Gerin *et al.* (1989) presented the results of a large case control study with 3,726 cancer patients. The odds ratio for the highest exposure group with adenocarcinoma of the lung was nearly significant at the 95% confidence level, and there was an apparent trend of incidence of this cancer with exposure. Nevertheless, the authors concluded that there was no persuasive evidence of an increased risk of any type of cancer among men exposed to the exposure levels of formaldehyde cited by Blair *et al*. (1986) (Table 1). The study did not consider cancers of the nasal cavity, of the brain, or of leukemia. Bertazzi *et al.* (1989) presented an extension of a previous study (Bertazzi *et al.*, 1986) which had detected elevated lung cancer among 1,332 workers in a resin manufacturing plant

subject to formaldehyde exposure. In the extended study with more accurate estimates of exposure, the lung cancer rate was not elevated above expected for those exposed to formaldehyde (Bertazzi *et al.*, 1989). Linos *et al.* (1990) reported elevated rates of follicular non-Hodgkin's lymphoma and of acute myeloid leukemia among embalmers and funeral directors in a population-based case control study. The investigators did not attribute these tumors to formaldehyde exposure. Malker *et al.* (1990) found significantly elevated rates of incidence of nasopharyngeal cancer among workers in fiberboard plants and among book binders, both being subject to formaldehyde exposure.

Four recent occupational studies have investigated the relationship of formaldehyde exposure to histological changes, some of which are potentially precancerous lesions, in the nasal mucosa. Holmstrom *et al.* (1989) found that workers exposed to well-defined levels of formaldehyde developed significant changes in the middle turbinate, while those exposed to both formaldehyde and wood dust did not. Boysen *et al.* (1990) found in nasal biopses that workers exposed to formaldehyde showed a significantly higher degree of metaplastic alterations. Edling *et al.* (1988) found significant histological differences in the nasal mucosa of formaldehyde workers compared to unexposed workers but found no histological differences between those exposed to formaldehyde and those exposed to formaldehyde and wood dust. Berke (1987) found no statistical relationship between exfoliated nasal cells in formaldehyde-exposed workers and control groups. Thus, these studies provide some indication of possible histologic change due to formaldehyde exposure in humans, consistent with results in animals.

Animal Studies

A study sponsored by the Chemical Industry Institute for Toxicology (CIIT) has provided the most quantitatively useful evidence for the carcinogenicity of formaldehyde (Swenberg *et al.*, 1980a, b; Kerns *et al.*, 1983). This study used 120 male and 120 female Fischer-344 rats in each dose group, including a clean air group. The adjusted tumor incidences (adjusted for competing causes of death, including scheduled interim sacrifices) for squamous cell carcinomas in the nasal passages of males and females combined, when exposed to 0, 2.0, 5.6, or 14.3 ppm formaldehyde for 6 hours/day, 5 days/week for up to 24 months, were 0/156, 0/159, 2/153 and 94/140 (U.S. EPA, 1987). In an analogous study on mice, two mice in the high dose group (14.3 ppm) developed squamous cell carcinomas, a finding that was not statistically significant but was thought to be biologically significant due to the absence of this tumor in control animals and to concurrence with rat studies. Kerns *et al.* (1983) also reported benign tumors, including polypoid adenomas and squamous cell papillomas. Swenberg *et al.* (1980a, b) described a number of additional lesions in the nasal turbinates of rats exposed to formaldehyde for 18 months, including rhinitis, epithelial dysplasia and hyperplasia, squamous hyperplasia, and cellular atypia that occurred in a dose-related manner. Other inhalation studies (Albert *et al.*, 1982; Tobe *et al.*, 1985) have provided positive evidence for the carcinogenicity of formaldehyde.

Recent investigations of chronic toxicity have shown formaldehyde administered orally for 24 months to be carcinogenic in Sprague-Dawley rats but not in Wistar rats. Soffritti *et al.* (1989), using six exposure groups each of 50 male and 50 female Sprague-Dawley rats, with drinking water concentration of 10 to 1500 mg/L formaldehyde, reported increases in the percent of animals bearing leukemias and gastrointestinal neoplasias at the higher exposures. Til *et al.* (1989), using

three exposure groups, each of 70 male and 70 female Wistar rats, with drinking water concentrations of 20 to 1900 mg/L, reported numerous pathological changes at the highest exposure level, but no evidence of carcinogenicity at any level. Tobe *et al.* (1989), using three exposure groups, each of 20 male and 20 female Wistar rats, with drinking water concentrations of 200 to 5000 mg/L, also reported pathological changes at the highest exposures level but no significant increases in the incidence of any tumor in these small treatment groups. In a letter to the editor, Feron *et al.* (1990) questioned the conclusions and some methods of Soffritti *et al.* (1989).

Other types of exposures have produced a spectrum of results. Watanabe *et al.* (1954) presented a brief preliminary report of experimentally inducing sarcomas by repeated injections of an aqueous solution of formaldehyde in rats. Muller *et al.* (1978) induced a preneoplastic lesion of the oral mucosa by repeated exposure to formalin solution in rabbits. Homma *et al.* (1986) found that formalin solution repeatedly administered in transplanted rat bladders did not promote formation of tumors. Takahashi *et al.* (1986) found that formalin solution in diet did promote stomach tumors in Wistar rats. Iversen *et al.* (1988) found that topical skin application of formaldehyde solution in mice did not promote the formation of skin tumors.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The International Agency for Research of Cancer (1987) has reviewed the evidence for carcinogenicity and found it to be limited in humans and sufficient in animals. U.S. EPA (1987) has classified formaldehyde in Group B1, probable human carcinogen. The U.S. Occupational Safety and Health Administration (U.S. OSHA, 1987) has concluded that "formaldehyde should be regarded as an occupational carcinogen," based upon animal and human studies. Considering these previous determinations, along with the evidence of carcinogenicity, OEHHA staff (OEHHA, 1992) concluded that formaldehyde is a probable carcinogen and meets the definition of a "toxic air contaminant": an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

Formaldehyde is carcinogenic in rodents, as described above, producing squamous cell carcinomas in the nasal passages of male and female rats and male mice. Several different types of potentially precancerous abnormalities, including polypoid adenomas and squamous cell papillomas, have also been observed. The epidemiological evidence, while suggestive of a risk of human cancer due to formaldehyde exposure, was considered insufficient for risk assessment purposes on its own. OEHHA (1992) found the tumor incidence data in rats reported by Kerns *et al.* (1983) and used by U.S. EPA (1987) to be the most appropriate for use in developing a quantitative risk assessment.

Methodology

In developing a spectrum of predictions of cancer risk to humans, the OEHHA (1992) assessment applied a pharmacokinetic interpolation of the molecular dosimetry data to the animal cancer bioassay data of Kerns *et al.* (1983). The analysis used the linearized multistage procedure (GLOBAL86), and the procedure developed by Moolgavkar and others, which takes into account the proliferation of premalignant cells due to the formaldehyde exposure. Both models derive upper confidence limits (UCL) for excess cancer risk and extrapolate the risk to humans by means of three different scaling factors. Two scaling factors take into account the contact mechanism of carcinogenesis. However, they do so in different ways. One uses only a generic calculation in terms of body mass. The other takes specific account of comparative data on DNA binding in rats and monkeys to adjust the metabolic rate for humans; it assumes humans respond as do monkeys and uses the data of Casanova *et al.* (1989; 1991). The third scaling factor follows the default option of the California carcinogen guidelines (CDHS, 1985), which calculates the adjustment for rat exposures to obtain the equivalent human exposure on the basis of intake rate divided by body surface area.

Table 2: Formaldehyde inhalation bioassay data used to estimate cancer risk to rats

Exposure (ppm HCHO)[a]	Rate of DNA Binding[b] (pmol/mg-hr)	Lifetime Equivalent Metabolic Exposure[b] (ppm)	Incidence of Nasal Squamous Carcinomas[c]
0	0	0	0/156 (0%)
2	2.5	0.54	0/159 (0%)
5.6	15.9	3.4	2/153 (1.3%)
14.3	74.8	16.	94/140 (67.5%)

Source: adapted from OEHHA (1992)
[a]Fischer 344 rats inhaled indicated concentrations of formaldehyde gas 6 hours per day, 5 days per week for 24 months.
[b]Details on how these estimates were obtained are presented in OEHHA (1992)
[c]Based on data partially reported in Kerns *et al.* (1983). Numerator and denominator are those used by U.S. EPA (1987).

For the best value of UCL on unit risk for a lifetime of exposure, the OEHHA staff selected 7×10^{-3} ppm^{-1} (6.0×10^{-6} ($\mu g/m^3$)$^{-1}$), based on molecular dosimetry data in a three-stage model and using the standard surface-area scaling factor, 1.2. The range of calculated values of UCL on unit risks is 0.3×10^{-3} ppm^{-1} to 40×10^{-3} ppm^{-1} (0.25×10^{-6} to 33×10^{-6} ($\mu g/m^3$)$^{-1}$).

In a review of epidemiological studies for workers exposed to formaldehyde the study by Blair *et al.* (1986) was selected as the most reliable for quantitative comparisons. That study, the largest and best documented study available, evaluated mortality in a cohort of more than 26,000 workers. The observed risk of death by lung cancer in exposed workers was 15×10^{-3} over their career. Based on extrapolation of rat cancer risk predictions to humans for a 40-hour work week for 20 years and an exposure level of 1.0 ppm, the prediction of 95% upper confidence limits on respiratory tract cancer was 32×10^{-3} for the three-stage tissue-dose model with generic contact

scaling factor. Thus, the upper range of human cancer risk predictions from the rat bioassay data (Kerns *et al.*, 1983) was consistent with the occupational exposure cancer risk data.

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HEXACHLOROBENZENE

CAS No: 118-74-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	284.8
Boiling point	323-326°C
Melting point	231°C
Vapor pressure	1.09 E-05 mm Hg at 20°C
Air concentration conversion	1 ppm = 11.65 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 5.1 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.8 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from potency derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

No adequate epidemiological studies of cancer in people exposed to hexachlorobenzene (HCB) are available. The only reported study found increases in porphyria, neurological, dermatological and orthopedic disorders and thyroid enlargement among 161 individuals (63 women, 98 men) studied out of a group of approximately 4000 who had suffered hexachlorobenzene poisoning 25 years previously as a result of eating HCB-treated wheat seed (Peters *et al.*, 1982, 1983). No increases in cancer incidence were reported; however, it should be noted that the methodology used in the study was not designed to evaluate excess cancer occurrence.

Animal Studies

A number of feeding studies have been conducted in hamsters, rats and mice. Hexachlorobenzene has been found to induce tumors in the liver, adrenal gland, thyroid gland, parathyroid gland, kidney, lymphoid tissue and endothelial tissue.

Cabral *et al.* (1977) fed male and female Syrian golden hamsters diets containing 0, 50, 100 or 200 mg/kg diet HCB for the life of the animals. Treatment group sizes for the control, low-dose, mid-dose and high-dose groups were 40, 30, 30 and 59, respectively, for males and 40,30,30 and 60, respectively, for females. Treatment-related increases in the incidence of liver tumors (hepatomas, hemangioendotheliomas) and thyroid adenomas were observed in both males and females. Tumor incidence data is listed in Table 1.

Table 1. Hexachlorobenzene-induced tumor incidence in male and female Syrian golden hamsters (Cabral *et al.*, 1977)

Dietary HCB concentration (mg/kg diet)	Calculated daily intake (mg/kg-day)	Tumor type	Tumor incidence	
			males	females
0	0	hepatomas	0/40	0/39
50	4		14/30	14/30
100	8		26/30	17/30
200	16		49/57	51/60
0	0	hemangioendotheliomas	0/40	0/39
50	4		1/30	0/30
100	8		6/30	2/30
200	16		20/57	7/60
0	0	thyroid adenomas	0/40	0/39
50	4		0/30	2/30
100	8		1/30	1/30
200	16		8/57	3/60

Outbred Swiss mice were fed diets containing 0, 50, 100, or 200 mg/kg hexachlorobenzene for 101-120 weeks; all surviving animals were killed at 120 weeks (Cabral *et al.*, 1979). Initial group sizes were 50 animals/sex for the control and 200 mg/kg diet groups, and 30/sex for the 50, 100 and 300 mg/kg diet groups. A dose-response related increase in the incidence of liver tumors (unspecified histological type) was noted in both male and female animals. An increased incidence of liver tumors was also found in a group of 30 males and 30 females fed diet containing 300 mg/kg diet HCB for 15 weeks followed by observation until 120 weeks. Tumor incidence data is listed in Table 2.

Table 2. Hexachlorobenzene-induced liver tumors in male and female Swiss mice (Cabral *et al.*, 1979)

Dose group (mg/kg diet)	Tumor incidence	
	males	females
0	0/47	0/49
50	0/30	0/30
100	3/29	3/30
200	7/44	14/41
300 (15 weeks exposure)	1/16	1/26

Smith and Cabral (1980) exposed female Agus and Wistar rats to diets containing 100 mg/kg HCB for up to 90 or 75 weeks, respectively. An increased incidence of liver tumors (histological type

not specified) due to HCB exposure was observed in both Agus and Wistar rats; tumor incidence was 14/14 and 4/6, respectively, compared to 0/12 and 0/4, respectively, in the control animals.

Male and female Syrian golden hamsters were exposed to diet containing 0, 200 or 400 mg/kg diet HCB for 90 days; 25-50 animals/group were sacrificed on the 91st day (Lambrecht *et al.*, 1982). The remaining 25 animals/group were placed on control diet and sacrificed at 6 week intervals up to 1 year. Hepatoma incidence in the 200 and 400 mg/kg diet groups was 1/13 and 1/20, respectively, for males and 1/15 and 1/7, respectively for females. No hepatomas were noted in 43-50 control animals for each sex.

Male and female Sprague-Dawley rats (94/sex/group) were fed diets containing 0, 75 or 150 mg/kg diet HCB for up to 2 years (Lambrecht *et al.*, 1983a, b, 1986). Four animals/sex/group were killed at 0, 1,2,3,4,8,16,32 and 64 weeks. Treatment-related increases in the incidence of hepatic tumors (hepatomas, hemangiomas, hepatocarcinomas and bile duct adenomas/carcinomas) and renal-cell adenomas were noted in both male and female animals. Tumor incidence data is noted in Table 3.

Table 3. Hexachlorobenzene-induced hepatic tumors in male and female Sprague-Dawley rats (Lambrecht *et al.*, 1983 a,b; 1986)

Dose group (mg/kg diet)	Tumor type	Tumor incidence	
		males	females
0	hepatoma/hemangioma	0/54	0/52
75		10/52	23/56
150		11/56	35/55
0	hepatocarcinoma	0/54	0/52
75		3/52	36/56
150		4/56	48/55
0	bile duct adenoma/carcinoma	0/54	1/52
75		2/52	19/56
150		2/56	29/55
0	renal-cell adenomas	7/54	1/52
75		41/52	7/56
150		42/56	15/55

Arnold *et al.* (1985) conducted two studies on the effects of chronic feeding of HCB in Sprague-Dawley rats. In the first study, male and female Sprague-Dawley rats were fed diets containing 0, 0.32, 1.6, 8 or 40 mg/kg diet HCB for 3 months after weaning. Group sizes were 40/sex except for the control and high-dose groups (64 and 66/sex, respectively). After 3 months, the F_0 rats were bred and 50 pups (F_1) of each sex were randomly selected from each group. The F_1 generation animals were fed their parent's diet from weaning for their lifetime (130 weeks). A significant positive trend was noted in the incidence of parathyroid adenomas in males ($p < 0.01$); the incidence in high-dose males was also significantly greater than controls ($p < 0.05$). A significant positive trend was also noted in the incidence of adrenal pheochromocytomas in both

males and females ($p < 0.05$ and $p < 0.01$, respectively); the incidence in high-dose females was also significantly greater than controls ($p < 0.01$). Tumor incidence data is listed in Table 4.

Table 4. Hexachlorobenzene-induced tumor incidence in the male and female exposed F_1 progeny of exposed F_0 Sprague-Dawley rats (Arnold *et al.*, 1985)

Dose group (mg/kg diet HCB)	Average dose[1]	Tumor type	Tumor incidence	
			males	females
0	0	parathyroid adenomas	2/48	
0.32	0.01		4/48	
1.6	0.07		2/48	
8	0.35		1/49	
40	1.72		12/49	
0	0	adrenal pheochromocytomas	10/48	2/49
0.32	0.01		12/48	4/49
1.6	0.07		7/48	4/50
8	0.35		13/49	5/49
40	1.72		17/49	17/49

1. As reported by CDHS (1988)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results from the following 3 studies provide the basis for cancer potency derivation. Cabral *et al.* (1977) treated groups of male and female Syrian golden hamsters with hexachlorobenzene in feed over the lifetime of the animals. Significant dose-related increases in hepatomas were observed in both sexes (see Table 1). Lambrecht *et al.* (1983a, b; 1986) exposed male and female Sprague-Dawley rats to hexachlorobenzene in feed for 2 years. Treated male and female rats exhibited significant increases in the incidence of hepatomas and renal-cell adenomas. Female rats also demonstrated significant increases in the incidence of hepatocellular carcinomas. No hepatocellular carcinomas were noted in control animals (see Table 3). Arnold *et al.* (1985) also observed a significant dose related increase in the occurrence of adrenal pheochromocytomas in the male and female and parathyroid adenomas in the male exposed F_1 progeny of exposed F_0 Sprague-Dawley rats (see Table 4).

Methodology

Cancer potency values are based on the most sensitive site, species and study demonstrating carcinogenicity of a particular chemical, unless other evidence indicates that the value derived from that data set is not appropriate (CDHS, 1985). For hexachlorobenzene, similar cancer potencies were derived using data from several tumor sites in different test species. Cancer potency factors (q_1^*) were derived by applying a linearized multistage procedure (CDHS, 1985) to the dose-response data for induction of hepatomas in male Syrian golden hamsters (Cabral *et al.*,

1977), and hepatocellular carcinomas (Lambrecht *et al.*, 1983a, b) and pheochromocytomas (Lambrecht *et al.*, 1983a, b; Arnold *et al.*, 1985) in female Sprague-Dawley rats. Surface area scaling was employed to transform animal cancer potency factors to human cancer potency factors. Assumed body weight values used for humans, hamsters and mice were 70 kg, 0.1 kg and 0.035 kg, respectively (CDHS, 1988). Lambrecht *et al.* (1983a, b) reported average body weights of 0.265 kg for female Sprague-Dawley rats; unpublished data cited by US EPA (1985) indicates that average body weights of female Sprague-Dawley rats in the study by Arnold *et al.* (1985) were 0.353 kg. A human cancer potency value (q_{human}) of 1.7 $(mg/kg\text{-}day)^{-1}$ was calculated from the male hamster hepatoma incidence data (Cabral *et al.*, 1977) and the female rat hepatocellular carcinoma incidence data (Lambrecht *et al.*, 1983a, b). A human cancer potency value of 1.8 $(mg/kg\text{-}day)^{-1}$ were calculated from female rat pheochromocytomas incidence data (Lambrecht *et al.*, 1983a, b; Arnold *et al.*, 1985). On the basis of the results stated above, a cancer potency of 1.8 $(mg/kg\text{-}day)^{-1}$ was selected for hexachlorobenzene (CDHS, 1988). The unit risk factor was derived by OEHHA/ATES from the low dose exposure cancer potency value using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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HEXACHLOROCYCLOHEXANE (TECHNICAL GRADE)

CAS No: 608-73-1

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	290.9
Boiling point	288°C (α-HCH); 323.4°C (γ-HCH)
Melting point	158°C (α-HCH); 113°C (γ-HCH)
Vapor pressure	0.02 mm Hg @ 20°C (α-HCH)
Air concentration conversion	1 ppm = 11.9 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.1E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 4.0 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by CDHS (1988)]

III. CARCINOGENIC EFFECTS

Human Studies

The International Agency for Research on Cancer (IARC) concluded in 1987 that the evidence for carcinogenicity of hexachlorocyclohexane (HCH; all isomers) was inadequate in humans. However, U.S.EPA (1988) designated α-HCH and technical grade HCH as B2 (probable human) carcinogens. Several case reports suggest an association between HCH isomers, including β- and γ-HCH, and exposure and leukemia, aplastic anemia, liver cancer, soft-tissue sarcomas, and lung cancer (IARC, 1987). In all of these case reports, the exposures are not well documented. In addition, exposures to other chemicals, including some pesticides, probably occurred in these cases.

Animal Studies

The incidence of liver tumors in male and female mice has been shown to be increased in two studies of technical HCH (Kashyap *et al*., 1979; Hanada *et al*., 1973). Hanada *et al*. (1973) exposed male and female dd mice (10-11 per group; 14 controls) to 100, 300, or 600 mg/kg diet of α-, β-, γ-, or technical HCH for 32 weeks, followed by a 6-week period without chemical exposure. The incidence of hepatomas was significantly increased in animals treated with increasing doses of α-, or technical HCH (Table 1).

In the study by Kashyap *et al*. (1979), Swiss mice (30/sex/group) were exposed to 0 or 100 mg/kg diet for 80 weeks. Mice were also exposed to technical HCH by gavage (10 mg/kg/day) or skin painting (0.25 mg in 0.1 mg olive oil). A significant increase in liver hepatocarcinomas and lymphoreticular tumors of type B was observed in mice exposed to technical HCH in the diet or by gavage (Table 2).

Table 1. Liver hepatoma incidence in dd mice treated with hexachlorocyclohexane (HCH) (Hanada *et al*. (1973).

HCH Isomer	Sex	Hepatoma incidence mg/kg diet HCH 100	300	600
α-HCH	males	1/8	7/7	7/7
	females	0/8	2/3	6/8
β-HCH	males	0/9	0/8	0/8
	females	0/9	0/8	0/4
γ-HCH	males	0/10	0/9	3/4
	females	0/8	0/7	1/3
Technical HCH	males	0/10	4/4	4/4
	females	0/8	3/5	5/5

Table 2. Tumor incidence in mice treated with technical hexachlorocyclohexane (HCH) (Kashyap *et al*., 1979).

HCH Treatment Group	Sex	Animals/ group	Liver tumors	Total tumors
Control	m	25	4	9
	f	26	1	5
100 mg/kg/day (diet)	m	23	16	22
	f	25	9	21
10 mg/kg/day (gavage)	m	26	12	17
	f	28	7	16
0.25 mg/0.1 mg (olive oil) (gavage)	m	25	5	11
	f	18	3	7

m = male, f = female

Wolff *et al*. (1987) reported on the carcinogenic effects of γ-HCH in several strains of mice. Female yellow, black, and pseudoagouti mice (36-96 per group) were exposed to 0 or 160 ppm γ-HCH in the diet for up to 24 months. Different response rates were observed between strains, indicating significant genetic variability in response to γ-HCH. In yellow mice, a significant increase in the incidence of Clara cell hyperplasia, papillary lung tumors and hepatocarcinomas and adenomas was observed (Table 3). The higher incidence of tumors in the obese yellow mice indicate that bioaccumulation of γ-HCH in obese animals may influence carcinogenicity. Similar experiments were not done using technical HCH.

Table 3. Tumor incidence in yellow mice exposed to γ-HCH (Lindane) (Wolff *et al*., 1987)

Concentration of γ-HCH (ppm)	Lung Tumors	Liver Adenomas
0	4/95	8/93
160	18/95	33/94

Thorpe and Walker (1973) showed an increase in liver tumors of male CF1 mice fed 400 ppm γ-HCH for 110 weeks, compared with controls. The time-weighted dose was estimated as 52 mg/kg/day by US EPA (1988). In this experiment, control mice exhibited an incidence of 11/45 for liver tumors, compared to 27/29 for the 52 mg/kg/day group.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The studies by Hanada *et al*. (1973) and Kashyap *et al*. (1979) both show a carcinogenic effect on the liver in mice. The study by Nagasaki *et al*. (1975) also showed a positive carcinogenic effect in mice, but these data were considered less reliable since the tumor incidence was zero in all but the highest dose group, where it was 100%. In addition, the study by Kashyap *et al*. (1979) was among those of the longest duration available for HCH (80 weeks). The potency values from the Kashyap *et al*. (1979) and Hanada *et al*. (1973) studies are the same for liver tumors in mice. Therefore, these studies were used by CDHS (1988) to determine the cancer potency for HCH.

Methodology

A linearized multistage procedure was used to estimate the cancer potency of technical HCH from the Kashyap *et al*. (1979) and Hanada *et al*. (1973) data in male Swiss mice (Crump *et al*., 1982). The concentrations of technical HCH given in the feed were 0 or 100 mg/kg diet (Kashyap *et al*., 1979), and 0, 100, 300, or 600 mg/kg diet (Hanada *et al*., 1973). The tumor incidence data are shown in Tables 1 and 2 above. The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value for HCH.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. The default lifespan for mice is 104 weeks. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 4.0 $[mg/kg\text{-}day]^{-1}$ was derived (CDHS, 1988). An airborne unit risk factor of 1.1E-3 $(\mu g/m^3)^{-1}$ was calculated by OEHHA/ATES from the q_{human} value using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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HYDRAZINE

CAS No: 302-01-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	32.05
Boiling point	113.5°C
Melting point	2.0°C
Vapor pressure	14.44 mm Hg @ 25°C
Air concentration conversion	1 ppm = 1.31 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.9 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 1.7 E+1 $(mg/kg\text{-}day)^{-1}$
[Calculated by US EPA (1991) from the male rat nasal cavity tumor data of MacEwan *et* al. (1980) using a linearized multistage procedure (Global 82), extra risk]

Oral Cancer Potency Factor: 3.0 E+0 $(mg/kg/day)^{-1}$
[Calculated by US EPA (1991) from the male mouse liver tumor data of Biancifiori (1970) using a linearized multistage procedure (Global 82), extra risk]

III. CARCINOGENIC EFFECTS

Human Studies

A published letter (Roe, 1978) presented mortality data from two hydrazine manufacturing plants (belonging to one of nine companies in the trade). This study included 423 workers employed at one plant between 1963 and 1975 (151 workers) and at a second plant (272 workers) between 1945 and 1970. Five cancer deaths were reported (three of the stomach, one prostatic and one neurogenic). A follow-up study of this cohort extended the observation period to 1982 (Wald *et al*., 1984). The only excess cancer mortality was the result of two lung cancer cases in the highest exposure group (relative risk = 1.2, 95% confidence interval 0.2 - 4.5). The author concluded that neither group of workers demonstrated an increased risk of cancer associated with occupational exposure to hydrazine. No other studies on human hydrazine exposure have been published.

Animal Studies

Several studies have tested the ability of hydrazine sulfate administered by gavage or in drinking water to induce cancer. Lung adenomas and adenocarcinomas and liver hepatomas and hepatocarcinomas were observed in both mice and rats. These studies have been reviewed by IARC (1974) and US EPA (1988). Lung tumors, reticulum-cell sarcomas and myeloid leukemias have also been observed to occur in mice exposed to hydrazine by intraperitoneal injection (Juhász *et al*., 1966; Kelly *et al*., 1969; Mirvish *et al*., 1969). MacEwan *et al*. (1981) reported that

inhalation exposure to hydrazine induced lung adenomas in mice, nasal cavity tumors and thyroid adenocarcinomas in rats and nasal polyps in hamsters.

V. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Inhalation

MacEwan *et al*. (1981) exposed C57BL/6 mice, F344 rats, Syrian golden hamsters and beagle dogs to hydrazine vapor (97% pure) by inhalation for 6 hours/day, 5 days/week for 1 year followed by a variable observation period (12-38 months). Exposure levels were 0.05, 0.25 and 1.0 ppm for mice, 0.05, 0.25, 1.0 and 5.0 ppm for rats, 0.25, 1.0 and 5.0 ppm for hamsters and 0.25 and 1.0 ppm for dogs. Lung adenomas were reported in 12/379 female mice ($p < 0.05$) exposed to 1.0 ppm hydrazine. Male and female rats demonstrated nasal cavity tumors after exposure to 1.0 ppm (11/98 and 4/97, respectively) and 5.0 ppm (72/99 and 36/98, respectively). Male rats also developed thyroid adenocarcinomas (13/99) after exposure to 5.0 ppm hydrazine. Nasal polyps were induced in male hamsters exposed to 5.0 ppm hydrazine (16/160, $p < 0.01$). No significant tumor increase was seen in either dog exposure group. This study was selected as the basis of a cancer potency factor for exposure to hydrazine by inhalation because it demonstrated a dose response, used a relevant exposure route and used hydrazine vapor instead of hydrazine sulfate.

Oral

Biancifiori (1970) administered hydrazine sulfate by gavage to groups of 24 to 30 8-week-old CBA/Cb/Se mice of each sex at doses of 0.0, 0.14, 0.28, 0.56, or 1.13 mg/day, 6 days/week for 25 weeks. Animals were observed throughout their lifetimes. Liver carcinomas were induced in a dose-related manner in both sexes and lung metastases were observed in some of the mice treated with 1.13 mg/kg/day (Table 1). Pulmonary tumors were reportedly present in many of the treated mice, but incidences were not reported because the purpose of the study was to describe hepatic tumors.

Table 1. Tumor incidence in male CBA/Cb/Se mice exposed by gavage to hydrazine sulfate (Biancifiori, 1970)

Administered dose (ppm)	Human equivalent dose (mg/kg)/day[1]	Liver tumor incidence
0	0	3/30
0.14	0.044	1/26
0.28	0.103	7/25
0.56	0.222	12/25
1.13	0.403	15/25

1. Human equivalent dose calculated by US EPA (1991).

Methodology

Inhalation

A linearized multistage procedure (Global 82) was used to calculate a slope factor of 1.7 E+1 $(mg/kg/day)^{-1}$ from the male F344 rat nasal cavity adenoma and adenocarcinoma incidence data of MacEwan *et al*. (1980). Male F344 rats were the most sensitive species and sex to the carcinogenic effects of inhaled hydrazine. Administered doses were 1.0 and 5.0 ppm; human equivalent doses were 0.01 and 0.05 mg/kg/day. Human equivalent doses were calculated on the basis of a 365 day treatment and an experimental period of 910 days. Rat body weight was assumed to be 350 g, and the animal lifespan was assumed to be 910 days. Calculation of the unit risk from the slope factor assumed a body weight of 70 kg and an inspiration rate of 20 m^3/day. EPA has stated that the unit risk should not be used if the air concentration exceeds 2 $\mu g/m^3$, since above this concentration the unit risk may not be appropriate.

Oral

A linearized multistage procedure (Global 82) was used to calculate a slope factor of 3.0 E+0 $(mg/kg/day)^{-1}$ from the male CBA/Cb/Se mouse liver tumor incidence data of Biancifiori (1970) (Table 1). Human equivalent doses were calculated to reflect a treatment period of 175 days and an experimental period of 607 days, the mean length of the experiment for each treatment group. Mouse body weight was assumed to be 0.03 kg and the animal lifespan was assumed to be 730 days.

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Wald N, Boreham J, Doll R and Bonsall J. 1984. Occupational exposure to hydrazine and subsequent risk of cancer. Br J Ind Med 41:31-41.

LEAD AND LEAD COMPOUNDS (INORGANIC)

CAS No.: 7439-92-1

I. PHYSICAL AND CHEMICAL PROPERTIES

Molecular weight	207.2 (Budavari, 1989)
Boiling point	1740° C (Budavari, 1989)
Melting point	327.4° C (Budavari, 1989)
Vapor pressure	1.77 mm Hg at 1000° C (Budavari, 1989)
Air concentration conversion	not available

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor:	1.2 E-5 $(\mu g/m^3)^{-1}$	
Slope Factor:	(inhalation)	4.2 E-2 $(mg/kg\text{-}day)^{-1}$
	(oral)	8.5 E-3 $(mg/kg\text{-}day)^{-1}$

[Calculated by OEHHA (1997) from rat kidney tumor incidence data (Azar *et al.*, 1973) using a linearized multistage procedure.]

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiological studies and case reports of people occupationally exposed to lead provide some evidence of carcinogenicity but are not convincing due to lack of controlling for confounders such as smoking and to the simultaneous exposure of some workers to known human carcinogens including arsenic and cadmium. These studies have been reviewed by several agencies (IARC, 1980; U.S. EPA, 1986; 1989a; 1989b; ATSDR, 1990).

The epidemiologic study by Selevan *et al.* (1985) suggested that human cancer may be induced in the same organ in which cancer is induced in animals. A cohort of 1,987 lead smelter workers was studied. The study confirmed previous reports of occupationally-induced, chronic, fatal renal disease after long term exposure to lead and yielded a Standardized Mortality Ratio (SMR) of 204 for kidney cancer, but the numbers were small (6 cases observed) and the SMR for kidney cancer was not statistically significant.

Recently the study has been updated to include 11 years of follow-up and 363 additional deaths (Steenland *et al.*, 1992). No additional deaths from nonmalignant kidney disease had occurred but 3 additional deaths from kidney cancer had occurred. The updated SMR from kidney cancer was 193 (9 total kidney cancer deaths, 95% confidence interval (CI = 0.88, 3.67), i.e., not statistically significant at the 5% level). The SMR for kidney cancer for those with the highest lead exposure was statistically significant (SMR = 239 based on 8 cancers, 95% CI = 1.03, 4.71). The study suffers from lack of detailed data on lead exposure levels and from potential confounding exposures to cadmium, arsenic, and tobacco smoke.

In an epidemiologic study of 7,121 deceased California plumbers and pipefitters, Cantor *et al.* (1986) found increased cancer incidence for all neoplasms and for cancers of several sites including the respiratory system, kidney, and stomach. In addition to lead, these workers were exposed to carcinogens such as asbestos and chromium. Since excess mesotheliomas were observed (16 observed, 2 expected), asbestos exposure likely contributed to the observed increase in stomach and respiratory system cancer. Asbestos, chromium, and cigarette smoking are likely contributors to lung cancer but are not generally considered causes of kidney cancer.

There are 2 case reports of renal cancer in men occupationally exposed to toxic levels of lead (Baker *et al.*, 1980; Lilis, 1981). Baker *et al.* (1980) thought that the histology in the renal tumor in their case report was similar to that of kidney tumors in lead-exposed animals. Despite the long history of human lead exposure and the chronic nephropathy induced by lead, the data on lead-induced, human renal cancer is not definitive.

In regard to induction of cancer in organs other than the kidney, the largest occupational cohort studied for lead-induced cancer included approximately 6,800 employees of 6 lead smelters and recycling plants and 10 battery manufacturing plants in the United States (Cooper and Gaffey, 1975; Cooper, 1976; Kang *et al.*, 1980; Cooper, 1981; Cooper *et al.*, 1985; Cooper, 1988). Statistically significant increases in cancer have been reported for total malignant neoplasms in lead production workers (Cooper and Gaffey, 1975), total malignant neoplasms and cancers of both the digestive tract and the respiratory tract in lead production workers and in battery workers (Kang *et al.*, 1980), no sites (Cooper, 1981; 1988), and total malignancies in the battery workers (Cooper at al., 1985) principally due to cancers of the respiratory and digestive tracts. In these studies several factors including cigarette smoking could not be ruled out as confounders.

Ades and Kazantzis (1988) studied 4,293 men at a zinc-lead-cadmium smelter in Great Britain. An effect of lead exposure on lung cancer was noted but lead exposure was highly correlated with exposure to arsenic, a known respiratory carcinogen, and no data on cigarette smoking were reported.

Fu and Boffetta (1995) have conducted a meta-analysis of the published studies on cancer and workplace exposures to inorganic lead compounds. The studies include the 2 case reports, 16 papers dealing with cohort studies, and 7 papers dealing with case-control studies. The meta-analysis showed a statistically significant, excess relative risk of cancer overall (RR = 1.11, 95% CI = 1.05-1.17), of stomach cancer (RR = 1.33, CI = 1.18-1.49), of lung cancer (RR = 1.29, CI = 1.10-1.50), and of bladder cancer (RR = 1.41, CI = 1.16=1.71). The relative risk for kidney cancer did not reach statistical significance (RR = 1.19, CI = 0.96-1.48). A separate analysis of studies involving workers heavily exposed to lead found higher relative risks for stomach cancer (RR = 1.50, CI = 1.23-1.43, based on 4 studies) and lung cancer (RR = 1.42, CI = 1.29-1.62, based on 4 studies). The meta-analysis is further indication of a relationship between lead exposure and cancer, but it is limited by the paucity of information in the various studies on confounders such as cigarette smoking, dietary habits, and other occupational carcinogens at many of the workplaces studied (Fu and Boffetta, 1995).

There are corroborative findings relevant to the potential of lead to be both an initiator and a promoter of carcinogenicity (Goyer, 1992). Results of these studies will not be discussed here.

Animal Studies

There are a large number of carcinogenicity studies in rodents in which lead compounds were administered by the oral route, either in feed or in drinking water. Although other types of tumors are occasionally seen, the principal finding has been kidney tumors, both benign and malignant, in rats. Important studies are summarized in Table 1.

No long-term studies in animals to investigate carcinogenicity due to lead inhalation have been conducted. Intratracheal instillation of lead oxide was employed in one study of cancer (Kobayashi and Okamoto, 1974). No tumors were seen in 20 hamsters after 10 intratracheal instillations of 1 mg of lead oxide, which gave a comparatively low total dose of 10 mg. In that study, however, simultaneous administration of lead with benzo[a]pyrene (10 instillations of 1 mg), which by itself also did not cause tumors, did act to produce lung tumors. Lead might be acting as a promoter or co-carcinogen for benzo[*a*]pyrene-initiated carcinogenicity.

Table 1: Kidney tumors induced by lead compounds

Author(s)	Pb Compound	Species	Sex	Route	Time[a]	Concentration	Total Lead Dose (g)	Tumor Incidence
van Esch and Kroes (1969)	subacetate	mouse	M	diet	24 mo	0.1%	2	6/26
			F			0.1%	2	2/25
van Esch and Kroes (1969)	subacetate	hamster	M, F	diet	24 mo	0.1%	7	M 0/22, F 0/24
						0.5%	35	M 0/22, F 0/24
Schroeder *et al.* (1970)	nitrate	rat	M	water	life	25 ppm	0.5	0/52
Zawirska and Medras (1968)	acetate	rat	M	po / feed	18 mo	3 mg/day,	1	58/94
			F			then 4 mg/day	1	14/32
Nogueira (1987)	acetate	rat	M	feed	6 mo	0.5%	9	0/12
						1.0%	17	9/10
Azar *et al.* (1973)	acetate	rat	F	diet	24 mo	0-2000 ppm	0-26	up to 13/20
Boyland *et al.* (1962)	acetate	rat	M	diet	12 mo	1.0%	34	15/16
Kasprzak *et al.* (1985)	subacetate	rat	M	feed	18 mo	1.0%	38	13/29
Koller *et al.* (1985)	acetate	rat	M	water	18 mo	2600 ppm	38	13/16
van Esch *et al.* (1962)	subacetate	rat	M, F	diet	24 mo	0.1%	10	M 5/12, F 6/13
			M, F			1.0%	97	M 6/7, F 7/9
					24 mo			
Mao and Molnar (1967)	subacetate	rat	M	diet	life	1.0%	97	31/40

[a] Time is in months unless otherwise noted.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The U.S. EPA, IARC, and the State of California have all determined that, based on animal studies, lead is a carcinogen. The relevant animal studies have been reviewed by U.S. EPA and IARC (IARC, 1980; 1987; U.S. EPA, 1986; 1989a; 1989b). U.S. EPA has classified lead and lead compounds in class B2, probable human carcinogens. This conclusion is based on sufficient animal evidence and inadequate human evidence. IARC has concluded: "There is sufficient evidence that lead subacetate is carcinogenic to mice and rats and that lead acetate and lead phosphate are carcinogenic to rats." There are inadequate human data. IARC classifies lead in Group 2B, possibly carcinogenic to humans.

The quantitative cancer risk assessment is based on the best available animal data set for risk assessment, male rat kidney tumors. The U.S. EPA Air Quality Criteria for Lead document (U.S. EPA, 1986; 1989a) examined lead's carcinogenicity but it also did not contain a formal quantitative risk assessment. OEHHA relied extensively on these U.S. EPA documents in the preparation of its quantitative cancer risk assessment.

Methodology

A large number of animal studies have shown kidney tumors following oral exposure to lead compounds (Tables 1), but there are no studies of carcinogenicity due to lead inhalation. The best tumor dose-response data for use in quantitative cancer risk assessment are those of Azar *et al.* (1973). In the Azar *et al.* study, lead as lead acetate was given to groups of male and female rats in the feed at concentrations of 0, 10, 50, 100, 500, 1000, and 2000 ppm (nominal concentrations) for 2 years. Kidney tumors, mainly adenomas, were seen in a dose-dependent relationship in the 3 highest dose groups in males. Tumors were also seen in the 2000 ppm dose group in females (7/20 or 35%). Cancer risk at ambient levels was estimated by extrapolating at least 5 orders of magnitude from these data by means of the best fitting linearized multistage model.

The data used to calculate cancer risk from the rat kidney tumors (Azar *et al.*, 1973) are given in Table 2. Doses were first converted to human equivalent doses (HED) (Anderson *et al.*, 1983). Using the computer software GLOBAL86 (Howe *et al.*, 1986), a linearized multistage model was fit to the male kidney tumor dose-response data. The male rat kidney tumor data yielded a maximum likelihood estimate (MLE) for q_1 (the linear or slope term, which relates the probability of cancer to the dose of carcinogen administered in the equation for the multistage model) of 0 $(mg/kg/day)^{-1}$, an MLE for q_2 of $2.5 \times 10^{-3} (mg/kg/day)^{-2}$, and an Upper 95% Confidence Limit (UCL) on q_1 (also known as q_1^* and as the cancer potency) of 8.5×10^{-3} $(mg/kg/day)^{-1}$.

Available human data indicate that approximately 50% of inhaled lead is absorbed compared to approximately 10% of ingested lead (summarized by Owen, 1990). If the percentage of lead absorbed by inhalation is similar for rats and humans and if the standard assumption that an average adult human has a body weight of 70 kg and an average air intake of 20 m^3 per day is used, an oral intake of 1 mg/kg/day lead is equivalent to an inhalation exposure of 3,500 $\mu g/m^3$ for 24 hr. Using

the latter units, the 95% UCL for q_1 equals 2.4×10^{-6} $(\mu g/m^3)^{-1}$, which assumes equivalent absorption by the 2 routes. If there is approximately 5 times higher absorption by the respiratory tract compared to the gastrointestinal tract (Owen, 1990), the inhalation risk can be multiplied by 5 and the corrected inhalation unit risk is 1.2×10^{-5} $(\mu g/m^3)^{-1}$.

To derive a range of risks, the study by Koller *et al.* (1985), which showed the greatest sensitivity to lead's carcinogenicity, was selected. In that study, 13 out of 16 male rats drinking water containing 2600 ppm lead acetate developed renal tumors, compared to 0 of 10 in controls. The resulting human equivalent dose (HED) was calculated as 60.1 mg/kg-day. Using the GLOBAL86 program, an MLE for q_1 of 0.0279 $(mg/kg\text{-}day)^{-1}$ and a 95% UCL, q_1^* of 0.0455 $(mg/kg\text{-}day)^{-1}$ were obtained. The latter potency was divided by 3500 to obtain a preliminary inhalation unit risk of 1.3×10^{-5} $(\mu g/m^3)^{-1}$, which, when corrected for the 5-fold greater absorption by inhalation compared to ingestion in humans (Owen 1990), yielded a final inhalation unit risk of 6.5×10^{-5} $(\mu g/m^3)^{-1}$.

Therefore, the 95% UCL obtained for the range of inhalation unit risks is 1.2×10^{-5} $(\mu g/m^3)^{-1}$ to 6.5×10^{-5} $(\mu g/m^3)^{-1}$. The best value of the cancer unit risk for air was selected as 1.2×10^{-5} per $\mu g/m^3$.

Table 2: Kidney tumors in rats fed lead[a]

Lead in food (ppm)		Animal dose (mg/kg-day)	HED[b] (mg/kg-day)	Number of rats[c]		%	% died
Added	Measured			exp[d]	tumors		
0	3	0.225	0.038	20[f]	0	0	50
0	5	0.39	0.067	100	0	0	37
10	18	1.40	0.238	50	0	0	36
50	62	4.78	0.818	50	0	0	36
100	141	10.88	1.86	50	0	0	36
500	548	42.27	7.22	50	5	10	52
1000[e]	1130	79.65	13.6	20[f]	10	50	50
2000[e]	2102	162	27.2	20[f]	16	80	80

[a] Data from Azar *et al.* (1973).
[b] Human Equivalent Dose = daily dose $\times (70/0.35)^{1/3}$.
[c] Among similar size groups of female rats, kidney tumors were seen only in 7 of 20 animals in the 2000 ppm group.
[d] Number of animals exposed to indicated level of lead in food.
[e] The rate of body weight gain was depressed in both groups. Since mortality was not increased in the 1000 ppm group, it can be considered a Maximally Tolerated Dose (MTD).
[f] The groups with only 20 rats per dose level were also studied for 2 years but were begun several months after the other dose groups.

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LINDANE (γ-Hexachlorocyclohexane)

CAS No: 58-89-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	290.9
Boiling point	323.4°C
Melting point	113°C
Vapor pressure	9.4E-6 mm Hg @ 20°C
Air concentration conversion	1 ppm = 11.9 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.1E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.1 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor reported by US EPA (1987)]

III. CARCINOGENIC EFFECTS

Human Studies

The International Agency for Research on Cancer (IARC) concluded in 1987 that the evidence for carcinogenicity of hexachlorocyclohexane (HCH; all isomers) was inadequate in humans. Several case reports suggest an association between lindane exposure and leukemia, aplastic anemia, liver cancer, soft-tissue sarcomas, and lung cancer (IARC, 1987). In all of these case reports, the exposures are not well documented. In addition, exposures to other chemicals, including some pesticides, probably occurred in these cases.

Animal Studies

Wolff *et al*. (1987) reported on the carcinogenic effects of lindane in several strains of mice. Female yellow, black, and pseudoagouti mice (36-96 per group) were exposed to 0 or 160 ppm lindane in the diet for up to 24 months. Different response rates were observed between strains, indicating significant genetic variability in response to lindane. In yellow mice, a significant increase in the incidence of lung and liver tumors was observed (Table 1). The higher incidence of tumors in the obese yellow mice indicate that bioaccumulation of lindane in obese animals may influence carcinogenicity.

Thorpe and Walker (1973) showed an increase in liver tumors of male CF1 mice fed 400 ppm lindane for 110 weeks, compared with controls. The time-weighted dose was estimated as 52 mg/kg/day by US EPA (1988). In this experiment, control mice exhibited an incidence of 11/45 for liver tumors, compared to 27/29 for the 52 mg/kg/day group.

Table 1. Tumor incidence in yellow mice exposed to γ-HCH (lindane) (Wolff *et al.*, 1987)

Dietary Concentration of γ-HCH (ppm)	Tumor Type and Incidence	
	Lung Carcinomas	Liver Adenomas
0	4/95	8/93
160	18/95	33/94

A study by Goto *et al.* (1972) showed a positive effect of lindane on cancer in mice. However, this experiment used only 10 animals per treatment group and was of a short duration. The NCI (1977) study on male mice showed a significant increase in cancer incidence in mice exposed to 80, but not 160 ppm γ-HCH. The absence of a clear dose-response precluded this data from use in determining the cancer potency for lindane.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The US EPA (1988) selected the study by Thorpe and Walker (1973) as the basis for the cancer potency for lindane. This was considered to be the best study for development of a cancer potency factor for lindane because of the large sample size of mice surviving for a full lifespan, and the large numbers of tumors in the treatment group. Thorpe and Walker (1973) showed an increase in liver tumors in male CF1 mice fed 400 ppm lindane in the diet for 110 weeks, compared with controls. Control mice exhibited an incidence of 11/45 for liver tumors, compared to 27/29 for the lindane-treated group ($p < 0.01$). Some lung metastases were also reported in the male and female mice treated with lindane.

Methodology

A linearized multistage procedure was used to estimate the cancer potency of lindane from the Thorpe and Walker (1973) data in male CF1 mice (Crump *et al.*, 1982). The concentrations of lindane given in the feed were 0 or 160 ppm. The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value for lindane.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 1.1 $[mg/kg\text{-}day]^{-1}$ was reported (US EPA, 1987). An airborne unit risk factor of 3.1E-4 $(\mu g/m^3)^{-1}$ was calculated from the q_{human} value by OEHHA/ATES using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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METHYL *TERT*-BUTYL ETHER (MTBE)

CAS No: 1634-04-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 2001)

Molecular weight	88.15
Boiling point	55.2°C
Melting point	-108.6°C
Vapor pressure	250 mm Hg at 25°C
Air concentration conversion	1 ppm = 3.6 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.6 E-7 $(\mu g/m^3)^{-1}$
Slope Factor: 1.8 E-3 $(mg/kg\text{-}day)^{-1}$
[Cancer slope factor (CSF) derived by OEHHA (1999a) from the geometric mean of the potency estimates obtained for male rat kidney adenomas and carcinomas (Chun *et al*. 1992), male rat Leydig interstitial cell tumors and female rat leukemia and lymphomas (Belpoggi *et al*. 1995, 1998) using potency values derived from the lower 95% confidence limit on the 10% tumor dose (LED_{10}) with pharmacokinetic adjustments; inhalation unit risk factor derived from the CSF by OEHHA (1999b).]

III. CARCINOGENIC EFFECTS

Human Studies

No studies regarding the carcinogenic effects of human exposure to MTBE were found in an earlier search by ATSDR (1996) or more recently by OEHHA (1999a).

Animal Studies

Oral

Male and female Sprague-Dawley rats (60/sex/group) were exposed to MTBE by gavage at doses of 0, 250 or 1,000 mg/kg body weight/day, four days/week for 104 weeks (Belpoggi *et al*., 1995, 1997, 1998). Animals were maintained until natural death; the last animal died at 174 weeks of age. A dose-related increase in the combined incidence of lymphomas and leukemia was observed in female rats (Table 1). The authors reported that the increase was highly significant ($p < 0.01$) in the high-dose group and marginally significant in the low-dose group, when analyzed using a log-ranked test. When analyzed using the Fisher exact test, the combined incidence of lymphomas and leukemia in high-dose females was significantly different from controls at the $p = 0.001$ level. Historical control incidence rates in this laboratory for lymphomas and leukemias (combined) was < 10% in female Sprague-Dawley rats (Belpoggi *et al*., 1995). Testicular Leydig cell tumor incidence was also significantly increased [$p = 0.05$, using a prevalence analysis for nonlethal tumors (Hoel and Walburg 1972)] in high-dose males.

Table 1: Tumors in Sprague-Dawley Rats exposed to MTBE by gavage (Belpoggi *et al*., 1995, 1997, 1998)

Tumor site	Tumor type	Tumor incidence Dose (mg/kg/day)		
Females		0	250	1000
Hemolymphoreticular tissues (including mesenteric lymph nodes)	Lymphomas and leukemias (Belpoggi *et al*., 1995)	2/58[b] (3.4%)	6/51[b] (11.8%)	12/47[b,c,d, e] (25.5%)
	Lymphomas and leukemias of lymphoid origin (Belpoggi *et al*., 1998)	2/58[b] (3.4%)	7/51[b] (13.7%)	12/47[b,d,e] (25.5%)
Males				
Testes	Leydig interstitial cell tumors (Belpoggi *et al*., 1995)	2/26[f] (7.7%)	2/25[f] (8.0%)	11/32[f, g, h] (34.4%)
	Leydig interstitial cell adenomas (Belpoggi *et al*., 1998)	3/26[f] (11.5%)	5/25 [f] (20.0%)	11/32[f, h] (34.4%)

[a] Administered in olive oil, four days per week, for 104 weeks.
[b] Number of lesion-bearing animals/total alive at 56 weeks of age, when the first leukemia was observed.
[c] Incidence relative to control group was significant ($p < 0.01$) using a log-ranked test (Mantel 1966, Cox 1972), as reported by Belpoggi *et al*. (1995).
[d] Incidence relative to control group was significant by the Fisher exact test ($p = 0.001$).
[e] Dose-related trend was significant by the Cochran-Armitage trend test ($p < 0.01$).
[f] Number of lesion-bearing animals/total alive at 96 weeks of age, when the first Leydig cell tumor was observed.
[g] Incidence relative to control group was significant ($p = 0.05$) level using prevalence analysis for nonlethal tumors (Hoel and Walburg 1972), as reported by Belpoggi *et al*. (1995).
[h] Incidence relative to control group was significant by the Fisher exact test ($p < 0.05$).

A pathology review was later published (Belpoggi *et al*., 1998) in which slides from the original study were re-examined, and diagnostic criteria reviewed. This was undertaken by an independent panel of the Cancer Research Centre (where the study authors were based), assisted by an outside pathologist. Tumor incidences according to the review are also presented in Table 1. Both observed types of tumor were reexamined:

Testicular tumors: Diagnosis was carried out according to criteria developed by the National Toxicology Program (NTP), and adenomas and hyperplasia were reported separately. In addition, adenomas were further characterized as having single or multiple histiotypes, and the number of

multifocal adenomas in each dose group was reported. The results confirmed the diagnosis of the Leydig cell tumors as adenomas, as initially reported. According to the NTP diagnostic criteria, the incidence of Leydig cell adenomas was 3, 5, and 11 in the control, low- and high-dose groups, respectively, compared to the originally reported incidences of 2, 2, and 11 in control, low- and high-dose animals. The review indicated that all four multifocal adenomas observed occurred in the high-dose group. No dose-related increase in atrophy or testicular tissue degeneration was observed. Therefore, the tumors were not considered likely to be secondary to cell death.

Lymphoid tumors: The cell type of origin and tumor sites were reported. All neoplasms were of lymphoid origin. Corrected incidences were 2, 7 and 12 in the control, low- and high-dose groups, respectively, compared to the previously reported incidence data of 2, 6 and 12 in the same groups. Cancers were classified as lymphoblastic lymphomas, lymphoblastic leukemias and lymphoimmunoblastic lymphomas. The latter category was the most prevalent, accounting for 1, 6 and 8 of the tumors observed in the respective dose groups. The data on site distribution indicated that most animals with lymphoid cancers were affected at multiple sites. The tissues involved in treated animals were lung, liver, spleen and lymph node, and "other", with the lung being the most commonly affected site in treated animals.

Inhalation

Male and female Fischer 344 rats (50 animals/sex/group) were exposed to target concentrations of 0, 400, 3000, or 8000 ppm MTBE by inhalation (actual concentrations of 403, 3023, or 7977 ppm) (Chun *et al*., 1992; Bird *et al*., 1997). The animals were exposed for 6 hours/day, 5 days/week for 24 months, except for the mid- and high-dose males, which were terminated at 97 and 82 weeks, respectively, due to a dose-dependent increased mortality rate from chronic progressive nephropathy. Low-dose males also experienced an increase in nephropathy that was associated with a slight increase in mortality and a decrease in survival. Survival times for females were not significantly different between exposed and control rats. However, there were slightly more deaths due to chronic progressive nephropathy in the mid- and high-dose females than in the low-dose and control females. Exposure-related increases in kidney and liver weights were reported in mid- and high-dose females, but not in males. Chun *et al*. (1992) concluded that the maximum tolerated dose (MTD) was exceeded in both sexes at high- and mid-dose levels, based on increased mortality. Other observed effects of MTBE exposure included anesthetic effects in rats of both sexes in the mid- and high-dose groups.

A detailed histopathology examination was performed on all animals in the control and high-dose groups, and on all animals that died or were sacrificed moribund. Only a limited histopathology examination was performed on non-moribund animals from the low- and mid-dose groups that survived to terminal sacrifice; for males, only the liver, kidneys, testes and gross lesions were evaluated, while for females, only the liver and gross lesions were examined microscopically (Bird *et al*. 1997).

At the request of the MTBE Task Force, Experimental Pathology Laboratories, Inc. (1993) reevaluated the histopathologic slides of kidneys from all male and female rats used in the Chun *et al*. (1992) study, and confirmed the study pathologist's conclusion that MTBE increased the severity of chronic progressive nephropathy in rats of both sexes. No histopathologic reevaluation

of the kidney tumors was performed. In males, a statistically significant increase in renal tubular adenoma and carcinoma combined) was observed in the mid-dose group (Table 2). In high-dose males renal tubular adenomas were increased, however, this increase did not reach statistical significance (Table 2). The sensitivity of the bioassay to detect a dose-related increase in renal tumors in the high-dose group is likely to have been reduced by the high rate of early mortality, and the early termination of this treatment group at week 82. Despite the reduced sensitivity of the bioassay, a statistically significant increase in Leydig interstitial cell testicular tumors was observed in mid- and high-dose males, with a clear dose-response evident (Table 2). Historical laboratory control values for Leydig testicular tumors in Fischer rats ranged from 64 to 98% (Bird *et al*. 1997).

Table 2: Tumor incidence in male Fischer 344 rats exposed to MTBE by inhalation (Chun *et al*., 1992; Bird *et al*., 1997)

Tumor site and type		Concentration (ppm)			
		0	400	3000	8000
Kidney	renal tubular adenoma	1/35[c]	0/32[c]	5/31[c]	3/20[c]
	renal tubular carcinoma	0/35[c]	0/32[c]	3/31[c]	0/20[c]
	renal tubular adenoma and carcinoma (combined)	1/35[c]	0/32[c]	8/31[c,d]	3/20[c]
		(3%)	(0%)	(26%)	(15%)
Testes	Leydig interstitial cell tumors	32/50	35/50	41/50[e]	47/50[f]
		(64%)	(70%)	(82%)	(94%)

[a] Mid- and high-dose animals were terminated at 97 and 82 weeks, respectively, due to a dose-dependent increased mortality rate from chronic progressive nephropathy.
[b] Administered as MTBE vapor six hours per day, five days per week.
[c] Survival-adjusted tumor incidence rates were used to attempt to control for excess early mortality in the mid- and high-dose groups (US EPA, 1995c).
[d, e, f] Incidence relative to control group was significant by the Fisher Exact test ([d] $p < 0.01$, [e] $p < 0.05$, [f] $p < 0.001$).

In female Fischer 344 rats exposed to MTBE vapor, a single rare renal tubular cell adenoma was observed in one mid-dose animal; no treatment-related increases in tumor incidence were observed (Chun *et al*. 1992, Bird *et al*. 1997). However, MTBE treatment of females was associated with several nonneoplastic kidney lesions. Both female and male rats exposed to MTBE experienced a dose-related increase in mortality from chronic progressive nephropathy. Increases in microscopic kidney changes indicative of chronic nephropathy were seen in all treated males and in mid- and high-dose females. All treated males had increases in the severity of mineralization and interstitial fibrosis of the kidney, while increases in mild to moderate glomerulosclerosis, interstitial fibrosis, and tubular proteinosis were observed in females.

Groups of 50 male and 50 female eight-week old CD-1 mice were exposed to 0, 400, 3000 or 8000 ppm MTBE vapor by inhalation (corresponding to analytical mean concentrations of 402, 3014 or 7973 ppm or 1442, 10816, or 28843 mg/m^3) (Burleigh-Flayer *et al*., 1992; Bird *et al*., 1997). The animals were exposed for six hours/day, five days/week for 18 months. Increased mortality and decreased mean survival time were observed only for male mice in the high-dose group. A slightly

increased frequency of obstructive uropathy, a condition that occurs spontaneously in this mouse strain, was observed in high-dose males. However, deaths due to the condition were within the range noted for historical controls. Body weight gain and absolute body weights were decreased in high-dose males and females. Dose-dependent increases in liver weights were observed in both sexes. Kidney weights were increased in high-dose females and in low- and mid-dose males.

Burleigh-Flayer *et al*. (1992) concluded that the MTD was exceeded in both sexes at the high-dose level. Other observed effects of MTBE exposure included anesthetic effects in mice of both sexes in the mid- and high-dose groups.

A detailed histopathology examination was performed on all animals in the control and high-dose groups, and on all animals that died or were sacrificed moribund. Only a limited histopathology examination was performed on non-moribund animals from the low- and mid-dose groups that survived to terminal sacrifice; for males, only the liver, spleen and submandibular lymph nodes were evaluated, while for females, only the liver, uterus and stomach were examined microscopically (Bird *et al*. 1997).

In females, a statistically significant increased incidence of hepatocellular adenomas was observed in the high-dose group (Table 3). The incidence of hepatocellular adenomas and carcinomas (combined) was also increased in high-dose females; however, only two hepatocellular carcinomas were reported, one each in the low- and high-dose groups. In males, a statistically significant increase in hepatocellular carcinomas was observed in the high-dose group (Table 3). Bird et al. (1997) noted that the combined incidence of adenomas and carcinomas in high-dose males was similar to the historical incidence for male CD-1 mice of 33%. However, after correcting for the number of animals alive at 49 weeks, when the first hepatocellular adenoma was observed in males, the incidence in the high-dose group was 43%
(16/37, see Table 3), representing a clear increase above the cited historical incidence in male CD-1 mice. Burleigh-Flayer et al. (1992) concluded that the increased incidence of liver tumors in the high-dose groups (adenomas in females and carcinomas in males) could be attributed to MTBE exposure. The ability of this study to detect increases in tumor incidence was likely decreased by the shortened study length (18 versus 24 months).

Table 3: Tumor incidence in CD-1 mice exposed to MTBE by inhalation (Burleigh-Flayer *et al*. 1992, Bird *et al*. 1997)

Tumor site and type	Dose[b] (ppm)			
	0	400	3000	8000
Females				
Liver hepatocellular adenoma	2/50	1/50	2/50	10/50[c]
hepatocellular carcinoma	0/50	1/50	0/50	1/50
hepatocellular adenoma and carcinoma (combined)	2/50	2/50	2/50	11/50[d]
Males				
Liver hepatocellular adenoma	11/47[e]	11/47[e]	9/46[e]	12/37[e]
hepatocellular carcinoma	2/42[f]	4/45[f]	3/41[f]	8/34[c,f]
hepatocellular adenoma and carcinoma (combined)	12/47[e]	12/47[e]	12/46[e]	16/37[e]

[a] Male mice in the high-dose group experienced early mortality.
[b] Administered as MTBE vapor six hours per day, five days per week.
[c,d] Incidence relative to control group was significant by the Fisher Exact test ([c] $p < 0.05$, [d] $p < 0.01$).
[e] Number of lesion-bearing animals per total alive at 49 weeks, when the first hepatocellular adenoma was observed.
[f] Number of lesion-bearing animals per total alive at 63 weeks, when the first hepatocellular carcinoma was observed.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

No human studies suitable for derivation of a cancer potency for MTBE have been reported. However, there is evidence for the carcinogenicity of MTBE at multiple sites in both sexes of rats (oral and inhalation exposure) (Chun *et al*., 1992; Belpoggi *et al*., 1995, 1997, 1998; Bird *et al*., 1997) and mice (inhalation exposure) (Burleigh-Flayer *et al*. 1992; Bird *et al*., 1997). These studies provide data from which cancer potency values can be derived.

Methodology

OEHHA (1999a) derived cancer slope factors (CSFs) from the rat oral and inhalation exposure (Chun *et al*., 1992; Belpoggi *et al*., 1995, 1997, 1998; Bird *et al*., 1997) and mouse inhalation exposure (Burleigh-Flayer *et al*. 1992; Bird *et al*., 1997) tumor incidence data (Table 4). The CSF is a potency value derived from the lower 95% confidence limit on the 10% dose that is predicted to give a 10% tumor incidence (LED_{10}). A multistage polynomial was used to fit data in the observable range. The CSF is equal to $0.1/LED_{10}$, in units of $(mg/kg\text{-}day)^{-1}$. For the curve fitting to estimate the LED_{10}, a $p \geq 0.05$ criterion for the Chi-squared goodness of fit statistic of the optimized polynomial was employed. Interspecies scaling for oral doses (and internal doses calculated from a single-species pharmacokinetic model) was based on $(body\ weight)^{3/4}$.

For inhalation exposures OEHHA and other risk assessors have in the past used an assumption of equivalence between different species of exposures to a given atmospheric concentration. This provides roughly similar scaling in effect, due to the way that breathing rate and related parameters affecting uptake scale with body weight. More recently, physiologically-based pharmacokinetic (PBPK) modeling has been seen as a preferable approach to both dose estimation and interspecies scaling of inhalation exposures, where data are available to support this. Since pharmacokinetic data are available for MTBE in the rat, the modeling approach was feasible in this case for that species only.

Due to the lack of a clear mode of action of TBA or other MTBE metabolites in MTBE-induced carcinogenesis in experimental animals, OEHHA treated the parent compound MTBE as the cause of the observed effects in animal studies for the purpose of determining dose metrics. In order to estimate internal doses of MTBE, in addition to simple continuous applied doses, a simplified PBPK model was employed. This model was based on both the Borghoff *et al*. (1996a) model, in that it has five compartments for MTBE and five compartments for TBA, and the Rao and Ginsberg (1997) model with its MTBE metabolic parameters and slowly perfused compartment/blood partition coefficient for TBA. The PBPK model employs compartments loosely representing "Fat, Liver, Kidneys, Muscle, and rapidly perfused tissues termed as Vessel Rich Group (VRG)". The model's fundamental structure was based on that developed by Hattis *et al*. (1986) for perchloroethylene and was formulated in Stella software (ithink v. 3.0.6b for the Power Macintosh, High Performance Systems Inc., Hanover, New Hampshire 03755). The model units for the whole animal were moles, L, moles/L, hour, moles/hour, L/hour, and ppm in alveolar air. Simulations of up to 32 hours were run at approximately 1,000 steps per simulated hour, using the Runge-Kutta four computation method. The model parameters were obtained from Borghoff *et al*. (1996a) or Rao and Ginsberg (1997). In addition to simulations of the pharmacokinetic data of Miller *et al*. (1997) with a model 0.22 kg rat, simulations of cancer bioassay doses were conducted assuming 0.35 kg for female and 0.5 kg for male lifetime average body weights. Physiological and metabolic parameters were scaled to these body weights as described in Borghoff *et al*. (1996a).

The data for kidney tumors in the high dose (8,000 ppm) male rats in the study by Chun *et al*. (1992) were excluded so that an adequate fit could be obtained. Results in the inhalation studies (Chun *et al*. 1992, Burleigh-Flayer *et al*. 1992) were effectively the same (within a factor of two) for the different sites in rats and mice, except that the potency for testicular interstitial cell tumors in male rats is about five times higher (Table 4a). Comparison between different routes and experiments for the rat was facilitated by examining the data calculated using the pharmacokinetic model to convert the inhalation exposures to equivalent oral doses. In this case it was apparent that all the results are comparable, with the testicular interstitial cell tumors in the Chun *et al*. (1992) males again showing a slightly higher value than those found at other sites or in the testis in the Belpoggi *et al*. (1995, 1997, 1998) oral study.

Carcinogen risk assessment guidelines used by OEHHA normally recommend selection of human cancer potency estimates based on the most sensitive site and species, unless there is evidence to indicate that the most sensitive site(s) are not relevant to human cancer induction, or represent data sets with unusually wide error bounds. As an alternative, where several equally plausible results

are available and are sufficiently close to be regarded as concordant, the geometric mean of all such estimates may be used.

Pharmacokinetic modeling, which would allow the comparison of different routes and correct for nonlinearities in the relationship between applied and internal dose, was not available for the mouse. Therefore, the potency estimates obtained in the rat were preferred for risk assessment purposes. Because the results in rats and mice are comparable, the use of the rat data was consistent with the policy of selecting appropriately sensitive species as the basis for the estimate of potency in humans.

Table 4: Dose Response Parameters for MTBE Carcinogenicity Studies

a) Inhalation studies - ppm in air as dose metric

Species	Sex	Tumor site and type	LED_{10} (ppm)	CSF (ppm^{-1})
mouse[a]	female	hepatocellular adenoma + carcinoma	320	3.2×10^{-4}
	male	hepatocellular adenoma + carcinoma	140	7.0×10^{-4}
rat[b]	male	renal tubular cell adenoma + carcinoma	240	4.2×10^{-4}
		testicular interstitial cell tumors	46	2.2×10^{-3}

[a] Burleigh-Flayer *et al*. 1992, Bird *et al*. 1997
[b] Chun *et al*., 1992; Bird *et al*., 1997.
Assumed: Data reassessment by U.S. EPA (1994c, 1995c).
Duration correction based on $(t_e/t_l)^3$: t_l = 104 weeks for both rats and mice.
Interspecies correction: ppm equivalency.

b) Rat oral and inhalation studies - Equivalent oral dose as dose metric

Route	Sex	Tumor site and type	LED_{10} (mg/kg-day)	CSF $(mg/kg/day)^{-1}$
Inhalation[a]	Male	Male renal tubular cell adenoma + carcinoma	55	1.8×10^{-3}
		Testicular interstitial cell tumors	11	8.7×10^{-3}
Gavage[b]	Male	Leydig cell tumors		
		Original 1995 report	76	1.38×10^{-3}
		Revised 1998 data	64	1.55×10^{-3}
	Female	Leukemia/lymphoma		
		Original 1995 report	49	2.03×10^{-3}
		Revised 1998 data	48	2.09×10^{-3}

[a] Chun *et al*., 1992.
[b] Belpoggi *et al*., 1995, 1998.
Assumed: Data reassessment by U.S. EPA (1994c, 1995c) for Chun *et al*. (1992) study.
Table 4 (continued): Dose Response Parameters for MTBE Carcinogenicity Studies

Duration correction based on $(t_e/t_l)^3$: t_l = 104 weeks for rats.

Interspecies correction: $BW^{3/4}$.

In terms of the relevance to human cancer and the mechanism of the observed effects, the results of the studies by Chun *et al*. (1992) and Burleigh-Flayer *et al*. (1992) are limited by the relatively severe mortality seen in the highest dose groups, and the less-than lifetime exposure given the mice and the male rats. These experimental flaws are not so severe as to exclude the use of the data in risk assessment, nor more prohibitive than the experimental flaws associated with many studies on other compounds that have been successfully used for this purpose. There are, however, additional problems in the case of the testicular interstitial cell tumors observed in male rats by Chun *et al*. (1992). The study authors stated that the control incidence of these tumors was lower than the historical incidence observed in animals from the colony from which these experimental animals were obtained. In view of this, the slightly divergent value for the potency estimate obtained with this data set was regarded with lower confidence than the other values obtained in this analysis, and was not included in the determination of a recommended potency.

In view of the closeness of the other values obtained in the rat, and their similar confidence levels, the preferred value for the cancer potency was therefore the geometric mean of the potency estimates obtained for the male rat kidney adenomas and carcinomas combined (1.8×10^{-3} $(mg/kg\text{-}day)^{-1}$) (Chun *et al*. 1992), and the male rat Leydig interstitial cell tumors (1.55×10^{-3} $(mg/kg\text{-}day)^{-1}$) and the leukemia and lymphomas in female rats (2.09×10^{-3} $(mg/kg\text{-}day)^{-1}$) (Belpoggi *et al*. 1995, 1998) (Table 4b). The combined use of these data yields an estimated CSF of 1.8×10^{-3} $(mg/kg\text{-}day)^{-1}$.

Since a pharmacokinetic model was not available for MTBE uptake, distribution and metabolism in humans, default assumptions were used to extrapolate from risk estimates in the experimental animals (rats) to the human situation. The CSF was calculated as an "oral equivalent" potency, in mg/kg-day. It is assumed that low oral doses would be essentially 100% absorbed, so no correction is required when this potency is used to estimate risks from oral exposures. However, in order to estimate risks from inhalation exposures it is necessary to have an estimate of the percentage uptake of inhaled MTBE at low atmospheric concentrations. OEHHA (1999b) assumed that humans absorb 50% of inhaled MTBE at low doses. This estimate is derived primarily from the 42 to 49% respiratory uptake observed among 10 healthy male volunteers inhaling 5 to 50 ppm for 2 hours (Nihlen *et al.,* 1998). This represents a small study of short duration exposures to relatively high concentrations of MTBE, and respiratory uptake among humans inhaling low concentrations for long periods is unknown. As absorption at low concentrations may in some cases be greater than that at higher concentrations, the assumption of 50% absorption should be viewed as a best estimate rather than an upper-bound estimate or health-protective assumption.

On this basis, and assuming a 70 kg human inhaling 20 m^3 per day, the oral CSF described above was converted to an inhalation potency estimate (cancer unit risk factor or URF) of 9.3×10^{-7} ppb^{-1}, or 2.6×10^{-7} $(\mu g/m^3)^{-1}$ (OEHHA, 1999b).

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4, 4'-METHYLENE BIS(2-CHLOROANILINE) (MOCA)

CAS No: 101-14-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	267.15
Boiling point	not available
Melting point	110°C
Vapor pressure	1.3×10^{-5} mm Hg at 60°C
Air concentration conversion	1 ppm = 10.9 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.3 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.5 E+0 $(mg/kg\text{-}day)^{-1}$
[Female beagle dog urinary bladder tumor data (Stula *et al*., 1977) , contained in Gold *et al*. (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

IARC (1993) has reviewed several descriptive studies on the potential carcinogenic effects of 4, 4'-methylene bis(2-chloroaniline) (MOCA) in humans. An epidemiological study by Ward *et al.* (1990) examined cancer incidence in workers employed at a chemical plant in Michigan where MOCA was produced between 1968 and 1979. All 532 workers employed in 1968-79 and an additional 20 workers employed in 1980-81 who had possible exposure due to plant site contamination were included. Median duration of employment was 3.2 months. Quantitative exposure was not available; however, worker exposure may have been substantial, since worker urinary levels of MOCA several months after the end of production ranged up to 50,000 µg/l. Telephone interviews were conducted with 452 workers, and 385 participated in a urine screening examination. Three asymptomatic bladder tumors were identified. The screening procedure was supplemented for some workers with cytoscopy after a 28-year old worker was found to have a non-invasive papillary transitional-cell tumor. Low-grade papillary transitional cell carcinoma was diagnosed in 2 of 200 examined workers; one was less than 30 years old. Mean interval time from first exposure to study initiation was 11.5 years, while the latency period for most bladder carcinogens is about 20 years (Ward *et al*., 1990). This finding increases the concern that MOCA is a human bladder carcinogen, since bladder carcinoma in young men is very uncommon. A limitation of this study was that expected numbers of bladder tumors could not be calculated, as no data exists on the incidence of bladder tumors diagnosed by cytoscopy in an asymptomatic nonexposed population.

Animal Studies

Male and female HaM/ICR mice and male Charles River CD-1 rats (25/sex/species/group) were exposed to MOCA hydrochloride in the diet for 18 months by Russfield *et al*. (1975). Mice were fed diet containing 0, 1000 or 2000 mg/kg diet MOCA hydrochloride; rats were fed diet containing 0, 500 or 1000 mg/kg diet MOCA hydrochloride. Surviving animals were killed after 24 months; about 55% of the control and treated animals were still alive at 20-22 months. Hemangiomas or hemangiosarcomas were noted in 0/10 control, 3/13 low dose and 8/20 high dose male mice; hepatomas were noted in 0/20 control, 9/21 low dose and 7/14 high dose female mice ($p < 0.01$, Fisher exact test), and in 0/22 control, 1/22 low dose and 4/19 high dose rats ($p < 0.05$, Cochran-Armitage trend test).

Male and female Charles River CD rats (50/sex/group) were fed diet containing 0 or 1000 mg/kg diet MOCA in a standard diet (23% protein) for life (Stula *et al*., 1975). Average experiment duration was 80 weeks for treated and control males, 89 weeks for female controls and 78 weeks for treated females. Six animals from each group were killed for an interim evaluation at one year. Lung carcinomas were observed in 21/44 treated males ($p < 0.05$, χ^2 test) and in 27/44 treated females ($p < 0.05$, χ^2 test); a lung squamous-cell carcinomas was also observed in one treated male and female. Pleural mesotheliomas occurred in 4/44 treated males and 2/44 treated females. Hepatocellular adenomas and carcinomas occurred in 3/44 and 3/44 treated males and 2/44 and 3/44 treated females, respectively. No lung tumors, pleural mesotheliomas or hepatocellular adenomas and carcinomas were noted in control animals.

Male Charles River CD rats were fed a "protein-adequate" diet containing 0, 250, 500 or 1000 mg/kg diet MOCA (group sizes 100, 100, 75 and 50, respectively) for 18 months followed by a 32 week observation period (Kommineni *et al*., 1979). MOCA exposure was associated with decreased survival; mean survival time was 89, 87, 80 and 65 weeks for controls, low-dose, mid-dose and high-dose animals, respectively. Dose-related increases in the incidences of lung tumors, mammary adenocarcinomas, Zymbal gland adenocarcinomas and hepatocellular carcinomas were noted. Tumor incidence data is listed in Table 1.

Table 1. 4, 4'-methylene bis(2-chloroaniline) (MOCA)-induced tumor incidence in male Charles River CD rats (Kommineni *et al*., 1979)

Tumor type	dietary MOCA (mg/kg diet)			
	0	250	500	1000
lung tumors	1/100	23/100	28/75	35/50
Zymbal gland carcinomas	1/100	8/100	5/75	11/50
mammary adenocarcinomas	1/100	5/100	8/75	14/50
hepatocellular carcinomas	0/100	3/100	3/75	18/50

Stula *et al*. (1977) exposed a group of 6 female beagle dogs to a daily dose of 100 mg MOCA by capsule 3 days/week for 6 weeks, then 5 days/week for up to 9 years. A second group of 6 females served as untreated controls. One treated dog died at 3.4 years of age because of an infection. The other animals were killed at 8.3-9 years. Transitional-cell carcinomas of the urinary bladder

occurred in 4 of 5 treated dogs ($p < 0.025$, Fisher exact test), and a composite tumor (transitional-cell carcinoma/adenocarcinoma) of the urethra was noted in one dog. No urinary tract tumors were noted in the untreated controls. Tumor incidence data is listed in Table 2.

Table 2. 4, 4'-Methylene bis(2-chloroaniline) (MOCA) -induced urinary bladder tumor incidence in female beagle dogs (Stula *et al*., 1977)

Average Dose[1] (mg/kg-day)	Tumor Incidence[2]
0	0/6
7.31	4/5

1. Doses as reported by Gold *et al*. (1984).
2. Tumor incidences as reported by Gold *et al*. (1984)

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Results from a number of studies using Charles River CD and Wistar rats, as well as female beagle dogs, are listed in Gold *et al*. (1984). 4, 4'-Methylene bis(2-chloroaniline) induced papillary transitional cell carcinomas of the urinary bladder in dogs, whereas the liver was the most common target site in the rat studies. Dogs are more sensitive to the carcinogenic effects of the compound than rats. The compound is similar in structure to benzidine, a human bladder carcinogen, which appears to be significantly more potent in humans than rodents. Results from the Stula *et al*. (1977) dog study are used as the basis of potency estimation, despite the small numbers of animals used, because dogs may be better predictors of human carcinogenicity of this compound than rodents (Cal/EPA, 1992). Dose-response data are listed in Table 2.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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METHYLENE CHLORIDE

CAS No: 75-09-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	84.9
Boiling point	39.7°C
Melting point	-95.1°C
Vapor pressure	400 mm Hg @ 24°C
Air concentration conversion	1 ppm = 3.47 mg/m^3@ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.0 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 3.5 E-3 $(mg/kg\text{-}day)^{-1}$
[Calculated from female mouse lung tumors (NTP, 1986; Mennear *et al.* 1988) using a linearized multistage procedure (CDHS, 1989) with high-to-low dose adjustment for saturation of mixed-function oxidase pathway (Cohn, 1987)]

III. CARCINOGENIC EFFECTS

Human Studies

Friedlander and colleagues studied male workers employed by the Eastman Kodak Company at its film-making operation in Rochester, New York, where MC was used as the primary solvent (Friedlander *et al.,* 1978; Hearne and Friedlander, 1981; Friedlander *et al.,* 1985; Friedlander *et al.*, 1986; Hearne *et al.,* 1987). Industrial hygiene surveys conducted there had found airborne levels of methylene chloride in the workroom ranging, in general, from 30 to 100 ppm. As control groups, the researchers considered (1) males in New York State not including New York City and (2) other males employed by Kodak in Rochester.

The 1985 report from Friedlander and colleagues noted that overall, significantly fewer deaths occurred during the follow-up period (January 1964 through December 1984) than expected based on the New York State data (165 vs. 231.1) (Friedlander *et al.,* 1985). However, the number of deaths among exposed workers was similar to that of the other Kodak employees (165 versus 167.0). Thirty-nine deaths from malignant neoplasms were observed in the exposed cohort compared to 54.7 expected based on the New York State data or 43.2 expected on the basis of the data from other Kodak employees. The follow-up rate for this report was 94% for the exposed cohort (N = 751). The above findings are consistent with the "healthy worker effect," in which working populations tend to experience lower mortality than the general population.

Using the statistical tests (one-tailed and two-tailed test of significance) employed by investigators, no cancer site displayed a significantly elevated death rate. Nevertheless, eight deaths from pancreatic cancer were observed compared to 3.0 expected based on the New York data or 2.6

based on the other employees' data. Although this finding was not considered significant based on the two-tailed test described above, an exact one-sided Poisson test using the other Kodak employees as the control group yields a *p*-value of 0.0053 and suggests a possible relationship between the exposure to MC and pancreatic cancer mortality. Somewhat smaller-than-expected death rates were observed for certain tumor sites, including the colon, and the genital and urinary organs; these observations did not reach statistical significance.

In the subset of the cohort which had been exposed for a minimum of 20 years by 1964 (N = 252), fewer cancer deaths were observed than expected when compared to the New York State data (23 vs. 33.8) or to the data from other Kodak employees (23 versus 27.0). A slight excess of deaths from pancreatic neoplasms was observed here (4 compared to 1.9 or 1.6 expected). None of these differences were statistically significant.

Hearne *et al.* (1987) presented estimates of exposure and expanded the exposed cohort to 1,013 men who had at least one year of experience in the methylene chloride operation between January 1964 and December 1970. The 1985 report analyzed approximately 14,000 person-years of follow-up in the exposed cohort; this 1987 report presented data from 19,465 person-years, with a follow-up of 99% for the exposed cohort. Again, compared to either control group, no statistically significant difference was found between observed and expected deaths for respiratory or hepatic cancer mortality, based on a one-sided test, ($p < 0.05$). Among the nonhypothesized outcomes, workers exposed to methylene chloride still experienced more than a two-fold greater rate of mortality from pancreatic cancer (8 observed vs. 3.2 or 3.1 expected [New York State or Kodak controls]). The SMR for this site was 2.5 with 95% confidence limits of 1.1 to 4.9. DHS staff calculations indicate an exact one-sided Poisson test using the New York State data as a control yields a p-value of 0.017. Thus, the results still suggest a possible relationship between exposure to methylene chloride and pancreatic cancer mortality. Exposure estimates were based on over 1,200 area and task-specific air samples collected between 1945 and 1986 and more than 900 full-shift personal samples collected between 1980 and 1986. The majority of the cohort was exposed to peak concentrations of 500 ppm, an average of three times per day, 10 or 40 days per year (depending on occupational classification). Other solvents, including 1,2-dichloropropane and 1,2-dichloroethane, were present at lower levels in the workroom.

In a series of reports, Ott and colleagues (Ott *et al.,* 1983a-e) evaluated the health of employees working at a fiber production plant in Rock Hill, South Carolina, where methylene chloride was used as a part of a solvent system. Workers in this plant were exposed to a mixture of methylene chloride and methanol from one process and to acetone from a second process. Median 8-hr TWA methylene chloride concentrations in the plant's two main work areas were 140 and 475 ppm. Health evaluations of these employees were compared to those of employees at a similar fiber production plant in Narrows, Virginia, where only acetone was used. One of the reports (Ott *et al.,* 1983b) evaluated whether exposure to methylene chloride was associated with cancer deaths. Smoking habits were not considered in the analyses, although age was controlled for. Compared with United States death rates, no excess mortality from any cause in either cohort was evident. A slight deficit in the observed deaths from cancer was noted among exposed white workers (7 observed versus 12.4 expected). The cancer mortality experience of the methylene chloride-exposed workers was not significantly difference from that of the reference cohort. One death

from pancreatic cancer was reported in the exposed cohort. Of the seven cancer deaths in the reference cohort, one was from pancreatic cancer (Bond, 1988).

This study is of somewhat limited usefulness for evaluating the association between exposure to methylene chloride and cancer mortality. It had limited power to detect increases in malignancy rates in the exposed cohort. In that cohort, only 54 deaths, of which 7 were from cancer, were reported. There were no deaths observed among the 108 nonwhite women in the study. In addition, the follow-up period was probably insufficient for any carcinogenic effects from exposure to methylene chloride to be manifested.

The International Agency for Research on Cancer (IARC, 1986) in their review of epidemiological studies has concluded that no excess risk of death from malignancies was observed, but noted that the studies had a limited power to detect excess risk. To date, epidemiological studies on methylene chloride do not provide sufficient evidence either to prove or disprove human carcinogenicity.

Animal Studies

There are five long-term rodent bioassays examining the effects of inhaling methylene chloride: two using Spartan/Sprague-Dawley (SD) rats (Burek *et al.,* 1984; U.S. EPA, 1985a; Nitschke *et al.,* 1988a), one with Fischer-344 (F344) rats (NTP, 1986), one using $B6C3F_1$ mice (NTP, 1986), and one with Ela:Eng (Syr) Syrian hamsters (Burek *et al.,* 1984). The NTP (1986) studies have also been published by Mennear *et al.* (1988). Bioassays exposing animals orally to MC in drinking water were conducted in F344 rats and $B6C3F_1$ mice (Serota *et al.,* 1986a-b).

In the Dow (1980) study, male and female Spartan/Sprague-Dawley (SD) rats were exposed to methylene chloride by inhalation for two years (Burek *et al.,* 1984; U.S. EPA, 1985a). During the first two months of the study, there was an outbreak of sialodacryoadenitis virus infection involving control and exposed rats. This virus has been associated with acute inflammation and diffuse coagulative necrosis of the parotid and submandibular salivary glands and Harderian gland (Burek *et al.,* 1984). The carcinogenic end points in this study were sarcomas arising in the cervical/salivary gland area in males and benign mammary tumors in both sexes (Table 1). Burek *et al.* (1984) suggested that the combinations of this viral infection and exposure to high concentrations of methylene chloride may have been associated with the salivary gland tumors. However, F344 rats exposed to higher methylene chloride concentrations and the same viral agent did not develop similar tumors in the salivary gland region (NTP, 1986; Mennear *et al.,* 1988). Control and exposed female rats all had a high incidence (above 80%) of benign mammary tumors.

Table 1: Methylene chloride-induced tumor incidence in Sprague-Dawley rats.

Dose (ppm)	Cervical/salivary gland region sarcomas in male rats	Benign mammary tumors	
		Males	Females
0	1/93 (1%)	7/95 (7%)	79/96 (82%)
500	0/94	3/95 (3%)	81/95 (85%)
1500	5/91 (5.5%)	7/95 (7%)	80/96 (83%)
3500	11/88 (12.5)*	14/95 (15%)	83/97 (86%)
HLC[1]	0-2%	10%	80%

[1] historical laboratory control

* $p < 0.05$, Fisher's exact probability test

In another inhalation study at Dow (Nitschke *et al.,* 1988a) 90 SD rats/sex were exposed to 0 (control), 50, 200, and 500 ppm methylene chloride, 6 hours/day, 5 days/week, for 20 (males) or 24 (females) months. Most of the animal husbandry conditions were similar to the previous rat study with the exception that the animals were housed in conventional animal rooms overnight and during the weekends instead of in the chamber rooms. Female rats kept in chambers 100% of the time were reported to have higher incidence of mammary tumors than in conventional animal rooms (Nitschke *et al.,* 1988a). In this study there was a nonsignificant increase in the number of benign mammary tumors per tumor bearing female rat. No other tumors were observed.

An inhalation study of Ela:Eng (Syr) Syrian hamsters (Burek *et al.,* 1984) were performed under similar experimental exposure conditions as the 1980 Dow SD rat study. In males, there were no exposure-related increases in mortality rates, although mortality was high at 24 months (82% in control, 85% in the 3500 ppm exposed group). In females, control animals had 100% mortality at 24 months which was higher than any of the exposed groups (90.3% in the 3500 ppm exposed group). No exposure-related neoplasms or nonneoplastic lesions were observed.

The strongest evidence for the carcinogenicity of methylene chloride to rodents was provided by the NTP inhalation bioassays (NTP, 1986; Mennear *et al.,* 1988). Fifty F344 rats and $B6C3F_1$ mice of both sexes/group were exposed to methylene chloride for 6 hours/day, 5 days/week for 102 weeks (concentrations: 0, 1000, 2000, and 4000 ppm for rats; 0, 2000, and 4000 ppm for mice). Rats were housed in cages in exposure chambers and remained in the chambers during nonexposure periods.

Under the conditions of the study, benign mammary tumors were induced in F344 rats, and the female rats exhibited a dose-related response ($p < 0.001$) (Table 2). NTP interpreted the incidence of benign mammary tumors in female rats as "clear evidence of carcinogenicity" and in male rats as "some evidence of carcinogenicity". An elevated incidence of leukemia was observed in the 2000 ppm and 4000 ppm exposed female rats but this was also observed in controls. NTP considered this to be "equivocal".

Table 2: Methylene chloride-induced benign mammary tumors incidence in F344/N rats.

Dose (ppm)	Benign mammary tumors	
	males[1]	female[2]
0	1/50 (2%)	5/50 (10%)
1000	1/50 (2%)	11/50 (22%)
2000	4/50 (8%)	13/50 (26%)
4000	9/50 (18%)*	23/50 (46%)*
Historical Control (Fibroadenoma)		
a. Laboratory	0%	16%
b. NTP	3%	28%

[1] Fibroadenoma, adenoma, or fibroma.
[2] Fibroadenoma or adenoma.
* ($p < 0.001$), Fisher exact test.

In $B6C3F_1$ mice, exposure to methylene chloride by inhalation (NTP, 1986; Mennear *et al.,* 1988) was associated with an increased incidence and multiplicity of alveolar and bronchiolar tumors (adenoma and carcinoma) in the lungs of both sexes. The incidence and multiplicity of liver tumors were also increased in both sexes. Male mice had an increased incidence of hepatocellular carcinomas and of adenomas or carcinomas (combined) at the high exposure level, while female mice had dose-related increases in hepatocellular adenoma and hepatocellular carcinoma (Table 3). The survival in both male and female high dose groups was significantly ($p < 0.001$, trend test) decreased as compared to controls.

Table 3: Methylene chloride-induced neoplasms incidence in $B6C3F_1$ mice

Dose (ppm)	Alveolar /Bronchiolar Adenomas(A) or Carcinomas(C) or combined		Hepatocellular Adenomas or Carcinomas or combined	
	Male	Female	Male	Female
0	A: 3/50(6%)	2/50(4%)	10/50(20%)	2/50(4%)
	C: 2/50(4%)	1/50(2%)	13/50(26%)	1/50(2%)
	A, C 5/50(10%)	3/50(6%)	22/50(44%)	3/50(6%)
2000	A: 19/50(38%)*	23/48(48%)*	14/49(29%)	6/48(13%)
	C: 10/50(20%)	13/48(27%)*	15/49(31%)	11/48(23%)*
	A, C 27/50(54%)*	30/48(63%)*	24/49(49%)	16/48(33%)*
4000	A: 24/50 (48%)*	28/48(58%)*	14/49(29%)	22/48(46%)
	C: 28/50(56%)*	29/48(60%)*	26/49(53%)	32/48(67%)*
	A, C 40/50(80%)*	41/48(85%)*	33/49(67%)	40/48(83%)*
Historical control	A: --	--	13%	1%
a. Laboratory	C: --	--	15%	4%
	A, C 31%	10%	28%	5%
b. NTP	A: --	--	10%	4%
	C: --	--	21%	5%
	A, C 17%	7%	30%	8%

* Fisher Exact Test ($p < 0.001$).

Significant treatment-related increases in the combined incidence of liver tumors, hepatocellular adenomas, and carcinomas occurred in $B6C3F_1$ mice exposed orally (via drinking water), 7 days/week for two years, to lower doses (125 and 185 mg/kg/day) of methylene chloride, but were not significant in the highest (250 mg/kg/day) exposure group (Serota *et al.*, 1986b). Serota *et al.* (1986a) considered the increase in liver tumors (combined neoplastic nodule and hepatocellular carcinoma) in rats to be insignificant because the incidence was within the laboratory's historical control range.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Methylene chloride has been observed to induce lung (alveolar and bronchiolar) and liver (hepatocellular adenoma or carcinoma) tumors in both sexes of $B6C3F_1$ mice and subcutaneous sarcomas of the ventral cervical-salivary gland region in male Sprague-Dawley rats, as described above. CDHS (1989) decided that the tumor incidence data from studies by Dow (1980), NTP (1986) and Mennear *et al.* (1988) were suitable for use in developing a quantitative risk assessment.

Methodology

DHS staff used female mouse lung tumor incidence (the most sensitive sex, species and tumor site of the 1986 NTP inhalation bioassay) to calculate the low-dose risk from exposure to MC. DHS staff fitted several low-dose risk assessment models to the mouse lung tumor data, including the multistage (GLOBAL82 and GLOBAL86), time-dependent multistage (Weibull 82), probit, logit, Weibull, gamma multihit, and two-stage models. DHS staff also applied a physiologically based pharmacokinetic model to estimate the internal dose. This model adjusts the expected exposure concentration and suggests lower human risks than predicted by an unadjusted or applied dose approach. The application of the pharmacokinetic approach to risk assessment is based on information developed by the U.S. EPA (1987) and U.S. Consumer Product Safety Commission (Cohn, 1987). DHS staff recommended that the range of risks for ambient exposures to methylene chloride be based on the upper 95% confidence limit predicted from fitting either the multistage (GLOBAL82) model or the time-dependent multistage (Weibull 82) model to the animal data. The unit risk for a lifetime of continuous exposure to methylene chloride is 0.3 to 3×10^{-6} $(\mu g/m^3)^{-1}$. The lower estimate (0.3×10^{-6} $(\mu g/m^3)^{-1}$) incorporates a complete pharmacokinetic adjustment as calculated by U.S. EPA (1987). DHS staff believe that the complete pharmacokinetic adjustment retains considerable uncertainty. In contrast, the applied dose value (3×10^{-6} $(\mu g/m^3)^{-1}$) does not incorporate any pharmacokinetic information with the likely result of overestimating the risk. The high-to-low dose adjustment used by the U.S. Consumer Product Safety Commission (Cohn, 1987) generates a risk of 4×10^{-6} ppb^{-1} which incorporates information regarding saturation of the mixed-function oxidase pathway. After reviewing the full range of values, DHS staff concluded that the most likely estimate of the risk of methylene chloride exposure is the adjusted value of 4×10^{-6} ppb^{-1} (1.0×10^{-6} $(\mu g/m^3)^{-1}$).

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4, 4'-METHYLENEDIANILINE

CAS No: 101-77-9

4, 4'-METHYLENEDIANILINE DIHYDROCHLORIDE

CAS No: 13552-44-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

4, 4'-Methylenedianiline

Molecular weight	198.26
Boiling point	398-399 °C at 768 mm Hg
Melting point	91.5-92 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 8.109 mg/m^3

4, 4'-Methylenedianiline didihydrochloride

Molecular weight	271.21
Boiling point	not available
Melting point	not available
Vapor pressure	not available
Air concentration conversion	1 ppm = 11.09 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.6 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.6 E+0 $(mg/kg\text{-}day)^{-1}$
[Male mouse liver tumors (NTP, 1983), contained in Gold *et al*. (1987) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 4,4' methylenedianiline in humans are known to exist.

Animal Studies

Griswold *et al*. (1968) exposed a group of 20 female Sprague-Dawley rats to 30 mg 4,4' methylenedianiline dihydrochloride in 1 ml sesame oil by gavage every third day for 30 days (total dose, 300 mg/rat). The animals were then observed for a further 9 months. A group of 140 female Sprague-Dawley rats served as a negative control group. No treatment-related increase in tumor incidence was noted; however, the study duration was short, a small number of animals was used,

only one sex was used, and the primary aim of the study was to develop methods of detecting carcinogens inducing mammary tumors.

The potential carcinogenicity of 4,4' methylenedianiline dihydrochloride was studied using Fischer 344 (F344) rats and B6C3F$_1$ mice (50 animals/sex/species/group) (NTP, 1983). Animals were exposed to 150 mg/l or 300 mg/l 4,4' methylenedianiline dihydrochloride in drinking water for 103 weeks followed by one week without treatment. Untreated control groups were included. A significantly increased incidence of thyroid follicular-cell adenomas was observed in male and female mice and rats. Significantly increased incidences of hepatocellular adenomas and carcinomas were observed in female and male mice; increased incidences of neoplastic nodules were observed in male rats. Tumor incidence data is listed in Table 1.

Table 1 4,4' methylenedianiline dihydrochloride-induced tumor incidences in male and female F344 rats and B6C3F$_1$ mice (NTP, 1983; Weisburger *et al*., 1984)

Sex/species	Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
male mice	control	0	thyroid tumors	0/50
	low dose	24.5		3/50
	high dose	49.5		16/50
	control		liver tumors	17/50
	low dose			43/50
	high dose			37/50
female mice	control	0	thyroid tumors	0/50
	low dose	29.4		1/50
	high dose	59.1		15/50
	control		liver tumors	4/50
	low dose			15/50
	high dose			23/50
male rats	control	0	thyroid tumors	1/50
	low dose	7.36		4/50
	high dose	14.7		10/50
	control		hepatic neoplastic nodules	1/50
	low dose			12/50
	high dose			25/50
female rats	control	0	thyroid tumors	0/50
	low dose	8.41		5/50
	high dose	16.7		22/50

1. Doses as reported by Gold *et al*. (1987).
2. Tumor incidences as reported by Gold *et al*. (1987).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

4, 4'-Methylenedianiline

The potency for this compound was derived from the potency for the dihydrochloride using a molecular weight conversion:

$$q_h \text{ (anhydrous)} = q_h \text{ (hydrate)} \times \frac{\text{MW (hydrate)}}{\text{MW (anhydrous)}}$$

where q_h is the human potency and MW is the molecular weight. This conversion assumes that the intake of equivalent moles of the two forms of the chemical (e.g. the anhydrous and hydrate forms) results in equivalent concentrations of the active species *in vivo*.

4, 4'-Methylenedianiline didihydrochloride

Results are listed for the drinking water studies by NTP (1983) in male and female B6C3F$_1$ mice and F344 rats. Significant increases in tumors of the liver or thyroid or both are observed for all sex/species combinations tested, with male mice the most sensitive. The cancer potency listed is based on the combined incidence of benign and malignant liver tumors in male mice (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. Analysis of the data set using the computer program TOX_RISK (Crump *et al*., 1991) indicated that inclusion of the high dose group resulted in a *p*-value of = 0.05 based on the chi-square goodness-of-fit test, indicating non-linearity. Following procedures described by US EPA (Anderson *et al*., 1983), the high dose group was excluded from the analysis to correct for the poor fit (Cal/EPA, 1992). A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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MICHLER'S KETONE (4,4′-BIS(DIMETHYLAMINO) BENZOPHENONE)

CAS No: 90-94-8

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	268.35
Boiling point	>360 °C (with decomposition)
Melting point	172 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 10.98 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.5 E-4 ($\mu g/m^3)^{-1}$
Slope Factor: 8.6 E-1 (mg/kg-day)$^{-1}$
[Female rat liver tumor data (NCI, 1979), contained in Gold *et* al. (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of Michler's ketone in humans are known to exist.

Animal Studies

Male and female Fischer 344 (F344) rats and B6C3F$_1$ mice were fed diets containing Michler's ketone (NCI, 1979). Mice were fed diets containing 1250 or 2500 mg/kg diet Michler's ketone for 78 weeks; animals were then maintained on control diet for an additional 13 weeks. Rats were fed diets containing 250 or 500 mg/kg diet Michler's ketone for males, 500 or 1000 mg/kg diet for females; the treatment period for both sexes was 78 weeks. The treatment period was followed by an observation period; 28 weeks for male rats and high dose female rats and 29 weeks for low dose female rats. Treatment group sizes were 50 animals/sex/species/group; control group sizes were 20 animals/sex/species.

Significant dose-related increases in incidences of liver tumors (hepatocellular adenomas and carcinomas) were observed in rats and female mice, and of hemangiosarcomas in male mice. Tumor incidence data is listed in Table 1.

Table 1. Michler's ketone-induced tumor incidence in male and female F344 rats and $B6C3F_1$ mice (NCI, 1979)

Sex/species	Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
Male mice	control	0	hemangiosarcomas	0/20
	low dose	128		5/50
	high dose	257		20/50
Female mice	control	0	liver tumors	0/20
	low dose	139		41/50
	high dose	278		49/50
Male rats	control	0	liver tumors	0/20
	low dose	7.4		17/50
	high dose	14.4		43/50
Female rats	control	0	liver tumors	0/20
	low dose	18		46/50
	high dose	37		48/50

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The results from feeding studies by NCI (1979) are listed by Gold *et al*. (1984). Rats are more sensitive than mice to induction of tumors due to exposure to Michler's ketone, with male and female rats having similar sensitivity. The cancer potency factor for Michler's ketone was derived from dose-response data for liver tumors in female rats as listed in Table 1 (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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NAPHTHALENE

CAS No: 91-20-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 2003 except as noted)

Molecular weight	128.2
Boiling point	218ºC
Melting point	80.5 ºC
Vapor pressure	0.078 Torr @ 25ºC (Sonnenfeld *et al*., 1983); 0.10 Torr @ 27ºC (CRC, 1994)
Air concentration conversion	1 ppm = 5.24 mg/m^3 (NIOSH, 2004)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.4 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 1.2 E-1 $(mg/kg\text{-}day)^{-1}$

[Male rat nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma incidence data (NTP, 2000), linearized multistage procedure (OEHHA, 2004).]

III. CARCINOGENIC EFFECTS

Animal Studies

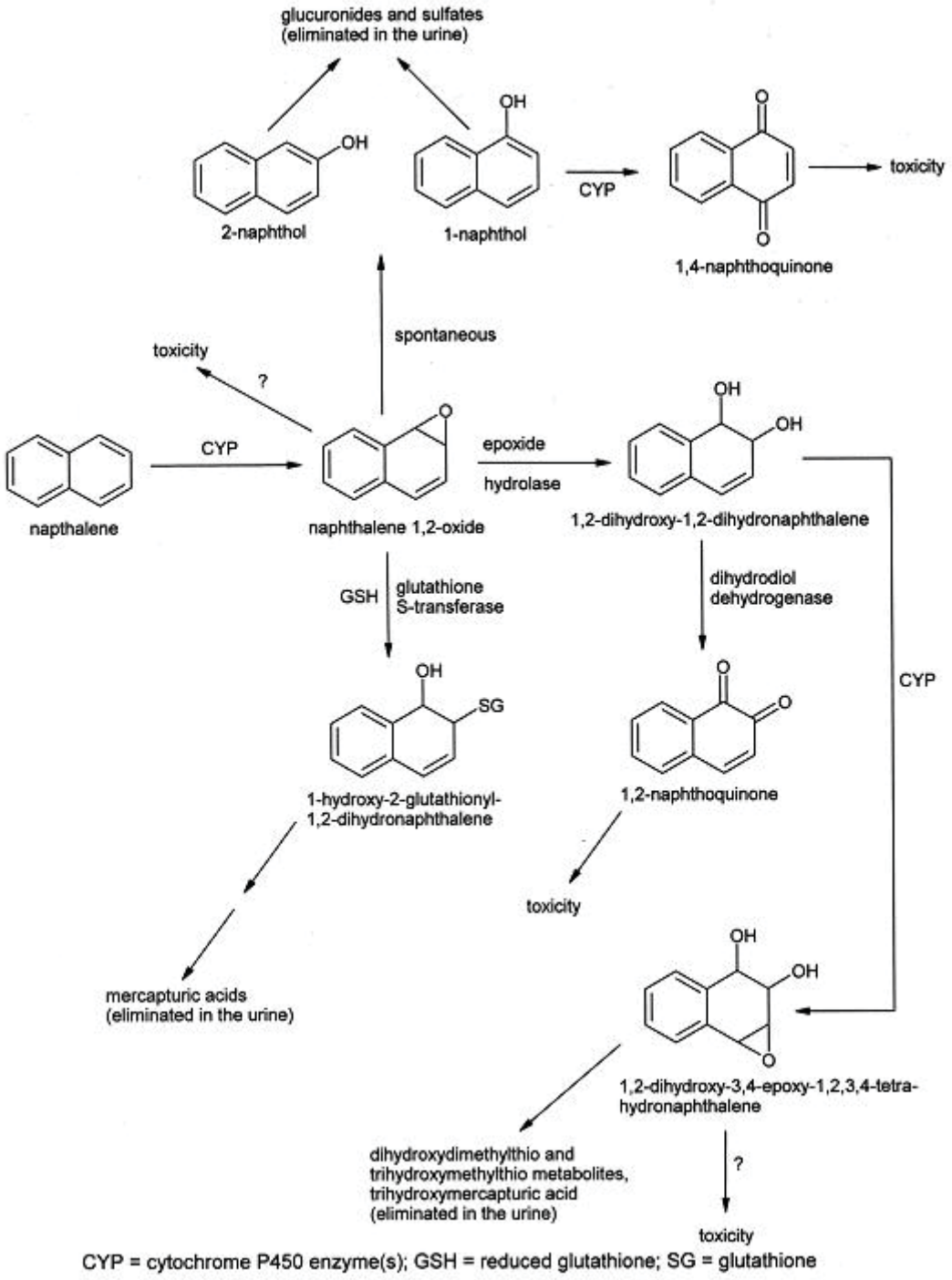
Metabolism

The metabolism of naphthalene is similar to that of many other aromatic hydrocarbons (reviews: Buckpitt and Franklin, 1989; Buckpitt *et al*., 2002). Initially, naphthalene is oxidized by the cytochrome P450 monooxygenase system to naphthalene oxide enantiomers. The primary site of metabolism is the liver, but oxidation also occurs in the lung and kidneys. Naphthalene oxides may be converted to dihydrodiols by epoxide hydrolase enzymes. Naphthalene oxides also undergo nonenzymatic conversion to 1-naphthol, which may be subsequently conjugated or may be further oxidized to 1,4-naphthoquinone. The naphthalene dihydrodiols may also be oxidized to 1,2-naphthoquinone (Figure 1). Toxicity is apparently related to protein binding by quinone metabolites and/or their participation in redox cycles leading to oxidative stress including DNA damage (O'Brien, 1991).

Naphthalene-1,2-epoxide

Naphthalene is oxidized to two reactive enantiomers: (1*R*, 2*S*)-naphthalene oxide and (1*S*, 2*R*)-naphthalene oxide. A purified cytochrome P450 monooxygenase (CYP2F2) from mouse liver that metabolized naphthalene rapidly with high stereoselectivity was used to clone and sequence the cDNA coding for a 50-kDa protein present at high levels in mouse lung and liver. The protein had 82% sequence homology to a cDNA cloned earlier from human lung (Ritter *et al*., 1991). The human CYP2F1 and mouse CYP2F2 were expressed in HEPG2 cells and yeast,

Figure 1. Metabolic Scheme for Naphthalene (adapted from ATSDR, 2003)



CYP = cytochrome P450 enzyme(s); GSH = reduced glutathione; SG = glutathione

respectively. In human lymphoblastoid cells the CYP2F1 demonstrated a naphthalene turnover of about 0.035 nmol/min/nmol P450 or about 0.1% the rate observed with the mouse CYP2F2 (Shultz *et al*., 1999). The human CYP2F1 showed slight stereopreference in the generation of the (1S, 2R) naphthalene oxide enantiomer (7.7:1). The recombinant mouse CYP2F2 exhibited a high degree of stereoselectivity for the (1*R*, 2*S*) enantiomer (66:1), a very high V_{max} (107 nmol/min/nmol P450), and a low K_m (3 μM). This high level of activity is consistent with the metabolism and toxicity of naphthalene in the mouse lung. CYP2F2 also oxidizes a number of other substrates including the lung toxicant 1-nitronaphthalene.

Characterization of naphthalene oxidizing P450 enzymes in the rat lung has been more difficult. A CYP2F4 has been cloned from the rat lung that has 93 percent identity with the mouse and 83 percent with the human (Buckpitt *et al*., 2002). While further characterization of the rat enzyme is necessary the results overall indicate that there are likely major interspecies catalytic differences in these CYP2F enzymes between mouse, rat and human. It is unknown whether CYP2F is responsible for the activation of naphthalene in the nasal epithelium. The rate of metabolism appears to be substantial in olfactory postmitochondrial supernatants from mice and rats, 87 and 43.5 nmol/min/mg protein, respectively (Buckpitt *et al*., 1992). However, there are several P450 isoforms in the nasal mucosa including CYP1A2, 2A, 2B, 2C, 2G1, 2J, and 3A and catalytic activities with naphthalene have not been established.

In contrast to the results of *in vitro* enzymatic studies, Willems *et al*. (2001) estimated overall kinetic parameters for intact mice and rats exposed to concentrations of naphthalene that were used in the NTP cancer bioassays in conjunction with a physiologically based pharmacokinetic model. The V_{max} (nmol/min/mg microsomal protein) and Km (μM) for the male and female rats were 16.5, 6.0; and 24.6, 3.2, respectively. For male and female mice the V_{max} and K_m values were 38.7, 1.5; and 54.8, 5.8, respectively. While some differences in metabolic saturation capacity are apparent the values overall are similar. The overall metabolic capacities based on these figures are higher than indicated by in vitro derived kinetic parameters of Quick and Shuler (1999). The values above do not distinguish between lung and liver.

1-Naphthol

Naphthalene-1,2-epoxide undergoes spontaneous rearrangement to 1-naphthol. Like naphthalene, 1-naphthol is subject to oxidation by cytochrome P450 enzymes. Wilson *et al.* (1996) studied the metabolism and cytotoxicity of naphthalene and 1-naphthol in vitro with human hepatic microsomes and mononuclear leukocytes (MNL). 1-Naphthol was observed to be more toxic than naphthalene (49.8 ± 13.9% vs. 19.0 ± 10.0% cell death; $p < 0.01$). CYP2E1-induced rat liver microsomes increased the metabolism of naphthalene giving increased yields of both naphthalene dihydrodiol and 1-naphthol. The cytotoxicity of naphthalene but not 1-naphthol was increased by CYP2E1 induction. The metabolites of 1-naphthol; 1,2-naphthoquinone and 1,4-naphthoquinone were directly toxic to MNL and depleted glutathione to one percent of control level. Both quinone metabolites of 1-naphthol were also genotoxic to human lymphocytes. Buckpitt *et al.* (1986) found that in vivo, reactive metabolites from [^{14}C]-1-naphthol became covalently bound to proteins in lung, liver, and kidney, but that the amount of binding was similar to that seen after administration of naphthalene. Zheng *et al.* (1997) incubated murine Clara cells with naphthalene and found adducts generated from the 1,2-quinone metabolite vs. the 1,2-epoxide had a ratio of 32:1. They found no evidence for formation of the 1,4-naphthoquinone. Conversely, Doherty *et al.* (1985)

incubated microsomal preparations and reported that the reactive metabolite generated from 1-naphthol was not trapped by ethylene diamine, which reacts rapidly with 1,2-naphthoquinone, and hence was likely 1,4-naphthoquinone. Current thinking is that 1-naphthol is oxidized to 1,4-naphthoquinone, which binds to proteins and plays a role in toxicity of naphthalene, particularly in the lung (Buckpitt *et al*., 2002).

1,2-Dihydroxy-1,2-dihydronaphthalene

Naphthalene-1,2-oxides are hydrolyzed to naphthalene dihydrodiols via epoxide hydrolase (EH). While generally less toxic than their parent epoxides dihydrodiols may serve as precursors to diol epoxides that represent ultimate carcinogens for many PAHs. The formation of diol epoxides and/or diepoxides from naphthalene is supported by the observation of trihydroxytetrahydro-urinary metabolite derivatives (Buckpitt *et al.*, 2002). In addition to this microsomal oxidation of naphthalene dihydrodiol, a competing biotransformation by cytosolic dihydrodiol dehydrogenase (DD) generates the catechol, 1,2-dihydroxynaphthalene, which is readily autoxidized to 1,2-naphthoquinone. During oxidation via single electron steps reactive oxygen species (ROS) are generated together with an *o*-semiquinone anion radical intermediate. 1,2-Naphthoquinone can be reduced by NAD(P)H back to the catechol creating a futile redox cycle, which may lead to oxidative stress including DNA damage (Penning *et al*. 1999). As noted above, studies in isolated Clara cells indicated that the 1,2-naphthoquinone was the major metabolite, which bound covalently to proteins (Zheng *et al*., 1997). 1,2-Naphthoquinone is mutagenic in the Salmonella microsome assay (Bolton *et al*., 2000) and forms *N*-7 adducts with deoxyguanosine *in vitro* (McCoull *et al*., 1999). 1,2-Naphthoquinone is a key metabolite in naphthalene toxicity and may be more important in humans than rodents due to the higher activities of both EH and DD in human tissues compared to rodent tissues (Penning *et al*., 1999; Kitteringham *et al*., 1996)

Conjugates of Naphthalene Metabolites

Naphthalene-1,2-oxides are converted via the action of glutathione *S*-transferase to 1-hydroxy-2-glutathionyl-1,2-dihydronaphthalenes. These are subsequently converted to mercapturic acids (*S*-conjugates of *N*-acetyl-L-cysteine) and excreted in the urine (25 to 35 percent of dose in mice and rats). In mice the ratio of diastereomeric mercapturates derived from (1*R*, 2*S*):(1*S*, 2*R*) epoxide ranged from 1:1 to 3:1 (1-200 mg/kg i.p.) whereas in rats the ratio was always less than 1:1 at all doses. For inhalation exposures the ratios were higher in mice 6:1 to 3:1 (15-100 ppm) but unchanged in rats (Pakenham *et al*., 2002). Three mercapturic acids have been identified in urine derived from the following conjugates: 1) 1*S*-hydroxy-2*S* –glutathionyl; 2) 1*R*-hydroxy-2*R*-glutathionyl; and 3) 1*R*-glutathionyl-2*R*-hydroxy. There are no substantial species differences in the percentage of dose eliminated as diastereomeric mercapturates. The ratios of the diastereomers do vary with species, administration route and dose level. Overall these urinary mercapturates appear to provide a useful biomarker of internal naphthalene dose.

Alternative biomarkers are the albumin and hemoglobin cysteinyl adducts of naphthalene-1,2-oxide, 1,2- and 1,4-naphthoquinones formed after administration of naphthalene to rats (Waidyanatha *et al*., 2002). In human coke oven workers only albumin adducts of 1,2-naphthoquinone (1,2-NQ-Alb) were found to significantly exceed background levels seen in control steel industry workers (Waidyanatha *et al*., 2004). This study also observed that 1,2-NQ-Alb levels were significantly correlated with urinary levels of naphthalene, 1-naphthol, 2-naphthol,

and 1-pyrenol but negatively correlated with age, suggesting a diminished cytochrome P450 metabolism of about three percent/year.

The conjugation of dihydroxy dihydronaphthalene metabolites and their subsequent urinary elimination as mercapturates is generally considered a detoxication process. However, reaction of these metabolites with glutathione may give rise to naphthoquinone thioethers that possess a variety of toxic properties (Monks and Lau, 1992,1998; Monks *et al*., 1992). ESR studies have shown the formation of GSH-conjugated semiquinone free radicals of 3-(glutathion-*S*-yl)-1,4-naphthoquinone and 2,3 (di-glutathion-*S*-yl)-1,4-naphthoquinone in rat hepatocytes (Takahashi *et al*., 1987; Rao *et al*., 1988). Conjugation of naphthoquinones with glutathione or *N*-acetylcysteine may exacerbate redox cycling by reducing the redox potential of the conjugate vs. the parent quinone. Quinone thioethers participate in protein crosslinking; serve as substrates for DT-diaphorase, or as inhibitors of important enzymes such as NADP-linked 15-hydroxyprostglandin dehydrogenase and glutathione sulfotransferase (GST). Some quinone thioethers also exhibit nephrotoxicity as indicated by menadione 3-thioethers (e.g., 2-methyl-3-(*N*-acetylcysteine-*S*-yl)-1,4-naphthoquinone) induction of renal proximal tubular necrosis in rats (Lau *et al*., 1990).

In addition to the GST mediated conjugation of naphthalene dihydrodiols and naphthoquinones, 1-naphthol and 2-naphthol arising from the rearrangement of the naphthalene-1,2-oxides are also conjugated and eliminated in the urine as sulfates and glucuronides.

Genotoxicity

Naphthalene

Naphthalene has been tested for genotoxicity in a variety of *in vitro* and *in vivo* genotoxicity assays. Those studies have recently been reviewed by ATSDR (2003). Naphthalene has not demonstrated genotoxicity in *Salmonella* reverse mutation assays. Those studies are listed in Table 1. All studies were performed in the presence and absence of metabolic activation (rat liver S9), and were negative.

Naphthalene has also been tested in other bacterial mutation assay systems. These studies are listed in Table 2. All studies were performed in the presence and absence of metabolic activation (rat liver S9), and were negative except for the study by Arfsten *et al*. (1994). This study used Vibrio fischeri (strain M169) in a Mutatox assay (reversion to luminescence). Naphthalene was negative in the absence of S9, but positive in the presence of S9.

Naphthalene has not demonstrated genotoxicity activity in mammalian *in vitro* DNA damage and gene mutation assays. The relevant studies are listed in Table 3.

Table 1. Naphthalene *Salmonella* reverse mutation studies

Test strains	Reference
TA98, TA100, TA1535, TA1537	McCann *et al*., 1975
TM677	Kaden *et al*., 1979
TA98, TA100, TA1535, TA1537	Florin *et al*., 1980
TA1537, TA1538	Gatehouse, 1980
TA98, TA100, UTH8413, UTH8414	Connor *et al*., 1985
TA98, TA100, TA1535, TA1537, TA1538	Godek, 1985
TA98, TA100, TA1535, TA1537	Mortelmans *et al*., 1986
TA98, TA1535	Narbonne *et al*., 1987
TA98, TA100	Bos *et al*., 1988
TA98, TA100, TA1535, TA1537	NTP, 1992a
TA98, TA100, TA1535, TA1537	Sakai *et al*., 1985

Table 2. Other naphthalene bacterial mutation studies

Assay system	Species/strain	Reference
SOS response	*E. coli* K12 inductest (λ lysogen GY5027; *uvr*B-, *env*A-)	Mamber *et al*., 1984
	S. typhimurium TA1535/p5K1002 (uMuC-IacZ)	Nakamura *et al*., 1987
SOS chromotest assay	*E. coli* PQ37 (*sfi*A::*lac*Z fusion)	Mersch-Sundermann *et al*., 1993
Pol A- or Rec	*E. coli* WP2/WP10 (*uvr*A-, *rec*A-)	Mamber *et al*., 1983
	E. coli WP2/WP67 (*uvr*A-, *pol* A-)	
	E. coli WP2/WP3478 (*pol* A-)	
Mutatox (reversion to luminescence)	*Vibrio fischeri* M169	Arfsten *et al*. 1994

Table 3. Naphthalene mammalian *in vitro* DNA damage and gene mutation studies

Assay	Cell type	Reference
Alkaline elution (single strand DNA breaks)	Rat primary hepatocytes	Sina *et al*., 1983
Unscheduled DNA synthesis	Rat primary hepatocytes	Barfknecht *et al*., 1985
hprt and *tk* loci mutations	Human MCL-5 B-lymphoblastoid cells	Sasaki *et al*., 1997

Naphthalene also induced chromosomal aberration in CHO cells in the presence but not absence of rat liver S9. Additionally, naphthalene caused an increase in the frequency of CREST micronuclei (indicative of chromosomal breakage) in human MCL-5 B-lymphoblastoid cells (Sasaki *et al*., 1997). These cells express microsomal epoxide hydrolase (EH) and CYP1A2, CYP2A3, CYP3A4, and CYP2E1 P450 isoforms.

Data on the induction by naphthalene of DNA damage *in vivo* are mixed. Single strand DNA breaks (measured by alkaline elution) were not induced in hepatocytes from female rats exposed to a single oral dose of naphthalene (Kitchin *et al*., 1992, 1994). In contrast, naphthalene caused DNA fragmentation in liver and brain tissue from rats given daily oral doses for up to 120 days (Bagchi *et al*., 1998), mice given single oral doses (Bagchi *et al*., 2000, 2002), and *p*53-deficient mice given single oral doses (Bagchi *et al*., 2002).

Naphthalene did not cause micronucleus induction (indicative of chromosomal damage) in the bone marrow cells of mice exposed to naphthalene either by gavage (Harper *et al*., 1984) or by intraperitoneal injection (i.p.) (Sorg, 1985). However, naphthalene was reported to induce chromosomal aberrations in preimplantation mouse embryos in an abstract by Gollahon *et al*. (1990).

Naphthalene has been demonstrated to have genotoxic effects in nonmammalian assay systems. Delgado-Rodriguez *et al*. (1995) reported positive results for naphthalene in the *Drosophila melanogaster* wing spot assay. This assay detects both somatic mutations and mitotic recombination in cells of the wing imaginal discs, based on the induced loss of heterozygosity for two recessive wing cell markers. Micronuclei induction was reported in the erythrocytes of salamander larvae (*Pleurodeles waltl*) exposed to naphthalene in their tank water (Djomo *et al*., 1995).

Naphthalene metabolites

1-Napthol, 2-Napthol

1-Naphthol was reported to be negative in the *Salmonella* reverse mutation assay by McCann *et al*. (1975) (test strains TA98, TA100, TA1535 and TA1537) and Norbonne *et al*. (1987) (test strains TA98 and TA1535). 1-Naphthol and 2-napthol also did not induce UDS in primary rat hepatocytes (Probst *et al*., 1981).

1,2-Napthoquinone

Flowers-Geary *et al*. (1996) tested 1,2-napthoquinone in the *Salmonella* reverse mutation assay (test strains TA97a, TA98, TA100 and TA104) in the presence and absence of rat liver S9. 1,2-Napthoquinone caused a 2.5-fold increase in revertants in strain TA104, a strain that is sensitive to oxidative DNA damage, compared to controls. 1,2-Napthoquinone also caused SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996).

1,4-Napthoquinone

1,4-Napthoquinone was negative in the *Salmonella* reverse mutation assay (test strains TA98, TA100, TA1535 and TA1537) (Sakai *et al*., 1985). 1,4-Napthoquinone also did not induce mutations at either the *hprt* or *tk* loci in the human B-lymphoblastoid MCL-5 cell line (Sasaki *et al*., 1997). However, 1,4-napthoquinone did cause SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996), and caused a significant increase in the frequency of

both $CREST^+$ (indicative of chromosomal loss) and total micronuclei in the human B-lymphoblastoid MCL-5 cell line (Sasaki *et al*., 1997).

Naphthalene 1,2-epoxide

Naphthalene 1,2-epoxide did not cause SCEs in human lymphocytes in the absence of metabolic activation (Wilson *et al*., 1996).

Naphthalene Atmospheric Reaction Products

Naphthalene is one of the more abundant PAH air pollutants in California. Atmospheric naphthalene occurs partially in the vapor phase and enters into rapid gas-phase reactions with hydroxyl radical (HO, daytime) and nitrate radicals (NO_3, nighttime). Reaction products include 1-nitronaphthalene (1NN), 2-nitronaphthalene (2NN), 1-hydroxy-2-nitronaphthalene (1H2NN), and 2-hydroxy-2-nitronaphthalene (2H2NN). Sasaki *et al*. (1997) evaluated the genotoxicity of these reaction products in the human B-lymphoblastoid MCL-5 cell line. 2-Nitronaphthalene caused a significant increase both in the mutation frequency at the thymidine kinase (*tk*) locus, and in the total micronucleus number (indicative of chromosomal damage).

The above data indicate that naphthalene generally has not been shown to cause gene mutations, but has been demonstrated to cause chromosomal damage and may cause DNA damage. The naphthalene metabolite 1,4-napthoquinone also causes chromosomal damage, and 1,2-napthoquinone causes both gene mutations and chromosomal damage, as does the atmospheric reaction product 2-nitronaphthalene.

Cancer Bioassays

The National Toxicology Program (NTP) conducted inhalation cancer studies of naphthalene using male and female $B6C3F_1$ mice (NTP, 1992). Animals were exposed to 0 (70 males, 69 females), 10 (69 males, 65 females) or 30 ppm naphthalene (135 males, 135 females) for 6 hours/day, 5 days/week for 104 weeks.

The survival rates of exposed female mice were similar to that of controls (86%, 88% and 76% for controls, 10 and 30 ppm exposure groups, respectively). However, survival of male control mice was significantly less than that of exposed male mice (37%, 75% and 89% for controls, 10 and 30 ppm exposure groups, respectively). NTP stated that the reduced control survival was due to wound trauma and secondary infections due to fighting among the group-housed mice.

Almost all of the male and female mice in the NTP 1992 mouse inhalation studies demonstrated an increased incidence of nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 4).

Table 4. Incidence of nonneoplastic nasal lesions in male and female $B6C3F_1$ exposed to naphthalene by inhalation for 104 weeks (NTP, 1992).

Lesion type	Sex	Naphthalene concentration		
		0 ppm	10 ppm	30 ppm
respiratory epithelium hyperplasia	male			
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade[a]		0	2.6	2.8
olfactory epithelium metaplasia				
overall rate		0/70 (0%)	66/69 (96%)	134/135 (99%)
average severity grade[a]		0	2.5	2.6
respiratory epithelium hyperplasia	female			
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade[a]		0	2.5	2.7
olfactory epithelium metaplasia				
overall rate		0/69 (0%)	65/65 (100%)	135/135 (100%)
average severity grade[a]		0	2.5	2.4

a: Average severity grade based on 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were observed in male $B6C3F_1$ mice. Alveolar/bronchiolar adenoma or carcinoma incidences in the male mice as cited by NTP were 7/70, 17/69 and 31/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The increased tumor incidences observed for the 10 and 30 ppm groups were significant when a pairwise comparison to control was performed using the Fisher exact test (p = 0.019 and 0.016 for the 10 and 30 ppm groups, respectively). However, NTP noted that an evaluation of the dose-response trend (p = 0.530) and pairwise comparisons between the controls and exposure groups (p = 0.212 and 0.394 for the 10 and 30 ppm exposure groups, respectively) using a logistic regression test indicated a lack of statistical significance. This was explained by NTP as being the result of the early control mortality due to fighting which lowered considerably the number of control animals at risk of developing lung tumors. NTP also noted that the alveolar/bronchiolar adenoma and carcinoma incidence (adjusted rate 26% in the high dose group) was within the historical control range for male $B6C3F_1$ mice (total incidence 19.7%, range 10-30%). NTP therefore concluded that the marginally increased alveolar/bronchiolar adenoma and carcinoma incidence in the male mice was more likely to be related to survival difference between exposed and control groups, than directly related to naphthalene exposure.

Increased incidences of alveolar/bronchiolar adenomas and carcinomas were also observed in female $B6C3F_1$ mice. The incidences of alveolar/bronchiolar adenoma or carcinoma, combined, in the female mice as cited by NTP were 5/69, 2/65 and 29/135 for controls, and the 10 and 30 ppm exposure groups, respectively. The tumors were primarily adenomas; one carcinoma was observed in high dose female mice. The increased tumor incidence in the 30 ppm exposure group females was statistically significant when compared to controls. NTP concluded that this provided *some evidence* of carcinogenicity.

These results were generally considered at the time to provide only equivocal evidence of carcinogenic activity, when considered in conjunction with earlier studies by various routes, which, although of lower power, also had negative or equivocal results (Adkins *et al*., 1986; Kennaway, 1930; Schmahl, 1955). However, the observation of possible tumor responses in the

mice prompted the National Toxicology Program to undertake naphthalene inhalation cancer studies in rats.

NTP (2000) exposed groups of 49 male and female Fischer 344N (F344) rats to naphthalene by inhalation at concentrations of 0, 10, 30 or 60 ppm for 6.2 hours/day, five days/week for 105 weeks. Survival of the male and female exposure groups were similar to that of controls.

These studies found clear evidence of carcinogenic activity in male and female rats, based on increased incidences of rare tumors, respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose, in both sexes. Respiratory epithelial adenoma incidence occurred with a positive dose-response trend in male rats and was significantly increased in all exposed male rat groups. Male rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 6/49, 8/48 and 15/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Respiratory epithelial adenoma incidences in female rats exposed to 30 or 60 ppm were also increased, but the increase in the 60 ppm animals was not significant, and the increase in the 30 ppm animals was of borderline significance ($p = 0.053$ by Poly-3 test). Female rat respiratory epithelial adenoma incidence as cited by NTP was 0/49, 0/49, 4/49 and 2/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

Olfactory epithelial neuroblastomas occurred in males exposed to 30 and 60 ppm naphthalene and in all dose groups of naphthalene-exposed females. Neuroblastoma incidences occurred with positive dose-response trends in males and females. The incidence in females exposed to 60 ppm was significantly greater ($p < 0.001$ by Poly-3 test) than that in controls. Male rat olfactory epithelial neuroblastoma incidence as cited by NTP was 0/49, 0/49, 4/48 and 3/48 for controls, and the 10, 30 and 60 ppm exposure groups, respectively. Female rat olfactory epithelium neuroblastoma incidence as cited by NTP was 0/49, 2/49, 3/49 and 12/49 for controls, and the 10, 30 and 60 ppm exposure groups, respectively.

NTP also noted that nasal olfactory epithelial neuroblastomas and nasal respiratory epithelial adenomas have not been observed in male or female control rats in the NTP historical control database for animals fed NIH-07 feed in 2-year inhalation studies or in the more recent, smaller database for control rats fed NTP-2000 feed. Additionally, almost all of the male and female mice in the NTP 1992 inhalation studies demonstrated increased nasal respiratory epithelium hyperplasia and olfactory epithelium metaplasia (Table 1). These tissue types correspond to the tumor sites observed in rats exposed to naphthalene by inhalation.

Human Studies

Although a number of reports exist which describe non-cancer health effects in humans (OEHHA, 2000), no studies of carcinogenic effects in humans were identified.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Unit risk values for naphthalene were calculated based on data in female mice, male rats and female rats from the studies of NTP (1992, 2000). The mouse lung alveolar/bronchiolar adenoma or carcinoma incidence data, rat nasal respiratory epithelial adenoma data and nasal olfactory epithelial neuroblastoma data used to calculate unit risk values are listed in Tables 5, 6 and 7, respectively.

Table 5. Incidence of lung alveolar/bronchiolar adenoma or carcinoma in female $B6C3F_1$ mice exposed to naphthalene via inhalation (from NTP, 1992)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
0	0	0	5/67 (7)	$p < 0.001$
10	9.36	12.3	2/61 (3)	$p = 1$
30	28.1	36.8	29/129[e] (22)	$p < 0.01$

[a] Average concentration calculated by multiplying chamber concentration by six hours/24 hours, 5 days/7 days and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the female mouse breathing rate (0.038 m^3/day) divided by the female mouse body weight (0.029 kg). The dosing period of 104 weeks was equivalent to the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

[c] Effective rate. Animals that died before the first occurrence of tumor (day 471) were removed from the denominator.

[d] The *p*-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The *p*-value listed next to the control group is the result of trend tests conducted by NTP (1992) using the logistic regression, life table, and Cochran-Armitage methods (all three methods produced the same result).

[e] One carcinoma was observed in the high dose group.

Table 6. Incidence of nasal respiratory epithelial adenoma in male F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
0	0	0	0/44 (0)	$p < 0.001$
10	9.67	5.69	6/42 (14)	$p < 0.05$
30	29.0	17.1	8/44 (18)	$p < 0.01$
60	58.0	34.1	15/41 (37)	$p < 0.001$

[a] Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7 days, and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the male rat breathing rate (0.262 m^3/day) divided by the male rat body weight (0.445 kg). The dosing period of 105 weeks was at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

Table 6 (cont.). Incidence of nasal respiratory epithelial adenoma in male F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

[c] Effective rate. Animals that died before the first occurrence of tumor (day 552) were removed from the denominator.

[d] The *p*-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher exact test. The p-value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

Table 7. Incidence of nasal olfactory epithelial neuroblastoma in F344/N rats exposed to naphthalene via inhalation (from NTP, 2000)

Chamber Concentration (ppm)	Average Concentration[a] (mg/m^3)	Lifetime Average Dose[b] (mg/kg-day)	Tumor Incidence[c] (%)	Statistical Significance[d]
Males				
0	0	0	0/49 (0)	$p = 0.027$
10	9.67	5.69	0/48 (0)	$p = 1$
30	29.0	17.1	4/48 (8)	$p = 0.056$
60	58.0	34.1	3/48 (6)	$p = 0.117$
Females				
0	0	0	0/49 (0)	$p < 0.001$
10	9.67	6.82	2/49 (4)	$p = 0.247$
30	29.0	20.4	3/49 (6)	$p = 0.121$
60	58.0	40.9	12/48 (25)	$p < 0.001$

[a] Average concentration calculated by multiplying chamber concentration by 6.2 hours/24 hours, 5 days/7days, and 5.24 mg/m^3/ppm.

[b] Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the rat breathing rate (males: 0.262 m^3/day; females: 0.182 m^3/day) divided by the rat body weight (males: 0.445 kg; females: 0.258 kg). The dosing period was at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

[c] Effective rate. Animals that died before the first occurrence of tumor (males, day 399; females, day 429) were removed from the denominator.

[d] The p-value listed next to dose groups is the result of pairwise comparison with controls using the Fisher Exact test. The p-value listed next to the control group is the result of the Poly-3 trend test, as reported by NTP (2000).

Methodology

The default approach, as originally delineated by CDHS (1985), is based on a linearized form of the multistage model of carcinogenesis (Armitage and Doll, 1954). Cancer potency is estimated from the upper 95% confidence bound, q_1^*, on the linear coefficient q_1 in a model relating lifetime probability of cancer (p) to dose (d):

$$p = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \ldots)] \quad (1)$$

The parameter q_1^* is estimated by fitting the above model to dose response data using MSTAGE (Crouch, 1992).

For a given chemical, the model is fit to one or more data sets. The default approach is to select the data for the most sensitive species and sex. For carcinogens that induce tumors at multiple sites, or at the same site but arising from different cell types, in a particular species and sex, cancer potency is taken to be the sum of potencies from the different sites or cell types. This approach assumes that tumors arising at different sites or from different cell types are independent. Because of the statistical uncertainty in individual estimates of potency, the terms are summed statistically as follows. A distribution of estimates corresponding to the 0.1 through 99.9 percentiles of the linear term (q_1) of the multistage model (Equation 1) is generated for each treatment-related tumor site in a given species and sex using the computer program MSTAGE (Crouch, 1992), modified to tabulate percentile values. (Distributional values stem from the assumption that twice the log likelihood function is χ^2 distributed). The discretized distributions were used to obtain a distribution of the sum of q_1s for each site affected by the chemical using Monte Carlo simulation (100,000 trials; Crystal Ball 2000 software, Decisioneering, Inc., Denver, Colorado). The upper 95 percent confidence bound on the summed q_1s is taken as q_1* for the combined tumor sites.

To estimate animal potency, q_{animal}, the parameter q_1* is adjusted to account for short duration of an experiment by assuming that the lifetime incidence of cancer increases with the third power of age. However, the durations of the studies examined here (NTP, 1992; 2000) were at least the standard lifespan of the test animals (104 weeks for rodents), so this correction was not required. Thus, for the calculations based on the NTP (1992; 2000) studies, q_1* is equivalent to q_{animal}.

Interspecies extrapolation from experimental animals to humans is normally based on the following relationship (Anderson *et al.*, 1983), where bw_h and bw_a are human and animal body weights, respectively, and potency (e.g., q_{animal}) is expressed on a dose per body weight basis:

$$q_{human} = q_{animal} \times \left(\frac{bw_h}{bw_a} \right)^{\frac{1}{3}} \qquad (2)$$

Alternatively, when performing calculations based on applied dose in terms of air concentrations, the assumption has sometimes been made that air concentration values are equivalent between species (CDHS, 1985). However, using the interspecies scaling factor shown above is preferred because it is assumed to account not only for pharmacokinetic differences (*e.g.*, breathing rate, metabolism), but also for pharmacodynamic considerations. Therefore, lifetime average doses in mg/kg-day were determined (details provided below) and used in the calculation of q_{animal} in $(mg/kg\text{-}day)^{-1}$. The interspecies scaling factor was applied to q_{animal} to obtain q_{human} in $(mg/kg\text{-}day)^{-1}$. Unit risk in $(mg/m^3)^{-1}$was determined from q_{human} in $(mg/kg\text{-}day)^{-1}$ by applying a conversion factor (the ratio of human breathing rate [20 m^3/day] to human body weight [70 kg]).

Male and female rats (NTP, 2000) were exposed 6.2 hours/day, five days/week for 105 weeks. Female mice (NTP 1992) were exposed six hours/day, five days/week for 104 weeks. Average concentrations during the dosing period were calculated by multiplying the reported chamber concentrations by 6 or 6.2 hours/24 hours, five days/seven days and 5.24 mg/m^3/ppm. The average body weight of female mice was estimated to be approximately 0.029 kg based on data for controls reported by NTP (1992). The average body weights of male and female rats were calculated to be

0.445 kg and 0.258 kg, respectively, based on data for controls reported by NTP (2000). Inhalation rates (I) in m^3/day for mice and rats were calculated based on Anderson *et al.* (1983):

$$I_{mice} = 0.0345 \times (bw_{mice}/0.025)^{2/3} \quad (3)$$

$$I_{rats} = 0.105 \times (bw_{rats}/0.113)^{2/3} \quad (4)$$

Breathing rates were calculated to be 0.038 m^3/day for female mice, 0.262 m^3/day for male rats, and 0.182 m^3/day for female rats. Lifetime average doses were determined by multiplying the average concentrations during the dosing period by the appropriate animal breathing rate divided by the corresponding animal body weight. The dosing periods in the NTP studies were at least the standard lifespan of the test animals (104 weeks for rodents), so no correction for less than lifetime exposure was required.

An alternative dose description approach, using pharmacokinetic analyses based on models described in the literature (Willems *et al.*, 2001; Quick and Shuler, 1999; Sweeney *et al.*, 1996; Frederick *et al.*, 1998, 2001; NTP, 2000) was evaluated. Although no data were available on the metabolism of naphthalene by rodent nasal tissues, simulations were conducted using parameters for rats and mice assuming either lung-like or liver-like scaling. The model predictions evaluated included amounts of naphthalene metabolized in each of the seven nasal compartments and their sum and the areas under the concentration × time curves (AUCs) for the olfactory and ventral respiratory compartments. Since all of these metrics appeared linear and in relative proportion to the applied doses, they did not indicate any substantial difference from the default potency analysis. If the assumptions used are correct, the concentrations used in the NTP studies were below those at which saturation of metabolism or other pharmacokinetic effects become important in the nasal and lung regions.

Application of an uptake rate for naphthalene was also considered. NTP (2000) estimated inhalation uptakes of 22 to 31 percent for rats and 65 to 73 percent for mice based on pharmacokinetic data and PBPK modeling. However, in the subsequent publication of NTP's PBPK modeling of inhaled naphthalene, uptakes are estimated to be 85 to 94 percent in rats and 92 to 96 percent in mice (Table 3, Willems *et al.*, 2001). Until more reliable estimates become available we assume there are no significant differences in uptake between mice and rats used in the NTP bioassay. Also we assume similar uptake in humans exposed to low levels of naphthalene.

Table 8 provides the q_{animal}, q_{human} and unit risk values, calculated using the linearized multistage procedure as described above, based on data for female mice (NTP, 1992) and male and female rats (NTP, 2000).

U.S. EPA (2003) and others (*e.g.* Gaylor *et al.*, 1994) have more recently advocated a benchmark dose method for estimating cancer risk. This involves fitting an arbitrary mathematical model to the dose-response data. A linear or multistage procedure is often used, although others may be chosen in particular cases, especially where mechanistic information is available which indicates that some other type of dose-response relationship is expected, or where another mathematical model form provides a better fit to the data. A point of departure on the fitted curve is defined: for animal carcinogenesis bioassays this is usually chosen as the lower 95% confidence bound on the

dose predicted to cause a 10% increase in tumor incidence (LED_{10}). Linear extrapolation from the point of departure to zero dose is used to estimate risk at low doses either when mutagenicity or other data imply that this is appropriate, or in the default case where no data on mechanism are available. The slope factor thus determined from the experimental data is corrected for experimental duration and interspecies extrapolation in the same way as the q_1^* adjustments described for the linearized multistage procedure. In the exceptional cases where data suggesting that some other form of low-dose extrapolation, such as the assumption of a threshold, is appropriate, a reference dose method with safety factors as required may be used instead.

Table 8. Cancer potency and unit risk values for naphthalene derived using the linearized multistage procedure based on data from NTP (1992) and NTP (2000).

Sex, Species	Site, Tumor Type	q_{animal} $(mg/kg\text{-}day)^{-1}$	q_{human}[a] $(mg/kg\text{-}day)^{-1}$	Human Unit Risk Value[b] $(mg/m^3)^{-1}$	Goodness-of-Fit Test[c]
Female mice	Lung alveolar/bronchiolar adenoma/carcinoma	0.004382	0.059	0.017	$p = 0.1428$
Male rats	Nasal respiratory epithelial adenoma	0.01919	0.10	0.030	$p = 0.4192$
	Nasal olfactory epithelial neuroblastoma	0.004651	0.025	0.0072	$p = 0.4224$
	All naphthalene-related tumor sites in male rats	0.02219	0.12	0.034	NA[d]
Female rats	Nasal olfactory epithelial neuroblastoma	0.007636	0.049	0.014	$p = 0.6342$

[a] The interspecies extrapolation was applied to q_{animal} in $(mg/kg\text{-}d)^{-1}$ to determine q_{human} $(mg/kg\text{-}day)^{-1}$, as described above.

[b] Unit risk was determined by multiplying the human cancer potency in $(mg/kg\text{-}day)^{-1}$ by the human breathing rate divided by human body weight, as described above.

[c] A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

[d] Not applicable.

The benchmark dose methodology was applied to the tumor incidence data for naphthalene in the NTP (1992; 2000) studies. Genetic toxicology results for naphthalene are mixed: *Salmonella* reverse mutation assays were generally negative, but some test results with eukaryotic systems *in vivo* or *in vitro* were positive (NTP, 2000). However, it was considered on balance that the weight of evidence, including metabolism to 1-naphthol via an epoxide intermediate (NTP, 1992, citing Bock *et al.*, 1976 and others; NTP, 2000), and the reactivity of naphthoquinones to cellular components (Zheng *et al*., 1997) favors the interpretation that the mechanism of naphthalene carcinogenicity likely involves a reactive metabolic intermediate which causes direct damage to DNA. A low dose linearity assumption is therefore appropriate when extrapolating from the point of departure to obtain an estimate of the cancer risk at low doses.

Model fits, points of departure and unit risks calculated using the benchmark methodology and U.S. EPA's Benchmark Dose Software version 1.3 are shown in Table 9. In all three cases, the model used was either a multistage polynomial, or a quantal linear model, which is identical to the multistage procedure in cases where the higher terms are not significant.

Table 9: Unit risk and human cancer potency values for naphthalene based on NTP (1992) and NTP (2000), derived using benchmark methodology.

Sex, Species	Site, Tumor Type	Model Fit:[a]	LED_{10} [ED_{10}] (mg/m^3)	Animal Unit Risk Value[b] $(mg/m^3)^{-1}$	Human Unit Risk Value[c] $(mg/m^3)^{-1}$ [Human Cancer Potency][c] $(mg/kg\text{-}d)^{-1}$
Female mice	Lung alveolar/bronchiolar adenoma/carcinoma	$\chi^2 = 1.42$ $p = 0.23$	17.1 [22.8]	0.0058	0.017 [0.059]
Male rats	Nasal respiratory epithelial adenoma	$\chi^2 = 2.82$ $p = 0.42$	9.3 [12.5]	0.0108	0.028 [0.099]
	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 2.82$ $p = 0.42$	38.5 [67.6]	0.0026	0.0068 [0.024]
	All naphthalene-related tumor sites in male rats	NA	8.1[d] [10.6]	0.012	0.031 [0.11]
Female rats	Nasal olfactory epithelial neuroblastoma	$\chi^2 = 1.73$ $p = 0.63$	18.1 [26.4]	0.0055	0.014 [0.050]

[a] A *p*-value of greater than 0.05 for the chi-square goodness-of-fit test indicates an adequate fit.

[b] Animal unit risk was calculated using the relationship $0.1/LED_{10}$.

[c] The interspecies extrapolation from rodent unit risks to human unit risks was based on the $(mg/kg\text{-}d)^{-1}$ equivalents of the animal unit risks, as described above. The following parameters were used to derive the $(mg/kg\text{-}day)^{-1}$ equivalents of the animal unit risks: bw_{animal} = 0.029 kg for female mice, 0.445 kg for male rats, and 0.258 kg for female rats; I_{animal} = 0.038 m^3/d for female mice, 0.262 m^3/d for male rats and 0.182 m^3/d for female rats. Human cancer potency was derived by applying the interspecies scaling factor to the $(mg/kg\text{-}day)^{-1}$ equivalents of the animal unit risks. The interspecies scaling factor is $(bw_{human}/bw_{animal})^{1/3}$, or 13.4 for female mice, 5.4 for male rats, and 6.5 for female rats. Human unit risks were then derived by multiplying human cancer potency by the human breathing rate (20 m^3/day) divided by the human body weight (70 kg).

[d] The LED_{10} in mg/m^3 for the multi-tumor analysis in rats was calculated by assuming a linear dose response relationship: $LED_{10} = -\ln(0.9)/(q_{animal} \times [I_{animal}/bw_{animal}])$. By inspection, this assumption appears reasonable for the dose-response curves considered, in the ED_{10} range. For the current case, q_{animal} is the cancer potency in rats ($0.02219\ [mg/kg\text{-}day]^{-1}$) generated using the multi-tumor analysis described above, I_{animal} is the breathing rate in male rats (0.262 m^3/day), and bw_{animal} is the male rat body weight (0.445 kg).

Using either of these methodologies, the 95% upper confidence bound on the unit risk value for purposes of calculating cancer risks associated with exposure to naphthalene is in the range 0.014-0.034 $(mg/m^3)^{-1}$, based on the incidence data in female mice and male and female rats from the NTP (1992; 2000) studies.

The male rat was the most sensitive sex and species tested by NTP (1992; 2000) in the inhalation carcinogenesis studies of naphthalene. NTP considered the increased incidences of nasal respiratory epithelial adenoma and nasal olfactory epithelial neuroblastoma, which are rare tumors, to provide clear evidence of the carcinogenic activity of naphthalene. The unit risk value of 0.034 $(mg/m^3)^{-1}$, or 3.4×10^{-5} $(\mu g/m^3)^{-1}$, based on the tumor incidence data in male rats, is therefore considered the most appropriate for use in risk assessment.

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NICKEL AND NICKEL COMPOUNDS

CAS No: 744-02-0 (nickel)

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	59 (nickel)
Boiling point	2730°C
Melting point	1455°C
Vapor pressure	not applicable
Air concentration conversion	not applicable

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.6 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 9.1 E-1 $(mg/kg\text{-}day)^{-1}$

[Calculated from Ontario nickel refinery sinter plant worker lung cancer mortality data (Chovil *et al.,* 1981; Roberts *et al.,* 1984; Muir *et al.,* 1985), using excess relative risk estimates (CDHS, 1991)].

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiologic studies of the carcinogenic effects of nickel generally center around cohort studies of refinery workers which have found increased risk of lung cancer and nasal sinus cancer. While other types of studies have been done, cohort studies of nickel refinery workers warrant special consideration for use in quantitative risk assessment in view of the high cancer risks detected and the availability of at least some exposure information. There are many studies of welders, for example, but exposure data are limited and the increase in lung cancer risks may have been more attributable to chromium than to nickel (Stern, 1983). A comprehensive quantitative risk analysis was undertaken by the US Environmental Protection Agency (US EPA) for the 1986 Health Assessment Document (US EPA, 1986). The following sections review the lung cancer findings for the cohort studies used in the US EPA risk assessment, and their subsequent follow-up by the International Committee on Nickel Carcinogenesis in Man (ICNCM). These studies also report increased risk of nasal cancer. However, the excess number of lung cancers were generally much greater than the excess numbers of nasal cancers.

Enterline and Marsh (1982) studied a West Virginia cohort consisting of 1,855 workers employed by the International Nickel Company (INCO) at a nickel refinery and alloy manufacturing plant in Huntington, West Virginia. Cohort members had at least one year service prior to 1948 when the calcining operations ceased. The cohort was followed to the end of 1977. Among the subset of 266 men that worked in the nickel refinery there were 113 deaths, eight of which were from lung cancer. The corresponding lung cancer standardized mortality ratio (SMR) for 20 or more years after first exposure was only 1.12 (90% confidence limits 0.56 and 2.02). Air levels of nickel were in the range of 0.01-5.0 mg/m^3.

The ICNCM (1990) reported a further five years of follow-up of this cohort up to 1982. Among workers hired before 1947 and who had 15 or more years since first exposure in calcining, there were eight lung cancer deaths yielding an SMR estimate of 1.15 (90% confidence limits 0.57 - 2.07). Two nasal cancer deaths were reported with 0.9 expected.

The Ontario cohort involved refinery workers at Copper Hill, Ontario (Chovil *et al.,* 1981; Roberts *et al.,* 1984; Muir *et al.,* 1985). A subcohort with high exposure to nickel consisted of 495 workers with five or more years' work history at a sinter plant operated by the INCO between 1948 and 1963. Workers were followed up from 1963 to the end of 1978, a minimum of 15 years. Eighty-five cohort members died during the follow-up period, of which 37 were lung cancer deaths. The SMR for lung cancer was 8.71, (90% confidence limits 6.49 and 11.45).

The ICNCM (1990) reported a further follow-up of this cohort up to 1984. There were a total of 63 lung cancer deaths and 6 nasal cancer deaths among the Copper Cliff sinter plant workers with 15 or more years since first exposure. Of the 63 lung cancer deaths, 33 had five or more years of exposure and yielded an SMR of 7.89 (90% confidence limits 5.78 - 10.56). For those who commenced work prior to 1952, the SMR estimate was 8.55 (90% confidence limits 6.15 - 11.59). These estimates are close to the SMR of 8.71 obtained from the earlier report by Chovil *et al.* presented above. Exposure data were given by Roberts *et al.* (1984) who estimated a level of 400 mg/m^3 in 1950, falling to 100 mg/m^3 "toward the end of the plant's productive life in 1958." Calculations based on the data give an average of 158 mg/m^3 for measurements made before 1952 and an average level of 73 mg/m^3 for measurements made after 1952.

Studies of a Welsh nickel refinery cohort involved 967 refinery workers, some of whom started working as early as 1910 at a nickel refinery in Clydach Wales, operated by INCO. Several publications provide some form of dose-response data. These studies include: 1) Doll et al. (1977) in which lung cancer mortality is presented by year of first employment; 2) Peto et al. (1984) study which categorized the cohort members by duration of exposure in the calcining furnaces, and 3) analyses by various exposure variables by Breslow and Day (1987) and Kaldor et al. (1986).

The Doll et al. (1977) study followed the employees up until the end of 1971 and calculated man-years at risk from 1934-1971. There were 689 total deaths in the cohort, including 145 lung cancer deaths, yielding a lung cancer SMR of 5.28 (90% CI: 4.58-6.03). The risk of lung cancer was increased in workers exposed before 1930.

The Peto *et al.* (1984) study updated dates of first employment in cases where additional information had come to light since the publication of the Doll *et al.* (1977) paper. Employees were classified into low and high exposure groups based on the number of years each employee spent at the furnaces or in the copper sulfate work areas. The lung cancer SMR for the low exposure group was 3.7 while that for the high exposure group was 14.0. Again, this paper provided no nickel exposure data. The ICNCM reported further follow-up of the cohort to 1984. There were 172 lung cancer deaths and 74 nasal cancer deaths among those hired prior to 1930. The overall lung cancer SMR was 3.93 (90% confidence limits 3.45-4.46). For those men hired before 1920, the SMR was 5.49 (90% confidence limits 4.57-6.60). This is the only cohort in which the excess number of nasal cancer deaths (73.6) was significant in number compared to the

excess number of lung cancer deaths (128.2). In the other cohorts reviewed, the excess number of lung cancer cases are much larger than the excess number of nasal cancers.

Exposure data are available for the Welsh cohort from a paper by Morgan (1985) in which a historical description of nickel monitoring at Clydach was presented. The main problem with the exposure data for this cohort is that the earliest data are for 1932. There were no measurements prior to 1930 when exposures were said to be higher.

Studies on a Norwegian nickel refinery worker cohort were described in two papers (Magnus *et al.,* 1982; Pedersen *et al.,* 1973). This cohort involved 2,247 refinery workers employed for at least three years prior to 1969 in Kristiansand, Norway. Follow-up was accomplished to the end of 1979, for a minimum of 10 years. Eighty-two lung cancer cases were observed, giving an SMR of 3.73 and 90% confidence limits 3.08 and 4.48. SMRs unadjusted for smoking habits ranged from 2.28 for those followed up for 3-14 years, to 4.30 for those followed up for more than 35 years. Exposure data for this site were estimated by the US EPA based on INCO estimates of exposure at the Clydach Wales plant. No exposure data were available from the Norwegian smelter itself. The US EPA (1986) took as their exposure estimate the range from 3 to 35 mg Ni/m^3 assuming air levels would be similar to those in the Welsh smelter, and further assumed exposure over one quarter of a lifetime as no record of number of years worked was provided for the Norwegian cohort.

The ICNCM report presented further follow-up of this cohort to 1984. There were 77 lung cancer cases among the refinery workers with an SMR estimate of 2.62 (90% confidence limits 2.15 - 3.16). Three deaths from nasal cancer were reported with 0.66 expected. It was thought that the excess lung cancer risk among electrolysis workers should be attributed to soluble nickel exposure. No actual measurements of exposure were presented.

Table 1 presents a range of exposure estimates and confidence limits around the SMRs for the West Virginia, the Ontario, the Welsh and the Norwegian cohort studies. The ICNCM follow up studies of these cohorts did not alter the relative magnitude of the precision estimates. The precision of the relative risk estimates is assessed by the upper confidence limit of the excess relative risk estimate divided by the point estimate (Table 1).

Table 1: Lung cancer risks in four cohort studies (from US EPA, 1986)

Study	Number of subjects	Lung cancer deaths	Lung cancer SMR	90% confidence limits(CI)	Ratio of upper CI of (SMR-1) to (SMR-1)*
W. Virginia	1855	8	1.12	0.56, 2.02	8.5
Ontario	495	37	8.71	6.49, 11.45	1.4
Wales	967	145	5.28	4.58 – 6.03	1.2
Norway	2247	82	3.73	3.08, 4.48	1.3

* Note: the (SMR-1) is an estimate of excess relative risk

The Ontario cohort study was determined to be the most appropriate for quantitative cancer risk assessment (CDHS, 1991). The main reason for choosing this cohort was that it was the only cohort with actual measurements of exposure associated with a relevant causal period with sufficient

latency (available from 1948 on which is the most relevant period for the lung cancer deaths ascertained between 1963 and 1978).

Animal Studies

At the time that the CDHS (1991) report "Health Risk Assessment for Nickel" was written, inhalation exposure cancer bioassays had only examined insoluble forms of nickel for their carcinogenic potential. Likewise, only soluble forms of nickel had been evaluated by oral administration. Evidence of carcinogenic potential following inhalation exposure has been shown for nickel subsulfide (Ni_3S_2) and suggested for nickel carbonyl. Inhalation exposure to metallic nickel resulted in very poor survival which was too short to properly evaluate carcinogenicity. Many rats exposed to metallic nickel by inhalation developed squamous metaplasia and peribronchial adenomatoses (Hueper and Payne, 1962). Exposure to metallic nickel by intratracheal instillation produced a significant increase in lung tumor incidence. Pott *et al.* (1987) administered nickel powder in a total intratracheal dose of 6 or 9 mg Ni/animal to female Wistar rats. Following treatment, animals were maintained for up to an additional 2.5 years. The lung tumor incidence in the saline control, 6 mg Ni (0.3 mg Ni × 20), and 9 mg Ni (0.9 mg × 10) treatment groups were 0%, 26% and 25%, respectively. The results from the nickel oxide (NiO) studies assessing the carcinogenicity of nickel oxide following inhalation exposure are negative in hamsters (Wehner *et al.,* 1975, 1981) and inconclusive in rats (Takenaka *et al.,* 1985; Glaser *et al.,* 1986; Horie *et al.,* 1985). These studies were also plagued by very poor survival rates. Intratracheal instillation of nickel oxide resulted in lung tumor induction. Female Wistar rats received a total dose of 0, 50, or 150 mg Ni/animal. Animals were maintained for an additional 2.5 years. The lung tumor incidence in the saline control, 50 (5 mg Ni × 10) and 150 mg Ni (15 mg Ni × 10) were 0%, 27% and 32%, respectively (Pott *et al.,* 1987).

Two studies produced positive results in evaluating the pulmonary tumorgenicity of nickel subsulfide, one utilizing inhalation exposure (Ottolenghi *et al.*, 1974) and the other, intratracheal administration. In the Ottolenghi *et al.* study 122 male and 104 female Fischer-344 rats were exposed to 0.97 mg/m^3 nickel subsulfide via inhalation for 78-80 weeks (6 hours/day, 5 days/week). The control group, 120 male and 121 female rats, was exposed to clean air. The study design included two subtreatments in a 2^4 factorial arrangement. The two subtreatments were: 1) pre-exposure for one month to 0.97 mg/m^3 nickel subsulfide 6 hr/day, 5 day/week; and 2) injection of the pulmonary infarction agent, hexachlorotetrafluorobutane. Animals found moribund or succumbing during the study and those killed at the end of the observation period (i.e., 30 weeks) were necropsied. A small number of animals (18 in the exposed and 26 in the control groups) were not examined because of autolysis or cannibalism.

The results demonstrated a significant increase in lung tumors (adenomas, adenocarcinomas, squamous cell carcinomas, and fibrosarcomas) in the exposed 110 male and 98 female rats examined as compared with the control 108 male and 107 female rats examined (Table 2).

Table 2: Neoplastic Changes in Lungs of Rats Exposed to Nickel Subsulfide

Tumors	Controls		Nickel Subsulfide Exposed	
	Males	Females	Males	Females
Adenoma	0/108 (0%)	1/107 (1%)	8/110 (7%)[b]	7/98 (7%)[a]
Adenocarcinoma	1/108 (1%)	0/107 (0%)	6/110 (5%)[a]	4/98 (4%)[b]
Squamous cell carcinoma	0/108 (0%)	0/107 (0%)	2/110 (2%)	1/98 (1%)
Fibrosarcoma	0/108 (0%)	0/107 (0%)	1/110 (1%)	0/98 (0%)

[a] $p < 0.01$, Fisher's Exact Test
[b] $p < 0.05$, Fisher's Exact Test

The two subtreatments, described above, did not alter the effects produced by nickel subsulfide treatment (Ottolenghi *et al.*, 1974). Sex of the exposed animal also did not appear to alter the effects produced by the nickel subsulfide treatment. The study results of Ottolenghi *et al.* (1974) for nickel subsulfide were selected for a quantitative risk assessment.

At the time the CDHS (1991) risk assessment was conducted, injection administration (intramuscular) was the only route of exposure from which data were available for comparison of carcinogenic potency of a variety of nickel compounds. Injection studies by Sunderman (1984) and Skaug *et al.* (1984) demonstrate that based on the incidence of sarcomas produced at the injection site, nickel subsulfide and nickel oxide possess approximately equal potency following this route of exposure. None of the studies utilizing oral exposure has produced evidence of carcinogenic potential. However, it should be noted that only nickel sulfate ($NiSO_4$) has been adequately evaluated in rats by this route.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Nickel subsulfide has been observed to induce lung tumors in male and female rats (Ottolenghi *et al.,* 1974). This study and the Ontario cohort study (Chovil *et al.,* 1981; Roberts *et al.,* 1984; Muir *et al.,* 1985; ICNCM, 1990), which demonstrated an increased risk of lung cancer associated with occupational nickel exposure in humans, were determined to be the most appropriate for use in developing a quantitative cancer risk assessment

Methodology

In the Ottolenghi *et al.* study, the low survival rate of the nickel subsulfide exposed group was examined. Mortality was about the same during the first year of study between the control and the nickel-exposed group but in the 76th week when the first tumor was observed, the exposed group had a 6% higher mortality rate (23% vs. 17% for controls). Taking this into account, the subsequent mortality [(0.94 (exposed group mortality) – 0.23) × 208 (animals examined) = 148 animals] in the nickel-exposed group due to tumors was 29/148 or approximately 20%. Based on these assumptions, nearly all of the differences (0.32 – 0.06 = 0.26) in the survival between control animals (32%) and the treated animals (6%) can be explained by lung tumor mortality. The animal

data was adjusted for continuous lifetime exposure [(979 μg/m^3)(6/24 hr)(5/7 day)(78/110) = 122.8 μg/m^3]. This was used to calculate the human equivalent exposure level using an inspiration rate of 20 m^3/day and a 70 kg body weight. The CDHS staff (CDHS, 1991) used a multistage model (GLOBAL86), fitted to adjusted data from this inhalation bioassay to yield a maximum likelihood estimate of carcinogenic potency of 2×10^{-4} (μg/m^3)$^{-1}$) nickel subsulfide (or 28×10^{-4} (μg Ni/m^3)$^{-1}$). The upper 95% confidence limit of the estimated carcinogenic potency is 28×10^{-4} (μg/m^3)$^{-1}$ nickel subsulfide [or 38×10^{-4} (μg Ni/m^3)$^{-1}$].

The risk quantification was also conducted using epidemiological data from worker studies. The Ontario cohort study (Chovil *et al.,* 1981; Roberts *et al.,* 1984; Muir *et al.,* 1985; ICNCM, 1990) was determined to be the most appropriate for quantitative cancer risk assessment due primarily to the fact that it was the only cohort study with exposure measurements available for a sufficiently early time period. The West Virginia cohort (Enterline and Marsh 1982; ICNCM, 1990) was rejected for use in the quantitative risk assessment due to the imprecision associated with the low SMR reported, which could have been due to confounding factors. The Norwegian cohort (Magnus *et al.,* 1982; Pedersen *et al.,* 1973) was deemed unsuitable for risk assessment due to the absence of nickel exposure data from the refinery. The Welsh cohort (Doll *et al.,* 1977; Peto *et al.,* 1984; Breslow and Day, 1987; Kaldor *et al.,* 1986; ICNCM, 1990) was also not used in the quantitative risk assessment because exposure measurements were not available for a relevant time period.

A relative risk model was chosen as the most appropriate method for linear extrapolation to low dose lifetime exposure. The excess risk from nickel exposure among smokers was assumed to be the same as among non-smokers. SMR values were plotted against cumulative exposure, and the slope of the linear regression of the data was 9.22. The 95% confidence limit of the slope was 11.26. This upper limit was corrected to 11.85 for the fraction of the study group lost to follow-up. The exposure was adjusted for an equivalent lifetime exposure [(8 hr/24 hr) × (5 days/7 days) × (48 weeks/52 weeks)]. The excess relative risk estimate for lifetime exposure at 1 mg/m^3 was 5.04. Considering the background lifetime mortality risk of 0.051 for Ontario at the time of the cohort study follow-up, the upper bound for lifetime added risk for exposure to 1 mg/m^3 was 2.57×10^{-1} (or 2.57×10^{-4} (μg/m^3)$^{-1}$). The average of the unit risk is 2.1×10^{-4} (μg/m^3)$^{-1}$ (257 × 9.22/11.26) using the actual SMR rather the upper confidence limit. Therefore, based on the human studies the range of unit risk is approximately 2.1 - 2.6×10^{-4} (μg/m^3)$^{-1}$. Using the human and animal data, the range of cancer risks is from 2.1 to 37×10^{-4} (μg/m^3)$^{-1}$. The best value chosen for the upper bound of risk was 2.6×10^{-4} (μg/m^3)$^{-1}$, derived from the human lung cancer incidence data. CDHS (1991) noted that the epidemiological studies indicate both soluble and insoluble nickel compounds are carcinogenic. However, the available epidemiologic data were inadequate to develop separate unit risk factors for different nickel compounds.

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N-NITROSODI-N-BUTYLAMINE

CAS No: 424-16-3

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	158.2
Boiling point	116° C @ 14 mm Hg
Melting point	0.5° C
Vapor pressure	Not found
Air concentration conversion	1 ppm = 6.46 mg/m^3 @ 25° C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.1 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 1.1 E+1 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by CDHS (1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There is no direct evidence that links nitrosamines, including N-Nitrosodi-n-butylamine (NDBA), to human cancer. The US EPA (1980) and IARC (1978) concluded that the epidemiological studies to date were inadequate to establish a valid causal relationship between nitrosamine exposure and human cancer. The US EPA (1980) also concluded that it was highly improbable that humans are refractory to the carcinogenic effects of nitrosamines considering the number of animal species that show increased tumor incidence following nitrosamine exposure. IARC (1978) concluded: "Although no epidemiological data were available, N-nitrosodi-n-butyl amine should be regarded, for practical purposes, as if it were carcinogenic to humans."

Animal Studies

Groups of DB rats were exposed to NDBA in the drinking water for an unspecified duration (Druckrey *et al.*, 1967). The rats were exposed to 10, 20, 37, and 75 mg/kg-day NDBA. The incidence of liver carcinomas and adenomas was 2/10, 4/10, 13/16, and 4/4, respectively. No data on control rats were reported. Median time-to-tumor decreased from 540 days at 10 mg/kg-day, to 150 days at 75 mg/kg- day.

Male Wistar rats (15 total) were exposed for 24 weeks to 0.05% NDBA in drinking water (Okajima *et al*., 1971). Nine animals served as controls. At the end of this period, the 12 remaining animals were necropsied and found to have papillomas of the bladder. Eleven of the 12 animals had bladder carcinomas, 4 had hepatocellular carcinomas, and all had papillomas or carcinomas of the esophagus. The increased incidence of bladder carcinomas and esophageal tumors was statistically significant compared to the control group ($p < 0.001$). The incidence of liver carcinomas was not statistically different from controls ($p < 0.083$). A group of 20 male Fischer-F344 rats was exposed

to 5.4 mg NDBA by gavage twice per week for 30 weeks (Lijinsky and Reuber, 1983). Six animals survived to 100 weeks. Three animals survived to the end of the 108-week experiment. All treated animals were necropsied and examined for tumors. Of the 20 treated animals, liver carcinomas were observed in 12, lung carcinomas and adenomas in 9 and 4, respectively, forestomach cancer in 10, and bladder cancer in 7. In the control group of 20 male rats, one animal had a lung carcinoma, but no carcinomas of the esophagus, liver, or bladder were observed. The incidences of liver, lung, forestomach, and bladder carcinomas were significantly increased over controls ($p < 0.001$, 0.004, 0.001, and 0.004, respectively.).

A group of 42 male Fischer F344 rats were exposed to 0.005% (50 ppm) NDBA for 4 weeks (Imaida and Wang, 1986). The rats were followed for 100 weeks post-exposure and the median survival was 93 weeks. Esophageal cancer (unspecified type) was found in 26 of the 42 animals and esophageal papillomas in 7 of 42. In addition, liver nodules and carcinomas were found in 8/42 and 3/42 treated animals, respectively, and forestomach papillomas and carcinomas occurred in 6/42 and 2/42 treated animals, respectively. Bladder papillomas and carcinomas were found in 5/42 and 2/42 treated animals, respectively. In the control group of 39 animals, 2 animals had liver nodules, but no other tumors were detected.

Tsuda *et al*. (1987) exposed groups of 20 male F344 rats to 650, 1250, and 2500 ppm NDBA in the diet for 2 weeks. The rats were then fed an untreated diet for 50 weeks, at which time the animals were killed and necropsied. The incidence of bladder papillomas and hepatocellular carcinomas are shown in Table 1.

Table 1. Tumor incidence in male F344 rats exposed to diet containing N-nitrosodi-n-butylamine (NDBA) (Tsuda *et al*., 1987)

NDEA Treatment Group (ppm in diet)	Bladder papillomas	Hepatocellular carcinomas
650	2/20	0/20
1250	2/20	2/20
2500	4/20	4/20

Male ICR mice (39 total) were exposed to 50 ppm NDBA in the diet for 12 months (Takayama and Imaizumi, 1969). Of the surviving 33 mice at the end of 12 months, 27/33 had squamous cell carcinomas of the forestomach, 15/33 developed liver tumors (5 trabecular hepatomas and 10 adenomas), 8/33 developed lung adenomas, and 4/33 developed esophageal papillomas. Examination of the 28 surviving control animals revealed that 2 had lung adenomas, but no other tumors were reported in the controls.

Male or female C57Bl/6 mice (50/sex/group) were exposed to 60 or 240 ppm NDBA in their drinking water from age 10-12 weeks until moribund or dead (Bertram and Craig, 1970). The incidence of bladder carcinomas in males was 17/47 and 36/45 at the 60 and 240 ppm concentrations, respectively. Females developed bladder carcinomas with an incidence of 2/42 and 8/45 for the 60 and 240 ppm groups, respectively. Esophageal papillomas were found in 45/47 and 40/42 for males and 40/42 and 45/45 for females at the 60 and 240 ppm groups, respectively. No data on unexposed controls were presented.

Male and female Syrian Golden Hamsters (5/sex/group) were given single doses of 400, 800, or 1600 mg/kg NDBA by gavage; groups of 20 males or females served as controls (Althoff *et al*., 1973). Animals were observed for their lifespan and were killed when moribund. Mean survival times were affected in a dose-dependent manner; controls survived 63.5 weeks, and low-, medium- and high-dose groups survived for 59.6, 54.5, and 49.3 weeks, respectively. Respiratory neoplasms (unspecified type) were found in 0/40, 3/10, 5/10 and 7/10 hamsters of the control, low-, medium-, and high-dose groups, respectively. In a later study, Althoff *et al*. (1974) exposed groups of 20 (10/sex/group) male and female Syrian Golden hamsters to 0, 29, 58, 116, 232, or 464 mg/kg NDBA once pre week for life. In this study, the incidences of respiratory neoplasms were 0/20, 0/20, 0/19, 2/16, 12/20, and 8/16 for the groups exposed to 0, 29, 58, 116, 232, or 464 mg/kg NDBA, respectively.

Several studies in mice, rabbits and hamsters have shown NDBA to be carcinogenic following subcutaneous injection (Fuji *et al*., 1977; Flaks *et al*., 1973; Althoff *et al*., 1973 & 1974; Reznik *et al*., 1976; Cohen *et al*., 1975).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The study by Bertram and Craig (1970) was used by CDHS (1988) to derive the cancer potency for NDBA. The upper bound estimate of cancer potency from these data is the most reliable upper bound from dose-response data in sensitive species.

The upper 95% bound on the multistage polynomial could not be determined from the tumor incidence data in two other studies: Althoff *et al*. (1974) and Okajima *et al*. (1971). The potency estimates from the studies by Lijinsky and Reuber (1983), and Takayama and Imaizumi (1971) both rely on assumptions about the time of sacrifice and corrections for less than lifetime dosing.

Methodology

The study by Bertram and Craig (1970) showed that several organ sites developed tumors in both sexes of mice exposed to NDBA. The combined incidence of bladder and esophageal neoplasms in male mice was used for the potency estimate. These data depict the most reliable dose-response for the most sensitive site and species.

A linearized multistage procedure was used to estimate the cancer potency of NDBA from the Bertram and Craig. (1970) data in male C57Bl/6 mice (Crump *et al*., 1982). The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. In this case, the scaling factor was equal to 1. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 10.8 $[mg/kg\text{-}day]^{-1}$ was calculated for NDBA (CDHS, 1988). An airborne unit risk factor of 3.1E-3 $(\mu g/m^3)^{-1}$ was calculated by OEHHA/ATES from the q_{human} value using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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N-NITROSO-N-METHYLETHYLAMINE

CAS No: 10595-95-6

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	88.13
Boiling point	163ºC
Melting point	not available
Vapor pressure	not available
Conversion factor	1 ppm = 3.61 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.3 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 2.2 E+1 $(mg/kg\text{-}day)^{-1}$
[Female rat hepatocellular carcinoma data (Druckrey *et al*., 1967), one hit model, time to tumor incidence (US EPA, 1993), cross-route extrapolation]

III. CARCINOGENIC EFFECTS

Human Studies

No human carcinogenicity data on specific exposure to N-nitroso-N-methylethylamine (NMEA) have been reported. Human exposure to nitrosamines is usually the result of exposure to complex mixtures containing these compounds (e.g. cutting oils, tobacco products). Carcinogenicity data on these mixtures are of limited use in evaluating the carcinogenicity of individual nitrosamines because of the presence of other potentially confounding substances.

Animal Studies

Fifteen female BD rats were exposed to N-nitroso-N-methylethylamine (NMEA) in drinking water at doses of approximately 1 mg/kg/day (4 rats) or 2 mg/kg/day (11 rats). Exposure was continuous for the lifetime of the animals. Nine of 15 animals developed hepatocellular carcinomas and one developed a fibrosarcoma of the vagina. Average tumor induction times and total doses to produce tumors in 50% of the animals were 500 days and 0.42 g/kg for the low dose group and 360 days and 0.75 g/kg for the high-dose group. No control group was included in this study (Druckrey *et al*., 1967; reviewed in IARC, 1974 and US EPA, 1993).

DMEA also induced hepatocellular carcinomas (19/20 animals), hemangiosarcomas (17/20) and cholangiocarcinomas (3/20) with accompanying lung metastases and esophageal papillomas and carcinomas in male and female Fisher 344 (F344) rats (20/group) (Lijinsky and Reuber, 1981). Study animals received drinking water (20 ml/day/rat) containing 150 mg/l DMEA 5 days/week (tap water provided on the untreated days) for 30 weeks (total dose 450 mg) with lifetime observation. Untreated control groups were not included in this study. In a similar study, male F344 rats dosed with 600 µg or 3000 µg DMEA/week (6 or 30 mg/l in drinking water 5 days/week)

for 30 weeks developed tumors. Three of 20 animals in the low-dose group developed hepatocellular carcinomas; 12 of 20 animals in the high-dose group developed hepatocellular carcinomas, nasal tumors and esophageal papillomas (Lijinsky and Reuber, 1980). This study also did not include an untreated control group. Liver (hepatocellular carcinoma; 9/20 animals) and nasal cavity (4/20 animals) tumors were also observed in male F344 rats exposed to drinking water containing 30 mg/l NMEA 5 days/week for 30 weeks (total dose 90 mg) (Lijinsky *et al.*, 1982). In contrast, the only possibly exposure-related increase in tumor incidence seen in female F344 rats receiving drinking water containing 6 mg/l NMEA was an increased leukemia incidence (18/20 treated compared to 12/20 controls) (Lijinsky *et al.*, 1983).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The study by Druckrey *et al*. (1967) in which 9 of 15 female BD rats exposed to 1 mg/kg/day (4 rats) or 2 mg/kg/day (11 rats) DMEA in drinking water over their lifetime developed hepatocellular carcinomas was chosen as the basis of a cancer potency factor for NMEA. This study used lifetime exposure to NMEA in a sensitive species and sex.

Methodology

A one-hit model was fitted to time-to-tumor data from the study by Druckrey *et al*. (1967). The dose associated with a lifetime risk of 0.5 was calculated as follows:

$$d = Ck/(t_{50})^n = \frac{88.1\ mg/mmol * 0.81 \times 10^{-4}\ mmol/kg/day}{(728)^{2.3}}$$

where C is the conversion between mmol and mg, k is an empirically derived constant (carcinogenicity index) (Druckrey *et al*., 1967), t_{50} is the median time of tumor induction in days, and n is an empirically generated representative value for dialkylnitrosamines (Druckrey *et al.*, 1967). This relationship was derived from experimental data from studies on a number of different N-nitroso compounds. The one-hit model was used to derive a cancer potency factor of 3.72 $(mg/kg/day)^{-1}$. Adjusting this factor by the cube root of the human body weight/assumed rat body weight ratio $[(70\ kg/0.35\ kg)^{1/3}]$ results in a human cancer potency factor of 2.2 E+1 $(mg/kg/day)^{-1}$. A unit risk of 6.3 E-3 $(\mu g/m^3)^{-1}$ was then calculated by OEHHA/ATES from the cancer potency factor (20 m^3/day inspiration rate).

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N-NITROSODI-N-PROPYLAMINE

CAS No: 621-64-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	130.12
Boiling point	not available
Melting point	not available
Vapor pressure	0.086 mm Hg @ 20°C
Air concentration conversion	1 ppm = 5.3 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.0 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 7.0 E+0 $(mg/kg\text{-}day)^{-1}$
[calculated from a cancer slope factor derived by US EPA (1986)]

III. CARCINOGENIC EFFECTS

Human Studies

No studies addressing the carcinogenicity of N-nitrosodi-n-propylamine to humans have been conducted.

Animal Studies

Druckrey *et al.* (1967) treated a total of 48 BD rats (sex unspecified) orally with N-nitrosodi-n-propylamine at concentrations of 4, 8, 15, or 30 mg/kg body weight 7 days per week for life (16, 16, 15, and 1 animal, respectively). An untreated control group was not included in the study, although background tumor incidence was reported to be negligible. Liver carcinoma incidence was 14/16, 15/16, 15/15, and 1/1 in the four dose groups, respectively. Tumor induction time was dose-related. The authors also note tumors of the esophagus (8/48) and tongue (6/48).

Lijinsky and Taylor (1978, 1979) exposed 15 male Sprague-Dawley rats to N-nitrosodi-n-propylamine in drinking water 5 days/week for 30 weeks at 1.8 mg/day resulting in a daily dose of 5.1 mg/kg-day. No control group was included. Liver carcinomas (9/15), esophageal papillomas (6/15) and carcinomas (8/15), and nasal adenocarcinomas (8/15) were observed among exposed rats.

Lijinsky and Reuber (1981) exposed 20 Fischer 344 rats (sex unspecified) to N-nitrosodi-n-propylamine in drinking water at 0.9 mg/day for 5 days/week for 30 weeks resulting in a daily dose of 2.6 mg/kg-day. No control group was included. Esophageal carcinomas (20/20) and forestomach tumors (12/20) developed in exposed animals.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

US EPA (1986) based its selection of a cancer potency on a study which demonstrates induction of liver tumors by N-nitrosodi-n-propylamine. US EPA (1986) used the data from Druckrey *et al.*(1967) in the induction of hepatocellular carcinoma in BD rats exposed to N-nitrosodi-n-propylamine in drinking water to calculate a cancer potency value.

Methodology

The high tumor incidence in all the N-nitrosodi-n-propylamine treated animals suggests time-dependent analysis is more appropriate than multistage analysis in the derivation of a cancer potency value. A dosage estimate for use in deriving the cancer potency value was based on the following relationship, where d is the daily dose, C is the mmol to mg conversion factor (130.2 mg/mmol), k is an empirically derived constant estimated from a plot of k versus the number of carbon atoms for lower di-N-alkylnitrosamines ($k=1.7 \times 10^4$ mmol/kg-day), t_{50} is the median time of tumor induction, and n is a representative value for dialkylnitrosamines (n=2.3; Druckrey *et al.*, 1967):

The resulting daily dose estimate was 0.578 mg/kg-day. Applying this estimate to a rearrangement of the one-hit model gave an animal cancer potency value (q_{animal}) of 1.2 $(mg/kg\text{-}day)^{-1}$.

$$q_{animal} = -\ln(0.5/day) / d$$

Conversion of the q_{animal} to a human cancer potency estimate (q_{human}) was made based on the following relationship, where bw_h is the assumed human body weight (70 kg) and bw_a is the assumed experimental animal body weight (0.35 kg):

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

The resulting estimate of q_{human} was 7.0 $(mg/kg\text{-}day)^{-1}$.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 2.0 E-3 $(\mu g/m^3)^{-1}$.

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N-NITROSODIETHYLAMINE

CAS No: 55-18-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	102.1
Boiling point	175-177° C
Melting point	Not found
Vapor pressure	0.86 mm Hg @ 20° C
Air concentration conversion	1 ppm = 4.2 mg/m^3 @ 25° C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.0 E-2 $(\mu g/m^3)^{-1}$
Slope Factor: 3.6 E+1 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by CDHS (1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There is no direct evidence that links nitrosamines, including N-nitrosodiethylamine (NDEA), to human cancer. The US EPA (1980) concluded that the epidemiological studies to date were inadequate to establish a valid causal relationship between nitrosamine exposure and human cancer. The US EPA (1980) also concluded that it was highly improbable that humans are refractory to the carcinogenic effects of nitrosamines considering the number of animal species that show increased tumor incidence following nitrosamine exposure.

Animal Studies

A number of qualitative studies were conducted in a range of species, including rats, mice, hamsters, guinea pigs, rabbits, dogs, and monkeys (Yamamoto *et al*., 1972; Druckrey *et al*., 1967; Magee *et al*., 1976; Rajewski *et al*., 1966; Tomatis, 1973). In addition to these studies, a number of later studies show evidence of quantitative dose-response relationships.

Drinking water containing a range of concentrations from 0.033 to 16.896 ppm NDEA was administered to male and female Colworth rats (60/sex/group) for their natural lifespan (Peto *et al*., 1982; 1984). The control groups consisted of 240 rats/sex. Nearly all animals exposed to the high dose of NDEA died from tumors of the liver or esophagus (Table 1). Other sites showing an increase in tumors included the lower jaw, stomach, kidney, ovaries, seminal vesicles, and nasopharynx.

Table 1. Liver tumors in Colworth male and female rats exposed to drinking water containing N-nitrosodiethylamine (NDEA) (Peto *et al*., 1982)

NDEA concentration (ppm)	Observed deaths from liver tumors	
	males	females
0	1/240	1/240
0.033	1/60	0/60
0.066	0/60	0/60
0.132	5/60	1/60
0.264	2/60	1/60
0.528	4/60	3/60
1.056	8/60	23/60
1.584	14/60	37/60
2.112	7/60	38/60
2.640	17/60	47/60
3.168	17/60	42/60
4.224	26/60	42/60
5.280	26/60	43/60
6.336	30/60	47/60
8.448	25/60	55/60
16.896	44/60	49/60

A later analysis by Peto *et al.* (1984) showed that, in rats, the initial age of contact with NDEA was important in determining the probability of liver cancer. Young rats were much more susceptible than adults to NDEA-induced liver neoplasia than adults exposed for an equal amount of time.

Lijinsky *et al.* (1981) administered NDEA in the drinking water to 11 groups of female Fisher-344 rats (20 per group) for varying durations, up to 104 weeks. The animals were observed for their lifespan. The treatment groups are shown in Table 2.

Table 2. Treatment groups, concentrations, and durations of N-nitrosodiethylamine (NDEA) exposures in female Fisher 344 rats (Lijinsky *et al*., 1981).

Treatment Group	NDEA concentration(mg/l)	Treatment duration (weeks)
Control	0	104
1	113	17
2	45	22
3	18	30
4	7	30
5	2.8	30
6	1.1	30
7	1.1	60
8	0.45	30
9	0.45	60
10	0.45	104

Treatment related increases in the incidence of tumors were observed in the liver, forestomach, esophagus and tongue. The incidence of tumors in the animals exposed to the lowest concentration are shown in Table 3.

Table 3. Tumor incidence in female rats exposed to 0.45 mg/l N-nitrosodiethylamine (NDEA) in drinking water for 0, 30, 60, or 104 weeks (Lijinsky *et al*., 1981).

Treatment duration (weeks)	Total dose (mg) of NDEA	Esophageal carcinoma or papilloma	Forestomach papilloma	Tongue carcinoma	Liver carcinoma	Liver carcinoma or hyperplastic nodule
0	0	0/20	0/20	0/20	0/20	1/20
30	1.35	1/20	1/20	1/20	1/20	6/20
60	2.70	3/20	2/20	2/20	6/20	11/20
104	4.68	13/20	5/20	2/20	4/20	7/20

Habs and Schmahl (1980) exposed male Sprague-Dawley rats (90 per group) to 0 or 0.1 mg/kg/day NDEA in the drinking water 5 times weekly until natural death of the animals. Another group of rats received NDEA followed by a 25% solution of ethanol. Liver tumors (histological type unspecified) were observed in 0/82 controls and 36/82 NDEA-treated rats, respectively. The rats receiving ethanol in addition to NDEA showed a liver tumor incidence of 4/59. A similar pattern was seen for the development of esophageal tumors. Rats exposed to NDEA alone developed esophageal tumors (type unspecified) at a rate of 33/82. Rats exposed to NDEA and ethanol developed esophageal tumors in 18/59 cases, and controls had a tumor incidence of 0/82.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The studies by Peto *et al*. (1982; 1984) were used by CDHS (1988) to derive the cancer potency for NDEA. These studies utilized relatively large numbers of animals (60-240 per group) over a wide dose range. The Peto *et al*. (1982) study contained more information about the dose-response at the low range of experimental doses than the other studies described. Therefore, the cancer potency for NDEA was calculated from the Peto *et al*. (1982) study even though the calculated value is lower than other potency estimates from Lijinsky *et al*. (1981) or Habs and Schmahl (1980).

Methodology

The study by Peto *et al*. (1982) showed that several organ sites developed tumors in both sexes of rats exposed to NDEA. The incidence of hepatocellular neoplasms (histological designation unknown) in males resulted in the highest potency value when only the 6 lowest doses were considered. Water consumption by male rats was reported by Peto *et al*. (1984) to be 41 mL/kg/day. Low-dose group mortality did not differ significantly from that observed in the control group, therefore no time corrections were applied to the calculation.

A linearized multistage procedure was used to estimate the cancer potency of NDEA from the Peto *et al*. (1982) data in male Colworth rats (Crump *et al*., 1982). The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. In this case, the scaling factor was equal to 1. An estimated value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the body weight of human or animal, in this case, 450 grams for male rats.

Using these relationships, a human cancer potency (q_{human}) of 36 $[mg/kg\text{-}day]^{-1}$ was calculated for NDEA (CDHS, 1988). An airborne unit risk factor of 1.0E-2 $(\mu g/m^3)^{-1}$ was calculated by OEHHA/ATES from the q_{human} value using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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N-NITROSODIMETHYLAMINE

CAS No: 62-75-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	74.1
Boiling point	151°C
Melting point	unknown
Vapor pressure	2.7 mm Hg @ 20°C
Air concentration conversion	1 ppm = 3.08 mg/m^3 @ 20°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.6 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 1.6 E+1 $(mg/kg\text{-}day)^{-1}$
[calculated from a cancer potency value derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiological studies correlating exposure to N-nitrosodimethylamine (NDMA) and human cancers are inadequate to establish a causal relationship.

Animal Studies

Terracini *et al* .(1967) exposed male and female MRC Porton rats to feed containing 0, 2, 5, 10, 20 or 50 ppm for up to 120 weeks. Daily dose rates were calculated based upon observed food consumption rates of 15 g/day. Most survivors were sacrificed at 104 weeks, with the exception of an unspecified number of animals which were sacrificed at 120 weeks. Liver tumor incidence data were grouped into those which occured among animals surviving greater than or less than 60 weeks. No liver tumors were reported among 29 untreated animals, four of which died before 60 weeks. Combined incidence data of liver tumors among female rats dying at any time during the course of the experiment were 0/18, 4/62, 2/5, 15/23, and 10/12 for the 2, 5, 10, 20, and 50 ppm dose groups, respectively. Significant dose-related increase in incidence of liver tumors and mortality in female rats was reported (level of significance not stated).

Terracini *et al.* (1973) exposed 4-5 week old female BALB/c mice to 3 ppm NDMA in drinking water for up to 80 weeks in a two generation study. The first generation treated group consisted of 62 animals and the second generation treated group consisted of 66 animals. Among first and second generation animals, an increased incidence of lung tumors was found (first generation:

44/62 treated vs. 20/62 control, $p = 10^{-5}$; second generation: 44/66 treated vs. 15/69 control, $p = 10^{-8}$).

Terao *et al.* (1978) exposed 4 week old male Wistar rats to feed containing NDMA and/or sterigmatocystin (STG) for 54 weeks. Five exposure groups included 10 ppm STG alone, 10 ppm STG and 1 ppm NDMA, 1 ppm STG and 10 ppm NDMA, 10 ppm NDMA, and a group receiving basal diet alone. Animals were sacrificed at 69 weeks with the exception of one animal from each group sacrificed after 5 weeks of exposure. Rats in all groups exposed to NDMA showed an increased incidence of Leydig-cell tumors ($p = 0.002$). Liver tumors were not observed in the group receiving NDMA alone; however, liver tumor incidence was elevated in the group receiving STG alone and in the group receiving 10 ppm STG and 1 ppm NDMA.

Arai *et al.* (1979) exposed 6 week old male and female Wistar rats (7 to 17/sex/group) to feed containing 0, 0.1, 1 or 10 ppm NDMA for 96 weeks. Food intake was monitored. Treated female animals were found to have a higher incidence of nodular hyperplastic liver lesions ($p < 0.05$, Fisher's exact test).

Griciute *et al.* (1981) treated 8 week old male and female C57BL mice with NDMA (0.03 mg) and/or ethanol (0.8 ml) in 0.2 ml water by intragastric intubation for 50 weeks. Control animals received only water. Survivors were sacrificed at 80 weeks. No liver tumors developed in animals receiving ethanol alone. Among animals receiving NDMA alone, the incidence of malignant liver tumors was increased over controls (14/37 treated males, $p = 10^{-5}$; 16/29 treated females, $p < 10^{-6}$). Among animals receiving both NDMA and ethanol, the incidence of forebrain olfactory neuroepithelioma was increased (24/66 treated vs. 0/66 control; $p < 0.001$). No tumors of this type were observed in animals receiving NDMA or ethanol alone.

Peto *et al.* (1982, 1984) exposed Colworth rats (60/sex/group) to NDMA in drinking water at 15 concentrations ranging from 0.033 to 16.896 ppm for life. A group of 240 animals receiving only drinking water served as controls. Additional treatment groups of 6 animals/group were sacrificed at 6 and 12 months. Water consumption for male and female rats was 41 ml/kg and 72 ml/kg, respectively. Among exposed animals, the incidence of fatal liver neoplasms (see Table 1) was significantly increased over controls ($p \leq 0.005$) and the increase was found to be dose-related. Other tumors with trends toward increased incidence include tumors of the lung ($p = 0.004$), skin ($p = 0.001$), lymphatic/hematopoietic tissues ($p = 0.032$), and seminal vesicles ($p = 0.004$).

Lijinski and Reuber (1984) exposed 7-8 week old female Fischer 344 rats (20/group) to NDMA in drinking water at concentrations of 13 and 5.5 mg/l for 5 days/week for 30 weeks. Animals were observed for life. Hepatocellular carcinomas, hemangiosarcomas, and neoplastic nodules were observed in treated animals. Significantly increased incidence of liver tumors of all types was observed in both low-dose (14/20 treated vs. 2/20 controls; $p = 10^{-4}$) and high-dose (19/20 treated vs. 2/20 controls; $p < 10^{-5}$) animals.

Table 1. Liver tumor incidence data in Colworth rats exposed to NDMA in drinking water (Peto *et al.*, 1982).

dose level (ppm)*	fatal liver tumor incidence	
	male	female
0	1/240	1/240
0.033	1/60	1/60
0.066	3/60	0/60
0.132	3/60	2/60
0.264	3/60	3/60
0.528	3/60	5/60
1.056	5/60	5/60
1.584	3/60	27/60
2.112	13/60	33/60
2.640	27/60	44/60
3.168	33/60	48/60
4.224	36/60	53/60
5.280	46/60	52/60
6.336	49/60	51/60
8.448	55/60	55/60
16.896	59/60	58/60

*Colworth rats (48/sex/group) were exposed to NDMA in drinking water for life.

Druckrey *et al.*(1967) exposed BD rats (sex unspecified) to NDMA by inhalation twice per week for 30 minutes. One group of 6 rats received 100 ppm NDMA and a group of 12 rats received 50 ppm NDMA. No information on a control group was reported. Tumors of the nasal turbinates were reported at the time of death in 4/6 and 8/12 of the high- and low-dose groups, respectively. Three animals in the low-dose group died prematurely (time unspecified). No liver tumors were reported in either group.

Moiseev and Benemansky (1975; reviewed in IARC, 1978) exposed Balb/c mice and Wistar rats to NDMA by inhalation at concentrations of 0.005 and 0.2 mg/m^3. Exposure duration was 17 months for mice and 25 months for rats. At 0.2 mg/m^3 NDMA, tumors of the lung, liver, and kidney were reported to arise earlier and in greater numbers than in control animals (IARC, 1978). Exposures to the lower concentration did not result in significantly increased incidence in tumors over controls.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Five studies showing tumor induction in animals by NDMA have been deemed appropriate by CDHS (1988) for the development of cancer potency values. The values derived from the studies are presented in Table 2. The methodologies used to derive the values are described below as well as the rationale for selection of the OEHHA unit risk value for NDMA.

Methodologies

Peto *et al* .(1982, 1984) derived potency values from the incidence data of fatal liver tumors in male and female Colworth rats. The cumulative risk was calculated based on the assumption that the risk increases with the seventh power of exposure duration and the observation that a dose of 1.0 μg/kg-day results in a 0.03-0.04% incidence of liver tumors at two years. Estimated cancer potency at low doses (q_{animal}) was found to be 0.29 and 0.4 $(mg/kg\text{-}day)^{-1}$ for male and female rats, respectively. Peto *et al.* (1982,1984) also scaled these potencies up by a factor of 7 to account for calculated increased risk from the observation that median experimental animal lifespan was beyond 2 years in this study. Conversion to human potency values (q_{human}) was based on the body weight scaling relationship described below, with an assumed human body weight (bw_h) of 70 kg and experimental animal body weights (bw_a) of 450 and 250 g for male and female rats:

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

The resulting q_{human} values were 12 and 16 $(mg/kg\text{-}day)^{-1}$ from the male and female rat data, respectively.

Dose rate estimates of 0.82 mg/kg-day for female BALB/c mice receiving NDMA in drinking water in the study by Terracini *et al.* (1973) were based on a US EPA (1988) reference animal body weight value and water consumption rate (CDHS, 1988). Using a multistage procedure, experimental potencies (q_1^*) derived from this dose rate using the incidence of lung tumors in F_0 and F_1 generation animals were 1.5 and 1.6 $(mg/kg\text{-}day)^{-1}$, respectively. Potency in animals (q_{animal}) was estimated assuming cancer incidence increases with the third power of age, with T_e the experimental duration and T the natural lifespan of the animals (104 weeks):

$$q_{animal} = q_1^* \times (T/T_e)^3$$

Further conversion to human cancer potencies with a body weight scaling factor were made as described for Peto *et al.*(1982,1984) resulting in human potency estimates (q_{human}) of 49 and 53 $(mg/kg\text{-}day)^{-1}$ from the F_0 and F_1 generation mouse tumor incidence data, respectively.

High- and low-dose rates estimates of 0.80 and 0.35 mg/kg-day in the study by Lijinsky and Reuber (1984) were based on US EPA (1988) animal body weight reference values in the induction of liver tumors in Fischer 344 rats (CDHS, 1988). Using the Crouch (1983) correction for variable dosing and a multistage procedure, the animal cancer potency estimate (q_{animal}) was 5.8 $(mg/kg\text{-}day)^{-1}$. Using the body weight conversion factor as described in the Peto *et al.* (1982, 1984) potency derivation (bw_a=0.229 kg; US EPA, 1988), the resulting q_{human} was 39 $(mg/kg\text{-}day)^{-1}$.

Dose rate estimates to MRC Porton rats exposed to NDMA in diet in the study by Terracini *et al.* (1967) were made by the method of Crouch (1983) to account for variable dosing during the course of the experiment (CDHS, 1988). Using liver tumor incidence among female rats surviving less than 60 weeks, a q_{animal} value of 5.8 $(mg/kg\text{-}day)^{-1}$ was derived from a multistage procedure. Conversion to q_{human} based on a body weight scaling factor resulted in a potency value of 34 $(mg/kg\text{-}day)^{-1}$. Using liver tumor incidence of animals surviving more than 60 weeks resulted in a q_{human} value of 7.6 $(mg/kg\text{-}day)^{-1}$; however, this value may be an underestimate because of early mortality in exposed animals.

Arai *et al.* (1979) estimated an NDMA dose rate of 0.018 and 0.033 mg/kg-day for male and female Wistar rats, respectively, based on experimentally reported food consumption rates and animal body weights. Applying a multistage procedure to the incidence of liver fibrosarcoma in male rats resulted in an estimated q_{animal} of 5.0 $(mg/kg\text{-}day)^{-1}$. Similarly, a q_{animal} for incidence of liver cancer in female rats was found to be 3.8 $(mg/kg\text{-}day)^{-1}$. The resulting q_{human} values from each of these tumor types were 29 and 25 $(mg/kg\text{-}day)^{-1}$, respectively.

Terao *et al.* (1978) demonstrated induction of Leydig-cell tumors in male Wistar rats fed diet containing NDMA. Dose rate calculations of 0.736 mg/kg-day were based on US EPA (1988) reference food intake and body weight values (CDHS, 1988). The dose correction method of Crouch (1983) was applied to account for variable dosing during the course of the experiment. Applying a multistage procedure to the tumor incidence data resulted in a q_{animal} value of 5.8 $(mg/kg\text{-}day)^{-1}$. The corresponding q_{human} value based on the body weight conversion factor was 31 $(mg/kg\text{-}day)^{-1}$.

Table 2. Cancer potencies derived from animal studies.

Study	Tumor type	Sex	q_{human} $(mg/kg\text{-}day)^{-1}$
Peto *et al.*(1982,1984)	fatal liver tumor	male	12
		female	16
Terracini *et al.*(1973)	lung tumor	F_0 female	49
		F_1 female	53
Lijinsky and Reuber (1984)	liver tumor	female	39
Terracini *et al.*(1973)	liver tumor	female	34
Arai *et al.* (1979)	liver fibrosarcoma	male	29
	liver cancer	female	25
Terao *et al.*(1978)	Leydig cell tumor	male	31

Cancer potency estimates from these studies range from 12 to 53 $(mg/kg\text{-}day)^{-1}$. Of these, the q_{human} values from the Peto *et al.* (1982,1984) study were derived from the experiment with the lowest daily dose rate and the data, therefore, are most appropriate for performing a low-dose risk extrapolation. Although the q_{human} values from Peto *et al.* (1982,1984) are lower than those derived from other studies, the fact that this study was conducted at low-doses and demonstrated sensitive and significant induction of liver tumors indicates it is useful for estimation of the cancer potency of NDMA. Furthermore, the other studies were neither as large in scale nor as long in duration, suggesting potency estimates from these studies may be overly conservative and not as representative of the true value. The most sensitive q_{human} value of 16 $(mg/kg\text{-}day)^{-1}$ derived from Peto *et al.* (1982,1984) was therefore adopted as a cancer potency value.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 4.6 E-3 $(\mu g/m^3)^{-1}$.

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N-NITROSODIPHENYLAMINE

CAS No: 86-30-6

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	198.2
Boiling point	66.5°C
Melting point	unknown
Vapor pressure	0.1 mm Hg @ 25°C
Air concentration conversion	1 ppm = 8.1 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.6 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 9.0 E-3 $(mg/kg\text{-}day)^{-1}$
[calculated from a cancer potency value derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There are no human carcinogenicity studies available for N-nitrosodiphenylamine (NDPA).

Animal Studies

Seven day-old $B6C3F_1$ and $B6AKF_1$ mice (18/sex/group) were treated with 1000 mg NDPA in dimethyl sulfoxide per kg body weight by oral gavage for 4 weeks (initial dose was not adjusted) (NTIS, 1968; Innes *et al.*, 1969). Mice were then exposed to 3769 ppm NDPA in feed to 79 weeks of age. Animals were observed for a total of 18 months. Among male $B6C3F_1$ mice, 6/15 surviving animals developed hepatomas versus 1/17 matched controls. Among female $B6AKF_1$ mice, 3/18 developed lung adenomas versus 0/17 matched controls. No statistically significant increases in tumor incidence were reported by NTIS (1968). However, a re-analysis of incidence data by current methodology showed significant increases in hepatoma incidence among male $B6C3F_1$ mice (p =0.027 by Fisher's exact test) and borderline significant increase in lung adenoma incidence among female $B6AKF_1$ mice (p =0.07) (CDHS, 1988).

$B6C3F_1$ mice (50/sex/group) were fed diet containing NDPA at two dose levels (NCI, 1979; Cardy *et al.*, 1979). Male mice received either 10000 or 20000 ppm NDPA in their diet for 101 weeks. The low- and high-dose groups of female mice received 5000 or 10000 ppm NDPA , respectively, for 38 weeks, no NDPA for 3 weeks, then 1000 or 4000 ppm NDPA for 60 weeks. Groups of 20 mice/sex fed only standard diet served as controls. No statistically significant increases in tumor incidence were observed over controls. Some incidence of epithelial hyperplasia of the urinary bladder was noted which was not seen in control animals.

Six-week old Fischer 344 rats (50/sex/group) were exposed to diet containing 1000 or 4000 ppm NDPA for 100 weeks, with groups of 20 rats/sex serving as controls (NCI, 1979; Cardy *et al.*, 1979). No dose-related increase in mortality was observed in male mice; however, a dose-related increase in mortality was observed in females (p =0.024). Among male rats, incidence of transitional-cell carcinoma of the urinary bladder was 16/45 in the high-dose group, 0/46 in the low-dose group, and 0/19 in the control animals (p =0.001). Among female rats, incidence of transitional-cell carcinoma of the bladder was 40/49 in the high-dose group, 0/46 in the low-dose group, and 0/18 in the control animals ($p < 0.001$). Among male mice, a dose-related trend in increased incidence of fibroma of the subcutis and skin was observed (p =0.003).

Argus and Hoch-Ligeti (1961) treated 25 male Wistar rats (92 g average body weight) with 1070 μg NDPA in 1 ml of 1.1% aqueous methylcellulose by oral gavage for 45 weeks, 5 days per week. At 53 weeks, all rats were alive and upon autopsy, no tumors were observed.

Druckrey *et al.* (1967) exposed 20 BD rats (sex unspecified) to NDPA in drinking water at a daily dose of 120 mg/kg body weight. No tumors were observed within 700 days.

Iverson (1980) applied 0.1 ml of 1% NDPA in acetone weekly for 20 weeks to the interscapular skin of 16 male and 24 female hairless hr/hr Oslo mice. Appropriate control mice were not included. Upon necropsy at 80 weeks, lung adenomas were observed in three of the 14 male survivors.

Boyland *et al.* (1968) injected 6-7 week old male CB rats (24/group) intraperitoneally with 2.5 mg NDPA in polyethylene glycol 400 once per week for six months. Control animals were injected with vehicle alone. After 2 yrs of observation, one of five treated and one of ten control rats which survived the treatment had hepatomas. One treated rat also had a pituitary adenoma.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Two animal studies described above are adequate for the derivation of cancer potency values for N-nitrosodiphenylamine. The studies initiated by Cardy *et al.* (1979) and Innes *et al.* (1969) were conducted with adequate numbers of animals and with appropriate controls such that statistically significant increases in tumor incidence were established. Cardy *et al.* (1979) report increased incidence of transitional-cell carcinomas of the bladder in male and female Fischer 344 rats. Innes *et al.*(1969) report increased incidence of hepatomas in male $B6C3F_1$ mice. The derivation of cancer potency values from these studies and the selection of a reference unit risk value are described below.

Methodology

Dosage estimates of NDPA from the Cardy *et al.* (1979) study were made based on reference body weights of 0.380 and 0.229 kg and daily food consumption rates of 0.030 and 0.021 kg for male and female mice, respectively. The resulting daily dosage calculations are 79 and 92 mg/kg-day for males and females, respectively, for the groups fed 1000 ppm in their diet, and 316 and 368 mg/kg-day for males and females, respectively, for the groups fed 4000 ppm in their diet. Fitting a multistage procedure to the incidence data for transitional-cell carcinoma of the bladder gives upper 95% confidence bounds on the cancer potency (q_1^*) of 0.00050 and 0.00048 $(mg/kg\text{-}day)^{-1}$ for male and female rats, respectively (Crump and Howe (1984)).

Calculation of the cancer potency for animals (q_{animal}) can be made using q_1^* and the following relationship, where T is the natural lifespan of the animal (104 weeks) and T_e is the experimental duration (100 weeks):

$$q_{animal} = q_1^* \times (T/T_e)^3$$

The resulting q_{animal} values of 0.00056 and 0.00050 $(mg/kg\text{-}day)^{-1}$ for male and female rats, respectively, can be converted to human cancer potency values (q_{human}) based on the following relationship, where bw_{animal} is the assumed body weight for the test species and bw_{human} is the assumed human body weight (reference values from US EPA (1986)):

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

The resulting estimates of q_{human} are 0.0032 and 0.0034 $(mg/kg\text{-}day)^{-1}$.

Daily dosage estimates for animals from the Innes *et al.*(1969) study were made with estimates of food consumption rates of 12% and 13% for male and female mice, respectively, based on Gold *et al.* (1984). During the oral gavage dosing period (days 7 to 28) it is assumed that a linear threefold increase in body weight occurs. The method of Crouch (1983) was used to account for variable dosing during the study period. Calculations of daily dosage are 444 and 476 mg/kg-day for male and female mice, respectively. Fitting the linear model below to the significant tumor incidence data for hepatomas in male $B6C3F_1$ mice results in a cancer potency estimate (q_{animal}) of 0.0046 $(mg/kg\text{-}day)^{-1}$. In this relationship, D is the estimated daily dose, $p(T_e)$ is the probability of dying with a tumor at time T_e, and A is the background (control) tumor incidence.

[redacted]mal

Conversion of the q_{animal} to q_{human} is achieved as described for the Cardy *et al.* (1979) data, with an assumed experimental animal body weight (bw_{animal}) of 0.03 kg. The resulting q_{human} for this study is 0.061 $(mg/kg\text{-}day)^{-1}$.

Selection of a reference cancer potency value comes from identification of the most sensitive site, species, sex and study, in the absence of evidence that the data are not representative. The Innes *et al.*(1969) study presents the highest, and thus most sensitive, cancer potency value of 0.061 $(mg/kg\text{-}day)^{-1}$. The lower 95% confidence bound on the Innes *et al.* (1969) potency value

also exceeds the potency values derived from Cardy *et al.* (1979) indicating the mouse strain used by Innes *et al.* (1969) may be more sensitive. The small number of animals used in this preliminary study, however, suggests the possibility this value may be overly conservative. The two q_{human} values for NDPA in male and female rats derived from Cardy *et al.* (1979) are close, 0.0032 and 0.0034 $(mg/kg\text{-}day)^{-1}$, respectively. Since these data were derived from a large, thorough study, the development of a reference cancer potency value should include these values. The potency estimate was therefore derived from the geometric mean of the three q_{human} values described above according to the approach of Anderson *et al.* (1983). The resulting reference q_{human} is 0.009 $(mg/kg\text{-}day)^{-1}$.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 2.6 E-6 $(\mu g/m^3)^{-1}$.

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p-NITROSODIPHENYLAMINE

CAS No: 156-10-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	198.24
Boiling point	not available
Melting point	144-145 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 8.1 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.3 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 2.2 E-2 $(mg/kg\text{-}day)^{-1}$
[Male rat liver tumor data (NCI, 1979), contained in Gold *et al.* (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of *p*-nitrosodiphenylamine in humans are known to exist.

Animal Studies

Male and female Fischer 344 rats and $B6C3F_1$ mice were fed diets containing technical grade *p*-nitrosodiphenylamine (73% active material, 25% water, unspecified impurities). Rats were fed diets containing 2500 or 5000 mg/kg diet *p*-nitrosodiphenylamine for 78 weeks, followed by a 27 week observation period. Mice were fed diets containing either 5000 mg/kg diet *p*-nitrosodiphenylamine for 40 weeks, then 2500 mg/kg diet for 17 weeks, or 10000 mg/kg diet for 40 weeks, followed by control diet for 7 weeks, then 5000 mg/kg for 10 weeks. Both dose groups were then maintained on control diet for an additional 35 weeks. Treatment groups consisted of 50 animals/sex/species/group; matched control groups consisted of 20 animals/sex/species and were kept under observation for 105 and 92 weeks for rats and mice, respectively. Rat survival was 90, 86 and 92% for males and 85, 84 and 92% for females in the control, low-dose and high-dose groups, respectively. Mouse survival was 85, 88 and 60% for males and 90, 84 and 52% for females in the control, low-dose and high-dose groups, respectively. Significant increases in the incidence of liver tumors (neoplastic nodules, hepatocellular adenomas and carcinomas) was noted in treated male mice and rats. Tumor incidence data is listed in Table 1.

Table 1. *P*-Nitrosodiphenylamine-induced liver tumor incidence in male Fischer 344 rats and B6C3F$_1$ mice (NCI, 1979)

Species	Dose group	Average dose[1] (mg/kg-day)	Tumor incidence[2]
rat	control	0	0/20
	low dose	74.3	10/50
	high dose	149	19/50
mouse	control	0	2/20
	low dose	316	22/50
	high dose	587	12/50

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The results of NCI (1979) feeding studies of *p*-nitrosodiphenylamine in male and female F344 rats and B6C3F$_1$ mice are listed by Gold *et al.* (1984). NTP (1991) characterizes the studies in male rats and male mice as positive. Significant increases in malignant liver tumors were observed in males of both species, with rats displaying greater sensitivity to the compound. However, survival was significantly reduced in the study in male mice, so the apparently lower sensitivity of these animals may have been due to the fact that they were at risk for a shorter time period than the rats. Cancer potency is based on the dose-response data for liver tumors in male rats as seen in Table 1 (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

California Environmental Protection Agency (Cal/EPA) 1992. Expedited Cancer Potency Values and Proposed Regulatory Levels for Certain Proposition 65 Carcinogens. Office of Environmental Health Hazard Assessment, Reproductive and Cancer Hazard Assessment Section, Berkeley, CA.

Gold L, Sawyer C, Magaw R, Backman G, de Veciana M, Levinson R, Hooper N, Havender W, Bernstein L, Peto R, Pike M and Ames B. 1984. A Carcinogenic Potency Database of the standardized results of animal bioassays. Environ Health Perspect 58:9-319.

Hazardous Substance Data Bank (HSDB) 1994. National Library of Medicine, Bethesda MD (CD-ROM Version). Micromedix, Inc., Denver CO, Edition 22.

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N-NITROSOMORPHOLINE

CAS No: 59-89-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	116.11
Boiling point	224-225 °C (@ 747 mm Hg)
Melting point	29 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 4.75 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.9 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 6.7 E+0 $(mg/kg\text{-}day)^{-1}$
[Female hamster respiratory tract tumor data (Ketkar *et al.*, 1983), contained in Gold *et al.* (1987) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of N-nitrosomorpholine in humans are known to exist.

Animal Studies

IARC (1978) reviewed a study in which 58 male NMRI mice were exposed to N-nitrosomorpholine in drinking water at a concentration of 100 mg/l for the life of the animals (Bannasch and Müller, 1964; Müller, 1964). Increases in liver tumor incidence were noted; 16/58 animals developed hepatocellular adenomas. Treated animals also developed "numerous" lung adenomas and one lung squamous cell carcinoma was observed. Only 1/17 controls developed lung adenomas.

Male and female MRC rats (15 animals/sex) were exposed to *N*-nitrosomorpholine in drinking water (100 mg/l; total dose 500 mg) for 50 weeks, then observed for the life of the animal (Garcia and Lijinsky, 1972). Male and females displayed increased incidences of liver tumors (hepatocellular carcinomas and hemangioendotheliomias; 13/15 and 13/14 in males and females, respectively) and nasal cavity tumors (9/15 and 5/14 in males and females, respectively). The study did not report that a control group was included.

Male and female Syrian golden hamsters (20/sex/group) were given weekly subcutaneous injections of *N*-nitrosomorpholine, which were one-fifth, one-tenth or one-twentieth of the LD50 of *N*-nitrosomorpholine, for life (Haas *et al*., 1973). Males received 24.6, 49.2 or 98.4 mg/kg body

weight; females recieved 28.1, 56.2 or 112.4 mg/kg body weight. Treatment-related increases in the incidence of respiratory tract tumors (primarily in the nasal cavity and trachea) were observed.

Lijinsky *et al*. (1976) exposed male Sprague-Dawley rats (30/exposure group) to *N*-nitrosomorpholine in drinking water (8 or 40 mg/l, 5 days/week; total dose 0.21 and 1.05 mM, respectively) for 30 weeks. The animals were then observed for the remainder of their lifetime. Hepatocellular (benign or malignant) tumors were reported in 11/30 and 16/30 low- and high-dose animals, respectively. Hemangioendothelial tumors were reported in 1/30 and 2/30 low- and high-dose animals, respectively. The authors did not report the inclusion of a control group in this study.

Ketkar *et al*. (1983) exposed male and female Syrian golden hamsters (30/sex/group) to 0.001, 0.005 or 0.01% *N*-nitrosomorpholine in the drinking water for life; untreated control groups (50 animals/sex) were also included. Increased incidences of benign and malignant tumors of the respiratory tract (primarily papillary polyps, papillomas and epidermoid carcinomas of the larynx and trachea) and the gastrointestinal tract (primarily liver tumors, including hepatocellular adenomas and carcinomas) were observed in both males and females. No corresponding tumors were observed in the control groups. Tumor incidence data is listed in Table 1.

Table 1. *N*-nitrosomorpholine-induced tumor incidence in male and female Syrian golden hamsters (Ketkar *et al*., 1983)

Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
male controls	0	respiratory tract	0/50
male low dose	1.2		8/29
male mid dose	6		13/29
male high dose	12		21/30
male controls	0	liver tumors	0/50
male low dose	1.2		4/29
male mid dose	6		9/29
male high dose	12		18/30
female controls	0	respiratory tract	0/50
female low dose	1.36		14/28
female mid dose	6.82		16/30
female high dose	13.6		22/30
female controls	0	liver tumors	0/50
female low dose	1.36		0/28
female mid dose	6.82		2/30
female high dose	13.6		6/30

1. Doses as reported by Gold *et al.* (1987).
2. Tumor incidences as reported by Gold *et al.* (1987).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al*. (1987) list results from a drinking water study in male and female Syrian Golden hamsters (Ketkar *et al.*, 1983). Tumors of the respiratory system and liver were observed at significant levels in both sexes; females were slightly more sensitive than males. Cancer potency for N-nitrosomorpholine is based on tumors of the respiratory system, the more sensitive site, in female hamsters (see Table 1) (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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Lijinsky W, Taylor HW and Keffer LK. 1976. Reduction of rat liver carcinogenicity of 4-nitrosomorpholine by Â-deuterium substitution. J Natl Cancer Inst 57:1311-1313.

Müller, H-A. 1964. Morphologische untersuchungen zur wirkung von *N*-nitrosomorpholin auf die lunge der maus. Z Krebsforsch 66:303-309.

N-NITROSOPIPERIDINE

CAS No: 100-75-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	114.15
Boiling point	217 °C (@ 721 mmHg)
Melting point	not available
Vapor pressure	not available
Air concentration conversion	1 ppm = 4.7 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.7 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 9.4 E+0 $(mg/kg\text{-}day)^{-1}$
[Rat liver tumors (Eisenbrand *et al*., 1980), contained in Gold *et* al. (1987) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of *N*-nitrosopiperidine in humans are known to exist.

Animal Studies

Takayama (1969) fed 33 male ICR mice diets containing 50 mg/kg *N*-nitrosopiperidine for a period of 12 months; a 30 animal untreated control group was included. An increased incidence of forestomach (squamous cell carcinoma), liver and lung (adenomas) tumors were observed in treated animals when compared to controls. Tumor incidence data is listed in Table 1.

Table 1. *N*-nitrosopiperidine-induced tumor incidence in male ICR mice (Takayama, 1969)

Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
controls	0	forestomach liver lung	0/30 0/30 2/30
treated	6	forestomach liver lung	18/33 6/33 10/33

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al*. (1984).

Male and female Sprague-Dawley rats were exposed to *N*-nitrosopiperidine in the drinking water 5 days/week for the life of the animals at exposure levels of 0, 0.024, 0.12, 0.6 and 3 mg/kg body

weight (group sizes were 40, 78, 75, 34 and 34 animals, respectively) (Eisenbrand *et al.*, 1980). Significant increases were noted in the incidence of esophageal squamous cell carcinomas and liver tumors (hemangioendotheliomas, and hepatocellular adenomas and carcinomas). Tumor incidence data is listed in Table 2.

Table 2. Induction of liver tumors in male and female Sprague-Dawley rats by *N*-nitrosopiperidine (Eisenbrand *et al.*, 1980)

Dose group $(mg/kg/day)^{-1}$	Average dose[1] $(mg/kg/day)^{-1}$	Tumor incidence[2]
0	0	0/40
0.024	0.0171	3/78
0.12	0.0857	5/75
0.6	0.429	16/34
3.0	2.14	11/34

1. Doses as reported by Gold *et al.* (1987).
2. Tumor incidences (males and females combined) as reported by Gold *et al*. (1987).

Adamson and Sieber (1982) exposed male and female rhesus and cynomolgus monkeys to *N*-nitrosopiperidine by gavage (400 mg/kg body weight, 5 days/week; average dose 279 and 280 mg/kg-day for cynomolgus and rhesus monkeys, respectively); male and female rhesus monkeys were also exposed to *N*-nitrosopiperidine by intraperitoneal injection (40 mg/kg body weight; average dose 5.59 mg/kg). Exposure and total experimental (exposure and untreated observation period) durations were 90 and 92 months, respectively for cynomolgus monkeys, 8 and 9 years, respectively for rhesus monkeys exposed by gavage, and 91 and 93 months, respectively, for rhesus monkeys exposed by intraperitoneal injection. Increased incidences of hepatocellular carcinomas were found in treated cynomolgus monkeys (5/5 compared to 0/38 in controls), rhesus monkeys exposed by gavage (6/7 compared to 0/32 in controls) and in rhesus monkeys exposed by intraperitoneal injection (3/5 compared to 0/32 in controls).

Ketkar *et al*. (1983) exposed male and female Syrian golden hamsters (30/sex/treatment group; 50/sex for controls) to 0, 0.006, 0.025 or 0.05% *N*-nitrosopiperidine in drinking water for the life of the animals. Increased incidences were noted for respiratory tract tumors (papillary polyps, papillomas and epidermoid carcinomas of the larynx, pharynx and trachea), liver tumors (hepatocellular adenomas and carcinomas, cholangiomas and cholangiocarcinomas) and digestive tract tumors (forestomach and colon adenocarcinomas). Tumor incidence data is listed in Table 3.

Table 3. *N*-Nitrosopiperidine-induced tumor induction in male and female Syrian golden hamsters (Ketkar *et al.*, 1983)

Dose group (% *N*-nitrosopiperidine)	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
male		respiratory tract	
0	0		0/50
0.006	7.2		5/30
0.025	30		10/30
0.05	60		15/30
female		respiratory tract	
0	0		0/50
0.006	8.18		4/30
0.025	34.1		6/30
0.05	68.2		10/30
male		liver tumors	
0	0		0/50
0.006	7.2		1/30
0.025	30		2/30
0.05	60		10/30
female		liver tumors	
0	0		0/50
0.006	8.18		1/30
0.025	34.1		2/30
0.05	68.2		4/30
male		digestive tract	
0	0		0/50
0.006	7.2		0/30
0.025	30		4/30
0.05	60		13/30
female		digestive tract	
0	0		0/50
0.006	8.18		4/30
0.025	34.1		5/30
0.05	68.2		7/30

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al.* (1984, 1987) list results from drinking water studies in male and female Syrian Golden hamsters, feeding studies in male ICR mice, feeding studies in rhesus and cynomolgus monkeys, intraperitoneal studies in rhesus monkeys (combined data for males and females), and drinking water studies in Sprague-Dawley rats (combined data for males and females). N-Nitrosopiperidine induced liver tumors in all species and strains. Hamsters are the least sensitive of the species tested. The majority of treated primates developed liver tumors, including all cynomolgus monkeys given the compound in feed. Rats and mice exhibit sensitivity similar to primates. Because treatment groups in the primate studies are small and incidences observed are high, accurate estimates of cancer potency cannot be obtained from these studies. Of the dose-response data available for rats and mice, the highest quality data set is reported by Eisenbrand *et al.* (1980) for liver tumors in Sprague-Dawley rats. Cancer potency is derived from this data set (Table 2) (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. Analysis of the data set using the computer program TOX_RISK (Crump *et al*., 1991) indicated that inclusion of the high dose group resulted in a *p*-value of 0.05 based on the chi-square goodness-of-fit test, indicating non-linearity. Following procedures described by US EPA (Anderson *et al*., 1983), the high dose group was excluded from the analysis to correct for the poor fit (Cal/EPA, 1992). A unit risk factor of 6.0 E-6 $(\mu g/m^3)^{-1}$ was derived by OEHHA/ATES from the human q_1^* using an inspiration rate of 20 m^3/day.

V. REFERENCES

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N-NITROSOPYRROLIDINE

CAS No: 930-55-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	100.1
Boiling point	214°C
Melting point	not available
Vapor pressure	not available
Air concentration conversion	1 ppm = 4.10 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.0 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 2.1 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by US EPA/IRIS (1994) from rat liver tumor data (Preussmann *et al*. 1977) using a linearized multistage procedure]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of *N*-nitrosopyrrolidine in humans are known to exist. Human exposure to nitrosamines occurs through contact with complex mixtures (e.g., metal cutting fluids) containing these compounds. US EPA (1994) states that data from such exposures are of limited use in evaluating the carcinogenicity of individual nitrosamines due to potential confounding by other substances present in the mixtures.

Animal Studies

Druckrey (1967) (reviewed by IARC, 1978) exposed 25 BD rats to drinking water containing *N*-nitrosopyrrolidine; average doses were 5 or 10 mg/kg body weight-day. After 150 days of treatment, the doses were increased to 10 or 20 mg/kg-day. Hepatocellular carcinomas were noted in 23/25 animals; the average induction time for the low- and high-dose animals was 470 and 290 days, respectively.

Male and female MRC rats (15/sex) were exposed to *N*-nitrosopyrrolidine in drinking water 5 days/week at a concentration of 200 mg/l for 67 weeks (mean total dose 1340 mg/animal, mean daily intake 16 mg/kg) (Greenblatt and Lijinsky, 1972a). After treatment, animals were observed for an additional 105 weeks. An untreated control group of 35 animals/sex was also included. An increased incidence of liver tumors (primarily hepatocellular carcinomas) were observed in both males (12/12) and females (13/13); 7/12 male rats also developed papillary mesotheliomas of the testes. No liver or testicular tumors were noted in the corresponding controls.

Greenblatt and Lijinsky (1972b) exposed male and female (30/sex/group) Swiss mice to 0 or 0.01% *N*-nitrosopyrrolidine in drinking water 5 days/week for 26 weeks (average dose 7-8 mg/kg-day). All surviving animals were killed after 38 weeks. Animals were only histopathologically evaluated for lung adenomas. Lung tumor incidence in treated mice was not significantly increased when compared to controls; however, the duration of treatment was short and the mean survival time of the treated animals was only 12 weeks, with the primary cause of mortality being liver necrosis.

Male and female Sprague-Dawley rats (14 males, 15 females) were exposed to 200 mg/l *N*-nitrosopyrrolidine in drinking water 5 days/week for 50 weeks (total dose 1000 mg/animal), then observed for the remainder of their lifetime (Lijinsky and Taylor, 1976). An increased incidence of hepatocellular tumors was noted in both males (12/14) and females (13/15). No liver tumors were reported in the control group (group size not reported).

Exposure of male and female Sprague-Dawley rats to N-nitrosopyrrolidine in drinking water at levels of 0, 0.3, 1, 3 or 10 mg/kg body weight for the life of the animals resulted in significant increases in the incidence of hepatocellular tumors (adenomas and carcinomas) (Preussmann *et al*., 1977). Tumor incidence data is listed in Table 1. Equal numbers of male and female animals were used.

Table 1: Incidence of hepatocellular carcinomas and adenomas in male and female Sprague-Dawley rats treated with *N*-nitrosopyrrolidine via drinking water (Preussmann *et al*., 1977)

Number of animals/ group[1]	*N*-nitrosopyrrolidine exposure level (mg/kg-day)	Human equivalent dose[2] (mg/kg-day)	Hepatocellular tumor incidence[1]
61	0	0	0/61
60	0.3	0.051	3/60
62	1.0	0.17	17/62
38	3.0	0.51	31/38
24	10	1.70	14/24

1. Males and females combined.
2. Calculated by US EPA (1994).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Tumor incidence data from the study by Preussmann *et al*. (1977) were the basis of cancer potency factor derivation. There were significant increases in the incidence of hepatocellular carcinomas and adenomas (see Table 1). Overall, there appeared to be dose-related increases in hepatocellular tumors, as well as shorter latency periods with increasing dose.

Methodology

Cancer potency values are based on the most sensitive site, species and study, in the absence of other evidence indicating that such a value is not appropriate (CDHS, 1985). Based on the dose-response data for hepatocellular tumors in male and female Sprague-Dawley rats, the cancer potency factor for *N*-nitrosopyrrolidine was calculated to be 2.1 $(mg/kg\text{-}day)^{-1}$ using a linearized multistage procedure with surface area scaling for conversion of the rat administered dose to a human equivalent dose (US EPA, 1994). A unit risk factor was calculated from the cancer potency factor by OEHHA/ATES using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day. US EPA has stated that the unit risk should not be used if air concentrations exceed 20 $\mu g/m^3$, since above this concentration the unit risk may not be appropriate.

V. REFERENCES

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PARTICULATE MATTER FROM DIESEL-FUELED ENGINES

CAS No: not available

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	not applicable
Boiling point	not applicable
Melting point	not applicable
Vapor pressure	not applicable
Air concentration conversion	not applicable

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.3 E-4 - 1.5 E-3 $(\mu g/m^3)^{-1}$ (measured as particulate matter)[Scientific Review Panel unit risk "reasonable estimate" = 3.0 E-4 $(\mu g/m^3)^{-1}$.]
Slope Factor: 1.1 E+0 $(mg/kg\text{-}day)^{-1}$
[Human occupational exposure lung tumor incidence (Garshick *et al*. (1987a, 1988), estimated exposure concentrations (Woskie *et al*., 1988a,b), relative risk model (OEHHA, 1998); human occupational exposure lung tumor incidence, meta-analysis (OEHHA, 1998).]

III. CARCINOGENIC EFFECTS

Human Studies

The epidemiological evidence concerning the carcinogenicity of diesel exhaust primarily involves cancers of the lung and bladder. The review of human diesel exhaust-exposure cancer studies in the document entitled *Health Risk Assessment For Diesel Exhaust* written for the Toxic Air Contaminant (TAC) program (OEHHA, 1998) focuses first on studies of lung cancer (Sections 6.2.1 and 6.2.2) and then turns to those of bladder cancer (Section 6.2.3). The evidence for causation of lung cancer was then assessed using criteria for causal inference from epidemiological studies (Section 6.2.4). The evidence linking diesel exposure and bladder cancer was not as extensive or compelling, and is discussed in the diesel exhaust TAC document but not in this summary. Because there are no epidemiological studies involving industrial hygiene measurements concurrent with the exposures of the study populations, exposure has typically been defined by the surrogate measures of usual occupation or job classification within an industry.

Review Of Lung Cancer Studies

The question of whether diesel exhaust causes lung cancer has been addressed by both industry-based cohort and case-control studies as well as population-based studies of lung cancer. In Section 6 of the diesel exhaust TAC document (OEHHA, 1998), the review of the lung cancer studies was divided into five parts focusing on studies of: (1) truck drivers, (2) transport and equipment workers, (3) dock workers, (4) railway workers, and (5) other miscellaneous occupations involving diesel exhaust exposure. This summary will focus on the railway workers studies, which were

used to derive the range of human cancer risks associated with diesel exhaust exposure. A summary of all occupational studies evaluating the relationship between diesel exhaust exposure and lung cancer is provided in Table 1.

Studies Of Lung Cancer Among Railway Workers

In 1959, Kaplan studied lung cancer mortality among employees of the Baltimore and Ohio Railroad. This railroad initiated locomotive dieselization in 1935, completing this process by 1958. Workers employed at any time between 1953 and 1958 were eligible for entry into the cohort; 154 deaths from primary cancers of the lung or bronchus were identified. Exposure was categorized into three groups by job type. The lung cancer SMR for the most exposed group, relative to the general population, was 0.875. The limited number of years of exposure to diesel exhaust for some members of the cohort and the abbreviated follow-up time do not allow for sufficient latency to be informative regarding the relationship of diesel exhaust exposure to lung cancer. In addition, no data on smoking were available.

In the Third National Cancer Survey discussed above, Williams *et al*. (1977) found a nonsignificant increased risk for railroad workers among lung cancer patients, OR = 1.40, based on 12 cases (no confidence intervals reported).

Howe *et al*. (1983) carried out a mortality study of 43,826 male pensioners of the Canadian National Railroad. The cohort consisted of all male pensioners who were alive at the beginning of 1965. Subjects were followed until 1977, by which time 933 deaths from respiratory cancer (trachea, bronchus and lung) had been recorded. Occupations at the time of retirement were classified as "nonexposed", "possibly exposed" or "probably exposed". Analysis restricted to individuals retiring after 1950 (n = 897 cases) yielded relative risks of 1.00, 1.20 ($p = 0.013$), and 1.35 ($p < 0.001$) for the three exposure groups, respectively (test for trend: $p < 0.00l$). There was little change in these effect estimates when individuals involved in locomotive maintenance (and who therefore may have been exposed to asbestos) were excluded from the analysis (n = 69).

This study also found coal dust to be associated with lung cancer, with a similar increasing trend with degree of exposure. Because of a high degree of overlap between exposures to coal dust and to diesel exhaust, the authors could not separate the effects of the two. However, since there is evidence from animal and human studies for the carcinogenicity of diesel exhaust, but such evidence does not exist for coal dust, the apparent effect of coal dust was more likely to have been due to confounding by diesel exhaust, rather than vice versa. No smoking information was available for this study, although there were increasing trends with degree of diesel exposure for mortality from emphysema (SMRs = 1.00, 1.35, and 1.44) and other smoking-related cancers combined (SMRs = 1.00, 1.08, and 1.16). The authors suggested that since the results were based on internal comparisons little variation in smoking would be expected among the different diesel exposure groups.

Garshick *et al*. (1987a) carried out a case-control study of lung cancer in U.S. railroad workers. Cases comprised 1,256 lung cancer deaths occurring between 1981 and 1982 in the population of active or retired railroad workers who had had 10 years or more of railroad service and were born in 1900 or later. Two controls who had died of causes other than cancer, suicide or accident were

matched to each case by dates of birth and death. Next of kin were interviewed to obtain information about the decedents, including their smoking habits. Job codes were obtained from the Railroad Retirement Board, and an industrial hygiene survey was used to classify the degree of diesel exposure for each job type. Jobs were dichotomously categorized as exposed or not exposed to diesel exhaust.

Garshick *et al*. considered exposure to diesel exhaust to have begun in 1959, since the transition from steam to diesel-powered locomotives took place mainly in the 1950s, and was nearly complete in 1959. Years of diesel exhaust exposure to death or retirement were totaled for each worker. The analysis separated those workers who died at age 65 (retirement age) or older (921 cases and 1,748 controls) from those workers <64 years at death (335 cases and 637 controls). Analysis by logistic regression showed no effect of diesel exhaust in the workers in the older age category, who had substantially less diesel exposure than those in the younger category. For example, 36% of cases and 43% of controls had no exposure in the younger group, while 52% of cases and 53% of controls had no exposure in the older group. Furthermore, 35% of cases and 26% of controls had more than 19 years of diesel exposure in the younger group, while only 3% of cases and controls had more than 19 years of diesel exposure in the older group.

In the group whose members were younger than 64 years old at time of death, the analysis by Garshick *et al*. showed evidence of an exposure-response relationship with an OR of 1.41 (95% C.I. = 1.06-1.88) for 20 or more years of exposure (diesel-years) after adjusting for smoking and asbestos exposure. Excluding exposure occurring within five years of death, the OR for 15 or more years of cumulative diesel exposure was 1.43 (95% C.I. = 1.06-1.94). For workers with 5 to 14 years of cumulative exposure, the OR was 1.07 (95% C.I. = 0.69-1.66) relative to a reference category of 0 to 4 diesel exposure years.

Garshick *et al.* (1988) also conducted a retrospective cohort study of U.S. railroad workers. Eligible for inclusion in the cohort were white males aged 40 to 64 years, who started work between 1939 and 1949 and were still employed in 1959 in designated jobs. Follow-up extended through 1980. Jobs with recognized asbestos exposure were not included in the job codes selected for study, although some of the selected occupations had at least some potential for asbestos exposure. The cohort consisted of 55,407 men, among whom there were 19,396 deaths, including 1,694 attributable to lung cancer. Diesel exhaust exposure was characterized based on their 1959 job group. Career paths were found to be very stable in the railways, such that a worker aged 40-44 with a diesel-exposed job in 1959 was likely to have a diesel-exposed job 20 years later; similarly a nonexposed person in 1959 was likely to have a nonexposed job 20 years later.

The youngest workers in 1959 had the longest potential duration of diesel exposure in the cohort. In a proportional-hazards model these workers had the highest estimated relative risks for lung cancer associated with diesel exhaust exposure: the relative risk for the group aged 40-44 in 1959 was 1.45 (95% C.I. = 1.11-1.89); for the group aged 45-49 the relative risk was 1.33 (95% C.I. = 1.03-1.73); for the group aged 50-54, 1.12 (95% C.I. = 0.88-1.42); for the group aged 55-59, 1.18 (95% C.I. = 0.94-1.50); and for the group aged 60-64, 0.99 (95% C.I. = 0.74-1.33). Though the results were statistically significant only for the two youngest groups, there was a decreasing trend with increasing age in 1959 (except for the 55-59 year age group), implying declining risk with decreasing duration of exposure.

When exposure to diesel over the last five years before death was excluded, a relationship was apparent between lung cancer risk and duration of exposure. The group with greater than 15 years of cumulative exposure had a RR for lung cancer of 1.72 (95% C.I. = 1.27-2.33); for those with 10 to 14 years of exposure the RR was 1.32 (95% C.I. = 1.13-1.56); for 5 to 9 years, 1.24 (95% C.I. = 1.06-1.44); and for 1-4 years, 1.20 (95% C.I. = 1.01-1.44). All of these results are statistically significant.

Although no smoking information was available for the cohort, the previous case-control study of railway workers by the same group (Garshick *et al*., 1987a) reported that little change occurred in the estimates of diesel exhaust effect due to adjustment for smoking habits and asbestos exposure (unadjusted OR = 1.39, 95% C.I. = 1.05-1.83; adjusted OR = 1.41, 95% C.I. = 1.06-1.88). In this analysis, the larger percentage of workers whose pack-year history was unknown (23% of cases and 22% of controls) was treated as a separate category of smoking. In additional analyses using only those workers for whom the investigators had detailed smoking data (n = 758), the ORs for 20 years of diesel exposure ranged from 1.50-1.53, adjusted for asbestos exposure and several specifications of cigarette smoking history. These models included pack-years as a single continuous variable, as two independent variables (cigarettes per day and years of smoking), or as a categorical variable classified in terms of the number of years the study subject had stopped smoking prior to death. These analyses suggested that the diesel exhaust-lung cancer odds ratios were not confounded by cigarette smoking in this population. Moreover, in a group of railroad workers previously surveyed for asbestos exposure (Garshick *et al*., 1987b) there was no difference in smoking prevalence between workers with and without diesel exhaust exposure (data not presented).

It should be noted that the case-control and the cohort studies by Garshick *et al*. involved different study populations: The case-control study (Garshick *et al*. 1987a) contained cases and controls who had died in 1981 and 1982, whereas the cohort study (Garshick *et al*., 1988) involved deaths occurring up to 1980. Thus, they may be considered different tests of the hypothesis of an association between lung cancer and diesel exhaust exposure, although this does not exclude the possibility of a common bias shared by the two studies, such as exposure to chemicals transported by rail or to suspended dusts and debris.

In the American Cancer Society prospective mortality study mentioned above (see Section 6.2.1.1, OEHHA, 1998), Boffetta *et al*. (1988) found an age- and smoking-adjusted RR of 1.59 (95% C.I. = 0.94-2.69) for lung cancer mortality in railroad workers. This estimate was based on only 14 lung cancer deaths.

Swanson *et al*. (1993) also examined the industrial category of railroad workers in their case-control study of lung cancer. The smoking-adjusted odds ratios for white males (67 cases) were 1.2 (95% C.I. = 0.5-2.7) for 1-9 years of employment and 2.4 (95% C.I. = 1.1-5.1) for more than 10 years of employment (χ^2 test for trend: $p < 0.05$). Elevated, but nonsignificant, smoking-adjusted ORs were also associated with the 31 lung cancer cases occurring in African-American railroad workers, OR = 2.6 (95% C.I. = 0.8-7.9) for 1-9 years and OR = 2.7 for $\geq$ 10 years of employment (95% C.I. = 0.6-12.1).

Nokso-Koivisto and Pukkala (1994) compared the incidence of lung cancer among locomotive drivers to the total Finnish population. The retrospective cohort consisted of the 8,391 members of the Finnish Locomotive Drivers' Association from 1953 until 1991 (retired drivers remain members until death). After excluding 302 members for lack of personal identification information, an overall standardized incidence ratio (SIR) of 0.86 (95% C.I. = 0.75-0.97) was found (236 cases). The overall incidence for all cancer sites was also lower than expected, SIR 0.95 (95% C.I. = 0.89-1.01) but the incidence of mesothelioma (SIR 4.05, 95% C.I. = 1.75-7.97) and oral cavity/pharyngeal cancers (SIR 1.75, 95% C.I. = 1.02-2.80) were significantly increased. Prior to the 1970s Finnish drivers trained for 2 years in railroad workshops, where significant exposure to asbestos occurred routinely during steam engine maintenance, with little, if any, diesel exposure. Only drivers working after this period had the potential for substantial exposure to diesel exhaust, and the electrification of the railroad in the 1970s and 1980s may also have reduced the proportion of the cohort's person-years that truly reflect exposure to diesel exhaust. No data on smoking within the cohort were available, though a cross-sectional study of locomotive drivers in 1976 showed that the prevalences of current smokers (40%), ex-smokers (34%), and never-smokers (26%) were similar to those in the Finnish population as a whole.

All three population-based case-control studies found elevated risks for lung cancer in railroad workers (Williams *et al*., 1977; Boffetta *et al*., 1988; Swanson *et al*., 1993); however, only the study by Swanson *et al*. (1993) found a statistically significant increase, with a smoking-adjusted OR of 2.4 (95% C.I. = 1.1-5.1) for workers with ten or more years of employment. This study also found evidence of a significant exposure-response relationship for the 67 cases observed in white railroad workers. Williams *et al.* (1977) and Boffetta *et al.* (1988) had relatively fewer railroad workers (12 and 14 cases respectively) and no information on duration of exposure.

In the railroad industry-based studies, three of the larger studies identified statistically significant increases in relative risk (Howe *et al*., 1983; Garshick *et al*., 1987a; Garshick *et al*., 1988). The large cohort reported on by Howe *et al.* (1983) found elevated risks for individuals categorized as "probably" and "possibly" exposed to diesel exhaust, but without adjustment for smoking or duration of employment, the underlying risk is uncertain. In both the case-control and cohort studies by Garshick *et al.*, 1987a, 1988), significantly increased risks were associated with long-term employment in diesel-related railroad jobs. Both studies had substantial exposure assessment, sufficient latency, and duration of employment data, and the case-control investigation also controlled for potential confounding by smoking and by asbestos exposure. In contrast, the study by Nokso-Koivisto *et al*. (1994), found no increase in lung cancer risk among Finnish locomotive engineers, though the description of the cohort indicates the earlier cases were unlikely to have experienced any diesel exposure.

Studies Of Lung Cancer Among Truck Drivers

The studies that have examined the lung cancer risk to truck drivers are summarized in Table 1. These studies have consistently reported small increases in lung cancer relative risk. However, the studies suffer from various deficiencies, including small numbers of subjects, inadequate adjustment for confounding, and crude exposure assessments, usually based on occupational classification. Most of the earlier studies did not adjust for smoking. Because of evidence that truck drivers have a higher smoking prevalence (Wynder and Higgins, 1986), individual studies that do not account for smoking generally provide limited evidence regarding carcinogenicity. Before 1988, the two studies that took smoking into account, Williams *et al.* (1977) and Hall & Wynder (1984), had ORs of 1.4 - 1.5, which were not statistically significant. The third study that accounted for smoking (Damber and Larsson, 1985, 1987), only found significantly elevated risks in truck drivers who smoked after stratifying on age (i.e., only for those > 70 years old at diagnosis). However, in the follow-up study, after analyzing for duration of employment (20 or more years), elevated but nonsignificant risks were observed for all professional drivers combined (Damber and Larsson, 1987).

By comparison, the majority of studies published since 1988 have adjusted for smoking to varying degrees. Of the smoking-adjusted population based studies, two of four found statistically significant increases in the relative risk for lung cancer associated with occupation as a truck driver, especially in individuals employed for 10 or more years (Hayes *et al*. 1989; Swanson *et al.* 1993). In addition, both studies reported some evidence of a positive trend between increased duration of employment and risk for lung cancer. Although both found statistically significant trends ($p < 0.05$), the only stratum with statistically significant relative risk estimates was that including 20 or more years' employment as a truck driver, with ORs of 1.5 (95% C.I. = 1.0-2.3) and 2.5 (95% C.I. = 1.1-4.4), reported by Hayes *et al*. (1989) and Swanson *et al*. (1993), respectively.

Three of the six more recent industry-specific studies adjusted for smoking, at either the individual (Benhamou *et al*. (1988) and Steenland *et al.* (1990)) or group level (Pfluger and Minder 1994). The two studies of professional drivers, a portion of which included truck drivers, found significantly elevated estimates of relative risk with smoking-adjusted ORs of 1.42 (95% C.I. = 1.07-1.89) and 1.48 (95% C.I. = 1.30-1.68) (Benhamou *et al*., 1988 and Pfluger and Minder, 1994, respectively). The one smoking-adjusted study focusing on trucking, Steenland *et al*. (1990), found elevated relative risk estimates for several occupational and duration of employment categories; however, the only statistically significant risk estimate found was for diesel truck drivers with greater than 34 years of exposure, (OR = 1.89; 95% C.I. = 1.04-3.42).

While several population-based studies enrolled a large number of subjects overall (Williams *et al.* 1977; Milne *et al.*, 1983; Hall and Wynder, 1984; Damber and Larsson, 1987; Boffetta *et al.* 1988), the actual numbers of subjects occupationally exposed to diesel exhaust (considered here as truck drivers) were small. Of the larger, general population studies (Hayes *et al*., 1989; Benhamou *et al*., 1988; Boffetta *et al.*, 1990; Swanson *et al.*, 1993) and industry- or occupation-specific studies (Ahlberg *et al*., 1981; Rafnsson and Gunnarsdottir, 1991; Guberan *et al.*, 1992; Hansen *et al*., 1993; Pfluger and Minder, 1994; Steenland *et al*., 1990) with greater numbers of truck drivers, significantly elevated smoking-adjusted risk estimates were limited mainly to the case-control studies described above (Hayes *et al*., 1989; Benhamou *et al*., 1988; Steenland *et al*.,

1990; Swanson *et al*., 1993; Pfluger and Minder, 1994). Although several industry-specific cohort studies found significantly elevated risks associated with truck or professional driving, with SMRs ranging between 1.33 and 2.14, all lacked smoking data.

Studies Of Lung Cancer Among Transport Workers

Table 1 summarizes the studies that have examined the lung cancer risk to truck drivers. Most studies of transportation workers are limited by small sample size, lack of smoking data, or limited follow-up. None of the three studies of London transportation workers, drivers or garage workers, (Raffle, 1957; Waller, 1981; Rushton *et al*., 1983) obtained information on smoking. In addition, two lacked sufficient follow-up (Raffle, 1957; Rushton *et al*., 1983), excluded retirees, or suffered from small sample size (Raffle, 1957; Waller, 1981). Of the other European studies focusing on bus company employees (Edling *et al*., 1987; Netterström, 1988; Gustavsson *et al*., 1990), only Gustavsson *et al*. (1990) found an elevated risk for lung cancer, with an overall SMR of 1.22 (95% C.I. = 0.71-1.96). However, in the more detailed nested case-control analysis using conditional logistic regression, estimated RRs increased with the cumulative diesel-exhaust exposure index, as noted above.

Of the three studies reporting increased risks for heavy equipment operators (Wong *et al*., 1985; Boffetta *et al*., 1988; Hayes *et al*., 1989), only the RR reported by Boffetta *et al*. (1988) was statistically significant (RR = 2.6; 95% C.I. = 1.12-6.06). However, this estimate was based on only five lung cancer deaths. The large industry-specific cohort study of Wong *et al*. (1985) did not find an elevated risk for lung cancer among unionized heavy equipment operators (SMR = 0.99; 95% C.I. = 0.88-1.10). A subset of individuals retiring at age 65 did have a significantly elevated risk, but a group excess in emphysema deaths (SMR = 2.75; 95% C.I. = 2.09-3.55) and the absence of smoking data suggest that the increased risk may have been related more to tobacco use than to diesel exhaust exposure.

Table 1: Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Menck and Henderson, 1976 USA	Cohort Truck drivers	109	SMR 1.65	$p < 0.01$	Included 2,161 lung cancer cases identified from death certificates in white males, aged 20 to 64, from 1968 through 1970, and 1777 incident cases of lung cancer reported to LA County Cancer Surveillance Program for 1972 - 73. Occupational information obtained from death certificates or hospital admission sheets/medical records represented the last occupation and industry of employment. No data on smoking.
Decoufle *et al*. 1977 USA	Case-control Truck or tractor driver ≥ 5 years as truck, bus or taxi driver	 56 50	OR 1.07 0.89	 N.S. N.S.	Hospital-based study of 6,434 cancers cases admitted to Roswell Park Memorial Institute between 1956 and 1965. Controls were patients admitted with non-neoplastic disease. Occupation and smoking data obtained by questionnaire. Crude adjustment for smoking. Inadequate latency.
Williams *et al*. 1977 USA	Case-control Transportation Industry Truck drivers Railroad workers Truck Industry	 38 22 12 13	RR 1.17 1.52 1.40 1.34	 N.S. N.S. N.S. N.S.	Study examined cancer incidence and its relation to occupation and industry based on the U.S. 3rd National Cancer Survey. The number of cases of cancer at various sites were compared with that of cases at all other sites combined. Occupational history (main and recent employment) and data on smoking were obtained by interview (n = 7,518). IARC noted the potential bias in this study due to the relatively low level of response to the questionnaire (57%). Results were controlled for tobacco use, alcohol consumption, race, education and geographic location.
Leupker and Smith, 1978 USA	Cohort Total cohort Age 50-59	 34 not given	SMR 1.21 1.37	 N.S. $p < 0.001$	Death certificates for a 3-month period in 1976 in the Central States Teamster population were examined. Comparison group was the US male population and was not adjusted for race. No data on smoking. Authors noted the follow-up was short. Retirees and members with lapsed benefits were excluded. 48,358 members were eligible in the 50-59 age group.
Ahlberg *et al*. 1981 Sweden	Cohort All truck drivers* Stockholm truck drivers#	 161	RR 1.33 1.62	 1.13-1.56 1.15-2.28	Cohort consisted of 34,027 Swedish drivers considered to be exposed to diesel exhaust identified from the 1960 national census. Reference population consisted of blue-collar workers from the same census thought to have had no exposure to petroleum products or chemicals (n=686,708). No data on smoking; however, a study of 470 professional drivers in Stockholm found that 78% of fuel truck drivers and 31% of other truck drivers smoked compared to 40% in the Swedish population (citing unpublished study). # Subset of all non-fuel tank drivers. *Does not include fuel tank drivers.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Milne *et al.* 1983 USA	Case-control		OR		Study compared lung cancer deaths with mortality from all other cancers in Alameda County between 1958 and 1962 to investigate possible associations between lung cancer and occupation. Data on cause of death and occupation were obtained from death certificates. No data on smoking or the types of vehicle engines. Results reported are for males. *Results in parentheses are ORs with potential occupationally related cancer removed from the control population. Significant risk estimates only observed when compared with control group before such cancers removed.
	Occupational groups:				
	All transport operatives	36	1.3 (1.1)*	N.S.	
	Bus drivers	4		$p < 0.05$*	
	Truck drivers	23	3.5 (2.8)*	$p < 0.05$*	
	Other transport	7	1.6 (1.3)*	N.S.	
	Industry groups:				
	Railroad	34	0.7 (0.6)*	N.S.	
			0.8 (0.8)*		
Hall and Wynder, 1984 USA	Case-control		OR		Study consisted of 502 men with histologically confirmed primary lung cancer (20 to 80 years old) and matched control patients in 18 hospitals in six cities. Controls with tobacco-related diseases were excluded. Patients were interviewed between December 1980 and November 1982. Smoking data were obtained. Occupations were grouped either dichotomously as exposed to diesel exhaust (warehousemen, bus drivers, truck drivers, railroad workers, heavy equipment operators) or unexposed. Exposure categorization also conducted by NIOSH-based occupational classifications with job title classified as having "probable" exposure to diesel exhaust as either "high" (10 cases), "moderate" (16 cases) or "little or none" (476 cases). No significantly elevated risks were reported in this latter analysis (data not shown here). See also Boffetta *et al*., 1990. *Compared DE exposed to unexposed within each smoking category.
	<u>Usual employment:</u>				
	Total diesel-exposed	45	2.0	1.2-3.2	
	- adjusted for smoking		1.4	0.8-2.4	
	<u>Selected occupations:</u>				
	Truck drivers				
	Railroad workers				
	Heavy equipment	22	1.4	0.7-2.6	
	repairmen &	5	2.6	0.5-12.8	
	operators	10	3.5	1.0-11.8	
	- adjusted for smoking		1.9	0.6-5.5	
	<u>Smoking & DE exposure:</u>				
	Non & ex-smokers	10	1.46*	0.9-2.3	
	≤ 20 cigarettes/day	10	0.82*	0.5-1.4	
	> 20 cigerettes/day	7	1.30*	0.8-2.1	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Boffetta *et al.*, 1990 USA	Exposure by occupation:		OR		Study consisted of 2584 histologically confirmed lung cancer cases and 5009 controls derived from 18 hospitals in six cities. Controls were patients with current non-tobacco-related diseases matched by age, hospital and year of interview. Exposure was assessed by occupational titles and self-reported exposure to diesel exhaust. Results were adjusted for smoking, education and asbestos exposure by logistic regression. Occupations were classified as having probable, possible or no diesel exhaust exposure. Exposure prevalence was low. Only 15.6% of the controls were ever in an exposed job and 6.4% were considered probably exposed. Self-reported exposure to diesel exhaust had consistently higher point estimates of risk than those based on occupational classification, suggesting the possibility of recall bias. See also Hall and Wynder, 1984. *Duration of employment data only available for 23 cases and 27 controls of all patients classified as truck drivers (114 cases and 176 controls).
	"Possible" exposure	240	0.92	0.76-1.10	
	"Probable" exposure	210	0.95	0.78-1.16	
	By duration:				
	"Probable" DE				
	1-15 years	4	0.52	0.15-1.86	
	16-30 years	15	0.70	0.34-1.44	
	31+ years	17	1.49	0.72-3.11	
	Truck driver*				
	1-15 years	4	1.83	0.31-10.73	
	16-30 years	12	0.94	0.41-2.15	
	30+ years	7	1.17	0.40-3.41	
	Self-reported exposure:		1.21	0.73-2.02	
	By duration				
	1-15 years	11	0.90	0.40-1.99	
	16-30 years	12	1.04	0.44-2.48	
	31+ years	12	2.39	0.87-6.57	
Damber and Larsson, 1985 Sweden	Case-control		OR		Study included 604 male patients with lung cancer from the 3 most northern counties in Sweden (all new cases reported to the Swedish Cancer Registry in 1972 to 77 who had died at least one year before the start of the study in 1979). Matched controls were drawn from the national registry for causes of death. Living controls were also used. Data on occupational and smoking habits were obtained by questionnaire. Study focused on professional drivers, most of whose vehicles had diesel engines. Investigators noted that drivers had considerably higher average tobacco consumption than nondrivers. Authors stated that the study suggests a synergistic interaction between smoking and occupational exposure. See also Damber and Larsson 1987. Risk estimates presented for portion of cohort with date of birth after 1900. [#] Subset of all drivers. * Compared to nondrivers. ** Compared to nondrivers/nonsmokers, where "nonsmokers" included ex-smokers who had quit for at least 10 years.
	By age of diagnosis:				
	Professional drivers				
	<70 years	40	1.00*	0.66-1.50	
	≥70 years	23	3.15*	1.66-6.00	
	Truck drivers[#]				
	<70 years	22	0.83*	0.50-1.40	
	≥70 years	13	5.70*	2.22-14.67	
	By age & smoking status:				
	Drivers/ Nonsmokers**				
	<70 years	NG	1.9	0.5-5.5	
	≥70 years	NG	4.5	1.1-16.4	
	Drivers/Smokers**				
	<70 years	NG	6.0	3.5-10.3	
	≥70 years	NG	20.8	9.4-46.0	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Damber and Larsson, 1987 Sweden	Case-control Professional drivers Years worked ≥1 ≥20 Adjusted for smoking ≥1 ≥20	 72 37 72 37	OR 1.3 1.5 1.0 1.2	 0.9-1.9 0.9-2.6 0.7-1.5 0.6-2.2	Study consisted of 600 men with lung cancer in northern Sweden reported to the Swedish Cancer Registry from 1972 through 1977 and dead before the start of the study (1979). Cases were matched with both dead and living controls. Results reported here are for comparisons with dead controls. Results with living controls were in good agreement. See Damber and Larsson (1985) for study focused on professional drivers only.
Boffetta *et al*. 1988 USA	Prospective Cohort Self-reported as DE: All DE exposed By duration exposure: 1-15 years 16+ years DE & smoking status*: nonsmokers ex-smokers current smokers Occupation: Railroad worker Truck driver Heavy equipment By occupation & DE: Truck/exposed Truck/nonexposed	 174 7 85 78 14 48 5 18** 18**	RR 1.18 1.05 1.21 1.73 11.06 19.82 1.59 1.24 2.60 1.22 1.19	 0.97-1.44 0.80-1.39 0.94-1.56[#] 0.60-4.95 6.27-19.53 11.20-35.07 0.94-2.69 0.93-1.66 1.12-6.06 0.77-1.95 0.74-1.89	Included 461,981 males, aged 40 to 79, participating in the American Cancer Society's Prospective Mortality Study in 1982. Follow-up for two years. Exposure assessment was based on self-reported (questionnaire) occupation and diesel exhaust exposure. Investigators stated that, although the sample was large, it was comprised of volunteers, who were healthier and were less frequently exposed to important risk factors such as smoking and alcohol. Reference population included men with no reported exposure or likely occupational exposure to diesel exhaust. Results were adjusted for smoking and other occupational exposures (asbestos, coal and stone dust, coal tar pitch, and gas exhaust). See Hall and Wynder, 1984. *Smoking data not available for all subjects. **Diesel exhaust exposure data not available for all truck drivers. [#]Test for trend reported by investigators as $0.05 < p < 0.10$.
Benhamou *et al*. 1988 France	Case-control Motor vehicle mechanic Transport equipment operators Professional drivers	 65 157 128	RR 1.06 1.35 1.42	 0.73-1.54 1.05-1.75 1.07-1.89	Study consisted of 1,334 histologically confirmed lung cancer cases and 2,409 controls matched on sex, age, hospital admission and interviewer. Study was conducted between 1976 and 1980. Results were adjusted for smoking and are limited to males. Occupation was determined by questionnaire (interview). The types of motor vehicle engines worked with were not specified. No evidence of increased risk with increased duration of exposure (years employed).

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Hayes *et al.* 1989 USA	Case-control Pooled Analysis Truck Drivers		OR		The study is a pooled analysis of three case-control studies conducted between 1976 and 1983 in Florida, New Jersey, and Louisiana. Total
	< 10 yrs employed	161	1.0	0.8-1.3	eligible cases = 2,291 and controls = 2,570. All occupational
	≥ 10 yrs employed	112	1.5	1.1-2.0	data were recoded from original interviews. No specific information regarding
	Heavy Equipment				
	< 10 yrs employed	7	1.5	0.4-5.3	diesel exposure or engine type. ORs were adjusted for birth
	≥ 10 yrs employed	10	2.1	0.6-7.1	cohort(<1910, 1910-19, 1920-29, 1930+), usual daily
	Bus Drivers				cigarette use, and state.
	< 10 yrs employed	23	1.1	0.6-2.1	
	≥ 10 yrs employed	24	1.7	0.8-3.4	
Steenland *et al*. 1990 USA	Case-control Occupation data: 1) Teamster records data		OR		Study consisted of 1,086 lung cancer cases and 1,085 controls among truck drivers in the Central States Teamsters Union. Information on work history was obtained from next of kin and union records.
	Long-haul driver		1.27	0.83-1.93	Subjects died in 1982-83 after applying for pensions, which
	Short-haul driver		1.31	0.81-2.11	required at least 20 years of union membership. Subjects
	2) Next-of-kin data				were classified according to the job category in which they
	Truck driver, diesel		1.42	0.89-2.26	worked the longest. Union data provided no information on
	Truck driver, gasoline		1.22	0.79-1.88	the type of truck driven. 90% of union long-haul drivers were also identified as diesel truck drivers by next of kin.
	Truck driver, both		1.25	0.81-1.95	Results were adjusted for smoking and asbestos exposure. Smoking data obtained by
	Duration employment after 1959*: 1) Teamster records data				next-of-kin interview used in both types of exposure classification. Steenland *et al*. (1992) summarized results from a recent industrial hygiene survey of exposure to diesel exhaust in the trucking industry, and found that elemental carbon measurements were generally consistent with the
	Long-haul driver				
	1-11 years	162	1.08	0.68-1.70	results; i.e., mechanics had the highest exposure and the
	12-17 years	228	1.41	0.90-2.21	highest risks, followed by long-haul and local drivers.
	≥18 years	213	1.55	0.97-2.47	Authors noted that exposure to asbestos may account for some of the observed effects in mechanics, but its
	2) Next-of-kin data				confounding effect was probably small. Study results for
	Diesel truck driver				truck mechanics and dock workers were elevated but not
	1-24 years	48	1.27	0.70-2.27	significant.
	25-34 years	72	1.26	0.74-2.16	*Study also presented risk estimates for duration of
	≥35 years	56	1.89	1.04-3.42	employment inclusive of the pre-1959 work era for both job ascertainment categories and for majority of job classifications.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Burns and Swanson 1991 USA	Case-control		OR		Occupational and smoking histories were obtained by telephone interview for 5,935 incident lung cancer cases and 3,956 incident colon and rectal cancer controls diagnosed between 1984 and 1987 and reported to the Detroit cancer registry. The smoking- and race-adjusted OR for all drivers (238 cases, 86 controls) was 1.88 (95% C.I. = 1.37-2.58), while drivers of "heavy trucks" (166 cases, 48 controls), maintained a higher risk even after adjustment for smoking, OR = 2.31 (95% C.I. = 1.56-3.42). Mechanics also had a significantly elevated OR for lung cancer (OR = 1.72, 95% C.I. = 1.15-2.59). The types of the vehicle engines were not specified. Results were adjusted for smoking. See Swanson *et al.* 1993.
	Drivers (white)	187	2.40	1.65-3.48	
	All drivers (race adj.)	238	1.88	1.37-2.58	
	Railroad workers	14	1.27	0.45-3.53	
Swanson *et al*. 1993 USA	Case-control Occupation and duration: 1) White males		OR		Cases and controls were from OCISS (see Burns and Swanson, 1991 for description of subjects). Incident lung cancer cases among black and white males, aged 40 to 84, from 1984 through 1987 are included in this report. Controls were colon and rectal cancer cases. Information on occupation, smoking, medical history were obtained by telephone interview. Results were adjusted for age at diagnosis, race and smoking. *Test for trend $p \leq 0.05$.
	Heavy truck drivers				
	0 years	88	1.0	Reference	
	1-9 years	78	1.4	0.8-2.4*	
	10-19 years	38	1.6	0.8-3.5*	
	20+ years	121	2.5	1.1-4.4*	
	Light truck drivers				
	0 years	88	1.0	Reference	
	1-9 years	46	1.7	0.9-3.3	
	10+ years	36	2.1	0.9-4.6	
	Railroad workers				
	0 years	73	1.0	Reference	
	1-9 years	27	1.2	0.5-2.7	
	10+ years	40	2.4	1.1-5.1	
	2) Black males				
	Heavy truck drivers				
	0 years	12	1.0	Reference	
	1-9 years	27	2.7	0.8-9.2	
	10-19 years	16	1.9	0.5-7.2	
	20+ years	16	2.1	0.5-9.2	
	Railroad workers				
	0 years	15	1.0	Reference	
	1-9 years	22	2.6	0.8-7.9	
	10+ years	9	2.7	0.6-12.1	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Truck Drivers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Rafnsson and Gunnarsdottir 1991 Iceland	Cohort Truck drivers Duration employment: <2 years 2-10 years 11-30 years >30 years	24	SMR 2.14 2.70 2.46 0.68 2.32	1.37-3.18 0.74-6.92 0.99-5.08 0.01-3.76 0.85-5.04	Cohort consisted of truck and taxi drivers in Reykjavik followed from 1951 to 1988. National mortality rates were used as for comparison. Information on truck drivers was obtained from their union. No data on smoking or type of vehicle engines used. No trend of increased risk with increased follow-up time was observed.
Guberan *et al*. 1992 Switzerland	Cohort Professional drivers	77	SMR 1.50	1.23-1.81	Cohort identified from vehicle license records of professional drivers required to obtain special license during the period from 1949 to 1961. Excluding individuals born prior to 1900, 1,726 drivers were eligible. Lung cancer cases identified from death and tumor registries through 1986. No smoking data obtained. Approximately 1/3 to 1/4 of professional drivers were reported to be long-haul truck drivers. Death rates compared to regional mortality rates. A significant ($p < 0.02$) upward trend in lung cancer mortality with time from first exposure was also observed: SMRs = 0.67, 1.18, 1.30, 1.35, and 2.59 for 0-14, 15-24, 25-34, 35-44, and ≥ 45 years, respectively (no confidence intervals reported).
Hansen 1993 Denmark	Cohort Age on Nov. 9, 1970 15-29 30-39 40-44 45-49 50-54 55-59 60-64 65-74 Total	 0 3 3 11 12 19 22 6 76	SMR 1.96 0.56 1.17 1.10 2.29 2.27 2.60 1.60	 0.40-5.73 0.12-1.64 0.58-2.09 0.57-1.93 1.38-3.58 1.42-3.44 0.95-5.65 1.26-2.00	Cohort consisted of 14,225 truck drivers followed for a 10-year period. Comparisons were made with another cohort of unskilled laborers. Members of the cohort were identified from the file of a nationwide census conducted in 1970. Self-reported occupation, trade, industry and employment on the day of the census were recorded. The study was comprised of unskilled male laborers 15 to 74 years old who were occupationally active on the day of the census. 627 truck drivers and 3,811 members of the control cohort died within the 10 years. No data on smoking. Diesel engines have comprised most of Danish fleet of trucks since the late 1940s.
Pfluger and Minder, 1994 Switzerland	Case-control Professional drivers - smoking adjusted	284	OR 2.27 1.48	1.99-2.58 1.30-1.68	Mortality of Swiss professional drivers (truck, bus and taxi drivers) was determined from death certificates and compared to census data to obtain occupation and age-specific death rates. No individual smoking data were available, but an indirect adjustment was conducted based on occupation specific mortality rates.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust. OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Transport (i.e., bus) and Equipment Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Raffle 1957 England	Cohort Overall Bus & trolley drivers Age 55-64	 96 30	SMR 1.4	 N.S.	Cohort consisted of deaths, retirements and transfers due to lung cancer in London transport employees (bus and trolley workers, bus engineers), aged 45 to 64 years, in jobs with presumably different exposures to exhaust fumes in 1950 to 1954. Only cases arising during exposure employment were considered. Rates were compared to lung cancer mortality in other company employees. Diesel buses had been gradually introduced since the 1930s. At the end of WWII only 15% of the buses still used petrol. All had been replaced by 1950. Consequently, the duration of exposure of some workers to DE might have been short. No data on smoking. See also Waller 1981.
Waller 1981 England	Cohort All workers Bus drivers Bus conductors Engineers, garages Engineers, central works Motormen and guards	 667 259 130 177 42 59	SMR 0.79 0.75 0.75 0.90 0.66 0.87	 NP NP NP NP NP NP	Cohort consisted of lung cancer deaths and retirements or transfers due to lung cancer in men, aged 45 to 64, employed within five categories of London Transport employees. Mortality was compared to men in Greater London. The study covered 25 years ending in 1974, thus including some of the data described by Raffle (1957). No data on smoking. Those who retired at age 65 or left earlier were not followed up, thus limiting the extent of case ascertainment.
Rushton *et al.* 1983 England	Cohort	 102	SMR 1.01	 $p = 0.94$	Cohort consisted of 8,684 men employed as maintenance workers in 71 bus garages in London for at least one year from 1967 to 1975. Follow-up through 1975. No data on smoking. Authors noted short follow-up period (average of 6 years). Lung cancer mortality was compared with the male population of England and Wales. The all-cause mortality was significantly lower than expected based on London residence.
Buiatti *et al.* 1985 Italy	Case-control Transportation Taxi driving Train conductors	 45 20 7	OR 1.1 1.8 1.4	 0.7-1.6 1.0-3.4 0.5-3.9	Study consisted of 340 confirmed cases in males (and 817 controls) in Florence, diagnosed from 1981 through 1983 in the regional general hospital and a referral center for lung cancer. Controls were matched on sex, age, date of admission and smoking, and were from the same hospital. Diesel exhaust exposure was assessed by questionnaire for all jobs held for more than one year.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio, NP = not presented.

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Transport (i.e. bus) and Equipment Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Wong *et al.* 1985 USA	Cohort Total By Duration <5 years 5-9 years 10-14 years 15-19 years ≥20 years All retired members Normal retired members	 309 10 25 53 58 163 155 86	SMR 0.99 0.45 0.75 1.08 1.02 1.07 1.64* 1.30**	 0.88-1.10 N.S. N.S. N.S. N.S. $p = 0.05$ $p < 0.01$ $p < 0.05$	Cohort consisted of 34,156 male members of a heavy construction equipment operators union for at least one year from 1964 through 1978. Mortality experience was compared with that of the US white male population. Partial work history was available for some cohort members through the union. A random sample of union members was surveyed to determine smoking habits, and no significant difference between members and the general population was found. Work groups evaluated were considered to have high exposure to diesel exhaust (scraper operator, bulldozer operator, backhoe operator and loader operator) or low exposure (mechanical maintenance workers and engineers). Overall mortality in the cohort was less than that in the U.S. male population (SMR 0.81, 95% C.I. 0.79-0.84). Workers were also categorized by job title and potential exposure, but no significant risks were observed. Analysis of retirees found an excess risk for lung cancer* and emphysema. *Includes also retirements due to ill health. *Normal retirees are those workers retired at or over 65 and early retirees who reached 65.
Edling *et al.* 1987 Sweden	Cohort Bus company employees Bus drivers Bus garage workers Clerks	 6 5 1 0	SMR 0.67 0.69	Not presented	Cohort consisted of 694 bus garage employees followed from 1951 through 1983. Men were divided into three exposure categories (clerks, bus drivers and bus garage workers). Clerks were assumed to have had the lowest exposure to diesel exhaust and bus garage workers the highest. Authors stated that the power of the study to detect specific cancers was limited. No data on smoking.
Netterstrom 1988 Denmark	Cohort Bus drivers	 15	SMR 0.87	 0.48-1.43	Cohort of 2,465 Danish bus drivers from three companies during the period 1978 to 1984. Cases were identified through death and cancer registries. Death rates were compared with national rates. No data on smoking were available. Mean value for employment duration among the lung cancer cases was 30 years

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Transport (i.e. bus) and Equipment Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Gustavsson *et al*. 1990 Sweden	Cohort Total (deaths) DE exposure index: 0-10* 10-30 >30 Nested case-control (20 incident cases) 0-10* 10-20 20-30 >30	 17 5 5 7 5 2 3 10	SMR 1.22 0.97 1.52 1.27 RR 1.0 1.34 1.81 2.43	 0.71-1.96 Reference 1.09-1.64 1.20-2.71 1.32-4.47	Cohort consisted of 695 bus garage workers employed as mechanics, servicemen or hostlers for at least six months in five bus garages in Stockholm between 1945 and 1970. A nested case-control study was performed within the cohort. Follow-up was through 1986. No data on smoking although no large variation in smoking habits was expected within the cohort. Exposure to diesel exhaust and asbestos were assessed based on time period-specific data on job tasks. Lung cancer cases were identified through tumor and death registries. In the cohort analysis regional rates were used for comparison. *Cumulative exposure index values (unitless).
Gustafsson *et al*. 1986 Sweden	Cohort Deaths Incident cases	 71 89	SMR 1.29 SIR 1.53	 1.02-1.63 1.24-1.80	Cohort consisted of 6,071 Swedish dockworkers first employed before 1974 for at least six months. The group was followed from January 1961 through January 1981. Cancer morbidity was determined among 6,071 dockworkers who had been alive and without cancer in January 1961. Comparison group was Swedish male population. Diesel trucks were introduced into Swedish ports in the late 1950s and became prevalent during the 1960s. No data on smoking. See Emmelin *et al*. (1993) for results from the follow-up study. Employment as a dockworker was the only information on diesel exhaust exposure used in the analysis.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Transport (i.e. bus) and Equipment Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Emmelin *et al*. 1993 Sweden	Case-control Exposure variable: Machine time high* Fuel consumption high* Exposed time high* Exposure & Smoking: Machine time medium high smoker Fuel consumption medium high smoker Exposed time medium high smoker	 14 15 19 	OR 1.3 1.7 2.9 1.8 2.9 5.7 1.5 2.9 5.5 2.7 6.8 6.2	 0.3-5.6** 0.5-5.9** 0.8-10.7** 0.5-6.6** 0.6-14.4** 2.4-13.3** 0.5-4.8** 0.7-11.5** 2.4-12.7** 0.6-11.3** 1.3-34.9** 2.6-14.6**	Study was a nested case-control of lung cancer among Swedish male dockworkers in the cohort studied by Gustafsson *et al*. (1986). 154 referents were matched to 50 cases on port and date of birth. Indices of exposure to diesel exposure were derived from employment records and records of annual fuel consumption by diesel vehicles. Three different exposure classifications were created: "machine time", "fuel consumption" and "exposed time". Information on smoking was obtained from questionnaires and interviews with foremen or workers who had worked with subjects. Response rate for mailed questionnaires was low (67%) but information from the interviews was available for 95% of the subjects. Some ex-smokers were classified as never smokers. No exposure level ("low", "medium", or "high") was significant for any DE exposure scheme (only "high" strata reported here). Comparisons based on exposure and smoking tended to find more elevated risks. Investigators noted that the increase in the OR for both smoking and exhaust exposure indicate that smoking does not explain the results from the exposure-only models, and that there may be an interaction between smoking and exhaust exposure. No information on asbestos exposure, which was said to have decreased by the 1970s. See also Gustafsson *et al*. (1986). * "Low" exposure category used for reference comparison. **Note: authors reported confidence intervals at 90% level.
Kaplan 1959 USA	Cohort Total Most likely exposed	 154 49	SMR 0.80 0.875	 0.68-0.94 N.S.	Cohort consisted of 6,506 deaths among railroad workers from the Baltimore and Ohio Railroad Relief Department between 1953 and 1958. Subjects were categorized into 3 groups by exposed to diesel exhaust and compared with national lung cancer mortality rates. IARC noted that since the changeover to diesel engines began in 1935 and was 95% completed by 1959 (Garshick *et al*. 1988), few, if any, of the lung cancer deaths could have occurred in workers with more than 10 years of exposure to diesel exhaust. No data on smoking.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Railroad Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence Interval[a] or *P*-Value	Comments
Howe *et al.* 1983 Canada	Cohort		SMR		Study consisted of 43,826 males of the Canadian National Railway Co. retired and alive in 1965 and followed until 1977. No data on smoking. However, authors note that this may not be crucial since conclusions were based on internal comparisons where no large variation in smoking habits was likely. It was also noted that certain smoking-related deaths were elevated. The results remained unchanged when individuals likely to have been exposed to asbestos were excluded from the analysis.
	Entire cohort	933	1.06	0.99-1.13	
	Retired after 1950	897			
	Exposure to DE				
	"nonexposed"	239	1.00		
	"possibly" exposed	407	1.20	$p = 0.13$	
	"probably" exposed	279	1.35	$p < 0.001$	
Garshick *et al.* 1987a USA	Case-control	1256	OR		Study consisted of Railroad Retirement Board registrants (1,256 cases and 2,385 matched controls) who died between March 1981 and February 1982. Subjects were active and retired workers with at least 10 years work experience. Persons who died from cancer, suicide, accidents or unknown causes were excluded as controls. Results were adjusted for smoking and asbestos exposure. The baseline study year was 1959, when diesel engines had nearly replaced all steam engines. Consequently, few of these workers were exposed to asbestos. Personal exposure was assessed by industrial hygiene sampling in 39 job categories. Job titles were used to dichotomize subjects into exposed and unexposed groups (Woskie *et al*., 1988a,b). See also Garshick *et al*. (1988). *Shopworkers had the highest levels of asbestos exposure. **These results excluded exposure occurring within 5 years before death. The shortest exposure category, 0 to 4 years, was used as a reference group.
	Age (years)				
	≤64	335	1.41	1.06-1.88	
	≥65	921	0.91	0.71-1.17	
	DE Exposure:				
	Diesel-years ≤ 64 worker				
	5-19		1.02	0.72-1.4	
	≥20		1.64	1.18-2.2	
	Diesel-years > 65 worker				
	5-19		0.95	0.79-1.13	
	≥20		0.94	0.56-1.59	
	Minus shopworkers*				
	≥20 years of exposure		1.55	1.09-2.21	
	Years of cumulative DE exposure:**				
	5-14		1.07	0.69-1.66	
	≥15		1.43	1.06-1.94	

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer Studies Among Railroad Workers

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or *P*-Value	Comments
Garshick *et al*. 1988 USA	Cohort By Age in 1959 w/ DE: 40-44 45-49 50-54 55-59 60-64 Minus those w/ asbestos exposure 40-44 45-49 By Years DE Exposure:* 1-4 years 5-9 years 10-14 years ≥ 15 years Minus those w/ asbestos exposure 1-4 years 5-9 years 10-14 years ≥ 15 years	1694	RR 1.45 1.33 1.12 1.18 0.99 1.57 1.34 1.20 1.24 1.32 1.72 1.34 1.33 1.33 1.82	 1.11-1.89 1.03-1.73 0.88-1.42 0.94-1.50 0.74-1.33 1.19-2.06 1.02-1.76 1.01-1.44 1.06-1.44 1.13-1.56 1.27-2.33 1.08-1.65 1.12-1.58 1.10-1.60 1.30-2.55	Cohort consisted of 55,407 white male railroad workers aged 40-64 exposed to little or no asbestos who had started work between 1939 and 1949 and had worked 10 to 20 years after 1959. Follow-up through 1980. Industrial hygiene data were used to categorize jobs as exposed or unexposed. No data on smoking; however, authors noted that there was no difference in smoking habits by job title in comparison studies of current workers (see Garshick *et al*. 1987). Diesel exhaust exposure in the US railroad industry occurred after WWII. The approximate midpoint of dieselization was in 1952 and by 1959, 95% of the locomotives were diesel-powered. Workers aged 40 to 44 in 1959 were the group with the longest possible duration of exposure. Most workers with potential asbestos exposure were excluded, though some did have potential exposure to asbestos (shopworkers and hostlers). Analyses were done with and without these groups. Exposure was assessed from samples of respirable dust taken in 1980s (Woskie *et al*. 1988a). Mean exposure levels suggested a five-fold range of exposure between clerks and shopworkers (Woskie *et al*. 1988b). These values confirmed the assignment of categories of diesel exhaust exposure in the present study and Garshick *et al*. 1987. * Excluding exposure to diesel exhaust over the 4 years preceding the year of death
Nokso-Koivisto and Pukkula, 1994 Finland	Cohort Total	236	SIR 0.86	 0.75 – 0.97	Cohort consisted of 8,391 members of the Finnish Locomotive Drivers' Association from 1953 to 1991 (including retirees). Information was not available for 302 members. No smoking data were available. The overall incidence for all cancer sites was lower than expected when compared to national rates (SIR = 0.95).

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer (Additional Studies Other Than Those Listed In Above Categories)

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or *P*-Value	Comments
Wegman and Peters, 1978 USA	Case-control Total study Transportation equipment operatives - Registry derived - Combination w/ registry data	91 8 5	OR 8.67 1.26	 NP NP	Tumor registry-based study of oat cell carcinoma during 1965 to 1972. Cancer controls identified from same registry. Smoking data collected but not used in analysis (94% cases and 78% controls smoked). Two methods used to classify occupation, registry-derived or combination of registry and next-of-kin questionnaire data. Number of cases classified as transportation equipment operatives decreased from 8 to 5 between two methods.
Coggon *et al.* 1984 England	Case-control Total DE exposed High DE exposure	 172 32	RR 1.3 1.1	 1.0-1.6 0.7-1.8	Study included all men 40 years of age in England and Wales who had died of tracheobronchial cancer from 1975 through 1979. A job exposure matrix was constructed in which occupations were grouped according to likely exposure to each of nine known or putative carcinogens. Occupational information abstracted from the death certificates. No information on smoking. IARC noted the limitations of information on death certificates, the young age of the subjects, short exposure and latency times, and the lack of data on smoking and other potential confounders.
Lerchen *et al.* 1987 USA	Case-control Diesel exhaust fumes - adjusted for smoking Diesel engine mechanics - adjusted for smoking	 7 5	OR 0.6 1.0	 0.2 – 1.6 0.2 – 2.0	Population-based case-control study of 506 patients diagnosed between January 1980 and December 31, 1982, and reported to the New Mexico tumor registry (333 males and 173 females). Data on lifetime occupation and smoking were obtained by personal interview and self-reported history of exposure to specific agents. Matched controls were selected randomly from the telephone directory or for persons over 65 from the roster of participants in a health insurance plan. Only seven males reported exposure to diesel exhaust.
Magnani *et al.* 1988 England	Cohort All DE exposure	 NP	SMR 1.07	 1.04 – 1.10	General population-based cohort analysis of death certificate and census survey information on 31,925 men with lung cancer between 1970-72. No smoking data were available. A job-exposure matrix was developed for several potential carcinogens, including diesel exhaust.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. NP = not presented. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Table 1 (continued): Epidemiological Studies of Exposure to Diesel Exhaust and Lung Cancer (Additional Studies Other Than Those Listed In Above Categories).

Reference	Study Design, Population, and Exposures	Cases or deaths	Effect Measure	Confidence[a] Interval or *P*-Value	Comments
Siemiatycki *et al*. 1988 Canada	Case-control Lung cell types among DE exposed: Oat cell Squamous cell Adenocarcinoma Other Total DE-exposed occupations minus mining:	 34 81 28 34 177 70	OR 1.1 1.2 0.9 1.0 1.1	 0.8-1.5** 1.0-1.5** 0.6-1.2** 0.8-1.4** 0.8-1.5**	This population-based case-control study provided information on the association between several cancer types and 10 types of exhaust and combustion products. Interviews were carried out for 3,726 cancer patients, aged 35 to 70, diagnosed in any of 19 participating Montreal area hospitals. Each type of cancer was a case series; reference groups were selected from among the other cancer patients interviewed. Results reported are adjusted for smoking, socioeconomic status, ethnic group and several other potential confounders. Authors noted that the excess lung cancers were concentrated among mine and quarry workers. **Authors reported 90% confidence intervals.
Bender *et al*. 1989 USA	Cohort State highway workers	 NP	SMR 0.69	 0.52 – 0.90	Cohort consisted of Minnesota highway workers employed for a minimum of one year and working at least one day after January 1, 1945. Mortality was compared to state rates. No data were available on smoking. Overall mortality was significantly lower than the expected, SMR = 0.83 (95% C.I. = 0.73-0.94).
Kauppinen *et al*., 1993 Finland	Case-control Engine exhaust exposure: Any exposure ≥ 1 month 1 month - 5 years > 5 years	 8 5 3	OR 1.7 0.39 2.21	 0.55-5.20** 0.05-2.94** 0.65-7.48**	Nested case-control study of woodworkers in Finland consisted of 136 lung cancer cases diagnosed between 1957 to 1982 and 408 matched controls. Original cohort consisted of 7,307 workers from 35 factories. Multiple chemical exposures were analyzed for, including engine exhaust (combination of diesel and gasoline engines). Smoking, age, and other chemical exposures were adjusted for; however, only a small number of individuals were categorized as having been exposed to engine exhaust. **Authors reported 90% confidence intervals.

[a] 95% Confidence intervals unless noted. N.S.= Not significant. No confidence intervals or *p*-values reported in original study. NP = not presented. DE = Diesel Exhaust, OR = Odds Ratio, RR = Relative Risk, SIR = Standardized Incidence Ratio, SMR = Standardized Mortality Ratio

Animal Studies

Section 6.1 (Animal Studies) of the diesel exhaust TAC document (OEHHA, 1998) describes the results of diesel exhaust inhalation carcinogenicity bioassays performed using mice, rats, hamsters and monkeys. The studies in rats provided the only clear and unequivocal evidence of diesel exhaust-induced carcinogenicity in animals.

The results of eleven animal cancer bioassays of inhalation of diesel exhaust alone were available at the time the document entitled *Health Risk Assessment For Diesel Exhaust* was written for the Toxic Air Contaminant (TAC) program (OEHHA, 1998). None of the four studies with either (a) exposure periods of less than 7 hours/day, 5 days/week for 24 months or (b) particulate exposure concentrations of less than 2.2 mg/m^3 (Karagianes *et al*., 1981; White *et al*., 1983; Lewis *et al*., 1986, 1989; Takemoto *et al*., 1986) gave positive results for carcinogenesis of diesel exhaust. The seven studies that presented positive results are as follows: Brightwell *et al*., 1986, 1989; Heinrich *et al*., 1986; Ishinishi *et al*., 1986a; Iwai 1986; Mauderly *et al*., 1987a; Heinrich *et al*., 1995; Nikula *et al*., 1995. Results of these studies are described in detail in the diesel exhaust TAC document (OEHHA, 1998).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The diesel exhaust TAC document (OEHHA, 1998) stated that the results of the epidemiological analyses described above are consistent with a positive association between occupational exposure to diesel exhaust and an increased risk of developing lung cancer. The diesel exhaust TAC document reviewed the evidence for causality in the association between diesel exhaust and cancer of the lung. The following criteria for causal inference were considered: (1) the consistency of the findings; (2) the strength of the associations; (3) the possibility that findings are due to bias; (4) the likelihood that findings are due to chance; (5) evidence for exposure-response relationships; (6) temporality of the associations; and (7) biological plausibility of a causal association.

Chapter 6 of the diesel exhaust TAC document provided evidence consistent with a causal relationship between occupational diesel exhaust exposure and lung cancer. A lengthy discussion of causal inference, including the strengths and limitations of the underlying data, can be found in Section 6.2.4 of that document. The key findings relating lung cancer and occupational exposure to diesel exhaust are as follows: the majority of studies examining the diesel exhaust-lung cancer association have reported elevated estimates of relative risk, many of which are statistically significant. The consistency of these findings is unlikely to be due to chance. Moreover, with the possible exception of some studies that did not take smoking into account, the results are unlikely to be explained by confounding or bias. This is reinforced by the results of a meta-analysis undertaken by OEHHA staff (summarized below, and presented in detail in Appendix C of the diesel TAC document (OEHHA, 1998)), in which statistically significant pooled estimates of relative risk persisted through numerous subset and sensitivity analyses. The most important potential confounder is cigarette smoking, which was measured and controlled for in multiple studies: in the meta-analysis the pooled relative risk estimate for studies that adjusted for smoking

was 1.43 (95% C.I. = 1.31-1.57). In addition, several studies provide evidence of exposure-response relationships. The strength of the associations reported is typically within the range considered "weak" in epidemiology (i.e., estimates of relative risk between 1 and 2); nonetheless, this is not a bar to causal inference as long as other criteria are met, as discussed in Section 6.2.4 of the diesel exhaust TAC document. The temporal relationship between exposures and lung cancer is consistent with a causal relationship.

Additionally, the basic hypothesis -- that occupational exposure to diesel exhaust causes human lung cancer -- is highly plausible biologically. The evidence can be briefly summarized as follows: (1) Diesel exhaust has been shown to induce lung and other cancers in laboratory animal studies (Brightwell *et al*. 1989; Heinrich *et al*. 1986a; Iwai *et al*. 1986; Mauderly *et al*. 1987a); (2) Diesel exhaust has been shown to contain highly mutagenic substances, including polycyclic aromatic hydrocarbons and nitroaromatic compounds (Ball *et al*., 1990; Gallagher *et al*., 1993; Nielsen *et al*., 1996; Sera *et al*., 1994); (3) Diesel exhaust contains many substances which occur in recognized complex mixtures of human respiratory carcinogens, including cigarette smoke and coke oven emissions (IARC, 1989); and (4) Diesel exhaust contains known and probable human carcinogens.

Therefore, a reasonable and very likely explanation for the increased risks of lung cancer observed in the occupational epidemiological studies is a causal association between diesel exhaust exposure and lung cancer.

Results based on the human data and those based on the animal data are both subject to uncertainty. The principal uncertainties in using the rat data are their application to humans in terms of response, the choice of dose-response model to extrapolate the risk to environmental concentrations, the presence or absence of a threshold for response, and the range of dose extrapolation involved. While there are issues surrounding the quantitation of worker exposure to diesel exhaust, the uncertainty of extrapolating from one species (rat) to another (human) is avoided by using the epidemiological data to estimate risk to humans from diesel exhaust exposure. OEHHA preferred, on balance, to use the epidemiological data in order to estimate risk to humans from diesel exhaust exposure. Therefore, only the unit risk estimates based on human data were included in the final range of cancer unit risks associated with exposure to particulate matter from diesel-fueled engines in the diesel exhaust TAC document (OEHHA, 1998). OEHHA included quantitative risk assessment data based on rat studies in Appendix G of the diesel exhaust TAC document (OEHHA, 1998) for informational purposes.

<u>Quantitative Meta-Analysis on the Relationship of Occupational Exposure to Diesel Exhaust and Lung Cancer</u>

A meta-analysis was conducted to summarize and help interpret the published reports examining the relationship of lung cancer and exposure to diesel exhaust (OEHHA, 1998). A meta-analysis systematically combines the results of previous studies in order to generate a quantitative summary of a body of research and to examine the sources of variability among studies (for review see Petitti, 1994). The variability, or heterogeneity, of results among studies may exist due to numerous factors, including differences in study design, exposures experienced by study subjects,

methods and accuracy of exposure ascertainment, length of follow-up, and control of confounders (such as smoking).

As described in OEHHA (1998), 30 studies, contributing a total of 39 effect estimates, were utilized in the meta-analysis. The pooled relative risks for lung cancer from all 39 risk estimates combined varied with the statistical model used, 1.04 (95% C.I. = 1.02-1.06) under the fixed-effects model and 1.33 (95% C.I. = 1.21-1.46) with the random-effects model. However, significant evidence of heterogeneity was found (DerSimonian and Laird Q-statistic = 214.59, 38 d.f., $p < 0.001$). Heterogeneity in this context refers to large between-study variability. The presence of heterogeneity undermines the validity of the pooled estimates, and suggests the need for additional analysis to identify the sources of heterogeneity. As discussed in detail in Appendix C of OEHHA (1998), this involved deriving pooled estimates for a variety of subsets of the reports.

Through subset analysis, several factors were identified which strongly influenced both the magnitude and the degree of heterogeneity of the pooled risk estimates: (1) whether or not a study adjusted for smoking, (2) study design (3) the exposure assessment, as developed from occupational categories, (4) the presence of selection bias, as manifested by an observed "healthy worker effect", and other study characteristics (See Appendix C of OEHHA (1998)). By stratifying the meta-analysis on whether the risk estimates accounted for smoking, the effect of failure to control for this exposure on the pooled estimate became readily apparent. Not only did the positive association between diesel-exhaust exposure and lung cancer persist, but the pooled risk estimate increased to 1.43 (95% C.I. = 1.31-1.57, random-effects model) with little evidence of heterogeneity among the 12 studies controlling for smoking.

The case-control studies (15 included in the meta-analysis) gave a summary estimate of 1.44 (95% C.I. = 1.33-1.56), again with little evidence of heterogeneity, while the estimate based on the results of the cohort studies remained heterogeneous. The lower pooled RR estimate and substantial heterogeneity obtained from the cohort subanalysis was probably due at least in part to failure to adjust for smoking, as only one of sixteen cohort studies controlled for this confounder, while most case-control studies did (11 of 14 studies, accounting for 17 of the 20 case-control risk estimates).

The "healthy worker effect" (HWE - here based on significantly lower than expected all-cause mortality) is a manifestation of selection bias related to hiring and retention of workers who are typically healthier than the general population, resulting in spuriously lower risk estimates for a variety of illnesses, including those potentially related to occupational exposures. Subsetting the cohort studies into those with and those without an obvious healthy worker effect markedly reduced the degree of heterogeneity in the group without the HWE (Q-statistic = 11.190, 9 d.f., $p = 0.27$), and produced an increase in the magnitude of the pooled relative risk (RR = 1.52, 95% C.I. = 1.36-1.71-1.78, random-effects model). In contrast, those studies whose results were characterized by the presence of a HWE continued to show substantial heterogeneity, and the pooled risk estimates declined. Thus, selection bias is likely to have played a role in the heterogeneity observed among the cohort studies. Selection bias results from choosing a study sample that is not representative of the entire population that could have been studied, and can distort the measure of effect (e.g., relative risk or odds ratio) (Rothman, 1986). With respect to exposure assessment, statistically significant pooled estimates of elevated risk lacking evidence of

heterogeneity were identified in several occupational subgroup analyses, both with and without additional stratification for smoking. Prior to stratifying by adjustment for smoking, the occupational subgroups involving trucking (pooled RR = 1.47, 95% C.I. = 1.33-1.63), the railroad industry (random-effects pooled RR = 1.45, 95% C.I. = 1.08-1.93), mechanics and garage workers (random-effects pooled RR = 1.35 (95% C.I. = 1.03-1.78), general transportation and professional drivers (random-effects pooled RR = 1.45, 95% C.I. = 1.31-1.60) gave risk estimates greater than the overall pooled risk estimate. The pooled RR estimates for trucking and general transportation and professional drivers showed little to no evidence of heterogeneity; however, estimates for the railroad industry demonstrated considerable heterogeneity (Q statistic = 30.90, $p < 0.001$).

Further stratification of the occupational subgroup analysis by adjustment for smoking produced a large impact on the pooled risk estimates, with all smoking-adjusted subgroup estimates displaying little evidence of heterogeneity and leading to increased risk estimates in all but one of the occupational categories. Pooled risk estimates by occupation in smoking-adjusted studies showed little evidence of heterogeneity for several occupations under both models, including truck drivers (random-effects pooled RR = 1.53, 95% C.I. = 1.20-1.94), railroad workers (random-effects pooled RR = 1.68, 95% C.I. = 1.28-2.19), and diesel mechanics and garage workers (random-effects pooled RR = 1.25, 95% C.I. = 0.87-1.80). The pooled estimates for the heavy equipment operators and dock workers and for the railroad industry studies adjusting for smoking displayed the most dramatic changes relative to the occupational analysis without smoking stratification. Among the former subgroup, the pooled risk estimate changed from 1.28 (random-effects model, 95% C.I. = 0.99-1.66) to 2.43 (95% C.I. = 1.21-4.88). Among the railroad industry studies, the pooled risk estimate also increased substantially (from 1.45 to 1.68, 95% C.I. = 1.28-2.19). In both subgroups, the pooled smoking-adjusted estimates showed little evidence of heterogeneity, though these estimates were based on two studies in the former instance and three in the latter. However, the other two heavy equipment operator and dock worker studies and the other three railroad industry studies that were not adjusted for smoking still displayed evidence of heterogeneity (Q-statistics = 2.933, 1 d.f., $p = 0.09$, and 21.517, 2 d.f., $p < 0.001$, respectively).

The meta-analysis also identified evidence of exposure-response relationships in the subgroup analyses based on duration of employment. However, as noted in OEHHA (1998), this analysis was hampered by the absence of duration-specific risk estimates in approximately one-half the studies. While the initial analysis conducted on all the included studies resulted in elevated pooled risk estimates for strata in which exposure durations were greater than 10 years relative to those with less than 10 years of exposure or for which the exposure durations were not clear from the published reports, there was still significant evidence of heterogeneity for several of the duration strata. In contrast, the analysis utilizing only estimates from the smoking-adjusted studies showed some evidence of an exposure-response gradient without evidence of statistical heterogeneity. The summary risks for all three exposure-duration strata were: RR = 1.39 (95% C.I. 1.19-1.63) for $<$ 10 years (based on three estimates from two studies), RR = 1.64 (95% C.I. = 1.40-1.93) for 10 $\leq$ to $<$ 20 years (11 estimates from 6 studies), and RR = 1.64 (95% C.I. = 1.26-2.14) for $\geq$ 20 years (four estimates from four studies). The pooled risk estimate for those studies for which the exposure duration was not clear in the published reports was 1.24 (95% C.I. = 1.00-1.54) (six estimates from four studies) (see Table C-4 in Appendix C of OEHHA (1998)).

These results were robust to a variety of sensitivity analyses. In an analysis of potential publication bias, however, there appeared to be a modest increase in the RR estimates with increasing sample size (reflected in a decreased standard error of the estimates). Publication bias, or the increased likelihood or preference for the publication of statistically significant results compared to nonsignificant or null results, may potentially distort pooled risk estimates. Publication bias is generally attributed to journal editorial policies that prefer "positive" results, so that small, statistically nonsignificant studies are less likely to be published than large, statistically nonsignificant studies (Greenland, 1994). However, it should be noted that the studies with the smallest standard errors were almost exclusively cohort studies that did not adjust for smoking and which also had a clear HWE, suggesting that other significant biases are likely to have played a role in creating an appearance of publication bias. Therefore, although publication bias cannot be ruled out, the inclusion of numerous studies of varying sample sizes and statistically insignificant findings, as well as the uncontrolled confounding and likely selection bias affecting many of the larger cohort studies, make it unlikely that the result of this meta-analysis can be completely explained by publication bias.

In summary, the meta-analysis indicated a consistent positive association between occupations involving diesel exhaust exposure and the development of lung cancer. Although substantial heterogeneity existed in the initial pooled analysis, stratification on several factors identified a persistent positive relationship. The major sources of heterogeneity included: (1) whether or not a study adjusted for smoking, (2) study design (3) the exposure assessment, as developed from occupational categories, (4) and the presence of selection bias, as manifested by an observed healthy worker effect. Taking these factors into account tended to increase the estimates of relative risks of lung cancer from occupational exposure to diesel exhaust.

Another independently conducted meta-analysis of diesel exhaust exposure and lung cancer produced remarkably similar results, with an overall pooled relative risk estimate of 1.33 (95% C.I. = 1.24-1.44) (Bhatia *et al*., 1998). In that analysis, the study inclusion and exclusion criteria were somewhat different than those used by OEHHA staff, so that 23 studies were included. Consequently, the results of some of their subset analyses differed from those described in OEHHA (1998). In addition, those authors used only a fixed-effects model to derive pooled risk estimates, and did not focus on explorations of sources of heterogeneity. Nevertheless, Bhatia and co-workers also found a persistent positive relationship between diesel exhaust exposure and lung cancer that could not be attributed to potential confounding by cigarette smoking. Moreover, in the narrower group of studies in their report, they identified a positive exposure-response relationship in studies stratified by exposure duration.

Table 2. Studies Included in Meta-analysis of Diesel Exhaust Exposure and Lung Cancer

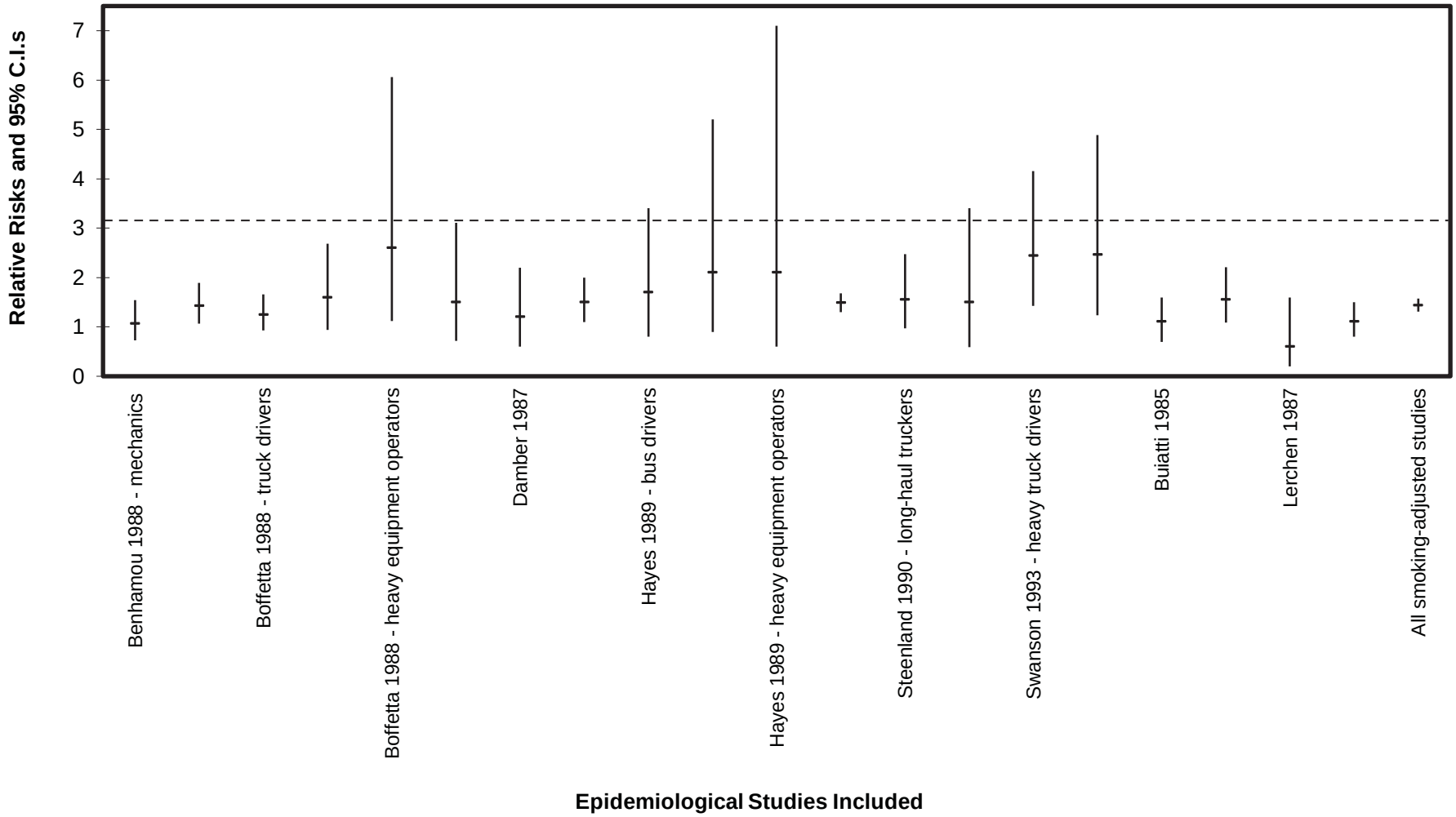
Study (year)	Design (Location)*	Occupation or Exposure Group	Smoking Adjusted	RR	C.I.
Ahlberg *et al.* (1981)	Cohort (†)	Truck drivers	no	1.33	1.13-1.56
Balarajan & McDowall (1988)	Cohort (†)	Truck drivers	no	1.59	1.00-2.53[a]
Bender *et al.* (1989)	Cohort (‡)	Highway maintenance	no	0.69	0.52-0.90
Benhamou *et al.* (1988)	Case-control (†)	Professional drivers	yes	1.42	1.07-1.89
Buiatti *et al.* (1985)	Case-control (†)	Transportation general	yes	1.1	0.7-1.6
Benhamou *et al.* (1988)	Case-control (†)	Mechanics	yes	1.06	0.73-1.54
Boffetta *et al.* (1988)	Cohort (‡)	Truck drivers	yes	1.24	0.93-1.66
	Cohort (‡)	Railroad workers	yes	1.59	0.94-2.69
	Cohort (‡)	Heavy equipment operators	yes	2.60	1.12-6.06
Boffetta *et al.* (1990)	Case-control (‡)	Probable DE $\geq$ 30 yr	yes	1.49	0.72-3.11
Coggon *et al.* (1984)	Case-control (†)	Diesel exhaust exposed group	no	1.1	0.7-1.8
Damber & Larsson (1987)	Case-control (†)	Professional drivers	yes	1.2	0.6-2.2
Edling *et al.* (1987)	Cohort (†)	Bus drivers	no	0.69[b]	0.2-1.6[b]
Garshick *et al.* (1987)	Case-control (‡)	Railroad workers $\geq$ 20 yrs[c]	yes	1.55	1.09-2.21
Garshick *et al.* (1988)	Cohort (‡)	Railroad workers $\geq$ 15 yrs[c]	no	1.82	1.30-2.55
Guberan *et al.* (1992)	Cohort (†)	Professional drivers	no	1.50	1.23-1.81[e]
Gustafsson *et al.* (1986)	Cohort (†)	Dock workers	no	1.32	1.05-1.66
Gustvasson *et al.* (1990)	Nested case-control (†)	Bus garage workers > 20 yr[d]	no	1.49[d]	1.25-1.77[d]
Hansen (1993)	Cohort (†)	Truck drivers	no	1.6	1.26-2.0
Hayes *et al.* (1989)	Case-control (‡)	Truck drivers $\geq$ 10 yr	yes	1.5	1.1-2.0
	Case-control (‡)	Bus drivers $\geq$ 10 yr	yes	1.7	0.8-3.4
	Case-control (‡)	Mechanic (excl auto) $\geq$ 10 yr	yes	2.1	0.9-5.2
	Case-control (‡)	Heavy equip. operators $\geq$ 10 yr	yes	2.1	0.6-7.1
Howe *et al.* (1983)	Cohort (‡)	Railroad workers probably exposed	no	1.35	1.13-1.61[a]
Lerchen *et al.* (1987)	Case-control (‡)	Diesel exhaust grouped	yes	0.6	0.2-1.6
Magnani *et al.* (1988)	Death certificate study (†)	Diesel exhaust grouped	no	0.97	0.95-1.00
Menck & Henderson (1976)	Cohort (‡)	Truck drivers	no	1.65	1.13-2.40[a]
	Cohort (‡)	Mechanic (excl auto)	no	3.32	1.35-8.18[a]
Nokso-Koivisto & Pukkala(1994)	Cohort (†)	Railroad workers	no	0.90[d]	0.79-1.04[d]
Pfluger & Minder (1994)	Case-control (†)	Professional drivers	yes	1.48	1.30-1.68
Rafnsson & Gunnarsdottir (1991)	Cohort (†)	Truck drivers $\geq$ 30 yr	no	2.32	0.85-5.04
Rushton *et al.* (1983)	Cohort (†)	Bus garage workers/mechanics	no	1.01	0.82-1.22
Siemiatycki *et al.* (1988)	Case-control (‡)	Diesel exhaust grouped	yes	1.1	0.8-1.5[e]
Steenland *et al.* (1990)	Case-control (‡)	Truck drivers $\geq$ 18 yr	yes	1.55	0.97-2.47
	Case-control (‡)	Truck mechanic $\geq$ 18 yr	yes	1.50	0.59-3.40
Swanson *et al.* (1993)	Case-control (‡)	Heavy truck drivers $\geq$ 20 yr	yes	2.44[d]	1.43-4.16[d]
	Case-control (‡)	Railroad workers $\geq$ 10 yr	yes	2.46[d]	1.24-4.89[a]
Wegman & Peters (1978)	Case-control (‡)	Transportation equip. operators	no	2.39[b]	0.70-8.05[b]
Wong *et al.* (1985)	Cohort (‡)	Heavy equip. operators $\geq$ 20 yr	no	1.07	1.00-1.15[a]

[a] Calculated from p-value.
[b] Calculated from data presented in publication.
[c] Risk estimates excluding shop workers.
[d] Pooled risk estimates from two racial or duration categories.
[e] 90% confidence intervals originally presented within study.

DE = diesel exhaust
RR = risk ratio
C.I.= 95% confidence interval.

* Location: (†)Europe, (‡)North America

Figure 1: Estimates of Relative Risks for Smoking-Adjusted Studies of Diesel Exhaust Exposure and Lung Cancer



Methodology

The complex and potentially variable mix of chemical species in the condensed phase and the vapor phase of diesel exhaust, required the measure of exposure related to carcinogenic risk to be specified. The most commonly used measure of exposure is atmospheric concentration of particles in $\mu g/m^3$. That measure is obtained from the mass of particles collected on a filter per volume of the air that flowed through the filter. On the basis of its relation to health studies and its general practicality, that measure was used in the diesel exhaust TAC document cancer risk assessment (OEHHA, 1998).

OEHHA used two approaches to employing epidemiological studies for diesel exhaust quantitative risk assessment. The first approach used the overall relative risks derived from the meta-analysis along with an overall range of exposure for all the studies. The second approach focused upon the railroad worker studies in developing the range of unit cancer risks.

Meta-analysis-Derived Cancer Unit Risks

The results of the meta-analysis provide information useful in bracketing the broadest likely range of plausible carcinogenic potencies for diesel exhaust. The pooled relative risk values derived from the 12 epidemiological studies in the meta-analysis which adjusted for smoking were 1.44 (95% C.I. 1.32 -1.56) for the fixed effects model and 1.43 (95% C.I. 1.31 -1.57) for the random effects model. The magnitude of these relative risks provide information on the potential magnitude of the cancer risk associated with diesel exhaust exposure. For the random effects model the upper 95% confidence limit on excess relative risk is 0.57.

None of the studies in the meta-analysis provide direct measurements of exposure concentration over the time of their follow up. Therefore, to the extent that the meta-analysis can be used to bracket the carcinogenic potency of diesel exhaust, the exposures of the various study populations need to be reconstructed. Hammond (1998) has reviewed the available industrial hygiene survey literature on the occupations considered in the meta-analysis (bus garage workers, mechanics, truck drivers, heavy equipment operators, railroad workers) and provided estimates of the plausible possible ranges of workplace exposures of diesel exhaust respirable particulate matter for those occupations. Because of the overall limitations in the data, the estimated ranges for each occupational subgroup of interest are particularly broad. The lowest plausible estimate of occupational exposure for any such subgroup is 5 $\mu g/m^3$ (heavy equipment operators). The highest plausible estimate of any occupational subgroup is 500 $\mu g/m^3$ (bus garage workers, railroad workers, mechanics). The total range of plausible exposures for the different populations therefore varies 100-fold. Using these air concentrations and the assumption that workers inhaling $10m^3$ of air per work shift were exposed to them for over 45 year period for a 70 year lifetime, it is possible to characterize a bracket of risks compatible with the results of the meta-analysis:

q_1^* = Excess relative risk × CA lifetime lung cancer risk.
Air concentration × exposure factor × intermittency factors × duration of exposure/lifetime
= 0.57 × 0.025
(5 or 500 μg/m^3) × 10 m^3/shift/20m^3 /d × 5d/7d × 48wk/52wk × 45 yrs/70yrs

Therefore, the results of the meta-analysis bracket lung cancer risks up to approximately 1.3×10^{-4} $(\mu g/m^3)^{-1}$ (assuming all the worker populations in the meta-analysis were exposed to 5 μg/m^3) to 1.3×10^{-2} $(\mu g/m^3)^{-1}$ (assuming all the workers populations in the meta-analysis were exposed to 500 μg/m^3). As these assumptions establish the extreme bounds of probable exposures, and such calculations based upon a meta-analysis are novel and subject to further possible refinements, these results are not incorporated into the range of risks. However, these results do bracket the carcinogenic potencies which would be consistent with the results of the meta-analysis and the broadest range of exposure estimates.

A more plausible range can be estimated by determining the 90% confidence interval (CI) of the range of risks. For the meta-analysis the range of concentrations thought to be plausible by Hammond (personal communication) was 5 to 500 μg/m³ with a mean of about 200 μg/m³, which corresponds to a unit risk of 3.3×10^{-4} $(\mu g/m^3)^{-1}$. Using that concentration range as the 98% CI for a shifted lognormal distribution fixes the geometric standard deviation at 1.22 with a shift of the origin of the distribution by 330 μg/m³. The 90% CI for this distribution of concentration is [52.5 to 356.5 μg/m³], corresponding to a 90% CI for the distribution of unit risk of [1.6×10^{-4} to 1.2×10^{-3} $(\mu g/m^3)^{-1}$].

Railroad Worker Study-Derived Cancer Unit Risks

Quantitative relationships were also developed between lung cancer risk and exposure to diesel exhaust for two nation-wide studies of lung cancer rates in U. S. railroad workers. These relationships provided additional values for the range of risk to the general California population. The first, Garshick *et al*. (1987a), is a case-control study. Using a logistic regression, that study determined the coefficient of the logistic relationship of the odds of lung cancer for duration of the workers' exposure to diesel exhaust. The coefficient determined in that study was used to estimate lifetime unit risks for exposure of the general population. The second study, Garshick *et al*. (1988), is a cohort study. Using a proportional hazards model, that study calculated the relative hazard of lung cancer for increasing duration of worker exposure. However, those numerical results have not been supported by Garshick (1991); so instead of using them to derive lifetime unit risks for the general population, new analyses were performed with the individual data, upon which that study is based, to determine a linear relationship of lung cancer hazard for worker exposure to diesel exhaust.

The term hazard was used for a prediction of incidence (cancers per year per population) resulting from a model. Relative hazard is generally called relative risk in epidemiological model work, and the term, relative risk, was used in the context of the epidemiology results. The lifetime inhalation unit risk, often simply called unit risk, is defined as the probability of contracting lung cancer from a 70-year exposure to a unit concentration (1 μg/m^3) of diesel exhaust.

The unit risks ultimately derived for the general population assume that the mass concentration of particles governs the risk of diesel exhaust, regardless of the particular type of diesel engine or fuel. The resulting estimate of risk entails uncertainties due primarily to the limited exposure information available and to the choice of models and data used in the analysis.

These two studies are among a number of studies establishing excess relative risk of lung cancer among workers exposed to diesel exhaust. These two studies were specifically selected for the quantitative risk assessment because of their general excellence, their apparent finding of a relationship of cancer rate to duration of exposure and because of the availability of measurements of diesel exhaust among such railroad workers from the early 1980's in other studies. The case-control study appears to have an advantage in obtaining direct information on smoking rates, while the cohort study has an advantage of smaller confidence intervals of the risk estimates.

Estimating Cumulative Exposure

The risk relationships developed for the case-control study and the initial analyses for the cohort study used cumulative atmospheric exposure to diesel exhaust particles as the effective dose. The use of cumulative exposure, defined as the area under the curve (AUC) of concentration versus time, required a specification of the temporal pattern of exposure concentration. However, direct measurements of exposure concentration over the time of the follow up were not available.

Therefore, the calculations required reconstruction of the exposure history in order to determine cumulative exposure. The reconstruction was undertaken using (1) personal exposure measurements on railroad workers just after the end of the follow-up period in that study, (2) historical data on the dieselization of locomotives in the United States, and (3) descriptive information. The analysis included workers on trains and excluded shop workers from the original cohort because of mixed exposures, including no exposure to an unknown number in this group.

Exposure Measurements In The Early 1980s

Woskie *et al*. (1988b) estimated national average concentrations of respirable particulate matter (RSP) for 13 job-groups. These concentrations were obtained by temperature correction of measurements of respirable particulate matter (RSP) made in 1982-1983 in the northern region of the United States, as reported in Woskie *et al*. (1988a). The investigators adjusted these concentrations to remove the portion of RSP attributable to environmental tobacco smoke (ETS). The average values of the ETS-adjusted RSP for the principal categories of workers are listed in Table 3 for exposed and unexposed workers.

Table 3: Number of Workers in the Exposure Categories and the Cohort Averages of the Worker Exposure Concentration Following the Garshick *et al.* (1988) Cohort Study.

Exposure status	Career group	Number of workers	Subsequent exposure concentration[a] ($\mu g/m^3$)
Uncertain	Shopworkers	12,092	141(those exposed)
Exposed[b]	Engineers, firemen	11,005	71
	Brakemen, conductors, hostlers	18,285	89
Unexposed[c]	Clerks	10,475	33
	Signalmen	3548	58

[a] Exposures reported by Woskie *et al* (1988b) for these career groups, based on measurements of ETS-adjusted RSP, circa 1982-3.

[b] For all exposed workers in the table, except for those shopworkers who were exposed, the temporal exposure patterns are assumed to be the same, and the concentrations are close to each other; so a simple population-weighted average for the two career groups characterizes the average concentration for the exposed group, train workers, circa 1982-83:

$$(11{,}005 \times 71 + 18285 \times 89) / (11005 + 18285) = 82\ \mu g/m^3$$

[c] For all unexposed workers (background) in the table except for those shopworkers who were unexposed, the concentrations are close to each other; so a simple population-weighted average for the two groups characterizes the average background concentration, circa 1982-83:

$$(10475 \times 33 + 3548 \times 58) / (10475 + 3548) = 39\ \mu g/m^3.$$

Reconstruction Of The Time Course Of Concentration

In order to estimate the time course of the exposure factors for the cohort, it was necessary to make assumptions about time trends of nationwide average concentration breathed by the workers. The exposure measurements made just after the follow-up period constitute a baseline for the reconstruction. The reconstruction of the time course of concentration proceeds by developing an exposure factor to multiply these baseline values. The analyses below explore the effect of alternative patterns of exposure concentration and baseline values.

Dieselization of the U.S. railroads began after the Second World War ended in 1945. The exposure of the railroad workers up until 1981 can be divided into two periods: (1) an initial period of increasing dieselization of U.S. locomotives from 1945 until mostly completed in 1959 and (2) a subsequent period of a moderate rate of addition of locomotives that were less smoky.

Woskie *et al*. (1988b) reported data showing a linear rise of percent dieselization with time in the first period from 1945 to 1959. They reported data from the Bureau of Labor Statistics showing that by 1947 fourteen percent of locomotives were diesel, by 1952 fifty-five percent were diesel, and by 1959 ninety-five percent were diesel. This linear rise of dieselization may be expected to have produced a linear rise of the national average exposure concentration around the trains. This linear rise is used in all the more realistic exposure patterns.

The exposure of workers on trains would then generally have declined as the newer, less smoky locomotives replaced the older, smokier locomotives on the main lines. To quantify the anecdotal information of greater smokiness of locomotives in the period before 1960, the national average exposure concentration was assumed to decline linearly in the second period, 1960-1980, to the baseline measured in 1982-3. The decline assumed from 1959 to 1980 is consistent with the report of sharp decreases of emissions of new engines between the 1970's and the 1980's. Emissions from naturally aspirated four-stroke engines declined from 2.1-3.0 g/kW-hr in the 1970's to 0.25 -0.6 g/kW-hr in the 1980's (Sawyer and Johnson, 1995).

In order to bracket the exposure of the railroad workers to diesel exhaust a variety of patterns of exposure are considered. The patterns are characterized by two components: a) the extent of change from 1959 to 1980 in diesel exhaust exposure, expressed as a ratio, and b) the average exposure concentration for the workers on trains measured in the Woskie *et al*. (1988a) study (i.e., the baseline). The alternate ratios are as follows: a) a ratio of 1 suggested and used in Crump *et al*. (1991) as more realistic than the Garshick *et al*. (1987a, 1988) assumption of constant concentration from 1959-1980 and none before that; b) a ratio of 2 suggested by K. Hammond to allow for a modest peak in 1959; c) a ratio of 3 allowing for more peak, a scaled down version of the exposure factor of 10 that Woskie *et al*. (1988b) reported for exposure concentration of shopworkers to nitrogen dioxide in enclosures including engine test sheds; and d) a ratio of 10, peak of the magnitude of values for the engine test sheds. The alternate baselines of exposure concentrations are as follows: 1) 40 $\mu g/m^3$, obtained by subtracting the background measurement of the unexposed workers from the measurement of the train workers, rounded down; 2) 50 $\mu g/m^3$, which also subtracted background from the train worker measurements but rounded up to allow somewhat for measurements of workers on trains not having as much exposure to non-diesel exhaust background particulate as the clerks; and 3) 80 $\mu g/m^3$, obtained by assuming that the entire ETS-adjusted RSP of the train workers is diesel exhaust while the clerks are considered unexposed to diesel exhaust (0 concentration).

The specific alternative patterns of linear decline (if any) of concentration from 1959 through 1980 are:

1. no decline, constant at the baseline values of 50, a ramp (1,50) pattern suggested and used in Crump *et al*. (1991).
2. declining 3-fold from a peak of 150 to a baseline of 50, a roof (3,50) pattern, the preferred pattern in this report;
3. declining 10-fold from a peak of 500 to a baseline of 50, a roof (10,50) pattern, suggested in information submitted by the Engine Manufacturers Association;
4. declining, 2-fold from a peak of 80 to a baseline of 40, a roof (2,40) pattern suggested by K. Hammond, one of the investigators in the Woskie *et al*. study; and
5. declining 3-fold from a peak of 240 to a baseline of 80, a roof (3,80) pattern, a variant on Pattern 3 for not subtracting background ETS-adjusted RSP in the exposed group while still maintaining
6. unexposed workers at zero concentration.

Calculation Of Cumulative Exposure

The estimate of the time course permits calculation of the overall average cumulative exposure for the cohort for each year of the follow-up period, 1959-1980. The cumulative exposure factor was calculated as the area under the curve (AUC) of the exposure factor (EF, ratio of concentration to baseline concentration) for successive years. Cumulative exposure is the cumulative exposure factor times the baseline value.

Intermittency Correction

The equivalent exposure duration for non-continuous exposure was scaled on the basis of volume of air breathed. Exposure durations are calculated to have the same cumulative yearly intake of the substance as produced by continuous inhalation of 20 m^3/day at the concentration of the substance breathed in. Assuming that the average exposed member of the cohort inhales 10 m^3 during an 8-hour working day implies an adjustment factor of 10/20 to multiply the exposure concentration to account for ventilation rate not equaling the standard human daily inhalation of 20 m^3/day. Adjusting for the discontinuous work week and work year yields additional adjustment factors of 5/7 for exposure days per week and 48/52 for weeks per year, all to multiply the exposure measure. In order to take account of the non-continuous work exposure, the resulting overall multiplicative factor on exposure duration is

$$(10/20)(5/7)(48/52) = 0.33.$$

Determining Lifetime Unit Risk From The Relative-Risk Slope

The analyses below calculate the relationship between relative risk (relative hazard) and duration of exposure. The relative risk is the prediction of the ratio: incidence (yearly death rate per population) of lung cancer due to diesel exhaust divided by the background incidence of lung cancer. In the principal modeling of both sets of epidemiological data, reported below in this chapter, relative risks are fitted linearly to duration of exposure. From that slope, an estimate of the slope with respect to cumulative exposure for the specific alternative patterns of occupational exposure considered is obtained by modifying the duration scale for the slope. The approximation for this modification is simply to multiply the duration scale by the overall area under the curve (AUC) of the desired pattern and to divide by the total duration of exposure in the analysis.

Approximations may often be used to determine lifetime unit risk from this slope, but the present work will, for consistency and accuracy, use life-table calculations for that determination. This calculation starts with a background life table for lung cancer in California. For each unit risk to be calculated, a modification of that table is constructed in a way that includes the predicted effect of a lifetime exposure to 1 unit of concentration, 1 $\mu g/m^3$ in the present calculations. The predicted effect is incorporated by multiplying the background lung cancer incidence for each age interval in the table by the relative risk (relative hazard) for that age interval. The relative risk is (1+ excess relative risk due to exposure). The excess relative risk due to exposure for unit concentration is the slope of relative risk with concentration, obtained from the epidemiological analyses. Using the general model based on cumulative exposure, as in the present calculations, the excess relative risk requires the slope coefficient per concentration-year to be multiplied by the age in years for

each age group in the table and to be divided by the intermittency factor. Any ages that fall within the number of years of detection lag prior to the target age have zero excess relative hazard. The modified table is completed in the manner of the original table. The lifetime unit risk is then the following difference: the probability of lung cancer at the target age in the table modified by exposure less the probability at the same age in the original table.

Use of the Garshick *et al*. (1987a) Case-Control Study to Estimate Unit Risk

The first study used to estimate lung cancer risk due to diesel exhaust exposure is the case-control study of U.S. railroad workers by Garshick *et al*. (1987a). For this case-control study Garshick *et al*. (1987a) collected 15,059 US railroad worker death records for 1981. They matched each of 1256 lung-cancer cases with 2 other deaths, each of those having nearly the same date of birth and death. For each of the controls, death was due to a specified natural cause with no mention of cancer on the death certificate. For each subject, Garshick *et al*. (1987a) determined years in a job with diesel exposure, asbestos exposure and smoking history. Taking into account the effect of age, their analysis used multivariate conditional logistic regression to determine the relationship between lung cancer and duration of exposure to diesel exhaust. For workers with more than 20 years exposure and for exclusion of shopworkers, they calculated the odds ratio was 1.55 (95% CI = 1.09, 2.21) with a referent category of 0 to 4 years work in a job exposed to diesel exhaust.

From the odds ratio for a 20 year duration of exposure, the coefficient of increase with duration of exposure was estimated by assuming a linear rise over the 20 years. Using a calculation similar to that used by Garshick *et al.* with shopworkers included, the slope coefficient for the odds ratio is 0.022 (90% C.I. = 0.0071, 0.037) year^{-1}. Because the odds ratio approximates relative risk (Breslow and Day, 1980, pp. 69-73), this value is approximately the rate of increase of relative risk (relative hazard) and is used in a life table to obtain the lifetime unit risk. The modified life table calculation for unit concentration (1 μg/m^3) for 5-yr. lag from carcinogenesis to death is in Table 7-1 of the diesel exhaust TAC document (OEHHA, 1998). The resulting unit risks are presented in Point I in Table 7-3 of the diesel exhaust TAC document. The highest values in that set are for the assumption that workers on trains have a ramp (1,50) pattern of exposure. The 95% UCL for lifetime unit risk is 2.4×10^{-3} (μg/m^3)$^{-1}$, with an MLE of 1.4×10^{-3} (μg/m^3)$^{-1}$. For the roof (3,50) pattern of exposure, the procedure is similar, but the exposure scale is increased by the ratio 65/22, representing the ratio of area under the EF of the roof to the area under the EF of the block. The resulting 95% UCL for lifetime unit risk is 1.0×10^{-3} (μg/m^3)$^{-1}$, with an MLE of 6.2×10^{-4} (μg/m^3)$^{-1}$. The lowest values in the set are for the roof (10,50) pattern of exposure. Using a similar approach, multiplying the exposure scale by the AUC ratio of 191/22, the 95% UCL for lifetime unit risk is 3.6×10^{-4} (μg/m^3)$^{-1}$, with an MLE of 2.1×10^{-4} (μg/m^3)$^{-1}$.

Using the slope coefficient for the analysis including shopworkers, reported in Garshick *et al*. (1987a), McClellan *et al.*(1989) previously calculated the expected increase in U.S. lung cancer deaths per year for each μg/m^3 of diesel exhaust exposure for two alternative exposure concentrations, 125 μg/m^3 and 500 μg/m^3, constant from 1959-1980. Mauderly (1992a) used these death rates to estimate unit risks, finding expected values of 1.2×10^{-3} (lifetime-μg/m^3)$^{-1}$ and 2.9×10^{-4} (lifetime μg/m^3)$^{-1}$, respectively. These values are close to the higher MLE values just given. Even though the higher concentrations assumed by McClellan *et al*. would tend to produce lower unit risks, the effect of using the more accurate life table method has a counteracting effect.

Use of the Garshick *et al*. (1988) Cohort Study to Estimate Unit Risk

The second study selected to estimate lung cancer risk due to diesel exhaust exposure was the retrospective cohort study of U. S. railroad workers by Garshick *et al*. (1988). The present analysis uses the individual data collected for that study in new calculations to determine slopes for the relationship of incidence to cumulative exposure. The analysis uses reconstructions of exposure, the ramp and the roof exposure patterns, to adjust the slope obtained from the analysis that is implemented with duration of exposure as the measure of exposure.

Further material on the cohort is developed in Appendices D, E, F of the diesel exhaust TAC document (OEHHA, 1998). Appendix E contains references to correspondence cited in this chapter. (The original unpublished documents referred to in Appendix E are available on request from the California Air Resources Board, Stationary Source Division or from the U.S. EPA docket for the Health Assessment Document for Diesel Emissions at the National Center for Environmental Assessment, Washington, DC. 20460 (1997)).

Description of the Original Study

The cohort consisted of 55,407 railroad workers, who were aged 40-64 in 1959 and who had started railroad service 10-20 years earlier; 1694 lung cancers were identified. The unexposed group in the cohort, the clerks and signal tenders, constituted 25.3% of the whole cohort. To develop the original data set, Garshick *et al*. (1988) obtained the following information for each individual in their cohort of railroad workers for the follow-up years of 1959-1980: cause of death by death certificate, the primary job classification for each year, and months worked in that classification in each year. In addition, the investigation obtained the age at the start of follow-up in 1959, total service months and, for those workers who began work after 1946, the date of starting work. From these data Garshick *et al*. calculated the elapsed time of exposure for each individual from 1959 up to each follow-up year or up to the four years before each follow-up year.

Relative Risk Analysis

Because of much uncertainty about the proportion of shop workers exposed to diesel exhaust, OEHHA decided to exclude them from the analysis, as suggested by the study authors and other participants at the Diesel Exhaust Workshop, January, 1996. Garshick (1991) had previously called attention to dilution of the effect of diesel exhaust on the shop workers because of the inclusion of shopworkers in that cohort who had no true exposure. The original study obtained risk estimates both with and without the shop workers, and found the results changed very little. The exclusion of shop workers simplifies the analysis in that lung burden calculations are not needed because the exposures of other exposed workers, namely train workers, are sufficiently low that lung burden may be assumed essentially proportional to atmospheric exposures. Exposure measurements for 1982-83 (Woskie *et al*. 1988a), just after the end of the follow-up period, show that train workers considered here all experienced approximately the same average concentration of diesel exhaust (for example, 50 $\mu g/m^3$, rounded, for use in determining unit risk in this work). The present work uses years with any month of exposure time, excluding the four years previous to each year of observation as the average lag time from carcinogenesis to death. This calculation

of exposure time starts in 1952 and continues yearly through 1980, the end of follow-up. It extends 7 years back from 1959, the start of follow-up, to account on the average for the assumed linear rise of exposure from 1945 to 1959. The unexposed workers are assigned zero exposure time throughout.

The OEHHA analysis uses two programs in the EPICURE software package, which is designed for several standard kinds of epidemiological analysis. The first program, DATAB, reduces the individual data to cells with each desired variable having a single value for the cell. The cells are designated by a set of numbers, one for each categorical variable to determine the category number of that variable. The second program, AMFIT, determines parameters of a model to provide a best fit of the data using Poisson regression, a maximum likelihood procedure (Breslow and Day, 1987). The calculation approach is described in more detail for the closely related calculations using general models, in Appendix D of the diesel exhaust TAC document (OEHHA, 1998).

The assumptions not otherwise specified here are essentially those of Garshick *et al*. (1988). For example, all years of the study are included, and their rather irregular boundary points on years of exposure are used.

The OEHHA analysis explored the fit and other characteristics of a number of forms of a general model. The model that appeared to be most satisfactory is the one with linear and quadratic continuous covariates, age and calendar year. The slope calculated for relative risk (relative hazard) per year of exposure is 0.015 (95% CI: 0.0086 to 0.022) $year^{-1}$. The slope divided by the intermittency correction (0.33) and the assumed constant concentration (e.g., 50 $\mu g/m^3$ for 29 years) and multiplied by attained age provides the excess relative hazard to determine the increase of lung cancer rates for the lifetable calculation of the unit risk. The resulting unit risks are presented in Point II in Table 4, and closely parallel the results for the case-control study (Point I). The highest values in that set are for the assumption that workers on trains have a ramp (1,50) pattern of exposure. For the ramp pattern the result is a 95% UCL of 1.8×10^{-3} $(\mu g/m^3)^{-1}$ and a MLE of 1.3×10^{-3} $(\mu g/m^3)^{-1}$. For the roof (3,50) pattern of exposure, the procedure is similar, but the exposure scale is increased by the ratio 65/29, representing the ratio of area under the EF of the roof to the area under the EF of the ramp. The result is a 95% UCL of 8.2×10^{-4} $(\mu g/m^3)^{-1}$ and a MLE of 5.7×10^{-4} $(\mu g/m^3)^{-1}$. The lowest values in the set are for the roof (10,50) pattern of exposure. Using a similar approach, multiplying the exposure scale by the AUC ratio of 191/29, the 95% UCL for lifetime unit risk is 2.8×10^{-4} $(\mu g/m^3)^{-1}$, with an MLE of, 1.9×10^{-4} $(\mu g/m^3)^{-1}$.

Table 4: Values from Unit Risk for Diesel Exhaust from Using Hazard Slope on Exposure Measure in California Life-Table. Garshick *et al*. (1987a, 1988) Studies of U.S. Railroad Workers.

	q1 $(\mu g/m^3)^{-1}$ MLE	q1 $(\mu g/m^3)^{-1}$ 95% UCL
I. Case-Control study (1987a) using published slope coefficient for hazard on years of exposure to diesel exhaust (Section 7.3.3)		
A. Adapted to ramp (1,50) pattern of exposure	1.4×10^{-3}	2.4×10^{-3}
B. Adapted to roof (2,40) pattern of exposure	1.1×10^{-3}	1.8×10^{-3}
C. Adapted to roof (3,50) pattern of exposure	6.2×10^{-4}	1.0×10^{-3}
D. Adapted to roof (3,80) pattern of exposure	3.9×10^{-4}	6.6×10^{-4}
E. Adapted to roof (10,50) pattern of exposure	2.1×10^{-4}	3.6×10^{-4}
II. Cohort study (1988) using individual data to obtain a slope for hazard on years of exposure to diesel exhaust (Section 7.3.4) Continuous covariates: (attained age and calendar year) or (age-at-start-of study and calendar year)		
A. Adapted to ramp (1,50) pattern of exposure	1.3×10^{-3}	1.8×10^{-3}
B. Adapted to roof (2,40) pattern of exposure	9.9×10^{-4}	1.4×10^{-3}
B. Adapted to roof (3,50) pattern of exposure	5.7×10^{-4}	8.2×10^{-4}
D. Adapted to roof (3,80) pattern of exposure	3.6×10^{-4}	5.1×10^{-4}
E. Adapted to roof (10,50) pattern of exposure	1.9×10^{-4}	2.8×10^{-4}
III. Cohort study (1988) applying time varying concentrations to individual data to obtain a slope of hazard on exposure (from Appendix D)		
A. Ramp (1,50) pattern of exposure		
1. General multiplicative model with age-at-start-of-study and U.S. rates as categorical covariates	1.2×10^{-3}	1.9×10^{-3}
2. 6th/7-stage model with age-at-start-of study as categorical covariate	2.4×10^{-4}	3.8×10^{-4}
B. Roof (3,50) pattern of exposure		
1. General multiplicative model with age-at-start-of-study and U.S. rates as categorical covariates	5.1×10^{-4}	7.2×10^{-4}
2. 6th/7-stage model with age-at-start-of-study as categorical covariate	8.1×10^{-5}	1.3×10^{-4}
3. 7th/7-stage model with age-at-start-of-study as categorical covariate	1.0×10^{-4}	1.5×10^{-4}

Discussion of Results

The investigation of the forms of the model using Poisson regression explored the use of categorical covariates, calendar year and age-at-start-of-follow-up that verified the categorical trend with exposure that Garshick *et al*. (1988) had obtained for relative hazard by using a Cox regression with calendar year as the principal time scale and age-at-start-of-follow-up as a covariate. This result was an elevated relative risk (relative hazard) for the middle durations of exposure and an apparent rise at the highest exposure, albeit with large error bars. Crump (1997) found by direct comparison a close correspondence of results for this Poisson regression and a Cox regression that replicated Garshick *et al*.

The investigation also explored the use of a general model with the categorical covariates, calendar year and attained age, that verified the categorical results for relative risk in Crump *et al*. (1991) and Crump (1997). This result showed a rise and then an apparent fall of relative risk for increasing exposure. Age and calendar year are important determinants of lung cancer rate, and Crump (1997) has argued that this choice should be used for covariates because it is the most accurate in characterizing background rates and, further, that a fall of relative risk at the higher exposure, obtained for this choice of covariates, is not consistent with an exposure response.

It should be kept in mind that the categorical trends of the relative risk with duration of exposure are all used to represent a large cloud of observed points of incidence as a function of duration of exposure. Appendix F of the diesel exhaust TAC document (OEHHA, 1998) indicates that the discrepancy between the results of Garshick *et al*. and of Crump *et al*. may be more apparent that real. The slopes for the relative risk are significant for both these choices of covariate, but the slope for the use of calendar year and age-at-start is about twice that for the use of calendar year and attained age. The latter slope is larger, though less significant, than the identical slope obtained in the present analysis using continuous forms of either pair of covariates. The use of the continuous form of the covariates appears to have a salutary effect on reducing the variance of the slope estimate. This choice allows some flexibility, but not a lot, in describing time trends.

Conclusion

Based on the human data, the principal finding of the diesel exhaust TAC document quantitative risk assessment is a range of lifetime unit risk (95% UCL) as shown in the right-hand column of Table 4 above. The lowest value in the range is 1.3×10^{-4}, and the highest value is 2.4×10^{-3}. The geometric mean unit risk obtained from these end points of the range of values is 6×10^{-4} (lifetime-$\mu g/m^3)^{-1}$. The geometric mean provides information on the central tendency of the range and is not to be confused with a best estimate identified from the available calculations. The lower end of the range is the rounded value for both forms of multistage model using the roof exposure pattern for the data of the Garshick *et al*. (1988) cohort study of U.S. railroad workers. OEHHA concluded that incorporation of the roof exposure pattern and biologically-based analyses improved the unit risk estimates. Consequently, unit risk values incorporating this information, those at the lower end of the range, provide more scientifically defensible values. The upper end of the range is obtained using the published results of the Garshick *et al*. (1987a) case-control study for US railroad workers. The Scientific Review Panel concluded in their findings that a reasonable estimate of the cancer unit risk is 3×10^{-4} $(\mu g/m^3)^{-1}$.

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PENTACHLOROPHENOL

CAS No: 87-86-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995)

Molecular weight	266.35
Boiling point	309°C
Melting point	190°C
Vapor pressure	0.00011 mm Hg @ 25°C
Air concentration conversion	1 ppm = 10.9 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 4.6 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 1.8 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by CDHS (1991) from male mouse liver tumor data (NTP, 1989) using a linearized multistage procedure.]

III. CARCINOGENIC EFFECTS

Human Studies

No human epidemiological studies were located that were adequate to evaluate a possible association between exposure to pentachlorophenol and cancer.

Animal Studies

The National Toxicology Program (NTP, 1989) conducted 2 studies on the carcinogenic effects of lifetime exposure of mice to pentachlorophenol (PeCP). In these studies, $B6C3F_1$ mice were exposed to dietary PeCP, as either the technical grade or EC-7 grade, for 2 years. The technical grade PeCP contained polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) as contaminants in significantly higher concentrations than the EC-7 grade. The NTP found dose-related increases in liver and adrenal medullary tumors in male and female mice, and an increase in hemangiosarcomas in the females exposed to the EC-7 grade (Table 1). The incidence of liver neoplasms and hemangiosarcomas was higher in female mice exposed to technical grade PeCP. The incidence of hemoangiosarcomas in female mice was 0/35, 3/50, and 6/50 for the 0, 100, and 200 ppm PeCP groups, respectively.

Table 1. Tumor incidence in male and female $B6C3F_1$ mice exposed to technical or EC-7 grade pentachlorophenol (PeCP) (NTP, 1989).

Grade of PeCP	Sex	Adrenal gland/medullary tumor incidence (benign and malignant pheochromocytomas) Dietary concentration (ppm)[a] 0	100	200	600	Liver tumor incidence (hepatocellular adenomas and carcinomas) Dietary concentration (ppm)[a] 0	100	200	600
EC-7	males	1/34	4/48	21/48	45/49	6/35	19/48	21/48	34/49
	females	0/35	2/49	2/46	38/49	1/34	4/50	6/49	31/48
Technical	males	0/31	10/45	23/45	NT	7/32	26/47	37/48	NT
	females	2/33	2/48	1/49	NT	3/33	9/49	9/50	NT

a) EC-7 dose levels for males and females were 0, 18, 37 and 118 mg/kg-day and 0, 17, 34 and 114 mg/kg-day, respectively.
NT = not tested

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The NTP (1989) study in mice was used as the basis for the cancer potency for PeCP. This represents the only adequate, long-term, positive study of the carcinogenic effects of PeCP. The NTP study shows that PeCP exposure in mice results in several types of tumors in males and females. In the NTP (1989) study, mice (35-50 per group) were exposed to 0, 100, 200, or 600 mg/kg diet PeCP (EC-7 grade) for 2 years. In a parallel series of experiments, mice were exposed to 0, 100, or 200 mg/kg diet of technical grade PeCP. Results of these bioassays included a significant incidence of liver and adrenal neoplasms in male and female mice exposed to PeCP. In addition, female mice exhibited a significant increase in hemangiosarcomas. Although there were trace amounts of PCDD's and PCDF's in the EC-7 grade, the amount of these contaminants was determined by CHDS (1991) to be insufficient to result in the observed tumor incidence.

Methodology

A linearized multistage procedure was used to estimate the cancer potency of EC-7 grade PeCP from the NTP (1989) liver tumor (hepatocellular adenomas and carcinomas) data in male $B6C3F_1$ mice (Crump *et al.*, 1982). The concentrations of PeCP given in the feed were 0, 100, 200, or 600 mg/kg diet. The tumor incidence data are shown in Table 1. The 95% upper confidence bound on the dose-response slope was used to derive the human cancer potency value for PeCP.

The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. The default lifespan for mice is 104 weeks. In this case, the lifetime scaling factor is equal to 1. An estimated value for the human cancer potency was determined using the

relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse).

Using these relationships, a human cancer potency (q_{human}) of 1.8E-2 $[mg/kg\text{-}day]^{-1}$ was derived (CDHS, 1991). An airborne unit risk factor of 4.6E-6 $(\mu g/m^3)^{-1}$ was calculated from the q_{human} value by OEHHA/ATES using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

V. REFERENCES

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PERCHLOROETHYLENE

CAS No: 127-18-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	165.83
Boiling point	121ºC
Melting point	-19 ºC
Vapor pressure	18.47 mm Hg @ 25ºC
Air concentration conversion	1 ppm = 6.78 mg/m^3 @ 25ºC

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 5.9 E-6 $(\mu g/m^3)^{-1}$
Slope Factor: 2.1 E-2 $(mg/kg\text{-}day)^{-1}$
[Male mouse hepatocellular adenoma and carcinoma incidence data (NTP, 1986), cancer risk estimate calculated using a linearized multistage procedure and PBPK model dose adjustment (CDHS, 1991).

III. CARCINOGENIC EFFECTS

Human Studies

Epidemiological studies of perchloroethylene (PCE) exposure have been reviewed by Reichert (1983) and by the U.S. EPA (1985). Blair *et al*. (1979) analyzed the death certificates of 330 union laundry and dry-cleaning workers (out of a cohort of 10,000). Of 330 decedents, 279 had worked solely in dry-cleaning establishments. Increased mortality from cancers of the respiratory tract, cervix, and skin was documented, and when all malignancies were evaluated together, the number of observed deaths was significantly greater than expected ($p < 0.05$). Although an excess of liver cancer and leukemia was also observed, these increases were not statistically significant.

In an expanded study, Blair *et al.* (1990) reported on mortality among 5,365 dry cleaning union members. Statistically significant excesses of cancer of the esophagus and cervix and non-significant excesses for cancer of the larynx, lung, bladder, and thyroid were reported. Lack of PCE exposure data and lack of accounting for potential confounding factors, such as economic status, tobacco, or alcohol use, prevents any firm conclusion as to the association of PCE exposure and excess cancer.

Katz and Jowett (1981) analyzed the mortality patterns of 671 white female laundry and dry-cleaning workers. Occupational codes listed on the certificates did not distinguish between the two types of work. Data on the duration of employment were not available, nor were the investigators able to determine to which solvent(s) the individuals were exposed. Smoking history was not known. A significant increase in risk of death from cancer of the kidneys ($p < 0.05$) and genitals ($p < 0.01$) was documented. An excess risk from skin and bladder cancer was also found; however, neither increase was statistically significant.

Other studies of laundry and dry-cleaning workers have also reported an increased risk of death from cervical cancer (Blair *et al.,* 1979; Kaplan, 1980); however, these investigators have not compared mortality data by low-wage occupation. Although not definitive, the findings of Katz and Jowett (1981) suggest that factor(s) other than (or in addition to) solvent exposure are important contributors to cervical cancer.

Kaplan (1980) completed a retrospective mortality study of 1,597 dry-cleaning workers exposed to PCE for at least one year (prior to 1960). The solvent history of approximately half of the dry-cleaning establishments was known. The inability of Kaplan to quantify solvent exposure adds an important confounding variable to the study (Kaplan, 1980). The mean exposure concentration of individuals to PCE was calculated to be 22 ppm for dry-cleaning machine operators and 3.3 ppm for all other jobs. Kaplan found an elevated SMR (182) for malignant neoplasms of the colon (11 observed deaths, 6.77 to 6.98 expected deaths). In addition to colon cancer, malignant neoplasms of the rectum, pancreas, respiratory system, urinary organs, and "other and unspecified sites (major)" were observed (Kaplan, 1980). Although the relatively small cohort in this study limits conclusions about the carcinogenic potential of PCE, the study (Kaplan, 1980) results suggest a relationship between colon cancer and solvent exposure.

A group of Danish laundry and dry-cleaning workers was identified from the Danish Occupational Cancer Register (Lynge *et al.,* 1990). From cancer incidence data for a 10-year period, a significant excess risk was found for primary liver cancer among 8,567 women (standardized incidence ratio 3.4, 95% confidence interval 1.4-7.0). No case of primary liver cancer was observed among 2,033 men, for whom the expected value was 1.1. Excess alcohol consumption did not appear to account for the excess primary liver cancer risk for women. However, no data was available on actual exposures of the study group to PCE or other chemicals.

Duh and Asal (1984) studied the cause(s) of mortality among 440 laundry and dry-cleaning workers from Oklahoma who died during 1975 to 1981. Smoking histories were not available and separation of the two groups by occupation was not possible. NIOSH reported that, although 75% of dry-cleaning establishments in the U.S. use PCE, Oklahoma may be unique in that petroleum solvents account for more that 50% of total solvents used during this period (NIOSH, 1980). Analysis of deaths due to cancer showed an increase for cancers of the respiratory system, lung, and kidney.

Brown and Kaplan (1987) conducted a retrospective, cohort-mortality study of workers employed in the dry-cleaning industry to evaluate the carcinogenic potential from occupational exposure to PCE. The study cohort consisted of 1,690 members of four labor unions (located in Oakland, Detroit, Chicago, and New York City). Individuals selected for the study had been employed for at least one year prior to 1960 in dry-cleaning shops using PCE as the primary solvent. Complete solvent-use histories were not known for about half of the shops included in the study. Because petroleum solvents were widely used by dry cleaners prior to 1960, most of the cohort had known or potential exposure to solvents other than PCE (primary, various types of Stoddard solvents). The investigators also identified a subcohort of 615 workers who had been employed only in establishments where PCE was the primary solvent. The PCE exposure in shops included in the study was evaluated independently (Ludwig *et al.,* 1983). The geometric mean of time-weighted-

average exposures was 22 ppm PCE for machine operators and approximately 3 ppm for other workers.

In summary, a statistically significant excess of deaths from urinary tract cancer was observed in those workers that were potentially exposed to both PCE and petroleum solvents. Individuals employed in shops where PCE was the primary solvent did not have an increased risk of mortality from kidney or bladder cancer. Although these findings do not rule out PCE as the causative agent of urinary tract cancer, the data suggest that other factors or agents may have contributed to the development of neoplastic disease. CDHS stated in the Toxic Air Contaminant document "Health Effects of Tetrachloroethylene" that until studies are completed that include a thorough analysis and quantification of PCE exposures, epidemiological studies will not be useful for the assessment of the human health risks of PCE (CDHS, 1991).

Animal Studies

Two lifetime bioassays have been completed on PCE (NCI, 1977; NTP, 1986). Additionally, three other studies have addressed the question of PCE carcinogenicity (Rampy *et al.,* 1978; Theiss *et al.,* 1977).

The National Cancer Institute (NCI) conducted a study in which B6C3F_1 mice and Osborne Mendel rats were administered PCE in corn oil by gavage, 5 days/week for 78 weeks (NCI, 1977). The time-weighted average daily doses of PCE were 536 and 1072 mg/kg for male mice, 386 and 722 mg/kg for female mice, 471 and 941 mg/kg for male rats, and 474 and 949 mg/kg for female rats. PCE caused a statistically significant increase in the incidence of hepatocellular carcinomas in mice of both sexes and both dosage groups ($p < 0.001$). The time to tumor development was considerably shorter in treated than in control mice. In untreated and vehicle control mice, hepatocellular carcinoma were first detected at about 90 weeks. In comparison, hepatocellular carcinomas in male mice were detected after 27 weeks (low dose) and 40 weeks (high dose) and in female mice after 41 weeks (low dose) and 50 weeks (high dose) (Table 1). The median survival times of mice were inversely related to dose. For control, low dose and high dose male mice, their median survival times were 90 weeks, 78 weeks and 43 weeks, respectively; for female mice, their median survival times were 90 weeks, 62 and 50 weeks, respectively. Early mortality occurred in all groups of rats dosed with PCE. NCI (1977) determined that the early mortality observed in rats in this bioassay were inappropriately high and because the optimum dosage was not used, the rat results preclude any conclusions regarding the carcinogenicity of PCE in rats. In addition, the PCE used in the NCI mouse and rat bioassays had a purity of 99%, with epichlorohydrin (ECH) used as a stabilizer. It has been suggested that the presence of this contaminant may have directly contributed to tumor induction.

The most definitive study of the carcinogenic potential of PCE was conducted by Battelle Pacific Northwest Laboratories for the National Toxicology Program (NTP, 1986). In this experiment, B6C3F_1 mice and F344/N rats were exposed to 99.9% pure PCE by inhalation, 6 hours/day, 5 days/week for 103 weeks. Mice were exposed to concentrations of 0, 100, or 200 ppm; rats were exposed to concentrations of 0, 200, or 400 ppm. Both exposure concentrations produced significant increases in mononuclear cell leukemia in female rats (incidence in control, 18/50 animals; in rats receiving 200 ppm, 30/50; and in rats receiving 400 ppm, 29/50). Treated male

rats also developed mononuclear cell leukemia in greater numbers than controls (controls, 28/50 animals; 200 ppm, 37/50; 400 ppm, 37/50) [Table 1]. Male rats (at the 200 ppm and 400 ppm PCE exposure levels) exhibited an increased incidence of both renal tubular-cell adenomas and adenocarcinomas. Although the increases were not statistically significant, they appeared to be dose-related.

Brain glioma (a rare tumor of neuroglial cells) was observed in one male control rat and in four male rats that were exposed to 400 ppm PCE (NTP, 1986). This increase was not statistically significant. However, because the historical incidence of these tumors is quite low (0.2% at Battelle Laboratories), the increased incidence in treated animals in this study is noteworthy. Both concentrations of PCE produced a statistically significant increase of hepatocellular carcinomas in treated mice of both sexes ($p < 0.001$). The incidence of these carcinomas in male mice was as follows: controls, 7/49 animals; low-dose, 25/49; and high-dose, 26/50. The incidence of hepatocellular carcinomas in treated female mice was: controls; 1/48 animals; low-dose, 13/50; high-dose, 36/50. Hepatocellular adenomas occurred in both sexes of mice and at both concentrations of PCE (Table 1). The incidence of adenomas was not statistically significant. However, the combined incidence of hepatocellular adenomas and hepatocellular carcinomas was significant. In males, the combined incidence was: controls, 16/49 animals; low-dose 31/49; ($p = 0.002$); adenomas and carcinomas was: controls, 4/48 animals; low-dose, 17/50 ($p = 0.001$); and high-dose, 38/50 ($p < 0.001$).

Table 1: PCE-induced tumor incidence in mice and rats

Study	Species	Sex	Concentration or dose	Tumor response	
				Type[a]	Incidence
NCI, 1977	Mice	Males	0 mg/kg-d	HC	2/17
			536 mg/kg-d	HC	32/49*
			1072 mg/kg-d	HC	27/48*
		Females	0 mg/kg-d	HC	2/20
			386 mg/kg-d	HC	19/48*
			772 mg/kg-d	HC	19/48*
NTP, 1986	Mice	Males	0 ppm	HC; HAC	7/49 ; 16/49
			100 ppm	HC; HAC	25/49*; 8/49(NS)
			200 ppm	HC: HAC	26/50*; 18/50(NS)
		Females	0 ppm	HC; HAC	1/48 ; 3/48
			100 ppm	HC; HAC	13/50*; 6/50(NS)
			200 ppm	HC; HAC	36/50*; 2/50(NS)

[a] HC = hepatocellular carcinomas; HAC = hepatocellular adenoma; ML = mononuclear cell leukemia.
*$p < 0.001$, Fisher Exact Test; **Probability level, Life Table Analysis. NS = not statistically significant

The NTP (1986) determined that, under the conditions of the study, there was "clear evidence of carcinogenicity" of PCE for male F344/N rats, "some evidence of carcinogenicity" of PCE for female F344/N rats, and "clear evidence of carcinogenicity" of PCE for both sexes of $B6C3F_1$ mice. IARC reevaluated the evidence of carcinogenicity of PCE in 1987 using data from the NTP study and concluded that there was sufficient evidence that PCE is carcinogenic to animals (IARC, 1987). Other studies on PCE included those by Rampy *et al.* (1978) and Theiss *et al.* (1977). Rampy *et al.* (1978) exposed male and female Sprague-Dawley rats to PCE by inhalation (300 or

600 ppm) 6 hours/day, 5 days/week for 12 months. Animals were subsequently observed for 18 months. Pathological changes in the liver or kidney were not observed. Theiss and coworkers studied the ability of PCE to induce lung adenomas in A/St male mice (Theiss *et al.,* 1977). Animals 6 to 8 weeks old were given 80, 200, or 400 mg/kg of PCE in tricaprylin (intraperitoneally) three times a week. Each group received 14, 24, or 48 injections. Treated animals did not exhibit a significant increase in the average number of lung tumors when compared to controls.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Perchloroethylene has been observed to induce mononuclear cell leukemia in male and female rats and liver tumors in male and female mice (NTP, 1986). CDHS (1992) decided that the tumor incidence data from this study were suitable for use in developing a quantitative risk assessment.

Methodology

Results from the 1986 NTP inhalation study were used as the basis for estimating the carcinogenic risk of PCE to humans. In this bioassay, PCE was 99.9% pure, and animals were exposed 6 hours/day, 5 days/week for 103 weeks. The mice in the 100 and 200 ppm dose groups were exposed to a time-weighted-average (TWA) of 16 and 32 ppm, respectively (e.g., 100 ppm × 6 hours/24 hours × 5 days/7 days). Similarly, rats in the 200 and 400 ppm dose groups were exposed to a TWA of 33 and 66 ppm, respectively.

The CDHS staff used the metabolized dose, adjusted to continuous lifetime exposure, to calculate the carcinogenic potency of PCE (CDHS, 1992). There are several uncertainties using this approach: 1) It was assumed that oxidative metabolism leads to the production of carcinogenic metabolites but the ultimate carcinogen(s) has not been well characterized. The metabolism of PCE is not well quantified in humans, and 20-40% of the absorbed PCE has not been accounted for. 2) The pharmacokinetic models used do not account for individual differences in metabolism and storage. The body burden depended on factors such as age, sex, exercise or workload, body mass, adipose tissue mass, pulmonary dysfunctional states, and individual differences in the intrinsic capacity to metabolize PCE.

Two pharmacokinetic models, the steady-state and the PB-PK approaches were used. They incorporated an 18.5% estimated applied dose as the fraction of the dose that is metabolized in humans. For the low-dose PCE risk assessment, the Crump multistage polynomial (Crump, 1984) was chosen. This model, rather than a time dependent form of the multistage model, was chosen because most tumors were discovered only at the time of sacrifice, and survival in this study was relatively good. The cancer potency values derived using the two different pharmacokinetic approaches using the 1986 NTP rat and mouse studies ranged from 0.12 - 0.95 $(mg/kg\text{-}d)^{-1}$. When expressed as a function of human applied dose the values obtained ranged from 0.0025 to 0.093 $(mg/kg\text{-}d)^{-1}$. Using an estimated human weight of 70 kg, estimated breathing rate of 20 m^3/day and the PCE conversion factor of 1 ppb = 6.78 $\mu g/m^3$, the cancer unit risk values for PCE ranged

from 0.2 - 7.2 × 10^{-5} $(ppb)^{-1}$. After considering the quality of the cancer bioassays and the uncertainty of human metabolism, CDHS (1992) decided that the best value for the PCE cancer unit risk was 4.0 × 10^{-5} $(ppb)^{-1}$ [5.9 × 10^{-6} $(\mu g/m^3)^{-1}$]. This value is derived from the tumor incidence data for the most sensitive species, sex, and tumor site, male mouse hepatocellular adenomas or carcinomas (NTP, 1986).

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POLYCHLORINATED BIPHENYLS (PCBs)

CAS No: 1336-36-3 (all congeners)

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1995 except as noted)

Molecular weight	154 - 499
Boiling point	Unknown
Melting point	340-375°C
Vapor pressure	4.06E-4 mm Hg @ 25°C (Aroclor 1242)
	7.71E-5 mm Hg @ 25°C (Aroclor 1254)
Air concentration conversion	1 ppm = 10.87 mg/m^3 (Aroclor 1242)
	1 ppm = 13.33 mg/m^3 (Aroclor 1254)

II. HEALTH ASSESSMENT VALUES

1) TEF_{WHO-97} Scheme (For use with 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) unit risk factor in cases where measurements or estimates are available for PCB congeners [Table 2 of Appendix A].)

Congener		TEF_{WHO-97}	Unit Risk $(\mu g/m^3)^{-1}$	Slope Factor $(mg/kg/day)^{-1}$
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin		1.0	3.8 E+1	1.3 E+5
77	3,3',4,4'-TCB	0.0001	3.8 E-3	1.3 E+1
81	3,4,4',5-TCB	0.0001	3.8 E-3	1.3 E+1
105	2,3,3',4,4'-PeCB	0.0001	3.8 E-3	1.3 E+1
114	2,3,4,4',5-PeCB	0.0005	1.9 E-2	6.5 E+1
118	2,3',4,4',5-PeCB	0.0001	3.8 E-3	1.3 E+1
123	2',3,4,4',5-PeCB	0.0001	3.8 E-3	1.3 E+1
126	3,3',4,4',5-PeCB	0.1	3.8 E+0	1.3 E+4
156	2,3,3',4,4',5-HxCB	0.0005	1.9 E-2	6.5 E+1
157	2,3,3',4,4',5'-HxCB	0.0005	1.9 E-2	6.5 E+1
167	2,3',4,4',5,5'-HxCB	0.00001	3.8 E-4	1.3 E+0
169	3,3',4,4',5,5'-HxCB	0.01	3.8 E-1	1.3 E+3
189	2,3,3',4,4',5,5'-HpCB	0.0001	3.8 E-3	1.3 E+1

[TCDD unit risk factor: linearized multistage procedure (GLOBAL79), fitted to male mouse hepatic adenoma and carcinoma data (NTP, 1982), body weight scaling, cross-route extrapolation (CDHS, 1986).

PCB TEQs are added to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) TEQs for calculation of risk assessment values. In analyses of data that lack measurements of individual PCB congeners, it may be necessary to assess cancer risk using the unit risk factor for unspeciated PCB mixtures.]

2) Unspeciated PCB mixtures

Unit Risk Factor:	5.7 E-4 $(\mu g/m^3)^{-1}$	(For use in cases where food chain exposure, sediment or soil ingestion, dust or aerosol inhalation, dermal exposure (if an absorption factor has been applied to reduce the external dose), presence of dioxin-like, tumor-promoting, or persistent congeners in other media or early-life exposure (all pathways and mixtures) is expected.)
	1.1 E-4 $(\mu g/m^3)^{-1}$	(For use in cases where ingestion of water-soluble congeners, inhalation of evaporated congeners or dermal exposure (if no absorption factor has been applied to reduce the external dose) is expected.)
	2.0 E-5 $(\mu g/m^3)^{-1}$	(For use in cases where congeners with more than four chlorines comprise less than one-half percent of total PCBs)

[Calculated from a cancer potency factor derived by US EPA/IRIS (1996c) from rat liver tumor data (Norback and Weltman, 1985; Brunner *et al*., 1996), using a linear-quadratic multistage procedure and ¾ power body weight scaling to calculate an ED_{10} (estimated dose associated with 10 percent increased incidence), and its lower bound, LED_{10}.]

III. CARCINOGENIC EFFECTS

Human Studies

The evidence for the human carcinogenicity of PCBs has been determined by IARC (1987) to be limited, due to concurrent exposures of test subjects to other chemicals, and to the small numbers of individuals examined. A study of workers heavily exposed to Aroclor 1254 (54% chlorine, by weight) for 9 years, showed 2 out of 31 heavily exposed workers developed malignant melanoma, while 1 out of 41 less heavily exposed workers developed this tumor (Bahn *et al.*, 1976; Lawrence, 1977). The expected number of melanomas in a population this size was 0.04. IARC (1978) concluded that there was suggestive evidence of carcinogenicity in humans.

Brown and Jones (1981) and Brown (1987) found an increase in the mortality caused by liver or biliary passage cancer (5 observed, 1.9 expected) in 2567 US workers exposed to Aroclor 1254 during the manufacture of capacitors. Four of the 5 deceased workers were female.

Bertazzi *et al*. (1982) reported on a study of capacitor manufacturing workers in Italy. Workers were exposed to mixtures of PCB congeners containing 54% chlorine prior to 1964, and 42% chlorine after that time. In these workers, significant increases in the incidence of cancers of the digestive, lymphatic, and hematopoietic systems were observed in both male and female workers.

An expanded study was later conducted by Bertazzi *et al*. (1987) who recorded cancer mortality in 2100 male and female workers from 1946 to 1982. Cancers of the gastrointestinal tract were significantly increased in male workers (6 observed, 2.2 expected) and hematopoietic cancers were significantly increased in female workers (4 observed, 1.1 expected).

PCB content in human fat tissues has been correlated with the occurrence of stomach, colon, pancreas, ovary, and prostate cancers (Unger *et al*., 1982; 1984).

A large population of people in Japan were exposed to PCBs from contaminated cooking oil (Umeda, 1984). In these patients, a 5-fold increase in liver cancer was reported, but a dose-response was not established. In a cohort of 887 male PCB-exposed patients with "Yusho" disease, Kuratsune *et al*. (1986) found an increase in mortality from malignant tumors (33 observed, 15.5 expected). Deaths from malignant liver and lung tumors were particularly high (9 observed, 1.6 expected; 8 observed, 2.5 expected, respectively). Female Yusho patients (n = 874) did not show the increase in cancer mortality.

Animal Studies

Early studies by Kimura and Baba (1973) and Ito *et al*. (1974) did not demonstrate carcinogenicity of PCBs in male or female rats orally exposed to highly chlorinated (60% by weight) PCBs in the diet for up to 77 weeks. In this study, 20 rats (10/sex) were exposed to diets ranging from 38.5 to 462 ppm PCBs. Ten rats (5/sex) served as experimental controls. Each rat was exposed to a unique treatment regimen that differed by amount of PCB ingested and duration of exposure. Female rats exhibited several benign adenomatous lesions in the liver, but no statistical comparisons were made. In these studies, small sample sizes (10 per group), less than lifetime exposure, and excess deaths unrelated to PCB exposure prevented definitive conclusions about the carcinogenicity of PCBs.

The NCI (1978) conducted a 2-year bioassay on male or female Fisher-344 rats (24 per group) exposed to Aroclor 1254 in the diet. Concentrations of PCBs in the feed were 0, 25, 50, or 100 ppm. A significant increase in the numbers of lymphomas and leukemias was observed in the male rats. The NCI did not conclude that these hematological tumors were treatment related. The incidence of hepatocarcinomas in the male rats was 0/24, 1/24, and 3/24 for the 0, 50, and 100 ppm groups, respectively. In other tissues, such as the stomach, jejunum, and cecum, rare tumors were found. The incidence of these tumors, while not statistically significant, was considered to be treatment-related due to the low incidence of these tumors in historical controls. The NCI concluded that PCBs were capable of inducing proliferative lesions in the liver, but were not carcinogenic to rats in this bioassay.

A reanalysis of the NCI data by Morgan *et al*. (1981) found that the incidence of focal metaplasia in the stomach increased in a dose-dependent fashion with a 6, 10, 17, and 35% occurrence in the 0, 25, 50, and 100 ppm groups, respectively. The incidence of stomach adenocarcinomas was significantly higher than in historical controls (4% vs. 0.03%, $p < 0.001$). With this reanalysis, the authors concluded that Aroclor 1254 was carcinogenic.

A chronic dietary exposure to Aroclor 1260 in rats was reported by Kimbrough *et al*. (1975). In this study, 200 young rats were fed 0 or 100 ppm Aroclor 1260 for 94 weeks. Actual dosages of PCB were estimated to be 11.6 mg/kg/day for the first week, 6.1 mg/kg/day at 3 months, and 4.3 mg/kg/day at 20 months. The time-weighted average dose was estimated to be 4.42 mg/kg/day (U.S. EPA, 1985). Almost all treated rats developed liver nodules (170/184). In addition, the incidence of hepatocellular carcinomas was highly significantly increased over controls (1/173 for controls vs. 26/184 for treated rats; $p < 1.0E-6$). Neoplastic nodules and total neoplastic lesions were also highly significantly increased over concurrent controls.

Schaeffer *et al*. (1984) reported results from a 2-year bioassay in rats using 2 PCB mixtures: Clophen A 30 (30% chlorine by weight) and Clophen A 60 (60% chlorine by weight) in the diet. Groups consisted of approximately 140 male weanling rats exposed to 100 ppm of either Clophen A 30 or Clophen A 60. A significant increase in the percentage of hepatocellular carcinomas was seen in the rats treated with Clophen A 60 (62%), but not with Clophen A 30 (3%). Hepatocarcinomas were observed in 2% of control animals. Preneoplastic lesions were not observed before 71 weeks.

The incidence of hepatocarcinoma was increased in rats exposed to Aroclor 1260 in a 2-year study by Norback and Weltman (1985). In this study, 70 male or female rats were exposed to 100 ppm Aroclor 1260 in the diet for 16 months, followed by 50 ppm in the diet for 8 months. The time-weighted average doses were calculated to be 5 mg/kg/day for the male rats, and 4.2 mg/kg/day for the females. The hepatocarcinoma incidence in control rats living 18 months or longer was 1.2% (1/81). The treated rats had an incidence of 95% (45/47) for the females, and 15% (7/46) for the males. The combined (male and female) tumor incidences were significantly higher in treated rats compared to controls. The authors also reported an increase in incidence of cholangiomas, but these lesions were designated by CDHS as benign, since no specific discussion of their malignancy was given.

Brunner *et al*. (1996) fed male and female Sprague-Dawley rats (50 animals/sex/Aroclor dose group) diets containing 25, 50 or 100 ppm Aroclor 1260 or 1254; 50 or 100 ppm Aroclor 1242; or 50, 100 or 200 ppm Aroclor 1016 for 104 weeks. Control groups were also included (100 animals/sex). Surviving animals were killed at 104 weeks. Statistically significant increases in liver adenoma or carcinoma incidence were observed in female rats for all Aroclor mixtures tested, and in male rats for Aroclor 1260 (Table 1).

In mice, two studies indicate that PCBs are carcinogenic, particularly with respect to hepatocarcinomas. Kimbrough and Linder (1974) exposed groups of 50 male BALB/cj mice to Aroclor 1254 at 0 or 300 ppm in the diet for 11 months, or for 6 months with a 5 month recovery period. The incidences of hepatoma were 0/34, and 0/24 for the 11- and 6-month control groups, respectively. The treated animals had incidences of 9/22, and 1/24 for the 11- and 6-month groups, respectively.

Ito *et al*. (1973) exposed groups of 12 mice to 500 ppm Kaneclor 500 (54% chlorine) in the diet for 32 weeks. No liver lesions were seen in 6 untreated controls. The incidence of hepatocellular carcinoma in the treated mice was 5/12 and the incidence of liver nodules was 7/12.

Table 1. Liver tumor incidence in Sprague-Dawley rats exposed to PCBs (Brunner *et al*., 1996; contained in US EPA, 1996a)

Mixture	Exposure level (ppm)	Tumor incidence Females	Males
Aroclor 1260	0	**1/85 (1%)	**7/98 (7%)
	25	10/49 (20%)	3/50 (6%)
	50	11/45 (24%)	6/49 (12%)
	100	24/50 (48%)	10/49 (12%)
Aroclor 1254	0	**1/85 (1%)	7/98 (7%)
	25	19/45 (42%)	4/48 (8%)
	50	28/49 (57%)	4/49 (8%)
	100	28/49 (57%)	6/47 (13%)
Aroclor 1242	0	**1/85 (1%)	7/98 (7%)
	50	11/49 (24%)	1/50 (2%)
	100	15/45 (33%)	4/46 (9%)
Aroclor 1016	0	**1/85 (1%)	7/98 (7%)
	50	1/48 (2%)	2/48 (4%)
	100	6/45 (13%)	2/50 (4%)
	200	5/50 (10%)	4/49 (8%)

**Statistically significant trend ($p < 0.05$) by Cochran-Armitage trend test.

In addition to the above studies describing the carcinogenicity of PCB mixtures, there are also some reports demonstrating the cancer promoting activity of dioxin-like PCB congeners.

Haag-Gronlund *et al*. (1997) studied the potential of 2,3',4,4',5-pentachlorobiphenyl (PCB 118) to promote liver tumors in female Sprague-Dawley rats using a two-stage initiation/promotion bioassay. In this study, animals were initiated by administrating N-nitrosodiethylamine after partial hepatectomy. The promotion began five weeks later by the subcutaneous administration of PCB 118 at six dose levels (10, 40, 160, 640, 2500 and 10000 µg/kg body weight) once/week for 20 weeks. Animals were also exposed to 40, 640 or 10000 µg/kg/week once/week for 52 weeks. After 20 weeks, the number of liver foci/cm^3, measured as foci positive for glutathione-S-transferase, was significantly increased in the two highest dose groups. The percentage of liver volume occupied by foci was not significantly increased after 20 weeks. However, after 52 weeks of treatment, the highest dose of PCB 118 significantly increased both the percentage of liver volume occupied by foci and the number of foci/cm^3.

According to Ito *et al.* (1992), the fraction of the liver occupied by foci (volume fraction) can be used as an approximate measure of the total amount of preneoplastic (initiated) cells in the liver available for further neoplastic development. Based on the volume fraction of liver occupied by foci, a relative potency of less than 0.00002 was estimated for PCB 118 when compared to TCDD liver foci development potency after 20 weeks of treatment. This value is less than the TEF_{WHO-97}

value of 0.0001 derived by the World Health Organization for PCB 118 (van den Berg *et al*., 1998). However, a relative potency of 0.0001 was estimated for EROD-induction activity of PCB 118. This value is exactly the TEF_{WHO-97} value proposed for PCB 118. Haag-Gronlund *et al.* (1997) concluded that co-planar *mono-ortho* PCBs, such as PCB 118, could act as tumor promoters by enhancing the growth of initiated cells into preneoplastic lesions.

Following a similar experimental design, Bager *et al*. (1995) treated female Sprague-Dawley rats with either 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) or 3,3',4,4',5-pentachlorobiphenyl (PCB 126) five weeks after a partial hepatectomy and initiation with the administration of nitrosodiethylamine. The test substances were administered by weekly subcutaneous injection for 20 weeks. Dosage of tested chemicals were 5000 + 1 and 3.16 + 10 µg/kg body weight/week for PCB 153 and PCB 126, respectively. A binary mixture of PCB 153 and PCB 126 was also tested with dosage of 5000 + 1, 5000 + 3.16 and 5000 + 10 µg/kg/week, respectively. Combined administration of both PCBs elicited a more than additive interaction on the formation of gamma-glutamyl-transpeptidase-positive hepatic foci. In addition, co-exposure to PCB 153 and PCB 126 caused a dose-dependent reduction in PCB 153-induced hepatic CYP2B1/2B2. The authors concluded that PCB 126 and PCB 153 can promote altered liver foci growth. They also suggested that the more than additive interaction between PCB 153 and PCB 126 could be due to PCB tested at greater than environmentally relevant levels and/or to different mechanistic pathways.

In contrast, Dean *et al*. (2002) described antagonistic interactions between 3,3',4,4',5-pentazchlorinated biphenyl (PCB 126), a co-planar PCB, and 2,2',4,4',5,5'-hexachlorinated biphenyl (PCB 153), a *di-ortho* PCB, in the promotion of liver carcinogenicity in Fischer 344 rats. Using a medium term (8-week) bioassay for promoters of hepatocarcinogenesis, Dean *et al* (2002) monitored the placental form of glutathione-S-transferase-positive (GST-P+) liver cell foci as preneoplastic markers in female Fischer 344 rats. Animals were treated with an initiator (diethylnitrosamine) and 21 days later partially hepatectomized. Fourteen days after initial DEN administration, animals were exposed by gavage to test chemicals three times weekly through the remainder of the 8-week study. PCB 126 and PCB 153 were administered at a dosage of 0.1, 1.0 and 10, and 10, 100, 1000, 5000, and 10000 µg/kg body weight, respectively. Combined exposure consisted of 0.1 + 10, 1 + 100, 10 + 1000, 10 + 5000, and 10 + 10000 µg/kg PCB 126 and PCB 153, respectively. Results from this experiment demonstrated a dose-dependent increase in liver and adipose tissue concentrations of PCB 126 and PCB 153. Hepatic PCB 153 levels were significantly increased ($p < 0.01$) after combined exposure to PCB 126 and PCB 153. A significant ($p < 0.01$) dose-dependent increase in GST-P+ foci area and number was observed in PCB 126- and PCB 153-treated animals. However, PCB 153 exhibited promotive activity of hepatic foci formation, but at levels 1000 to 5000 × that of PCB 126. Combined exposure to PCB 126 and PCB 153 resulted in an antagonistic response of GST-P+ focus formation ($p < 0.001$) for both foci area and number. A less than additive effect was observed at all 5 PCB 126/PCB 153 dose combinations. Even the lowest combination dosage of 0.1 + 10 µg/kg of PCB 126 and PCB 153, respectively, elicited an antagonistic response based on the formation of liver foci. This is particularly interesting since these doses approach environmentally relevant levels. The antagonistic hepatic tumor promotional response seen after treatment with a combination of PCB 126 and PCB 153 was partly attributed to toxicokinetic interactions. One possible interaction of the PCB153/PCB 126 mixture could be the competitive inhibition of PCB 126 binding to the *Ah* receptor by PCB 153 which has a weak efficacy and a weak affinity for the *Ah* receptor.

Nevertheless, a PCB 153/PCB 126 combination ratio of 1000 could contain enough PCB 153 to inhibit PCB 126 binding to the *Ah* receptor and thus elicit an antagonistic effect. It is probable that the specific congeners in the mixture, the dose level of the individual congeners, route of administration, duration of exposure, and species and strain of animal affect such toxicokinetic interactions (Dean *et al.*, 2002). PCB 153 tumor promotive activity was attributed to a different mechanistic pathway than the *Ah* receptor-mediated pathway responsible for the mode of toxicity demonstrated by PCB 126.

The liver tumor promotive activity of PCB 126 was compared to that of TCDD (2,3,7,8-tetrachloro-*p*-dioxin) by Hemming *et al.* (1995) using an initiation/promotion experimental design. Female Sprague-Dawley rats were partially hepatectomized and initiated by the administration of nitrosodiethylamine. Five weeks later, the promotion treatment started with a weekly administration of PCB 126, TCDD or a mixture of the two substances. The promotion treatment continued for 20 weeks. The results of this study, based on increased development of gamma-glutamyl-transpeptidase-positive altered hepatic foci, indicated that PCB 126 had a liver tumor promotive activity of approximately 10% of that elicited by TCDD. Thus, a relative potency of 0.1 was estimated for PCB 126 based on its relative tumor promotive activity in comparison to that of TCDD. This value is identical to the TEF_{WHO-97} value for PCB 126 used in the WHO-97 TEF scheme (van den Berg *et al.*, 1997). Treatment to a binary mixture of PCB 126 and TCDD elicited an additive response for tumor promotive effects. These results support the additivity assumption of the TEF scheme for the tumor promotive activity of TCDD and dioxin-like compounds. Therefore, these results suggest that PCB 126 and TCDD elicit their liver tumor promotive effects through the *Ah* receptor-mediated pathway.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Several studies (Kimbrough *et al.*, 1975; Schaeffer *et al.*, 1984; Norback and Weltman, 1985; Brunner *et al.*, 1996) have demonstrated increased liver tumor incidence in rats exposed to PCB mixtures. The mixtures found to induce liver tumors range in chlorine content from 60% (Aroclor 1260; (Norback and Weltman, 1985; Brunner *et al.*, 1996)), through 54% (Brunner *et al.*, 1996), to 41% (Brunner *et al.*, 1996). Female Sprague-Dawley rat liver tumor incidence data for Aroclor 1260 from the study by Norback and Weltman (1985) were chosen by US EPA as the basis of a cancer potency value for "high risk and persistence" PCB exposures. Female Sprague-Dawley rat liver tumor incidence data for Aroclor 1242 and 1016 from the study by Brunner *et al.* (1996) were chosen by US EPA as the basis of cancer potency values for "low risk and persistence" and "lowest risk and persistence" PCB exposures, respectively.

However, although PCB in many cases enters the environment as commercial formulations containing a relatively defined mixture of specific PCB congeners, the accumulation and retention of specific PCB congeners in various environmental matrices, wildlife, and humans do not directly reflect the PCB profile of the commercial mixtures. It is therefore important to consider the biological fate and activity of individual PCB congeners when assessing the risk that PCBs pose to human health (ATSDR, 2000). For cancer risk assessment, PCB toxicity is believed to be

elicited through the *Ah* receptor-mediated pathway (dioxin-like PCBs). Thus, the TEF_{WHO-97} methodology (Appendix A) can be used to sum the cancer risk associated with exposure to dioxin-like PCB congeners, chlorinated dioxins and chlorinated furans.

US EPA (1991) examined the toxic effects, including cancer, of four structural classes: dioxin-like PCBs, *ortho*-substituted PCBs, hydroxylated metabolites, and sulfonated metabolites. Different mechanisms of carcinogenicity were discussed for dioxin-like and other PCBs. It was concluded that congener toxicity could not be characterized by chlorine content alone. Chlorine content was formerly regarded by some scientists as correlated with cancer risk. Recently, however, Aroclor 1254 was found to be a more potent liver tumor inducer than Aroclor 1260, which was only slightly more potent than Aroclor 1242 (Brunner *et al*., 1996). This suggests that both the number and position of chlorines are a more useful indicator of cancer potency than total chlorine content. Accordingly, this difference can also partly be explained by the greater proportion of PCB 126 congener found in Aroclor 1254 (0.02 % in one of the tested lots) compared to no detectable level of PCB 126 in Aroclor 1260. The toxicological potency of PCB congener 126 in PCB mixtures is well documented and a TEF value of 0.1 (0.1 times the toxicity of TCDD) was attributed to this congener by the World Health Organization in 1997 (van den Berg *et al*., 1998).

Based on their predominant mechanism of toxicity, PCB congeners can be divided into two groups: dioxin-like and non dioxin-like PCBs. There is a large body of research indicating that dioxin-like (co-planar) PCB congeners elicit their toxicological effects through *Ah* receptor binding. *Ah* receptor-mediated PCB toxic effects include: induction of cytochrome P450 1A1/1A2/2B1, and phase II conjugation enzymes, body weight wasting, thymic atrophy and porphyria, and possibly cancer (ATSDR, 2000).

The WHO expert committee proposed the inclusion of dioxin-like PCBs in the TEF_{WHO-97} methodology (van den Berg *et al*., 1998) because of 1) similarities between the spectrum of effects in animals exposed to some PCB mixtures and congeners and that produced by 2,3,7,8-TCDD (Ah receptor-mediated pathway) and 2) the presence of a relationship between the binding affinities of co-planar PCBs for the *Ah* receptor and their potency in producing health effects in rodents such as body weight wasting and inhibition of immunological responses to sheep red blood cells (SRBCs) (ATSDR, 2000; Harper et al., 1993; Safe 1990, 1994). A description of the TEF methodology and its use is provided in Appendix A of this document.

Methodology

Use of the TEF Methodology to Calculate Cancer Risk

Humans are exposed to complex and varying environmental mixtures containing PCBs, PCDDs and PCDFs. However, limited toxicological data are available for these complex mixtures and many of their components (ATSDR 1998; 2000; Safe 1990, 1994; van den Berg *et al*. 1998). Therefore, toxicological equivalents (TEQs) of dioxin-like PCBs in the tested environmental mixture are added up to TEQs for PCDDs and PCDFs using the TEF_{WHO-97} scheme (Appendix A). A cancer risk factor can then be estimated from the product of the calculated total TEQs of the mixture and the risk factor of TCDD (3.8 E+1 $(\mu g/m^3)^{-1}$). This methodology shall be used when

concentration data are available for the PCB congeners listed in Section II (Health Assessment Values) of this summary (also listed in Appendix A of this document).

Use of PCB Mixture Unit Risk Factors for the Calculation of Cancer Risk

Lack of PCB speciation data would require the use of the previously developed unit risk factors for PCB mixtures in cancer risk assessments.

The September 1996 US EPA document "PCBs: Cancer Dose-Response Assessment and Application to Environmental Mixtures" (US EPA, 1996a) differed from the prior US EPA PCB risk assessment (US EPA, 1988) which was also used for Proposition 65 purposes in that it included data from a new study of rats fed diets containing Aroclor 1260, 1254, 1242, or 1016 which found statistically significant, dose-related, increased incidences of liver tumors from each mixture (Brunner *et al.*, 1996). Earlier studies used in the previous US EPA IRIS listing for PCBs found high, statistically significant incidences of liver tumors in rats ingesting Aroclor 1260 or Clophen A 60 (Kimbrough *et al.*, 1975; Norback and Weltman, 1985; Schaeffer *et al.*, 1984) in addition to partial lifetime studies which found precancerous liver lesions in rats and mice ingesting PCB mixtures of high or low chlorine content. However, the studies used for the previous IRIS listing had no data available indicating that PCBs with chlorine contents of less than 60% induced frank tumors.

The new US EPA PCB risk assessment document also used dose scaling to humans using a factor based on the ¾ power of relative body weight. Cancer potency is described by an ED_{10} (estimated dose associated with 10 percent increased incidence) and its lower bound, LED_{10}. These measures are expressed as equivalent human doses. Formerly, upper-bound slopes were calculated by the linearized multistage procedure; these were reported as "q1*"s. The LED_{10} method and the linearized multistage procedure give similar upper-bound slopes; for example, for female rats fed Aroclor 1254, the LED_{10} method and the linearized multistage procedure give upper-bound slopes of 1.5 and 1.6 per mg/kg-d, respectively. These changes conform to the 1996 draft US EPA cancer risk assessment guidelines (US EPA, 1996b).

The range of potency values is summarized in Table 2. It is based primarily on the range for Aroclor 1260, 1254, 1242, and 1016 in female Sprague-Dawley rats (Brunner *et al.*, 1996), but considers the other available studies also.

Table 2. Range of human potency and slope estimates (from US EPA, 1996a)

	ED_{10} (mg/kg-d)	LED_{10}[a] (mg/kg-d)	Central slope[b] $(mg/kg\text{-}d)^{-1}$	Upper-bound slope[c] $(mg/kg\text{-}d)^{-1}$
Highest observed potency	0.046	0.086	1.2	2.2
Lowest observed potency	1.4	2.4	0.04	0.07

[a]95% lower bound on ED_{10}.
[b]Computed as $0.10/ED_{10}$ and rounded to one significant digit.
[c]Computed as $0.10/LED_{10}$ and rounded to one significant digit.

The new upper-bound slopes are lower than the previous estimate of 7.7 per mg/kg-d average lifetime exposure (U.S. EPA, 1988). The previous estimate was derived from female rats in the

Norback and Weltman (1985) study; the new estimate from the same study is 2.2 per mg/kg-d. This difference is attributable to three factors, each responsible for reducing the slope by approximately one-third: a rat liver tumor reevaluation (Moore *et al*., 1994), use of the new cross-species scaling factor (U.S. EPA, 1996b), and not using a time-weighted average dose. The difference between the highest observed new upper-bound slope (2.2 per mg/kg-d) and the lowest (0.07 per mg/kg-d) is entirely attributable to the availability of tests on several commercial mixtures (Brunner *et al*., 1996). This 30-fold range in potency reflects differences in commercial mixture composition, as reflected in Table 3.

Table 3. Typical composition (%) of some commercial Aroclor PCB mixtures (from US EPA, 1996a)

	1016	1242	1248	1254	1260
Mono-CBs	2	1	—	—	—
Di-CBs	19	13	1	—	—
Tri-CBs	57	45	21	1	—
Tetra-CBs	22	31	49	15	—
Penta-CBs	—	10	27	53	12
Hexa-CBs	—	—	2	26	42
Hepta-CBs	—	—	—	4	38
Octa-CBs	—	—	—	—	7
Nona-CBs	—	—	—	—	1
Deca-CB	—	—	—	—	—

US EPA designated three reference points for each range (see Table 4): a "high risk" point based on studies of Aroclor 1260 and 1254, which give the highest observed potencies; a "low risk" point, based on the study of Aroclor 1242; and a "lowest risk" point, based on the study of Aroclor 1016. The "high risk" point is used for exposure pathways associated with environmental processes that tend to increase risk, including early life exposure. The "low risk" point is used for exposure pathways resulting in mid-range risk. The "lowest risk" point is used in cases where congener or isomer analyses verify that congeners with more than four chlorines comprise less than one-half percent of total PCBs.

Table 4. Tiers of human potency and slope estimates for environmental mixtures (from US EPA, 1996)

HIGH RISK AND PERSISTENCE				
ED_{10}	LED_{10}	Central slope	Upper-bound slope	Criteria for use
0.086	0.067	1.0	2.0	Food chain exposure Sediment or soil ingestion Dust or aerosol inhalation Dermal exposure, if an absorption factor has been applied to reduce the external dose Presence of dioxin-like, tumor- promoting, or persistent congeners in other media Early-life exposure (all pathways and mixtures)
LOW RISK AND PERSISTENCE				
ED_{10}	LED_{10}	Central slope	Upper-bound slope	Criteria for use
0.38	0.27	0.3	0.4	Ingestion of water-soluble congeners Inhalation of evaporated congeners Dermal exposure, if no absorption factor has been applied to reduce the external dose
LOWEST RISK AND PERSISTENCE				
ED_{10}	LED_{10}	Central slope	Upper-bound slope	Criteria for use
2.4	1.4	0.04	0.07	Congener or isomer analyses verify that congeners with more than 4 chlorines comprise less than 1/2% of total PCBs

Less-than-lifetime exposure induced statistically significant increased incidences of liver tumors in female rats fed Aroclor 1260, 1254, and 1242 (Brunner *et al.*, 1996). This result was most pronounced for Aroclor 1260, where tumor incidences at the highest dose were higher for a 12-month exposure than for a 24-month lifetime exposure. Only Aroclor 1016 showed no significant increases from less-than-lifetime exposure. The earlier less-than-lifetime studies in rats and mice suggest that less-than-lifetime exposure can quickly induce high incidences of early stages of tumor development (Kimbrough *et al.*, 1972; Ito *et al.*, 1973, 1974). With further exposure, these can progress to malignancy (Kimbrough *et al.*, 1975; Norback and Weltman, 1985). Tumor incidences from less-than-lifetime exposure were sometimes lower (Kimbrough and Linder, 1974), and sometimes similar (Rao and Banerji, 1988), to those from full lifetime exposure to PCBs.

Infants can be highly exposed to PCBs during pregnancy and lactation (Dewailly *et al.*, 1991, 1994). The accumulation of PCBs in human adipose tissue creates a store for subsequent release of PCBs into the bloodstream and then into the fetal circulation. During the postpartum period, PCBs are mobilized from adipose stores, transferred into human milk, and delivered to the neonate via nursing (Dewailly *et al.*, 1991). This pathway may account for a substantial fraction of neonatal exposure to dioxin-like and other PCBs.

Additionally, normal fetal development depends on the timing and rate of release of T3 and T4. Some evidence indicates that PCBs can alter normal T3 and T4 metabolism, thereby disturbing thyroid function and provoking secondary impacts on organogenesis during development. Any estrogenic/anti-estrogenic, androgenic/anti-androgenic, or other hormonal activity of PCB mixtures has the possibility of altering the development of reproductive organs or the urogenital tract, potentially causing cancer or other adverse effects through a mechanism different from those causing liver cancer (US EPA, 1996b).

Few studies, however, have investigated early-life sensitivity. In human infants, glucuronidative mechanisms are not fully developed; additionally, some breast-fed infants experience an inhibition of glucuronyl transferase activity, further reducing PCB metabolism and elimination (Calabrese and Sorenson, 1977). In animals, Aroclor 1260 induced high incidences of liver tumors when fed to 5-week-old rats for a short time (Rao and Banerji, 1988). On the other hand, acute perinatal dosing with Aroclor 1254 promoted nitrosamine-initiated lung and liver tumors in mice but did not induce cancer in the offspring when administered alone (Anderson *et al.*, 1983, 1986, 1994). A study of polybrominated biphenyls (PBBs) found that perinatal exposure enhanced susceptibility to liver tumors for female rats also exposed as adults and increased the incidence of liver tumors in male and female mice not further exposed as adults (NTP, 1993). Because of the potential magnitude of early-life exposures, the possibility of greater perinatal sensitivity, and the likelihood of thyroid and hormone-dependent development perturbation, it is reasonable to conclude that early-life exposures may be associated with increased risks; this would indicate using the "high-risk" potency estimates for early-life exposure.

US EPA has listed three different cancer potency values for PCBs; the choice of which factor to use depends on the physical form of the PCBs and the expected route of exposure. However, US EPA states that the "high risk and persistence" factor (2.2 $(mg/kg\text{-}day)^{-1}$) should be used when an "early-life exposure (all pathways and mixtures)" exposure scenario is expected. Since this is the most likely exposure scenario expected for Hot Spots emissions, this is the appropriate cancer potency factor to list for PCBs in the Hot Spots cancer document, with the exception of PCB emissions in cases where 1) an early-life exposure scenario is not expected, and ingestion of water-soluble congeners, inhalation of evaporated congeners or dermal exposure (if no absorption factor has been applied to reduce the external dose) is anticipated - “low risk” category, or 2) congener or isomer analyses verify that congeners with more than four chlorines comprise less than one-half percent of total PCBs - "lowest risk and persistence" category. For these cases, this would support use of the “low risk” or "lowest risk and persistence" cancer potency value (0.4 $(mg/kg\text{-}day)^{-1}$ and 0.07 $(mg/kg\text{-}day)^{-1}$, respectively).

Inhalation unit risk factors of 5.7 E-4 $(\mu g/m^3)^{-1}$ ("high risk and persistence"), 1.1 E-4 $(\mu g/m^3)^{-1}$ ("low risk") and 2.0 E-5 $(\mu g/m^3)^{-1}$ ("lowest risk and persistence") were calculated from the cancer potency values 2.0 $(mg/kg\text{-}day)^{-1}$, 0.4 $(mg/kg\text{-}day)^{-1}$ and 0.07 $(mg/kg\text{-}day)^{-1}$, respectively, using the default parameters of 70 kg human body weight and 20 m^3/day breathing rate.

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POTASSIUM BROMATE

CAS No: 7758-01-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	167.01
Boiling point	not available
Melting point	434 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 6.8 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.4 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 4.9 E-1 $(mg/kg\text{-}day)^{-1}$
[Male rat kidney tumor data (Kurokawa *et al*., 1983), contained in Gold *et al.* (1987) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of potassium bromate in humans are known to exist.

Animal Studies

Male and female Wistar rats (90/sex/group) (Fisher *et al*., 1979) and "Theiller's Original" strain mice (60/sex/group) (Ginocchio *et al*., 1979) were fed diets consisting of 79% bread crumbs made from untreated flour, or flour containing 50 mg/kg or 75 mg/kg potassium bromate. Mice and rats were fed treated diet for 80 and 104 weeks, respectively. No significant increase in tumor induction was reported; however, IARC (1986) noted that bromates are substantially degraded during bread baking.

Male and female Fischer 344 (F344) rats were exposed to 0, 250 or 500 mg/l potassium bromate in drinking water for 100 weeks (Kurokawa *et al*., 1983). Group sizes were 52-53/sex/group. Total doses were 9.6 and 21.3 mg/kg body weight for low and high dose males, respectively, and 9.6 and 19.6 mg/kg body weight for low and high dose females, respectively. Significant increases in the incidences of renal andenomas and adenocarcinomas were noted in both males and females; significant increases were also noted in the incidence of peritoneal mesotheliomas in males, and of thyroid tumors in females. Tumor incidence data is listed in Table 1.

Table 1. Potassium bromate-induced tumor incidences in male and female Fisher 344 rats (Kurokawa *et al*., 1983)

Sex	Dose group	Average dose[1] (mg/kg-day)	Tumor type	Tumor incidence[2]
male	control	0	kidney tumors[3]	3/53
	low dose	12.4		32/53
	high dose	22.5		46/52
	control	0	peritoneal mesotheliomas	6/53
	low dose	12.4		17/52
	high dose	22.5		28/46
female	control	0	kidney tumors[3]	0/47
	low dose	14.2		28/50
	high dose	28.3		39/49
	control	0	thyroid tumors	3/52
	low dose	14.2		10/52
	high dose	28.3		12/52

1. Doses as reported by Gold *et al.* (1987).
2. Tumor incidences as reported by Gold *et al.* (1987).
3. Adenomas and adenocarcinomas

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al*. (1987) list the results from positive drinking water studies in male and female F344 rats (Kurokawa *et al*., 1983) and from negative feeding studies in male and female Wistar rats (Fisher *et al*., 1979) and "Theiller's Original" mice (Ginocchio *et al*., 1979). Male and female rats are of similar sensitivity. Cancer potency is based on results from Kurokawa *et al.* (1983). The dose-response data for renal adenomas and adenocarcinomas in male rats are listed in Table 1 and are the basis for the cancer potency for potassium bromate (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

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1,3-PROPANE SULTONE

CAS No: 1120-71-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	122.1
Boiling point	180°C at 30 mm Hg
Melting point	31°C
Vapor pressure	not available
Air concentration conversion	1 ppm = 5.01 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 6.9 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 2.4 E+0 $(mg/kg\text{-}day)^{-1}$
[Male rat cerebellar malignant glioma tumor data (Ulland *et al*., 1971; Weisburger *et al*., 1981), contained in Gold *et al*. database (1984), expedited Proposition 65 methodology (Cal/EPA, 1992)]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 1,3-propane sultone on humans are known to exist.

Animal Studies

Several studies exist on the potential carcinogenic effects of 1,3-propane sultone in animals. These studies have been reviewed by IARC (1974).

BD rats (12/group, sex unspecified) were given 30 mg/kg body weight 1,3-propane sultone as a 3% aqueous solution weekly by gavage (Druckrey *et al*., 1970). Four of 10 survivors developed tumors between days 248 and 377; tumors noted were 1 glial-mesodermal mixed tumor, 1 advential cell sarcoma of the brain, 1 nephroblastoma and 1 subcutaneous spindle cell sarcoma.

Local sarcomas resulting in mortality were induced in all of 18 BD rats given weekly subcutaneous injections of 15 mg/kg 1,3-propane sultone in water (total dose 225 mg/kg) between 208 and 387 days. Additionally, single subcutaneous injections of 30 or 100 mg/kg produced local sarcomas at the injection site resulting in mortality in 12/18 animals and 18/18 animals, respectively (Druckrey *et al*., 1970). In the same study, BD rats were given 1,3-propane sultone as a 1% solution in arachis oil by subcutaneous injection weekly at doses of 15 or 30 mg/kg. Mortality resulted from local sarcomas which developed (myosarcomas and fibrosarcomas) at the site of injection (7/12 and 11/11 rats in the low and high-dose groups, respectively) within 217-360 days (total dose up to 390 mg/kg) (Druckrey *et al*., 1968, 1970).

Weekly intravenous injections of a 2% solution of 1,3-propane sultone in water at doses of 10, 20 or 40 mg/kg (total doses 300, 570 and 560 mg/kg, respectively) were administered to groups of 10 BD rats (the 40 mg/kg group treatment was suspended after 16 weeks due to tail vein sclerosis) (Druckrey *et* al., 1970). Three animals in the 40 mg/kg group died of tumors after 280-410 days (sarcoma of the mediastinum with right lung and kidney metastases, glial-mesodermal mixed tumor of the brain, neurosarcoma); 2/12 and 3/8 animals respectively in the 10 and 20 mg/kg groups died of tumors (10 mg/kg group: ganglioneuroma, neurocytoma; 20 mg/kg group: nephroblastoma, ileocaecal carcinoma, glial-mesodermal mixed tumor of the brain and mammary carcinoma) after 381-492 days. In the same study, a single intravenous injection of 1,3-propane sultone (150 mg/kg) induced tumors at various sites resulting in mortality within 459 days in 10/32 BD rats. Single intravenous injections of 20 or 60 mg/kg 1,3-propane sultone administered to pregnant BD rats on gestation day 15 resulted in malignant neurogenic tumors in 3/25 offspring born to the 20 mg/kg group, and in malignant tumors in 4/14 offspring (2 neurogenic tumors, 1 pancreatic tumor, 1 ovarian tumor) born to the 60 mg/kg group.

Female ICR/Ha Swiss mice (30/group) given weekly subcutaneous injections of 0.3 mg 1,3-propane sultone in 50 µl distilled water developed tumors in 21/30 mice at the injection site (1 papilloma, 7 adenoacanthomas, 12 sarcomas, 1 undifferentiated carcinoma) within 63 weeks, compared to 0/30 controls after 78 weeks (Van Duuren *et al.*, 1971).

Male and female Charles River CD rats (26/sex/group) were exposed to an aqueous solution of 1,3-propane sultone by gavage twice weekly at doses of 28 mg/kg body weight for 60 weeks and 56 mg/kg for 32 weeks (Ulland *et al.*, 1971; Weisburger *et al.*, 1981). Control groups (32/sex) were also included; however, only 6 animals/sex were killed and necropsied at 61 weeks. Tumor types induced by 1,3-propane sultone are listed in Table 1.

Table 1: 1,3-propane sultone-induced tumor incidences in male and female CD rats

Exposure group (mg/kg)	28		56	
Sex	Male	Female	Male	Female
Tumor type				
Breast	1/26	7/26	1/26	13/26
Glioma	12/26	15/26	16/26	13/26
Ear duct	1/26	0/26	3/26	3/26
Leukemia	0/26	2/26	4/26	3/26
Intestinal adenocarcinoma	4/26	1/26	3/26	1/26
Miscellaneous	5/26	7/26	4/26	6/26

One female control died of a cerebral glioma after 33 weeks, and a pituitary chromophobe adenoma was discovered in a female control. No other control animal tumor incidences were reported.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The carcinogenicity bioassay using male and female Charles River CD rats exposed to 1,3-propane sultone by gavage (Ulland *et al.*, 1971; Weisburger *et al.*, 1981) demonstrated that 1,3-propane sultone induced tumors in both sexes at multiple sites; the sensitivity of both sexes was similar. The dose-response data for cerebellar malignant glioma incidence in male rats, the most sensitive site in males, was chosen as the basis for a cancer potency factor.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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PROPYLENE OXIDE

CAS No: 75-56-9

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	58.08
Boiling point	34.2°C
Melting point	-112°C
Vapor pressure	543 mm Hg @ 25°C
Air concentration conversion	1 ppm = 2.37 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 3.7 E-6 $(\mu g/m^3)^{-1}$
[Calculated by US EPA (1995) from male rat nasal cavity hemangioma/hemangio-sarcoma data (NTP,1985) using a linearized multistage procedure, extra risk.]

Oral Cancer Potency Factor: 2.4 E-1 $(mg/kg/day)^{-1}$
[Calculated by US EPA (1995) from female Sprague-Dawley rat forestomach squamous cell carcinoma tumor data (Dunkelberg, 1981), using a linearized multistage procedure, extra risk.]

III. CARCINOGENIC EFFECTS

Human Studies

Theiss *et al*. (1981) conducted a retrospective cohort study of 602 active and former employees who had worked for 6 months or more in one of 8 German alkylene oxide production plants. The workers had been exposed to alkylene oxides (propylene oxide and ethylene oxide) as well as to other chemicals such as dichloropropane and epichlorohydrin. No ambient propylene oxide concentrations were reported and the study included workers employed as early as 1928; propylene oxide production did not begin until 1959. No significant difference was observed between the observed and expected numbers of cancer deaths.

Animal Studies

Exposure to propylene oxide by gavage at dose levels of 0, 15 or 60 mg/kg twice weekly for 150 weeks has been shown to induce forestomach tumors (primarily squamous cell carcinomas) in female Sprague-Dawley rats (Dunkelberg, 1982). Female NMRI mice treated with 0.1, 0.3, 1.0 or 2.5 mg propylene oxide once a week for 95 weeks via subcutaneous injection demonstrated a dose-dependent increase in injection site tumors (mostly fibrosarcomas) (Dunkelberg, 1984). Subcutaneous injection of a total dose of 1500 mg/kg propylene oxide over 325 days in rats (sex and strain not specified) resulted in the induction of injection site sarcomas (8/12 and 3/12 rats

receiving propylene oxide in oil and water vehicle, respectively) (Walpole, 1958). However, the study did not include an appropriate control group.

F344 rats and B6C3F$_1$ mice (50/sex/dose) were exposed to 0, 200 or 400 ppm (0, 475 or 950 mg/m^3) of propylene oxide for 6 hours/day, 5 days/week for 102 weeks (NTP, 1985; Renne *et al*., 1986). In rats, positive trends were demonstrated for papillary adenomas of the nasal turbinate epithelium (males and females), thyroid gland C-cell adenomas or carcinomas (females) and keratoacanthomas (males). A significantly increased incidence of endometrial stromal polyps and sarcomas combined was noted for all doses. However, the NTP decided that only the nasal epithelial tumors were treatment-related because the other tumors were either relatively common (thyroid) or were of low incidence relative to historical controls. In mice, the low-dose group was killed due to an inadvertent overdose 19 weeks after the initial start date. New groups of low-dose mice of both sexes were started, but additional control groups were not included. Hemangiomas (males) and hemangiosarcomas (both sexes) were significantly increased at the high dose. One squamous cell carcinoma and one papilloma were observed in the nasal cavity of 2 high-dose males; nasal cavity adenocarcinomas were reported in 2 high-dose females. Although not statistically significant, historical controls have demonstrated an extremely low incidence of these tumor types. A significant dose-related trend and incidence of mammary gland adenocarcinomas was observed in high-dose females.

Cpb:WU Wistar rats (100/sex/group) were exposed to 0, 30, 100 or 300 ppm (0, 71, 238 or 713 mg/m^3) propylene oxide for 6 hours/day, 5 days/week for 123-124 weeks (Reuzel and Kuper, 1983; Kuper *et al*. , 1988). No nasal cavity tumors were observed; however, significant increases in degenerative changes and neoplasia of the olfactory and respiratory epithelium were noted in both sexes of all exposure groups. Significant increases were also found in mammary gland adenocarcinomas in high-dose females. Squamous-cell carcinomas of the nose, larynx/pharynx and trachea, and adenocarcinoma of the larynx/pharynx and lungs were reported in 5 high-dose males. Although not statistically significant, none of these tumor types were reported in control males.

Male F344 rats exposed to 0, 100 or 300 ppm propylene oxide for 7 hours/day, 5 days/week for 104 weeks displayed a significant increase in nasal epithelium hyperplasia in the high-dose group (Lynch *et al*., 1984). The incidence of adrenal pheochromocytomas was also increased.

V. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Studies by Dunkelberg (1981) and NTP (1985) demonstrated that oral and inhalation exposure, respectively, to propylene oxide can result in increased animal tumor incidence. In the study by Dunkelberg (1981), female Sprague-Dawley rats exposed to total average doses of 0, 2714 or 10,798 mg/kg-day propylene oxide for 150 weeks demonstrated a significant increase in forestomach squamous cell carcinoma tumor incidence (0/100, 2/50 and 19/50 for control, low-dose and high-dose animals, respectively). These data were used to calculate an oral cancer potency factor for propylene oxide. In the NTP carcinogenicity study (1985), F344 rats and B6C3F$_1$ mice (50/sex/dose) were exposed to 0, 200 or 400 ppm (0, 475 or 950 mg/m^3) of propylene

oxide for 6 hours/day, 5 days/week for 102 weeks. High-dose rats exhibited an increased incidence of nasal papillary adenomas (2/50 for males, 3/50 for females), suggesting a carcinogenic response. However, these increases were not significant when compared to controls (0/50 for both sexes), making them unsuitable for carcinogenicity risk estimation. The incidence of nasal cavity hemangiomas or hemangiosarcomas in mice was 10/50 and 5/50 in the high-dose males ($p = 0.001$) and females ($p = 0.028$), respectively. These data, from a study where adequate numbers of animals of both sexes were treated for a lifetime, demonstrate that inhalation exposure to propylene oxide results in respiratory tract carcinogenicity. The male rat hemangioma/hemangiosarcoma data was used as the basis for a inhalation unit risk factor.

Methodology

Oral Cancer Potency Factor

Transformed animal doses (0, 2.58 and 10.28 mg/kg/day) and human equivalent doses (0, 0.44 and 1.76 mg/kg-day) were calculated from the administered doses using a rat body weight of 0.35 kg, a human body weight of 70 kg, 1029 days as the length of the exposure, and 1050 days as the length of the experiment and animal lifespan. A human oral cancer potency factor of 2.4 E-1 $(mg/kg/day)^{-1}$ was calculated using the linearized multistage procedure developed by Kenneth Crump and adopted by US EPA (1980).

Inhalation Unit Risk Factor

Transformed animal doses (0, 55 and 110 mg/kg/day) were calculated from administered doses assuming 50% pulmonary absorption, 0.03 kg mouse body weight, 0.039 m^3/day as the daily inhalation volume for mice and an exposure duration and length of experiment of 103 weeks. The absorption factor is consistent with that observed for epichlorohydrin in rat respiratory tract (Stott and McKenna, 1984). Human equivalent doses were 0, 4.15 and 8.3 mg/kg/day. The transformed animal dose level was used to calculate an animal slope factor of 9.3E-4 $(mg/kg/day)^{-1}$ using the linearized multistage procedure developed by Kenneth Crump and adopted by US EPA (1980). A human unit risk factor of 3.7 E-6 $(\mu g/m^3)^{-1}$ was determined using an animal body weight of 0.03 kg, a human body weight of 70 kg and an animal lifetime of 103 weeks. US EPA has stated that the unit risk should not be used if the air concentration exceeds 3 mg/m^3, since above this concentration the unit risk may not be appropriate.

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1,1,2,2-TETRACHLOROETHANE

CAS No: 79-34-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	167.9
Boiling point	146.5ºC
Melting point	-44ºC
Vapor pressure	9 mm Hg @ 30ºC
Air concentration conversion	1 ppm = 6.87 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 5.8 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 2.0 E-1 $(mg/kg\text{-}day)^{-1}$
[Calculated by US EPA from NCI (1978) female mouse hepatocellular carcinoma tumor data using a linearized multistage procedure, extra risk]

III. CARCINOGENIC EFFECTS

Human Studies

Norman *et al*. (1981) studied Army personnel assigned to treat chemical warfare protective equipment with material dissolved in tetrachloroethane. Of 3859 workers assigned to this process, 1099 whites and 124 blacks had probable exposure to the solvent. No statistically significant excess cancer mortality was noted. Slight excesses were noted for leukemia (SMR = 272, based on 4 deaths) and cancer of the genital organs (SMR = 158, based on 3 deaths).

Animal Studies

Theiss *et al*. (1977) exposed groups of 20 male A/st mice to 1,1,2,2-tetrachloroethane in tricaprylin by intraperitoneal injection (3/week) at doses of 80 mg/kg body weight, 200 mg/kg or 400 mg/kg. All survivors (10, 15 and 5 at the three doses, respectively) were killed 24 weeks after the first injection. The average number of tumors/animal were not significantly increased in the treated mice (0.3, 0.5 and 1.0 at the three doses, respectively compared to 0.27 for controls). However, the poor survival of treated animals and inadequate length of observation made this study unusable for a determination of the carcinogenicity of 1,1,2,2-tetrachloroethane.

$B6C3F_1$ mice (50 male, 50 female) were treated with technical grade (90% pure) 1,1,2,2-tetrachloroethane in corn oil by gavage 5 days/week (NCI, 1978). Low-dose and high-dose mice initially received 100 and 200 mg/kg body weight/day, respectively. Doses were increased to 150 and 300 mg/kg respectively at 18 weeks, 200 and 400 mg/kg at 21 weeks and 150 and 300 mg/kg at 26 weeks. Total duration of exposure was 78 weeks. Animals were killed 12 weeks after

exposure termination. The low and high time-weighted average doses for males and females was 142 and 282 mg/kg/day, respectively. Control groups (20 male, 20 female) were left untreated or given corn oil alone for 78 weeks, and were then killed after 90 weeks. Only 1 high-dose male survived to 90 weeks, compared with 34% of the females. The incidence of hepatocellular carcinoma was positively correlated with dose level ($p < 0.001$) in both male and female mice; tumor incidence in males was 1/18 for vehicle-treated controls, 13/50 for the low-dose group and 44/49 for the high-dose group. The respective tumor incidence for females was 0/19, 30/48 and 43/47.

Osborne-Mendel rats (50 male, 50 female) were treated with technical grade 1,1,2,2-tetrachloroethane in corn oil by gavage 5 days/week (NCI, 1978). High-dose animals initially received 100 mg/kg body weight/day. In males, doses were increased to 130 mg/kg at 14 weeks, followed by 9 cycles of 4 weeks at 130 mg/kg followed by 1 week treatment-free starting at 32 weeks. In females, the dose was reduced at 25 weeks to 80 mg/kg, then followed at 32 weeks by the cyclic dosing protocol described for males (dose level 80 mg/kg). The duration of the cyclic dosing for both males and females was 45 weeks. Low-dose rats were initially exposed to 50 mg/kg/day. Doses were increased for males to 65 mg/kg at 14 weeks; doses were decreased for females to 40 mg/kg at 25 weeks. The total duration of exposure for both dose groups was 78 weeks, followed by 32 weeks without treatment. The low and high time-weighted average doses were 62 and 108 mg/kg/day for males and 43 and 76 mg/kg/day for females. Control groups (20 male, 20 female) were left untreated or given corn oil alone for 78 weeks; all surviving control and exposed animals were killed at 110 weeks. No significant increases in tumor incidence for any tumor type were noted in exposed animals. However, 2 of 49 high-dose males developed hepatocellular carcinomas and another developed a neoplastic nodule, compared with 0/20 vehicle controls.

Detailed reviews of these studies have been performed by IARC (1979) and US EPA (1980).

V. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Data from the bioassay of 1,1,2,2-tetrachloroethane by NCI (1978) was selected as the basis of a cancer potency factor because it demonstrated a dose-responsive induction of carcinogenicity after exposure to 1,1,2,2-tetrachloroethane in both sexes of a susceptible species ($B6C3F_1$ mice). Tumor incidence data from the most sensitive sex was used (hepatocellular carcinomas in females).

Methodology

The linearized multistage procedure developed by Kenneth Crump and adopted by US EPA (1980) was used to calculate a slope factor of 2.0 E-1 $(mg/kg/day)^{-1}$ from the NCI (1978) female $B6C3F_1$ mouse hepatocellular carcinoma incidence data. Calculation of the unit risk from the slope factor assumed a body weight of 70 kg and an inspiration rate of 20 m^3/day. US EPA has stated that the unit risk should not be used if the air concentration exceeds 200 $\mu g/m^3$, since above this concentration the unit risk may not be appropriate.

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THIOACETAMIDE

CAS No: 62-55-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	75.14
Boiling point	not available
Melting point	113-114 °C
Vapor pressure	not available
Air concentration conversion	1 ppm = 3.1 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.7 E-3 $(\mu g/m^3)^{-1}$
Slope Factor: 6.1 E+0 $(mg/kg\text{-}day)^{-1}$
[Female mouse liver tumor data (Gothoskar *et al.*, 1970), contained in Gold *et al*. (1984) database, expedited Proposition 65 methodology (Cal/EPA, 1992), with cross-route extrapolation.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of thioacetamide in humans are known to exist.

Animal Studies

Male albino rats (10/group) were exposed to thioacetamide in the diet for 18 months (Fitzhugh and Nelson, 1948). Dietary thioacetamide levels were 0, 50, 100, 250, 500 or 1000 mg/kg diet. Animals exposed to 1000 mg/kg diet thioacetamide survived for less than one month; animals exposed to 250 or 500 mg/kg diet thioacetamide also had increased mortality. One animal in the 500 mg/kg diet exposure group developed a hepatocellular carcinoma (the number of surviving animals in this group was unspecified). One animal of the 6 survivors in the 50 and 100 mg/kg diet exposure groups developed a hepatocellular adenoma. No liver tumors were observed in the control animals.

Gupta (1955, 1956) exposed 150 male and female Wistar rats to 32 mg/kg diet thioacetamide in the diet for more than 23 weeks; an untreated control group of 50 animals was included in the study. Bile duct tumors (unspecified type) were observed in 18/36 animals killed between 9 and 23 weeks; no liver tumors were noted in the control animals. Liver tumor metastases to the ovaries were noted in 4/5 animals treated for 47 weeks or longer.

Male and female Swiss mice were fed diet containing 0.03% thioacetamide for 65 weeks (89 mice total); an untreated control group was included in the study (Gothoskar *et al*., 1970). Interim

sacrifices were performed at 6, 9 and 13 months. An increased incidence of liver tumors (hepatomas) was noted in both males and females. Tumor incidence data is listed in Table 1.

Table 1. Thioacetamide-induced hepatoma incidence in male and female Swiss mice (Gothoskar *et al.*, 1970)

Dose group	Average dose[1] (mg/kg-day)	Tumor incidence
male control	0	0/6
male treated	36	6/6
female control	0	0/6
female treated	39	6/7

1. Doses as reported by Gold *et al.* (1984).
2. Tumor incidences as reported by Gold *et al.* (1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Gold *et al.* (1984) list results from the study of thioacetamide by Gothoskar *et al.* (1970) in male and female Swiss mice. A total of 89 mice of both sexes were fed a diet containing 0.03% thioacetamide for 6, 9, 13 or 17 months. The group studied for 17 months consisted of 12 control mice (6 male and 6 female) and 13 treated mice (6 male and 7 female). Hepatomas were seen in all treated male mice, precluding estimation of the upper bound on potency in these animals. Females were slightly less sensitive; six of the seven dosed female mice developed hepatomas. Because this is the only dose-response data available in Gold *et al.*, the data for the females are used to derive potency (see Table 1). The value presented here may be an underestimate, but is the best value currently available (Cal/EPA, 1992).

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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Hazardous Substance Data Bank (HSDB) 1994. National Library of Medicine, Bethesda MD (CD-ROM Version). Micromedix, Inc., Denver CO, Edition 22.

TOLUENE DIISOCYANATE
CAS No: 26471-62-5

2,4-TOLUENE DIISOCYANATE
CAS No: 584-84-9

2,6-TOLUENE DIISOCYANATE
CAS No: 91-08-7

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	174.15
Boiling point	251°C at 760 mm Hg
Freezing point	22°C (pure toluene-2,4-diisocyanate (IARC, 1985); 7.2°C (pure toluene-2,6-diisocyanate (IARC, 1985)
Vapor pressure	0.01 mm Hg at 20°C
Air concentration conversion	1 ppm = 7.12 mg/m^3 (IARC, 1985)

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.1 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 3.9 E-2 $(mg/kg\text{-}day)^{-1}$
[Male rat subcutaneous fibroma/fibrosarcoma tumor data (NTP, 1986) contained in Gold *et* al. database (1990), expedited Proposition 65 methodology (Cal/EPA, 1992)]

III. CARCINOGENIC EFFECTS

Human Studies

A case report reviewed by IARC (1985) described a 47-year old nonsmoking spray painter with a lung adenocarcinoma who had been exposed to TDI and 4,4'-methylenediphenyl diisocyanate for 15 years and had a 10-year history of lung disease thought to be caused by isocyanate exposure (Mortillaro and Schiavon, 1982).

Hagmar *et al*. (1993) performed a cohort based case-referent study to assess any potential cancer risk associated with occupational exposure to TDI or methylene diphenyldiisocyanate. The subjects were 7023 workers employed during the period 1958 to 1987 in nine Swedish polyurethane foam manufacturing plants. Odds ratios were adjusted with respect to matching factors (age at risk, calendar year at risk, sex, and plant), calculated from a conditional logistic regression model. An association was found between intermediate/high exposure to isocyanates and prostate cancer (OR 2.96, 90% confidence interval (90% CI) 0.45-19.4); however, this association was not statistically significant. An association between isocyanate exposure and colon cancer (OR 3.25, 90% CI 0.5 - 21.3) was also not statistically significant. Study limitations included a small number of subjects (102 controls, 155 in the intermediate/high exposure group), a lack of quantitative exposure characterization, and a lack of control for potential confounding factors (tobacco smoking, etc.).

Sorahan and Pope (1993) conducted a historical prospective cohort study in order to determine specific mortality and site specific cancer morbidity among workers employed in factories that produce polyurethane foams, and to determine if any part of the experience may be due to occupation, and in particular to exposure to diisocyanates. The subjects were 8288 male and female production employees from 11 factories in England and Wales with some employment in the period 1958-79, and with a minimum period of employment of six months.

Standardized mortality ratios (SMRs) for all causes and all neoplasms were 97 (expected deaths (Exp) 844, observed deaths (Obs) 816) and 88 (Exp 251, Obs 221) respectively, compared to the general population of England and Wales. The exposed women demonstrated statistically significant increased incidences of death due to pancreatic cancer (Exp 2.2, Obs 6, SMR 271, 95% CI 100-595, $p < 0.05$) and lung cancer (Exp 9.1, Obs 16, SMR 176, 95% CI 100-285, $p < 0.05$). Similar cancer mortality increases were not noted for the male workers. Statistically significant increases in tumor incidences among women were found for cancers of the larynx (Exp 1.6, Obs 3, SRR 1024, 95% CI 105-755, $p < 0.05$) and kidney (Exp 0.9, Obs 4, SRR 449, 95% CI 122-1146, $p < 0.05$). Incident cancers of the lung and pancreas among women were also in excess, although these findings were not independent of the findings for mortality. Poison regression did not indicate that ever having been employed in jobs attracting either higher or lower exposure to isocyanates was a risk factor for the mentioned cancers. A nested case-control design was used to investigate any associations with nine other occupational exposures. No statistically significant association was found. This study did not include any corrections for cigarette smoking-induced cancer morbidity or mortality; therefore, cigarette smoking must be considered a potential confounder. Other study limitations included a lack of quantitative exposure characterization.

Schnorr *et al*. (1996) evaluated cancer mortality among United States workers exposed to TDI in the manufacture of polyurethane foam. This cohort mortality study included 4611 men and women employed in four polyurethane foam plants for at least three months between the late 1950s and 1987. The mortality experience of the cohort was compared with that of the general United States population. Industrial hygiene data indicated that air concentrations in 1984-5 were below the current United States standard of 0.04 mg/m^3 but exceeded the standard before 1980. Mortality from rectal cancer (SMR 2.78, 95% CI 0.57-8.13) and non-Hodgkin's lymphoma (SMR 1.54, 95% CI 0.42-3.95) were increased, but not significantly. There was one male breast cancer (SMR 18.52). However, breast cancer was not increased in women (SMR 0.74). No other cancer category had an increased number of deaths compared with the general population. Only non-Hodgkin's lymphoma and Hodgkin's disease showed a possible relation with time since first employment and no cancer death category showed a strong relation with duration of employment. The authors noted that the cohort was young, had few deaths and a short follow up, rendering the findings inconclusive.

Animal Studies

The National Toxicology Program (NTP) (1986) exposed male and female Fischer 344 344/N rats and B6C3F$_1$ mice (50/sex/exposure group) to commercial grade TDI (86% 2,4 isomer; 14% 2,6 isomer) in corn oil by gavage 5 days/week for 106 and 105 weeks, respectively. Rat exposure groups were 30 or 60 mg/kg for males and 60 or 120 mg/kg for females. Mouse exposure groups were 120 or 240 mg/kg body weight for males and 60 or 120 mg/kg for females. Vehicle control groups (50/sex/species) were included.

A dose-dependent reduction in survival occurred in treated rats; 36/50 (72%) controls, 14/50 (28%) low-dose and 8/50 (16%) high-dose males, and 36/50 (72%) controls, 19/50 (38%) low-dose and 6/50 (12%) high-dose females survived to study termination (108 weeks). A treatment-related induction of subcutaneous fibromas and fibrosarcomas was noted in males and females. The combined fibroma/fibrosarcoma incidence was 3/50 (6%) in controls, 6/50 (12%) in low-dose and 12/50 (24%) in high-dose males, and 2/50 (4%) in controls, 1/50 (2%) in low-dose and 5/50 (10%) in high-dose females. Fibromas and fibromasarcomas occurred in male and female rats with a statistically positive trend, and the incidence in both high-dose males and females was significantly greater than controls. Increased mammary gland tumor incidence in female rats was found to be significant in both the low- and high-dose groups by life table and incidental tumor analysis. The first mammary tumor was seen in an animal dying at week 84; the survival-adjusted mammary tumor incidences were 17/45 (38%) for controls, 25/36 (69%) for low-dose and 21/28 (75%) for high-dose animals. Increased incidences of pancreatic acinar cell adenomas with a statistically significant trend were observed in male rats; the incidence in the high-dose group was significantly greater than that in the controls. Pancreatic acinar nodular hyperplasia incidence was also increased in male rats in a dose-dependent manner (control, 0%; low-dose, 4%; high-dose, 8%). A statistically significant trend was noted for pancreatic islet cell adenoma incidence in both male and female rats; the incidences were significantly greater than those in the controls in both dose groups for females, and in the high-dose group for males. A significant dose-related increase in hepatic neoplastic nodule incidence in high-dose female rats was also noted.

Survival of high-dose male mice was reduced; 26/50 (52%) animals in this group were still alive at study termination (week 107) compared to 46/50 (92%) controls and 40/50 (80%) in the low-dose group. No statistically significant increase in tumor incidence was noted in treated male mice. A statistically significant positive trend was observed in the incidence of hemangiomas and hemangiosarcomas (in liver, ovaries or peritoneum), lymphomas, and hepatocellular adenomas and carcinomas in female mice. Overall hemangioma and hemangiosarcoma incidence was 0/50 in controls, 1/50 (2%) in the low-dose group and 5/50 (10%) in the high-dose group. Pairwise comparisons between the control and high-dose groups also indicated a significantly increased tumor incidence in the high-dose group. Combined hepatocellular adenoma and carcinoma incidence was 4/50 (8%) in controls, 5/50 (10%) in the low-dose group and 15/50 (30%) in the high-dose group. Tumor incidence in the high-dose group was significantly greater than in the controls. Overall lymphoma incidence was 10/50 (20%) in controls, 17/50 (34%) in the low-dose group, and 16/50 (32%) in the high-dose group; high-dose group tumor incidence was significantly greater than controls.

Male and female CD-1 mice and Sprague-Dawley CD rats (120/sex/group) were exposed to 0, 0.05 or 0.15 ppm (0, 0.36 or 1.07 mg/m^3) industrial-grade TDI (approximately 80% 2,4 isomer, 20% 2,6 isomer) by inhalation for 6 hours/day, 5 days/week, for 104 (mice), 108 (female rats) or 110 (male rats) weeks (Loeser, 1983). No treatment-induced increase in tumor incidence was noted in rats or mice. However, the rat histopathological evaluation was incomplete. Also, NTP (1986) noted that the exposure levels used corresponded to daily gavage doses of less than 1 mg/kg, and may not have been adequate doses to detect a potential carcinogenic response.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The NTP carcinogenicity study (1986) demonstrated that TDI induced tumors in several species (rats and mice), in both sexes in at least one of those species, at multiple sites. The male rat subcutaneous fibroma/fibrosarcoma tumor data was chosen as the basis of a cancer potency factor because it was the most sensitive endpoint in the most sensitive of the responsive species and sexes tested.

Methodology

Expedited Proposition 65 methodology (with cross-route extrapolation) was used to derive a cancer potency factor. A unit risk factor was then calculated by OEHHA/ATES from the cancer potency factor using a reference human body weight of 70 kg and an inspiration rate of 20 m^3/day.

V. REFERENCES

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1,1,2-TRICHLOROETHANE (VINYL TRICHLORIDE)

CAS No: 79-00-5

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB (1995) except as noted)

Molecular weight	133.42
Boiling point	113.8°C at 760 mm Hg
Melting point	-36.5°C
Vapor pressure	23 mm Hg at 25°C
Air concentration conversion	1 ppm = 5.55 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 1.6 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 5.7 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated by US EPA/IRIS (1980, 1994) from male mouse hepatocellular carcinoma tumor data (NCI, 1978), using a linearized multistage procedure, extra risk.]

III. CARCINOGENIC EFFECTS

Human Studies

No studies on the potential carcinogenic effects of 1,1,2-trichloroethane in humans are known to exist.

Animal Studies

The carcinogenicity of 1,1,2-trichloroethane in rats and mice was studied by the National Cancer Institute (NCI, 1978). Groups of 50 male and female $B6C3F_1$ mice (5 weeks of age) and Osborne-Mendel rats (6 weeks of age) were exposed to technical-grade 1,1,2-trichloroethane (92.7% pure, impurities unspecified) by gavage on 5 consecutive days/week for 78 weeks of a 90-91 week (mice) or 111-113 week (rats) experimental period.

Low and high dose mice received 150 and 300 mg/kg body weight, respectively, for 8 weeks, followed by 200 and 400 mg/kg, respectively, for 70 weeks, followed by 12-13 weeks without treatment, after which the experiment was terminated. The time-weighted average doses were 195 and 390 mg/kg, respectively. Untreated control and vehicle control groups were included (20 animals/sex/group).

Low and high dose rats received 35 and 70 mg/kg body weight, respectively, for 20 weeks, followed by 50 and 100 mg/kg, respectively, for 58 weeks, followed by 34-35 weeks without treatment, after which the experiment was terminated. The time-weighted average doses were 46 and 92 mg/kg, respectively. Untreated control and vehicle control groups were included (20 animals/sex/group).

No statistically significant increase in 1,1,2-trichloroethane-induced tumor incidence was noted in either male or female rats. Increases in hepatocellular carcinoma incidence were noted in all male and female mouse 1,1,2-trichloroethane-exposed treatment groups. The Fisher exact test comparing tumor incidences of dosed to control groups and the Cochran-Armitage test for positive dose-related trend indicated a highly significant association ($p < 0.001$) between hepatocellular carcinomas and 1,1,2-trichloroethane exposure. A positive dose-related association between 1,1,2-trichloroethane exposure and adrenal gland pheochromocytoma incidence in male and female mice was also indicated by the Cochran-Armitage test ($p = 0.003$ for males, $p < 0.001$ for females). Fisher exact tests confirmed these results for high dose female mice ($p = 0.006$) but not for other mouse treatment groups. Mouse tumor incidence data is listed in Table 1.

Table 1. 1,1,2-Trichloroethane-induced B6C3F$_1$ mouse tumor incidence data (NCI, 1978)

Treatment group[1] (mg/kg/day)	Time-weighted[2] average dose (mg/kg/day)	Human equivalent dose[2] (mg/kg/day)	Tumor incidence[3]
			hepatocellular carcinomas
males			
vehicle control	0	0	2/20
low dose	139	9.3	18/49
high dose	279	18.6	37/49
females			
vehicle control	0	0	0/20
low dose	139	9.3	16/48
high dose	279	18.6	40/45

1. Low and high doses: 150 and 300 mg/kg body weight, respectively, for 8 weeks, followed by 200 and 400 mg/kg, respectively, for 70 weeks, followed by 12-13 weeks without treatment.
2. Doses as reported by US EPA (1994).
3. Tumor incidences as reported by US EPA (1994).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

The NCI (1978) carcinogenicity bioassay of 1,1,2-trichloroethane indicated that 1,1,2-trichloroethane induced tumor formation in male and female B6C3F$_1$ mice. The cancer potency value is based on the dose-response data for hepatocellular carcinomas in male mice.

Methodology

Doses are time-weighted averages adjusted for frequency of exposure (5 of 7 days/week) (US EPA, 1994). Weight of the mice was assumed to be 0.033 kg. A linearized multistage procedure was used to calculate a slope factor of 5.7 E-2 $(mg/kg/day)^{-1}$ and a unit risk value of 1.6 E-5 $(\mu g/m^3)^{-1}$ from the NCI (1978) male mouse hepatocellular carcinoma incidence data. US EPA has stated that the unit risk should not be used if the air concentration exceeds 600 $\mu g/m^3$, since above this concentration the unit risk may not be appropriate.

V. REFERENCES

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U.S. Environmental Protection Agency 1994. Integrated Risk Assessment System: 1,1,2-Trichloroethane. Office of Health and Environmental Assessment, Washington, DC.

TRICHLOROETHYLENE

CAS No: 79-01-6

I. PHYSICAL AND CHEMICAL PROPERTIES (Fan, 1988)

Molecular weight	131.4
Boiling point	87.7° C
Melting point	-72.8° C
Vapor pressure	77 mm Hg @ 25° C
Air concentration conversion	1 ppm = 5.37 mg/m^3 @ 25° C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor:	2.0 E-6 ($\mu g/m^3$)$^{-1}$
Slope Factor:	7.0 E-3 (mg/kg-day)$^{-1}$

[Male mouse hepatocellular adenoma and carcinoma incidence, female mouse lung adenocarcinoma and malignant lymphoma incidence (Bell *et al.*, 1978; Henschler *et al.*, 1980; Fukada *et al.*, 1983; Maltoni *et al.*, 1986). Cancer unit risks calculated using a linearized multistage procedure, metabolized dose of TCE determined using a physiologically-based pharmacokinetic (PBPK) model, geometric mean of unit risks (CDHS, 1990a).]

III. CARCINOGENIC EFFECTS

Human Studies

Hardell *et al.* (1981) conducted a retrospective study of 169 men, aged 25 to 85 years in Umea, Sweden, between 1974 and 1978 for histologically confirmed malignant lymphoma. Sixty cases had Hodgkin's disease and 109 had non-Hodgkin's lymphoma. Seven cases and three controls reported high-grade exposure to TCE (odds ratio of 7.88). When compared with 162 cases and 335 controls that were not exposed to high-grade levels of TCE, but including those persons exposed to other chemicals and low-grade levels of TCE, the odds ratio dropped to 4.8, but remained significant ($p < 0.05$, chi-squared test). This study yielded an estimate of the relative risk of developing malignant lymphoma that is more than seven times greater for those who recall a high-grade exposure to TCE compared with those that report no exposure to phenoxy acids, chlorophenols, or organic solvents.

Imperial Chemical Industries conducted a retrospective study of 95 primary liver cancer cases diagnosed between 1951 and 1977 in England (Paddle, 1983). Paddle calculated the expected number of cases of primary liver cancer among workers from 1951 to 1977 to be about 0.3.

Axelson *et al.* (1978) who performed a mortality analysis of a cohort of workers occupationally exposed to TCE from 1955 to 1975, revealed 49 deaths from all causes (62 were expected using Swedish national death-rates). No significant elevated risk of tumor-related deaths was observed. The study size was probably too small to detect a positive association between exposure to TCE

and specific cancer deaths. Therefore, an upper bound on potential cancer risk of TCE to humans cannot be estimated on the basis of data from this study.

US EPA (1985) reported an historical cohort study by Malek *et al.* (1979) of 57 dry cleaners who used TCE as a cleaning solvent. Exposure to TCE was confirmed by urine analyses of the metabolite trichloroacetic acid (TCA). The follow-up time ranged from 5 to 50 years with a median greater than 20 years. The 6 cases of cancer observed were not significantly ($p < 0.05$) different from the number expected in the general population. The small size of the cohort severely limited the power of the study to detect a significant increase in cancer incidence.

Tola *et al.* (1980) established a cohort of 2117 workers (1148 men, 969 women) who had been occupationally exposed to TCE at some time between 1963 and 1976. The observed number of deaths (58) was lower than those expected (84.3). The percentage of deaths attributable to cancer among the workers (11/58 = 19%) was slightly greater than expected, but the difference was not significant ($p > 0.05$). The results from this study did not demonstrate an increased tumor incidence among workers exposed to TCE relative to that of the general Finnish population. Several limitations, such as unknown duration of exposure to TCE and exposure to other organic solvents, prevent a firm conclusion.

Shindell and Ulrich (1985) studied a cohort of 2,646 people who had worked at least 3 months between 1957 and 1983 at a facility that used TCE as a degreasing agent. The cohort showed a healthy worker effect (Standard Mortality Ratio = 0.79 for all causes of death) and much lower levels of heart disease and hypertension than the general population.

There are a number of cohort studies on workers exposed to TCE as a dry-cleaning solvent. Use of TCE as a dry-cleaning solvent began in the 1930's and waned in the 1960's (Waters *et al.*, 1977). Cohort studies of dry-cleaning workers have been reviewed in the past (IARC, 1979; Apfeldorf and Infante, 1981). The value of these studies is greatly limited by an undefined exposure to TCE and is confounded by exposure to other dry-cleaning agents such as tetrachloroethylene, carbon tetrachloride, and petroleum solvents.

Significant ($p < 0.05$) increases in the incidence of cancers of the lung, cervix, and skin contributed to an overall significant excess of cancer deaths among 330 deceased laundry and dry-cleaning workers (Blair *et al.*, 1979). This cohort also showed a slight increase in leukemia, liver, and kidney cancer, and a deficit of breast cancer compared to that expected. The authors warn that the cohort mortality pattern may reflect inherent biases, such as socioeconomic status and smoking, and should be interpreted cautiously.

Katz and Jowett (1981) reported a significant elevated risk for cancers of the kidney ($p < 0.05$) and genitals ($p < 0.01$) in a cohort of 671 deceased white female laundry and dry-cleaning workers. The cohort also exhibited smaller excesses of lymphosarcoma, bladder cancer, and skin cancer. An increase in cervical cancer disappeared when compared to low-wage controls.
A mortality analysis of a cohort of metal platers and polishers revealed significantly ($p < 0.05$) higher proportionate mortality ratios for esophageal and liver cancer deaths relative to a general white male population (Blair, 1980). The positive results were, however, confounded by

occupational exposure to known carcinogens, including chromium, nickel, and other metals, along with acids and other solvents.

Animal Studies

The National Cancer Institute (NCI, 1976) study was the first major long-term cancer bioassay of TCE. TCE was administered by gavage 5 days/week for 78 weeks to B6C3F$_1$ mice and Osborne-Mendel rats of both sexes (n = 50). The industrial grade of TCE used contained 1,2-epoxybutane (0.19%), ethyl acetate (0.04%), epichlorohydrin (0.09%), N-methylpyrrole (0.02%), and diisobutylene (0.03%) as stabilizers.

Male mice received initial daily doses of 2000 mg/kg of body weight in the high-dose group and 1000 mg/kg in the low-dose group. Dose levels were increased during the course of the study resulting in corresponding experimental time-weighted average (TWA) doses of 2339 and 1169 mg/kg. Initial doses to female mice were 1400 and 700 mg/kg, and the corresponding experimental TWA doses were 1739 and 869 mg/kg. For rats, high-dose groups of both sexes initially received 1300 mg/kg, but a lower TWA dose of 1097 mg/kg. The low-dose groups initially received 650 mg/kg, but a lower TWA dose of 549 mg/kg.

Higher incidences of hepatocellular carcinoma in mice were statistically significant in both high- (31/48, $p < 0.001$) and low-dose (26/50, $p = 0.004$) males and high-dose females (11/47, $p = 0.008$) relative to the matched controls.

In contrast to the positive results in the mouse study, analysis of tumor incidences in rats showed no significant difference in specific or total tumors between treated and control groups.

Questions have been raised about the possible impact of the epichlorohydrin (ECH) impurity in the TCE used. While it is possible that ECH contributed to the observed increased tumor incidence in TCE-exposed mice in the NCI (1976) bioassay, it is unlikely that ECH was responsible for all or most of the increased incidence observed. US EPA (1985) also noted that TCE-treated animals in the NCI (1976) experiments were housed in the same rooms as animals treated with other compounds but considered it unlikely that other compounds were responsible for the observed response.

To address the question of contaminant effects on the results of the 1976 NCI mouse study, the National Toxicology Program (NTP, 1983) repeated the carcinogenicity studies in B6C3F$_1$ mice and F344/N rats. The TCE contained no epichlorohydrin and was stabilized with 8 ppm diisopropylamine. Treated mice and high-dose rats received 1000 mg/kg TCE 5 days/week. Low-dose rats received 500 mg/kg TCE 5 days/week. The dosing period lasted 103 weeks.

The incidences of renal tubular-cell adenocarcinoma in male rats dosed with TCE were not significantly different from controls. However, high-dose male rats that survived until the end of the experiment exhibited a statistically significant higher incidence (3/16) of renal tubular-cell adenocarcinoma than the study controls (0/33) or F344/N male rats historical vehicle gavage controls (3/748). The NTP (1983) considers these results equivocal and "inadequate to evaluate the presence or absence of a carcinogenic response" of these rats to TCE. Significantly higher

incidences of hepatocellular carcinoma in dosed male mice (30/50, $p < 0.001$) and dosed female mice (13/49, $p < 0.05$) relative to those of their controls (8/48 and 2/48, respectively) confirmed the positive results of the 1976 NCI mouse study. Dosed female mice were also found to have a statistically significant ($p < 0.05$) increase in the incidence of hepatocellular adenomas (8/49) relative to that of the controls (2/48). This bioassay provided evidence that epichlorohydrin is not needed to induce hepatocarcinogenesis in $B6C3F_1$ mice.

In another NTP study (1988), 4 strains of rat (ACI, August, Marshall, and Osborne-Mendel) received high (1000 mg/kg) or low (500 mg/kg) daily doses of TCE in corn oil by gavage 5 days/week for 103 weeks. The TCE used contained no epichlorohydrin. Test groups consisted of 50 animals of each sex. An increased incidence of renal tubular cell tumors was observed in dosed animals, and an increased incidence of interstitial cell tumors of the testes was observed in dosed Marshall rats. Results of audits conducted in 1983 and 1984, revealed problems with the laboratory conducting the study (NTP, 1988) making interpretation of the bioassay results difficult.

Bell *et al.* (1978) reported the results of a study in which Charles River rats (120/group) and $B6C3F_1$ mice (140/group) were exposed to TCE vapor at concentrations of 100, 300, or 600 ppm for 6 hours/day, 5 days/week, for 104 weeks. Animals were sacrificed upon termination of treatment. The test chemical was greater than 99% pure but contained impurities such as diisobutylene, butylene oxide, ethyl acetate, N-methylpyrrole, and epichlorohydrin.

The incidences of hepatocellular carcinoma in male mice exposed to TCE at concentrations of 100 ppm (28/95), 300 ppm (31/100), and 600 ppm (43/97) were statistically significant ($p < 0.05$, $p = 0.03$, and $p < 0.001$, respectively) when compared to controls (18/99). The level of significance increased when the incidences of both hepatocellular carcinoma and hepatocellular adenoma combined in treated versus control mice are compared by the Fisher exact test. Female mice exposed to TCE at a concentration of 600 ppm exhibited a significant ($p < 0.05$) increase in the incidence of hepatocellular adenomas and hepatocellular carcinomas combined (17/99) relative to that of the controls (8/99). No statistically significant increase in the incidence of any other tumor type was observed among the treated rats. An audit revealed marked deficiencies and flaws in both the rat and mouse studies. According to US EPA (1985), the usefulness of these bioassays is limited by deficiencies in their conduct.

Van Duuren *et al.* (1979) exposed Ha/ICR male and female mice to purified TCE by 3 different routes: skin application, subcutaneous injection, and gavage. No significant increase in any tumor was observed in treated animals by any route of administration.

Henschler *et al.* (1980) exposed 3 species of rodents (Han:NMRI mice, and Syrian hamsters) to concentrations of pure amine-based TCE at 100 and 500 ppm for 6 hours/day, 5 days/week, for 78 weeks. Neither rats, hamsters, nor male mice had significantly increased tumor incidence. Dosed female mice, however, exhibited significantly ($p < 0.05$) higher incidences of malignant lymphoma relative to that of the controls which may be due to immunosuppression by TCE or some other nonspecific agent (US EPA, 1985).

In a study by Fukuda *et al.* (1983), female Sprague-Dawley rats and female ICR mice were exposed to concentrations of 50, 150, and 450 ppm of reagent grade TCE for 7 hours/day, 5 days/week, for

104 weeks (49-51 animals/test group). Chemical analysis revealed the test sample TCE, 99.824% pure, to contain impurities such as carbon tetrachloride, benzene, epichlorohydrin, and 1,l,2-trichloroethane in the vapor phase. The incidence of lung adenocarcinomas among mice in the 2 higher exposure groups (150 ppm, 8/50; 450 ppm, 7/46) was significantly ($p < 0.05$) higher than that of the controls (1/49), but the incidence was not dose-related. The incidence of total lung tumors (adenomas and adenocarcinomas combined) in exposed mice was not significantly different from that of the controls. Statistical analysis of the tumor incidences among rats showed no significant increases or trends.

Henschler *et al.* (1984) tested different samples of TCE with or without epichlorohydrin (ECH) and/or 1,2-epoxybutane, in groups of 50 5-week-old male or female ICR/Ha-Swiss mice. Treated animals received TCE, with or without epoxides, by corn oil gavage 5 days/week for 18 months. Males received 2400 mg/kg, while females received 1800 mg/kg. All doses were reduced after the 40th week giving an experimental TWA daily doses of 1900 mg/kg for males and 1400 mg/kg for females.

Mice dosed with pure TCE did not exhibit a statistically significant increase in the incidence of any tumor type. The administration of TCE with 0.8% ECH or both 0.25% ECH and 0.25% 1,2-epoxybutane was associated with a significant ($p < 0.05$) increase in forestomach papillomas or carcinomas in both sexes. These predicted increased risks from ECH more than account for the observed increased incidence of forestomach tumors cited above. Thus, results from this study support the hypothesis that ECH may be the proximate cause of increased tumor incidence observed in some studies of rodents exposed to ECH-stabilized TCE.

Maltoni *et al.* (1986) reported the results of a series of 8 TCE carcinogenicity experiments performed between 1976 and 1983, using mice and rats. In bioassay BT301, TCE was administered by stomach tube to Sprague-Dawley rats (30/sex/group) at dose levels of 50 or 250 mg/kg, 4 to 5 days/weeks, for 52 weeks. No significant increase in any tumor was observed in treated animals. This was probably due to the dosing period of 52 weeks which was less than a potential lifetime exposure.

Two short-term inhalation bioassays were conducted by Maltoni *et al.* (1986) with Sprague-Dawley rats (BT302) and Swiss mice (BT303). The animals were exposed to 100 or 600 ppm TCE for 7 hours/day, 5 day/weeks, for 8 weeks. No statistically significant effect was observed.

Bioassays BT304-bis were two similar long-term inhalation experiments whose results were combined and evaluated together. Sprague-Dawley rats were exposed to either 100, 300 or 600 ppm TCE for 7 hours/day, 5 days/week, for 104 weeks. A statistically significant, exposure-related increase in the incidence of Leydig cell tumors of the testes was observed in treated rats: 31/130 at 600 ppm, 30/130 at 300 ppm and 16/130 at 100 ppm, compared to 6/135 in the control group. Five of 260 rats exposed to 600 ppm TCE developed kidney adenocarcinomas that, although lacking statistical significance, must be considered biologically significant due to their rarity.

In experiment BT305, Swiss mice were exposed to TCE at a concentration of 100, 300 or 600 ppm for 7 hours/day, 5 days/week, for 78 weeks. Males exposed to the 2 higher levels showed statistically significant increases in the incidence of pulmonary tumors (27/90 at 600 ppm, 23/90

at 300 ppm) relative to that of the control group (11/90). Males exposed to 600 ppm TCE also had a higher frequency of hepatomas (13/90, $p < 0.05$) than that of controls (4/90). Females did not show any significant response to TCE exposure in this bioassay.

Bioassays BT306 and BT306-bis were both conducted with B6C3Fl mice under similar exposure conditions as above. A dose-related increase in the incidence of pulmonary tumors was observed in females but was significant ($p < 0.05$) only at 600 ppm (15/90) relative to the control group (4/90).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Based on their designation of "limited evidence" of carcinogenicity in animals and "inadequate evidence" of carcinogenicity in humans, IARC (1984) determined that TCE cannot be classified as to its carcinogenicity to humans. US EPA placed TCE in Group B2, a probable human carcinogen (US EPA, 1985). CDHS staff reviewed the literature and disagreed with IARC's conclusion. CDHS considers TCE to be carcinogenic and not to have a threshold for carcinogenicity (CDHS, 1990a).

A quantitative risk assessment for TCE was conducted by CDHS (1990a) using the dose-response data for carcinogenicity from four inhalation studies in mice (Bell *et al.*, 1978; Henschler *et al.*, 1980; Fukada *et al.*, 1983; Maltoni *et al.*, 1986).

Methodology

The metabolized dose for TCE for each of the studies evaluated was determined using a physiologically-based pharmacokinetic model (PBPK) and used for the calculation of carcinogenic potency. Because absorbed TCE is completely metabolized, metabolized dose mirrors applied dose. The metabolized dose of TCE was included because it takes into account uptake and distribution factors. The data obtained for uptake and distribution factors are in good agreement with experimental results obtained with human volunteers. Interspecies variation was accounted for by utilizing surface area scaling.

Carcinogenic responses in the inhalation studies included increased incidences of hepatocellular carcinoma and adenoma in male mice and increased incidences of lung adenocarcinomas and malignant lymphomas in female mice. Since most tumors were discovered at the time of sacrifice rather than at the time of their appearance, the GLOBAL79 and GLOBAL86 computer programs for the linearized multistage modes, without a time-to-tumor factor, were used for the low-dose risk assessment. The above adjustments to the animals' exposure results in a lifetime time-weighted average dose, either applied or metabolized. The range of 95% upper confidence limit (UCL) potency estimates (q_1*) obtained using the human equivalent applied and metabolized doses and the tumor incidences in the four inhalation studies notes above is 0.006 to 0.098 $(mg/kg\text{-}day)^{-1}$. Based on the same data, the individual risk for a 70-year lifetime exposure of a 70 kg person breathing 20 m^3 per day of ambient air containing 1 $\mu g/m^3$ (0.19 ppb) of TCE is 8×10^{-7} to 1×10^{-5}. A best estimate of the unit risk was obtained by taking the geometric mean of the unit

risks from the four inhalation studies. From the metabolized dose approach a unit risk of 2.0×10^{-6} $(\mu g/m^3)^{-1}$ was obtained, and from the applied dose a unit risk of 3.0×10^{-6} $(\mu g/m^3)^{-1}$ was obtained. CDHS (1990b) chose the cancer unit risk value of 2.0×10^{-6} $(\mu g/m^3)^{-1}$ calculated using the metabolized dose approach as the "best value" for TCE inhalation cancer unit risk.

Table 1: Dose-response data used by CDHS (1990a) in quantitative risk assessment for trichloroethylene exposure[1]

Study Species / sex Strain	Tumor Type	Daily experimental applied concentration	LTWA Metabolized Dose[2] (mg/kg-day)	Tumor Incidence[3]
Bell *et al.,* 1978	hepatocellular	0 ppm – 6 hr	0	20/99
Mice (male)	carcinoma or	100 ppm – 6 hr	42.3	35/95
$B6C3F_1$	adenoma	300 ppm – 6 hr	127	38/100
		600 ppm – 6 hr	254	53/97
Henschler *et al.*, 1980	malignant	0 ppm – 6 hr	0	9/29
Mice (female)	lymphoma	100 ppm – 6 hr	33.2	17/30
Han:NMRI		500 ppm – 6 hr	166	18/28
Fakuda *et al.*, 1983	lung	0 ppm – 7 hr	0	1/49
Mice (female)	adenocarcinoma	50 ppm – 7 hr	25.8	3/50
ICR		150 ppm – 7 hr	77.4	8/50
		450 ppm – 7 hr	232	7/46
Maltoni *et al.*, 1986	malignant	0 ppm – 7 hr	0	4/90
Mice (male)	hepatoma	100 ppm – 7 hr	35.3	2/90
Swiss		300 ppm – 7 hr	106	8/90
		600 ppm – 7 hr	212	13/90

[1]Source: CDHS (1990a).
[2]Lifetime, time-weighted-average metabolized dose.
[3]Tumor incidence denominator excludes animals dying before the occurrence of the first corresponding tumor type observed in the NCI (1976) and NTP (1983) studies. See CDHS (1990a) for more detail.

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2,4,6-TRICHLOROPHENOL

CAS No: 88-06-2

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	197.5
Boiling point	246°C
Melting point	69°C
Vapor pressure	0.012 mm Hg @ 25°C
Air concentration conversion	1 ppm = 8.00 mg/m^3 @ 25°C

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.0 E-5 $(\mu g/m^3)^{-1}$
Slope Factor: 7.0 E-2 $(mg/kg\text{-}day)^{-1}$
[Calculated from a cancer potency factor derived by RCHAS/OEHHA (CDHS, 1988)]

III. CARCINOGENIC EFFECTS

Human Studies

There are no human carcinogenicity studies available for 2,4,6-trichlorophenol.

Animal Studies

Innes *et al.* (1969) administered 100 mg/kg body weight 2,4,6-trichlorophenol by oral gavage to two F_1 generation strains of mice, B6C3F_1 (C57BL/6 × C3H/Anf) and B6AKF_1 (C57BL/6 × AKH) (18/sex/strain) from day 7 to 28 of life, without adjusting the initial dose to account for weight gain. After 28 days, 2,4,6-trichlorophenol was added to feed at 260 ppm for 74 weeks. Surviving animals were sacrificed at 78 weeks. Survival data and tumor incidence are reported in Table 1. Incidence of tumors of all types was found to be significantly increased only among treated B6C3F_1 males (p =0.004; Fisher's exact test). Incidence of reticulum cell tumors was also found to be significantly increased among pooled male and female treated B6C3F_1 animals (p =0.005). Pairwise comparison of summary incidence data shows an increased incidence of hepatomas among treated B6C3F_1 females (p =0.028) and reticulum cell sarcomas among treated B6C3F_1 males (p =0.021). Some results of this study were published separately (Bionetics Research Laboratories, 1968).

A lifetime feeding study of 2,4,6-trichlorophenol was conducted by the National Cancer Institute in two species, B6C3F_1 mice and F344 rats (NCI, 1979). Rats (50/sex/group) were treated with 5,000 or 10,000 ppm 2,4,6-trichlorophenol in feed for 106-107 weeks, plus a control group of 20 rats/sex given only standard feed. Tumor incidence data are presented in Table 2. Significant

increases in hematopoietic tumor (malignant lymphoma and monocytic leukemia) incidence was observed among males of both the low-dose ($p = 0.013$) and high-dose ($p = 0.002$) groups.

In the same study (NCI, 1979), $B6C3F_1$ mice were treated with 2,4,6-trichlorophenol in feed for 105 weeks. Male mice (50/group) were treated with 5,000 or 10,000 ppm 2,4,6-trichlorophenol. Female mice (50/group) were initially treated with diets containing 10,000 and 20,000 ppm 2,4,6-trichlorophenol; however, indications of reduced growth rate at 38 weeks led the investigators to reduce the level of compound to 2,500 and 5,000 ppm 2,4,6-trichlorophenol for the balance of the experiment, leading to time-weighted average concentrations of 5,214 and 10,428 ppm. The control group consisted of mice (20/sex) given the standard diet. Tumor incidence data are presented in Table 3. A significant increase in hepatoma incidence was observed among male mice in both treatment groups and among female mice in the high dose group ($p < 0.001$, Fisher's exact test).

In an effort to establish whether 2,4,6-trichlorophenol may be acting as a tumor initiator, Bull *et al.* (1986) treated female SENCAR mice (30/group) with 200 mg/kg 2,4,6-trichlorophenol by several routes of exposure, including gavage, intraperitoneal injection, subcutaneous injection, and dermal application, followed by dermal application of 1.0 μg 12-o-tetradecanoylphorbol-13-acetate three time per week for 20 weeks. No skin tumors were found when survivors were examined 52 weeks after first exposure.

Table 1. Survival and tumor incidence in male and female $B6AKF_1$ and $B6C3F_1$ mice treated with 2,4,6-trichlorophenol (Innes *et al.*, 1969).

		tumor incidence				
		$B6C3F_1$			$B6AKF_1$	
tumor type	treatment[1]	total	male	female	male	female
total tumors	treated	16/36	9/18[2]	7/18[3]	3/17	2/17
	control	30/166	22/79	8/87	16/90	7/82
hepatomas	treated	5/36[4]	3/18	2/18[5]	1/18	1/18
	control	17/166	17/79	0/87		
reticulum cell sarcomas	treated	6/36[6]	4/18[7]	2/18	0/18	1/18
	control	5/166	3/79	2/87		

[1] $B6C3F_1$ and $B6AKF_1$ mice were treated with 100 mg/kg body weight 2,4,6-trichlorophenol by oral gavage from day 7 to 28 of life, then fed diet containing 260 ppm 2,4,6-trichlorophenol for 74 weeks, at which time surviving animals were sacrificed.
[2] $p = 0.064$, Fisher's exact test.
[3] $p = 0.004$, Fisher's exact test.
[4] $p = 0.059$, Fisher's exact test.
[5] $p = 0.028$, pairwise comparison of summary incidence data.
[6] $p = 0.005$, Fisher's exact test.
[7] $p = 0.021$, pairwise comparison of summary incidence data.

Table 2. Tumor incidence in Fischer F344 rats treated with 2,4,6-trichlorophenol (NCI, 1979).

tumor type/ treatment[1]		tumor incidence male	tumor incidence female
total hemato-	control	4/20	3/20
poietic tumors	low-dose	25/50[2]	11/50
	high-dose	29/50[3]	13/50
malignant	control	1/20	0/20
lymphoma	low-dose	2/50	0/50
	high-dose	0/50	2/50
monocytic	control	3/20	3/20
leukemia	low-dose	23/50[4]	11/50
	high-dose	29/50[5]	11/50

[1] F344 rats were treated with diet containing 5,000 or 10,000 ppm 2,4,6-trichlorophenol for 106-107 weeks at which time animals were sacrificed.
[2] $p = 0.019$, Fisher's exact test.
[3] $p = 0.004$, Fisher's exact test.
[4] $p = 0.013$, Fisher's exact test.
[5] $p = 0.002$, Fisher's exact test

Table 3. Hepatoma incidence in $B6C3F_1$ mice treated with 2,4,6-trichlorophenol (NCI, 1979).

treatment[1]/hepatoma type		tumor incidence male	tumor incidence female
control	adenoma	3/20	1/20
	carcinoma	1/20	0/20
	total	4/20	1/20
low-dose	adenoma	22/49	12/50
	carcinoma	10/49	0/50
	total	32/49[2]	12/50[3]
high-dose	adenoma	32/47	17/48
	carcinoma	7/47	7/48
	total	39/47[2]	24/48[2]

[1] Male $B6C3F_1$ mice were treated with diet containing 5,000 or 10,000 ppm 2,4,6-trichlorophenol for 105 weeks, then sacrificed. Female $B6C3F_1$ mice were treated with diet containing 10,000 or 20,000 ppm 2,4,6-trichlorophenol for 38 weeks at which time diet concentrations were reduced to 2,500 or 5,000 ppm 2,4,6-trichlorophenol, respectively, until they were sacrificed at 105 weeks.
[2] $p < 0.001$, Fisher's exact test.
[3] $p = 0.059$, Fisher's exact test.

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Two studies, Innes *et al.* (1969) and NCI (1979), have been deemed adequate for the derivation of cancer potencies. Both demonstrate statistically significant increases in tumor incidence among 2,4,6-trichlorophenol exposed animal populations. Innes *et al.* (1969) show increased incidence of reticulum cell sarcomas in male $B6C3F_1$ mice and heptomas in female $B6C3F_1$ mice. The NCI (1979) study shows increased incidence of hepatomas in both male and female $B6C3F_1$ mice, and leukemia in male Fischer F344 rats. The US EPA estimate of cancer potency of 2,4,6-trichlorophenol derived from the rat study is lower than that for mice (US EPA, 1988 and below). Since selection of the potency value is made on the basis of the most sensitive species, site, and study in the absence of evidence that the data are not representative, tumor induction in $B6C3F_1$ mice has been chosen as the basis for derivation of a cancer potency value for 2,4,6-trichlorophenol.

Methodology

The multistage Doll-Armitage model polynomial was fit to tumor incidence data from Innes *et al.*(1969) and NCI (1979) (Armitage and Doll, 1954). Dosage estimates for the studies were based on food intake assumptions of 12% and 13% of body weight for male and female mice, respectively (Gold, 1984). In the NCI (1979) study, final dose values were calculated to be 1200 and 600 mg/kg-day for high- and low-dose females, and 1356 and 678 mg/kg-day for high- and low-dose males. In the Innes *et al.*(1969) study, dosage estimates were based on the method of Crouch to account for variation in dosing during the course of the experiment (Crouch, 1983). Dosage estimates were calculated to be 32.5 and 34.7 mg/kg-day for male and female mice, respectively. Using a multistage polynomial, the cancer potency was derived using the probability of dying with a tumor from a given dose and the background lifetime cancer incidence (Crump and Howe, 1984). The upper 95% confidence bound on the cancer potency was termed q_1^*. Estimates of q_1^* for tumor induction in $B6C3F_1$ mice are presented in Table 4.

Calculation of the cancer potency in animals (q_{animal}) can be made using q_1^* and the following relationship, where T is the natural lifespan of the animal (104 weeks) and T_e is the experimental duration (Innes *et al.*, T_e = 104 weeks; NCI, T_e = 78 weeks):

$$q_{animal} = q_1^* \times (T/T_e)^3$$

The resulting q_{animal} can be converted to human cancer potency (q_{human}) based on the following relationship, where bw_{animal} is the assumed body weight for the test species (Innes *et al.* (1969), bw_{animal} = 0.030 kg; NCI (1979), bw_{animal} = 0.04 kg-males and 0.035 kg-females) and bw_{human} is the assumed human body weight (70 kg):

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

Table 4. Derivation of cancer potencies from NCI (1979) and Innes *et al.*(1969).

study	tumor/group	q_1^* $(mg/kg\text{-}day)^{-1}$	q_{animal} $(mg/kg\text{-}day)^{-1}$	q_{human} $(mg/kg\text{-}day)^{-1}$	maximum likelihood estimate	LCB (95%)
NCI, 1979	hepatoma/ male	0.0017	12.1	0.021	0.016	0.004
	hepatoma/ female	0.0006	12.6	0.008	0.003	0
Innes, 1969	reticulum cell sarcoma/ male	0.035	0.035	0.47	0.2	0.05
	hepatoma/ female	0.021	0.021	0.28	0.11	0.03

LCB = Lower confidence bound.

The highest upper bound cancer potency for humans (q_{human}) was derived from the results of the Innes *et al.* (1969) study showing reticulum cell tumor induction in male $B6C3F_1$ mice. However, confidence in this value is reduced because the number of animals used in the study is small (18/group) and data were reported incompletely. Innes *et al.* (1969) and NCI (1979) both present data showing induction of hepatomas in female $B6C3F_1$ mice. However, lack of overlap between the 95% confidence bounds of the two potencies suggests there may be a greater sensitivity to this effect in the strain used by Innes *et al* (1969). Selection of a cancer potency value is made based on the most sensitive species, site, and study in the absence of evidence indicating the value is not representative (CDHS, 1985). On balance, the evidence favors neither the higher sensitivity of the Innes *et al.* (1969) study nor the high quality of the NCI (1979) study. For this reason, a method of Anderson (1983) was chosen for combining the results of these studies. The resulting cancer potency derived from the geometric mean of the four potencies shown in Table 4 is 0.07 $(mg/kg\text{-}day)^{-1}$.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 2.0 E-5 $(\mu g/m^3)^{-1}$.

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URETHANE

CAS No: 51-79-6

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1994)

Molecular weight	89.09
Boiling point	182-184°C
Melting point	48-50°C
Vapor pressure	0.36 mm Hg @ 25°C
Air concentration conversion	1 ppm = 3.64 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 2.9 E-4 $(\mu g/m^3)^{-1}$
Slope Factor: 1.0 E+0 $(mg/kg\text{-}day)^{-1}$
[Calculated from a potency factor derived by RCHAS/OEHHA (CDHS, 1989)]

III. CARCINOGENIC EFFECTS

Human Studies

There are no studies available directly linking urethane exposure to induction of cancer in humans. Urethane is, however, frequently present in alcoholic beverages, particularly brandy, whisky and wine, and IARC has recognized alcoholic beverages as carcinogenic to humans (IARC, 1988). Although other compounds present in alcoholic beverages may account for this effect, urethane may be a contributor to alcohol-related increases in cancer incidence.

Animal Studies

CDHS (1989) has identified nearly 200 studies which demonstrate the carcinogenicity of urethane in animals. Below are summaries of those determined to be most relevant in the establishment of the reference cancer potency value, with emphasis on studies performed by realistic routes of exposure and in multiple doses.

Pietra and Shubik (1960) exposed male and female Syrian golden hamsters (10/sex) to drinking water containing 0.2% urethane for life. Control animals (49 male and 14 female) received plain drinking water. Animals were autopsied at death. Among exposed animals, males showed increased incidence of dermal melanotic tumors (7/10 exposed, 1/49 control; $p < 0.01$, Fisher's exact test). Male and female animals showed an increased incidence of forestomach papillomas (males: 4/10 exposed, 0/49 control; females: 6/10 exposed, 0/14 control; $p < 0.01$). Other tumors noted in exposed animals but not control animals include single cases of thyroid adenoma and liver hemangiosarcoma in males, malignant lymphoma and bronchial adenoma in females.

Toth *et al.* (1961a) exposed Syrian golden hamsters (31 male; 30 female) to drinking water containing 0.2% urethane. Control groups of 54 male and 47 female received plain drinking water. Dosing began at 5 weeks and continued to 25 weeks at which point the drinking water urethane concentration was increased to 0.4%. At 40 weeks treatment was discontinued due to diarrhea among the animals. At 48 weeks, treatment with 0.4% urethane resumed, but was discontinued permanently at 50 weeks due to diarrhea. Survival was significantly decreased in exposed male and female hamsters. Among exposed animals, significant increases in incidence of dermal melanotic tumors (12/27 exposed males vs. 0/54 control males; 11/25 exposed females vs. 0/47 control females; $p \leq 10^{-6}$; Fisher's exact test), forestomach papillomas (22/27 exposed vs. 0/54 control, $p < 10^{-15}$, males; 18/25 exposed vs. 1/47 control; $p < 10^{-9}$, females) and carcinomas (3/27 exposed vs. 0/54 control; $p = 0.04$, males; 2/25 exposed vs. 0/47 control; $p = 0.1$, females), pulmonary adenomatosis (3/27 exposed vs. 0/54 control; $p = 0.04$, males), mammary tumors (3/25 exposed vs. 0/47 control; $p = 0.04$, females), hepatomas (3/27 exposed vs. 0/54 control; $p = 0.04$, males), and hepatic or splenic hemangiomas (5/27 exposed vs. 0/54 control; $p = 0.04$, males) were found.

Toth and Boreisha (1969) exposed Syrian golden hamsters (48 male and 52 female) to drinking water containing 0.1% urethane for life, beginning at 5 weeks of age. Control groups (100/sex) received plain drinking water. Survival was significantly decreased in exposed male and female animals. An increased incidence of dermal melanocytosis (26/49 exposed males vs. 1/88 control males; 25/41 exposed females vs. 0/79 control females; $p = 10^{-14}$; Fisher's exact test), forestomach papillomas (36/49 exposed vs. 6/88 control, $p = 10^{-15}$, males; 35/44 exposed vs. 2/84 control, $p = 10^{-20}$, females) and adenomatous polyps of the cecum (4/40 exposed vs. 0/79 control, $p = 0.01$, males; 7/33 exposed vs. 0/72 control, $p = 0.001$, females) was noted in both males and female animals. Among females an increased incidence of gall bladder papillomas, adrenal cortex carcinomas, thyroid carcinomas, ovarian carcinomas, vaginal carcinomas, and lung adenomatosis was observed ($p < 0.05$). Hemangiosarcoma incidence was increased in exposed males.

Tannenbaum *et al.* (1962) exposed Sprague-Dawley rats (15/group) to drinking water containing 0.1% urethane. Two "young" groups, one virgin females and the other males, were treated from age 7 weeks to 32 weeks. A third group of virgin females was treated for 14 weeks from age 32 weeks. Age and sex matched control groups of 15 each receiving plain drinking water were included in the study. Animals were autopsied at the time of natural death unless sacrificed when moribund. Survival was reduced in the treated groups. Incidence of Zymbal gland carcinoma was increased in treated "young" male rats (4/15 treated vs. 0/15 control, $p = 0.05$, Fisher's exact test) and female rats (3/15 treated vs. 0/15 control, $p = 0.11$). Control animals showed higher incidence of mammary tumors than treated animals, most likely because of the reduced survival of treated animals. Tumors noted among treated animals, but not control animals, include malignant lymphoma, sarcoma, and kidney tumors.

Schmähl *et al.* (1977) and Port (1976) report on exposure of rats and mice to urethane in drinking water. Tumor specific incidence data are reported by Port (1985). Male and female NMRI mice and Sprague-Dawley rats (40/sex/group) were exposed to urethane in drinking water from 8 weeks of age for their lifetime such that the daily dose rate was 0, 0.1, 0.5, 2.5, or 12.5 mg/kg body weight. Animals were given the appropriate dose in 20 ml drinking water. Animals were observed until their natural death. Among mice, significant increases in tumor incidence ($p < 0.1$ by Fisher's

exact test) were found for pulmonary adenoma in males (6/40, 9/40, 14/40 in the 0.5, 2.5, and 12.5 mg/kg dose groups, respectively vs. 0/40 controls), and pulmonary adenoma (12/40 treated with 12.5 mg/kg urethane vs. 5/40 controls), pulmonary carcinoma (5/40 treated with 12.5 mg/kg urethane vs. 0/40 controls), angiosarcoma of the liver (4/40 treated with 12.5 mg/kg urethane vs. 0/40 controls), and mammary carcinoma (4/40 treated with 12.5 mg/kg urethane vs. 0/40 controls) in females. For each of these tumor types, the trend toward increased incidence was found to be dose-related ($p < 0.01$ by Mantel-Haenszel trend test). Among female rats, a significant increase in the incidence of mammary carcinoma (1/40, 2/40, 9/40 in the 0.5, 2.5, and 12.5 mg/kg urethane dose groups, respectively vs. 0/40 in the 0.1 mg/kg urethane dose group, $p = 0.0011$) and the combined incidence of mammary adenoma and carcinoma was found (2/40, 3/40, 4/40, 13/40 in the 0.1, 0.5, 2.5, and 12,5 mg/kg urethane dose groups, respectively vs. 2/40 in the 0.1 mg/kg urethane dose group, $p = 0.0016$ by Fisher's exact test). The incidence data for the untreated control animals were lost. The trend was found to be dose-related ($p < 10^{-4}$ by Mantel-Haenszel trend test).

Klein *et al.* (1962) treated 7-8 day old $B6AF_1/J$ mice (C57BL/6 female × A/J male) with 2.8 or 5.5 mg urethane in 0.05 ml 0.1% dioctylester of sodium sulfosuccinic acid by oral gavage 3 times per week for 5 weeks. Control groups for the low-dose group included both males receiving vehicle only and males receiving no treatment. Males and females receiving no treatment served as a control for the high-dose group. Survivors of the treatment period comprised the study group and ranged from 39 to 57 animals. Survival in the treated groups was significantly lower than in controls. Among animals receiving the higher dose of urethane, treated males had a higher incidence of leukemias (32/42 treated vs. 0/38 vehicle controls, $p = 10^{-13}$; Fisher's exact test), lung adenomas (42/42 treated vs. 10/38 vehicle controls, $p = 10^{-12}$) and hepatoma (4/42 treated vs. 0/38 vehicle controls, $p = 0.07$) than animals receiving vehicle alone. Among animals receiving the lower dose of urethane, males showed a higher incidence of lung adenomas (40/41 treated vs. 3/40 untreated controls, $p = 10^{-17}$), hepatoma (23/41 treated vs. 0/40 untreated controls, $p = 10^{-8}$), and leukemias (19/41 treated vs. 0/40 untreated controls, $p = 10^{-6}$) relative to untreated control animals. In the same dose group, females showed a higher incidence of leukemias (16/40 treated vs. 1/57 controls, $p = 10^{-6}$) , lung adenomas (39/40 treated vs. 9/57 controls, $p = 10^{-6}$), hepatomas (5/40 treated vs. 0/57 controls, $p = 0.01$), and forestomach papillomas (3/40 treated vs. 0/57 controls, $p = 0.07$) relative to untreated control animals.

Della Porta *et al.*(1963a) exposed 5 groups of male and female CTM mice to drinking water containing 0.4% urethane in several exposure scenarios ranging from a total exposure time of 5 to 15 days. Effective group size was the number of survivors at 25 weeks for treated animals (range: 30-83 mice) and survivors at 45 weeks for controls (88 males and 99 females). Among all exposed animals there was a significant increase in the incidence of lung adenomas over control animals ($p < 10^{-8}$; Fisher's exact test). Among all exposed female mice, the incidence of lymphosarcoma was increased over controls ($p < 0.05$). Among all exposed male mice, the incidence of reticulosarcoma was increased over controls ($p < 0.04$). Other tumor types showing some significant increase ($p < 0.05$) in incidence over controls in some but not all exposure scenarios include lymphosarcomas and Harderian gland adenomas in male mice, and mammary gland adenocarcinoma, hepatoma, and Harderian gland adenomas in female mice.

Table 1. Tumor incidence in CTM mice exposed to drinking water containing 0.4% urethane (Della Porta *et al.*, 1963a).

	A*		B		C		D		E		control	
tumor type	male	female	male	female	male	female	male	female	male	female	male	female
lung adenoma	29/36	53/63	42/56	53/83	58/71	51/68	19/45	26/48	25/30	30/39	2/88	7/99
lympho-sarcoma	12/36	17/63	14/56	13/83	14/71	15/68	8/45	7/48	2/30	10/39	4/88	5/99
reticulo-sarcoma	3/36	4/63	6/56	1/83	8/71	6/68	3/45	2/48	3/30	2/39	0/88	7/99

*Exposure scenarios: A - two 10 day exposures, separated by 10 days; B - one 10 day exposure; C - three 5 day exposures, separated by 10 days; D - two 5 day exposures, separated by 10 days; E - one 5 day exposure.

Della Porta *et al.* (1963b) conducted another study similar to that described above, but exposed CTM mice (75 male and 108 female) to drinking water containing 0.4% urethane for 10 days or two 10-day periods separated by 10 days. Control animals received plain drinking water (130 males and 120 females). Surviving animals were sacrificed at 75 weeks. The effective group size was considered the size of the group at the time of appearance of the first malignant lymphoma. Among male and female mice in both treatment groups, the incidence of malignant lymphoma was elevated over control animals (15/40 in the 20-day treatment group, 19/61 in the 10-day treatment group, vs. 4/103 controls, $p < 10^{-4}$ by Fisher's exact test). Among female mice, the incidence of mammary gland tumors was increased over controls (21/70 in the 20-day treatment group vs. 15/108 controls,$p < 0.008$; 34/83 in the 10-day treatment group vs. 15/108 controls, $p = 10^{-4}$). Other tumors observed included lung adenomas, mammary carcinomas, liver angiosarcomas, hepatomas, Harderian gland adenomas, and forestomach and skin papillomas.

Della Porta *et al.* (1967) exposed four inbred mouse strains (C57BL, C3H, C3Hf, SWR) and one hybrid mouse strain ($B6C3F_1$) to drinking water containing 0.4% urethane. Five-week old male and female animals were exposed for 15-20 days in 5- or 10-day periods separated by 10 days, and 10-day old animals were exposed five times, once every other day. The effective group size for analysis of tumor incidence was considered the initial number of animals less those dying without tumors before the 25th week for urethane-exposed animals or the 45th week for control animals. The effective group size ranged from 30 to 158 animals for animals showing significant increases in tumor incidence. Among male and female $B6C3F_1$ mice exposed for 10 days, the incidence of Harderian gland tumors (45/51 exposed vs. 3/32 control, males; 44/81 exposed vs. 0/39 control, females; $p < 10^{-10}$, Fisher's exact test) and lung adenomas (23/51 exposed vs. 7/32 control, males; 18/81 exposed vs. 4/39 control, females; $p < 0.1$) was increased. Among female $B6C3F_1$ mice alone, the incidence of thymic lymphosarcoma (10/81 exposed vs. 0/39 control; $p = 0.02$) and mammary gland adenocarcinoma (23/81 exposed vs. 1/39 control; $p = 10^{-3}$) was also increased. Among male and female C3Hf mice exposed for 15 days, the incidence of lung adenoma (45/79 exposed vs. 3/30 control, males, $p = 10^{-5}$; 46/87 exposed vs. 12/62 control, females, $p = 10^{-4}$) and Harderian gland adenoma (32/79 exposed vs. 3/30 control, males, $p = 0.001$; 25/87 exposed vs. 2/62 control, females, $p = 10^{-4}$) was increased. Among female C3Hf mice alone, the incidence of mammary adenocarcinoma (36/87 exposed vs. 6/62 control; $p = 10^{-5}$), thymic lymphosarcoma (2/87 exposed vs. 0/39 control; $p = 0.02$), and hepatoma (29/87 exposed vs. 15/62 control; $p = 0.15$) was increased.

Innes *et al.* (1969) treated male and female $B6C3F_1$ and $B6AKF_1$ mice (24/sex) with 158 mg/kg body weight urethane (on day 7) by oral gavage from day 7 to 28 of life. Thereafter, animals were exposed to a concentration of 600 ppm urethane in their diet. Control groups (90/sex/strain)

received received vehicle alone, then normal diet. Surviving animals were sacrificed and autopsied between 78 and 88 weeks of age. Significant increases in the incidence of pulmonary adenomas or carcinomas and hepatomas were observed in treated animals of both sexes and strains (see Table 2, $p < 0.05$, Fisher's exact test). The incidence of angiomas was increased in male and female $B6AKF_1$ mice ($p < 0.01$). Harderian gland adenomas were increased in $B6C3F_1$ females and $B6AKF_1$ males and females ($p < 0.01$). Lymphomas were increased in $B6AKF_1$ male mice ($p < 0.01$).

Table 2. Tumor incidence data on two strains of mice exposed to urethane by oral gavage and in drinking water (Innes, 1969).

	$B6C3F_1$				$B6AKF_1$			
	male		female		male		female	
tumor type	treated	control	treated	control	treated	control	treated	control
pulmonary adenomas or carcinomas	6/20	5/79	6/23	3/87	15/22	10/90	17/19	3/82
Hepatomas	8/20	8/79	12/23	0/87	14/22	5/90	5/19	1/82
Angiomas					4/22	0/90	11/19	0/82
Harderian gland adenomas			5/23	4/87	11/22	0/90	7/19	0/82
lymphomas					6/22	1/90		

Tomatis *et al.* (1972) report on a study in which urethane treatment was used as a positive control in a study of DDT's long-term health effects. Male and female CF-1 mice (60/sex) were exposed continuously to drinking water containing 0.01% urethane for 6 generations. Control animals (60/sex) were given plain drinking water. Parent generation animals were sacrificed at 140 weeks and subsequent generations at 130 weeks. Survival among animals of both sexes was reduced by urethane treatment. For statistical purposes, group size was determined by the number of animals surviving at the time of the appearance of the first tumor of any type. Comparison of groups was made by combining data from all generations. Lung tumor incidence was found to be significantly increased in urethane treated male mice (261/314 exposed vs. 157/328 controls, $p = 10^{-21}$, Fisher's exact text) and female mice (181/241 exposed vs. 124/340 controls, $p = 10^{-20}$). Among treated males, lymphoma incidence was increased (100/314 exposed vs. 79/328 controls, $p = 0.02$) and among treated female mice, osteoma incidence was increased (55/241 exposed vs. 39/340 controls, $p < 0.001$).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Although IARC (1988) has recognized urethane as a possible human carcinogen, inadequate information relating cancer incidence to specific exposure levels precludes the development of a cancer potency value from human data. An abundant body of literature relating urethane exposure to the development of tumors in animals is available. Standard carcinogenesis models applied to the data, along with some data useful for making pharmacokinetic adjustments, permit quantitative estimates of cancer potency from the animal studies. Estimated potency values are summarized below along with the rationale for development of the reference unit risk value.

Methodology

Estimates of cancer potency from the available data on the carcinogenicity of urethane can be made based on the multistage procedure initially described by Armitage and Doll (1954). For studies in which variable dosing over time has occurred, a mathematical dosage modification was made based on the estimation procedures described by Crouch (1983) and Crump and Howe (1984). Several studies have been conducted which also permit making pharmacokinetic adjustments in the estimation of carcinogenicity. Specifically, a model has been developed describing the pharmacokinetics of urethane either administered continuously or in discrete increments (Mitchell and Gauthier Associates Inc., 1975). Urethane distribution in the body and kinetic constants for rats and mice have been determined from the studies of O'Flaherty and Sichak (1983) and Nomeir *et al.* (1989). When appropriate, a proportional correction factor was applied to the experimental dose rate to estimate the effective dose rate.

Estimates of human cancer potency (q_{human}) were estimated from derived animal values (q_{animal}) based on a scaling factor proportional to the third power of the human to experimental animal body weight ratio (bw_h and bw_a). The relationship is described as follows:

$$q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$$

Table 3 presents the estimated human cancer potency values from animal studies in which significantly increased in tumor incidences have been found, including the species studied, most sensitive site of tumor development, the multistage procedure applied to the incidence data, and whether a pharmacokinetic adjustment was applied. For details of the methodology and assumptions made in deriving the potency, see Salmon and Zeise (1991). A measure of the "expected" or "average" value of the potency (q_1) estimated from the experimental data, termed q_{bar} , is also presented. The q_{bar} is derived when there are a large number of positive data points and the probability mass function (arithmetic mean) becomes less meaningful. It is derived by numerical computation, using the same continuous, asymptotic distribution as when deriving the upper confidence limit. The q_{bar} value is derived as follows,

$$q_{bar} = \int_0^\infty f(q_1) \cdot q_1 \, dq_1$$

with $f(q_1)$ as the frequency distribution whose log-likelihood function follows a chi-square distribution.

The selection of a cancer potency value should be made on the basis of the most sensitive site, species, and study, in the absence of evidence that such a value is not representative. The hamster studies of Pietra and Shubik (1960) and Toth *et al.* (1961a, 1969) indicate the hamster may not be as sensitive as the mouse when individual tumor sites are compared. Although the limited data available do not rule out the possibility that the hamster may be the more sensitive species, data are not available to make quantitative comparisons of hamsters and mice. This, coupled with the fact that there are extensive studies on mice, suggests the mouse urethane studies are more appropriate than hamster studies in developing a cancer potency value. Only two studies in the rat are useful for deriving cancer potency values. The Tannebaum *et al.* (1962) study showing development of Zymbal's gland tumors is of limited use since there is no supporting evidence that this site of tumor development is the most sensitive. The Schmähl *et al.* (1977) study is also of questionable value because of incomplete reporting of tumor incidence in untreated animals, in spite of showing sensitive induction of mammary tumors in female rats. In light of these limits on the studies in hamster and rats, the body of data showing tumor induction in mouse has been deemed most appropriate for the development of a cancer potency value.

Calculation of the geometric mean of q_{human} and q_{bar} values from the most sensitive sites of malignancy development in the oral mouse studies resulted in values of 0.5 and 1.4 $(mg/kg\text{-}day)^{-1}$, respectively. The geometric mean of the q_{bar} values provides an estimate of the upper 95% confidence limit on the distribution of values. Calculation of the geometric mean of q_{human} and q_{bar} values from mouse studies where the lung was the most sensitive site of malignancy development resulted in values of 0.8 and 1.9 $(mg/kg\text{-}day)^{-1}$, respectively. These mean values, coupled with the q_{human} values from the sensitive multiple dose study by Schmähl *et al.* (1977), indicate the most plausible estimate of cancer potency for urethane falls in the range of 0.6 to 3.0 $(mg/kg\text{-}day)^{-1}$. Therefore, as a reasonable estimate to the cancer potency, 1.0 E+0 $(mg/kg\text{-}day)^{-1}$ has been adopted as a cancer potency value.

A unit risk value based upon air concentrations was derived by OEHHA/ATES using an assumed human breathing rate of 20 m^3/day, 70 kg human body weight, and 100% fractional absorption after inhalation exposure. The calculated unit risk value is 2.9 E-4 $(\mu g/m^3)^{-1}$.

Table 3. Cancer potency estimates from oral studies in animals (adapted from CDHS (1989)).

study/tumor	species/strain	sex	model*	q_{human} (95 %) $(mg/kg\text{-}day)^{-1}$	q_{bar} $(mg/kg\text{-}day)^{-1}$
Pietra (1960)	hamster/ Syrian G		MST		
skin melanotic tumor		M		0.19	0.11
forestomach papilloma		M		0.11	0.060
forestomach papilloma		F		0.18	0.094
Toth (1961a)	hamster/Syrian G		AD		
forestomach papilloma		M		0.13	0.091
forestomach papilloma		F		0.15	0.11
Toth (1969)	hamster/Syrian G		MST		
forestomach papilloma		M		0.10	0.077
forestomach papilloma		F		0.20	0.15
Tannenbaum (1962)	rat/Sprague-Dawley		MPK		
Zymbal gland carcinoma		M		0.12	0.061
Schmähl (1977)	rat/Sprague-Dawley		WPK		
mammary gland carcinoma		F		0.83	0.48
Toth (1961b)	mouse/Swiss		APK		
lung adenoma		M		3.8	3.0
lung adenoma		F		3.6	2.9
lymphoma		M		0.30	0.18
lymphoma		F		0.46	0.25
Tannenbaum (1962)	mouse/		APK		
lung alveolar cell tumor	DBA	M		0.9	0.61
mammary gland carcinoma	DBA	F		1.2	0.90
lung adenoma	DBA	M		0.4	0.29
lung adenoma	C3H	F		0.4	0.28
Klein (1962)	mouse/$B6AF_1$		APK		
lung adenoma		M		0.85	0.54
lung adenoma		F		0.76	0.48
leukemia		M		0.090	0.071
leukemia		F		0.10	0.066
Della Porta (1963a)	mouse/CTM		APK		
lung adenoma		M		0.62	0.17
lung adenoma		F		0.60	0.12
Della Porta (1963b)	mouse/CTM		APK		
malignant lymphoma		M		0.55	0.17
mammary gland		F		0.50	0.12
Della Porta (1967)	mouse/		APK		
Harderian gland tumor	$BC3F_1$	M		0.88	0.66
Harderian gland tumor	$BC3F_1$	F		0.32	0.25
Harderian gland tumor	C3Hf	M		0.22	0.15
Harderian gland tumor	C57BL	M		0.32	0.25
Harderian gland tumor	C57BL	F		0.23	0.19
mammary gland carcinoma	C3Hf	F		0.23	0.16
lung adenoma	C3H	M		0.18	0.13
lung adenoma	SWR	M		0.94	0.67
lung adenoma	SWR	F		1.0	0.76

Table 3 (continued). Cancer potency estimates from oral studies in animals [adapted from CDHS (1989)].

study/tumor	species/strain	sex	model*	q_{human} (95 %) $(mg/kg\text{-}day)^{-1}$	q_{bar} $(mg/kg\text{-}day)^{-1}$
Innes (1969)	mouse/		MPK		
liver hepatoma	$B6C3F_1$	M		0.40	0.23
liver hepatoma		F		0.61	0.27
lung adenoma	$B6AKF_1$	M		0.78	0.51
lung adenoma		F		1.5	0.99
Tomatis (1972)	mouse/CF-1		MPK		
lung adenoma		M		0.90	0.75
lung adenoma		F		0.67	0.55
Schmähl (1977)	mouse/NMRI				
lung adenoma		M	WPK	3.0	1.7
lung carcinoma		M	MPK	0.85	0.55
lung adenoma		F	WPK	1.9	-
lung carcinoma		F	MPK	0.56	0.12
			W1PK	3.0	1.7

*MST- multistage; APK - Armitage-Doll model; MPK - multistage with pharmacokinetic adjustment; WPK - Weibull time-dependent with pharmacokinetic adjustment; W1PK - Weibull linear in dose time-dependent model with pharmacokinetic adjustment.

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VINYL CHLORIDE

CAS No: 75-01-4

I. PHYSICAL AND CHEMICAL PROPERTIES (From HSDB, 1998)

Molecular weight	62.5
Boiling point	-13.37ºC
Melting point	-153.8°C
Vapor pressure	2660 mm Hg @ 25°C
Air concentration conversion	1 ppm = 2.56 mg/m^3

II. HEALTH ASSESSMENT VALUES

Unit Risk Factor: 7.8 E-5 ($\mu g/m^3$)$^{-1}$
Slope Factor: 2.7 E-1 (mg/kg-day)$^{-1}$
[Female mouse lung tumor incidence (Drew *et al*., 1983), extra risk calculated using a linearized multistage procedure (CDHS, 1990).]

III. CARCINOGENIC EFFECTS

Human Studies

In 1974, Creech and Johnson described three cases of angiosarcoma of the liver (LAS) among workers at the B.F. Goodrich Tire and Rubber Co. in Louisville, Kentucky. Because LAS is a very rare cancer (20-25 cases per year in the U. S.), the clustering of three cases in one vinyl chloride (VC) polymerization facility indicated an abnormally high incidence of this cancer. Based on this report, as well as data indicating that VC is carcinogenic in laboratory animals, multiple studies of workers exposed to this agent were conducted. By 1985, at least 15 epidemiologic studies relating VC exposure to the incidence of various cancers had been completed. A summary of the data from these studies is provided in Table 1.

Between 1961 and 1977, 23 cases of LAS were reported among approximately 20,000 VC workers in the U.S. (Lelbach and Marsteller, 1981; Spirtas and Kaminski, 1978). The expected incidence of LAS is 0.014 cases per 100,000 per year in the general population in the U.S. (Heath *et al*., 1975). Based on analysis of these data, the relative risk for developing LAS following VC exposure among this country's VC workers is 483.

The epidemiologic studies also demonstrate a strong and consistent association between VC exposure and primary cancer of the liver. All of the studies that assessed risk for primary liver cancer note a statistically significant increase in standardized mortality ratios (SMRs). The average relative risk for liver cancer among VC workers is five to six times greater than the incidence of that seen in the general population. The evidence strongly suggests that exposure to VC can cause liver cancer. All reports published to date indicate that the SMRs of exposed workers are elevated, and risk of liver cancer was seen to increase with both increased dose and a longer follow-up time.

Table 1: A Summary of Epidemiologic Data for Occupationally Exposed Vinyl Chloride Workers

Study	Place	Cohort	Deaths (%)	Exposure in years	SMR				
					All Sites	Liver (LAS)	Brain	Lung	Lymphoma
Tabershaw and Gaffey[1] (1974)	U.S.	8,384	352 (4.7)	> 1 10.2%>20 yrs	110	94[3] (6)	155[4]	112	106
Duck *et al.* (1975)	U.K.	2,122	152 (7.2)	> 0	96	93[3] (0)	--	103	--
Nicholson *et al.* (1975)	U.S.	257	24 (9.3)	> 5	231	- (3)	--	--	--
Ott *et al.*[2] (1975)	U.S.	594	79 (13.3)	> 0	81	- (0)	--	77	--
Byren *et al.* (1976)	Sweden	771	58 (75)	> 0	--	413[a] (2)	612[a]	168	--
Waxweiler *et al.* (1976)	U.S.	1,294	136 (10.5)	> 5 (f/u > 10 yrs)	149[a]	1,155[b] (11)	329[a5]	156	159
				> 5 (f/u > 15 yrs)	189[b]	1,606[b] -	498[a]	194[a]	176
Fox and Collier (1977)	U.K.	7,717	409 (5.3)	> 0 8% > 20 yrs	90.7	1,408[a] (2)	54.6	89.8	90.9
EEH (1975)[1]	U.S.	10,173	707 (6.9)	> 1 19.3%>20 yrs	104	75[3] (5)	203[a]	107	112
Buffler *et al.* (1979)	Texas	464	28 (6.0)	> 0	138	- (0)	--	208[a]	--
Bertazzi *et al.* (1979)	Italy	4,777	62 (1.3)	> 0.5	97	800[a] (3)	125	81	133
Masuda *et al.* (1979)	Japan	304	26 (8.5)	> 1	138	500[a] (0)	--	125	--
Weber *et al.* (1981)	Germany	7,021 production	414 (5.9)	> 0	112	1,523[b] -	162	--	214[a]
		4,007 processing	360 (9)	> 0	85	434[a] -	535[a]	--	34
		4,910 unexposed	417 (8.5)	n/a	83	401[a] -	184	--	77
Cooper[1] (1981)	U.S.	10,173	707 (6.9)	> 1	104	75[3] (8)	203[a]	107	112
Heldaas *et al.* (1984)	Norway	454	50 (11)	> 1	114	- (1)	--	180	--
					Relative Risk				
Theriault and Allard (1981)	Canada	451 exposed	59 (2.6)	> 5	1.48	6.25[a] (10)	--	.36	--
		871 unexposed	233 (26.8)	n/a					

Table 1 (continued): A Summary of Epidemiologic Data for Occupationally Exposed Vinyl Chloride Workers

LAS = angiosarcoma of the liver; f/u = follow-up
[1]The studies of Cooper and EEH are reanalyses of the Tabershaw and Gaffey Cohort
[2]SMR subjects also in the Tabershaw and Gaffey Cohort
[3]SMR is for the "digestive system cancer", not liver cancer
[4]SMR is for "other and unspecified cancer", 40% of which were brain cancer
[5]SMR is for cancer of CNS, not brain
[a] $p < 0.05$ [b] $p < 0.01$

The association between VC exposure and increased risk for other cancers is not as clear as that for liver cancer. Some evidence associates exposure to VC with increased mortality ratios for brain cancer, lung cancer, and lymphoma. Since these cancers appear more commonly in the general population than LAS and primary liver cancer, it becomes more difficult to show increased risk.

Workers exposed to VC appear to be at greater risk for brain cancer than do non-exposed populations. Of the six studies that assessed the risk of brain cancer, five showed a positive trend for increased risk of this cancer type following exposure to VC, with four demonstrating statistical significance ($p < 0.05$). Cancer risk increased an average of four times above that expected in the general population in those studies that exhibited a significantly increased risk. Of the two studies not showing a significant increase in risk for brain cancer, statistical power in the Bertazzi and associates study was only about 35% (Bertazzi *et al*., 1979), while that of Fox and Collier (1977) was approximately 80% (Beaumont and Breslow, 1981). In the Fox and Collier study, the number of deaths overall was low and, most importantly, a large percentage of workers in the cohort was very recently employed in the VC industry and thus had a short follow-up time. These factors may partially explain why this study failed to detect an association between VC exposure and brain cancer.

The evidence linking VC exposure with lung cancer remains inconclusive. Analyses of SMRs for cancer of the lung were performed in 12 studies. Of these, seven studies showed an increased risk for lung cancer, but only one was statistically significant at the 5% level (Buffler *et al*., 1979). This increased risk persisted after adjusting for personal smoking habits (for this particular cohort). However, this cohort was small and the study was unable to demonstrate an increased risk for any other cancer. The Waxweiler *et al*. (1976) cohort (which had a follow-up period greater than 15 years) also used a small group.

An association between VC exposure and lymphoma has not been established. Five studies evaluated the risk of lymphoma development among workers occupationally exposed to VC. Four of the studies showed a positive trend for lymphoma among VC workers, but statistical significance was noted only by Weber *et al.* (1981). However, the statistical power in all of these studies was less than 80% to demonstrate a relative risk of two, and less than 40% to show a relative risk of 1.5.

Animal Studies

Reviews of VC carcinogenicity data from exposed laboratory animals available at the time the document "*Health Effects of Airborne Vinyl Chloride*" (CDHS, 1990) was released include those by Kalmaz and Kalmaz (1984), IARC (1979), SRI (1983), Kuzmack and McGaughy (1975), and Purchase *et al*. (1987). Adequate experimental evidence exists to indicate that VC is carcinogenic in mice, rats, and hamsters when given orally and by inhalation. VC has been found to cause tumors in a dose-related manner at several sites, including liver, lung and mammary gland. The oncogenic response appears to be a function of the site, VC concentration, tumor type, species of animal, and route of administration.

Although some evidence of VC-induced carcinogenesis has been observed by all routes of administration and in all species tested, important discrepancies in the protocols of many studies have limited their usefulness in quantitative risk assessment. These discrepancies include the lack of appropriate control groups, insufficient exposure time, or incomplete histopathology of the animals. Studies that have been used previously in risk assessment include feeding studies (Feron *et al*., 1981; Til *et al*., 1983) and a series of inhalation studies (Maltoni *et al*., 1984).

Groups of 60-80 male and 60-80 female five-week old Wistar rats were fed polyvinyl chloride powder (10% of diet) with or without a high VC monomer content (0 to 4000 ppm) in the diet for their lifetimes (Feron *et al*., 1981). The actual doses of VC given to rats in the feed were 0, 1.7, 5.0, and 14.1 mg/kg/day.

Necrosis, centrilobular degeneration and mitochondrial damage were seen in the hepatic parenchyma of rats administered VC. Significantly increased incidences of liver and lung angiosarcomas and hepatocellular carcinomas were observed in both male and female rats. Tumor incidences are listed in Table 2. It is possible that underreporting of tumors at all sites occurred because of the incomplete histopathology performed and the fact that only the longest-surviving high-dose animals were chosen for complete histopathology.

As a follow-up to the study of Feron and co-workers (1981), groups of 100 male and 100 female Wistar rats (except for the top-dose group, which was composed of 50 animals of each sex) were fed polyvinyl chloride (up to 1% of diet) with a high content of VC monomer for up to 149 weeks (Til *et al*., 1983). Levels of VC administered in the powder were 0, 0.017, 0.17, and 1.7 mg/kg/day for 149 weeks. Actual oral exposure to VC monomer (calculated by measuring the evaporative loss of VC during the four-hour feeding periods, the rate of food intake, and the level of VC in the feces) was estimated to be 0.014, 0.13, or 1.3 mg VC/kg/day for the low, middle, and high dose groups, respectively.

The results of this study demonstrated increases in the incidences of hepatic foci or cellular alteration, neoplastic nodules, hepatocellular carcinomas, liver-cell polymorphism, and cysts in the highest dose group. Two females and one male in this group developed liver angiosarcomas. Females, but not males, of the low- and mid-dose groups developed a higher incidence of hepatic basophilic foci of cellular alteration. No pathologic effects in other organ systems were attributed to VC exposure (Til *et al*., 1983). Histopathology of all organs was not performed on all animals;

therefore, tumors not grossly observable or palpable could have been missed. Because of the shortcomings of the study, its utility for the evaluation of carcinogenic risk is limited.

Several researchers have investigated the potential carcinogenicity of VC administered by inhalation (Viola, 1977; Caputo *et al*., 1974; Keplinger *et al*., 1975; Lee *et al*., 1977; Hong *et al*., 1981; Suzuki, 1981; Groth *et al*., 1981; Drew *et al*., 1983; Maltoni *et al*., 1984; Bi *et al*., 1985). All experiments confirm the carcinogenicity of VC, although only a few of the studies are adequate for a quantitative evaluation of carcinogenic risk. This summary will concentrate on the studies (Drew *et al*., 1983; Maltoni *et al*., 1984; Bi *et al*., 1985) used by CDHS (1990) for quantitative risk assessment purposes.

Table 2: Tumor incidences in male and female Wistar rats exposed to dietary vinyl chloride (Feron *et al*., 1981).

	Incidence[1] Vinyl chloride (mg/kg-day)			
Tumor type/Sex	0	1.7	5.0	14.1
Liver angiosarcoma				
male	0/55	0/58	6/56*[2]	27/59***
female	0/57	0/58	2/59	9/57**
Hepatocellular carcinoma				
male	0/55	1/58	2/56	8/59**
female	0/57	4/58	19/59***	29/57***
Neoplastic nodules				
male	0/55	1/58	7/56**	23/59***
female	2/57	26/58***	39/59***	44/57***
Lung angiosarcoma				
male	0/55	0/58	4/56*	19/59***
female	0/57	0/58	1/59	5/57*
Abdominal mesotheliomas				
male	3/55	1/58	7/56	8/59
female	1/57	6/58*	3/59	3/57
Mammary tumors[3]				
female	3/57	2/58	5/59	9/57

[1] Number in denominator = number of animals necropsied.
[2] values marked with asterisks differ significantly from controls as determined using the Chi-square test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.
[3] Including mammary adenomas, adenocarcinomas and anaplastic carcinomas.

Bi *et al*. (1985) evaluated the tumorigenic potential of VC in male Wistar rats following inhalation exposure to 0, 10, 100 or 3000 ppm (six hours/day, six days/week) for up to 12 months. The incidence of liver angiosarcomas was 0/19, 0/20, 7/19 and 17/19 for the four exposure groups, and 0/19, 0/20, 2/19 and 9/20 for lung angiosarcomas, respectively. The incidence of liver angiosarcomas in the 100 and 3000 ppm groups was significantly greater than controls ($p = 0.004$, $p < 0.001$, respectively); the incidence of lung angiosarcomas in the 3000 ppm group was also significantly greater than controls ($p = 0.001$). This study probably underestimated the

carcinogenic potential of VC because of the less-than-lifetime exposure and the small number of animals per group.

Drew *et al*. (1983) examined the effect of age and exposure duration on VC oncogenicity in females of several different species of rodents. Groups of female CD-1 Swiss mice, $B6C3F_1$ mice, Fischer 344 rats, and Golden Syrian hamsters (n = 54 for mice, n = 56 for rats and hamsters) were exposed to VC for six hours/day, five days/week for six, 12, 18, or 24 months, beginning at eight weeks of age, and observed for their lifespans. Other groups were held until six or 12 months of age, exposed for six or 12 months, and then observed for the remainder of their lifespans. The exposures were conducted at a single dose level for each species; mice, rats and hamsters were exposed to 50, 100, and 200 ppm VC, respectively. All animals exposed to VC at age eight weeks (the start of the experiment) exhibited decreased survival relative to controls (Drew *et al.*, 1983). $B6C3F_1$ mice experienced the most significant life-shortening regardless of the age at which exposure was begun. No significant decrease in survival was observed in rats, hamsters, or Swiss mice initially exposed after six months of age. Other clinical signs of VC toxicity were not evident and liver necrosis was not observed.

In rats, exposure to VC was associated with hemangiosarcomas, mammary gland adenocarcinomas and adenomas, and hepatocellular carcinomas. The incidence of hemangiosarcomas was a function of the duration of exposure and age at start of exposure; the longer the exposure period the greater the incidence of hemangiosarcomas. A six-month exposure produced a low incidence of hemangiosarcomas and hepatocellular carcinomas only if begun early in life. One-year exposures produced a significant incidence of tumors, especially if begun early in life. The incidence of mammary gland adenocarcinomas and fibroadenomas was not always related to exposure duration, but the incidence was higher in rats whose exposure began at eight weeks of age. Hepatocellular carcinomas were induced in a dose-related manner in rats when exposures began at eight weeks. Tumor incidences in VC-exposed rats are listed in Table 3.

In hamsters, hemangiosarcomas, mammary gland carcinomas, stomach adenomas, and skin carcinomas were associated with VC exposure (Drew *et al*., 1983). The highest incidence of hemangiosarcomas and stomach adenomas occurred in animals exposed early in life for only six months. The highest incidence of mammary gland carcinomas was seen in animals exposed at an early age for up to twelve months. Exposure beginning at or after eight months of age resulted in a markedly lower tumor incidence, possibly because the lifespans of chronically exposed hamsters were significantly reduced to the point that late-appearing tumors would not be expressed. Tumor incidences in VC-exposed hamsters are listed in Table 4.

Mice, especially the $B6C3F_1$ strain, appeared to be the species most sensitive to the carcinogenic effects of VC (Drew *et al.*, 1983). Hemangiosarcomas and mammary gland carcinomas in both strains and lung carcinomas in Swiss mice were associated with VC exposure. In $B6C3F_1$ mice, exposure to VC for six months resulted in 60-70% incidence of hemangiosarcomas, regardless of the age at exposure initiation. The incidence of mammary gland carcinomas in $B6C3F_1$ mice was

Table 3: Tumor incidences in 100 ppm vinyl chloride-exposed female Fisher 344 rats (Drew *et al.*, 1983).

Tumor type	Length of Exposure (months)	LDE (ppm)[a]	Tumor incidence[b] (%)
Liver hemangiosarcomas	control	0	1/112 (0.9)
	6	4.5	4/76 (5.3)
	12	8.9	11/55 (20.0)***
	18	13.4	13/55 (23.6)***
	24	17.9	19/55 (34.7) ***
Mammary adenocarcinomas	control	0	5/112 (4.5)
	6	4.5	6/76 (7.9)
	12	8.9	11/56 (19.6) **
	18	13.4	9/55 (16.4) *
	24	17.9	5/55 (9.1)
Hepatocellular carcinomas	control	0	1/112 (0.9)
	6	4.5	3/75 (4.0)
	12	8.9	4/56 (7.1) *
	18	13.4	8/54 (14.8) ***
	24	17.9	9/55 (16.4) ***

[a] LDE = Lifetime Daily Exposure. [b] Value in parentheses is percent incidence.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Fisher's exact test)

Table 4: Tumor incidences in 200 ppm vinyl chloride-exposed female Golden Syrian hamsters (Drew *et al.*, 1983).

Tumor type	Length of Exposure (months)	LDE (ppm)[a]	Tumor incidence[b] (%)
Hemangiosarcomas (all sites)	control	0	0/143 (0)
	6	8.9	13/88 (14.8) ***
	12	17.9	4/52 (7.7) **
	18	26.8	2/103 (1.9)
Mammary carcinomas	control	0	0/143 (0)
	6	4.5	28/87 (32.2) ***
	12	8.9	31/52 (59.6) ***
	18	13.4	47/102 (46.1) ***
Skin carcinomas	control	0	0/133 (0)
	6	4.5	2/80 (2.5)
	12	8.9	9/47 (18.8) ***
	18	13.4	3/90 (3.3)

[a] LDE = Lifetime Daily Exposure. [b] Value in parentheses is percent incidence.
** $p < 0.01$; *** $p < 0.001$ (Fisher's exact test)

greatest when the animals were exposed early in life. Lower incidences of this tumor were seen when initial exposure occurred at a later age. In Swiss mice, exposure to VC at an early age resulted in the highest incidence of hemangiosarcomas, mammary gland carcinomas, and lung carcinomas, regardless of duration of exposure. Lower incidences of all tumors were observed in animals exposed later in life. Tumor incidences in VC-exposed mice are listed in Table 5.

Table 5: Tumor incidences in 50 ppm vinyl chloride-exposed female $B6C3F_1$ and CD-1 Swiss mice (Drew *et al.*, 1983).

Strain/Tumor type	Length of Exposure (months)	LDE (ppm)[a]	Tumor incidence[b] (%)
$B6C3F_1$			
hemangiosarcomas (all sites)	control	0	4/69 (5.8)
	6	2.23	46/67 (68.7) ***
	12	4.46	69/90 (76.7) ***
	18	--	--
mammary carcinomas	control	0	3/69 (4.3)
	6	2.23	29/67 (43.2) ***
	12	4.46	37/90 (41.1) ***
	18	--	--
CD-1 Swiss			
hemangiosarcomas (all sites)	control	0	1/71 (1.4)
	6	2.23	29/67 (43.3) ***
	12	4.46	30/47 (63.8) ***
	18	6.69	20/45 (44.4) ***
mammary carcinomas	control	0	2/71 (2.8)
	6	2.23	33/67 (49.3) ***
	12	4.46	22/47 (46.8) ***
	18	6.69	22/45 (48.9) ***
	control	0	9/71 (12.7)
	6	2.23	18/65 (27.7) *
	12	4.46	15/47 (31.9) *
	18	6.69	11/45 (24.4)

[a] LDE = Lifetime Daily Exposure. [b] Value in parentheses is percent incidence.
* $p < 0.05$; *** $p < 0.001$ (Fisher's exact test)

Maltoni and co-workers performed a series of chronic inhalation studies on rats, mice, and hamsters in the Bentivoglio Laboratories (BT) or the Bologna Institute of Oncology (Maltoni *et al.*, 1984). The investigators studied the effects of exposure to 14 concentrations of VC (1-30,000 ppm) in male and female rats and six concentrations of VC in male and female mice and male hamsters. In each experiment, animals were exposed to VC for four hours daily, five days per week for various durations, and observed for the rest of their lives. A number of the experimental procedures were not described or were inadequately described in the report by Maltoni *et al.* (1984). Details of the experimental protocol for the BT experiments are provided in Table 6.

Data on noncarcinogenic toxic effects of vinyl chloride were sparsely reported in the Maltoni BT experiments. Vinyl chloride appeared to be toxic at the higher concentrations, but reportedly the high mortality at these dose levels was due to a high incidence of vinyl chloride-induced tumors. The available information on survival, including Kaplan-Meier survival curves, indicates that vinyl chloride decreased survival in a dose-dependent manner.

Table 6: Experimental protocol for vinyl chloride inhalation studies performed by Maltoni *et al.* (1984).

Experiment number	Dose (ppm)	Exposure duration (weeks)[1]	Species/ strain	Starting exposure age (weeks)	animals/exposure concentration[2]
BT1	0, 50, 250, 500, 2500, 6000, 10000	52	rat/SD	13	30 M, 30 F
BT2	1, 100, 150, 200	52	rat/SD	13	60 M, 60 F (85 M, 85 F)
BT3	0, 50, 250, 500, 2500, 6000, 10000	17	rat/SD	12	30 M, 30 F
BT4	0, 50, 250, 500, 2500, 6000, 10000	30	mouse/ Swiss	11	30 M, 30 F (80 M, 70 F)
BT5	6000, 10000	1	rat/SD	19 (fetus)	30 F 13-29 M, F (no controls)
BT6	30000	52	rat/SD	17	30 M, 30 F (no controls)
BT7	0, 50, 250, 500, 2500, 6000, 10000	52	rat/ Wistar	11	30 M (40 M)
BT8	0, 50, 250, 500, 2000, 6000, 10000	30	hamster/ Syrian Golden	11	30 M (62 M)
BT9	0, 50	52	rat/SD	13	150 M, 150 F (50 M, 50 F)
BT14	6000, 10000	5	rat/SD	21 (parents)	6 F (no controls)
		5		1day (offspring)	21-22 M, F (no controls)
BT15	0, 1, 5, 10, 25	52	rat/SD	13	60 M, 60 F
BT4001	0, 2500	76	rat/SD	13	54 F (60 F)
		69		1 day	68 M, 68 F (158 M, 149F)
BT4006	0, 2500	15	rat/SD	1 day	60 M, 60 F

[1] Exposures were for four hours/day, 5 days/week. [2] Number in parentheses = number of control animals when not equal to number of animals in experimental groups.

In the Maltoni experiments, exposure to vinyl chloride was associated with an increased incidence of malignant tumors at a variety of tissue sites in all of the species tested. A summary of these tumor sites is provided in Table 7 (Maltoni *et al*., 1984). A direct relationship between exposure levels and tumor incidence was apparently demonstrated, although no statistical tests for trends were performed. Results of experiments on Sprague-Dawley rats exposed to vinyl chloride for 52 weeks were statistically analyzed using the Fischer exact probability test. A summary of the lowest concentrations at which a statistically significant excess of tumors was observed is given in Table 8. When adjusted to average lifetime exposure, the lowest concentration associated with tumor production is 0.06 ppm (1 ppm * 4/24 * 5/7 * 12/24 - 0.3 ppm).

Table 7: Tumors correlated to inhalation exposure to vinyl chloride in rats, mice, and hamsters in the BT experiments (Maltoni *et al*., 1984).

Tumors	Rat	Mouse	Hamster
Liver angiosarcomas	+	+	+
Hepatomas	+	(+)	
Encephalic neuroblastomas	+		
Lung adenomas		+	
Lymphomas/leukemias			(+)
Angiosarcomas at other sites	+	+	(+)
Zymbal gland epithelial tumors	+		
Nephroblastomas	+		
Cutaneous epithelial tumors	(+)	(+)	(+)
Mammary adenocarcinomas	+	+	
Forestomach papillomas, acanthomas	+	(+)	+

\+ Tumor incidence was statistically significant ($p < 0.05$) by the Fisher exact test.

(+) Association was not statistically significant, but was considered biologically significant.

Table 8: Lowest concentration of VC at which a significant incidence of tumors ($p < 0.05$) was reported by Maltoni *et al*. (1984) at specific sites in Sprague-Dawley rats.

Tumor type	Vinyl chloride concentration (ppm)
forestomach papilloma	30,000 (male, female)
Zymbal gland carcinoma	10,000 (male, female)
neuroblastoma	10,000 (female)
nephroblastoma	250 (female)
liver angiosarcoma	200 (male); 25 (female)
mammary adenocarcinoma	1 (female)

Experiment BT1

Most previous risk assessments have been based on the data from experiment BT1 (Maltoni *et al*., 1984). In this study, 30 Sprague Dawley rats of each sex were exposed to concentrations of vinyl chloride ranging from 50 to 10,000 ppm for four hours daily, five days per week for 52 weeks, beginning at 13 weeks of age. A positive control group received 2,500 ppm of vinyl acetate. After treatment the animals were observed for their lifespans up to 135 weeks. Survival of both males and females decreased in a dose-related manner, especially at concentrations above 500 ppm. Vinyl chloride was more toxic to females than to males in this experiment. Vinyl chloride was associated with an increased incidence of liver angiosarcomas in a dose-related fashion. These results are presented in Table 9 (Maltoni *et al.*, 1984). In addition to liver angiosarcomas, vinyl chloride (at concentrations above 2500 ppm) caused an increased incidence of zymbal gland carcinomas, nephroblastomas, hepatomas, and neuroblastomas. The incidence of liver angiosarcomas was probably underestimated at the higher exposure levels due to mortality resulting from tumors at other sites.

Table 9: Incidence of liver angiosarcomas (LAS) in male and female Sprague-Dawley rats exposed to 52 weeks to vinyl chloride (Maltoni *et al*., 1984)

Study	Exposure level (ppm)	LAS incidence[1]	corrected LAS incidence[2]

		male	female	male	female
BT1	0	0/30	0/30	0/22	0/29
	50	0/30	1/30	0/26	1/29
	250	1/30	2/30	1/28	2/26
	500	0/30	6/30	0/22	6/28
	2,500	6/30	7/30	6/26	7/24
	6,000	3/30	10/30	3/17	10/25
	10,000	3/30	4/30	3/21	4/25
BT2	0*	0/85	0/100	0/61	0/68
	100	0/60	1/60	0/37	1/43
	150	1/60	5/60	1/36	5/46
	200	7/60	5/60	7/42	5/44
BT6	30,000	5/30	13/30	5/22	13/24
BT9	0	0/50	0/50	0/29	0/38
	50	1/150	12/150	2/70	12/110
BT15	0	0/60	0/60	0/25	0/44
	1	0/60	0/60	0/48	0/55
	5	0/60	0/60	0/43	0/47
	10	0/60	1/60	0/42	1/46
	25	1/60	4/60	1/41	4/40
LAS incidence in historical controls		1/1179	2/1202	1/364	2/541

[1] Number in denominator - number of animals necropsied.
[2] Number in denominator - number of animals alive when first liver angiosarcoma was observed.

Experiment BT 15
Groups of 60 male and 60 female Sprague-Dawley rats were exposed to 0, 1, 5, 10, or 25 ppm of vinyl chloride for four hours daily, five days per week for 52 weeks, beginning at 13 weeks of age (Maltoni *et al.*, 1984). Following exposure the animals were observed for the remainder of their lives (up to 147 weeks). Available data,. including Kaplan-Meier survival curves, indicated that vinyl chloride did not affect survival at the concentrations tested.

No statistical analyses of mortality and body weight data were reported. Mortality was greater in the male control group than in the treated groups: the time at which 50% of the male control group had died was week 72, compared with week 100 in the 25-ppm vinyl chloride group. No explanation was given for this decreased survival. The incidence of mammary gland carcinomas in treated females was higher than in controls at all concentrations of vinyl chloride exposure. The differences from control values were statistically significant at concentrations of 1 ppm and above. The mammary gland adenocarcinoma incidence for this and the other relevant BT experiments are presented in Table 10.

Table 10: Incidence of mammary gland carcinomas in female Sprague-Dawley rats and Swiss mice exposed by inhalation to vinyl chloride (Maltoni *et al*., 1984)

Study No.	Experimental Dose Level (ppm)	Tumor Incidence[1]	Corrected Tumor Incidence[2]
BT1 (Rat)	0	0/30	0/29
	50	2/30	2/30
	250	2/30	2/27
	500	1/30	1/28
	2,500	2/30	2/25
	6,000	0/30	0/28
	10,000	3/30	3/29
BT2 (Rat)	0	2/60	2/100
	100	4/60	4/60
	150	6/60	6/60
	200	5/60	5/60
BT6 (Rat)	30,000	2/30	2/30
BT9 (Rat)	0	9/50	9/43
	50	59/150	59/142
BT15 (Rat)	0	6/60	6/60
	1	14/60	14/60
	5	22/60	22/60
	10	21/60	21/60
	25	16/60	16/60
Tumor Incidence in Historical Controls		100/1202	100/1202
BT4 (Mice)	0	1/80	1/67[3]
	50	12/30	12/30[3]
	250	13/30	13/29[3]
	500	10/30	10/28[3]
	2,500	9/30	9/30[3]
	6,000	9/30	9/28[3]
	10,000	14/30	14/28[3]
Tumor Incidence in Historical Controls		21/554	21/554[3]

[1] Number in denominator - number of animals examined.
[2] Number in denominator - number of animals alive when first malignant mammary tumor was observed (type unspecified).
[3] Number in denominator - number of animals alive when first mammary tumor was observed (type unspecified).

Experiment BT4

Thirty male and 30 female Swiss mice were exposed to 0, 50, 250, 500, 2,500, 6,000, or 10,000 ppm of vinyl chloride four hours daily, five days weekly for 30 weeks, beginning at 11 weeks of age (Maltoni *et al*., 1984). The study was terminated 81 weeks after the exposure period began. Vinyl chloride was highly toxic to both males and females, but males appeared more sensitive than females to the toxic effects of vinyl chloride. Survival decreased in a dose-related manner, although statistical analysis apparently was not performed on the data presented.

A very high incidence of lung adenomas was observed in vinyl chloride-treated male and female mice. A statistically significant increase in the incidence of liver angiosarcomas was seen in male and female mice exposed to vinyl chloride, but a dose response was not seen in the male animals. In addition, a high incidence of mammary gland adenocarcinomas occurred in treated female mice. These results are presented in Table 10 (data from Maltoni *et al*., 1984).

IV. DERIVATION OF CANCER POTENCY

Basis for Cancer Potency

Human occupational studies demonstrate a strong and consistent association between VC exposure and primary cancer of the liver. All of the studies that assessed risk for primary liver cancer note a statistically significant increase in standardized mortality ratios (SMRs). The average relative risk for liver cancer among VC workers is five to six times greater than the incidence of that seen in the general population. The evidence strongly suggests that exposure to VC can cause liver cancer. All reports published to date indicate that the SMRs of exposed workers are elevated, and risk of liver cancer was seen to increase with both increased dose and a longer follow-up time. CDHS (1990) decided that the Waxweiler *et al.* (1976) study was most suitable for quantitative risk assessment use.

Three sets of animal cancer bioassays (Drew *et al*., 1983; Maltoni *et al*., 1984; Bi *et al*., 1985) were also considered by CDHS (1990) to provide adequate data for quantitative risk assessment purposes. The Maltoni *et al*. experiments together provide an unusually large set of data on cancer incidence in both males and females rats over a large range of exposures at many concentrations - altogether fifteen groups beyond the four control groups. The Drew *et al.* experiments provide incidence data on female rodents for an unusual exposure protocol in that the duration varied for two or three groups beyond controls, while the concentration remained fixed for each species. The Bi *et al*. experiments provide incidence data on male rats for three exposures beyond controls. CDHS (1990) chose to develop cancer risk estimates for VC using both the human and animal data described above.

Methodology

Human-derived risk estimates

The review of the epidemiological studies strongly suggests a causal association between vinyl chloride and several different types of cancer, including liver, lung, and brain. However, none of the occupational cohort studies presented exposure data for a large enough cohort to derive a dose-response curve; so the CDHS (1990) analysis used historical industrial hygiene data to reconstruct a range of likely exposures, from which risk estimates can be extrapolated.

This risk analysis proceeds by selecting the Waxweiler *et al*. (1976) study of 1294 workers who experienced high sustained exposures to vinyl chloride and who were followed long enough (10 years) to develop substantial numbers of cancers that appeared to be related to the exposure. The retrospective estimates of Barnes *et al*. (1976) for the relevant industrial processes furnished

concentrations of the exposures of vinyl chloride, having an overall average value of 647 ppm. The analysis converts these annual average exposure estimates to a lifetime daily equivalent tissue exposure of 3.6 ppm on the assumption of a saturable metabolic process (Michaelis-Menten) leading to active carcinogens. This is based on extrapolated measurements of binding rates to macromolecules (Gehring *et al*. 1977). The seven liver cancer deaths reported for that cohort project to a lifetime risk of .039 (.089 upper confidence limit) per worker for liver cancers. That risk divided by the overall lifetime daily equivalent of effective exposure yields unit risk estimates for that malignancy.

The calculations provided the following upper confidence limits (UCL) on unit risks: 2.5×10^{-5} ppb^{-1} for liver cancers, and 4.5×10^{-5} ppb^{-1} for three sites of cancer combined, liver, lung and brain. Each of these three sites of cancer had a significantly elevated SMR when calculated for a 15-year follow up time. The unit risks calculated in this manner are about six times greater than would be calculated by using actual exposures instead of the effective exposures that take account of the metabolic saturation in the tissue.

Animal-derived risk estimates

The animal bioassay-based quantitative risk assessment analyses performed by CDHS (1990) used the computer program GLOBAL86 to calculate potential risks using a linearized multistage procedure that were associated with vinyl chloride exposure. Significant trends for liver angiosarcoma dominated the results of the modeling. All three analyses of female rats and two of the three analyses of male rats met the statistical criterion ($p > 0.05$) for goodness of fit of the dose-dependent response of liver angiosarcoma (LAS) to vinyl chloride. In addition, the following experimental groups met that criterion: lung carcinoma in the Swiss mice of Drew *et al*. (1983), lung angiosarcoma in the Wistar rats of Bi *et al*. (1985), and mammary tumors in both the Sprague Dawley rats of Maltoni *et al*. (1984) and the F-344 rats of Drew *et al*. (1985).

Table 11 gives unit risk estimates calculated by using the linearized multistage procedure for LAS and other tumor types from both male. and female rats and for female mice for inhalation experiments done by Maltoni *et al*. (1984), Bi *et al*. (1985), and Drew *et al*. (1983). The entries in Table 11 include all those instances in which an adequate fit of the data is achieved by the model using all data points for each species, sex, and tumor type at exposures not greater than 500 ppm, when practical. This exposure limitation tends to reduce the effects of the parent compound (including mortality) at the higher exposure levels. The analyses did include one higher exposure, the 3000 ppm exposure of Bi *et al*., which was retained in order to obtain an adequate number of exposure groups (four) to establish a clear trend.

The results of Table 11 do not include the analyses for angiosarcoma and mammary tumors in mice or the angiosarcoma, skin carcinoma, and mammary tumors in hamsters. The estimates for q_1^* for angiosarcomas and mammary tumors in mice were in the range of 20×10^{-5} to 50×10^{-5} ppb^{-1}, greatly elevated above those for rats while the estimates for those tumors in hamsters (6×10^{-5} and 1×10^{-4}) were about the same as the highest results in rats. None of these analyses met the stringent criteria for goodness of fit of the MLE as defined above, so they were not included in the tabulation of risk estimates.

Table 11: Risks of carcinogenicity from vinyl chloride exposure estimated from rodent data

Experiment	Strain/species, sex	Tumor	Rodent q_1^* (10^{-5} ppb^{-1})	Human[a] q_1^* (10^{-5} ppb^{-1})
Maltoni *et al*., (1984)				
BT-1,2 (≤ 500 ppm)	SD/rat, female	LAS	1.9	4.9
	SD/rat, female	mammary	1.4	3.7
BT-9, 15	SD/rat, female	LAS	6.7	18.0
	SD/rat, male	LAS	2.5	6.5
Bi *et al*. (1985)	Wi/rat, male	LAS	5.0	13.0
	Wi/rat, male	lung angiosarcoma	1.7	4.5
Drew *et al*. (1983)	F344/rat, female	LAS	3.2	8.4
	F344/rat, female	hepatocellular carcinoma	1.7	4.4
	F344/rat, female	mammary	1.6	4.2
	Sw/mouse, female	lung	6.9	20.0

[a] Determined by multiplying by the scaling factor on rodent dose.
SD = Sprague-Dawley; Wi = Wistar; F344 = Fischer 344; Sw = Swiss; LAS = liver angiosarcoma.

The effect of combining the BT (Maltoni *et al*. 1984) experiments was to lower the value of the resulting q_1^* by a modest amount. Thus BT-1 and BT-2 individually yielded values of 2.5×10^{-5} and 2.2×10^{-5} ppb^{-1} respectively, compared to 1.9×10^{-5} ppb^{-1} when combined. Also, BT-9 and BT-15 individually yielded values of 6.9×10^{-5} and 1×10^{-4} ppb^{-1}, compared to 6.7×10^{-5} ppb^{-1} when combined. The use of metabolized exposure rather than ambient exposure had the effect of increasing the values of q_1^* by about 30-50% in the BT-1 and BT-2 experiments. The effect on BT-9 and BT-15 was virtually negligible because of the much lower exposures experienced in those experiments.

Uncertainties in estimates of unit risk arise from uncertainties mentioned earlier about the accuracy of the model used to determine metabolized exposure. Departures from the present fit of the Michaelis-Menten model could cause calculations of risk to lose accuracy. Cumulative effects or different metabolism, for example, may cause the true risk to differ from that predicted. Nevertheless, uncertain as it is, the metabolic model appears much more likely to provide a more accurate measure of risk than does ambient exposure.

Final cancer unit risk calculation

Cancer risk estimates for VC derived from human and animal data provided the range of 95% UCLs on cancer unit risk for humans: from 2.5×10^{-5} to 2×10^{-4} ppb^{-1}. Because many of the tumors associated with vinyl chloride exposure (particularly LAS) exhibit a long latency period, exposure at an early age would produce a greater risk. The average latency period for the development of LAS in one study of occupationally exposed vinyl chloride workers was determined to be 22.1 years (Stafford, 1983). Drew *et al*. (1983) demonstrated that in rats, mice and hamsters, the highest incidence of neoplasms was observed when vinyl chloride exposure was started early in life.

Exposures early in life may produce up to a 10-fold greater incidence in tumors compared to exposures late in life.

Because of these considerations, CDHS decided that the best estimate of unit risk coincided with the top of the range, which was, when rounded, 2×10^{-4} ppb^{-1}, or 7.8×10^{-5} $(\mu g/m^3)^{-1}$. This is approximately the value obtained from the more recent Maltoni *et al*. experiments, with lower exposure concentrations than the previous experiments. That result is at the top of the range of six experiments that provided clear dose response relationships for liver cancer. The selected top of the range, 2×10^{-4} ppb^{-1} is also equal to the Drew *et al*. result for lung carcinoma in mice. That result is one of the lowest for mice. The other, higher results for mice are not explicitly reported in the present risk analysis because of scattering of points in each case not providing a clear exposure-response trend. The results for hamsters, not reported quantitatively for the same reason, were close to those for the rats. This approach was considered to provide adequately health protective estimates of human unit risks, which represent the 95% upper confidence limits for risk calculations.

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Attachment

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United States Environmental Protection Agency

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Provisional Peer-Reviewed Toxicity Values for

2,6-Dinitrotoluene
(CASRN 606-20-2)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2,6-DINITROTOLUENE (CASRN 606-20-2)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

2,6-Dinitrotoluene (also called 2,6-DNT) has several names, including toluene, 2,6-dinitro-; 2-methyl-1,3-dinitrobenzene or benzene, 2-methyl-1,3-dinitro; and 1-methyl-2,6-dinitrobenzene (U.S. EPA, 1990; IARC, 1996; NLM, 2011). The isomer 2,6-dinitrotoluene often occurs in a mixture with the isomer 2,4-dinitrotoluene (2,4-DNT), made by combining toluene with mixed nitric and sulfuric acids. The isomeric composition of dinitrotoluene (DNT) may vary, but technical grade DNT (CASRN 25321-14-6) refers to a mixture of approximately 76% 2,4-DNT and 19% 2,6-DNT. The remaining 5% is a combination of the remaining four dinitrotoluene isomers: 2,3-DNT, 2,5-DNT, 3,4-DNT, and 3,5-DNT. In the literature, this mixture is also called dinitrotoluene (isomers mixture), DNT or DNT 80/20. In this document, the name technical grade DNT (tgDNT) is used as a representative of this mixture composition (76% 2,4-DNT and 19% 2,6-DNT). DNT is used to make flexible polyurethane foams used in the bedding and furniture industries, and as a chemical intermediate in the production of toluene diamines and diisocyanates. DNT is also used to produce dyes, explosives, and propellants (IARC, 1996; ATSDR, 1998). The empirical formula for 2,6-DNT is $C_7H_6N_2O_4$, and its structure is shown in Figure 1. A table of the physicochemical properties of 2,6-DNT is provided in Table 1.

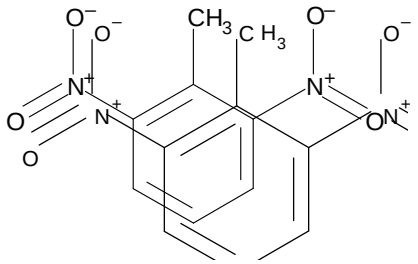

Figure 1. 2,6-Dinitrotoluene Structure

Table 1. Physicochemical Properties for 2,6-Dinitrotoluene (CASRN 606-20-2)[a]

Property (unit)	Value
Boiling point (ºC)	285
Melting point (ºC)	66
Density (g/cm^3)	1.2833 at 111ºC
Vapor pressure (mm Hg at 25ºC)	5.67×10^{-4}
pH (unitless)	ND
Solubility in water (mg/L at 20ºC)	180
Relative vapor density (air = 1)	6.28
Molecular weight (g/mol)	182.14

[a]Values from NLM (2011) and IARC (1996).

ND = No data.

IRIS has developed assessments for 2,4-DNT (approximately 98% 2,4-DNT and 2% 2,6-DNT; U.S. EPA,1993)and for a 2,4-/2,6-DNT mixture (various compositions of DNTs; U.S. EPA, 1990). There is also a PPRTV assessment for tgDNT (approximated as 76% 2,4-DNT and 19% 2,6-DNT; U.S. EPA, 2013). Table 2 provides a summary of available toxicity values from the U.S. Environmental Protection Agency (U.S. EPA) and other agencies/organizations for tgDNT, the 2,4-DNT and 2,6-DNT isomers, and the 2,4-/2,6-DNT mixture. A previous PPRTV for 2,6-DNT was posted in 2004 (U.S. EPA, 2004; see Table 2). The purpose of this PPRTV is to review and update the toxicity of 2,6-DNT (approximately 99% 2,6-DNT).

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Cancer							
IRIS/OSF	NV	NV	NV	6.8×10^{-1} per mg/kg-d	IRIS entry is for 2,4-/2,6-DNT mixture (various compositions of DNTs) with no CASRN; principal study used rats dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT to determine OSF	U.S. EPA (1990)	9-13-2012
IRIS/drinking water unit risk	NV	NV	NV	1.9×10^{-5} per µg/L	IRIS entry is for 2,4-/2,6-DNT mixture (various compositions of DNTs) with no CASRN; principal study used rats dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT to determine OSF	U.S. EPA (1990)	9-13-2012
HEAST	NV	NV	NV	NV	None	U.S. EPA (2003)	9-13-2012
IARC/cancer WOE	NV	NV	NV	NV	Group 2B—Possibly carcinogenic to humans for 2,4- and 2,6-DNT	IARC (1996)	9-13-2012
NTP	NV	NV	NV	NV	None	NTP (2011)	9-13-2012
Cal EPA/unit risk	NV	8.9×10^{-5} per $\mu g/m^3$	NV	NV	Data source was RCHAS-S	Cal EPA (2009)	9-13-2012
Cal EPA/OSF	NV	3.1×10^{-1} per mg/kg-d	NV	NV	Data source was RCHAS-S	Cal EPA (2009)	9-13-2012

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
ACGIH (cited in NLM, 2011)	NV	NV	NV	NV	Group A3—Confirmed animal carcinogen with unknown relevance to humans for tgDNT, 2,4- and 2,6-DNT	NLM (2011)	9-13-2012
Drinking Water/ cancer risk health advisory	5×10^{-3} mg/L	5×10^{-3} mg/L	5×10^{-3} mg/L	NV	None	U.S. EPA (2011a)	9-13-2012
Health effect assessment	2.3×10^{-1} per mg/kg-d[d] and 2.1×10^{-1} per mg/kg-d[e]	6.8×10^{-1} per mg/kg-d[f]	NV	NV	[d]Based on a 104-wk study in rats with increased incidence of liver tumors in males; [e]Based on a 104-wk study in rats with increased incidence of liver tumors in females; [f]Based on a 2-yr study in rats with increased incidence of combined mammary/hepatic tumors	U.S. EPA (1987)	2-6-2013
PPRTV	4.5×10^{-1} per mg/kg-d (screening p-OSF)	NV	NV	NV	Based on a $BMDL_{10HED}$ of 0.224 from a 104-wk study in rats with increased incidence of liver hepatocellular carcinomas, liver neoplastic nodules, mammary fibroadenomas and subcutaneous fibromas in males	U.S. EPA (2013)	4-3-2013

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
Noncancer							
ACGIH/TLV	0.2 mg/m^3	NV	NV	NV	NA	NLM (2011)	9-13-2012
ATSDR/acute oral MRL	NV	5×10^{-2} mg/kg-d	NV	NV	Toxicological profile for 2,4-DNT; based on neurotoxicity in dogs	ATSDR (1998)	11-21-2012
ATSDR/chronic or intermediate-duration oral MRL	NV	2×10^{-3} mg/kg-d[g]	4×10^{-3} mg/kg-d[h]	NV	[g]Chronic oral MRL for 2,4-DNT; based on neurotoxicity, Heinz bodies, and biliary tract hyperplasia in dogs; [h]Intermediate-duration oral MRLfor 2,6-DNT based on hematological effects of splenic extramedullary erythropoiesis and lymphoid depletion in dogs	ATSDR (1998)	11-21-2012
Cal EPA/REL	NV	NV	NV	NV	NA	Cal EPA (2012a, b)	8-1-2012
Drinking water	NV	2×10^{-3} mg/kg-d (1-d Health advisory) 1×10^{-1} mg/L (Drinking water equivalent level) 1×10^{0} mg/L (1- and 10-d Health advisory for a 10-kg child)	1×10^{-3} mg/kg-d (1-d Health advisory) 4×10^{-2} mg/L (Drinking water equivalent level) 4×10^{-1} and 4×10^{-2} mg/L (1- and 10-d Health advisory for a 10-kg child)	NV	NA	U.S. EPA (2011a)	2-6-2013

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
NIOSH/REL	1.5 mg/m^3	NV	NV	NV	TWA for 10-hr workday; document specifies CASRN for tgDNT but notes that various isomers of DNT exist	NIOSH (2007)	9-13-2012
OSHA/PEL	1.5 mg/m^3	NV	NV	NV	TWA for 8-hr workday	OSHA (2006)	9-13-2012
IRIS/Oral RfD	NV	2×10^{-3} mg/kg-d	NV	NV	Based on a 2-yr study in dogs dosed with 98% 2,4-DNT and 2% 2,6-DNT; critical effect of CNS neurotoxicity, Heinz bodies in erythrocytes, and hyperplasia of biliary tract	U.S. EPA (1993)	9-13-2012
IRIS/Inhalation RfC	NV	NV	NV	NV	None	U.S. EPA (1990)	9-13-2012
HEAST/ subchronic Oral RfD	NV	2×10^{-3} mg/kg-d	NV	NV	Based on a 2-yr study in dogs dosed with a mixture of 98% 2,4-DNT and 2% 2,6-DNT; critical effect of CNS neurotoxicity, Heinz bodies in erythrocytes, and hyperplasia of biliary tract	U.S. EPA (2003)	9-13-2012
Health effects assessment	NV	NV	NV	NV	NA	U.S. EPA (1987)	2-6-2013

Table 2. Summary of Available Toxicity Values for tgDNT (CASRN 25321-14-6), 2,4-DNT (CASRN 121-14-2), 2,6-DNT (CASRN 606-20-2), and 2,4-/2,6-DNT Mixture (no CASRN)[a]

Source/ Parameter[b,c]	tgDNT Value (approximately 76% 2,4-DNT and 19% 2,6-DNT)	2,4-DNT Value (approximately 98% 2,4-DNT and 2% 2,6-DNT)	2,6-DNT Value (approximately 99% 2,6-DNT)	2,4-/2,6-DNT Mixture Value (various compositions of DNTs)	Notes	Reference	Date Accessed
PPRTV	5×10^{-3} mg/kg-d (screening subchronic p-RfD)[i] 9×10^{-4} mg/kg-d (screening chronic p-RfD)[j]	NV	1×10^{-2} mg/kg-d (subchronic p-RfD)[k] 1×10^{-3} mg/kg-d (chronic p-RfD)[k]	NV	[i]Based on a $BMDL_{10HED}$ of 0.52 mg/kg-d for hepatic necrosis in a 26-week oral study in male rats; [j]Based on a $BMDL_{10HED}$ of 0.087 mg/kg-d for hepatic necrosis in a 104-week oral study in male rats; [k]Based on a NOAEL of 4 mg/kg-d for numerous health effects in a 13-wk oral study in male and female dogs	tgDNT is U.S. EPA (2013); 2,6-DNT is U.S. EPA (2004)	4-3-2013 and 2-6-2013
CARA HEEP	NV	NV	NV	NV	None	U.S. EPA (1994)	9-13-2012
WHO	NV	NV	NV	NV	None	WHO (2012)	8-1-2012

[a]No information was available from any source for 2,3-, 2,5-, 3,4-, and 3,5-DNT.
[b]Sources: Integrated Risk Information System (IRIS); Health Effects Assessment Summary Tables (HEAST); International Agency for Research on Cancer (IARC); National Toxicology Program (NTP); California Environmental Protection Agency (Cal EPA); American Conference of Governmental Industrial Hygienists (ACGIH); Agency for Toxic Substances and Disease Registry (ATSDR); National Institute for Occupational Safety and Health (NIOSH); Occupational Safety and Health Administration (OSHA); Chemical Assessments and Related Activities (CARA); Health and Environmental Effects Profile (HEEP); World Health Organization (WHO).
[c]Parameters: weight of evidence (WOE); reference dose (RfD); reference concentration (RfC); oral slope factor (OSF); minimum risk level (MRL); time-weighted average (TWA); reference exposure level (REL); permissible exposure limit (PEL); Reproductive and Cancer Hazard Assessment Section (RCHAS).
[d–k]See notes column for corresponding information.

NA = not applicable; NV = not available.

Literature searches were conducted on sources published from 1900 through February 2013 for studies relevant to the derivation of provisional toxicity values for 2,6-DNT CAS Number (606-20-2). The following databases were searched by chemical name, synonyms, or CASRN: ACGIH, ANEUPL, ATSDR, BIOSIS, Cal EPA, CCRIS, CDAT, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HERO, HMTC, HSDB, IARC, INCHEM IPCS, IPA, ITER, IUCLID, LactMed, NIOSH, NTIS, NTP, OSHA, OPP/RED, PESTAB, PPBIB, PPRTV, PubMed (toxicology subset), RISKLINE, RTECS, TOXLINE, TRI, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, and U.S. EPA TSCATS/TSCATS2. The following databases were searched for toxicity values or exposure limits: ACGIH, ATSDR, Cal EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 3 provides an overview of the relevant database for 2,6-DNT and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. The phrase, "statistical significance" used throughout the document, indicates a *p*-value of <0.05.

Table 3. Summary of Potentially Relevant Data for 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
2. Inhalation (mg/m^3)[a]								
Acute[c]	ND							
Short-term[d]	ND							
Long-term[e]	ND							
Chronic[f]	ND							
Animal								
1. Oral (mg/kg-d)[a]								
Short-term	6/0, Sprague-Dawley rat, gavage, 14 d	0, 4, 7, 14, 35, 68, 134 mg/kg-d	Mild anemia at 14 mg/kg-d, increased relative kidney weight at ≥7 mg/kg-d, decreased body weight at ≥35 mg/kg-d, increased ALT activity at ≥68 mg/kg-d, increased absolute and relative spleen weight at ≥68 mg/kg-d; decreased absolute and relative testes weight and absolute epididymides weight at 134 mg/kg-d; histopathological changes in various organs at ≥35 mg/kg-d	7	DU	14	Lent et al. (2012a)	PR

Table 3. Summary of Potentially Relevant Data for 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Subchronic	16/16, CD rat, dietary, 4 or 13 wk	0, 7, 35, 145 (M); 0, 7, 37, 155 (F) (Adjusted)	Lesions in the liver and spleen at ≥35 mg/kg-d, testicular atrophy and aspermatogenesis in males at 145 mg/kg-d	7	DU	35	Lee et al. (1976a)	NPR
Subchronic	16/16, Albino Swiss mouse, dietary, up to 13 wk	0, 11, 51, 289 (M); 0, 11, 55, 299 (F) (Adjusted)	Mortality at ≥51 mg/kg-d, decreased relative liver weight at ≥11 mg/kg-d in males, decreased relative kidney weight at 51 mg/kg-d in males	NDr	DU	11	Lee et al. (1976b)	NPR
Subchronic	**4/4, Beagle dog, oral (gelatin capsules), d/wk not reported, 4 or 13 wk**	**0, 4, 20, 100 (Adjusted)**	**Mortality, neurotoxicity, hematological effects, histopathology of liver, kidney, and testes, anemia, Heinz bodies, and methemoglobinemia at ≥20 mg/kg-d, splenic extramedullary hematopoiesis at ≥4 mg/kg-d**	**NDr**	**DU**	**4**	**Lee et al. (1976c)**	**PS, NPR**
Chronic	28/0, F344/Cr1BR rat, dietary, 26 or 52 wk	0, 7, 14 (Adjusted)	Decreased body weight, increased relative liver weight, increased ALT activity; all at ≥7 mg/kg-d	NDr	0.69 for increased relative liver weight at 52 wk	7	Leonard et al. (1987)	PR
Developmental	ND							
Reproductive	ND							
Carcinogenicity	30/0, F344 rat, diet (high in pectin, pectin-free, or 5% pectin), up to 12 mo	ADD: 0, 0.6−0.7, 3.0−3.5 HED: 0, 0.13–0.15, 0.63–0.74	Increase in hepatocellular carcinomas and neoplastic nodules in rats fed diet high in pectin content at 3.0−3.5 mg/kg-d	NA	NA	NA	Goldsworthy et al. (1986); Tumors were only observed in rats fed 2,6-DNT in diet high in pectin content	PR

Table 3. Summary of Potentially Relevant Data for 2,6-Dinitrotoluene (CASRN 606-20-2)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (Comments)	Notes[b]
Carcinogenicity	**28/0, F344/CrlBR rat, dietary, 52 wk**	**ADD: 0, 7, 14** **HED: 0, 1.9, 3.6**	**Increase in neoplastic nodules and hepatocellular carcinomas at 7 and 14 mg/kg-d**	**NA**	**0.25 for increased hepatocellular carcinomas**	**NA**	**Leonard et al. (1987)**	**PS, PR**
Carcinogenicity	26/26, A/J mouse, gavage, 2 d/wk, 12 wk	ADD: 0, 343.9, 857.1, 1714 HED: 0, 79, 198, 396	No increase in lung tumor incidence	NA	NA	NA	Stoner et al. (1984)	PR
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects. Values are also presented as a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure.
[b]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study; PR = Peer reviewed; NPR = Not peer reviewed.
[c]Acute = Exposure for 24 hr or less (U.S. EPA, 2002).
[d]Subchronic = Repeated exposure for >24 hr ≤30 d (U.S. EPA, 2002).
[e]Long-term = Repeated exposure for >30 d ≤10% lifespan (based on 70 yr typical lifespan) (U.S. EPA, 2002).
[f]Chronic = Repeated exposure for ≥10% lifespan (U.S. EPA, 2002).

DU = Data unsuitable, NA = Not applicable, NV = Not available, ND = No data, NDr = Not determined, NI = Not identified, NP = Not provided, NR = Not reported, NR/Dr = Not reported but determined from data, NS = Not selected, FEL = Frank effect level.

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of oral exposure to 2,6-DNT in humans have been identified.

Inhalation Exposures

No data on the effects of pure 2,6-DNT in humans following inhalation exposure are identified.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to 2,6-DNT have been evaluated in one short-term (Lent et al. 2012a), one subchronic (Lee et al., 1976), one chronic (Leonard et al., 1987), and three carcinogenicity studies (Goldsworthy et al., 1986; Leonard et al., 1987; Stoner et al., 1984).

Short-term Studies

Lent et al., 2012a

In a peer-reviewed study, Lent et al. (2012a) investigated the effects of short-term oral administration of various DNT isomers including 2,6-DNT in male rats. Groups of Sprague-Dawley rats (6/males/dose level) were gavaged with 2,6-DNT (>99% pure) at doses of 0, 4, 7, 14, 35, 68, 134 mg/kg-day for 14 days. All animals were observed twice a day for clinical signs of toxicity and morbidity. Body weight and food consumption were measured on Days 0, 1, 3, 7, and 14. Blood samples were collected for hematology and clinical chemistry tests at study termination prior to necropsy. Weights of the liver, spleen, kidneys, heart, brain, testes, and epididymides were recorded and relative organ weights were calculated. Various tissues underwent histopathological examination.

The study authors observed no clinical signs of toxicity. At study termination, absolute body weight exhibited a biologically relevant decrease (>10%) at 35, 68, and 134 mg/kg-day. This decrease in body weight was statistically significant at 134 mg/kg-day. Food consumption was also statistically significantly decreased at 134 mg/kg-day from Days 0 to 7. The study authors noted the following statistically significant changes in blood parameters: decreased hemoglobin and hematocrit in all dose groups, increased total and percent neutrophils at 68 and 134 mg/kg-day, increased monocytes and percent lymphocytes at 134 mg/kg-day, decreased albumin and total protein in all dose groups, decreased chlorine at 134 mg/kg-day, and increased ALT and AST at 68 and 134 mg/kg-day. Mild anemia was reported at 14 mg/kg-day. Relative kidney weight was statistically significantly increased at 14, 68, and 134 mg/kg-day. Relative spleen weight was statistically significantly increased at 68 and 134 mg/kg-day, and absolute spleen weight was statistically significantly increased at 134 mg/kg-day. Absolute and relative testes and absolute epididymides weights were statistically significantly decreased at 134 mg/kg-day. With respect to histopathological examination, the following changes were observed: tubular degeneration, multinucleated giant cell formation, and interstitial atrophy in the testes at 68 and 134 mg/kg-day and hepatocellular hyperplasia, oval cell hyperplasia, and hepatocellular hypertrophy were observed in the liver at ≥35 mg/kg-day. Mitotic activity, cell necrosis, and karyocytomegaly were also noted in the liver at ≥68 mg/kg-day. Proximal tubule degeneration and renal tubular basophilia were observed in the kidney at 134 mg/kg-day, as well as lymphoid hyperplasia at ≥68 mg/kg-day. Splenic extramedullary hematopoiesis was reported

at 68 and 134 mg/kg-day along with lymphoid depletion of the spleen at 134 mg/kg-day. The study authors did not report statistical analyses for any of the histopathological changes. Based on mild anemia and increased relative kidney weight, a LOAEL of 14 mg/kg-day is determined with a corresponding NOAEL of 7 mg/kg-day.

Subchronic Studies

Lee et al., 1976

Lee et al. (1976) conducted a series of tests investigating the subchronic oral toxicity of 2,6-DNT in rats, mice, and dogs. For the sake of clarity, in this document, the study is divided into three separate summaries (Lee et al., 1976) based on the species tested. These studies are not considered to be peer reviewed.

Lee et al., 1976a

Groups of CD rats (16/sex/dose level) were fed diets containing 2,6-DNT (>99% pure) at 0, 0.01, 0.05, or 0.25% for 4 or 13 weeks. These doses were calculated by the study authors to be equivalent to 0, 7, 35, or 145 mg/kg-day for males and 0, 7, 37, or 155 mg/kg-day for females. All animals were observed for clinical signs of toxicity and behavioral changes. Body weight was recorded weekly while food consumption was determined throughout the study. At 4, 8, 13, and/or 17 weeks, blood samples were collected for hematology and clinical chemistry tests. Of the 16 rats/sex/group, 4 were sacrificed at 4 weeks, and 4 were sacrificed at 13 weeks. Additionally, the treatment of 4 rats/sex/group was discontinued at the end of 4 and 13 weeks. These rats were kept for observation for 4 weeks and necropsied for examination at 8 and 17 weeks, respectively, to study the reversibility of any adverse effects and were examined for gross lesions. Weights of the liver, spleen, kidneys, heart, and brain were recorded. Relative organ weights were calculated. Various tissues were removed and stained for microscopic examination of lesions.

Lee et al. (1976a) reported that treatment with 2,6-DNT did not cause overt neuromuscular signs, but rats in the high-dose group (145 mg/kg-day [males], 155 mg/kg-day [females]) were less active and had rough coats and signs of malnutrition. There were no deaths during the study. Tables B.1 and B.2 provide treatment-related effects on body weights and absolute and relative organ weights. Body weights were markedly and consistently reduced at the mid- and high-doses of 35 and 145 mg/kg-day in males and 37 and 155 mg/kg-day in females throughout the exposure period. At 13 weeks, absolute body weights were 17 and 25% lower than controls in mid-dose males and females, respectively, and 53 and 38% lower than controls in high-dose males and females, respectively. Absolute body weights were also reduced in males and females at the low dose of 7 mg/kg-day for much of the study, but the difference from controls was less than 10% (9.8% in males and 5.5% in females).

Lee et al. (1976a) found that after 13 weeks, there were several statistically significant organ weight changes. Statistically significant increases in relative liver weight in males were observed in the liver at 7 mg/kg-day and at 145 mg/kg-day (but not at 35 mg/kg-day) and in females at 155 mg/kg-day. Absolute liver weight was statistically significantly increased in males at 35 and 145 mg/kg-day. Relative organ weights (spleen, kidneys, heart, and brain) were statistically significantly increased in males at 145 mg/kg-day. There were also statistically significant increases in absolute spleen, kidney, and heart weights in male rats at 145 mg/kg-day. Relative spleen weights were statistically significantly increased in females at 155 mg/kg-day,

and relative brain weights were statistically significantly increased at 37 and 155 mg/kg-day. Study authors also reported a statistically significant decrease in absolute heart weight in females at 155 mg/kg-day.

Lee et al. (1976a) observed no significant and/or consistent treatment-related hematological effects in the low-dose rats (7 mg/kg-day). A statistically significant decrease in reticulocyte count from baseline (baseline refers to data obtained from rats during pretreatment) was observed in control males and males at 7 and 35 mg/kg-day at 4 and 13 weeks (see Table B.3). A statistically significant increase in leukocyte count was observed in males at 35 mg/kg-day at Week 13. In addition, a statistically significant increase in erythrocyte count was observed in males at this dose group (35 mg/kg-day) at Weeks 4 and 13. This increase in erythrocyte count was considered as mild by the study authors and was not seen in male controls or males fed the low or high level of 2,6-DNT. Males at 145 mg/kg-day showed a decrease in erythrocyte count with a compensatory reticulocytosis and an increase in leukocyte count at 4 weeks; these parameters partially recovered at 8 (data not shown) or 13 weeks. Females fed 7 and 37 mg/kg-day had a statistically significant decrease in reticulocyte count at 4 and 13 weeks when compared to baseline. Females fed 37 mg/kg-day had a statistically significant increase in leukocytes at 13 weeks (see Table B.3). Females at 155 mg/kg-day showed an increase in leukocyte and reticulocyte count at 4 weeks; these parameters partially recovered at 13 weeks. A statistically significant increase in methemoglobin was seen in males at 145 mg/kg-day and in females at 155 mg/kg-day after 8 (data not shown) or 13 weeks; however, after recovery for an additional 4 weeks, this increase was no longer observed (data not shown). In summary, the only significant hematological changes from control were increased leukocytes (males), increased reticulocytes (males and females), and decreased erythrocytes (males) following 4 weeks of exposure to the high dose of 2,6-DNT (145 mg/kg-day [males] and 155 mg/kg-day [females]).

Lee et al. (1976a) reported extramedullary hematopoiesis in the spleen and liver; also, bile duct hyperplasia in the liver was observed in males at 35 mg/kg-day and in females at 37 mg/kg-day following 13 weeks of exposure (see Tables B.4 and B.5). Hemosiderosis was also observed in the spleen in males at 145 mg/kg-day and in females at 155 mg/kg-day at 13 weeks. Focal atrophy of the testes was observed in control and low dose males at 13 weeks. Retardation of spermatogenesis was observed at 13 weeks in mid-dose males, and atrophy of the testes and aspermatogenesis were observed in males at 145 mg/kg-day. In general, the effects on the testes, spleen, and liver were more frequent and more severe in the high-dose rats than in the mid-dose rats. Only partial recovery of the tissue lesions was seen after the 4-week recovery periods (data not shown).

Lee et al. (1976a) observed several effects in animals exposed to 2,6-DNT. Decreases in body weights (greater than 10%) were biologically significant in both males and females; however, study authors suggested that the decreases in body weight could be due to decreased food consumption. Therefore, decreased body weight could be due to the effect of 2,6-DNT on food palatability and not necessarily a systemic toxicological effect of the chemical. Additionally, hematological effects are noted but are only significantly different from control at the high-dose in animals exposed for 4 weeks, with no significant effects, as compared to control, at 12 weeks. Histopathology revealed lesions in the liver (i.e., hematopoiesis and bile duct hyperplasia) that were statistically significantly increased in males exposed to 35 mg/kg-day for 13 weeks and in females exposed to 37 mg/kg-day; incidence of bile duct hyperplasia was

statistically significant at 145 mg/kg-day in males and 155 mg/kg-day in females. Incidence of splenic hemosiderosis was statistically significantly increased as compared to controls in males exposed to 7 mg/kg-day for 13 weeks. However, at the next dose tested of 35 mg/kg-day, splenic hemosiderosis was not seen in any of the rats examined. While effects were also noted in the testes, atrophy observed in the control and low-dose group makes these effects difficult to interpret. Thus, based on the increased incidences of splenic and liver hematopoiesis in male rats, a LOAEL of 35 mg/kg-day is identified from this study with a corresponding NOAEL of 7 mg/kg-day.

Lee et al., 1976b

Lee et al. (1976b) fed groups of 16 male and 16 female albino Swiss mice a diet containing 0, 0.01, 0.05, or 0.25% 2,6-DNT (>99% pure) for up to 13 weeks. According to the authors, the corresponding intakes of test material were 0, 11, 51, or 289 mg/kg-day for males and 0, 11, 55, or 299 mg/kg-day for females. The basic design and procedure for this study were the same as that described for rats (Lee et al., 1976a); however, blood clinical chemistry tests in mice were not performed.

Lee et al. (1976b) reported no compound-related effects in mice at 11 mg/kg-day. However, several deaths occurred during the study, including 3 males in the control group (Week 12), 2 males in the low-dose group of 11 mg/kg-day (Weeks 1 and 3), 8 males and 1 female in the mid-dose group of 51 mg/kg-day (males) and 55 mg/kg-day (females), and 8 males and 6 females in the high-dose group of 289 mg/kg-day (males) and 299 mg/kg-day (females). Furthermore, all males in the high-dose group died before Week 9, while two of eight females in the high-dose group survived the full 13 weeks of feeding. The authors stated that in the mid- and high-dose groups, most of the deaths could be contributed to 2,6-DNT administration. The exact cause of death was not discussed, but most mice that died had low body weight, frequently with significant weight loss a week or two before death. In the mid- and high-dose groups, food consumption was lower than in controls. Blood analyses in the mid- and high-dose groups revealed a number of statistically significant changes relative to the controls at the respective time intervals; however, the authors stated that these changes were mild, inconsistent, and not related to 2,6-DNT exposure. The study authors also observed decreased absolute and relative liver weight (≥10%) at 11 mg/kg-day in male mice treated for 13 weeks. Absolute kidney weight in male mice was increased (≥10%) at 11 mg/kg-day and then decreased at 51 mg/kg-day. The biological significance of the observed decrease in absolute liver and kidney weights is unknown due to the accompanied increases in body weight that were biologically significant at 11 and 51 mg/kg-day in mice treated for 13 weeks. Relative kidney weight was biologically and statistically significantly decreased at 51 mg/kg-day in male mice treated for 13 weeks.

Lee et al. (1976b) noted marked aspermatogenesis in all males at 289 mg/kg-day, and depressed spermatogenesis was seen in one male from the mid-dose group (51 mg/kg-day) treated for 4 weeks and in two males from the low-dose group (11 mg/kg-day) treated for 4 or 13 weeks. Bile duct hyperplasia occurred in the only mouse that survived treatment with the high dose of 2,6-DNT for 13 weeks and in two mice fed the mid-dose for 13 weeks. Bile duct hyperplasia was also observed in two high-dose mice treated for 4 weeks and allowed to recover for 4 weeks, suggesting that this lesion developed slowly. The investigators indicated that extramedullary hematopoiesis in the liver and spleen was seen more often in mice treated with 2,6-DNT than in the controls and that generally, the incidence and severity were dose related.

No testicular lesions were observed in mice treated for 4 weeks and allowed to recover for 4 weeks. Because no high-dose males survived longer then Week 9, it is not known whether testicular lesions would have occurred in this dose group following 13 weeks of treatment. There was partial recovery of the bile duct hyperplasia after the 4-week recovery period, but extramedullary hematopoiesis continued to be observed in the liver and/or spleen. A LOAEL of 11 mg/kg-day is identified based on decreased relative liver weight. Because 11 mg/kg-day is the lowest dose tested, a NOAEL could not be determined.

Lee et al., 1976c

Lee et al. (1976c) is selected as the principal study for deriving the screening subchronic and chronic p-RfDs. Lee et al. (1976c) dosed groups of beagle dogs (4/sex/dose level) with 2,6-DNT (>99% pure) in gelatin capsules at doses of 0, 4, 20, or 100 mg/kg-day for 4 or 13 weeks. The dogs were evaluated in the same manner as in the rat study (Lee et al., 1976a) with the following exceptions: at the end of 4 and 13 weeks, one male and one female dog from each group were euthanized for necropsy; liver, spleen, kidneys, brain, adrenals, thyroid and gonads were examined and the weights recorded; the treatment for one male and one female dog from each group was discontinued at the end of 4 and 13 weeks and observed until necropsy at the end of 8 and 17 weeks, respectively; food consumption was recorded daily.

Lee et al. (1976c) observed the deaths of all dogs receiving 100 mg/kg-day of 2,6-DNT were between Weeks 2 and 8 (one dog died during the second week, and the last dog on this treatment died in the eighth week). The signs exhibited by these dogs consisted of listlessness, incoordination, lack of balance, pale gums, dark urine, and weakness (particularly of the hind limbs); tremors were seen occasionally. Terminal signs seen in some dogs included yellow gums and darkened sclera. Because of the severity of symptoms observed in the dogs exposed to 100 mg/kg-day of 2,6-DNT, they were placed on the reversibility study after 4 weeks and continued for 19 weeks (23 weeks total) before they were sacrificed. Similar symptoms were observed in dogs at 20 mg/kg-day but were less severe. Signs of toxicity in the mid-dose group (20 mg/kg-day) were not seen until Week 4. Two female dogs at 20 mg/kg-day died during Week 9. A Fisher's exact test comparing death in the control and mid-dose groups yielded a *p*-value of 0.233, indicating a nonstatistically significant difference. However, group sizes were too small for the statistical test to have much power to detect an effect and the deaths may have been compound-related, as gross necropsy showed emaciation and jaundice. No significant treatment-related effects occurred in dogs at 4 mg/kg-day (other than mild splenic extramedullary hematopoiesis in some dogs). However, animals at both 20 and 100 mg/kg-day showed clear signs of toxicity (neurological, hematological, and liver histopathology), and the incidence and severity of the effects were dose related. Extramedullary hematopoiesis in the spleen was observed at 4 mg/kg-day and appeared to be reversible depending upon the length of exposure and postexposure recovery period even at the higher doses.

Lee et al. (1976c) found no significant alterations in body weight in animals receiving 2,6-DNT at 4 mg/kg-day, but dogs at 20 mg/kg-day began to lose weight during Weeks 4 and 5, which correlated with the adverse effects previously noted. Dogs at 100 mg/kg-day lost weight from the first week of treatment. Food consumption correlated with weight changes. During the reversibility studies, the affected dogs quickly returned to normal food consumption rates.

Due to the mortality observed in one dog in the high-dose group during Week 2, the study authors collected blood samples from all surviving dogs in all dose groups at the end of Week 2, in addition to the scheduled analyses (Lee et al., 1976c). Control dogs and dogs at 4 mg/kg-day showed mild fluctuations in hematology and clinical chemistry parameters, which were not considered to be biologically significant by the study authors. However, significant effects were observed at 2 weeks in dogs exposed to 20 mg/kg-day, including anemia characterized by decreases in hematocrit and hemoglobin with a compensatory reticulocytosis. Small amounts of methemoglobin were seen at Week 8, and Heinz bodies were seen at Week 13. Serum alanine aminotransferase (ALT) activity was increased at Weeks 8 and 13. One of the females that died in Week 9 was severely anemic, with large amounts of Heinz bodies and methemoglobin, and elevated serum ALT, aspartate aminotransferase (AST), and alkaline phosphatase (AP). Blood analysis done on Week 2 in dogs at 100 mg/kg-day showed severe effects, including a 66% reduction in RBC and signs of immature erythrocytes. Also evident was leukocytosis, with an increased percentage of neutrophils and decreased percentage of lymphocytes, and increased serum AP and ALT activities. Laboratory data from dogs in the mid-dose group (20 mg/kg-day) treated for 4 or 13 weeks showed recovery after 4 weeks, but high-dose dogs (100 mg/kg-day) treated for 4 weeks did not recover until Week 19.

No significant alterations in organ weights were seen in dogs at 4 mg/kg-day compared to the control group (Lee et al., 1976c). Treatment-related histological alterations in dogs at both 20 and 100 mg/kg-day after 4 weeks of treatment included extramedullary hematopoiesis in the liver and spleen, bile duct hyperplasia, degeneration and/or subacute inflammation in the liver, and degeneration and/or depression of spermatogenesis in the testes. The incidence and severity of these lesions were generally dose related. Lymphoid depletion in the spleen and lymph node, and involution of the thymus were also seen in high-dose animals. A female dog from the low-dose group (4 mg/kg-day) had several graafian follicles but no corpora lutea. This female also had mild extramedullary hematopoiesis in the spleen. Because this effect was observed in dogs given this dose for 13 weeks and in dogs given higher doses, these alterations were considered to be compound related. Treatment up to 13 weeks with the mid- or high-dose of 2,6-DNT caused similar lesions in the liver and spleen. It also caused kidney effects consisting of dilated tubules, foci of inflammation, degeneration, yellow pigment, and/or casts in the tubules. The high-dose caused lesions in the testes, lymph nodes, and thymus. The effects observed in dogs treated with 20 mg/kg-day of 2,6-DNT at 13 weeks were usually more numerous and more severe than those seen at 4 weeks. Mild extramedullary hematopoiesis and lymphoid depletion in the spleen of some dogs at 4 mg/kg-day were also considered compound related by the study authors. Splenic extramedullary hematopoiesis was observed in all dogs treated at 4, 20, and 100 mg/kg-day for 13 weeks. In dogs treated for 4 weeks and allowed to recover, extramedullary hematopoiesis and adverse testicular effects were milder. Two dogs receiving 2,6-DNT at 100 mg/kg-day and allowed to recover for 19 weeks showed complete recovery. Dogs treated for 13 weeks did not show full recovery, as one dog in the mid-dose group still had various lesions in the liver, kidney, and testes, and a low-dose female dog still had minimal bile duct hyperplasia. In dogs treated for 13 weeks and allowed to recover for 4 weeks, splenic extramedullary hematopoiesis was not observed in any dose group. To determine whether treatment with 2,6-DNT causes an allergic reaction, the study authors measured its effects on serum IgE levels. The results revealed no apparent change in serum IgE concentration.

A LOAEL of 4 mg/kg-day is identified based on splenic extramedullary hematopoiesis. However, the biological significance of this endpoint is indefinite. Because 4 mg/kg-day is the lowest dose tested, a NOAEL cannot be identified.

Chronic Studies

Leonard et al., 1987

Leonard conducted a 1-year chronic toxicity and carcinogenicity study on pure 2,6-DNT and technical grade DNT. The unpublished study was designed to compare the hepatic carcinogenic potential of technical grade DNT, 2,6-DNT, and 2,4-DNT. The tumor data reported in this study are discussed separately under the carcinogenicity studies subheading below.

In a peer-reviewed study, Leonard et al. (1987) fed groups of 28 male Fischer (F344)/CrlBR rats a diet containing 2,6-DNT at doses of 0, 7, or 14 mg/kg-day for 52 weeks. The authors stated that purified 2,6-DNT was used, but the actual purity was not specified. Concentrations of 2,6-DNT were adjusted in each diet batch based on food consumption and average body weight to maintain the 2,6-DNT doses at the target levels. Rats were housed as four per cage, and average dietary consumption for each cage was determined weekly. Body weights were measured every 2 weeks throughout the study. The study authors sacrificed 4 animals in each group after 6 and 26 weeks of feeding and measured hepatic microsomal epoxide hydrolase (EH) and cytosolic DT-diaphorase (DTD) activities (these are considered to be phenotypic markers of neoplastic lesions). At the end of the treatment period, all surviving animals were sacrificed and necropsied, selected organs were weighed (liver and lungs), histopathological examination was performed on liver and lung tissue, and hepatic EH and DTD activities were measured. Serum enzyme activities (ALT and glutamyl transferase [GGT]) were also determined. Statistical evaluations were done using the F-test and Dunnett's test ($p \leq 0.05$). Hematology and clinical chemistry were not evaluated in rats sacrificed at 1 year.

Leonard et al. (1987) reported no treatment-related deaths. Statistically significant changes were seen in body weight, organ weight, and serum chemistry in rats that received 7 or 14 mg/kg-day 2,6-DNT at 26 or 52 weeks, including decreased body weight, increased absolute and relative liver weights and increased serum GGT activity (see Table B.11). These effects, however, were more pronounced at 52 weeks. For example, terminal body weights of the 7 and 14 mg/kg-day rats were decreased relative to the controls by 5 and 18% and 20 and 32% at Weeks 26 and 52, respectively. It is unclear from the study report if the observed decreased body weight was due to a systemic effect of 2,6-DNT or an effect of the chemical on food palatability and consumption. At 52 weeks, statistically significantly increased serum ALT activity was seen in both the 7- and 14-mg/kg-day dose groups, and statistically significantly increased serum gamma-glutamyl transpeptidase activity was seen at 14 mg/kg-day (see Table B.11). The study authors did not report the effects of 2,6-DNT exposure for 6 weeks on body weight, liver weight, or serum enzyme activities. Microscopic evaluation of the liver sections from animals that had received 52 weeks of dietary treatment revealed hepatocyte degeneration and vacuolation in the majority of these treated animals at all doses. These effects, however, did not appear to be dose-related and were also seen in controls. Over 90% of the treated animals had acidophilic and basophilic hepatocyte foci. Neither type of foci was apparent in the controls. The study authors also noted bile duct hyperplasia in most animals fed 2,6-DNT. No specific mention was made of nonneoplastic lesions in the lungs. A LOAEL of 7 mg/kg-day based on a biological (≥10% change) and statistically significant increase in relative liver weight observed at 12 months is identified from this study. Because 7 mg/kg-day is the

lowest dose tested, a NOAEL cannot be identified from this study. It is unclear if the noncancer liver effects observed in this study were due to the presence of hepatocellular tumors caused by 2,6-DNT treatment.

Developmental Studies

No studies were identified.

Reproductive Studies

The studies by Lee et al. (1976) report limited reproductive toxicological endpoints (e.g., aspermatogenesis). These studies are summarized in the *Subchronic Studies* section.

Carcinogenicity Studies

Goldsworthy et al., 1986

Goldsworthy et al. (1986) conducted a study to evaluate the effect of diets varying in pectin content on the induction of foci and hepatic tumor by 2,6-DNT. Six groups of 30 male F344 (CDF/CrlBR) rats were placed on one of three diets containing sufficient quantities of 2,6-DNT (purity of 99.9%) to produce daily doses of 0.6−0.7 or 3−3.5 mg/kg-day. The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2,6-DNT (see Table B.12). The study authors incorporated 2,6-DNT into the diets by premixing the 2,6-DNT into 100 grams of the test diet followed by blending the mixture. Body weight and food consumption were recorded monthly. The study authors screened the rats for the absence of viral titers throughout the study. Ten animals from each group were sacrificed at 3, 6, and 12 months, and the livers were evaluated histopathologically. Quantitative stereology was used to assess the number of hepatic foci per liver. Three markers commonly used to detect hepatic preneoplasia—GGT, ATP, G6P—were used to score and quantitate foci. The study authors performed statistical analysis on the number of foci per cm^3 liver using a Newman-Keuls multiple comparison test ($p \leq 0.05$).

Goldsworthy et al. (1986) reported no deaths in the control or treatment groups. Body weight was increased in all rats fed all three diets (all animals in the 6 groups) for 3, 6, and 12 months. All groups receiving the high-dose of 2,6-DNT (3.0−3.5 mg/kg-day) gained approximately 10% less weight than their respective controls at 12 months (data on body and liver weight were presented in a graphical format by the study authors and not in tables). Liver weights were not significantly altered throughout the study compared to the control groups, except for the high-dose NIH-DNT (3−3.5 mg/kg-day) treated group, which showed a marked increase in liver weights at 12 months. In the other dose groups, the liver weights remained constant, and the liver/body-weight ratio was decreased in rats throughout the treatment periods. The study authors reported that no changes in monthly food consumption were observed in treated rats during the treatment period (data were not shown in the study). No further information was provided regarding nonneoplastic effects.

Goldsworthy et al. (1986) examined the effect of the control and DNT-diets on the fraction of animals with foci (see Table B.13). The fraction of animals with hepatocyte foci (i.e., GGT, ATP, G6P) was increased in a dose- and time-dependent manner in animals administered 2,6-DNT in the test diet with NIH > AP > AIN. Animals fed AIN and AP diets, with or without 2,6-DNT, had few or no GGT foci throughout the study. At 12 months, the number of ATP and G6P foci was approximately equal in all DNT-treated groups, and the number of GGT foci in the

high dose NIH-DNT group (3−3.5 mg/kg-day) was impossible to quantitate accurately, which was explained by the study authors to be due to the presence of neoplastic nodules and hepatocellular carcinomas.

Hepatocellular carcinomas and neoplastic nodules were observed only in rats fed the NIH diet containing 2,6-DNT (statistical analysis was only done on the number of foci per cm^3 in the liver and not on the hepatocellular carcinomas and neoplastic nodules). At 12 months, the treatment group on the NIH diet that received the high dose of 2,6-DNT (3−3.5 mg/kg-day) exhibited a 100% incidence of hepatic foci, including 6/10 rats with hepatocellular carcinomas and 6/10 with neoplastic nodules; the low dose of 2,6-DNT (0.6−0.7 mg/kg-day) exhibited 3/10 rats with neoplastic nodules, each rat with a single nodule; and the control diet had no tumors or neoplastic nodules present in 10 rats. No tumors or neoplastic nodules were observed in rats receiving the control AIN diet with or without added pectin or added 2,6-DNT in these diets.

Based on these results, the study authors concluded that 2,6-DNT is a potent hepatocarcinogen in male F344 rats, and its carcinogenic potency differs depending on whether rats are fed an NIH or AIN (with or without pectin) diet. They stated that the carcinogenicity of 2,6-DNT would not have been observed in the study if tested only in the pectin purified diets and scored by the GGT marker alone.

Leonard et al., 1987

The carcinogenic study by Leonard et al. (1987) is selected as the principal study for deriving the oral slope factor (p-OSF). In a peer-reviewed study, Leonard et al. (1987) fed groups of 28 male Fischer (F344)/CrlBR rats a diet containing 2,6-DNT at doses of 0, 7, or 14 mg/kg-day for 52 weeks. Other details concerning the study methodology are presented in the *Chronic Studies* section.

Leonard et al. (1987) noted that administration of 2,6-DNT at doses of 7 and 14 mg/kg-day to male rats in the diet for 52 weeks resulted in an increased incidence of neoplastic lesions in the liver (see Table B.14). Neoplastic nodules were found in 18/20 rats at the low-dose (7 mg/kg-day) and 15/19 rats at the high-dose (14 mg/kg-day). Hepatocellular carcinomas, described as trabecular, occurred in 17/20 rats at 7 mg/kg-day and 19/19 rats at 14 mg/kg-day, and one tumor described as an adenocarcinoma was found in a rat at 7 mg/kg-day. Cholangiocarcinomas occurred in 2/20 rats at 7 mg/kg-day. Liver tumors metastasized to the lung in 3/20 rats at 7 mg/kg-day and 11/19 at 14 mg/kg-day. These results indicated that overall neoplastic nodule incidence paralleled tumor incidence, with the exception of the high-dose 2,6-DNT-treated group. According to the study authors, this difference is due to the extensive tumor involvement in the livers from the high-dose group.

Hepatic microsomal EH and cytosolic DTD activity are induced following treatment with a number of hepatocarcinogens and are considered to be phenotypic markers of neoplastic nodules (Leonard et al., 1987). The study authors noted a dose-related increase in EH activity in rats treated with 7 mg/kg-day and 14 mg/kg-day for 26 weeks to 380% and 520% of controls, respectively. This increase was sustained at similar levels from 6 weeks to 1 year (data in the study on hepatic microsomal EH and cytosolic DTD activity were presented in graphical format and not in a table). At 1 year, EH activity in the high-dose group was lower than that observed at 6 weeks and 26 weeks (220% of controls). The study authors attributed this change to the

extensive tumor burden in the livers from these animals. They also stated that this observation is consistent with the lower nodule incidence observed in these animals, and this provides indirect evidence to support the suggestion that EH elevations occur in the nodules but not the tumors. In contrast to EH activities, DTD activity was increased in the high-dose 2,6-DNT-treated rats at 6 weeks. This increase was enhanced to a somewhat greater extent in both 2,6-DNT-treated groups at 26 weeks. At 12 months, DTD activities were maximally enhanced in the low- and high-dose 2,6-DNT-treated animals to 420 and 650% of controls, respectively. The study authors stated that the increase in DTD activity appeared to be linked to the presence of nodules and tumors, particularly at 12 months.

In summary, administration of 2,6-DNT at oral doses of 7 and 14 mg/kg-day produced hepatocellular carcinomas in 85% and 100% of male rats, respectively. The majority of tumors had a trabecular pattern, and pulmonary metastases. Similar to the 2,6-DNT results, rats fed a diet containing 35 mg/kg-day technical grade DNT (equivalent to 7 mg/kg-day 2,6-DNT) yielded a positive hepatocarcinogenic response. The 2,6-DNT isomer induced hepatocellular carcinomas in twice as many animals as did the technical grade DNT, and 2,4-DNT was not hepatocarcinogenic when fed to rats at twice the high dose of 2,6-DNT (2 mg/kg-day) over the same time period. The study authors concluded that 2,6-DNT is a complete carcinogen, capable of both initiation and promotion, and the hepatocarcinogenicity of technical grade DNT is mainly due to 2,6-DNT.

Stoner et al., 1984

In a 12-week study, Stoner et al. (1984) administered 0, 1200, 3000, or 6000 mg/kg 2,6-DNT (98% purity) to A/J mice (26 mice/sex/dose group) for 2 days/week for 12 weeks by gavage. The corresponding duration-adjusted doses are 0, 342.9, 857.1, or 1714 mg/kg-day. The animals were sacrificed after 30 weeks, and the lungs were examined. Lung tumors, which appeared as white nodules on the surface, were counted and randomly sampled for histopathological evaluation and confirmation of adenoma. In addition, liver, kidneys, spleen, intestines, thymus, stomach, and endocrine glands were examined grossly. If gross lesions were observed, the organs were examined histologically for the presence of neoplasms. The lung tumor response (percentage of mice that developed lung tumors and the number of lung tumors per mouse) in experimental and control groups was compared by Student's t-test. No increase in lung tumor incidence or in the number of lung tumors per mouse, as compared to controls, was observed.

Ellis et al., 1979

Ellis et al. (1979) performed a 2 year carcinogenicity study in which Sprague Dawley rats (38/sex/dose), CD-1 Swiss mice (58/sex/dose), and beagle dogs (6/sex/dose) were treated with 2,4-DNT (approximately 98% 2,4-DNT and 2% 2,6-DNT) in the diet. The rat portion of the study was used as the principal study by IRIS to derive the p-OSF for the 2,4-/2,6-DNT mixture (various compositions of DNTs; U.S. EPA, 1990). Because the focus of this PPRTV is to review the toxicity of only 2,6-DNT, the Ellis et al. (1979) study is not considered as a potential principal study because of the low amount (2%) of 2,6-DNT that was in the test compound.

Inhalation Exposures

No studies were identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Studies on the genotoxicity, carcinogenicity by routes other than oral or inhalation, short-term toxicity, toxicokinetics, and mode of action/mechanism of 2,6-DNT are available. These are summarized in Tables 4A and 4B.

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Genotoxicity studies in prokaryotic organisms						
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	1000 µg/plate	+ (TA98, TA100, TA1535, TA1538)	+ (TA98, TA100, TA1535, TA1537, TA1538)		Couch et al. (1981)
Reverse mutation	*S. typhimurium* strains TA98, TA1537, TA1538	NR	− (TA98, 1537) + (TA1538)	− (TA98, TA1537, TA1538)		Ellis et al. (1978, as cited in ATSDR, 1998)
Reverse mutation	*S. typhimurium* strain TA100	50−1000 µg/plate	+	ND		Simmon et al. (1977)
Reverse mutation	*S. typhimurium* strains TA98, TA100	5−2000 µg/plate	−	−		Sayama et al. (1989, 1992)
Reverse mutation	*S. typhimurium* strains TA98, TA100, YG1021, YG1024, YG1026, YG1029, YG1041, YG1042	0−5 µM	− (TA98, TA100, YG1021) + (YG1024, YG1026, YG1029, YG1041, YG1042)	ND	Highest degree of mutagenicity in YG1041 and YG1042	Sayama et al. (1998)
Reverse mutation	*S. typhimurium* strains TA98, TA100	NR	+ (TA98, TA100)	+ (TA98) − (TA100)		Tokiwa et al. (1981)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Reverse mutation	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, TA100NR3	10−5000 µg	+ (TA100) − (TA98, TA1535, TA1537, TA1538, TA100NR3)	+ (TA100) − (TA98, TA1535, TA1537, TA1538, TA100NR3)		Spanggord et al. (1982)
Reverse mutation	*S. typhimurium* strains TA98, TA100	0.1−10 µmol/plate	− (TA98, TA100)	+ (TA98) − (TA100)	In TA98, it was negative with rat liver activation and positive with hamster liver activation and in TA100 it was negative with both rat and hamster liver activation	Dellarco and Prival (1989)
Reverse mutation	*S. typhimurium* strains TA98, TA1538	1−1000 nmol/plate	+ (weakly positive at highest doses)	+ (weakly positive at highest doses)		Whong and Edwards (1984)
Reverse mutation	*S. typhimurium* strains TA98, TA98NR, TA98/1,8-DNP_6, YG1021, YG1024	NR	+ (TA98, YG1021, YG1024, TA98/1,8-DNP_6) − (TA98NR)	ND		Einistoe et al. (1991)
Reverse mutation	*S. typhimurium* strains NR	NR	NT	+		Pearson et al. (1979, as cited by ATSDR, 1998)
Reverse mutation	S. typhimurium strains TA98, YG1021, YG1024, YG1041	10−100 µg/plate	+ (TA98, (YG1021 YG1024, YG1041)	ND		Hagiwara et al. (1993)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Reverse mutation	*S. typhimurium* strains TA98, TA100	50−500 µg/plate	− (TA98) + (TA100)	− (TA98) + (TA100)		George et al. (2001)
Reverse mutation	*S. typhimurium* strain TA100	1−150 µg/mL	−	ND		Padda et al. (2003)
Forward mutation	TM 677	500 µg/mL	+	+	2,6-DNT and technical-grade DNT	Couch et al. (1981)
SOS repair induction	ND					
Genotoxicity studies in nonmammalian eukaryotic organisms						
Mutation	ND					
Recombination induction	ND					
Chromosomal aberration	ND					
Chromosomal malsegregation	ND					
Mitotic arrest	ND					
Genotoxicity studies in mammalian cells—in vitro						
Mutation	Chinese hamster ovary/HGPRT	2.5 mM	−	−		Abernathy and Couch (1982)
Mutation	Chinese hamster ovary	NR	−	ND		Lee et al. (1976)
Mutation	P388 mouse lymphoma cells	1.6−1000 µg/mL	−	−	2,6-DNT and technical grade DNT	Styles and Cross (1983)
Morphological transformation	Syrian hamster embryo cells	up to 100 µg/mL	−	ND	2,6-DNT and technical grade DNT	Holen et al. (1990)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
Chromosomal aberrations	Human peripheral lymphocytes	0.002−0.10 mmol/L	+	ND		Huang et al. (1995)
Sister chromatid exchange (SCE)	ND					
DNA damage	Rat germ cells	0.000032−0.02 mmol/L	+	ND	DNA strand breaks showed a dose-response relationship	Yang et al. (2005)
DNA adducts	ND					
DNA repair	Primary rat hepatocytes	1×10^{-4}, 1×10^{-3} M	−	ND		Bermudez et al. (1979)
DNA repair	Primary rat hepatocytes	0.1, 1.0 mM	−	−		Butterworth et al. (1989)
DNA repair	Human hepatocytes	0.01−1.0 mM	−	−		Butterworth et al. (1989)
Unscheduled DNA synthesis	Rat spermatocytes	10−1000 µM	−	ND		Working and Butterworth (1984)
DNA and protein synthesis	Chinese hamster ovary cells	10−1000 µg/mL	+ (weak)	ND		Garrett and Lewtas (1983)
Genotoxicity studies in mammals—in vivo						
Chromosomal aberrations	CD rats (sex not reported)	35−37 mg/kg-d	+	ND		Lee et al. (1976)
Sister chromatid exchange (SCE)	ND					
DNA damage	Male Sprague-Dawley rats	35, 68, and 134 mg/kg-d (gavage)	+ (liver)	ND		Lent et al. (2012b)

Table 4A. Summary of 2,6-Dinitrotoluene Genotoxicity Studies

Endpoint	Test System	Dose Concentration[a]	Results[b]		Comments	References
			Without Activation	With Activation		
DNA adducts	Male F344 rats	1.2 mmol/kg i.p.	+	ND		La and Froines (1993)
Mouse biochemical or visible specific locus test	ND					
Dominant lethal	ND					
Unscheduled DNA synthesis	Male F344 rats	5−100 mg/kg (gavage)	+	ND		Mirsalis and Butterworth (1982)
Unscheduled DNA synthesis	Male F344 rats	20 mg/kg (gavage)	−	ND		Working and Butterworth (1984)
Micronucleus test	Male F344 rats	125, 250 mg/kg (gavage)	+ (liver) − (peripheral blood)	ND		Takasawa et al. (2010)
Micronucleus test	Male Sprague-Dawley rats	35, 68, and 134 mg/kg-d (gavage)	− (peripheral blood)	ND		Lent et al. (2012b)
Genotoxicity studies in subcellular systems						
DNA binding	ND					

[a]Lowest effective dose for positive results, highest dose tested for negative results.
[b]+ = Positive, ± = Equivocal or weakly positive, − = Negative, T = Cytotoxicity, NA = Not applicable, ND = No data, NDr = Not determined, NR = Not reported, NR/Dr = Not reported, but determined from data.

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	26/26 A/J mice were administered 2,4-DNT (92−95% pure, with the major impurity being 2,6-dinitrotoluene), 2,6-DNT (98% pure), and 2:l mixture of 2,4-DNT and 2,6-DNT by i.p. injection at doses of MTD (maximal tolerated dose), 0.5 MTD, and 0.2 MTD three times per week for 8 wk. The total doses were 0, 600, 1500, and 3000 (maximum tolerated dose, MTD) mg/kg bw for 2,4- and 2,6-DNT. For the 2:l mixture of 2,4-DNT and 2,6-DNT, the total doses were 0, 960, 2400, and 4800 (MTD) mg/kg bw. Animals were killed after 30 wk, and the lungs, liver, kidneys, spleen, intestines, thymus, stomach, salivary, and endocrine glands were examined grossly. If gross lesions were observed, they were examined histologically for the presence of neoplasms.	No increase in lung tumor incidence or in the number of lung tumors per mouse was observed compared to controls in all three compounds. No lesions were observed in any other organ site.	2,6-DNT, 2,4-DNT, and the 2:l mixture of 2,4-DNT and 2,6-DNT did not induce lung tumors in A/J mice when given by i.p. injection. According to the study authors, the inability of these dinitrotoluenes to induce lung tumors in A/J mice is expected because it is known that hepatocarcinogens are either inactive or only weakly active for lung tumor induction in strain A mice.	Stoner et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	Study authors performed a number of initiation-promotion liver foci assays in CDF (F344)/CrlBR rats using 4 initiation-promotion protocols. The study aimed to evaluate the relative initiating potential of each DNT isomer (2,4 and 2,6) and compare it with the initiating potential of technical grade DNT. A group of 8−10 male (F344)/CrlBR rats were administered by gavage a single dose of 75 mg/kg bw in corn oil of either technical grade DNT or 2,6-DNT (purity >99.4%), or 2,4-DNT (purity >99.4%) at 12 hr post partial hepatectomy. The numbers of gamma glutamyltranspeptidase-positive (GGT) foci were quantified.	Technical grade DNT was a weak initiator when administered as a single oral dose (75 mg/kg) at 12 hr post hepatectomy. Significant dose-related increases in the number of GGT^+ foci were observed compared with control rats administered 2,6-DNT. No initiating activity was demonstrated with 2,4-DNT.	2,6-DNT and technical grade DNT were weak tumor initiators with comparable initiating activity. Thus, the initiating activity of 2,6-DNT likely accounts for the initiating activity in technical grade DNT. 2,4-DNT was not a tumor initiator.	Leonard et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Carcinogenicity other than oral/inhalation	A rat hepatic initiation-promotion protocol, 8−10 male CDF (F344)/CrlBR rats were initiated with a single dose of 150 mg/kg bw diethylnitrosamine by i.p. injection and permitted to recover for 2 wk. After the recovery period, the animals were placed on diets containing: • 27 mg/kg-d of 2,4-DNT (99.4% pure) or • 2.8, 7 or 14 mg/kg-d 2,6-DNT (99.4% pure) or • 14 or 35 mg/kg-d technical grade DNT Rats receiving technical-grade DNT were killed after 3 or 6 wk of feeding and those receiving the purified isomers after 6 or 12 wk of feeding. Sections from three liver lobes of each animal were stained for gamma glutamyl transferase (GGT), and the number of GGT^+ foci per cm^3 was calculated.	Initiation-promotion assay: 1) Technical grade DNT: at 3 wk, dose-dependent increase in number of GGT^+ foci and foci volume; at 6 wk, time-dependent increase in the number of foci and foci volume relative to 3 wk treatment. 2) 2,6-DNT: time- and dose-dependent increase in the number of GGT^+ foci and foci volume at 6 wk 3) 2,4-DNT: time-dependent increase in the number of GGT^+ foci at 6 wk. Technical grade DNT, 2,4-DNT, and 2,6-DNT had hepatocyte foci-promoting activity.	2,6-DNT is a complete hepatocarcinogen while 2,4-DNT was a pure promoter.	Leonard et al. (1986)
LD_{50} studies	Rat (gavage) Mouse (gavage)	LD_{50} (rat) = 795 mg/kg to180 mg/kg LD_{50} (mouse) = 621 mg/kg to 807 mg/kg	LD_{50} (rat) = 795 to 180 mg/kg-d LD_{50} (mouse) = 621 to 807 mg/kg	Lee et al. (1975, as cited in ATSDR, 1998); Ellis et al. (1978, as cited in ATSDR, 1998); Vernot et al. (1977, as cited in ATSDR, 1998)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Male and female F344 rats were administered by gavage (^{14}C)-ring-labeled 2,6-DNT (99% radio chemically pure) at a dose of 10 mg/kg-bw. Urine and fecal samples were collected and analyzed for metabolites.	The major route of excretion of ^{14}C after a single dose was via the urine (males, 53.6 ± 2.6%; females, 54.0 ± 4.8%). Fecal excretion accounted for 17.9% (males) and 19.8% (females) of the dose. The urinary metabolites of 2,6-DNT identified were • 2,6-dinitrobenzyl (21.7%); • 2,6-dinitrobenzoic acid (21.1%); and • 2-amino-6-nitrobenzoic acid (14.0%). Results were compared with to those found after administration of 10 mg/kg bw 2,4-DNT to male and female F344 rats. The only major difference in the disposition of the two isomers was that no *N*-acetylaminonitrobenzoic acid was found after administration of 2,6-DNT in vitro. This may reflect steric hindrance to *N*-acetylation of an amino group adjacent to a methyl group.	Urine is the major route of 2,6-DNT excretion. Disposition of 2,4-DNT and 2,6-DNT is not the same	Long and Rickert (1982, as cited in IARC, 1996)
Metabolism/ toxicokinetic	Six male F344 rats received an i.p. injection of either sulfotransferase inhibitor: DCNP (2,6-dichloro-4-nitrophenol) or PCP (pentachlorophenol) (40 µmol/ kg). After 45 min, three of these animals were administered orally [3-^{3}H]-2,6-DNT at a dose of 28 mg/kg and were euthanized 12 hr later. Urine was collected for analysis of metabolites.	Prior administration of PCP had no significant effect on the excretion of the benzyl glucuronide or benzoic acid metabolites of 2,6-DNT. (The effects of DCNP on 2,6-DNT excretion were not tested.)	Prior administration of PCP had no significant effect on the excretion of urinary metabolites of 2,6-DNT.	Kedderis et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	The study authors examined metabolites formed by anaerobic incubation of 2,6-DNT or 2,4-DNT with intestinal microflora of male Wistar rats in vitro.	The metabolites formed with the incubation of 2,6-DNT were • 2-nitroso-6-nitrotoluene (reached peak at 2 hr of the anaerobic incubation); • 2-hydroxyl amino-6-nitrotoluene (reached peak at 5 hr); • 2-amino-6-nitrotoluene (reached peak at 6 hr); and • 2,6-diaminotoluene (reached peak at 12 hr). Two nitroazoxy compounds: 2,2'-dimethyl-5,5'-dinitroazoxybenzene and 4,4'-dimethyl-3,3'-dinitroazoxybenzene, in addition to known metabolites (nitrosonitrotoluenes, hydroxylaminonitrotoluenes, aminonitrotoluenes, and diaminotoluene), were detected in the incubation of 2,4-DNT with intestinal microflora.	2,6-diaminotoluene is the terminal intestinal metabolite of 2,6-DNT.	Sayama et al. (1993, as cited in ATSDR, 1998)
Metabolism/ toxicokinetic	Groups of six male F344 rats were pretreated with Aroclor 1254 at a dose of 25 mg/kg for 1 week and then administered 75 mg/kg 2,6-DNT in DMSO by gavage for 5 wk. Urine was collected for analysis. Interim sacrifices were carried out at 2 and 4 wk of treatment, and the liver, small intestine, large intestine, and cecum of each rat was excised at autopsy for analysis. Gastrointestinal enzyme activities were measured, and DNA adducts from liver were also determined.	A significant increase in the excretion of mutagenic urinary DNT metabolites was observed after the first week of Aroclor 1254 treatment, peaked at Wk 2 and then declined by nearly 25% at Wk 4. However, at the end of the treatment, a 4-fold increase in the formation of hepatic DNA adducts was observed. This increase in DNA adducts and decrease in urinary mutagens was due to the significant elevation in hepatic metabolism and to the increase in ß-glucuronidase activity in the small intestine and cecum at 4 wk.	Pretreatment of F344 rats with Aroclor 1254 significantly altered select intestinal enzyme activity, stimulated hepatic enzyme activity, accelerated the biotransformation and bioactivation of 2,6-DNT, and potentiated the formation of 2,6-DNT-derived DNA adducts in the liver. The authors noted that hepatic metabolism alone likely did not account for the potentiated bioactivation of 2,6-DNT.	Chadwick et al. (1993)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	2,6-DNT metabolism by human liver and male F344 rat liver subcellular fractions under aerobic (100% oxygen) and anaerobic (100% nitrogen) incubations conditions was examined.	Under aerobic conditions, • The major 2,6-DNT metabolite formed by hepatic microsomes was 2,6-dinitrobenzyl alcohol (2,6-DNBalc); and • Rates of 2,6-DNBalc formation by human and rat liver microsomes under aerobic conditions were 247 and 132 pmol/min per mg protein, respectively. Under anaerobic conditions, • 2-amino-6-nitrotoluene (2Am6NT) was the major metabolite; and • Rates of 2Am6NT formation by human and rat liver microsomes were 292 and 285 pmol/min per mg protein, respectively. Anaerobic reduction of 2,6-DNT and 2Am6NT by rat and human liver microsomes is mediated by cytochrome P-450. Liver cytosolic fractions also metabolized 2,6-DNT to 2Am6NT under anaerobic conditions.	The major metabolites isolated from microsomal fractions of human and rat liver preparations incubated with 2,6-DNT were 2,6-dinitrobenzyl alcohol and 2-amino-6-nitrotoluene.	Chapman et al. (1992, 1993)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Male Wistar rats (n = 6) were administered orally a single dose of 2,6-DNT or 2,4-DNT (75 mg/kg). Urine samples were collected after 24 hr and were analyzed for conjugated and unconjugated metabolites using high performance liquid chromatography.	The major urinary metabolite identified after oral administration of 2,6-DNT was 2,6-dinitrobenzyl glucuronide, which accounted for about 17.4% of the administered dose. Other metabolites identified were • 2,6-dinitrobenzyl alcohol (0.53%); • 2-amino-6-nitrotoluene (0.44%); and • 2,6-dinitrobenzoic acid (0.17%). Urinary excretion of oxidized and *N*-acetylated derivatives for 2,4-DNT was observed but not after administration of 2,6-DNT, demonstrating metabolic differences in male Wistar rats for the two isomers.	2,6-dinitrobenzyl glucuronide is the major urinary metabolite of 2,6-DNT. Metabolic differences for 2,4-DNT and 2,6-DNT exist in male Wistar rats. Metabolism of 2,6-DNT differs between two strains of rat (Wistar and F344 [Rickert et al., 1983]).	Mori et al. (1996)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	Ninety-nine Chinese workers exposed to dinitrotoluenes and 61 nonmatched, nonexposed controls working in the same factory manufacturing TNT were examined and subjected to questionnaires inquiring about exposure history and other health and lifestyle factors. Blood samples were collected, and the levels of hydrolyzable Hb adducts were determined. Female Wistar rats (n = 3) were administered a single dose of 0.5 mmol/kg of 2,6-DNT or 2,4-DNT by gavage. The rats were killed after 24 hr, and hydrolyzable hemoglobin (Hb) adducts were determined for each of the compounds investigated. The Hb adduct profile in rats was compared to those in Chinese workers.	Hydrolysis of Hb from rats dosed with 2,4-DNT yielded 4-amino-2-nitrotoluene (4A2NT), 24TDA, and 4-acetylamino-2-aminotoluene (4AA2AT). Hydrolysis of Hb from rats dosed with 2,6-DNT yielded three amines, 2-amino-6-nitrotoluene (2A6NT), 26TDA and 2-acetylamino-6-aminotoluene (2AA6AT) with 2A6NT being the predominant adduct. Hb adduct levels in rats dosed with 2,4-DNT were higher than adduct levels in rats dosed with 2,6-DNT. A similar Hb adduct pattern was found in Chinese workers exposed to dinitrotoluenes, although 2-acetylamino-6-aminotoluene (2AA6AT) was not found in the workers. 2A6NT was the predominant adduct in 2,6-DNT exposed workers, and 4A2NT was the predominant adduct in 2,4-DNT exposed workers.	Quantification of DNT-Hb adducts provided an effective biomarker of toxicity (inertia, somnolence, nausea, and dizziness) and could be used to estimate the risk associated with a particular exposure to DNT.	Jones et al. (2005)
Distribution and absorption/ toxicokinetic	Male F344 rats (CDF (F344)/CrlBR) (36/group) were administered gavage doses of 10 or 35 mg/kg of radiolabeled 2,6-DNT. Three rats from each group were killed at 1, 2, 4, 8, 12, 24, 48, 96, 192, and 384 hr after the dose. Livers and intestine were removed and analyzed for metabolites.	Increase in hepatic concentrations of radioactivity in male rats in 2 stages, with the first peak occurring l−2 hr and a second peak occurring 8−12 hr after the dose. The second peak was followed by a gradual decline up to 16 d and was thought to be the result of enterohepatic cycling.	The rapid disappearance of radioactivity from the first quarter of the small intestine of rats following the oral administration of uniformly [14C]-ring-labeled 2,4- or 2,6-DNT indicates rapid and fairly complete absorption.	Rickert et al. (1983)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Distribution and excretion/ toxicokinetic	Male A/J mice were given 1-, 10-, or 100-mg/kg doses of the radiolabeled [^{3}H] 2,6-DNT (2.5 μCi/mouse) by oral or i.p. exposure. The amount of ^{3}H in blood, liver, kidney, lung, and intestine was measured.	Distribution of radioactivity in the blood, liver, kidneys, lungs, and intestines was the same 8 hr after dosing, with very low levels of radioactivity in brain, heart, and spleen. Orally administered 2,6-DNT was eliminated primarily via urine (approximately 50% of the administered dose after 8 hr).	2,6-DNT was rapidly and extensively metabolized following both routes of administration. The liver and intestines appear to be the primary organ sites for metabolism.	Schut et al. (1983, as cited in U.S. EPA, 2011a, ORNL, 1995)
Mode of action/ mechanistic	Male F344 rats (CDF (F344)/CrlBR) (36/group) were administered by gavage doses of 10 or 35 mg/kg of radiolabeled 2,4- or 2,6-DNT. Three rats from each group were killed at 1, 2, 4, 8, 12, 24, 48, 96, 192, and 384 hr after the dose. Livers and intestine were removed and analyzed for metabolites. Hepatic covalent binding to RNA, DNA, and protein were measured. Also, intestinal disposition of [14C] dinitrotoluenes was determined.	DNT-related material is covalently bound to hepatic DNA, RNA, and protein after administration of either isomer. Covalent binding to each macromolecular species was proportional to dose. Terminal half-lives of radioactivity indicated that macromolecular damage produced by 2,6-DNT was no more persistent than that produced by 2,4-DNT. However, 2,6-DNT was 10 times more potent than 2,4-DNT in producing unscheduled DNA synthesis after 12 hr.	Results indicate that DNT-related material is covalently bound to hepatic DNA, RNA, and protein after administration of either isomer, but that the degree of binding after 2,6-DNT is greater than after 2,4-DNT.	Rickert et al. (1983)
Mode of action/ mechanistic	Six male F344 rats received an i.p. injection of either sulfotransferase inhibitor: DCNP (2,6-dichloro-4-nitrophenol) or PCP (pentachlorophenol). After 45 min, three of these animals were administered orally [3-^{3}H]-2,6-DNT at a dose of 28 mg/kg and killed 12 hr later. Livers were excised and minced, and covalently bound radiolabel to hepatic macromolecular and hepatic DNA was determined.	No signs of toxicity were observed in any of the animals receiving 2,6-DNT or sulfotransferase inhibitors at the doses administered. Prior administration of the sulfotransferase inhibitors DCNP or PCP resulted in a significant decrease in the hepatic macromolecular covalent binding of 2,6-DNT by 65 to 70%. Prior administration of the sulfotransferase inhibitors DCNP or PCP decreased the binding of 2,6-DNT to DNA by 95%.	These results suggest that a sulfotransferase-dependent pathway is responsible for the majority of the covalent binding of 2,6-DNT to hepatic DNA.	Kedderis et al. (1984)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic	Male F344 rats and Male A/J mice were administered 150 mg/kg of [3-^{3}H]-2,6-DNT and 2,4-DNT by i.p. The animals were killed by cervical dislocation after 12 or 24 hr (two animals/time point), and their liver, lungs, and small and large intestines were removed. Covalently bound radiolabel to DNA from these tissues was determined.	Treatment in F344 rat resulted in • a covalent binding of 2,6-DNT and 2,4-DNT to DNA of the liver; and • lower binding to DNA of the lungs and the intestine. Treatment of A/J mice resulted in • lower binding in the liver; • no detectable binding of 2,6-DNT in extrahepatic tissues; and • low amounts of binding of 2,4-DNT to lung and intestinal DNA.	Binding of 2,6-DNT to liver DNA requires its prior reductive metabolism, probably by intestinal microorganisms, and that the higher binding of 2,6-DNT in the F344 rat than in the A/J mouse may, in part, be responsible for the high susceptibility of the F344 rat to 2,6-DNT carcinogenesis.	Dixit et al. (1986)
Mode of action/ mechanistic	F344 rats were given 219 mg/kg of 2,6-DNT or 2,6-diaminotoluene by single i.p. injection. In another experiment, 2,6-DNT was also given to the animals by gavage. In both experiments, DNA adduct formation in the liver was determined.	Four adducts were detected following administration of 2,6-DNT. No adducts were observed following administration of 2,6-diaminotoluene. 2,6-DNT produced extensive hemorrhagic necrosis in the liver, whereas no evidence of hepatocellular necrosis was detected following administration of 2,6-diaminotoluene. No quantitative or qualitative differences in adduct formation were found when treatment occurred by gavage or i.p. injection.	The differences between the two compounds in both DNA binding and cytotoxicity were consistent with the differences in their carcinogenicity: 2,6-DNT is a potent hepatocarcinogen while 2,6-diaminotoluene is not carcinogenic.	La and Froines (1993); La and Froines (1992, as cited in ATSDR, 1998)

Table 4B. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mode of action/ mechanistic	Male B6C3F$_1$ mice (n = 5) were dosed orally with 50 mg/kg 2,6-DNT daily for 3 consecutive days. CD-1 mice (n = 4) were given a single oral dose of 75 mg/kg 2,6-DNT. F344 rats (n = 6) were treated orally with 75 mg/kg 2,6-DNT three times at biweekly intervals. DNA adduct formation in the liver was determined.	Two distinct hepatic DNA adducts were detected in B6C3F$_{1,}$ which differed from the four adducts observed in hepatic DNA from 2,6-DNT treated F344 rats. This difference in the number of adducts in B6C3F$_1$ mice in comparison with F344 rats was explained to be due to • the differences in dosing regimen; and • 80% of the dose administered to B6C3F$_1$ mice is excreted in feces.	Different number of adducts observed in mice compared to rats	George et al. (1996)
Immunotoxicity	ND			
Neurotoxicity	ND			

ND = No data

Tests Evaluating Carcinogenicity, Genotoxicity, and/or Mutagenicity

The in vitro mutagenicity or genotoxicity of 2,6-DNT has been evaluated in studies with bacterial and mammalian cell systems. It has shown mixed results in the *Salmonella typhimurium* Ames assay using several strains with and without metabolic activation, see Table 4A. It was negative in most studies in mammalian cell systems, including studies for mutations in Chinese hamster ovary cells (Abernathy and Couch, 1982; Lee et al., 1976), only weakly positive in a study by Garrett and Lewtas (1983) and negative in mouse lymphoma cells (Styles and Cross, 1983) and Syrian hamster embryo cells (Holen et al., 1990). It was also negative for DNA repair in rat and human hepatocytes (Bermudez et al., 1979; Butterworth et al., 1989) and for unscheduled DNA synthesis in rat spermatocytes (Working and Butterworth, 1984). Positive results were reported for chromosomal aberrations in human peripheral lymphocytes (Huang et al., 1995) and DNA strand breaks in rat germ cells (Yang et al., 2005).

2,6-DNT has also been tested in vivo in mutagenicity and genotoxicity studies, with mixed results. In rats, 2,6-DNT induced positive results for chromosomal aberrations (Lee et al., 1976), DNA adducts (La and Froines, 1993), DNA damage (Lent et al., 2012b), and micronuclei (Takasawa et al., 2010) in the liver, ,but was negative for unscheduled DNA synthesis (Working and Butterworth, 1984) and micronuclei in peripheral blood (Takasawa et al., 2010, Lent et al., 2012b).

In terms of tests evaluating the carcinogenicity of 2,6-DNT, Stoner et al. (1984) did not report an increase in lung tumor induction in mice exposed intraperitoneally (i.p.) to 2,6-DNT, 2,4-DNT, or a mixture of 2,4-DNT and 2,6-DNT. In hepatic tumor initiation-promotion protocols, both 2,6-DNT and technical grade DNT were reported to have tumor promoting and tumor initiating activity (Leonard et al., 1983). In contrast, 2,4-DNT was a hepatic tumor promoter but not a tumor initiator in the same in vivo hepatic initiation-promotion protocol. Leonard et al. (1986) reported that 2,6-DNT, technical grade DNT, and 2,4-DNT have hepatocyte foci promoting activity by the i.p. route. 2,6-DNT was approximately 10 times more potent than 2,4-DNT.

LD_{50} Toxicity Studies

Characteristic signs of 2,6-DNT toxicity in animals include central nervous system depression, respiratory depression, and ataxia (U.S. EPA, 1986, as cited in U.S. EPA, 2004). The following LD_{50} values were identified for 2,6-DNT (Lee et al., 1975; Ellis et al., 1978; Vernot et al., 1977, all as cited in ATSDR, 1998): 535 and 795 mg/kg for male and female CD rats, respectively; 180 mg/kg for male Sprague-Dawley rats; 621 and 807 mg/kg for male and female CD mice, respectively; and 1000 mg/kg for CF-mice.

Metabolism/Toxicokinetic Studies

Information on the toxicokinetics of 2,6-DNT is available in several reviews (ATSDR, 1998; U.S. EPA, 2004, 1987; OECD, 2004; Rickert et al., 1984, as cited in ATSDR, 1998) and is described in Table 4B. Results of the available studies indicate that DNT, including the isomer 2,6-DNT and technical grade DNT, is absorbed through the gastrointestinal tract, respiratory tract, and skin in most species. Metabolism of 2,6-DNT is believed to occur in the liver and the intestine. Urine appears to be the major route of 2,6-DNT excretion. The main urinary metabolites of 2,6-DNT are the corresponding dinitrobenzyl alcohol glucuronide, dinitrobenzoic acid, and aminonitrobenzoic acid (Rickert and Long, 1982, as cited in Mori et al., 1996). Figure 2 illustrates the proposed pathway for the metabolism of 2,6-DNT in rats from gastric absorption

to urinary excretion (Rickert et al., 1984, as cited in ATSDR, 1998; Sayama et al., 1993, as cited in ATSDR, 1998; Chapman et al., 1993; La and Froines, 1993). After 2,6-DNT is absorbed from the gastrointestinal tract, it is metabolized by the following steps: (1) Oxidation of the aliphatic methyl group of 2,6-DNT by hepatic cytochrome P-450 to form dinitrobenzyl alcohol, which is then conjugated with glucuronic acid, partially excreted in the bile and subsequently transferred to the intestine; (2) In the intestine, hydrolyzation of the glucuronide and reduction of one nitro group occur by intestinal microflora to form aminonitrobenzyl alcohol; (3) A portion of this metabolite is reabsorbed from the intestine and circulated back to the liver by enterohepatic circulation; and (4) In the liver, the amine group is *N*-hydroxylated by cytochrome P450 to form an unstable sulfate conjugate (Kedderis et al., 1984). The sulfate conjugate can decompose and form carbonium or nitrenium ions, which then can bind to hepatic macromolecules, leading to mutations and subsequently to liver tumors.

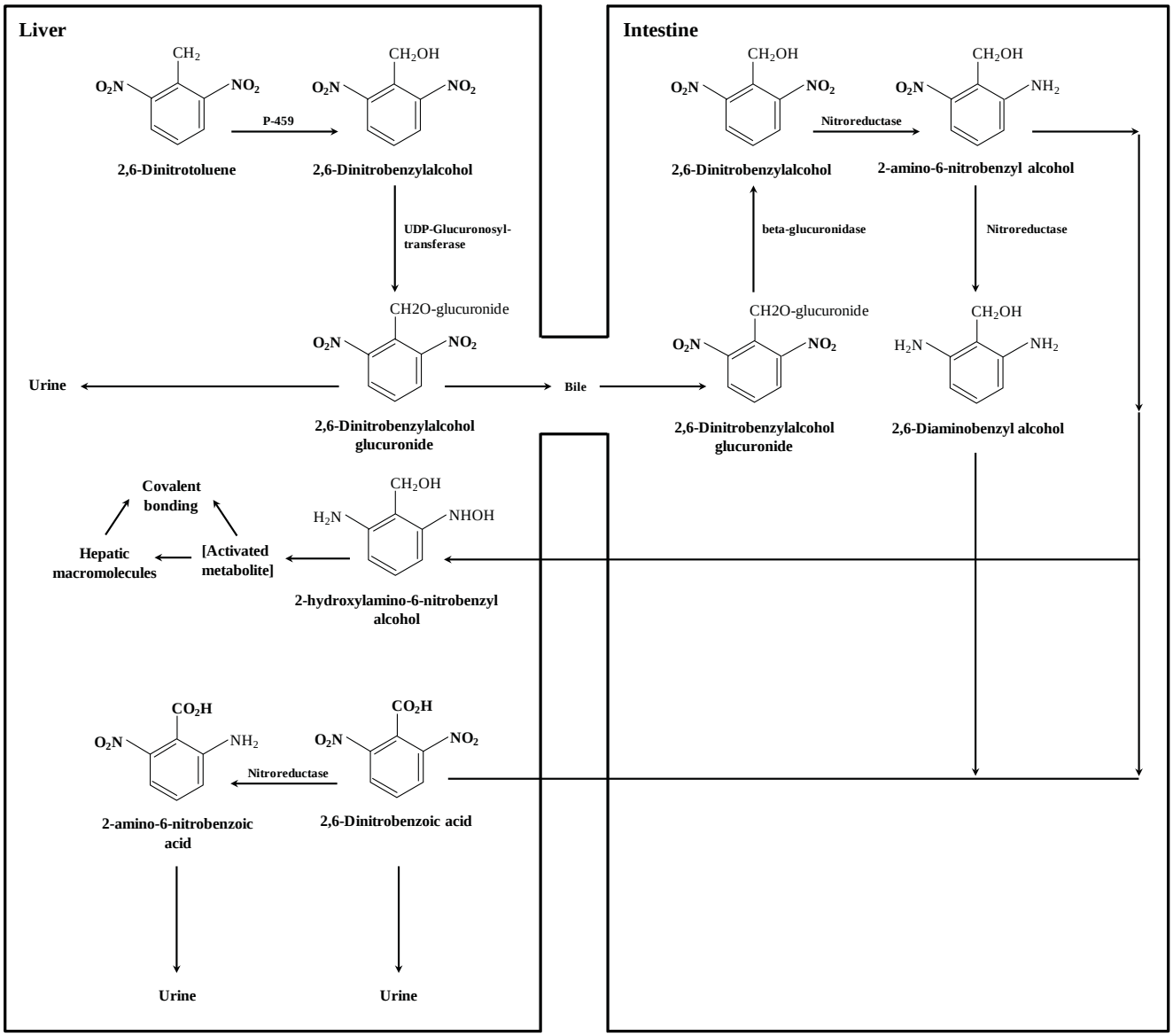

Figure 2. Metabolism Pathway of 2,6-DNT

Mode-of-Action/Mechanistic Studies

Ellis et al. (1979, as cited in ATSDR, 1998; U.S. EPA, 2004) described the mechanism by which DNT (in general) induced hematotoxicity in animals. DNT compounds or their metabolites can oxidize the ferrous ion in hemoglobin and produce methemoglobin. Hydroxylamine is probably the oxidizing species, because it is an intermediate in the reduction of nitrogen compounds to amines. Methemoglobin can form aggregates of hemoglobin degradation products called Heinz bodies, which are sensitive indicators of hemoglobin destruction. High levels of methemoglobin lead to the development of anemia, which is compensated by reticulocytosis. When reticulocytosis cannot compensate adequately, then frank anemia develops. This hematotoxic syndrome is a common effect of exposure to aromatic amines and most organic and inorganic nitrates.

The principal mechanism that is thought to be responsible for the genotoxicity of 2,6-DNT involves the bioactivation of 2,6-DNT to reactive metabolites, which are capable of covalent binding to hepatic macromolecules. As illustrated in Figure 2, conjugation, biliary excretion, microbial metabolism in the gut, and intestinal reabsorption are prerequisites to hepatic binding of DNT. Swenberg et al. (1983) demonstrated covalent binding of 2,6-DNT to rat hepatocyte RNA following oral dosing with 2,6-DNT, with hepatocytes of female rats showing slightly less binding than male rats. Rickert et al. (1983) reported similar hepatic binding of 2,6-DNT to protein, RNA, and DNA of rats. Hepatic binding may be greater for 2,6-DNT than for 2,4-DNT (Rickert et al., 1983). Diet (i.e., as it affects microbial activity and number) also may influence the degree to which binding of DNT metabolites occurs. Hepatic DNA adducts have been detected by ^{32}P-postlabeling technique in 2,6-DNT-treated male B6C3F$_1$ and CD-1 mice and F344 rats (George et al., 1996). Further information on these studies is provided in Table 4B.

Neurotoxicity

In animal studies, 2,6-DNT has been shown to affect the nervous system of mice and dogs (Lee et al., 1976b,c). Clinical signs in dogs have included incoordination and stiffness of the hind legs leading to complete paralysis, cerebellar vacuolation, hypertrophy, and focal gliosis, and cerebellar and brain stem hemorrhage. In mice, depression and hyperexcitability were observed, while some rats administered 2,6-DNT showed neuromuscular symptoms. Further details on this study are presented in Table 3 and in the *Subchronic Studies* section.

There are no data on the biochemical events involved in the toxicity of the nervous system.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 present a summary of noncancer reference and cancer values, respectively. IRIS data are indicated in the table, if available.

Table 5. Summary of Reference Values for 2,6 Dinitrotoluene (CASRN 606-20-2)

Toxicity Type (Units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Screening Subchronic p-RfD (mg/kg-d)	Dog/M and F	Increased incidence of splenic extramedullary hematopoiesis	3×10^{-3}	$LOAEL_{HED}$	3	1,000	Lee et al. (1976c)
Screening Chronic p-RfD (mg/kg-d)	Dog/M and F	Increased incidence of splenic extramedullary hematopoiesis	3×10^{-4}	$LOAEL_{HED}$	3	10,000	Lee et al. (1976c)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

NDr = Not determined.

Table 6. Summary of Cancer Values for 2,6 Dinitrotoluene (CASRN 606-20-2)

Toxicity type	Species/Sex	Tumor type	Cancer value	Principal study
p-OSF	F344 Rat/M	Hepatocellular carcinomas	1.5×10^{0} $(mg/kg\text{-}d)^{-1}$	Leonard et al. (1987)
p-IUR	NDr			

NDr = Not determined.

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

There are three subchronic-duration studies (Lee et al. 1976) presented in one report on 2,6-DNT in rats, mice, and dogs (see Table 3). Lee et al. (1976) is considered inadequate for p-RfD derivation because it is a nonpeer-reviewed and unpublished report. However, the Lee et al. (1976c) study is suitable for the derivation of a screening subchronic toxicity value. Appendix A provides details on the screening subchronic p-RfD.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

One chronic oral study in rats is available (Leonard et al., 1987), but it is not a comprehensive study and only investigated effects in the liver. Leonard et al. (1987) is not considered to derive the chronic p-RfD because it is unclear if the limited noncancer effects in the liver could be attributed to the carcinogenic effects of 2,6-DNT. The subchronic study in dogs by Lee et al. (1976c) (see discussion in the derivation of the subchronic p-RfD section above), is not used to derive the chronic p-RfD because it is a nonpeer-reviewed and unpublished report. However, the Lee et al. (1976c) dog study is used to derive the screening chronic p-RfD. Details are provided in Appendix A.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies were identified that could be used to derive provisional inhalation RfCs for 2,6-DNT. Available epidemiological studies consist primarily of occupational studies in which workers were exposed to the technical grade DNT mixture, which consists primarily of the 2,4-DNT isomer. In these studies, dermal as well as inhalation exposure was investigated, and exposure to 2,6-DNT was not quantified. No animal inhalation studies are available for 2,6-DNT.

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

The available data do not support derivation of any inhalation toxicity values.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

The available data do not support derivation of any inhalation toxicity values.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 7 identifies the cancer weight-of-evidence (WOE) descriptor for 2,6-DNT.

Table 7. Cancer WOE Descriptor for 2,6-DNT			
Possible WOE Descriptor	**Designation**	**Route of Entry (Oral, Inhalation, or Both)**	**Comments**
"Carcinogenic to Humans"	ND	ND	No human cancer studies on pure 2,6-DNT are available by any route of exposure.
"Likely to Be Carcinogenic to Humans"	NA	NA	The cancer weight of evidence does not meet the examples to be considered *"Likely to be Carcinogenic to Humans."*
"Suggestive Evidence of Carcinogenic Potential"	**Selected**	**Both[a]**	**As described below, 2,6-DNT is considered to have *"Suggestive Evidence of Carcinogenic Potential."***
"Inadequate Information to Assess Carcinogenic Potential"	NA	NA	There is evidence to assess the carcinogenic potential of 2,6-DNT.
"Not Likely to Be Carcinogenic to Humans"	NA	NA	Evidence of the carcinogenic potential of 2,6-DNT is available in animals.

[a]Although data on the carcinogenic effects of 2,6-DNT via the inhalation route are limited to human exposures to a DNT mixture, 2,6-DNT is considered to have *suggestive evidence of carcinogenic potential* by all routes of exposure based on EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), which indicates that for tumors occurring at a site other than the initial point of contact, the cancer descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses.

NA = Not applicable, ND = No data.

No human cancer studies of pure 2,6-DNT are available. Results from experimental animal studies showed that 2,6-DNT: (1) increased the incidence of hepatocellular neoplastic nodules and carcinomas in a chronic dietary exposure bioassay with male F344 rats (Leonard et al., 1987); (2) is a tumor initiator and promoter in rat liver using the in vivo hepatic initiation-promotion assay (Leonard et al., 1983, 1986); (3) is mutagenic in bacteria and induces DNA damage and mutations in mammalian cells in culture (Rickert et al., 1984, as cited in ATSDR, 1998; Sayama et al., 1998).

As stated in the EPA's cancer guidelines (U.S. EPA, 2005), one of the examples for a chemical to be considered to have *suggestive evidence of carcinogenic potential* is*: "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study that does not reach the weight of evidence for the descriptor 'Likely to Be Carcinogenic to Humans'."* Based on these guidelines and the carcinogenicity data from available animal studies, the WOE descriptor of *suggestive evidence of carcinogenic potential* is appropriate for 2,6-DNT.

EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) indicates that for tumors occurring at a site other than the initial point of contact, the cancer descriptor may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there are convincing toxicokinetic data that absorption does not occur by other

routes. Information available on the carcinogenic effects of 2,6-DNT demonstrates that tumors occur in tissues remote from the site of absorption. 2,6-DNT has been shown to be a hepatocarcinogen in rats in bioassays of various experimental designs by oral exposure. An excess of hepatobiliary cancer was found among munition workers exposed to dinitrotoluenes in which exposures are presumed to be predominantly inhalation with contributions from the dermal route. Information on the carcinogenic effects of 2,6-DNT via the dermal route in humans and animals is limited or absent. Data on the absorption of 2,6-DNT show that the chemical is readily absorbed via all routes of exposure, including oral, inhalation, and dermal. Therefore, based on the observance of liver tumors following oral exposure and absorption by all routes of exposure, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, 2,6-DNT is considered to have *suggestive evidence of carcinogenic potential* by all routes of exposure.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) define mode of action "…as a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation" (p. 1−10). Examples of possible modes of carcinogenic action for any given chemical include "…mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression" (p. 1−10).

The potential mode of action for 2,6-DNT is unclear. Table 4A summarizes the studies examining genotoxicity (e.g., clastogenicity, mutagenicity) of 2,6-DNT. 2,6-DNT was shown to be both positive and negative for mutagenicity in *S. typhimurium* strains. 2,6-DNT was not mutagenic in mammalian cell systems (i.e., chinese hamster ovary (CHO) cells and p388 mouse lymphoma cells). 2,6-DNT did not cause morphological transformations in Syrian hamster embryo cells but did induce chromosomal aberrations in human peripheral lymphocytes in vitro. 2,6-DNT also caused DNA damage in rats both in vitro (germ cells) and in vivo (liver) but was negative for DNA repair in rat and human hepatocytes. Assays of unscheduled DNA synthesis in rat spermatocytes and CHO cells showed a negative response under in vitro conditions. 2,6-DNT induced both chromosomal aberrations and DNA adducts in rats in vivo. Assays of unscheduled DNA synthesis were both positive and negative in rats. 2,6-DNT caused micronuclei formation in the liver of rats but not in the peripheral blood. Taken together, the available data do not provide a definitive conclusion regarding the mode-of-action for 2,6-DNT-induced carcinogenicity. Therefore, a detailed mode-of-action discussion for 2,6-DNT is precluded and a linear approach is applied as recommended by the U.S. EPA (2005).

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

As noted in Table 7, EPA concluded that there is *suggestive evidence of carcinogenic potential* for 2,6-DNT. The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) state: "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities. In each case, the rationale for the

quantitative analysis is explained, considering the uncertainty in the data and the suggestive nature of the weight of evidence. These analyses generally would not be considered Agency consensus estimates."

In this case, although there are no epidemiologic studies that have evaluated the carcinogenicity of 2,6-DNT in humans, its carcinogenicity has been evaluated in studies in both rats and mice. As highlighted in Table 3, these studies indicate that there are differing results regarding the carcinogenic potential of 2,6-DNT. However, the study by Leonard et al. (1987) is a well-conducted study showing evidence of increased incidence of hepatic carcinomas in male rats at multiple treatment levels. The data from this study are adequate to support a quantitative cancer dose-response assessment. Considering these data and the uncertainty associated with the suggestive nature of the tumorigenic response, EPA concluded that quantitative analyses may be useful for providing a sense of the magnitude of potential carcinogenic risk. Based on the weight of evidence, a dose-response assessment of the carcinogenicity of 2,6-DNT is deemed appropriate.

The study by Leonard et al. (1987) is selected as the principal study for deriving the p-OSF, with a cancer endpoint of hepatocellular carcinomas in male rats. This study is peer reviewed, examined a sufficient number of animals (28 per dose group), was well conducted, and was of sufficient duration (52 weeks). It was not stated whether the study was performed under GLP standards, but the study appears scientifically sound. Details are provided in the *Carcinogenicity Studies* section. The study by Goldsworthy et al. (1986) was not selected as the principal study because the reported carcinogenicity of 2,6-DNT was enhanced by the content of pectin in diet and was not solely related to pure 2,6-DNT. In this study, 2,6-DNT was provided in diets with varying pectin content, which is believed to promote or enhance 2,6-DNT-induced carcinogenesis. The hepatocellular carcinomas and neoplastic nodules were observed only in rats fed 2,6-DNT in diets high in pectin content (NIH-2,6-DNT), and, therefore, the reported liver tumor incidences cannot be used to derive a p-OSF for 2,6-DNT. The Stoner et al. (1984) study was not selected as the principal study because it was of insufficient duration (12 weeks) to determine carcinogenic effects.

In the study by Leonard et al. (1987), 28 male F344 rats were fed 2,6-DNT (purity unknown) in the diet for 1 year, and the results were compared with an untreated control group of 28 rats. 2,6-DNT induced hepatocellular carcinomas in 100% (19/19) of the high-dose rats (14 mg/kg-day) and 85% (17/20) of the low-dose rats (7 mg/kg-day), compared to no incidence (0/20) in controls. Statistical significance tests conducted by the EPA indicated that incidence of hepatocellular carcinomas was statistically significant at both the high- and low-dose groups compared to controls. The dose-response data for hepatocellular carcinomas in male rats (see Table 8 and B.14) can be used to derive a p-OSF for 2,6-DNT. Statistical analyses performed for these data were done by Fisher's Exact test.

Table 8 presents BMD input data for incidence of hepatocellular carcinomas in male rats exposed to 2,6-DNT orally for 1 year. The model result and BMD output text are provided in Appendix D.

Table 8. BMD Input for Incidence of Hepatocellular Carcinomas in the Male (F344) Crlbr Rat Exposed to 2,6-DNT Orally for 1 Year[a]

$Dose_{ADJ}$ (mg/kg-day)	Number of Subjects	Response (Hepatocellular Carcinoma)
0	20	0
7	20	17*
14	19	19*

[a]Leonard et al. (1987).
*$p < 0.001$ by Fisher's Exact Test performed by EPA.

Table 9 shows the BMD modeling results. Adequate model fit is obtained for the hepatocellular carcinoma incidence data using the multistage-cancer model. The modeling results yield a BMD_{10} of 2.7 mg/kg-day and a $BMDL_{10}$ of 0.25 mg/kg-day. This $BMDL_{10}$ was further converted from an animal dose to an HED and then used as the POD_{HED} to derive the p-OSF for 2,6-DNT.

Table 9. Goodness-of-Fit Statistics and BMD_{10} and $BMDL_{10}$ Values for Dichotomous Model for Hepatocellular Carcinoma in the Male (F344)/Crlbr Rat Exposed to 2,6-DNT Orally for 1 Year[a]

Multistage Cancer Model	Goodness-of-fit *p*-value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Hepatocellular carcinoma	1.0	18.91	2.7	0.25

[a]Leonard et al. (1987).
[b]Values >0.1 meet conventional goodness-of-fit criteria.

$$\begin{aligned} POD_{HED} &= BMDL_{10}\ (\text{mg/kg-day}) \times DAF \\ &= BMDL_{10}\ (\text{mg/kg-day}) \times (BW_a^{1/4} \div BW_h^{1/4}) \\ &= BMDL_{10}\ (\text{mg/kg-day}) \times (0.376^{1/4} \div 70^{1/4}) \\ &= 0.25\ \text{mg/kg-day} \times 0.27 \\ &= 0.068\ \text{mg/kg-day} \end{aligned}$$

Note: The BW_a of 0.376 kg is the mean body weight from the low-dose male group at Week 104 (see Table B.11).

$$\begin{aligned} \textbf{p-OSF} &= \mathbf{0.1 \div BMDL_{10HED}} \\ &= 0.1 \div 0.068\ \text{mg/kg-day} \\ &= \mathbf{1.5 \times 10^0\ (mg/kg\text{-}day)^{-1}} \end{aligned}$$

The p-OSF is 1.5×10^0 $(\text{mg/kg-day})^{-1}$, as calculated based on BMD modeling from Leonard et al. (1987).

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human and animal studies examining the carcinogenicity of 2,6-DNT following inhalation exposure are available located. Therefore, derivation of a p-IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

For the reasons noted in the main PPRTV document, it is inappropriate to derive a provisional subchronic or chronic p-RfD for 2,6-DNT. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

DERIVATION OF SCREENING PROVISIONAL ORAL REFERENCES DOSES

Derivation of Screening Subchronic Provisional RfD (Subchronic p-RfD)

The 13-week toxicity study in dogs (Lee et al., 1976c) is selected as the principal study for the derivation of the screening subchronic p-RfD. The critical effect is increased incidence of splenic extramedullary hematopoiesis in male and female dogs. No human studies are available on oral exposure to 2,6-DNT. One subchronic animal oral study is available (Lee et al., 1976), which, for the sake of clarity in this document, is divided into three separate study summaries based on the species tested: Lee et al. (1976a) in rats, (1976b) in mice, and (1976c) in dogs. Lee et al. (1976) is an unpublished and nonpeer-reviewed study. It is unclear if the study was performed according to Good Laboratory Practice (GLP) guidelines. The dog study (Lee et al. 1976c) is considered to have a small sample size as only one dog/sex/dose level were treated for 13 weeks in the control and low-dose group. At the mid-dose level, only two females and one male were treated for 13 weeks and two dogs/sex/dose level at the high dose. However, this study examined an adequate number of endpoints and has been previously used by both EPA and ATSDR to develop reference values (see Table 2) and is considered to be adequate for the derivation of screening oral reference values. Lee et al. (1976c) (dogs) is selected as the principal study because treatment-related effects observed in the dogs were more sensitive than effects observed in the rats (Lee et al. 1976a) and mice (Lee et al. 1976b). Study details are provided in the "Review of Potentially Relevant Data" section.

The most sensitive treatment-related effect observed in the Lee et al. (1976c) study was increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs. Splenic extramedullary hematopoiesis was observed in every dog treated at 4 (2/2), 20 (3/3), and 100 (4/4) mg/kg-day for 13 weeks compared to zero incidence in the controls (see Table B.8). These data could not be modeled by Benchmark Dose Software (BMDS version 2.1.2) due to the lack of a dose-response. Because these data were not amenable to BMD modeling, a NOAEL/LOAEL approach was employed to identify a potential point of departure (POD). For increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs, there was an increase at the low-dose group, identifying a LOAEL of 4 mg/kg-day. A Fisher's exact test comparing splenic extramedullary hematopoiesis in the control and treated groups indicated a nonstatistically significant difference. However, group sizes were too small for the statistical test to have much power to detect an effect.

Dogs are considered to be the most sensitive species to the toxicological effects of 2,6-DNT compared to rats and mice. For rats, the most sensitive potential POD is increased incidences of liver and splenic extramedullary hematopoiesis in male rats with a LOAEL of 35 mg/kg-day and a corresponding NOAEL of 7 mg/kg-day. In mice, decreased relative liver weight that was biologically significant (≥10%) is the most sensitive effect with a LOAEL of 11 mg/kg-day. Effects in dogs, rats, and mice were modeled by BMDS (version 2.1.2) for consideration of a potential POD when data were amenable to BMD modeling. Details of the modeling methods are provided in Appendix C. Potential PODs for dogs, rats, and mice are listed in Table A.1.

Of the toxicological effects observed in dogs, rats, and mice in the subchronic study by Lee et al. (1976), the most sensitive is increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs with a LOAEL of 4 mg/kg-day. The selection of increased incidence of splenic extramedullary hematopoiesis is supported by the observation that the severities of this effect increased with dose in dogs (see Table B.8). At 4 mg/kg-day in male and female dogs, the severity of splenic extramedullary hematopoiesis ranged from minimal to mild; at 20 mg/kg-day, the severity ranged from minimal to moderate; at 100 mg/kg-day, the severity ranged from marked to markedly severe. Furthermore, splenic extramedullary hematopoiesis was not present in male and female dogs that were treated with 2,6-DNT for 13 weeks and allowed to recover for 4 weeks, suggesting that this effect is treatment-related. Splenic extramedullary hematopoiesis was reported in rats gavaged with 2,6-DNT for 14 days at doses of 68 (1/6) and 134 (6/6) mg/kg-day, the incidence of this lesion in controls was not reported by the study authors (Lent et al., 2012a). Splenic extramedullary hematopoiesis was also observed in male and female dogs treated at 4 (1/2), 20 (2/2), and 100 (2/2) mg/kg-day for 4 weeks compared to zero incidence in controls. Splenic extramedullary hematopoiesis was also statistically significantly increased in both male and female rats treated for 13 weeks (see Tables B.4 and B.5). Further support for splenic extramedullary hematopoiesis as a critical effect following 2,6-DNT treatment is provided by hematological data in Tables B.3. 2,6-DNT statistically significantly increased reticulocytes (an indicator of hematopoiesis) in male and female dogs at 100 mg/kg-day following 2 and 4 weeks of treatment. Additionally, 2,6-DNT statistically significantly increased the amount of reticulocytes in male and female rats compared to controls at the highest dose tested following 4 weeks of exposure. **Therefore, the LOAEL of 4 mg/kg-day based on increased incidence of splenic extramedullary hematopoiesis in male and female dogs is chosen as the POD to derive a screening subchronic p-RfD.**

It is important to note that the selection of the LOAEL of 4 mg/kg-day for increased incidence of splenic extramedullary hematopoiesis in male and female beagle dogs as the POD would also be protective against the 2,6-DNT-induced mortality that was observed in mice and dogs. In mice, 8 of 16 males at the high-dose (289 mg/kg-day) died before Weeks 9, and 6 of 8 females died at the high-dose (299 mg/kg-day) before the end of the study. In addition, 8 of 16 males and 1 of 16 females died at the mid-dose (51 and 55 mg/kg-day, respectively), and 2 of 16 males died at the low-dose (11 mg/kg-day). The study authors stated that in the mid- and high-dose groups, most of the deaths could be contributed to 2,6-DNT administration. These data suggest an FEL of 51 mg/kg-day for the mouse study (Lee et al. 1976b). In dogs, all animals (2 males and 2 females) in the high dose group (100 mg/kg-day) died between Weeks 2 and 8, and 2 of 3 dogs (both females) in the mid-dose group (20 mg/kg-day) died during Week 9. These data suggest an FEL of 20 mg/kg-day for the dog study (Lee et al. 1976c).

Table A.1. Potential Subchronic PODs in Animals Following 13 Weeks of Treatment to 2,6-DNT

Effect	Sex/Species	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Comment
Decreased Body Weight	Males/Rats	7	35	13	Due to decreased palatability possibly due to chemical exposure
Increased Relative Liver Weight	Males/Rats	Not determined	7	Not run	No dose-response, effect most likely due to decreased body weight
Increased Relative Spleen Weight	Males/Rats	35	145	No fit	Effect most likely due to decreased body weight
Decreased Relative Kidney Weight	Males/Rats	Not determined	7	Not run	No dose-response, effect reverses at higher doses
Increased Relative Heart Weight	Males/Rats	35	145	Not run	No dose-response, effect most likely due to decreased body weight
Increased Relative Brain Weight	Males/Rats	35	145	No fit	Not a valid toxicological endpoint
Splenic Hemosiderosis	Males/Rats	Not determined	7	Not run	No dose-response
Splenic Hematopoiesis	Males/Rats	7	35	Not run	Data not suitable for BMD modeling
Liver Hematopoiesis	Males/Rats	7	35	Not run	Data not suitable for BMD modeling
Decreased Body Weight	Females/ Rats	37	155	6.4	Due to decreased palatability possibly due to chemical exposure
Increased Relative Liver Weight	Females/ Rats	Not determined	7	No fit	Effect most likely due to decreased body weight
Increased Relative Spleen Weight	Females/ Rats	37	155	48	Effect most likely due to decreased body weight
Increased Relative Kidney Weight	Females/ Rats	7	37	Not run	No dose-response, effect most likely due to decreased body weight
Decreased Relative Heart Weight	Females/ Rats	155	Not determined	Not Run	No dose-response
Increased Relative Brain Weight	Females/ Rats	7	37	3.1	Not a valid toxicological endpoint
Splenic Hemosiderosis	Females/ Rats	37	155	Not run	Data not suitable for BMD modeling
Splenic Hematopoiesis	Females/ Rats	7	37	Not run	Data not suitable for BMD modeling
Liver Hematopoiesis	Females/ Rats	37	155	Not run	Data not suitable for BMD modeling

Table A.1. Potential Subchronic PODs in Animals Following 13 Weeks of Treatment to 2,6-DNT

Effect	Sex/Species	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Comment
Decreased Relative Liver Weight	Males/Mice	Not determined	11	No fit	
Decreased Relative Kidney Weight	Males/Mice	11	51	Not run	LOAEL is 10-fold higher than LOAEL for splenic extramedullary hematopoiesis in dogs
Mortality	Males/Mice	11	51 (FEL)	Not run	
Mortality	Females/ Mice	55	299 (FEL)	Not run	
Liver Hematopoiesis	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Liver bile duct hyperplasia	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Liver degeneration	Both/Dogs	4	20	Not run	Data not suitable for BMD modeling
Splenic Hematopoiesis	Both/Dogs	Not determined	4	Not run	Data not suitable for BMD modeling
Mortality	Both/Dogs	4	20 (FEL)	Not run	

Dosimetric adjustment for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for dietary treatment to adjust for daily exposure. Dosimetric adjustment for 4 mg/kg-day is presented below.

$$(DOSE_{ADJ}) = DOSE_{Lee\ et\ al.,\ 1976c} \times [\text{conversion to daily dose}]$$
$$= 4\ \text{mg/kg-day} \times (\text{days of week dosed} \div 7)$$
$$= 4\ \text{mg/kg-day} \times (7 \div 7)$$
$$= 4\ \text{mg/kg-day}$$

In EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches may include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, EPA endorses body weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving a RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving a RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects or developmental endpoints. A validated human PBPK model for 2,6-DNT is not available for use in extrapolating doses from animals to humans. The selected critical effect of splenic extramedullary hematopoiesis was associated with the parent compound or a stable metabolite. Furthermore, this splenic effect is not a portal-of-entry or developmental effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for these effects.

Following U.S. EPA (2011b) guidance, the POD for splenic extramedullary hematopoiesis in adult animals is converted to a HED through application of a dosimetric adjustment factor (DAF^{1}) derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

[1]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Using a BW_a of 12 kg for dogs and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.63. Applying this DAF to the LOAEL identified for the critical effect in mature dogs yields a $LOAEL_{HED}$ as follows:

$$\begin{aligned} LOAEL_{HED} &= 4 \text{ mg/kg-day} \times DAF \\ &= 4 \text{ mg/kg-day} \times 0.63 \\ &= 3 \text{ mg/kg-day} \end{aligned}$$

The screening subchronic p-RfD for 2,6-DNT is derived as follows:

$$\begin{aligned} \textbf{Screening Subchronic p-RfD} &= \textbf{LOAEL}_{\textbf{HED}} \textbf{ ÷ UF}_{\textbf{C}} \\ &= 3 \text{ mg/kg-day} \div 1{,}000 \\ &= \textbf{3 × 10}^{\textbf{-3}} \textbf{ mg/kg-day} \end{aligned}$$

The UF_C of 1,000 is presented in Table A.2.

Table A.2 summarizes the UFs for the screening subchronic p-RfD for 2,6-DNT. Confidence in the screening value is by definition, low.

Table A.2. UFs for Screening Subchronic p-RfD of 2,6-DNT[a]

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of splenic extramedullary hematopoiesis (Lee et al., 1976c), a UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between dogs and humans following oral 2,6-DNT exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a DAF as outlined in the *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2,6-DNT in humans.
UF_L	3	A UF_L of 3 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL based on splenic extramedullary hepatopoiesis, for which the biological significance is not entirely clear.
UF_S	1	A UF_S of 1 has been applied because a subchronic-duration study was selected as the principal study.
UF_C	1,000	

[a]Lee et al. (1976c).

Derivation of Screening Chronic Provisional RfD (Chronic p-RfD)

The 13-week toxicity study in dogs (Lee et al., 1976c) is selected as the principal study for the derivation of the screening chronic p-RfD. The critical effect is increased incidence of splenic extramedullary hematopoiesis in male and female dogs. No human chronic studies are available for 2,6-DNT. There is an available chronic oral study in male rats by

Leonard et al. (1987) that investigated the carcinogenic and noncancer effects of only 2,6-DNT on the liver. The carcinogenic effects are described in the "Derivation of Provisional Oral Slope Factor" section. Regarding the noncancer effects, Leonard et al. (1987) reported increased absolute and relative liver weight, decreased body weight, and increased ALT activity (only at 12 months) in male rats at both 6 and 12 months of exposure to 2,6-DNT. Decreased body weight cannot be considered as a critical effect because it is not clear if this effect is due to direct treatment with 2,6-DNT or to a reduction in food consumption. Decreased food consumption was observed in rats in the subchronic study by Lee et al. (1976a), suggesting that decreased body weight in rats reported in the Leonard et al. (1987) study could be due to reduced food consumption. Because there were statistically and biologically significant changes in body weight, changes in absolute organ weights are not considered for potential POD selection. The data for increased relative liver weight were analyzed by the EPA's BMDS (version 2.1.2) continuous-variable models. For increased relative liver weight at 6 months, a LOAEL of 7 mg/kg-day based on a biologically (≥10% change) and statistically significant change is a potential POD. Following 12 months of treatment with 2,6-DNT, BMDS calculated a $BMDL_{10}$ of 0.69 mg/kg-day for increased relative liver weight. For increased ALT activity at 12 months, a LOAEL of 7 mg/kg-day based on a statistically significant change is a potential POD. Complete modeling methods and results are in presented Appendix C. The study authors also reported nonneoplastic lesions (e.g., bile duct hyperplasia, basophilic foci, etc) in the liver but presented no quantitative data for these effects that could be used to derive a chronic p-RfD.

Based on the BMD modeling results, the most sensitive effect following chronic exposure to 2,6-DNT appears to be increased relative liver weight (BMDL = 0.69 mg/kg-day). However, the chronic study by Leonard et al. (1987) is not a comprehensive study and only reports carcinogenic and noncancer effects in the liver, as well as evaluation of pulmonary metastases. It is also unclear if the reported noncancer effects in the liver may be due to the hepatocarcinogenic effects of 2,6-DNT because increased relative liver weight and increased ALT activity were observed at the same doses (7 and 14 mg/kg-day) as hepatocellular carcinomas following 12 months of exposure. Whereas increased relative liver weight was also observed at 6 months, the study authors did not report pathology results for this time period so it is possible that hepatocellular carcinomas may have been present. From the subchronic-duration study by Lee et al. (1976), it is clear that the spleen is a target organ for 2,6-DNT toxicity; the most sensitive subchronic effect from that study was increased incidence of splenic extramedullary hematopoiesis in dogs with a NOAEL of 4 mg/kg-day, which is more sensitive than splenic and liver effects observed in rats. Because the chronic-duration study by Leonard et al. (1987) did not investigate splenic effects in any species, the sensitivity of spleen toxicity following chronic 2,6-DNT exposure is unknown. Therefore, to protect against potential splenic effects from chronic 2,6-DNT exposure, the LOAEL of 4 mg/kg-day based on increased incidence of splenic extramedullary hematopoiesis in dogs from the subchronic-duration study by Lee et al. (1976c) is used as the POD to derive a screening chronic p-RfD. For the same reasons listed in the screening subchronic p-RfD discussion above, the study by Lee et al. (1976c) meets standards of study design and performance. Details are provided in the "Review of Potentially Relevant Data" section.

Dosimetric adjustment for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for dietary treatment to adjust for daily exposure. Dosimetric adjustment for 4 mg/kg-day is presented below.

$$\begin{aligned}(DOSE_{ADJ}) &= DOSE_{\text{Lee et al., 1976c}} \times [\text{conversion to daily dose}] \\ &= 4 \text{ mg/kg-day} \times (\text{days of week dosed} \div 7) \\ &= 4 \text{ mg/kg-day} \times (7 \div 7) \\ &= 4 \text{ mg/kg-day}\end{aligned}$$

Following U.S. EPA (2011b) guidance, the POD for splenic extramedullary hematopoiesis in adult animals is converted to a HED through application of a dosimetric adjustment factor (DAF^1) derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 12 kg for dogs and a BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.63. Applying this DAF to the LOAEL identified for the critical effect in mature dogs yields a $LOAEL_{HED}$ as follows:

$$\begin{aligned}LOAEL_{HED} &= 4 \text{ mg/kg-day} \times DAF \\ &= 4 \text{ mg/kg-day} \times 0.63 \\ &= 3 \text{ mg/kg-day}\end{aligned}$$

The screening chronic p-RfD for 2,6-DNT based on a $LOAEL_{HED}$ of 3 mg/kg-day for splenic extramedullary hematopoiesis in male and female dogs, is derived as follows:

$$\begin{aligned}\textbf{Screening Chronic p-RfD} &= \textbf{LOAEL}_{\textbf{HED}} \textbf{ ÷ UF}_{\textbf{C}} \\ &= 3 \text{ mg/kg-day} \div 10{,}000 \\ &= \textbf{3} \times \textbf{10}^{-4} \textbf{ mg/kg-day}\end{aligned}$$

The UF_C of 10,000 is presented in Table A.3.

Table A.3 summarizes the UFs for the screening chronic p-RfD for 2,6-DNT. Confidence in the screening value is by definition, low.

Table A.3. UFs for Screening Chronic p-RfD of 2,6-DNT[a]

UF	Value	Justification
UF_A	3	For the POD based on an increased incidence of splenic extramedullary hematopoiesis (Lee et al., 1976c), a UF_A of 3 ($10^{0.5}$) has been applied to account for uncertainty in characterizing the toxicodynamic differences between dogs and humans following oral 2,6-DNT exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a DAF as outlined in the *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	10	A UF_D of 10 has been applied because there are no acceptable two-generation reproductive toxicity or developmental toxicity studies via the oral route.
UF_H	10	A UF_H of 10 has been applied for inter-individual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of 2,6-DNT in humans.
UF_L	3	A UF_L of 3 has been applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL based on splenic extramedullary hepatopoiesis, for which the biological significance is not entirely clear.
UF_S	10	A UF_S of 10 has been applied to account for the extrapolation from less than chronic exposure.
UF_C	10,000	

[a]Lee et al. (1976c).

APPENDIX B. DATA TABLES

Table B.1. Body Weight and Absolute and Relative Organ Weight in Male CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	7	35	145
Male Rat—4 Wks				
Sample size	4	4	4	4
Body weight[b] (g)	391 ± 8	364 ± 18	306 ± 9*	225 ± 52*
Absolute liver[b] (g)	12.6 ± 0.6	12.7 ± 0.6	11.7 ± 0.5	9.2 ± 0.6*
Relative liver[b] (g organ/100 g body wt)	3.2 ± 0.1	3.5 ± 0.3	3.8 ± 0.1	4.1 ± 0.2*
Absolute spleen[b] (g)	0.81 ± 0.03	0.72 ± 0.03	0.64 ± 0.03	0.97 ± 0.09
Relative spleen[b] (g organ/100 g body wt)	0.2l ± 0.01	0.20 ± 0.02	0.2l ± 0.01	0.43 ± 0.03*
Absolute kidneys[b] (g)	3.26 ± 0.08	3.01 ± 0.12	3.04 ± 0.2l	2.29 ± 0.20*
Relative kidneys[b] (g organ/100 g body wt)	0.83 ± 0.01	0.83 ± 0.05	0.97 ± 0.05	0.02 ± 0.08
Absolute heart[b] (g)	1.34 ± 0.10	1.26 ± 0.03	1.06 ± 0.02*	0.79 ± 0.05*
Relative heart[b] (g organ/100 g body wt)	0.34 ± 0.02	0.35 ± 0.02	0.35 ± 0.00	0.35 ± 0.02
Absolute brain[b] (g)	2.08 ± 0.04	2.02 ± 0.09	2.05 ± 0.04	1.94 ± 0.07
Relative brain[b] (g organ/ 100 g body wt)	0.53 ± 0.02	0.56 ± 0.03	0.67 ± 0.02*	0.86 ± 0.02*
Male Rat—13Wks				
Sample size	4	4	4	4
Body weight[b] (g)	545 ± 2l	486 ± 24	45l ± 13*	256 ± 12*
Absolute liver[b] (g)	l5.4 ± 0.5	17.0 ± 1.3	10.7 ± 0.2*	8.9 ± 0.3*
Relative liver[b] (g organ/100 g body wt)	2.8 ± 0.0	3.5 ± 0.3*	2.4 ± 0.1	3.5 ± 0.1*
Absolute spleen[b] (g)	0.95 ± 0.06	1.02 ± 0.03	0.90 ± 0.10	0.65 ± 0.02*
Relative spleen[b] (g organ/100 g body wt)	0.17 ± 0.00	0.21 ± 0.01	0.20 ± 0.02	0.26 ± 0.02*
Absolute kidneys[b] (g)	3.09 ± 0.10	2.33 ± 0.18	3.05 ± 0.02	2.11 ± 0.07
Relative kidneys[b] (g organ/100 g body wt)	0.57 ± 0.03	0.48 ± 0.03	0.68 ± 0.02	0.83 ± 0.06*
Absolute heart[b] (g)	1.56 ± 0.09	1.60 ± 0.15	1.27 ± 0.01	0.99 ± 0.08*
Relative heart[b] (g organ/100 g body wt)	0.29 ± 0.01	0.33 ± 0.03	0.29 ± 0.00	0.39 ± 0.02*

Table B.1. Body Weight and Absolute and Relative Organ Weight in Male CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	7	35	145
Absolute brain[b] (g)	2.09 ± 0.10	2.17 ± 0.07	2.l8 ± 0.07	1.97 ± 0.05
Relative brain[b] (g organ/ 100 g body wt)	0.39 ± 0.03	0.45 ± 0.02	0.48 ± 0.00	0.78 ± 0.05*

[a]Lee et al. (1976a).
[b]Means ± SE.
*Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).

Table B.2. Body Weight and Absolute and Relative Organ Weight in Female CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	7	37	155
Female Rat—4 Wks				
Sample size	4	4	4	4
Body weight[b] (g)	232 ± 5	210 ± 7	194 ± 8*	157 ± 10*
Absolute liver[b] (g)	6.9 ± 0.3	6.5 ± 0.3	7.1 ± 0.2	6.0 ± 0.6*
Relative liver[b] (g organ/100 g body wt)	3.0 ± 0.1	3.1 ± 0.2	3.7 ± 0.1*	4.2 ± 0.2*
Absolute spleen[b] (g)	0.51 ± 0.04	0.54 ± 0.04	0.70 ± 0.08	0.46 ± 0.02
Relative spleen[b] (g organ/100 g body wt)	0.22 ± 0.02	0.26 ± 0.02	0.36 ± 0.05*	0.29 ± 0.02
Absolute kidneys[b] (g)	1.78 ± 0.04	1.62 ± 0.07	1.58 ± 0.10	1.44 ± 0.10*
Relative kidneys[b] (g organ/100 g body wt)	0.77 ± 0.03	0.77 ± 0.02	0.81 ± 0.04	0.91 ± 0.03*
Absolute heart[b] (g)	0.92 ± 0.08	0.86 ± 0.07	0.75 ± 0.04	0.58 ± 0.07*
Relative heart[b] (g organ/100 g body wt)	0.40 ± 0.04	0.41 ± 0.04	0.39 ± 0.03	0.37 ± 0.04
Absolute brain[b] (g)	1.88 ± 0.05	1.73 ± 0.03	1.82 ± 0.07*	1.86 ± 0.06
Relative brain[b] (g organ/ 100 g body wt)	0.81 ± 0.01	0.83 ± 0.02	0.94 ± 0.04	1.20 ± 0.07*
Female Rat—13Wks				
Sample size	4	4	4	4
Body weight[b] (g)	286 ± 11	270 ± 8	214 ± 17*	176 ± 9*
Absolute liver[b] (g)	7.9 ± 0.2	8.3 ± 0.6	7.1 ± 0.4	7.2 ± 0.2
Relative liver[b] (g organ/100 g body wt)	2.8 ± 0.0	3.1 ± 0.2	3.4 ± 0.3	4.1 ± 0.1*
Absolute spleen[b] (g)	0.58 ± 0.04	0.69 ± 0.09	0.54 ± 0.05	0.57 ± 0.06
Relative spleen[b] (g organ/100 g body wt)	0.20 ± 0.01	0.25 ± 0.03	0.26 ± 0.04	0.32 ± 0.02*
Absolute kidneys[b] (g)	1.65 ± 0.15	1.93 ± 0.07	1.71 ± 0.04	1.60 ± 0.08
Relative kidneys[b] (g organ/100 g body wt)	0.66 ± 0.01	0.62 ± 0.11	0.81 ± 0.06	0.91 ± 0.06*
Absolute heart[b] (g)	0.94 ± 0.03	0.86 ± 0.06	0.87 ± 0.06	0.66 ± 0.05*
Relative heart[b] (g organ/100 g body wt)	0.33 ± 0.01	0.32 ± 0.02	0.41 ± 0.03	0.38 ± 0.03
Absolute brain[b] (g)	1.88 ± 0.06	2.08 ± 0.07	2.00 ± 0.04	1.86 ± 0.06
Relative brain[b] (g organ/ 100 g body wt)	0.66 ± 0.03	0.77 ± 0.05	0.95 ± 0.06*	1.06 ± 0.03*

[a]Lee et al. (1976a).
[b]Means ± SE.
*Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).

Table B.3. Selected Hematology Parameters in the CD Rats Fed 2,6-DNT for 4 or 13 Weeks[a]

Parameter	Male Exposure Group, mg/kg-d			
	0	**7**	**35**	**145**
Male Rat—4 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	17.4 ± 1.7	19.5 ± 1.1	22.2 ± 1.2	31.5 ± 2.0*,**
Reticulocytes [b] (%)	1.39 ± 0.23*	1.73 ± 0.13*	0.85 ± 0.09*	9.33 ± 1.27*,**
Erythrocytes[b] ($\times 10^6$/MM^3)	7.15 ± 0.17	6.94 ± 0.03	7.78 ± 0.18*	5.80 ± 0.35**
Methemoglobin[b] (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Male Rat—13 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	20.7 ± 1.9	25.2 ± 4.9	27.4 ± 3.1*	25.0 ± 4.0
Reticulocytes[b] (%)	1.00 ± 0.12*	0.85 ± 0.20*	0.81 ± 0.16*	0.82 ± 0.26
Erythrocytes[b] ($\times 10^6$/MM^3)	6.81 ± 0.20	6.88 ± 0.57	7.76 ± 0.15*	7.40 ± 0.14*
Methemoglobin[b] (%)	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	3.0 ± 1.2*
Parameter	**Female Exposure Group, mg/kg-d**			
	0	**7**	**37**	**155**
Female Rat—4 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	19.3 ± 1.7	17.6 ± 1.6	22.7 ± 2.1	24.0 ± 3.1*
Reticulocytes[b] (%)	1.18 ± 0.20	1.07 ± 0.15*	0.85 ± 0.26*	5.24 ± 1.12**
Erythrocytes [b] ($\times 10^6$/MM^3)	6.92 ± 0.18	6.35 ± 0.31	6.78 ± 0.20	6.91 ± 0.22
Methemoglobin [b] (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6
Female Rat—13 Wks				
Leukocytes[b] ($\times 10^3$/MM^3)	17.5 ± 2.7	20.1 ± 2.0	26.4 ± 2.7*	20.5 ± 1.7
Reticulocytes[b] (%)	1.38 ± 0.16	1.24 ± 0.40*	1.13 ± 0.15*	0.89 ± 0.10
Erythrocytes[b] ($\times 10^6$/MM^3)	6.49 ± 0.44	6.35 ± 0.28	6.44 ± 0.24	7.06 ± 0.26
Methemoglobin[b] (%)	0.6 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 1.0*

[a]Lee et al. (1976a).
[b]Means ± SE.
*Significantly different from baseline (Dunnett's multiple comparison procedure).
**Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure).

Table B.4. Tissue Lesions in Male Rats Fed 2,6-DNT for 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	7	35	145
Sample size		4	4	4	4
Heart	Focal myocarditis	1[c]	0	0	0
Trachea	Tracheitis	1[d]	0	0	0
Lung	Chronic murine pneumonia	1[c]	0	0	0
Submaxillary Salivary gland	Sialadenitis	0	1[d]	0	0
Liver	Focal mononuclear infiltration	2[c]	2[c]	1[c]	1[c]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
	Bile duct hyperplasia	0	0	3[c]	4[c, *]
	Focal degeneration	0	0	0	1[c]
Kidney	Dilatation of pelvis and/or tubules	0	0	0	2[c]
Testis	Focal atrophy	1[d]	1[d]	0	0
	Degeneration, retardation of spermatogenesis	0	0	1[c]	0
	Atrophy and aspermatogenesis	0	0	0	4[d, *]
Spleen	Hemosiderosis	0	4[c, *]	0	4[d, *]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
Bone marrow	M/E ratio	- to 1.5	-	-	1.3−1.5

[a]Lee et al. (1976a).
[b]Four rats examined per dose group; data are expressed as number of rats exhibiting each type of lesion, except for bone marrow ratio in which the ratio in all four rats is provided as a range.
[c]Lesions were graded by the author as "present, minimal, or mild."
[d]Lesions were graded by the author as "moderate, marked, or severe."
[*]$p < 0.05$ by Fisher's Exact Test performed by EPA.

Table B.5. Tissue Lesions in Female Rats Fed 2,6-DNT for 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	7	37	155
Sample size		4	4	4	4
Liver	Focal mononuclear infiltration	2[c]	2[c]	2[c]	1[c]
	Extramedullary hematopoiesis	0	0	2[c]	4[c, *]
	Bile duct hyperplasia	0	0	3[c]	4[c, *]
	Focal degeneration	0	0	0	1[c]
Kidney	Microcalculi	1[c]	0	0	1[c]
	Focal mononuclear infiltration	1[c]	0	0	1[c]
Uterus	Infiltration of eosinophils	2[c]	0	0	0
Spleen	Hemosiderosis	2[c]	2[c], 2[d]	0	4[d, *]
	Extramedullary hematopoiesis	0	0	4[c, *]	4[c, *]
Bone marrow	M/E ratio	- to 1.6	-	-	- to 1.5

[a]Lee et al. (1976a).
[b]Four rats examined per dose group; data are expressed as number of rats exhibiting each type of lesion, except for bone marrow ratio in which the ratio in all four rats is provided as a range.
[c]Lesions were graded by the author as "present, minimal, or mild."
[d]Lesions were graded by the author as "moderate, marked, or severe."

Table B.6. Body Weight and Absolute and Relative Liver Weight in Male Albino Swiss Mice Fed 2,6-DNT for 13 Weeks[a]

Parameter	Exposure Group, mg/kg-d			
	0	11	51	289
Sample size	2	4	3	0
Body weight[b] (g)	27.5 ± 2.5	32.5±1.6	31.3±2.2	NA
Absolute liver[b] (g)	1.75 ± 0.46	1.51 ± 0.08	1.54 ± 0.08	NA
Relative liver[b] (g organ/100 g body wt)	6.3 ± 1.1	4.6 ± 0.1	4.9 ± 0.3	NA
Absolute kidneys[b] (g)	0.87 ± 0.01	1.01 ± 0.10	0.54 ± 0.06	NA
Relative kidneys[b] (g organ/100 g body wt)	3.19 ± 0.25	3.11 ± 0.22	1.73 ± 0.13*	NA

[a]Lee et al. (1976b).
[b]Means ± SE.
*Significantly different from corresponding control values at $p < 0.05$ (Dunnett's multiple comparison procedure).
NA=Not applicable.

Table B.7. Mortality in Male Albino Swiss Mice Treated with 2,6-DNT in Diet for 4 or 13 Weeks

Exposure Group, mg/kg-day	No. of Animals	No. of Mice Dying During Specified Weeks[a,b]				
		0− 4	5−8	9−13	14−17	Total[c]
Males						
0	16	0	0	3	0	3
11	16	2	0	0	0	2
51	16	6	0	1	1	8
289	16	0	6	2	0	8
Females						
0	16	0	0	0	0	0
11	16	0	0	0	0	0
55	16	1	0	0	0	1
299	16	1	3	2	0	6

[a]Number of dead animals.
[b]Number of animals includes mice treated for 4 and 13 weeks and necropsied and mice allowed to recover for 4 weeks.
[c]Calculated for this review from data reported in the study.

Source: Lee et al. (1976b)

Table B.8. Tissue Lesions in Male and Female Dogs Treated with 2,6-DNT for 4 or 13 Weeks[a]

Lesion Type[b]		Exposure group, mg/kg-d			
		0	4	20	100
4 weeks					
Sample Size		2	2	2	2
Lung	Focal inflammation	2	0	1	1
Liver	Extramedullary hematopoiesis	0	0	1	2
	Bile duct hyperplasia	0	0	1	0
	Degeneration	0	0	0	1
	Subacute inflammation	0	0	1	1
Testis	Degeneration and/or retardation of spermatogenesis	0	0	1	1
Ovary	Several graafian follicles, but no corpea lutea		1	1	1
Thyroid	Increase in parafollicular cells	0	1	2	1
Spleen	Extramedullary hematopoiesis	0	1	2	2
	Lymphoid depletion				1
Tonsil	Inflammation	0	1	0	1
Lymph Node	Lymphoid degeneration and depletion	0	0	0	1
Thymus	Involution	0	0	0	1
	Focal hyalinization of the corpuscles				1
Bone marrow	M/E ratio	1.3 to 1.4	1.1 to 1.3	1.1	- to 0.9
13 weeks					
Sample size		2	2	3	4
Liver	Extramedullary hematopoiesis	0	0	3	4
	Bile duct hyperplasia	0	0	2	2
	Focal degeneration	0	0	3	4
Kidney	Dilatation of pelvis and/or tubules	0	0	0	2
Testis	Atrophy and aspermatogenesis	0	0	0	2
Spleen	Extramedullary hematopoiesis	0	2[c]	3[d]	4[e]
Bone marrow	M/E ratio	1.3 to 1.5	1.3 to 1.5	- to 1.3	0.9 to 1.0

[a]Lee et al. (1976c).
[b]Data are expressed as number of dogs exhibiting each type of lesion, except for bone marrow ratio in which the ratio in dogs is provided as a range.
[c]Lesions were graded by the author as "minimal or mild."
[d]Lesions were graded by the author as "minimal, mild, or moderate."
[e]Lesions were graded by the author as "marked or markedly severe."

Table B.9. Mortality in Male and Female Dogs Treated with 2,6-DNT in Diet for 13 Weeks

Exposure Group, mg/kg-day	No. of Animals	No. of Dogs Dying During Specified Weeks[a]			
		0–4	5–8	9–13	Total[b]
Males/Females					
0	2	0	0	0	0
4	2	0	0	0	0
20	3	0	0	2	2
100	4	0	4	0	4

[a]Number of dead animals.
[b]Calculated for this review from data reported in the study.

Source: Lee et al. (1976c).

Table B.10. Reticulocyte Data in Dogs Treated with 2,6-DNT for up to 13 Weeks[a]

Parameter	Male/Female Exposure Group, mg/kg-day			
	0	4	20	100
2 Wks				
Reticulocytes [b] (%)	0.76 ± 0.07	1.10 ± 0.13	1.43 ± 0.32	16.99 ± 3.33*,**
4 Wks				
Reticulocytes [b] (%)	0.59 ± 0.08	0.98 ± 0.10	1.67 ± 0.43	6.23 ± 1.60**
8 Wks				
Reticulocytes[b] (%)	1.01 ± 0.24	0.88 ± 0.08	0.84 ± 0.30	No data
13 Wks				
Reticulocytes [b] (%)	0.56 ± 0.11	0.62 ± 0.0	1.91	No data

[a]Lee et al. (1976c).
[b]Means ± SE.
*Significantly different from baseline (Dunnett's multiple comparison procedure).
**Significantly different from the controls at the respective time interval (Dunnett's multiple comparison procedure).

Table B.11. Body Weight, Liver Weight, and Serum Enzyme Activities in the Male (F344)/CrlBR Rat Exposed to Oral 2,6-DNT for 26 and 52 Weeks[a]

Parameter	Exposure Group, mg/kg-d		
	0	7	14
Male Rat—6 Mo			
Sample size	4	4	4
Terminal body wt[b] (g)	395 ± 2	376 ± 9	316 ± 9*
Liver wt[b] (g)	9.62 ± 0.25	10.52 ± 0.47	11.42 ± 0.59*
Liver/body wt × 100[b]	2.43 ± 0.07	2.80 ± 0.07*	3.62 ± 0.08*
Serum activity			
ALT[b] (IU/liter)	69 ± 5	58 ± 3	48 ± 5
GGT[b] (IU/liter)	0.2 ± 0.2	1.2 ± 0.5	3.7 ± 0.6*
Male Rat—12 Mo			
Sample size	20	20	20
Terminal body wt[b] (g)	434 ± 3	356 ± 5*	297 ± 7*
Liver wt[b] (g)	10.30 ± 0.16	21.09 ± 0.74*	38.20 ± 2.14*
Liver/body wt × 100[b]	2.38 ± 0.04	5.99 ± 0.29*	13.19 ± 0.97*
Serum activity			
ALT[b] (IU/liter)	133 ± 14	230 ± 75*	1,044 ± 163*
GGT[b] (IU/liter)	3.8 ± 0.5	43 ± 18	205 ± 46*

[a]Leonard et al. (1987).
[b]Means ± SEM.
*Significantly different from corresponding control values at $p < 0.05$.

Table B.12. Treatment Protocol Adapted from Goldsworthy et al., 1986			
Treatment Group[a]	**No of Animals**	**Diet**	**Concentration of 2,6-DNT in the Diet, mg/kg-d[b]**
NIH	30	NIH-07	0
NIH-HD DNT	30	NIH-07	3−3.5
NIH-LD DNT	30	NIH-07	0.6−0.7
AIN	30	AIN-76A	0
AIN-HD DNT	30	AIN-76A	3−3.5
AIN-LD DNT	30	AIN-76A	0.6−0.7
AP	30	AIN-76A + 5% pectin	0
AP-HD DNT	30	AIN-76A + 5% pectin	3−3.5
AP-LD DNT	30	AIN-76A + 5% pectin	0.6−0.7

[a]The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2.6-DNT at either a high-dose (HD DNT) or low-dose (LD DNT).
[b]HED are 0, 0.13−0.15, and 0.63−0.74 mg/kg-day for the control, low-dose, and high-dose, respectively.

Table B.13. Effect of the Control and DNT-Diets on the Fraction of Animals with Hepatic Foci Identified by Three Phenotypic Markers[a]

Treatment Group[b]	Hepatic Phenotypic Markers		
	GGT	ATP	G6P
At 3 months			
NIH	2/10	6/10	5/10
NIH-LD DNT	6/10	10/10	9/10
NIH-HD DNT	9/10	10/10	8/10
AIN	0/10	4/10	1/10
AIN-LD DNT	0/10	7/10	5/10
AIN-HD DNT	0/10	7/10	7/10
AP	0/10	4/10	5/10
AP-LD DNT	0/10	8/10	7/10
AP-HD DNT	0/10	9/10	10/10
At 6 months			
NIH	2/10	5/10	5/10
NIH-LD DNT	9/10	8/10	10/10
NIH-HD DNT	8/10	10/10	10/10
AIN	0/10	4/10	3/10
AIN-LD DNT	0/10	7/10	4/10
AIN-HD DNT	3/10	10/10	10/10
AP	0/10	4/10	3/10
AP-LD DNT	0/10	9/10	6/10
AP-HD DNT	3/10	10/10	10/10
At 12 months			
NIH	10/10	10/10	9/10
NIH-LD DNT	10/10	10/10	10/10
NIH-HD DNT	10/10[c]	10/10[c]	10/10[c]
AIN	0/10	9/10	6/10
AIN-LD DNT	5/10	10/10	8/10
AIN-HD DNT	10/10	10/10	10/10
AP	3/10	9/10	9/10
AP-LD DNT	7/10	10/10	10/10
AP-HD DNT	10/10	10/10	10/10

[a]Goldsworthy et al. (1986).

[b]The diets used were NIH-07, an open formula cereal-based diet high in pectin content; AIN-76A, a purified pectin-free diet; or AP, which is AlN-76A supplemented with 5% pectin. These three diets served as the control diets for the addition of 2,6-DNT at either a high-dose (HD DNT) or low-dose (LD DNT).

[c]Livers exhibited multi foci, neoplastic nodules (6/10), and hepatocellular carcinomas (6/10). The presence of these lesions did not allow for the accurate quantitation of the foci in these livers.

Table B.14. Incidence of Neoplastic Lesions and Metastases in a 1-Year Oral Study of 2,6-DNT in the Male (F344)/Crlbr Rat[a]

Lesion Type	Exposure Group, mg/kg-d		
	0 (0)	7	14
Neoplastic nodules	0/20	18/20*	15/19*
Hepatocellular carcinoma:Trabecular	0/20	17/20*	19/19*
Adenocarcinoma	0/20	1/20	0/19
Cholangiocarcinoma	0/20	2/20	0/19
Pulmonary metastases	NA	3/20	11/19

[a]Leonard et al. (1987).
NA = not applicable (no primary tumors present).
*$p < 0.001$ by Fisher's Exact Test performed by EPA.

APPENDIX C. BMD OUTPUTS FOR THE SUBCHRONIC AND CHRONIC p-RfDs

MODELING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.1.2). For these data, all continuous models available within the software were fit using a BMR of 10% relative risk or 1 standard deviation. An adequate fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a homogeneous variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; *p*-value < 0.1), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the screening subchronic and chronic p-RfD.

APPENDIX D. BENCHMARK DOSE CALCULATIONS FOR THE ORAL SLOPE FACTOR

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence data is as follows. The Multistage-cancer model in the EPA benchmark dose software (BMDS) is fit to the incidence data using the extra risk option. The Multistage-cancer model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). An adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the BMDL from the best fitting Multistage-cancer model as judged by the goodness-of-fit p-value, is selected as the point of departure. In accordance with EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

MODEL-FITTING RESULTS FOR HEPATOCELLULAR CARCINOMAS IN MALE F344 RATS (Leonard et al., 1987)

Table B.14 shows the dose-response data on hepatocellular tumors in male F344 rats administered 2,6-DNT via the diet for 12 months (Leonard et al., 1987). Modeling was performed according to the procedure outlined above using BMDS version 2.1.2 with parameter restrictions for rats based on the duration-adjusted animal doses shown in Table 3. Model predictions are shown in Table 9. For incidence of hepatocellular carcinomas in both male rats, the multistage-cancer model provided an adequate fit (goodness-of-fit p-value >0.1). The 3-degree polynomial model yielded a BMD_{10} value of 2.7 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10}$) of 0.25 mg/kg-day for male rats. The fit of the multistage-cancer models to the hepatocellular carcinoma incidence data for male rats is shown in Table 9 and Figure D.1.

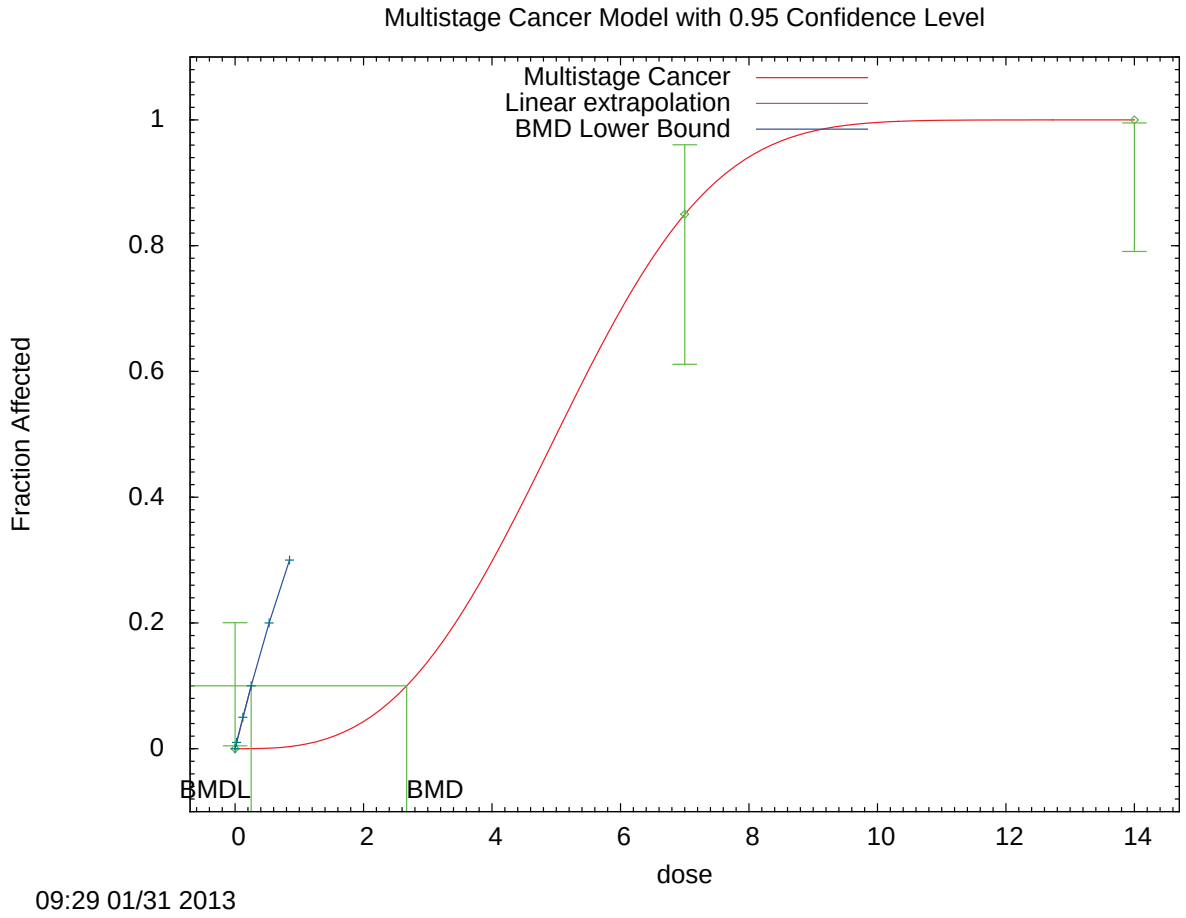

Figure D.1. Multistage Cancer Model for Hepatocellular Carcinomas in Male Rats (Leonard et al., 1987)

Text Output for Multistage Cancer Model for Hepatocellular Carcinomas in Male Rats (Leonard et al., 1987)

```
 ================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/Documents and Settings/JKaiser/Desktop/modeling
results/msc_26dnt_hcar_m_Msc3-BMR10.(d)
        Gnuplot Plotting File:  C:/Documents and Settings/JKaiser/Desktop/modeling
results/msc_26dnt_hcar_m_Msc3-BMR10.plt
                                                Thu Jan 31 09:29:24 2013
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

Observation # < parameter # for Multistage Cancer model.
   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Response
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =            0
                        Beta(3) = 3.92465e+016


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(3)

   Beta(3)           1



                                Parameter Estimates
```

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	0.00553091	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-8.45418	3			
Fitted model	-8.45419	1	9.74156e-006	2	1
Reduced model	-39.4517	1	61.995	2	<.0001

AIC: 18.9084

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	20	-0.000
7.0000	0.8500	17.000	17.000	20	0.000
14.0000	1.0000	19.000	19.000	19	0.002

Chi^2 = 0.00 d.f. = 2 P-value = 1.0000

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.67071

BMDL = 0.250402

BMDU = 3.13339

Taken together, (0.250402, 3.13339) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.399358

APPENDIX E. REFERENCES

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Attachment

1I

6

1,4-Dioxane; CASRN 123-91-1

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR 1,4-Dioxane

File First On-Line 08/22/1988

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	08/11/2010
Inhalation RfC (I.B.)	yes	09/20/2013
Carcinogenicity Assessment (II.)	yes	09/20/2013

I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Substance Name – 1,4-Dioxane
CASRN – 123-91-1
Section I.A. Last Revised – 08/11/2010

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iris/backgrd.html for an elaboration of these concepts. Because RfD values can be derived for the noncarcinogenic health effects of

substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

There was no previous oral RfD for 1,4-dioxane on IRIS.

I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure	UF	Chronic RfD
Liver and kidney toxicity **Chronic oral male rat study** **Kociba et al. (1974)**	NOAEL: 9.6 mg/kg-day	300	0.03 mg/kg-day

I.A.2. PRINCIPAL AND SUPPORTING STUDIES (ORAL RfD)

Liver and kidney toxicity were the primary noncancer health effects associated with exposure to 1,4-dioxane in humans and laboratory animals. Occupational exposure to 1,4-dioxane has resulted in hemorrhagic nephritis and centrilobular necrosis of the liver (Johnstone, 1959; Barber, 1934). In animals, liver and kidney degeneration and necrosis were observed frequently in acute oral and inhalation studies (JBRC, 1998a; Drew et al., 1978; David, 1964; Kesten et al., 1939; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935; Fairley et al., 1934). Liver and kidney effects were also observed following chronic oral exposure to 1,4-dioxane in animals (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Argus et al., 1965) [see summary Table 4-25 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013)].

In the available chronic studies, (Kociba et al., 1974) reported the most sensitive effects in the liver and kidney based on a NOAEL of 9.6 mg/kg-day and a LOAEL of 94 mg/kg-day in male Sherman rats. Kociba et al. (1974) reported toxic effects of hepatocellular degeneration and necrosis in the liver, while liver lesions reported in other studies (JBRC, 1998b; Argus et al., 1973) appeared to be related to the carcinogenic process. Kociba et al. (1974) also reported renal tubule epithelial cell degenerative changes and necrosis in the kidney which was supported by data in NCI (1978) and Argus et al. (1973); however, kidney toxicity was observed in these studies at higher doses. For degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. (1974) study represents the most sensitive effect and

dataset observed in a chronic bioassay. As a result, Kociba et al. (1974) was chosen as the principal study for the derivation of the RfD.

Kociba et al. (1974) conducted a 2-year study in which four groups of 6-8-week-old Sherman rats (60/sex/dose level) were administered 1,4-dioxane in drinking water at levels of 0 (controls), 0.01, 0.1, or 1.0% for up to 716 days. Based on water consumption and BW data for specific exposure groups, Kociba et al. (1974) calculated mean daily doses of 9.6, 94, and 1,015 mg/kg-day for male rats and 19, 148, and 1,599 mg/kg-day for female rats during days 114-198 for the 0.01, 0.1, and 1.0% concentration levels, respectively. Rats were observed daily for clinical signs of toxicity, and BWs were measured twice weekly during the first month, weekly during months 2-7, and biweekly thereafter. Water consumption was recorded at three different time periods during the study: days 1-113, 114-198, and 446-460. Blood samples were collected from a minimum of five male and five female control and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at termination. Each blood sample was analyzed for packed cell volume, total erythrocyte count, hemoglobin, and total and differential WBC counts. Additional endpoints evaluated included organ weights (brain, liver, kidney, testes, spleen, and heart) and gross and microscopic examination of major tissues and organs (brain, bone and bone marrow, ovaries, pituitary, uterus, mesenteric lymph nodes, heart, liver, pancreas, spleen, stomach, prostate, colon, trachea, duodenum, kidneys, esophagus, jejunum, testes, lungs, spinal cord, adrenals, thyroid, parathyroid, nasal turbinates, and urinary bladder).

Histopathological lesions were restricted to the liver and kidney from the mid- and high-dose groups and consisted of variable degrees of renal tubular epithelial and hepatocellular degeneration and necrosis (no quantitative incidence data were provided). Rats from these groups also showed evidence of hepatic regeneration, as indicated by hepatocellular hyperplastic nodule formation and evidence of renal tubular epithelial regenerative activity (observed after 2 years of exposure). These changes were not seen in controls or in low-dose rats. The authors determined a NOAEL of 9.6 mg/kg-day and a LOAEL of 94 mg/kg-day for 1,4-dioxane based on the liver and kidney effects in male rats.

Methods of Analysis. Kociba et al. (1974) did not provide quantitative incidence or severity data for liver and kidney degeneration and necrosis. Benchmark dose (BMD) modeling could not be performed for this study, and the NOAEL for liver and kidney degeneration (9.6 mg/kg-day in male rats) was used as the point of departure (POD) in deriving the RfD for 1,4-dioxane.

Other datasets and alternative POD values were also considered as the basis for the 1,4-dioxane RfD, including incidence data reported for cortical tubule degeneration in male and female rats (NCI, 1978) and liver hyperplasia (JBRC, 1998b). The $BMDL_{10}$ values of 22.3

mg/kg-day and 23.8 mg/kg-day from the (NCI, 1978) and (JBRC, 1998b) studies, respectively, are about double the NOAEL (9.6 mg/kg-day) observed by Kociba et al. (1974).

I.A.3. UNCERTAINTY FACTORS

UF = 300
= 10 (UF_A) × 10 (UF_H) × 1 (UF_S) × 1 (UF_L) × 3 (UF_D)

A default interspecies UF of 10 (UF_A) was used to account for pharmacokinetic and pharmacodynamic differences between rats and humans. Existing PBPK models could not be used to derive an oral RfD for 1,4-dioxane.

A default interindividual variability UF of 10 (UF_H) was used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, 1,4-dioxane.

An UF to extrapolate from a subchronic to a chronic (UF_S) exposure duration was not necessary (e.g., $UF_S = 1$) because the RfD was derived from a study using a chronic exposure protocol.

An UF to extrapolate from a LOAEL to a NOAEL (UF_L) was not necessary (e.g., $UF_L = 1$) because the RfD was based on a NOAEL. Kociba et al. (1974) was a well-conducted, chronic drinking water study with an adequate number of animals. Histopathological examination was performed for many organs and tissues, but clinical chemistry analysis was not performed. NOAEL and LOAEL values were derived from the study based on liver and kidney toxicity.

An UF of 3 for database deficiencies (UF_D) was applied due to the lack of a multigeneration reproductive toxicity study.

I.A.4. ADDITIONAL STUDIES/COMMENTS

The predominant noncancer effect of chronic oral exposure to 1,4-dioxane is degenerative effects in the liver and kidney. For degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. (1974) study represents the most sensitive effect and dataset observed in a chronic bioassay.

Kidney toxicity as evidenced by glomerulonephritis (Argus et al., 1965; Argus et al., 1973) and degeneration of the cortical tubule (CAA, 1990; NCI, 1978; Kociba et al., 1974) has also been observed in response to chronic exposure to 1,4-dioxane. Degenerative effects were observed in the kidney at the same dose level as effects in the liver (Kociba et al., 1974).

Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978) (mice only, dose ≥ 380 mg/kg-day) and JBRC (1998a) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-day in mice). JBRC (1998a)reported nasal inflammation in rats (NOAEL 55 mg/kg-day, LOAEL 274 mg/kg-day) and mice (NOAEL 66 mg/kg-day, LOAEL 278 mg/kg-day).

Studies in experimental animals have also found that relatively high doses of 1,4-dioxane (1,000 mg/kg-day) during gestation can produce delayed ossification of the sternebrae and reduced fetal BWs (Giavini et al., 1985).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF)

I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study - Medium
Data Base - Medium
RfD - Medium

The overall confidence in the RfD is medium. Confidence in the principal study (Kociba et al., 1974) is medium. The 2-year drinking water study is a well-conducted, peer-reviewed study that used 3 dose groups plus a control. The study had adequate group sizes (60 rats/sex/dose group) and investigated multiple target organs.

Confidence in the oral database is medium due to the lack of a multigeneration reproductive toxicity study.

Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfD is medium.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document – *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and the Executive Office of the President, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). **To**

review this appendix, exit to the Toxicological Review, Appendix A, Summary of External Peer Review and Public Comments and Disposition (PDF).

I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Substance Name — 1,4-Dioxane
CASRN — 123-91-1
Section I.B. Last Revised — 09/20/2013

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. The U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation is contained in Section II of this file.

I.B.1. CHRONIC INHALATION RfC SUMMARY

Critical Effect	Point of Departure*	UF	Chronic RfC
Atrophy and respiratory metaplasia of the olfactory epithelium **Chronic inhalation male rat study** **Kasai et al. (2009)**	LOAEL: 50 ppm LOAEL POD_{ADJ}: 8.9 ppm LOAEL POD_{HEC}: 32.2 mg/m^3	1,000	3×10^{-2} mg/m^3

*****Conversion Factors and Assumptions - Rats in the principal study were exposed for 6 hours/day, 5 days/week, for 104 weeks. The POD associated with intermittent exposure was adjusted to continuous exposure by multiplying the POD by 6/24 hours and 5/7 days resulting in the POD_{ADJ} of 8.9 ppm. This ppm value was converted to mg/m^3 using the chemical-specific conversion factor of 1 ppm = 3.6 mg/m^3. Additionally, the POD was adjusted to reflect the human equivalent concentration (HEC) by application of a chemical-specific dosimetric adjustment factor (DAF) of 1.12 resulting in the POD_{HEC} of 32.2 mg/m^3

I.B.2. PRINCIPAL AND SUPPORTING STUDIES

Four inhalation studies in animals were identified in the literature; two, 13 week subchronic studies in laboratory animals (Kasai et al., 2008; Fairley et al., 1934) and two, 2 year chronic studies in rats (Kasai et al., 2009; Torkelson et al., 1974). Nasal, liver, and kidney toxicity were the primary noncancer health effects of inhalation exposure to 1,4-dioxane in rodents (see summary Table 4-26 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013).

The chronic Kasai et al. (2009) study was selected as the principal study for the derivation of the RfC. Groups of male 6 week old F344/DuCrj rats (50/group) were exposed via inhalation to nominal concentrations of 0 (clean air), 50, 250, and 1,250 ppm (0, 180, 900, and 4,500 mg/m^3, respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 104 weeks (2 years) in whole body inhalation chambers (Kasai et al., 2009). At the time of death or at the end of the 2-years of exposure the authors examined multiple organ systems. Based on the noncancer database for 1,4-dioxane, this study demonstrated exposure concentration related effects for histopathological lesions at a lower concentration (50 ppm) compared to the subchronic Kasai et al. study (2008). The 2 year bioassay (Kasai et al., 2009) did not observe effects in both sexes, but the use of only male rats was proposed by the study authors as justified by data illustrating the absence of induced mesotheliomas in female rats

following exposure to 1,4-dioxane in drinking water (Yamazaki et al., 1994). Additionally, a similar pattern of effects was observed after oral exposure to 1,4-dioxane (Kano et al., 2009; JBRC, 1998b) as observed in the Kasai et al. (2009) 2 year inhalation study.

Nonneoplastic lesions from the Kasai et al. (2009) study that were statistically increased as compared to control were considered candidates for the critical effect. The candidate endpoints included centrilobular necrosis of the liver, squamous cell metaplasia of nasal respiratory epithelium, squamous cell hyperplasia of nasal respiratory epithelium, respiratory metaplasia of nasal olfactory epithelium, sclerosis in lamina propria of nasal cavity, and two degenerative nasal lesions, that is, atrophy of nasal olfactory epithelium and hydropic change in the lamina propria. Despite statistical increases at the low and mid exposure concentrations (50 and 250 ppm, respectively), incidences of nuclear enlargement of respiratory epithelium (nasal cavity), olfactory epithelium (nasal cavity), and proximal tubule (kidney) were not considered candidates for the critical effect, since nuclear enlargement as a specific morphologic diagnosis is not considered by EPA to be an adverse effect of exposure to 1,4-dioxane.

Methods of Analysis. Benchmark dose (BMD) modeling methodology (U.S. EPA, 2012) was used to analyze the candidate endpoints identified for 1,4-dioxane. BMDs and BMDLs were able to be determined for centrilobular necrosis, squamous cell metaplasia and hyperplasia of the respiratory epithelium, and hydropic change of lamina propria. Due to poor fit or substantial model uncertainty, BMD model results were inadequate for the following nasal lesions: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, the NOAEL/LOAEL approach was used to determine potential PODs for these endpoints.

Other endpoints in Kasai et al. (2009) were considered as alternative POD values in the derivation of the RfC, including incidence data reported for centrilobular necrosis in the liver and other respiratory effects. Alternative PODs are shown in Table 5-4 and Figure 5-5 of the *Toxicological Review for 1,4-Dioxane* (U.S. EPA, 2013).

I.B.3. UNCERTAINTY FACTORS

UF = 1,000

$= 3\ (UF_A) \times 10\ (UF_H) \times 1\ (UF_S) \times 10\ (UF_L) \times 3\ (UF_D)$

An interspecies UF of 3 (UF_A) was used for animal to human extrapolation to account for pharmacodynamic differences between species. This uncertainty factor is comprised of two separate areas of uncertainty to account for differences in the toxicokinetics and toxicodynamics of animals and humans. In this assessment, the toxicokinetic uncertainty was accounted for by the calculation of a HEC and application of a dosimetric adjustment factor as

outlined in the RfC methodology (U.S. EPA, 1994). As the toxicokinetic differences are thus accounted for, only the toxicodynamic uncertainties remain, and an UFA of 3 is retained to account for this uncertainty.

A default interindividual variability UF of 10 (UF_H) was used to account for variation in sensitivity within human populations because there is limited information on the degree to which humans of varying gender, age, health status, or genetic makeup might vary in the disposition of, or response to, 1,4-dioxane. A recent modeling study by Valcke and Krishnan (2011) assessed the impact of exposure duration and concentration on the human kinetic adjustment factor and estimated the neonate to adult 1,4-dioxane blood concentration ratio to be 3.2. Thus, a full factor of 10 was used to account for differences between adults and neonates, as well as other differences in gender, age, health status, or genetics that might result in a different disposition of, or response to, 1,4-dioxane.

An UF to extrapolate from a subchronic to a chronic (UF_S) exposure duration was not necessary (e.g., $UF_S = 1$) because the RfC was derived from a study using a chronic exposure protocol.

An UF of 10 (UF_L) was used to extrapolate from a LOAEL to a NOAEL because a LOAEL was used as the POD. A NOAEL for atrophy and respiratory metaplasia of the olfactory epithelium was not identified in the study by Kasai et al. (2009).

An UF of 3 for database deficiencies (UF_D) was applied due to the lack of a multigeneration reproductive toxicity study.

I.B.4. ADDITIONAL STUDIES/COMMENTS

Prior to the Kasai et al. (Kasai et al., 2009; Kasai et al., 2008) studies, two other studies were available for consideration in the derivation of inhalation toxicity values: one subchronic study (Fairley et al., 1934) and one chronic inhalation study (Torkelson et al., 1974). In the subchronic study, rabbits, guinea pigs, rats, and mice (3-6/species/group) were exposed to 1,000, 2,000, 5,000, or 10,000 ppm of 1,4-dioxane vapor for 1.5 hours two times a day for 5 days, 1.5 hours for one day, and no exposure on the seventh day. Animals were exposed until death occurred or were sacrificed after various durations of exposure (3-202.5 hours). Detailed dose-response information was not provided; however, severe liver and kidney damage and acute vascular congestion of the lungs were observed at concentrations ≥ 1,000 ppm. Kidney damage was described as patchy degeneration of cortical tubules with vascular congestion and hemorrhage. Liver lesions varied from cloudy hepatocyte swelling to large areas of necrosis.

Torkelson et al. (1974) performed a chronic inhalation study in which male and female Wistar rats (288/sex) were exposed to 111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years. Control rats (192/sex) were exposed to filtered air. No significant effects were observed

on BWs, survival, organ weights, hematology, clinical chemistry, or histopathology. Because Fairley et al. (1934) identified a free-standing LOAEL only, and Torkelson et al. (1974) identified a free-standing NOAEL only, neither study was sufficient to characterize the inhalation risks of 1,4-dioxane.

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

I.B.5. CONFIDENCE IN THE CHRONIC INHALATION RfC

Study—Medium
Database—Medium
RfC—Medium

The overall confidence in the RfC is medium. Confidence in the principal study (Kasai et al., 2009) is medium. The 2-year inhalation study is a well-conducted, peer-reviewed study that used 3 dose groups plus a control. The study had adequate group sizes (50 rats/dose group) and investigated multiple target organs; however, the study did only used male rats and did not investigate chronic effects in females.

Confidence in the database is medium due to the lack of supporting studies and a multigeneration reproductive toxicity study.

Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfC is medium.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

I.B.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC INHALATION RfC

Source Document—*Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013).

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and the Executive Office of the President, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). ***To review this appendix, exit to the toxicological review, Appendix A, EPA Response to Major External Peer Review and Public Comments (PDF).***

Agency Completion Date—09/20/2013

___I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name — 1,4-Dioxane
CASRN — 123-91-1
Last Revised — 09/20/2013

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per µg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

A cancer assessment for 1,4-dioxane via the oral route of exposure was posted on the IRIS databse in 2010. At that time, 1,4-dioxane was classified as a likely human carcinogen, based on the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). This update adds an inhalation unit risk (IUR) to that assessment, and the weight-of-evidence cancer classification remains the same.

A previous cancer assessment for 1,4-dioxane was posted on the IRIS database in 1988. At that time, 1,4-dioxane was classified as a B2 carcinogen (probable human carcinogen), based on inadequate human data and sufficient evidence of carcinogenicity in animals (induction of nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs). An oral cancer slope factor (CSF) of 1.1×10^{-2} (mg/kg-day)$^{-1}$ was derived from the tumor incidence data for nasal squamous cell carcinoma in male rats exposed to 1,4-dioxane in drinking water for 2 years (NCI, 1978). The linearized multistage extra risk procedure was used for linear low dose extrapolation. An inhalation unit risk (IUR) was not previously derived.

II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), 1,4-dioxane is characterized as "likely to be carcinogenic to humans." This characterization is based on the following findings: (1) inadequate evidence of carcinogenicity in humans, and (2) sufficient evidence in animals (i.e., hepatic tumors in multiple species [three strains of rats, two strains of mouse, and in guinea pigs]; mesotheliomas of the peritoneum, mammary, and nasal tumors have also been observed in rats following 2 years of oral exposure to 1,4-dioxane).

There is adequate evidence of liver carcinogenicity in several 2-year bioassays conducted in three strains of rats, two strains of mice, and in guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965). Additionally, tumors of the peritoneum (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), mammary (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), and nasal cavitiy (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Argus et al., 1973; Hoch-Ligeti et al., 1970), as well as kidney, Zymbal gland, and subcutaneous tissue (Kasai et al., 2009) have been observed in rats due to exposure to 1,4-dioxane. Studies in humans are inconclusive regarding evidence for a causal link between occupational exposure to 1,4-dioxane and increased risk for cancer; however, only two studies were available and these were limited by

small cohort size and a small number of reported cancer cases (Buffler et al., 1978; Thiess et al., 1976).

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of 1,4-dioxane via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. Information on the carcinogenic effects of 1,4-dioxane via the dermal route in humans and animals is absent. Based on the observance of systemic tumors following oral and inhalation exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, 1,4-dioxane is "likely to be carcinogenic to humans" by all routes of exposure.

A MOA hypothesis involving sustained proliferation of spontaneously transformed liver cells has some support from data indicating that 1,4-dioxane acts as a tumor promoter in mouse skin and rat liver bioassays (Lundberg et al., 1987; King et al., 1973). Dose-response and temporal data support the occurrence of cell proliferation and hyperplasia prior to the development of liver tumors (JBRC, 1998b; Kociba et al., 1974) in the rat model. However, the dose-response relationship for induction of hepatic cell proliferation has not been characterized, and it is unknown if it would reflect the dose-response relationship for liver tumors in the 2-year rat and mouse studies. Conflicting data from rat and mouse bioassays (JBRC, 1998b; Kociba et al., 1974) suggest that cytotoxicity may not be a required precursor event for 1,4-dioxane-induced cell proliferation. Liver tumors were observed in female rats and female mice in the absence of lesions indicative of cytotoxicity (Kano et al., 2009; JBRC, 1998b; NCI, 1978). Thus, data regarding a plausible dose response and temporal progression from cytotoxicity and cell proliferation to eventual liver tumor formation are not available. The MOA by which 1,4 dioxane produces liver, nasal, peritoneal (mesotheliomas), and mammary gland tumors is not conclusive, and the available data do not support any hypothesized carcinogenic MOA for 1,4 dioxane.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.8 (PDF)

II.A.2. HUMAN CARCINOGENICITY DATA

Human studies of occupational exposure to 1,4-dioxane were inconclusive to assess the evidence of carcinogenicity of 1,4-dioxane (see Section 4.1 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). In each case, the cohort size and number of reported cases were of limited size (Buffler et al., 1978; Thiess et al., 1976).

II.A.3. ANIMAL CARCINOGENICITY DATA

Three chronic drinking water bioassays provided incidence data for liver tumors in rats and mice, and nasal cavity, peritoneal, and mammary gland tumors in rats only (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974). With the exception of the NCI (1978) study, the incidence of nasal cavity tumors was generally lower than the incidence of liver tumors in exposed rats. The Kano et al. (2009) drinking water study was chosen as the principal study for derivation of an oral cancer slope factor (CSF) for 1,4-dioxane. This study used three dose groups in addition to controls and characterized the dose-response relationship at lower exposure levels, as compared to the high doses employed in the NCI (1978) bioassay. The Kociba et al. (1974) study also used three low dose exposure groups; however, the study authors only reported the incidence of hepatocellular carcinoma, which may underestimate the combined incidence of rats with adenoma or carcinoma. In addition to increased incidence of liver tumors, chosen as the most sensitive target organ for tumor formation, the Kano et al. (2009) study also noted increased incidence of peritoneal and mammary gland tumors. Nasal cavity tumors were also seen in high-dose male and female rats; however, the incidence of nasal tumors was much lower than the incidence of liver tumors in both rats and mice.

As described in detail in Section 4.2.1.2.6 and Appendix E of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013), the Japanese Bioassay Research Center conducted a 2-year drinking water study on the effects of 1,4-dioxane in both sexes of rats and mice. The results from that study were reported several times, once as conference proceedings (Yamazaki et al., 1994), once as a detailed laboratory report (JBRC, 1998b), and once as a published manuscript (Kano et al., 2009). As a result of the most recent publication (Kano et al., 2009), the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2010) was updated and the data in the new publication was considered. Although the data contained in the reports varied, the differences were minor and did not affect the conclusions of this assessment. The variations included: (1) the level of detail on dose information reported; (2) categories for incidence data reported (e.g., all animals or sacrificed animals); and (3) analysis of non- and neoplastic lesions.

A chronic bioassay of 1,4-dioxane by the inhalation route reported by Kasai et al. (2009) provides data adequate for dose response modeling and was subsequently chosen as the study for the derivation of an IUR for 1,4-dioxane. In this bioassay, groups of 50 male F344 rats were exposed to either 0, 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2

years (104 weeks). In male F344 rats, 1,4-dioxane produced a statistically significant increase in incidence and/or a statistically significant dose response trend for the following tumor types: hepatomas, nasal squamous cell carcinomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai et al., 2009).

II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978; Kociba et al., 1974; Torkelson et al., 1974; Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965). Liver tumors have been observed following drinking water exposure in male Wistar rats (Argus et al., 1965), male guinea pigs (Hoch-Ligeti and Argus, 1970), male Sprague Dawley rats (Argus et al., 1973; Hoch-Ligeti et al., 1970), male and female Sherman rats (Kociba et al., 1974), female Osborne-Mendel rats (NCI, 1978), male and female F344/DuCrj rats (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994), male and female B6C3F$_1$ mice (NCI, 1978), and male and female Crj:BDF$_1$ mice (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994). In the earliest cancer bioassays, the liver tumors were described as hepatomas (Argus et al., 1973; Hoch-Ligeti and Argus, 1970; Hoch-Ligeti et al., 1970; Argus et al., 1965); however, later studies made a distinction between hepatocellular carcinoma and hepatocellular adenoma (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994; NCI, 1978). Both tumor types have been seen in rats and mice exposed to 1,4-dioxane. Kociba et al. (1974) noted evidence of liver toxicity at or below the dose levels that produced liver tumors but did not report incidence data for these effects. Hepatocellular degeneration and necrosis were observed in the mid- and high-dose groups of male and female Sherman rats exposed to 1,4-dioxane, while tumors were only observed at the highest dose. Hepatic regeneration was indicated in the mid- and high-dose groups by the formation of hepatocellular hyperplastic nodules. Findings from JBRC (1998b) also provided evidence of liver hyperplasia in male F344/DuCrj rats at a dose level below the dose that induced a statistically significant increase in tumor formation.

Nasal cavity tumors were also observed in Sprague Dawley rats (Argus et al., 1973; Hoch-Ligeti et al., 1970), Osborne-Mendel rats (NCI, 1978), Sherman rats (Kociba et al., 1974), and F344/DuCrj rats (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998b; Yamazaki et al., 1994). Most tumors were characterized as squamous cell carcinomas. Nasal tumors were not elevated in B6C3F$_1$ or Crj:BDF$_1$ mice. JBRC (1998b) was the only study that evaluated nonneoplastic changes in nasal cavity tissue following prolonged exposure to 1,4-dioxane in the drinking water. Histopathological lesions in female F344/DuCrj rats were suggestive of toxicity and regeneration in this tissue (i.e., atrophy, adhesion, inflammation, nuclear enlargement, and hyperplasia and metaplasia of respiratory and olfactory epithelium). Some of these effects

occurred at a lower dose (83 mg/kg-day) than that shown to produce nasal cavity tumors (429 mg/kg-day) in female rats. Reexamination of tissue sections from the NCI (1978) bioassay suggested that the majority of nasal tumors were located in the dorsal nasal septum or the nasoturbinate of the anterior portion of the dorsal meatus. Nasal tumors were not observed in an inhalation study in Wistar rats exposed to 111 ppm for 5 days/week for 2 years (Torkelson et al., 1974).

Tumor initiation and promotion studies in mouse skin and rat liver suggested that 1,4-dioxane does not initiate the carcinogenic process, but instead acts as a tumor promoter (Lundberg et al., 1987; Bull et al., 1986; King et al., 1973) [see Section 4.2.3 in the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013)].

In addition to the liver and nasal tumors observed in several studies, a statistically significant increase in mesotheliomas of the peritoneum was seen in male rats from the Kano et al. (2009) (also (JBRC, 1998b; Yamazaki et al., 1994) and Kasai et al. (2009). Female rats dosed with 429 mg/kg-day in drinking water for 2 years also showed a statistically significant increase in mammary gland adenomas (Kano et al., 2009; JBRC, 1998b; Yamazaki et al., 1994) A significant increase in the incidence of these tumors was not observed in other chronic oral bioassays of 1,4-dioxane (NCI, 1978; Kociba et al., 1974). Additional statistically significant increases in other tumor types were observed in male F344 rats exposed to 0, 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2 years (104 weeks) including renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai et al., 2009).

II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

II.B.1. SUMMARY OF RISK ESTIMATES

II.B.1.1. Oral Slope Factor: 1×10^{-1} per mg/kg-day

The derivation of the oral slope factor 1×10^{-1} per mg/kg-day is based on the incidence of hepatocellular adenomas and carcinomas in female mice exposed to 1,4-dioxane in drinking water for 2 years (Kano et al., 2009). The dose metric used in the current estimate of the human equivalent dose (HED) is the applied or external dose because a PBPK model was determined not to be suitable for species extrapolation (see Appendix B of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). The rat $BMDL_{50}$ of 32.93 mg/kg-day represents the POD used to calculate the $BMDL_{50HED}$ of 4.95 mg/kg-day.

The oral slope factor is derived from the $BMDL_{50HED}$, the 95% lower bound on the exposure associated with a 50% extra cancer risk, by dividing the risk (as a fraction) by the $BMDL_{50HED}$, and represents an upper bound, continuous lifetime exposure risk estimate:

$BMDL_{50HED}$, lower 95% bound on exposure at 50% extra risk – 4.95 mg/kg-day

BMD_{50HED}, central estimate of exposure at 50% extra risk – 7.51 mg/kg-day

The slope of the linear extrapolation from the central estimate is 0.5/ (7.51 mg/kg-day) = 7×10^{-2} per mg/kg-day

The slope factor for 1,4-dioxane should not be used with exposures exceeding the point of departure ($BMDL_{50HED}$ = 4.95 mg/kg-day), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 1,4-dioxane.

II.B.1.2. Drinking Water Unit Risk*: 2.9×10^{-6} per µg/L

Drinking Water Concentrations at Specified Risk Levels

Risk Level	Lower Bound on Concentration Estimate*
E-4 (1 in 10,000)	35 µg/L
E-5 (1 in 100,000)	3.5 µg/L
E-6 (1 in 1,000,000)	0.35 µg/L

*The unit risk and concentration estimates assume water consumption of 2 L/day by a 70 kg human.

II.B.1.3. Extrapolation Method

Log-logistic model with linear extrapolation from the POD ($BMDL_{50HED}$) associated with 50% extra cancer risk.

The log-logistic model provided the best-fit to the female mouse liver tumor data Kano et al. (2009) female data as indicated by the AIC and *p*-value as was chosen as the best-fitting

model to carry forward in the analysis; however, this model resulted in a $BMDL_{10}$ much lower than the response level at the lowest dose in the study (Kano et al., 2009). Thus, the log-logistic model was also run for BMR values of 30 and 50%. Using a higher BMR value resulted in BMDL values closer to the lowest observed response data, and a BMR of 50% was chosen to carry forward in the analysis.

II.B.2. DOSE-RESPONSE DATA

Tumor Type – hepatocellular adenoma and carcinoma
Test Species – female BDF1 mouse
Route – Oral, drinking water
References – Kano et al. (2009)

Incidence of liver tumors in female BDF1 female mice exposed to 1,4-dioxane in drinking water for 2 years

Tumor	Dose (mg/kg-day)			
	0	66	278	964
Hepatocellular adenoma or carcinoma	5/50	35/50[a]	41/50[a]	46/50[a,b]

[a]Significantly different from control by Fisher's exact test ($p < 0.01$.)

[b]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

Source: Kano et al. (2009)

Oral Cancer Slope Factor (CSF) using linear low-dose extrapolation approach and interspecies extrapolation

Tumor	Dose groups modeled	BMD_{50} mg/kg-day	$BMDL_{50}$ mg/kg-day	BMD_{HED} mg/kg-day	$BMDL_{HED}$ mg/kg-day	Oral SF $(mg/kg\text{-}day)^{-1}$
Female mouse hepatocellular adenoma or	0, 66, 278, 964	49.88	32.93	7.51	4.95	0.10

Oral Cancer Slope Factor (CSF) using linear low-dose extrapolation approach and interspecies extrapolation	
carcinoma	mg/kg-day

II.B.3. ADDITIONAL COMMENTS

Supplementary information not required.

II.B.4. DISCUSSION OF CONFIDENCE

Relevance to humans. The oral CSF was derived using the tumor incidence in the liver of female mice. A thorough review of the available toxicological data available for 1,4-dioxane provides no scientific justification to propose that the liver adenomas and carcinomas observed in animal models following exposure to 1,4-dioxane are not plausible in humans. Liver adenomas and carcinomas were considered plausible outcomes in humans due to exposure to 1,4-dioxane.

Choice of low-dose extrapolation approach. The possibilities for the low-dose extrapolation of tumor risk from exposure to 1,4-dioxane, or any chemical, are linear or nonlinear, but is dependent upon a plausible MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity (Kano et al., 2009). MOA information that is available for the carcinogenicity of 1,4-dioxane has largely focused on liver adenomas and carcinomas, with little or no MOA information available for the remaining tumor types. In Section 4.7.3 of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013), hypothesized MOAs, other than a mutagenic MOA, were explored due to the lack of mutagenicity observed in genetic toxicology tests performed for 1,4-dioxane. The available evidence in support of the hypothesized MOAs for 1,4-dioxane is not conclusive. In the absence of a MOA(s) for the observed tumor types associated with exposure to 1,4-dioxane, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure.

In the studies evaluated (Kano et al., 2009; NCI, 1978; Kociba et al., 1974), the multistage model provided good descriptions of the incidence of a few tumor types in male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice (hepatocellular) exposed to

1,4-dioxane via ingestion (see Appendix D of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013) for additional details). However, the multistage model did not provide an adequate fit for female mouse liver tumor dataset based upon the following (U.S. EPA, 2012):

Goodness-of-fit *p*-value was less than 0.10 indicating statistically significant lack of fit;

AIC was larger than other acceptable models;

Observed data deviated substantially from the fitted model, as measured by their standardized χ^2 residuals (i.e., residuals with values greater than an absolute value of one).

By default, the BMDS software imposes constraints on the values of certain parameters of the models. When these constraints were imposed, the multistage model and most other models did not fit the incidence data for female mouse liver adenomas or carcinomas, even after dropping the highest dose group.

The log-logistic model was selected because it was the only model that provided an adequate fit to the female mouse liver tumor data (Kano et al., 2009). A BMR of 50% was used because it is proximate to the response at the lowest dose tested and the $BMDL_{50}$ was estimated by applying appropriate parameter constraints to the selected model, consistent with the BMD Technical Guidance Document (U.S. EPA, 2012).

The human equivalent oral CSF estimated from liver tumor datasets with statistically significant increases ranged from 4.2×10^{-4} to 1.0×10^{-1} per mg/kg-day, a range of about three orders of magnitude, with the upper and lower extremes coming from the combined male and female data for hepatocellular carcinomas (Kociba et al., 1974) and the female mouse liver adenoma and carcinoma dataset (Kano et al., 2009).

Dose metric. 1,4-Dioxane is known to be metabolized in vivo. However, it is unknown whether a metabolite or the parent compound, or some combination of parent compound and metabolites, is responsible for the observed carcinogenicity. If the actual carcinogenic moiety is proportional to administered exposure, then use of administered exposure as the dose metric is the least biased choice. On the other hand, if this is not the correct dose metric, then the impact on the CSF and IUR is unknown.

Interspecies extrapolation. An adjustment for cross-species scaling ($BW^{0.75}$) was applied to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the U.S. EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-logistic model applied to the female

mouse data, there is a reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear low-dose extrapolation), as indicated by the proximity of the $BMDL_{HED}$ (4.95 mg/kg-day) to the BMD_{HED} (7.51 mg/kg-day).

Bioassay selection. The study by Kano et al. (2009) was used for development of an oral CSF. This was a well-designed study, conducted in both sexes in two species with a sufficient number of animals per dose group. The number of test animals allocated among three dose levels and an untreated control group was adequate, with examination of appropriate toxicological endpoints in both sexes of rats and mice. Alternative bioassays (Kociba et al., 1974) are available and were fully considered for the derivation of the oral CSF.

Choice of species/gender. The oral CSF for 1,4-dioxane was derived using the tumor incidence data for the female mouse, which was thought to be more sensitive than male mice or either sex of rats to the carcinogenicity of 1,4-dioxane. While all data, from both species and sexes reported from the Kano et al. (2009) study, were suitable for deriving an oral CSF, the female mouse data represented the most sensitive indicator of carcinogenicity in the rodent model. The lowest exposure level (66 mg/kg-day [animal dose] or 10 mg/kg-day [HED]) observed a considerable and significant increase in combined liver adenomas and carcinomas. Additional testing of doses within the range of control and the lowest dose (66 mg/kg-day [animal dose] or 10 mg/kg-day [HED]) could refine and reduce uncertainty for the oral CSF.

Human population variability. The extent of inter-individual variability in 1,4-dioxane metabolism has not been characterized. A separate issue is that the human variability in response to 1,4-dioxane is also unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity across life stages is unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed carcinogenicity is a source of uncertainty.

II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

II.C.1. SUMMARY OF RISK ESTIMATES

II.C.1.1. Inhalation Unit Risk: 5×10^{-6} $(\mu g/m^3)^{-1}$

The derivation of the inhalation unit risk 5×10^{-6} per $\mu g/m^3$ is based on combined tumor incidence in male rats exposed to 1,4-dioxane in via inhalation for 2 years (Kasai et al., 2009). The dose metric used in the current estimate of the human equivalent concentration (HEC) is the applied or inhaled concentration because a PBPK model was determined not to be suitable

for species extrapolation (see Appendix B of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). The rat multitumor $BMCL_{10}$ of 30.3 ppm represents the POD used to calculate the $BMCL_{HEC}$ of 19.5 mg/m^3.

The inhalation unit risk is derived from the $BMCL_{HEC}$, the 95% lower bound on the exposure associated with a 10% extra cancer risk, by dividing the risk (as a fraction) by the $BMCL_{HEC}$, and represents an upper bound, continuous lifetime exposure risk estimate:

$BMCL_{10HEC}$, lower 95% bound on exposure at 10% extra risk — 19.5 mg/m^3

BMC_{10HEC}, central estimate of exposure at 10% extra risk — 26.0 mg/m^3

The slope of the linear extrapolation from the central estimate is 0.1/ (26.0 mg/m^3) = 4×10^{-6} per µg/m^3

The inhalation unit risk for 1,4-dioxane should not be used with exposures exceeding the point of departure ($BMCL_{10HEC}$ = 19.5 mg/m^3), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of 1,4-dioxane.

II.C.1.2. Extrapolation Method

Multi-tumor dose-response model with linear extrapolation from the POD ($BMCL_{10HEC}$) associated with 10% extra cancer risk. Statistically significant dose response trends for the increase in tumors with increasing dose was observed for the nasal cavity squamous cell carcinomas, hepatomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, and Zymbal gland adenomas. All of these tumors were considered to be of independent origin and included in the multi-tumor analysis.

II.C.2. DOSE-RESPONSE DATA

Tumor Types — multiple (nasal, liver, kidney, peritoneal, mammary gland, and Zymbal gland)
Test species — male F344 rats
Route — Inhalation
Reference — Kasai et al. (2009)

Incidence of tumors in F344 male rats exposed to 1,4-dioxane for 104 weeks (6 hours/day, 5 days/week)

Tumor Type	Animal Exposure (ppm)			
	0	50	250	1,250

Nasal cavity squamous cell carcinoma	0/50	0/50	1/50	6/50[a,b]
Hepatocellular adenoma or carcinoma[e]	1/50	2/50	4/50	22/50[a,c]
Renal cell carcinoma	0/50	0/50	0/50	4/50[a]
Peritoneal mesothelioma	2/50	4/50	14/50[c]	41/50[a,c]
Mammary gland fibroadenoma	1/50	2/50	3/50	5/50[d]
Mammary gland adenoma	0/50	0/50	0/50	1/50
Zymbal gland adenoma	0/50	0/50	0/50	4/50[a]
Subcutis fibroma	1/50	4/50	9/50[c]	5/50

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).

[b]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).

[c]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).

[d]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.05$).

[e]Provided via email from Dr. Tatsuya Kasai (JBRC) to Dr. Reeder Sams (U.S. EPA) on 12/23/2008 (2008). Statistics were not reported for these data by study authors, so statistical analyses were conducted by EPA.

Source: Kasai et al. (2009) and Kasai (2008)

Inhalation Unit Risk (IUR) using linear low-dose extrapolation approach and interspecies extrapolation

Tumor	Dose groups modeled	BMC_{10} mg/m^3	$BMCL_{10}$ mg/m^3	BMC_{HEC} mg/m^3	$BMCL_{HEC}$ mg/m^3	Inhalation Unit Risk $(\mu g/m^3)^{-1}$
Multiple – F344 male rats	0, 50, 250, 1,250 ppm (0, 180, 900, or 4,500 mg/m^3)	40.4	30.3	26.0	19.5	5.0×10^{-6}

II.C.3. ADDITIONAL COMMENTS

Supplementary information not required.

II.C.4. DISCUSSION OF CONFIDENCE

Relevance to humans. The derivation of the inhalation unit risk is based on the tumor incidence at multiple sites in male rats. There is no information on 1,4-dioxane to indicate that the observed rodent tumors are irrelevant to humans. Further, no data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans. In the absence of information to indicate otherwise and considering similar cell types are prevalent throughout the respiratory tract of rats and humans, the nasal, liver, renal, peritoneal, mammary gland, Zymbal gland and subcutis tumors were considered relevant to humans.

Choice of low-dose extrapolation approach. The possibilities for the low-dose extrapolation of tumor risk from exposure to 1,4-dioxane, or any chemical, are linear or nonlinear, but is dependent upon a plausible MOA(s) for the observed tumors. The MOA is a key consideration in clarifying how risks should be estimated for low-dose exposure. Exposure to 1,4-dioxane has been observed in animal models to induce multiple tumor types, including liver adenomas and carcinomas, nasal carcinomas, mammary adenomas and fibroadenomas, and mesotheliomas of the peritoneal cavity (Kano et al., 2009). MOA information that is available for the carcinogenicity of 1,4-dioxane has largely focused on liver adenomas and carcinomas, with little or no MOA information available for the remaining tumor types. In Section 4.7.3 of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013), hypothesized MOAs, other than a mutagenic MOA, were explored due to the lack of mutagenicity observed in genetic toxicology tests performed for 1,4-dioxane. The available evidence in support of the hypothesized MOAs for 1,4-dioxane is not conclusive. In the absence of sufficient information to support a MOA(s) for the observed tumor types associated with exposure to 1,4-dioxane, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure.

The BMDS multistage cancer model provided adequate fits for the tumor incidence data following a 2 year inhalation exposure to 1,4-dioxane by male rats (Kasai et al., 2009), thus the BMDS MS_Combo multi-tumor model was used to determine a $BMCL_{10}$.

Interspecies extrapolation. Differences in the anatomy of the upper respiratory tract and resulting differences in absorption or in local respiratory system effects are sources of uncertainty in the inhalation cancer assessment. However, since similar cell types are prevalent throughout the respiratory tract of both rats and humans, the tumors are considered biologically plausible and relevant to humans.

Statistical uncertainty at the POD. Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the multistage, multi-tumor model applied for the male rat inhalation dataset, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low dose extrapolation).

Bioassay selection. The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This was a well designed study, conducted in male rats with a sufficient number (N=50) of animals per dose group. Three dose levels plus an untreated control group were examined following exposure to 1,4-dioxane via inhalation for 2 years.

Choice of species/gender. Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kano et al. (2009) showed that male rats were more sensitive than female rats to the effects of 1,4-dioxane following oral administration; therefore, male rats were chosen to be studies in the 2 year bioassay conducted by the same laboratory (Kasai et al., 2009). The sensitivity and tumorigenic response of female rats or male or female mice following inhalation of 1,4-dioxane is unknown. Since female mice were the most sensitive gender and species examined in the Kano et al. (2009) oral study, female mice may also be more sensitive to the inhalation of 1,4-dioxane which would result in a greater risk.

Human population variability. The extent of inter-individual variability in 1,4-dioxane metabolism has not been characterized. A separate issue is that the human variability in response to 1,4-dioxane is also unknown. Data exploring whether there is differential sensitivity to 1,4-dioxane carcinogenicity across life stages is unavailable. This lack of understanding about potential differences in metabolism and susceptibility across exposed human populations thus represents a source of uncertainty. Also, the lack of information linking a MOA for 1,4-dioxane to the observed carcinogenicity is a source of uncertainty.

II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

II.D.1. EPA DOCUMENTATION

Source Document — *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013)

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and the Executive Office of the President, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2013). **To review this appendix, exit to the Toxicological Review, Appendix A, Summary of External Peer Review and Public Comments and Disposition (PDF).**

II.D.2. EPA REVIEW

Agency Completion Date Oral – **08/11/2010**

Agency Completion Date Inhalation – **09/20/2013**

II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — 1,4-Dioxane
CASRN — 123-91-1

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VII. Revision History

Substance Name — 1,4-Dioxane
CASRN — 123-91-1
File First On-Line — 08/22/1998

Date	Section	Description
08/22/1988	II.	Carcinogen summary on-line
08/11/2010	I., II., VI.	RfD and cancer assessment updated; RfC discussion added.
09/20/2013		Added RfC and inhalation cancer assessment.

VIII. Synonyms

Substance Name — 1,4-Dioxane
CASRN — 123-91-1
Section VIII. Last Revised — 09/20/2013

- 123-91-1
- diethylene dioxide
- diethylene oxide
- dioxane, 1,4-
- p-dioxane
- dioxane
- dioxyethylene ether
- diethylene ether
- 1,4-diethylene dioxide

Attachment

1I

7

EPA/635/R-18/211Fc
www.epa.gov/iris

Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Executive Summary

(CASRN 121-82-4)

August 2018

Integrated Risk Information System
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

RDX is a synthetic chemical used primarily as a military explosive. RDX releases have been reported in air, water, and soil, and exposure is likely limited to individuals in or around military facilities where RDX is or was produced, used, or stored. Oral exposure may occur from drinking contaminated groundwater or ingesting crops irrigated with contaminated water. Inhalation or dermal exposures are more likely in occupational settings.

Epidemiological studies provide only limited information on worker populations exposed to RDX; several case reports describe effects primarily in the nervous system following acute exposure to RDX. Animal studies of ingested RDX demonstrate toxicity, including effects on the nervous system, urinary system (kidney and bladder), and prostate.

Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on evidence of positive trends in liver and lung tumor incidence in experimental animals. There are no data on the carcinogenicity of RDX in humans.

ES.1. EVIDENCE FOR HAZARDS OTHER THAN CANCER: ORAL EXPOSURE

Nervous system effects are a human hazard of RDX exposure. Several human case reports and animal studies provide consistent evidence of an association between RDX exposure and effects on the nervous system, including findings related to the induction of seizures, abnormal electrical activity, convulsions, tremors, and a reduced threshold for seizure induction by other stimuli; behavioral effects that may be related to seizures such as hyperirritability, hyper-reactivity, and other behavioral changes. Mechanistic data support the hypothesis that RDX-induced seizures and related behavioral effects likely result from inhibition of gamma-aminobutyric acid (GABA)ergic signaling in the limbic system. Some investigators reported that unscheduled deaths in experimental animals exposed to RDX were frequently preceded by convulsions or seizures.

Urinary system effects are a potential human hazard of RDX exposure based largely on observations of histopathological changes in the kidney and urinary bladder of male rats exposed to RDX at doses higher than those associated with nervous system effects. The available evidence indicates that male rats are more sensitive than females, and rats are more sensitive than mice to RDX-related urinary system toxicity. There is suggestive evidence of male prostate effects associated with RDX exposure based on an increased incidence of suppurative prostatitis in male rats exposed to RDX in the diet for 2 years, in one of the few studies that evaluated the prostate. There is no known mode of action (MOA) for effects of RDX exposure on the urinary system or prostate, although there are studies indicating GABA helps regulate urinary system and prostate

function. Evidence for effects on other organs/systems, or developmental effects, was more limited than for the endpoints summarized above.

ES.1.1. Oral Reference Dose (RfD) for Effects Other Than Cancer

Organ-specific RfDs were derived for hazards associated with RDX exposure (see Table ES-1). These organ- or system-specific reference values may be useful for subsequent cumulative risk assessments that consider the combined effect of multiple agents acting at a common site.

Table ES-1. Organ/system-specific reference doses (RfDs) and overall RfD for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Effect	Basis	RfD (mg/kg-day)	Study exposure description	Confidence
Nervous system	Convulsions	4×10^{-3}	Subchronic	Medium
Urinary system	Kidney medullary papillary necrosis	1×10^{-2}	Chronic	Medium
Prostate	Suppurative prostatitis	8×10^{-4}	Chronic	Low
Overall RfD	**Nervous system effects**	**4×10^{-3}**	**Subchronic**	**Medium**

The overall RfD (see Table ES-2) is derived to be protective of all types of hazards associated with RDX exposure. Although the RfD for prostate effects results in a smaller value, it was not selected as the overall RfD due to uncertainties in the evaluation of this endpoint ("low confidence"). The effect of RDX on the nervous system was chosen as the basis for the overall RfD because nervous system effects were observed most consistently across studies, species, and exposure durations, and because they represent a sensitive human hazard of RDX exposure. Evidence for effects of RDX on the urinary system and prostate is more limited relative to the effects of RDX on the nervous system. Incidence of seizures or convulsions as reported in a subchronic gavage study (Crouse et al., 2006) was selected for deriving the overall RfD because this endpoint was measured in a study that was well conducted, used a test material of high purity (99.99%), and had five closely spaced dose groups that supported characterization of the dose-response curve. In contrast, most other studies used a technical grade with ~10% or more impurities. Benchmark dose (BMD) modeling was used to derive the point of departure (POD) for RfD derivation (expressed as the lower confidence limit on the benchmark dose [$BMDL_{05}$]). A 5% response level was chosen because of the severity of the endpoint.

Table ES-2. Summary of reference dose (RfD) derivation

Critical effect	Point of departure[a]	UF	Chronic RfD	Confidence
Nervous system effects (convulsions) 90-d F344 rat study Crouse et al. (2006)	$BMDL_{05-HED}$: 1.3 mg/kg-d	300	4×10^{-3} mg/kg-d	Medium

AUC = area under the curve; BMDL = benchmark dose lower confidence limit.
[a]A benchmark response (BMR) of 5% was used to derive the BMD and BMDL. The resulting POD was converted to a $BMDL_{05-HED}$ using a PBPK model based on modeled arterial blood concentration. The concentration was derived from the AUC of modeled RDX concentration in arterial blood, which reflects the average blood RDX concentration for the exposure duration normalized to 24 hr.

A PBPK model was used to extrapolate the $BMDL_{05}$ derived from a rat study to a human equivalent dose (HED) based on RDX arterial blood concentration, which was then used for RfD derivation.

The overall RfD, 4×10^{-3} mg/kg-day, was calculated by dividing the $BMDL_{05}$ expressed as a human equivalent dose ($BMDL_{05-HED}$) for nervous system effects by a composite uncertainty factor (UF) of 300 to account for extrapolation from animals to humans (3), interindividual differences in human susceptibility (10), and uncertainty in the database (10).

Because a subchronic-to-chronic uncertainty factor (UF_S) of 1 was applied to the POD based on evidence that nervous system effects (in particular convulsions) are more strongly driven by dose than duration of exposure, the RfD may be appropriate for assessing health risks of less-than-lifetime as well as chronic durations of exposure.

The overall confidence in the RfD is medium based on high confidence in the principal study (Crouse et al., 2006) and medium to low confidence in the database. Confidence in the database is reduced largely because of (1) differences in test material used across studies (i.e., differences in formulation and particle size that may have affected RDX absorption and subsequent toxicity), (2) uncertainties in the influence of oral dosing methods (in particular, based on evidence that bolus dosing of RDX resulting from gavage administration induces neurotoxicity at doses lower than administration in the diet), and (3) significant limitations in the available studies to fully characterize subconvulsive neurological effects as well as developmental neurotoxicity.

ES.2. EVIDENCE FOR HAZARDS OTHER THAN CANCER: INHALATION EXPOSURE

No studies were identified that provided useful information on the effects observed following inhalation exposure to RDX. Of the available human epidemiological studies of RDX, none provided data that could be used for dose-response analysis of inhalation exposures. The single experimental animal study involving inhalation exposure is not publicly available and was excluded from consideration due to significant study limitations, including small numbers of animals tested, lack of controls, and incomplete reporting of exposure levels. Therefore, the available health effects

literature does not support the identification of hazards following inhalation exposure to RDX nor the derivation of an inhalation reference concentration (RfC).

While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support development of an inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an RfC from the RfD.

ES.3. EVIDENCE FOR HUMAN CARCINOGENICITY

Under EPA's cancer guidelines (U.S. EPA, 2005a), there is *suggestive evidence of carcinogenic potential* for RDX. RDX induced benign and malignant tumors in the liver and lungs of mice (Parker et al., 2006; Lish et al., 1984) or rats (Levine et al., 1983) following long-term administration in the diet. The potential for carcinogenicity applies to all routes of human exposure.

ES.4. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

A quantitative estimate of carcinogenic risk from oral exposure to RDX was based on the increased incidence of hepatocellular adenomas or carcinomas and alveolar/bronchiolar adenomas or carcinomas in female B6C3F$_1$ mice observed in the carcinogenicity bioassay in mice (Lish et al., 1984). This 2-year dietary study included four dose groups and a control group, adequate numbers of animals per dose group (85/sex/group, with interim sacrifices of 10/sex/group at 6 and 12 months), and detailed reporting of methods and results (including individual animal data). The initial high dose (175 mg/kg-day) was reduced to 100 mg/kg-day at Week 11 due to high mortality.

When there is *suggestive evidence* of carcinogenicity to humans, EPA generally would not conduct a dose-response assessment and derive a cancer value. However, when the evidence includes a well-conducted study (as is the case with RDX), quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities (U.S. EPA, 2005a).

An OSF was derived that considered the combination of female mouse liver and lung tumors. In modeling these data sets, the highest dose group was excluded because of the initial high mortality (loss of almost half the mice in that dose group). BMD and benchmark dose lower confidence limit (BMDL) estimates were calculated that correspond to a 10% extra risk (ER) of either tumor. The $BMDL_{10}$ so derived was extrapolated to the HED using body-weight scaling to the ¾ power ($BW^{3/4}$), and an OSF was derived by linear extrapolation from the $BMDL_{10}$ expressed as an HED ($BMDL_{10-HED}$). The OSF is 0.08 per mg/kg-day, based on the liver and lung tumor response in female mice (Lish et al., 1984).

ES.5. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

An inhalation unit risk (IUR) value was not calculated because inhalation carcinogenicity data for RDX are not available. While inhalation absorption of RDX particulates is a plausible route of exposure, there are no toxicokinetic studies of RDX inhalation absorption to support an

inhalation model. Therefore, a PBPK model for inhaled RDX was not developed to support route-to-route extrapolation of an IUR from the OSF. Thus, a quantitative cancer assessment was not conducted for inhalation exposure.

ES.6. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Little information is available on populations that may be especially vulnerable to the toxic effects of RDX. Life stage, particularly childhood, susceptibility has not been well-studied in human or animal studies of RDX toxicity. In rats, transfer of RDX from the dam to the fetus during gestation and to pups via maternal milk has been reported; however, reproductive and developmental toxicity studies did not identify effects in offspring at doses below those that also caused maternal toxicity. Yet, based on the primary mode of action for RDX exposure-induced nervous system effects (GABA receptor antagonism), and the fact that GABAergic signaling plays a prominent role in nervous system development, a significant concern is raised regarding the potential for developmental neurotoxicity. In addition, data on the incidence of convulsions and mortality provide some indication that pregnant animals may be a susceptible population, although the evidence is inconclusive. Data to suggest that males may be more susceptible than females to noncancer toxicity associated with RDX are limited. Some evidence suggests that cytochrome P450 (CYP450) enzymes may be involved in the metabolism of RDX, indicating a potential for genetic polymorphisms in these metabolic enzymes to affect susceptibility to RDX. Similarly, individuals with epilepsy or other seizure syndromes that have their basis in genetic mutation to $GABA_A$ receptors (GABA receptors that are ligand-gated ion channels, also known as ionotropic receptors) may represent another group that may be susceptible to RDX exposure; however, there is no information to indicate how genetic polymorphisms may affect susceptibility to RDX.

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Attachment

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United States Environmental Protection Agency

EPA/690/R-06/001F
Final
10-23-2006

Provisional Peer Reviewed Toxicity Values for

Aluminum
(CASRN 7429-90-5)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
IUR	inhalation unit risk
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR ALUMINUM (CASRN 7429-90-5)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

This document has passed the STSC quality review and peer review evaluation indicating that the quality is consistent with the SOPs and standards of the STSC and is suitable for use by registered users of the PPRTV system.

INTRODUCTION

Verified toxicity values for aluminum (Al) and its compounds are unavailable on IRIS or HEAST (U.S. EPA, 2006, 1997), except for a chronic oral RfD of 4E-4 mg/kg-day for aluminum phosphide. However, occupational guidelines and standards have been established for a number of chemical and physical forms of Al, including, from ACGIH, 8-hour TWA-TLVs of 10 mg/m^3 for the compound as a metal dust or oxide, 5 mg/m^3 as "pyro" powders or welding fumes, and 2 mg/m^3 for soluble salts or organic forms of the metal (ACGIH, 1998). From NIOSH, 10-hour TWA-RELs of 10 mg/m^3 are specified for "total" Al dust versus 5 mg/m^3 for the respirable portion (NIOSH, 1994). NIOSH covers all other forms of the metal by identical values to those specified by ACGIH (ACGIH, 1998). OSHA PELs for Al include an 8-hour TWA value of 15 mg/m^3 for "total" metal dust, versus 5 mg/m^3 for the respirable portion (NIOSH, 1994). The U.S. EPA's CARA list (U.S. EPA, 1994) cites a HEA for Al (U.S. EPA, 1987), and ATSDR has updated its toxicological profile of the element (ATSDR, 1998).

The U.S. FDA (2000) has specified a maximum aluminum concentration of 25 mcg/L in large-volume parenterals (LVP) used in total parenteral nutrition (TPN). The FDA regulation applies to all LVPs used in TPN, including but not limited to parenteral amino acid solutions, highly concentrated dextrose solutions, parenteral lipid emulsions, sodium chloride and electrolyte solutions, and sterile water for injection.

Research papers pertinent to the potential toxicological and carcinogenic effects of Al were sought through computer searches of the HSDB, RTECS, MEDLINE and TOXLINE (and its subfiles) databases, covering the time period 1995-1999. The literature searches were conducted in June, 1999.

REVIEW OF PERTINENT DATA

The review by Stokinger (1981) gives an account of Al as an all-pervasive component of products that are central to the daily lives of most Americans. For example, the metal is a crucial part of manufactured products for the building, automobile and container industries, while Al as powder or flake is a component in a number of consumer products, such as paints, fireworks, etc. Al complexes and minerals are used in the brewing and paper industries, and as coagulants for water purification. Aluminum oxide finds application in abrasives, as a catalyst or absorbent, and as a component in fillers. Aluminum chloride is included in cosmetic formulations such as deodorants.

Human exposure to Al arises principally from food and water, through its widespread use in food additives, packaging and cooking utensils and Al-containing medications, particularly antacid, buffered aspirin, anti-ulcer and anti-diarrheal formulations (Marquis, 1989; Lione, 1985). Pennington and Schoen (1995) estimated daily Al intakes of 0.1-0.3 mg/kg-day for infants and children 6 months-6 years of age and 0.1-0.18 mg/kg-day for older children and adults, based on the FDA Total Diet Study (1993) and the U.S. Department of Agriculture Nationwide Food Consumption Survey (1987-1988). These data are in broad agreement with those of Wilhelm et al. (1995) who reported the dietary intake of Al in German children (living in the Duisberg area) as ranging from 0.008 to 0.11 mg Al/kg-day. In addition, these values are consistent with a range of 1-20 mg/day (0.014-0.3 mg/kg -day) for normal oral daily Al intake from food and water reported by other investigators (Ganrot, 1986; Iyengar et al., 1987; Wilhelm et al., 1990). However, users of Al-containing medications can ingest much larger amounts of the element, possibly as high as 840-5000 mg/day (12-71 mg/kg-day) from antacids, 126-728 mg/day (1.8-10.4 mg/kg-day) from buffered aspirins and 828 mg/day (11.8 mg/kg-day) from anti-ulcer compounds when taken at recommended dosages (Lione, 1985).

Toxicokinetics of Aluminum

There is a large amount of information available on the absorption, transfer from tissue to tissue and elimination of Al from the body, including data that have been amassed from studies on either human volunteers or laboratory animals. In general, the chemical appears to be poorly absorbed from the gastrointestinal tract, though the portion of the load that is retained will vary depending on the concentration, the chemical species administered, the fasting or fed state of the

host, gastrointestinal pH, animal model, etc. For example, Yokel and McNamara (1988) administered single oral doses of a number of Al compounds (both water soluble and insoluble) to New Zealand white rabbits and obtained absorbed proportions of the load ranging from 0.27% to 27%. Fractional uptake of Al in humans under normal conditions (i.e., with no intake of large quantities of Al from medicine) was estimated to be 0.1-0.3% assuming an intake of 20 mg Al/day (0.3 mg Al/kg-day) and urinary excretion of 20-50 μg Al/day (0.3-0.7 μg Al/kg-day) (Ganrot, 1986). However, little information is available on the actual mechanism by which the element and its compounds are transported across the brush border. (Wilhelm et al., 1990; Lione, 1985).

Although the overall extent of Al absorption is poor following oral exposure, there may be significant intake of the compound by those taking large amounts of Al compounds in patented remedies. As stated, absorption of Al is influenced by gastrointestinal conditions and content because Al can form various complexes with different solubilities and oxidation states depending on pH and interactions with dietary constituents. At low pH (3-5) in aqueous solutions, the soluble (ionic) forms of the Al prevail (Al^{3+}); at high pH (>8), Al in the form of soluble aluminum oxide is present; and at pH 5-8, the element is predominantly in the form of aluminum hydroxide, which is insoluble (van der Voet and de Wolff, 1986; Wilhelm et al., 1990). Ingested constituents that can influence absorption by forming complexes with Al include phosphate, fluoride, calcium, citrate and lactate. For example, Al is used to bind dietary phosphorus and decrease its absorption as a control for hyperphosphatemia, and citrate and lactate are complexing agents that can significantly increase Al absorption (Slanina et al., 1984, 1985, 1986; Partridge et al., 1989; Domingo et al., 1991; Ittel et al., 1991; Lione, 1985; Wihelm et al., 1990).

A number of recent reports of studies on the gastrointestinal absorption of Al have examined the influence of organic anions such as citrate. In general, the presence of such components appears to enhance the absorption of Al, within narrow limits. For example, Deng et al. (1998) administered a single oral dose of either distilled water, 2 mmoles/L aluminum chloride or 2 mmoles/L aluminum chloride plus 2 mmoles/L sodium citrate to six male Wistar rats/group. Animals were bled at 1, 2 and 4 hours after dosing, then terminated after 6 hours. Inductively coupled plasma (ICP) was used to measure Al concentrations in blood, bone (tibia), kidney, liver and the intestinal wall. Irrespective of treatment, the appearance of Al in the blood of dosed groups peaked after 1 hour, with the concentration of the element at higher levels in those animals receiving citrate in addition to aluminum chloride. In those animals receiving aluminum chloride alone, significant tissue concentrations of the element were restricted to the gastrointestinal wall. Those receiving citrate displayed measurable quantities of the element in several of the other monitored tissues, including bone.

Sutherland and Greger (1998a) used a similar dosing regimen to examine the kinetics of absorption and elimination of Al in male Sprague-Dawley rats that had received a single oral dose of 0, 0.25, 0.5 or 1 mmoles/L/kg body weight aluminum lactate in 1 mL of 16% citrate. Concentrations of Al in serum, liver, kidney or bone (tibia) were measured at various post-dosing time intervals up to 6 hours. Depending on the dose, absorption factors for Al of up to 4.2% of the administered dose were observed, with the greater proportion retained in bone. The authors reported a slower rate of absorption in those animals receiving Al at the higher doses, an

observation potentially indicating reduced gut motility and/or saturation of the transcellular absorption processes at the higher concentrations. Aluminum deposited in kidney and bone appeared to turn-over at a slower rate than in the liver.

The influence of citrate on the gastrointestinal absorption of Al in man was examined directly by Taylor et al. (1998) who administered a drink containing Al and citrate to three volunteers. Aluminum and citrate concentrations were monitored in serial blood and urine samples for up to 24 hours. The kinetics of citrate and Al differed markedly, the former peaking in plasma after 32 minutes, versus 87 minutes for Al. This suggests that Al probably does not cross the gastrointestinal barrier as the citrate. Furthermore, the authors reported that the overall extent of Al absorption had probably not exceeded 1% in their experiment, a finding that contrasts with the higher values reported by Sutherland and Greger (1998a) in Sprague-Dawley rats and by Deng et al. (1998) in Wistar rats.

As discussed in a report by Glynn et al. (1999), gastrointestinal absorption of Al from aqueous media will be almost impossible to predict, because of the likelihood that the element will become absorbed to food particles in the intestinal lumen. Accordingly, depending on the dose, mode of delivery and caloric state of the experimental animal (fed/fasted), significant amounts of aqueous forms of Al will be absorbed only when available binding sites on food have become saturated. This presents an inherently complex overall picture of the element's absorption since, additionally, the normal dietary content of Al will be substantial. Thus, it may be assumed that some sequestered Al will be absorbed along with non-sequestered water soluble forms of the element, while the rest will be retained within the gastrointestinal tract.

Sutherland and Greger (1998b) used their aluminum lactate in 16% citrate dosing regimen to examine the comparative importance of biliary versus urinary excretion of Al. Five to seven male Sprague-Dawley rats/group who had previously received an implanted bile cannula were treated by gavage. Another similarly-treated cohort of five animals/group were housed in metabolic cages immediately after dosing to provide 0- to 3-hour and 3- to 6-hour urine specimens. At termination, all animals were sacrificed and exsanguinated, and tissue, bile and urine samples were measured by graphite furnace atomic absorption spectroscopy. Among the key findings to emerge from this study was the incremental appearance of Al in bile as early as 15 minutes after dosing. However, overall amounts of Al were greater in the 3-hour urine samples than those that had accumulated in bile samples collected within a similar time frame. The fact that control rats excreted 3 times more Al in bile than in urine during the first 3 hours after dosing led the authors to conclude that, at low exposure to Al (in controls receiving Al solely from food), the liver is capable of excreting the element to the bile, a mechanism that becomes saturated as the level of Al administration becomes increased. Thereafter, urinary excretion becomes the primary route of elimination in circumstances of Al overload.

Aluminum can also be absorbed by inhalation as indicated by age-related deposition in the lungs of the general population and exposure-related increased blood and urine concentrations in workers exposed to Al (Bast-Pettersen et al., 1994; Sjogren et al., 1996; Hosovski et al., 1990; Wilhelm et al., 1990; U.S. EPA, 1987). Aluminum occurs primarily in particulate form in the ambient atmosphere and as various dusts and fumes during its production and use. Common forms of inhaled Al include aluminum oxide (alumina; Al_2O_3), pyro powders

(powder and flake Al-treated to reduce surface oxidation), Al welding fume and soluble salts (e.g., aluminum chloride and sulfate) (ACGIH, 1998).

Neurotoxicity as a Primary Toxicological Effect of Aluminum

One of the greatest health concerns regarding Al is its neurological effects. The first evidence for Al-induced neurotoxicity in humans was seen in patients who, as a result of receiving long-term hemodialysis for chronic renal failure, developed a degenerative neurological syndrome (dialysis dementia) characterized by the gradual loss of motor, speech and cognitive functions (Alfrey, 1993). This dementia, attributable to Al in the dialysate, is usually fatal within 6-9 months after the first clinical signs appear. In addition, many patients received high oral doses of Al to act as phosphate binders. Autopsies of these patients revealed increased concentrations of Al in the gray matter and cerebral spinal fluid (CSF) but no evidence of neurofibrillary degeneration (NFD) despite the elevated Al levels. Once the connection between Al and dialysis dementia was established, Al was removed from dialysis fluid and the incidence of dementia rapidly declined, thereby strengthening the argument that Al was a causal agent in dialysis dementia (Ganrot, 1986).

Amyotrophic Lateral Sclerosis (ALS) and Parkinson's Disease (PD) are other neurological diseases which have been associated with Al exposure. ALS is a progressive disease of the Central Nervous System (CNS) that is characterized by an accumulation of neurofibrillary tangles. In Guam, southern West New Guinea and parts of Japan, there is an unusually high prevalence of ALS and PD. This may be related to the natural abundance of Al coupled with the virtual lack of magnesium and calcium in the drinking water supplies and soil of these areas. In a study designed to evaluate effects of high Al and low calcium levels in the diet, much like the conditions associated with Guam and other similar areas, cynomolgus monkeys were placed on a low calcium diet either with or without supplemental Al and manganese (Garruto et al., 1989). Chronic calcium deficiency alone produced neurodegenerative effects, although neurofibrillary changes were most frequently seen in the monkeys on a low calcium diet supplemented with Al and manganese.

Though a cause and effect relationship between Al and three forms of chronic encephalopathy in humans: senile dementia of the Alzheimer type (SDAT, Alzheimer's Disease), endemic Amyotrophic Lateral Sclerosis (ALS) and endemic Parkinsonism-dementia (PD, a mixture of Parkinsonism and senile dementia) has been suggested, there is no firm evidence that it plays a causal role in the development of these diseases (Ganrot, 1986; Lione, 1985). The condition is degenerative and characterized by the progressive loss of speech, motor and cognitive functions, with death typically occurring within 1-6 months. Autopsies of patients revealed increased concentrations of Al in the gray matter and cerebral spinal fluid (CSF), though with no conclusive evidence of NFD or other neuropathological changes despite the elevated Al levels.

The neurotoxicity of Al is well documented in certain animal species. Aluminum induces a spectrum of behavioral abnormalities and brain neurofibrillary degenerative changes in rabbits and cats when injected intracranially or parenterally in high doses, though hamsters and monkeys are less sensitive (Ganrot, 1986; Lione, 1985). Such studies have been designed as models for

the possible neurotoxicological effects of Al in humans. However, it should be noted that the neurofibrillary changes in affected animals differ in morphological detail from those associated with SDAT. As discussed further in the Oral Toxicity section, oral doses of Al can also induce neurobehavioral effects in adult mice and rats and in their developing offspring. In general, such neurotoxic effects of Al appear to be more subtle than those induced through routes of administration that by-pass the gastrointestinal tract, perhaps reflecting the lower doses of Al reaching the brain.

Recent reports of studies on the effects of Al on neurotoxicity in animals have sought to define the biochemical mechanisms that are impaired when Al crosses the blood-brain barrier. However, a unifying concept has yet to emerge, though the passage of the element into various regions of the brain has been clearly demonstrated (Deloncle et al., 1995). Among the many biochemical functions and processes that appear to be perturbed by the presence of Al in the brain are the peroxidation status of biological membranes (Katyal et al., 1997; Deloncle et al., 1999), inhibition of the neuronal glutamate-nitric oxide-cyclic GMP pathway (Cucarella et al., 1998), and the marked reduction of protein- and non-protein-bound thiols and the specific activity of Na^{+}/K^{+} and Mg^{++} ATPases (Katyal et al., 1997). The relative importance of each of these mechanisms and how (or whether) they interact to bring about the observed physiological changes remains unclear.

Other Effects of Aluminum

Osteomalacia was frequently observed among long-term dialysis patients with neurological signs and is commonly attributed to Al overload (Ganrot, 1986; Lione, 1985). This bone condition is characterized by widened osteoid (unmineralized bone matrix) with no fibrosis, reduced mineralization rate, skeletal pain and a strong tendency for fractures, lack of response to vitamin D therapy and increased Al concentration in bone. Effects on bone histology and elevated bone Al levels have also been observed in patients with normal renal function who received total parenteral nutrition with Al-contaminated casein as a protein source, and in parenteral Al loading induced osteomalacia in rats and dogs (Lione, 1985).

There are a number of published reports of studies in which the carcinogenicity of aluminum compounds has been evaluated. These include oral exposure studies in which the compounds were made available to experimental animals in the drinking water or diet (Schroeder and Mitchener, 1975a,b: Oneda et al., 1994), and inhalation epidemiological studies, in which the incidence of tumor formation in persons exposed to aluminum-containing dusts and fumes in an occupational setting was compared to unexposed individuals (Spinelli et al., 1991; Thériault et al., 1984, 1990; Armstrong et al., 1986; Tremblay et al., 1995; Selden et al., 1997; Cullen et al., 1996; Dufresne et al., 1996; Ronneberg and Langmark, 1992). However, it has been generally concluded that the inferential association between exposure to Al and marginally increased incidences of tumors of the bladder and/or lung are confounded because of the co-exposure of subjects in such settings to other harmful and potentially carcinogenic substances, such as polycyclic aromatic hydrocarbons (PAHs and coal tar pitch volatiles (CTPV) (Ronneberg and Langmark, 1992). Therefore, the issue of the potential carcinogenicity of Al compounds remains uncertain.

Human Studies

Oral Exposure

Few reports have been identified that address the toxicological effects of Al in humans exposed orally. Furthermore, in a review, Reiber et al. (1995) pointed to the conflicting findings that have been reported when the incidence of neurological symptoms has been assessed in relation to Al exposure in either cross-sectional, ecological or case-control epidemiological studies. Among the more recent studies that have used this approach, Martyn et al. (1997) discussed the findings of a case-control study involving 441 men in England and Wales who were afflicted with either Alzheimer's disease, brain cancer, dementia or other neurological conditions. Assessing the historical exposure of these subjects failed to establish a link between Al in drinking water at the prevailing concentrations (below 0.2 mg/L) and the incidence of one or more of the conditions under investigation. No data were located regarding the oral carcinogenicity of aluminum compounds in humans.

Inhalation Exposure

Neurobehavioral effects were evaluated in a group of 87 Al foundry workers who were occupationally exposed to 4.6-11.5 mg/m^3 Al fumes and dust for a mean of 12.0 years [standard deviation (SD) 4.5 years, shortest exposure 6 years] compared to an unexposed control group (n=60) who were matched for age, job seniority and social status to exposed subjects (Hosovski et al., 1990). It is reported that environmental Al concentrations were measured for each worker separately during the winter and summer, implying that personal sampling may have been used and that the contributing concentrations are time-weighted averages. In certain places, the number of particles ranged as high as 329-1020/cm^2 air, and dust particle sizes were ≤1, 1-5 and ≤5 microns in 65.6, 26.6 and 7.6% of the samples, respectively. Tests of psychomotor ability (simple and complex reaction time, oculomotor coordination), intellectual ability (Wechsler intelligence, performance intelligence and verbal intelligence quotients and Wechsler subtests on information processing, memory, understanding, calculation, coding, picture completion, picture grouping, object assembling, assembling of cubes and common concepts) and cerebral damage (Bender visual motor test) were conducted. Performance of the exposed workers was found to be significantly ($p<0.02$) impaired on the complex reaction time, oculomotor coordination, memory, coding, picture completion and object assembling tests. However, the investigators noted that the performance deficits had no clinical manifestations, and that additional studies were probably needed to confirm the possibility of cerebral damage. The study yielded a lowest available non-duration adjusted LOAEL of 4.6 mg Al/m^3 for psychomotor and cognitive impairment during repeated 8-hour occupational exposures (Hosovski et al., 1990), that could be corrected for discontinuous exposure (10 m^3/20 m^3 and 5 days/7 days) to yield a $LOAEL_{HEC}$ of 1.64 mg/m^3 Al.

Aluminum oxide powders were administered to Canadian miners (mainly underground gold and uranium miners) in known exposures as a means of prophylaxis against silicosis (Stokinger, 1981; Rifat et al., 1990). Data in which more than 42 million Al treatments (≈150,000 man-years) had been given over a period of 27 years ending in 1971 were reviewed

by Stokinger (1981). The effectiveness of this treatment is uncertain but no lung damage or other ill effects (not specified) were observed. The powders (McIntyre powder) were prepared by grinding Al pellets so that 96% of the particles were ≤1.2 μm in diameter. During this process most of the particles became oxidized to aluminum oxide; the powder contained 85% aluminum oxide and 15% elemental Al. According to Stokinger (1981), recommended exposure concentrations were 30,000 particles of respirable size per cubic centimeter (ppcc) for 10 minutes/day or 10,000-20,000 ppcc for 20 minutes/day (total treatment days not indicated). Rifat et al. (1990) stated that the recommended exposure was to an Al dust concentration of 20,000-34,000 parts per ml air in the miners' changing rooms before each shift for 10 minutes. Stokinger (1981) reported that the 30,000 ppcc concentration corresponds to ≈350 mg/m^3, which is equivalent to an 8-hour average concentration of 2 mg/m^3. Based on the Stokinger (1981) data and the fact that one unspecified study used levels 30 times higher than advised, the TLV of 10 mg/m^3 is recommended for Al dust (ACGIH, 1998).

The increasing awareness of the potential neurotoxicity of Al has resulted in a number of investigations of the incidence of neurotoxicological symptoms in Al workers. Although treatment with McIntyre powder had not produced apparent adverse effects, a neurobehavioral evaluation of male miners (261 exposed to McIntyre powder, 346 unexposed) who started working between 1940 and 1979 (additional duration data not reported) was performed in 1988-1989 (Rifat et al., 1990). There were no significant differences between exposed and unexposed miners in reported diagnoses of neurological disorder. Results of cognitive testing (Mini-Mental State Examination for general cognitive function, Ravens colored progressive matrices test for reasoning and Symbol Digit Modalities Test for spatial perceptual accuracy and information processing), however, showed that the exposed group had significantly ($p \leq 0.001$) impaired performance on at least one test, and when all test scores were summed. Also, the likelihood of scores in the impaired range increased with duration of exposure.

A neurologic syndrome was described in Al smelting plant potroom workers (White et al., 1992). Twenty-five men were evaluated for suspected work-related neurologic illness based on findings in three patients studied previously. The average duration of employment was 18.7 years (SD, 3.6; range, 12-23 years), 15 of the patients were working at the time of evaluation, and 10 had taken early retirement or medical leave due to workplace-related symptoms (mean length of time since exposure was 1.3 years ranging from 0.2-5 years). Quantitative exposure level data were not reported, but 21 of the workers had been employed in the potroom prior to installation of fume hoods for a mean duration of 5.3 years (range 3-7 years). Symptoms most often reported by the patients were frequent loss of balance (88%), memory loss (84%) and joint pain (84%); other symptoms included dizziness (80%), numbness (80%), parasthesias (72%) and tremor (68%). Neurologic examinations showed mild to moderate signs of lack of coordination (tremor, dyssynergy of upper extremity limb movement or ataxia) in 84% of the patients. Neuropsychologic effects were evaluated in 21 of the patients using the Wechsler Adult Intelligence Scale-Revised (intellectual functioning), Wide Range Achievement Test-Revised (academic functioning), Halstead-Reitan Neuropsychological Test Battery (neuropsychological assessment) and Minnesota Multiphasic Personality Inventory (personality functioning). Memory function was assessed with the Wechsler Memory Scale (14 patients) and Wechsler Memory Scale-Revised (8 patients). The memory function evaluation showed mild to moderate impairment on subtests of immediate recall for verbal or visual information (70-75% of the

tested patients) and delayed verbal or visual recall (50-70%). Other effects included mild or moderate impairment on Halstead-Reitan tests of abstract reasoning and flexible thinking (42% of the tested patients), memory for tactile information (53%) and sustained attention and discrimination of tonal and speech patterns (44 and 64%, respectively). On the Wechsler memory and Halstead-Reitan tests, mild and moderate impairment was defined as scores 1.5-2 and ≥2 standard deviations below the mean of the normal population, respectively. Most (89%) of the patients tested with the Minnesota Multiphasic Personality Inventory had abnormally elevated scores (≥2 SDs above the population mean) indicative of clinical depression. Significant positive correlations were found between severity of incoordination (signs and symptoms) and degree of exposure (qualitative) before the introduction of the ventilation hoods.

White et al. (1992) noted two other studies that described neurologic problems among Al smelter workers. Thus, an evaluation of 444 electrolysis workers found neuropsychiatric changes in 123 (28%), "neurotic syndromes" in 89 (20%) and "slight pyramidal and cerebellar changes" in 39 (9%) (Langauer-Lewowicka and Braszczynska, 1983). In the second study, symptoms including mental confusion, concentration and memory problems were described in six potroom workers (Cawthon, 1988).

In another study of Al production workers, neuropsychological effects were assessed in 38 elderly men who had been exposed for at least 10 years exclusively in the potroom (n=14), foundry (n=8) or other manual labor departments of the same plant (n=16, control group) (Bast-Pettersen et al., 1994). The mean ages and employment durations of the groups were in the ranges of 62.5-63.5 and 19.2-19.6 years, respectively. The men were examined soon after or just before retirement in 1991. Limited environmental monitoring data indicates that the degree of Al exposure varied between the subgroups and over the years. Average annual total dust concentrations in the potroom were reduced significantly from 9.5 mg/m^3 in 1977 to 3.0 mg/m^3 in 1990. Aluminum levels were not specifically reported, but the average Al content in the total potroom dust was approximately 20% by weight; other constituents of the dust included fluoride and coal tar pitch components. Data from an Al uptake/excretion study of workers from the same plant indicated that the level of Al exposure was approximately 8 times higher in the potroom than in the foundry (0.48 and 0.06 mg/m^3, respectively) (Drablos et al., 1992). Medical examinations (including lung function, standard laboratory tests and serum and urine Al concentrations) and a neuropsychological test battery were performed. The battery assessed six mental functions (neuropsychiatric symptoms, motoric/sensoric, reaction time, psychomotor speed/efficiency, memory/learning and intelligence) using a questionnaire and 15 different objective tests. Some subtle deficits were found in potroom workers that were not considered to be indicative of a significant neurological syndrome. The findings in potroom workers included a subclinical tremor as indicated by results of a static steadiness test [time scores on one of two test indices were significantly worse in comparison with the control group (84% slower, p=0.03)], and possible tendencies (i.e., test results that were about 1 SD below normal mean values but not statistically significant) for increased risk of impaired visuospatial organization (Block Design subtest of the Wechsler Adult Intelligence Scale) and psychomotor tempo (one Halstead ReitanTrail Making test). Although these findings were not considered to be indicative of a neurologic syndrome, it was suggested that they may be early signs of CNS impairment. Additionally, the finding of a subclinical tremor seems to be consistent with the tremor and other

signs of incoordination observed in 84% of the patients in the White et al. (1992) study summarized above.

Studies of Al welders are consistent with those of Al smelter workers in indicating that occupational exposure to Al can be neurotoxic. CNS function was evaluated in 17 welders who had an average of 15 years (range 5-27 years) experience, with the last 4 years exclusively with Al (Hanninen et al., 1994). Most of the welders had equipment that ventilated the welding masks but the respiratory protection was not always used. The assessment included measurements of serum and urinary Al, neuropsychological tests (simple reaction time, three tests for psychomotor speed, two tests for visual and spatial ability, four memory tests and two verbal ability tests), a symptom questionnaire and neurological interview, quantitative electroencephalography (QEEG) and P-300 event-related auditory-evoked responses. Serum and urine Al levels were 3.5 and 8.5 times higher, respectively, than an unexposed reference population. The welders performed normally on the neuropsychological tests, although correlation analysis of test scores and exposure parameters showed weak negative associations between the four memory tests and urinary Al level and a positive association between the variability (standard deviation) of visual reaction times and serum Al levels. Analysis of the QEEG data showed that serum Al levels were positively correlated with the amount of delta and theta activity in the brain frontal region and negatively correlated with the amount of alpha activity in the frontal region. Results of this study (disturbances of memory and attention, QEEG changes similar to those in patients with Al encephalopathy) were interpreted as consistent with known CNS effects of Al, but insufficient for establishing a definite relationship between Al exposure and effects.

In another study of Al welders, CNS evaluations were performed on 38 men who had at least 5 years exposure (mean 17.1 years) and a control group of 44 railway track welders exposed to metal fumes other than Al (mean 13.8 years) (Sjogren et al., 1996). Limited monitoring data indicated that the median exposure to welding fumes was 10 mg/m^3 and that the Al content was 40% of the total fumes. Symptom questionnaires, psychological tests (simple reaction time, finger tapping speed and endurance, digit span, vocabulary, tracking, symbol digit coding, cylinders, olfactory threshold and Luria-Nebraska motor scale), neurophysiological indices [electroencephalography, P-300 auditory-evoked responses, brain-stem auditory evoked responses and diadochokinesis (ability to perform rapidly alternating movements with one limb)] and blood and urine Al levels were assessed. The blood and urine Al concentrations were approximately 3 and 7 times higher in the Al welders than in the controls, but there were no clear correlations between duration of exposure to Al and concentration of Al in blood or urine. The Al welders reported more acute CNS symptoms (e.g., concentration difficulties) and had decreased motor function in five tests (finger tapping in non-dominant hand, two tasks from the Luria-Nebraska motor scale, pegboard peg movement with dominant hand, amplitude of diadochokinesis in dominant hand) when compared to the control group. Urinary Al concentration was significantly correlated with acute CNS symptoms, but not with any of the performance measures. To further study possible dose-effect relationships of Al exposure, the Al welders were combined with the control group and divided into three exposure categories according to urinary Al levels, using the 50^{th} and 75^{th} percentiles as category dividers. The group with the highest mean urinary Al level had significantly more acute CNS symptoms and significantly reduced performance on one of the motor function tests (a Luria-Nebraska motor

scale task) when compared to the group with the lowest Al level. In an earlier study of 65 welders with ≥10 years of exposure to Al fumes, the highest exposure category (based on exposure duration) was 2.8 times more likely than unexposed workers to have three or more neuropsychiatric symptoms (Sjogren et al., 1990).

A body of epidemiological evidence has pointed to an increased incidence of cancers of various kinds in workers employed in the aluminum production industry. However, as discussed in a review by Ronneberg and Langmark (1992), the concern about potential cancer hazards in the aluminum industry has primarily arisen because of exposures to polycyclic aromatic hydrocarbons (PAHs) and coal tar pitch volatiles (CTPVs) rather than to Al *per se*. Thus, while a number of studies have provided inferential data linking occupationally exposed aluminum workers with an increased risk of developing tumors of the bladder or lung (Gibbs, 1985; Thériault et al., 1984, 1990; Armstrong et al., 1986; Spinelli et al., 1991; Pearson et al., 1993; Tremblay et al., 1995), it would be unwise to ascribe any excess tumor formation to the effects of Al in view of the concurrent exposure to well-documented carcinogenic PAHs such as benzo(a)pyrene. The issue is further complicated by the likely exposure of production workers to other substances such as fluorides, sulfur dioxide, aromatic amines and asbestos (Ronneberg and Langmark, 1992; Tremblay et al., 1995; Dufresne et al., 1996), and to the possible effects of cigarette smoking in affected individuals. Consequently, these studies have failed to provide direct evidence for the carcinogenicity of Al fumes and dusts.

Animal Studies

Oral Exposure

Numerous subchronic animal studies were located in the biomedical/toxicological literature but only those that define the threshold region of the oral dose-response relationship are summarized in this paper. A major limitation of many of the studies of Al toxicity is the lack of complete information on total dietary (e.g., food and drinking water) intake of Al and of other elements that are known to effect Al biokinetics and toxicity (e.g., calcium and magnesium). Estimated or reported dosages used in studies in which Al content of the basal diets are not reported must be assumed to underestimate the actual experimental dosages. The magnitude of the underestimate may be considerable. For example, a range of Al contents of 200-1200 mg Al/kg for commercial grain-based diets (Golub et al., 1992b) would provide 30-200 mg Al/kg bw-day in a subchronic or chronic mouse bioassay [based on U.S. EPA (1988) default values for body weight and food intake]. On this basis, studies in which complete dietary Al intakes were not reported or could not be estimated may provide some information about the hazards of oral exposure to Al but are inappropriate for establishing NOAELs or LOAELs for the critical effect of Al. NOAELs and LOAELs from studies that provide estimates of total Al dosages, or otherwise provide information relevant to determining the NOAEL/LOAEL boundary for the critical effect of Al are presented in Table 1 and are summarized below.

Systemic toxicity

Groups of 10 female Sprague-Dawley rats were administered aluminum nitrate nonahydrate in sugar-containing drinking water at doses of 360, 720 and 3600 mg/kg-day (26, 52

and 259 mg Al/kg bw-day, respectively) for 100 days (Domingo et al., 1987). A control group received sugar-containing distilled water only. Sugar had been added to the drinking water of all groups to reduce the taste-aversive effects of Al. The level of Al in the diet was not reported. Animals were housed in metabolic cages to facilitate the collection of fecal and urine samples. Food and water consumption were measured daily, body weights were noted weekly and blood samples were taken at monthly intervals and at termination to monitor clinical chemistry and hematological parameters. At termination, all animals were necropsied, and the weights of major organs (brain, heart, lungs, kidneys, liver and spleen) were monitored. Aluminum concentrations were measured in various tissues, pieces of which were processed for histopathological examination. A significant decrease ($p<0.05$) in body weight gain was observed in the 259 mg Al/kg-day group, attributed by the authors to decreased food intake. Overall, no consistent variations in hematological (hemoglobin, hematocrit) or clinical chemistry (SGOT, SGPT, alkaline phosphatase, urea, creatinine, total protein, cholesterol, glucose) parameters were observed. No histopathological alterations in the heart, liver, kidney, spleen, brain and cerebellum were observed. Interpretation of these data was complicated by the concurrent exposure of the rats to high doses of nitrate of up to 475 times the RfD for nitrate (1.6 mg nitrate-nitrogen/kg-day) which is based on methemoglobinemia in humans (U.S. EPA, 1999). Therefore, because of nitrate co-exposure, the absence from the study design of a food-restricted control group and uncertainty surrounding the contribution of Al in food, the apparent effect of Al on body weight gain cannot be conclusively attributed to Al alone.

Some recent studies have identified a number of potential toxicological responses in laboratory animals exposed orally to Al compounds in a subchronic or chronic dosing regimen. In most cases, however, only one dose level was employed in the study compared to controls, and since the amount of Al in the diet was not given, the resulting dose level represents an incremental dose of Al compared to that of controls as baseline. However, while these studies may offer inadequate quantitative dosimetric information for NOAEL/LOAEL identification and consequent RfD development, they provide an qualitative indication of a range of potential toxicological responses that might be induced in humans exposed to the element. For example, Garbossa et al. (1998) studied the potential for water-soluble Al to affect the erythropoietic integrity of late erythroid progenitor cells in the bone marrow. Three groups of five male Wistar rats/group were either (1) gavaged with citrate at a dose of 1.0 μm Al/g-day (27 mg/kg-day), 5 days/week, for 15 weeks, (2) had drinking water containing 100 mmol Al/L made available to them as the citrate for the same length of time or (3) maintained as controls. As calculated by the authors, the dose associated with the applied concentration of Al in drinking water approximated to 14-17 μmol/g-day (420 mg/kg-day). Rats had access to a standard chow diet, though with no indication of the baseline concentration of Al provided therein. At the end of the in-life phase of the study, all rats were sacrificed, and samples of blood were obtained for hematological investigation. Femoral bone marrow cells were flushed with physiological medium, stimulated with recombinant human erythropoietin, then monitored for the comparative incidence of colony-forming units-erythroid (CFU-E). Further tests were carried out to monitor the osmotic fragility and average life-span of erythrocytes from each test group. The animals in the group receiving Al at the higher dose showed decreased hematocrit, hemoglobin concentration, median osmotic fragility and erythrocyte life-span values compared to controls. The content of Al increased in the serum and bone of both exposed groups, the distribution of concentrations in bone correlating inversely with the extent of an animal's CFU-E development.

That Al in drinking water may have the ability to cause histopathological changes and altered hepatic enzyme activities was suggested by Basu et al. (1997) who made available aluminum chloride in drinking water to groups of eight male Sprague-Dawley rats at a dose of 50 mg/kg-day (10.1 mg Al/kg-day) for 40 days. Additionally, other groups of similarly-treated rats received drinking water containing either 0, 50, 100, 200 or 400 ppm (mg/L) added calcium (Ca), as the chloride. The authors reported increased specific activities of acid and alkaline phosphatases in liver 10,000 x g supernatants from Al-receiving animals versus controls, and in alkaline phosphatase activity in equivalent kidney preparations. The presence of Ca in the drinking water appeared to reverse these changes, plus the accompanying histopathological features associated with them.

Konishi et al. (1996) examined the ability of Al and Ca to cause opposite and potentially harmful effects in laboratory animals, in relation to the well-documented association between Al and the onset of osteomalacia. Male STD Wistar rats were divided into four groups (n=4), receiving either (1) a normal diet (Group I), (2) a normal diet supplemented with Al (Group II), (3) a Ca-deficient diet (Group III) or (4) a Ca-deficient diet with supplemental Al (Group IV), for 10 weeks. Blood samples were taken at termination, and then animals were perfused with paraformaldehyde/glutaraldehyde fixative. Levels of Ca, iron (Fe) and Al in serum and bone were measured by atomic absorption spectrophotometry, and sections of the resected right tibia were prepared for histopathological examination after decalcification in 5% formic acid in 10% formalin.

There were statistically-significant changes in body weight gain when those of groups 3 and 4 were compared to animals from groups 1 and 2, the values for the latter groups remaining constant from about 4 weeks of dosing. In discussing their histopathological findings, the authors described no decrease in the thickness of cortical bone in Group II compared to control, while bone specimen from Groups III and IV showed “an increase in osteoid as well as osteoblasts and osteoclasts”, in addition to other disturbances of ossification. Such effects were considered to suggest bone fragility, with changes being more marked in Group IV compared to III. The amount of Al in the tibia of exposed rats was significantly greater in Group II than in Group I, whereas the average levels in Groups III and IV showed a further increase in Al deposition, most notably in group IV. There were also differences among the groups in the concentration of Fe in bone (tibia), and in the concentrations of Al, Ca, Fe and the levels of parathyroid hormone in blood. The authors concluded that Ca deficiency appeared to potentiate the deposition of orally administered Al in bone, and the attendant inhibition of ossification. Iron deposition was also thought to play a role in the osteogenic disturbance, where Ca is deficient.

A histopathological investigation indicated profound changes in the cerebrovascular and neuronal integrity when male Long-Evans rats (n=9) were exposed for 52 weeks to 0.5 ppm aluminum fluoride in drinking water (Varner et al., 1998). This corresponded to an Al dose of 0.019 mg/kg-day, based on a default drinking water consumption of 0.057 L/day, and a default body weight of 0.472 kg for male Long-Evans rats (U.S. EPA, 1988). Duel control groups received either NaF (fluoride controls) or double distilled deionized water. Tissue levels of Al were measured in brain, liver and kidney by the use of a direct current plasma technique.

Animals receiving aluminum fluoride showed poor survival compared to the other groups, with 6/9 having died by week 48. The tissue concentrations of Al were increased in the brain and kidney compared to both the control groups, with Al-fluorescence being used to demonstrate that Al deposition was mostly in the vasculature. Morphological and histopathological changes due to treatment were apparent in the liver, kidney and spleen. Some changes in neuronal integrity were also evident in the hippocampus and neocortex. Other cytological changes in the brain were associated with chromatid clumping, pyknosis and vacuolation.

A report by Somova et al. (1997) describes a study in which 10 male Wistar rats/group received either 0, 5 or 20 mg/kg-day aluminum chloride by gavage in water for 6 months. At termination, all animals were exsanguinated, then subjected to a necropsy in which excised pieces of liver, kidney and cardiac and skeletal muscle were taken for histopathological examination. Pieces of brain were examined by electron as well as light microscopy, and all tissues were monitored for Al concentration by atomic absorption spectrophotometry. As tabulated by the authors, Al in plasma and all of the listed tissues was dose-dependently increased to levels that were statistically significantly greater than controls. However, though described in qualitative terms and illustrated photographically, the Al-induced lesions did not receive a quantitative treatment in the report. Thus, while at least some of the low dose rats displayed NFD (neuro fibrillar degeneration) of the hippocampal region of the brain, insufficient data are provided in the report to apply this observation to the identification of a NOAEL or LOAEL.

Dietary experiments

Six Beagle dogs/sex/group were fed a diet providing either, in males, 0, 118, 317 or 1034 mg/kg-day sodium aluminum phosphate (0, 3.4, 9.0 or 29.4 mg Al/kg-day, respectively) or, in females, 0, 112, 361 or 1087 mg/kg-day sodium aluminum phosphate (0, 3.2, 10.3 or 30.9 mg Al/kg bw-day, respectively), for 6 months (Katz et al., 1984). No information was available on the level of Al in the diet, and no compound-related effects on body weight gain, hematological and clinical chemistry parameters (parameters not specified) or histopathological endpoints (major organs and tissues examined) were observed. A highest NOEL of 30.9 mg Al/kg-day could be tentatively identified in this study, but this would not include the contribution of Al from the basal diet, nor reflect the identification of any toxicological effects, since the NOEL occurred at the upper limit of the dose-response curve.

Neurotoxicity

A number of studies have been reported in which neurotoxicological/neurobehavioral effects have been explicitly evaluated. In others, the effects of Al on neurological developmental have been addressed. For example, Golub et al. (1989) fed diets containing Al as the lactate at 25 (controls), 500 or 1000 mg Al/kg diet (3.3, 65 or 130 mg Al/kg-day) to groups of 15 female Swiss-Webster mice for 6 weeks (Golub et al., 1989). No mice were exposed to lactate alone. While no statistically significant differences in food intake or body weight gain were observed, mice fed the highest Al concentration gained less weight than the controls or low-dose group. As reported by the authors, a significant decrease (20%) in spontaneous motor activity (i.e., total, vertical and horizontal movement) was observed in the 130 mg Al/kg-day group. Activity in the

65 mg Al/kg-day group was not significantly different than the controls. Thus, the highest NOAEL is 65 mg Al/kg-day and the LOAEL is 130 mg Al/kg-day.

Neurobehavioral effects of aluminum lactate were evaluated in groups of 12 female N:NIH Swiss-Webster mice (4.5-5.5 weeks old) that were fed 25 (controls) or 1000 mg Al/g diet for 90 days (Golub et al., 1992a). Based on a food factor of 0.19 kg diet/kg body weight/day calculated using an algorithm relating food consumption to body weight (U.S. EPA, 1988) and reported body weight data (the time-weighted average weight is 25.4 g), the dosage in the treated mice is estimated to be 190 mg Al/kg bw-day. No mice were exposed to lactate alone. A neurobehavioral test battery used by Donald et al. (1989) was administered at the beginning of the experiment (day 0) and after 45 and 90 (±3) days, with motor activity evaluated at the latter two time points. Aluminum levels were measured in brain, femur and liver at the end of the exposure period.

Body weight was significantly increased in the treated mice but no exposure-related changes in food intake or overt signs of neurotoxicity were observed. Results of the neurobehavioral tests showed significantly decreased hindlimb grip strength at 90 days, decreased air puff startle response at 90 days and decreased auditory startle response at 45 days in the treated mice. Spontaneous motor activity was reduced at 90 days as indicated by decreased total activity counts, horizontal activity counts and percentage of intervals with high activity counts. Aluminum concentrations in the brain and liver were increased approximately 3-fold in the treated mice, but brain and liver lipid peroxidation indices were not altered.

Male Wistar rats (6-8 per group) were exposed continuously for 6 months to food containing 1.52 mg Al/kg (normal diet) or 1000 mg Al/kg as aluminum chloride with citrate (Florence et al., 1994). The average daily Al intake was estimated to be 0.13 or 84 mg Al/kg bw-day, assuming a body weight of 0.305 kg (arithmetic mean of default mature weight of male Wistar rats and the starting weight in this study of 0.11 kg) and a food intake of 0.026 kg food/kg bw-day, calculated using an algorithm relating food intake to body weight (U.S. EPA, 1988). The citrate content of the diet was in a 1:1 stoichiometric proportion to Al, therefore, the estimated daily intake was 598 mg/kg-day. Rats exposed to Al developed histopathological abnormalities in brain tissue, not specific to any brain region, characterized by extensive cytoplasmic vacuolization in astrocytes, swelling of astrocytic processes, particularly of astrocyte end-feet abutting blood vessels. Neurons also exhibited vacuolization and nuclear inclusions. Although no specific behavioral assays were reported, the investigators noted that "no significant behavioral changes were observed". Accordingly, the functional significance of the histopathological lesions is uncertain. The lesions appear to differ from the NFD observed with parenteral Al exposures (Kowall et al., 1989; Wakayama et al., 1993); or from exposures to Al in combination with calcium deprivation (Garruto et al., 1989; Kihira et al., 1995; Mitani, 1992). The LOAEL for histopathological changes in the brain was 84 mg Al/kg-day.

Male Sprague-Dawley rats (40 per group) were exposed in drinking water to 0, 50 or 100 mg Al/kg bw-day as aluminum nitrate with citric acid for 6.5 months beginning at 21 days of age, 8 months of age or 16 months of age (Domingo et al., 1996). The citric acid dosage was 355 or 710 mg/kg-day in the 50 or 100 mg Al/kg bw-day groups, respectively. Controls did not receive citric acid. Dietary Al intake was not reported; the rats were maintained on Panlab rat

chow. Animals from control and exposed groups were subjected to a number of neurobehavioral tests, and at termination, Al levels were measured in various excised regions of the brain. The authors observed the highest Al levels in the olfactory bulb and rhachidical bulb, while the cortex and thalamus were the regions showing the lowest Al content. However, compared to controls, there were no significant effects ($p>0.05$) of Al (with citric acid) on spontaneous motor activity (open-field) or passive avoidance operant training or performance (grid floor shock, light/dark shuttle box). Thus, the NOAEL was 100 mg Al/kg-day with citric acid; although this does not include the Al contribution from food. This study is listed on Table 1 because the NOAEL, although probably underestimated because of unreported Al intake from food, is still lower than the LOAELs from other studies.

Groups of six male albino rats were administered 0 or 25 mg Al/kg bw-day as aluminum nitrate in normal saline by gavage, 10% ethanol in drinking water, or 25 mg Al/kg bw-day by gavage combined with 10% ethanol in drinking water, 6 days/week for 6 weeks (Flora et al., 1991). The level of Al in the diet was not reported. Urinary Δ-aminolevulinic acid (ALA), blood ALA-dehydratase (ALAD), blood zinc protoporphyrin (ZPP), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in serum and liver and brain biogenic amines and their metabolites [dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), homovanillic acid (HVA) and 5-hydroxyindolacetic acid (5-HIAA)] were evaluated at the end of the treatment period. Treatment with Al alone caused significantly increased blood ALAD ($p<0.01$), decreased liver GPT ($p<0.05$), decreased brain DA ($p<0.01$), increased brain NE ($p<0.05$) and decreased brain 5-HT ($p<0.05$). Compared to treatment with Al alone, concurrent exposure to ethanol and Al produced significantly decreased ALAD, increased ALA, increased ZPP, increased liver GPT, increased serum GOT and increased brain HVA. Significant changes found only in the combined Al and ethanol group included increased serum GPT, increased brain NE and decreased brain 5-HT. Treatment with ethanol alone only inhibited blood ALAD. The rats were co-exposed to relatively high levels of nitrate [comparable to those in the Domingo et al. (1987) subchronic study], but it seems likely that some of the changes (i.e., effects on brain chemicals) are related to aluminum which is known to be neurotoxic. Because the toxicological significance of the changes is unclear due to lack of evaluation of neurobehavioral performance and other endpoints, there is uncertainty whether the 25 mg Al/kg-day dose is a NOAEL or a LOAEL, an uncertainty compounded by the absence of information about the level of Al in the basal diet.

Reproductive/developmental toxicity

A number of studies have been carried out to examine the effects of Al compounds on developmental toxicity, particularly their effects on postnatal neurobehavioral development. For example, Bernuzzi et al. (1989) exposed groups of 6-12 pregnant Wistar rats to aluminum chloride or aluminum lactate in the diet on gestational days 1 through 21. The rats received nominal daily doses of 0, 100, 300, 400 mg Al/kg as aluminum chloride or 0, 100, 200 or 400 mg Al/kg as aluminum lactate. No rats were exposed to lactate alone, and information regarding level of Al in the basal diet was not reported. On the average, there was a less than 10% decrease in maternal body weight gain and no effect on food or water intake. No significant difference in litter size was observed. However, postnatal mortality increased 55% and 26% in offspring of the rats exposed to 300 or 400 mg Al/kg-day, respectively. The offspring of dams

fed ≥300 mg Al/kg-day weighed significantly less than controls on postnatal day 1. Decreased body weight was also observed on postnatal days (PD) 4 and 14 in the offspring of rats fed 400 mg Al/kg-day as aluminum lactate. The following tests were used to assess neuromotor development (maturation): righting reflex, grasping reflex, negative geotaxis, suspension test and locomotor coordination. The tests were performed on PDs 4, 6, 9, 12 and 20, respectively. Impairment of neuromotor development (righting and grasping reflexes) was observed in the pups exposed to ≥200 mg Al/kg-day. Impaired grasping reflex was also observed in the 100 mg/kg-day aluminum lactate group. Offspring of rats fed 400 mg/kg-day also exhibited altered performance on the locomotor coordination test.

A follow-up study by the same research group found that ingestion of 400 mg Al/kg bw-day as aluminum lactate had no effect on postnatal mortality, body weight and righting and grasping reflex tests (Muller et al., 1990), although significant differences between control and exposure groups were noted in locomotor coordination and operant conditioning tests. Significant differences between controls and exposed groups in the negative geotaxis test were limited to those pups of dams treated during the second and third weeks of gestation, a finding interpreted by the authors to indicate the possibility of long-term effects on the central nervous system of trans-placenta exposure to Al during a later organogenic phase. According to Muller et al. (1990), the contradictions between this and their earlier study (Bernuzzi et al., 1989) could be related to environmental modifications. In particular, the mothers and pups were much more protected in the Muller et al. (1990) study than in the previous one because they were housed in plastic cages instead of wire mesh cages and received cotton to build nests. Body temperature of the pups, therefore, may have been more adequately maintained in the Muller et al. (1990) study. As discussed in this study, toxicity in pups can be confounded by insufficient body temperature, and delayed pup weight gain could explain the differences in neuromotor performance.

Muller et al. (1990) administered diets supplemented with 0 or 400 mg Al/kg bw-day as aluminum lactate to groups of 6-9 pregnant Wistar rats on days 1-7, 1-14 or 1-21 of gestation. No rats were exposed to lactate alone, and information regarding level of Al in the basal diet was not reported. Neuromotor development was assessed on postnatal days 4, 6, 9, 12 and 20 using tests of righting reflex, grasping reflex, negative geotaxis, suspension and locomotor coordination, respectively. Learning ability was also tested on PD 65 using operant conditioning. No effects on maternal body weight or food intake were observed in dams exposed on gestational days 1-7 or 1-14. In the dams exposed on gestational days (GD) 1-21, a significant decrease in maternal body weight (26 and 35%, respectively) was observed on days 16 and 19 of gestation. Decreased food intake was also observed on day 19 of gestation. No effects on litter size, postnatal mortality or postnatal body weight were observed. Impairment of neuromotor development ($p<0.05$) was observed in two of the five tests (negative geotaxis and locomotor coordination); no differences between the three treated groups were observed. For the operant conditioning test, there were significant differences ($p<0.05$) between the treated and control young rats. No differences between the three treated groups were observed. The LOAEL for developmental toxicity is 400 mg Al/kg-day, but this does not include the contribution of Al from the basal diet.

Groups of 10 pregnant Sprague Dawley rats were administered 180, 360 or 720 mg/kg-day aluminum nitrate nonahydrate by gavage (13, 26, 52 mg Al/kg bw-day) on GDs 6-14

(Paternain et al., 1988). A vehicle (water) only control group was used. The level of Al in the diet was not reported. Aluminum exposed dams gained significantly less weight than the controls. No significant effects on the numbers of litters, corpora lutea, total implants, live fetuses, resorptions or runt fetuses were observed. Significant decreases in fetal body weight and tail length were observed at all three Al doses; decreased fetal body length was also observed at the 52 mg Al/kg-day dose level. No dose-related external or visceral malformations were observed in the offspring. However, a significant increase in the incidence of skeletal malformations (delayed ossification, hypoplastic deformed ribs) was observed at all three treatment levels. In addition, the incidence of hematomas was significantly increased at the high dose. Because the rats were co-exposed to relatively high levels of nitrate [comparable to those in the Domingo et al. (1987) subchronic study], the effects of treatment cannot be conclusively attributed to Al alone, in the absence of a nitrate-exposed control group.

By contrast to the striking findings of potentially teratogenic effects of aluminum nitrate in Sprague-Dawley rats, as described above (Paternain et al., 1988), equivalent experiments by Domingo et al. (1989) in Swiss mice did not reveal any reproductive, developmental or teratogenic effects of Al, when administered to dams as the hydroxide. Domingo et al. (1989) administered by gavage 0, 66.5, 133 or 266 mg/kg-day aluminum hydroxide (0, 23.9, 47.8 or 95.5 mg Al/kg bw-day) to groups of 20 pregnant Swiss mice on GD 6-15. The level of Al in the diet was not reported. The dams were killed on GD 18. No compound-related effects were observed on maternal mortality, clinical signs, body weight, food intake or absolute or relative heart, lung, spleen, liver, kidney and brain weights. In addition, no compound-related effects were observed on numbers of implantations, resorptions, live and dead fetuses, sex ratio and the incidences of external malformations, internal soft-tissue defects or skeletal abnormalities. Therefore, this study identifies a NOEL of 95.5 mg Al/kg-day by default for reproductive, developmental and teratogenic toxicity in mice. However, neuromotor development was not assessed and the contribution of Al from the basal diet was not stated in the report.

A number of studies have been designed to evaluate the influence of citrate or lactate on the potential developmental toxicity of Al. For example, Gomez et al. (1991) exposed groups of 15-19 pregnant Sprague-Dawley rats to either distilled water (controls) or 133 mg Al/kg bw-day in the form of either aluminum hydroxide (384 mg/kg-day), aluminum citrate (1064 mg/kg-day) or aluminum hydroxide (384 mg/kg-day) concurrent with citric acid (62 mg/kg-day) by gavage on GD 6-15. The level of Al in the diet was not reported and no rats were exposed to citric acid alone. Terminations were performed on GD 20. Maternal and fetal evaluations showed exposure-related effects only in the group exposed to aluminum hydroxide and citric acid concurrently. Significant changes included reduced maternal body weight gain on GDs 6-20 (but not at sacrifice on day 20), reduced fetal body weight and some skeletal variations (increased delayed occipital and sternebrae ossification and increased absence of xiphoides). No effects were seen on maternal food consumption or clinical signs, maternal absolute or relative liver, kidney or brain weights, gravid uterine weight, corpora lutea/dam, implantations/litter, pre- or postimplantation loss/litter, viable or nonviable implants/litter, fetal sex ratio or fetal malformations (external, visceral or skeletal). This study identified a stand alone minimum LOAEL of 133 mg Al/kg-day for non-neurobehavioral developmental toxicity of aluminum hydroxide and aluminum citrate in rats. Although confidence in this LOAEL is low (because aluminum hydroxide administered concurrently with citric acid induced did developmental

effects and because the dose does not include a contribution of Al from the basal diet) the value is consistent with the developmental NOAEL of 95.5 mg Al/kg-day for aluminum hydroxide in mice (Domingo et al., 1989).

In a similar experimental protocol, groups of 11-13 pregnant female Swiss albino (CD-1) mice were administered 57.5 mg Al/kg bw-day as either aluminum hydroxide (166 mg/kg-day), aluminum lactate (627 mg/kg-day) or aluminum hydroxide (166 mg/kg-day) concurrent with lactic acid (570 mg/kg-day) by gavage on gestation days 6-15 (Colomina et al., 1992). Other groups were treated with lactic acid alone (570 mg/kg-day, equivalent to the amount in 627 mg/kg of aluminum lactate) or distilled water (controls). The level of Al in the diet was not reported. Fetal evaluations were performed on GD 18, including examinations for skeletal and visceral abnormalities in approximately two-thirds and one-third of the pups, respectively. The investigators noted that the dose of Al (57.5 mg/kg-day) is equivalent to ingestion of 3.5 g Al/day by a 60 kg person, which is higher than the usual quantities of Al ingested therapeutically for peptic disorders. Maternal body weight gain was significantly lower than control values in the aluminum lactate-treated mice when evaluated over GDs 6-9 (92%), 6-12 (55.6%) and 0-18 (38.5%) and in the mice treated with combined aluminum hydroxide and lactic acid evaluated over GDs 6-12 (37.8%), 6-15 (42.7%) and 0-18 (15.7%). The decreased maternal weight gain in the aluminum lactate group was accompanied by significantly reduced food consumption during gestation days 6-18. Significant developmental and/or teratological effects in the aluminum lactate group included 16% reduced fetal body weight ($p<0.01$) and increased incidences of cleft palate (13.2%, $p<0.05$), dorsal hyperkyphosis (i.e., excessive flexion of spine) (13.5%, $p<0.05$) and delayed parietal ossification (15.4%, $p<0.01$). These developmental effects were not observed in any of the control or aluminum hydroxide exposed pups, and the only other significant changes in the other groups were decreased maternal relative liver weight and delayed fetal parietal ossification in the lactic acid only exposure group. Other types of internal or skeletal malformations or variations were not found in any of the fetuses. Additionally, no effects were seen on maternal absolute or relative kidney weight, gravid uterine weight, numbers of implantation sites/litter, live or dead fetuses, resorptions, postimplantation loss/litter, litters with dead fetuses or fetal sex ratio in any of the groups. By analogy to the findings of the Domingo et al. (1989) and Gomez et al. (1991) studies, the lack of developmental effects of aluminum hydroxide at the tested dose could be related to low solubility and absorption.

In a more recent study, pregnant Swiss mice were administered gavage doses of 0 or 104 mg Al/kg bw-day as aluminum hydroxide on days 6-15 of gestation (Colomina et al., 1994). Dietary Al intake was not reported; the mice were maintained on Panlab rodent chow. Compared to controls, there were no effects ($p>0.05$) of Al on maternal body or organ weight, number of implantations per litter, number of resorptions per litter, number of dead fetuses per litter, percentage of positive post-implantation loss, sex ratio or fetal body weight per litter. Gross external, visceral or skeletal examination of fetuses revealed no abnormalities or developmental variations. Thus, the NOAEL for development effects from this study is 104 mg Al/kg-day, however, this does not include the Al contribution from food. Thus, based on this study and the previous study (Colomina et al., 1992), aluminum lactate appears to be more potent as a developmental toxicant in mice than the less water soluble aluminum hydroxide.

Groups of 16 pregnant Swiss-Webster mice were fed 25 (control group), 500 or 1000 mg Al/kg diet as aluminum lactate throughout gestation and lactation (Donald et al., 1989). The control diet was fed to pups that were selected for post-weaning neurobehavioral assessment. Reported maternal doses were 5, 100 and 200 mg Al/kg bw-day at the beginning of pregnancy and 10.5, 210 and 420 mg Al/kg bw-day near the end of lactation. No mice were exposed to lactate alone. There were no treatment-related changes in maternal survival, body weight (measured on GD 0 and 16 and PDs 0, 5, 10, 15 and 20), food intake, toxic signs or neurobehavior (evaluated after pups were weaned at PD 21 using the same test battery used for the pups and described below), or on litter size or postnatal growth and development in pups as assessed by body weight, toxic signs on PDs 0-55, and by crown-rump length on PDs 0 and 20. Neurobehavioral maturation was tested in two pups per litter on PDs 8-18 with a 12-item test battery (fore- and hindlimb grasp, fore- and hindpaw placement on sticks of 2 widths, vibrissa placing, visual placing, auditory and air puff startle, eye opening and screen grasp, cling and climb). A neurobehavioral test battery was administered to six pups per litter at age 25 days (4 days postweaning) or 39 days (fore- and hindlimb grip strengths, temperature sensitivity of tail, negative geotaxis, startle reflex to air puff and auditory stimuli) or age 21 and 35 days (foot splay). The pre-weaning neurobehavioral testing showed that a significant ($p=0.007$) number of pups in the high dose group had impaired vertical screen climb performance. The postweaning neurobehavioral assessment showed significantly ($p<0.05$) altered performance on several tests. These included decreased forelimb grip strength at age 39 days in the low dose group, increased hindlimb grip strength at age 25 days in both low and high dose groups, increased foot splay distance at age 21 days in both low and high dose groups and at age 35 days in the low dose group, and increased forelimb grip strength at age 25 days and decreased thermal sensitivity at age 25 and 39 days in the high dose group. There were no treatment-related changes in concentrations of Al in pup liver or bone (brain tissue was not analyzed).

In a more recent study of similar design by the same group of investigators, groups of 14 and 9 female Swiss Webster mice (6-8 weeks old) were fed 25 (control) or 1000 mg Al/g diet as aluminum lactate, respectively, during gestation and lactation (Golub et al., 1992b). The 1000 mg/g concentration was selected based on the demonstration of neurobehavioral effects in weanlings at this level (Donald et al., 1989). No mice were exposed to lactate alone. Using food intake and body weight values estimated from reported data, maternal doses are estimated to be approximately 4.3 and 174 mg Al/kg bw-day at the beginning of gestation and 4.8 and 607 at the end of the lactation period. At birth, litters were fostered either within or between groups to provide four groups of offspring that were exposed to excess Al via maternal diet during gestation, lactation, both or neither (i.e., 25 ppm during gestation and lactation, 1000 ppm during gestation and 25 ppm during lactation, 25 ppm during gestation and 1000 ppm during lactation, and 1000 ppm during gestation and lactation). Maternal effects included significantly ($p\leq0.015$) reduced (10-12%) body weight gain and food intake in the treated group during late pregnancy and lactation, and signs of neurotoxicity (hindlimb splaying and dragging) in one treated dam at postnatal day 21 (weaning); this dam had seizures and died 4 days later. No treatment-related effects on litter size, birth weight, crown-rump length, righting ability at birth, sex ratio or postnatal survival were observed. Both gestation-only and lactation-only exposure caused significantly ($p<0.05$) decreased body weight gain in the treated pups beginning on postnatal day 10; combined gestation and lactation exposure produced the greatest decrease (approximately 24% at weaning). Neurobehavioral testing using the same battery as Donald et al. (1989) was

performed at weaning on the dams and on a total of 12, 16, 12 and 6 pups (1 male and 1 female pup per litter) from the control, gestation-only, lactation-only and combined gestation and lactation groups, respectively. Results of this testing showed effects only in pups, including significantly decreased forelimb grip strength after gestation-only exposure, increased hindlimb grip strength after both gestation and lactation exposure, decreased temperature sensitivity after lactation-only exposure, and longer negative geotaxis latency after lactation-only exposure. In general, the findings of this study are consistent with those of Donald et al. (1989) in showing neurodevelopmental effects at the 1000 mg/kg dietary concentration, although intake dosages are dissimilar at the end of lactation. Using the dosage at the beginning of gestation, this study defines a LOAEL of 174 mg/kg-day for developmental effects.

The Donald et al. (1989) study differs from that of Golub et al. (1992b) in that offspring were not fostered, were tested at a later age (25 vs. 21 days), were allowed 4 days of recovery from the treated diet prior to testing, participated in other behavioral tests currently, and experienced no growth retardation. The effects found only in the cross-fostered groups in the Golub et al. (1992b) study (lower forelimb strength after gestation exposure and altered negative geotaxis latencies after lactation only exposure) were not observed by Donald et al. (1989). Increased footsplay was observed by Donald et al. (1989) but not by Golub et al. (1992b), perhaps due to an opposing effect of smaller pup body size in this study. Neither gestation or lactation exposure affected pup brain or liver Al concentrations, but lactation exposure caused significantly lower manganese and iron concentrations in liver and manganese concentrations in brain.

In a further extension of the two previous studies (Donald et al., 1989; Golub et al., 1992b), pregnant female Swiss-Webster mice were exposed continuously to a semi-purified diet containing 7 (control), 500 or 1000 mg Al/kg from the time of conception, through pregnancy and lactation (Golub et al., 1995). At weaning, pups were exposed to the same Al diet as their mothers (500 or 1000 mg Al/kg) until they were 150-170 days of age or were switched to the control diet (7 mg Al/kg) for the same time period. Based on reported dosages in previous studies by the same investigators, estimated daily dosages for mice exposed to 1000 mg Al/kg diet were as follows: 200 mg/kg bw-day in pregnant mice, 420 mg/kg-day in lactating mice and 130 mg/kg-day in offspring (Golub et al., 1994); doses for the mice exposed to 500 mg Al/kg diet were assumed to be approximately half of that of mice fed 1000 mg Al/kg, or 100 mg/kg-day in pregnant mice, 210 mg/kg-day in lactating mice and 65 mg/kg-day in offspring. Compared to the control diet, the Al diet had no effect on dam weight, gestation length, litter size, pup weight, offspring growth or organ weights. Operant conditioning (nose poke) of offspring for delayed spatial alternation or discrimination reversal tasks was initiated at 50 days of age and continued 5 days/week for a total of 35 sessions. A neurobehavioral test battery was conducted when the offspring were 150-170 days of age (forelimb and hindlimb grip strength, temperature sensitivity, negative geotaxis, air puff and auditory startle response). Maternal and pre-weaning exposure to 500 mg Al/kg significantly affected ($p<0.05$) operant training in the offspring, but not performance after training in delayed spatial alternation or discrimination reversal tasks (i.e., decreased number of training sessions to achieve the training criteria). This exposure also significantly decreased forelimb and hindlimb grip strength and puff startle response ($p<0.05$). Pre-weaning and combined pre- and post-weaning exposure to 1000 mg Al/kg significantly increased ($p<0.05$) incidence of cagemate aggression at the time behavioral

testing. No effects were observed on auditory startle response, temperature sensitivity or negative geotaxis in offspring. Histopathological examination of the brain and spinal cord revealed no treatment-related changes. Thus, the LOAEL for combined maternal and pre-weaning exposure on neurobehavioral effects in mice would approximate to 100 mg Al/kg-day (estimated daily maternal dosage).

Pregnant Charles River CD rats were administered gavage doses of 0, 250, 500 or 1000 mg Al/kg bw-day ("experiment A") or 0, 5, 25, 50, 250 or 500 mg Al/kg bw-day ("experiment B") as aluminum lactate in distilled water on GDs 5-15 (Agarwal et al., 1996). Dietary Al intake was not reported. Offspring were examined for body weight, anogenital distance, oestrus cycle regularity (after puberty), duration of pseudopregnancy induced by mechanical stimulation of the cervix, oocyte production induced by an injection of human chorionic gonadotropin, and male and female gonad weights. Aluminum had no effect on litter size and no consistent effects on birth weight were observed. For example, birth weights were decreased in male offspring from dams that received 250 mg Al/kg-day, but not at higher dosages, and the effect was observed only in experiment A. Female offspring birth weights decreased at certain dosage levels in experiment A and increased at these same dosage levels in experiment B. Similar inconsistencies between experiment A and B were observed for gonadal weights, anogenital distance, time to puberty (vaginal opening), duration of pseudopregnancy or numbers of superovulated oocytes. A significantly increased ($p<0.05$) number of abnormal oestrus cycle lengths (defined as less than 4 days or greater than 5 days) occurred in offspring from dams that received 250 mg Al/kg-day (in experiment A, the endpoint was not measured in experiment B). However, the effect was most pronounced in the first three oestrus cycles (of five observed) and not detected by the 5th cycle. Thus, the NOAEL for temporary disturbance of the oestrus cycle in offspring of dams administered Al is 250 mg Al/kg-day. NOAELs for all other reproductive endpoints in this study were 1000 mg Al/kg-day. These NOAELs do not include the contribution of Al in food.

In a three-generation study, Ondreicka et al. (1966) exposed initial groups of seven female and three male Dobra Voda mice to either 0 or 19.3 mg Al/kg bw-day as aluminum chloride in drinking water. The diet also contained 160 to 180 ppm Al, giving an estimated intake of 27-31 mg/kg-day based on default values for food consumption and body weight for chronic exposure of mice (U.S. EPA, 1988). Using this estimate, the total Al intakes (drinking water and food) were 27 mg/kg-day (controls) and 46.3 mg/kg-day (exposed group). The P_0 group produced three litters (designated F_{1a}, F_{1b} and F_{1c}) and the F_{1a} group produced two litters (designated F_{2a} and F_{2b}) from which the weanlings were exposed to Al in the drinking water starting at 4 weeks of age. There was no difference in body weight gain among the groups in the P_0 generation, a result that contrasted with the striking decrease in this parameter in the treated F_{1b}, F_{1c}, F_{2a} and F_{2b} groups. Though no effects on erythrocyte count, hemoglobin levels or histopathology of the liver, spleen and kidneys were observed in the P_0, F_1 or F_2 generations at the end of the study and no significant differences were seen in the number of litters or offspring between the exposed and control groups, the study identified a LOAEL of 46.3 mg Al/kg-day, based on the observed changes in body weight gain.

Other toxicological effects of aluminum

In a study designed to determine the effects of oral Al exposure on susceptibility to bacterial infection, female Swiss-Webster mice (13-14 per group) were exposed to a diet containing 25 (control), 500 or 1000 mg Al/kg as aluminum lactate during pregnancy, through lactation and for 10 days following weaning of the pups (Yoshida et al., 1989). Based on reported dosages in previous studies by the same investigators, estimated daily dosages for mice exposed to 1000 mg Al/kg diet are as follows: 200 mg/kg-day during pregnancy and 420 mg/kg-day during lactation; doses for the mice exposed to 500 mg Al/kg diet are assumed to be approximately half of that of mice fed 1000 mg Al/kg, or 100 mg/kg-day in pregnant mice and 210 mg/kg-day in lactating mice (Golub et al., 1994). At weaning, dams and pups were inoculated with a tail vein injection of *Listeria monocytogenes* and monitored for mortality for 10 days. In a separate experiment, female mice, 6 weeks of age, were exposed to the same dietary Al levels for 6 weeks and then inoculated with *L. monocytogenes*. Estimated Al dosages were 5, 98 or 195 mg Al/kg bw-day for the 25, 500 or 1000 mg Al/kg dietary levels, respectively, based on a default food factor of 0.195 kg diet/kg bw-day assuming a reference "subchronic" food intake and body weight for female B6C3F1 mice over the period from weaning to 90 days (U.S. EPA, 1988). Inoculation resulted in significantly greater ($p<0.025$) mortality in dams exposed to 500 or 1000 mg Al/kg diet compared to controls. There were no differences in mortality between the groups of inoculated pups or between groups of inoculated adult mice exposed to Al for 6 weeks. The LOAEL for pregnant mice was 100 mg Al/kg bw-day and the NOAEL for adult, non-pregnant mice was 195 mg Al/kg bw-day. Although the exposure duration in this study was only 7 weeks, it is included in Table 1 because it provides the only dose-response data on the effects of Al on resistance to pathogens.

Carcinogenicity studies

Schroeder and Mitchener (1975a) exposed 52 Long-Evans rats/sex/group to 0 or 5 ppm Al as potassium aluminum sulfate in drinking water for life. Based on default values for drinking water consumption and body weight for this strain of rat in a chronic study (U.S. EPA, 1988), these values are equivalent to Al doses of 0.472 and 0.67 mg/kg-day, for males and females, respectively. Study endpoints included body and heart weight; serum glucose, cholesterol and uric acid; and urinary protein, glucose and pH. All animals were necropsied at the time of natural death, and histological examinations were carried out on heart, lung, kidney, liver, spleen and gross tumors, for approximately 50% of the animals in the group. The only remarkable finding was a significant increase ($p<0.005$) in gross tumor incidence in exposed male rats [13/25 (52%) compared to 4/26 (15%) in controls], although the tumor sites were not reported. Six of the tumors in the exposed males (46% of total) were considered malignant compared to two malignant tumors (50% of total) in the male controls. There were no significant differences in tumor incidences between exposed and control females.

In another study by the same investigators, 54 Swiss mice/sex/group were exposed to drinking water containing 0 or 5 ppm Al as aluminum potassium sulfate for life (Schroeder and Mitchener, 1975b). Based on default values for drinking water consumption and body weight for B6C3F1 mice in a chronic study (U.S. EPA, 1988), these values approximate to Al doses of 1.2 mg/kg-day in both males and females. Study endpoints included body weight, gross pathology,

and some limited histology of the heart, lung, liver, kidney and spleen. The incidences of gross tumors were 15/41 (36.6%) and 11/38 (28.9%) in exposed and control males, respectively, and 19/41 (46.3%) and 14/47 (29.8%) in exposed and control females, respectively, differences that did not achieve statistical significance by Fisher's exact test, although incidences of multiple tumors and lymphoma leukemia were considered by the authors to be significantly increased in females ($p<0.025$ and $p<0.05$, respectively). However, a definitive assessment of aluminum carcinogenicity in both this and the rat study (Schroeder and Mitchener, 1975a) is precluded by the limitations of the pathology examinations and reporting.

In a more recent study, the tumorigenic potential of aluminum potassium sulfate was assessed in B6C3F1 mice chronically exposed in the diet (Oneda et al., 1994). Sixty animals/sex/group were fed a diet containing 0, 1.0, 2.5, 5.0 or 10.0% (w/w) for 20 months. These concentrations of aluminum potassium sulfate (as the dodecahydrate) are equivalent to 0, 569, 1422, 2844 and 5687 ppm Al. Using food factors calculated with an algorithm relating food consumption to body weight (U.S. EPA, 1988) and body weight data estimated from growth curves reported by the investigators, the dosages of aluminum are estimated to be 0, 95, 237, 483 or 1024 mg Al/kg-day in males and 0, 97, 242, 512 or 1110 mg Al/kg-day in females. Clinical signs, food consumption, and body weight were evaluated weekly. Hematology, clinical chemistry or urine endpoints were not assessed. Necropsies that included organ weight measurements and comprehensive histological examinations (including brain) were performed on all animals, including those that died during the course of the study. Survival rates were higher than control values in all treated male and female groups, ranging from 86.7-95.0% compared to 73.3% in males and 86.7-91.7% compared to 78.3% in females. No changes in food consumption were observed, but body weight gain was increased in both sexes at 95-97 and 237-242 mg Al/kg-day (weights were 10-23% higher than controls at end of study), was similar to controls in both sexes at 483-512 mg Al/kg-day, and decreased in both sexes at 1024-1110 mg Al/kg-day (11-16% lower than controls at end of study). There were no exposure-related increased incidences of tumors, other proliferative lesions or non-neoplastic lesions. In fact, the incidence of spontaneous hepatocellular carcinomas was significantly decreased in males at 1024 mg Al/kg-day (5.5% compared to 20.5% in controls, $p<0.01$).

Inhalation Exposure

Groups of 20 weanling Fischer 344 rats/sex and 20 weanling Hartley guinea pigs/sex were exposed to 0, 0.25, 2.5 or 25 mg/m^3 aluminum chlorhydrate [$Al_2(OH)_5Cl \geq x(H_2O)$] for 6 hours/day, 5 days/week for 6 months (Steinhagen et al., 1978). Analysis of the aluminum chlorhydrate by the investigators showed it to contain 24.5% Al, indicating that the animals were exposed to 0, 0.061, 0.61 and 6.1 mg Al/m^3. Body weights were measured weekly for the first 8 weeks and biweekly thereafter. At the end of the exposure period, 10 animals (5/sex) of each species were sacrificed for organ weight measurements (heart, lung, liver, kidney, spleen and brain) and histological examination of the lungs, liver and kidney. In addition, comprehensive histological examinations were performed on animals in the control and 6.1 mg AL/m^3 groups. The remainder of the animals was used for hematology evaluation (RBC, WBC, hematocrit and hemoglobin) and Al measurements in blood and tissues. Apparent effects of Al included multifocal granulomatous pneumonia in both species at ≥0.61 mg Al/m^3, significantly increased absolute and relative lung weights in both species, and decreased body weight gain in rats and

minimal lung edema in guinea pigs at 6.1 mg Al/m^3. The granulomatous reaction was characterized by foci of giant vacuoled particle-containing macrophages in the lungs and macrophages that did not appear to contain vacuoles or other evidence of phagocytized material in the peribronchial lymph nodes. There was a significant dose-related accumulation of Al in the lungs of both species at ≥0.061 mg Al/m^3. However, a NOAEL of 0.061 mg/m^3 could be identified for the onset of compound-induced histopathological effects.

In other studies, groups of 14-30 guinea pigs, rats and hamsters were exposed to fine metallic Al powders (pyro, atomized and flaked) at concentrations of 15, 30, 50 or 100 mg powder/m^3 air for 6 hours/day, 5 days/week for 6 months (Gross et al., 1973). Alveolar proteinosis occurred in exposed animals of all three species after 2 months of exposure, but fibrosis or other pulmonary changes did not develop. Similarly, groups of 23 or 46 rats and 48 hamsters were exposed to undetermined concentrations of Al fumes or Al powder (20% Al, 80% $Al(OH)_3$) for morning hours only or morning and afternoon for up to 20 months (Christie et al., 1963). Effects were similar for both forms of Al in both species, including initial increased alveolar macrophage proliferation followed by nodular hyalinized areas, with development of pneumonia but no fibrosis.

Exposure to 2.18 mg Al fibers/m^3 for 6 hours/day, 5 days/week for up to 86 weeks produced slightly increased alveolar macrophages and some irritation of the nasal passages in a group of 50 Alderly Park rats (Pigott et al., 1981). Finally, a study by Drew et al. (1974) observed the development of granulomatous nodules also developed in male hamsters that were exposed to 8 mg Al/m^3 of *Alchlor* (a propylene glycol complex of aluminum-chloride-hydroxide) for 6 hours/day, 5 days/week for 20 or 30 exposures. The alterations persisted at the longest post treatment observation (6 weeks) and consistently developed at the bifurcation of the bronchioloalveolar ducts, which is a likely site of particulate deposition.

DERIVATION OF A PROVISIONAL CHRONIC RfD FOR ALUMINUM

This survey of the toxicological effects of Al in rodents suggests that neurotoxicological and developmental (including neurodevelopmental) endpoints are among the most sensitive indicators of Al toxicity. However, as vehicles for the development of toxicity values such as a provisional chronic RfD, the latter group of studies are considered to be more appropriate, since the level of exposure to Al appears to be better characterized. In fact, neurobehavioral deficits have been observed in mice and rats exposed during various stages of development and in subchronic studies (Bernuzzi et al., 1989; Donald et al., 1989; Golub et al., 1989, 1992a, b, 1995; Muller et al., 1990), as described above. These deficits include impaired operant learning, changes in grip strength, altered startle response and impaired motor coordination. In addition, several studies have shown that oral Al can produce histopathological changes in the CNS, although the histopathological lesions have yet to be causally related to the neurobehavioral deficits. Thus, Florence et al. (1994) reported histopathological changes in the brain of rats exposed to dietary Al for 6 months, the changes including the appearance of vacuolation of the cell body and cell processes of astrocytes in the brain and swelling of astrocytic processes. In addition, more localized vacuolization of neurons in the brain also was observed. These changes

were observed in rats exposed to elevated Al in the diet and are distinct from the NFD that has been observed in rats, rabbits and monkeys maintained on elevated dietary Al in combination with reduced dietary calcium (Garruto et al., 1989; Kihira et al., 1994; Mitani, 1992; Yano et al., 1989; Yoshida et al., 1990) or in rabbits administered intracisternal or intraventricular injections of Al (Kowall et al., 1989; Wakayama et al., 1993). Interpretation of the low-calcium studies is complicated by the observation that NFD was observed in animals maintained on low-calcium diets without excess Al and was enhanced by the addition of excess Al to these diets (Garruto et al., 1989; Kihira et al., 1994). Furthermore, Al has been shown to inhibit the gastrointestinal absorption of calcium (Orihuela et al., 1996), an effect that may exacerbate the calcium deprivation induced by low calcium diets. Thus, it is not clear whether calcium deprivation enhances the neurotoxicity of Al or Al exacerbates the adverse effects of calcium deprivation.

Donald et al. (1989) and Golub et al. (1995) are co-principal studies that identify a LOAEL of 100 mg Al/kg-day for minimal neurotoxicity in the offspring of mice exposed to dietary aluminum lactate (soluble aluminum) during gestation and lactation. The neurotoxicity associated with this LOAEL is consistent with LOAELs from other developmental and subchronic neurobehavioral studies in mice and rats which used higher dietary dosages of aluminum lactate or aluminum chloride (Golub et al., 1989, 1992a,b; Bernuzzi et al., 1989; Muller et al., 1990). Of the above, Golub et al., (1995) is the only study in which a histopathological examination of the brain and spinal cord was conducted and no abnormalities were reported. The Florence et al. (1994) study indicates that histopathological abnormalities of the CNS can occur in rats exposed subchronically to 84 mg/kg-day; although this is lower than the LOAEL for neurobehavioral effects, it was not chosen as the principal study because the functional significance of the histopathological lesions are uncertain.

A number of studies were identified that, at face value, appeared to indicate LOAELs at lower doses than the 100 mg Al/kg-day value selected herein, for example, Paternain et al. (1988) and Colomina et al. (1992). However, in these as in many of the studies under consideration, insufficient information on dietary Al (Al content and/or feed type) was reported to permit a reliable estimation of the overall dose level to which the animals were subjected.

Other developmental studies with aluminum hydroxide and/or citrate in mice and rats identified a NOAEL which are equivalent (95.5 mg Al/kg-day), or a minimum LOAEL that was greater (133 mg Al/kg-day) than the 100 mg Al/kg-day critical LOAEL (Domingo et al., 1989; Gomez et al., 1991), an overlap potentially related to differences in effective doses due to variations in unreported Al dietary content and factors affecting absorption such as chemical form (e.g., the use of less absorbable aluminum hydroxide). In addition, the LOAEL of 43.3 mg Al/kg-day for decreased body weight gain in mice exposed to aluminum chloride for 180-390 days (Ondreicka et al., 1966) was thought be inappropriate for risk assessment due to the small sample size and to the poor reporting of study details. Aluminum nitrate caused alterations in levels of brain biogenic amines and hepatic and hematological indices in rats exposed to 21.4 mg Al/kg-day for 6 weeks (Flora et al., 1991). This dose is not a LOAEL because insufficient information is available to determine if the effects are adverse.

Therefore, the LOAEL of 100 mg Al/kg-day for minimal neurotoxicity in the offspring of mice (Donald et al., 1989, Golub et al., 1995) is selected as the basis for the provisional chronic

RfD. The LOAEL is considered minimal because the results of the postweaning neurobehavioral test battery indicate that performance deficits may be marginal. In particular, of the three observed effects (decreased forelimb and increased hindlimb grip strengths, increased hindlimb foot splay distance), one effect (increased grip strength) has unclear toxicological significance and two effects (increased grip strength and foot splay distance) did not persist after 2 weeks of no further exposure.

Application of an uncertainty factor (UF) of 100 (3 for use of a minimal LOAEL, 10 for interspecies extrapolation and 3 for intrahuman variability where the critical effects have been observed in a sensitive sub-group) results in a provisional RfD of

p-RfD = 1E-0 mg Al/kg-day.

The provisional RfD of **1E-0** mg Al/kg-day is approximately 3-fold higher than estimated normal daily Al intake of approximately 0.2-0.3 mg/kg-day (Iyengar et al., 1987; Ganrot, 1986; Wilhelm et al., 1990). Chronic users of medications such as antacids, buffered aspirins and antiulceratives would be expected to ingest much larger amounts of Al, possibly as high as 10-70 mg/kg-day. However, these subjects would not represent the most sensitive population (developing infants), as indicated by the animal data.

Low confidence is placed in the co-critical studies, because they only identify a LOAEL for a sensitive effect and evaluated comparatively small numbers of animals. Confidence in the data base is low because the most reliable supporting data for neurotoxicity of Al in humans are of limited general relevance (e.g., dialysis encephalopathy is manifested in patients with impaired renal function and excessive Al uptake from intravenous exposure). In fact, neurotoxicity remains to be assessed in animals chronically exposed to Al, and developmental morphology has not been adequately investigated in two animal species. These limitations in the Al data base do not increase uncertainty in the RfD; therefore, a data base uncertainty factor was not used. However, reflecting the low confidence in the co-critical studies, there is low overall confidence in the RfD.

DERIVATION OF A PROVISIONAL CHRONIC RfC FOR ALUMINUM

Al seems to be the most likely cause for the generally and consistently reported psychomotor and cognitive effects (particularly signs of impaired coordination) in Al production workers and welders (Bast-Pettersen et al., 1994; Rifat et al., 1990; Hosovski et al., 1990; White et al., 1992; Hanninen et al., 1994; Sjogren et al., 1990, 1996). In addition, there is strong evidence that Al is neurotoxic by other routes of exposure. Thus, a degenerative neurological syndrome (dialysis dementia) has been documented in humans with chronic renal failure, apparently due to an increased exposure to Al from dialysis treatment and/or ingestion of phosphate binding agents which contain Al (Alfrey, 1993). This syndrome is characterized by gradual loss of motor, speech and cognitive functions. Neurotoxicity, particularly neuromuscular effects such as decreased motor activity, startle responsiveness and grip strength, has also been observed in mice following subchronic oral exposure and in the offspring of mice and rats exposed orally during gestation and/or lactation. Based on this information, as well as evidence

that Al is absorbed by Al production workers and welders, the hypothesis that the occupational studies are indicative of a neurotoxic effect of Al appears to be justified. However, the only occupational study that has yielded suitable monitoring data is that of Hosovski et al. (1990), in which workers were exposed to presumed time-weighted average (TWA) concentrations of 4.6-11.5 mg Al/m^3 magnitude for an average of 12 years. Using 4.6 mg Al/m^3 as the LOAEL for psychomotor and cognitive impairment for an 8-hour occupational exposure (Hosovski et al., 1990) and corrections for discontinuous exposure (10 m^3/20 m^3 and 5 days/7 days), the $LOAEL_{HEC}$ is 1.64 mg/m^3. Applying an uncertainty factor of 300 for intrahuman variability (10), use of a LOAEL (10) and an incomplete database (3) yields a provisional RfC of

p-RfC = 1.64 mg/m^3/300 = 5E-3 mg/m^3.

The lack of inhalation developmental studies may increase uncertainty in the database because oral data in animals indicate that neurotoxic and morphological developmental effects may occur at lower doses than neurotoxicity in adults. Additionally, there is uncertainty related to the lack of corroborating data on air concentrations associated with neurotoxicity. Confidence in the critical study is low to medium because only a LOAEL was identified. Confidence in the database is medium because (1) there are no corroborating data on effect levels (NOAELs and additional LOAELs), (2) no data are available for developmental neurotoxicity by the inhalation route and (3) a well-designed two-generation reproduction study is lacking. Reflecting the low to medium confidence in the critical study and database, there is low to medium confidence in the provisional RfC.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR ALUMINUM

Weight-Of-Evidence Classification

A considerable number of epidemiological studies have examined the incidence of excess tumor formation in persons occupationally exposed to Al in the form of dusts or fumes. In general, a body of inferential evidence exists for an increase in cancer of the bladder and lung through such occupational exposure to Al, although conclusions linking these responses to the effects of Al are confounded by attendant co-exposure to other harmful emissions such as PAHs and by cigarette smoking. A 20-month exposure of B6C3F1 mice to Al potassium sulfate dodecahydrate in the diet at concentrations up to 10% w/w displayed no indication of compound-related carcinogenicity and, in general, no indication of adverse toxicological effects of any kind (Oneda et al., 1994). Similarly, the life-time exposure of Swiss mice and Long-Evans rats to 5 ppm Al as aluminum potassium sulfate in drinking water provided no convincing evidence for the carcinogenicity of Al compounds (Schroeder and Mitchener, 1975a,b). Gene reversion experiments on Al compounds resulted in negative results in *S. typhimurium* (Ahn and Jeffrey, 1994). Taking all of the evidence of Al carcinogenicity together, and in accordance with the U.S. EPA (2005) cancer guidelines, aluminum is classified as *inadequate information to assess carcinogenic potential*. The basis for this classification is insufficient evidence in epidemiological/occupational studies, lack of demonstrated carcinogenicity or mutagenicity in

available animal studies, lack of positive evidence of non-carcinogenicity and lack of mode of action data for aluminum.

Quantitative Estimates of Carcinogenic Risk

Due to insufficient data, a provisional oral slope factor and inhalation unit risk could not be developed.

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Table 1. Summary of oral toxicity data for aluminum[a]

Study	Type	Species	Al	Exposure Concentration (ppm)	Exposure Dosage (mg Al/kg-day)	Exposure Frequency and Duration	Critical Effect	NOAEL (mg Al/kg-day)	LOAEL (mg Al/kg-day)	FEL (mg Al/kg-day)
Ondreicka et al., 1966	Subchronic 3-gen dietary	Dobra Voda mice	chloride	--	27 (control), 46	Continuous, 180-390 days	Decreased body weight gain in F1 and F2.	--	46	--
Golub et al., 1989	Subchronic dietary	S-W mice	lactate	25 (control), 500,1000	3.3 (control), 65,130	Continuous, 6 weeks	Decreased spontaneous motor activity; decreased weight gain.	65	130	--
Golub et al., 1992a	Subchronic dietary	S-W mice	lactate	25 (control), 1000	190	Continuous, 90 days	Decreased hindlimb grip, decreased spontaneous motor activity, decreased startle response.	--	190	--
Florence et al., 1994	Subchronic dietary	Wistar rat	chloride (with citric acid)	1.52 (control), 1000	0.13 (control), 84	Continuous, 6 months	Histopathological changes in brain astrocytes and neurons.	--	84	--
Domingo et al., 1996	Subchronic drinking water	Sprague Dawley rats	nitrate (with citric acid)	--	0, 50, 100 (plus unreported dietary Al)	Continuous, 6.5 months	Operant conditioning and performance	100	--	--
Yoshida et al., 1989	Subchronic dietary	S-W mice	lactate	25 (control), 500, 1000	5 (control), 98, 195	Continuous, 7 weeks	Increased mortality from *L. monocytogenes* inoculation	195	--	--
Donald et al., 1989	Developmental dietary	S-W mice	lactate	25 (control), 500, 1000	5 (control), 100, 200	Continuous, gestation and lactation	Neurobehavioral effects.	--	100	--
Golub et al., 1992b	Developmental dietary	S-W mice	lactate	25 (control), 1000	4 (control), 174	Continuous, gestation and lactation	Neurobehavioral effects.	--	174	--
Golub et al., 1995	Developmental dietary	S-W mice	lactate	7, 500, 1000	1 (control), 100, 200	Continuous, gestation, lactation to maturity	Neurobehavioral effects.	--	100	--

Table 1. Summary of oral toxicity data for aluminum[a]

Study	Type	Species	Al	Exposure Concentration (ppm)	Exposure Dosage (mg Al/kg-day)	Exposure Frequency and Duration	Critical Effect	NOAEL (mg Al/kg-day)	LOAEL (mg Al/kg-day)	FEL (mg Al/kg-day)
Yoshida et al., 1989	Developmental dietary	S-W mice	lactate	25 (control), 500, 1000	4 (control), 100, 200	Continuous, gestation and lactation	Increased mortality of dams from *L. monocytogenes* inoculation	--	100	--

[a]Studies for which total dosages were reported or could be estimated (unless otherwise noted).

Attachment

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United States Environmental Protection Agency

EPA/690/R-08/016F
Final
6-12-2008

Provisional Peer Reviewed Toxicity Values for

Lithium
(CASRN 7439-93-2)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR LITHIUM (CASRN 7439-93-2)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and

circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Lithium (Li), an alkali metal, exists in two isotopic forms (^{7}Li and ^{6}Li) and is naturally present in soil and water. Lithium has numerous industrial and commercial uses including as a cell additive in electrolytic aluminum production, a catalyst of chemical reactors, a component of fluxes and brazing alloys, a component of batteries, specialized glass and ceramics, and a sanitizing agent for swimming pools, hot tubs and spas (Leonard et al., 1995; Moore, 1995). Lithium carbonate and lithium citrate are also used for the therapeutic treatment of psychiatric disorders, primarily in the acute and long-term maintenance treatment of bipolar mood disorders.

A reference dose (RfD) or reference concentration (RfC) for lithium are not available on the Integrated Risk Information System (IRIS) (U.S. EPA, 2007), the Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997), or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2004). The U.S. Environmental Protection Agency's (EPA) Chemical Assessments and Related Activities (CARA) (U.S. EPA, 1994) lists only Reportable Quantity (RQ) documents for lithium chromate and lithium hydride; the RQ documents (U.S. EPA, 1983, 1988) for these two compounds state that the data are not sufficient for derivation of an RQ as there are no subchronic or chronic studies. Neither the Agency for Toxic Substances Disease and Registry (ATSDR) (2006), the National Toxicology Program (NTP) (2006), the International Agency for Research on Cancer (IARC) (2006), nor the World Health Organization (WHO) (2006) has produced documents regarding lithium. The following sources were also consulted: Chemical Hazard Information Profiles (U.S. EPA, 1980), National Occupational Health Survey of Mining (NIOSH, 1990) and Information Profiles on Potential Occupational Hazards - Classes (NIOSH, 1978). Literature searches were conducted from 1965 to August 2006 in TOXLINE

(including NTIS and BIOSIS updates), CANCERLIT, MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS and TSCATS.

REVIEW OF PERTINENT LITERATURE

Human Studies

Oral Exposure

Overview of the Therapeutic Use of Lithium – Lithium carbonate, and more recently, lithium citrate have been used since 1949 in the treatment of bipolar affective (manic-depressive) disorder; thus, extensive clinical literature on the beneficial and adverse effects of lithium is available. Lithium is therapeutically used in the treatment of bipolar affective disorder as a sole therapy or in combination with other antidepressant drugs and in the treatment of schizophrenia in combination with anti-psychotic drugs. Although lithium is effective in the treatment of bipolar affective disorders, adverse effects are associated with therapeutic dose levels, resulting in a low therapeutic index (e.g., ratio of dose associated with therapeutic efficacy to dose associated with adverse effects). Thus, lithium is not simply prescribed by dose, but is monitored based on serum concentrations. For the treatment of bipolar disorder, the desired therapeutic serum concentrations range from 0.6 to 1.4 mmol Li/L, although concentrations of 0.8-1.0 mmol Li/L are generally accepted as providing optimal therapeutic effects (Physicians Desk Reference, 2006; Baldessarini and Tarazi, 2001).

Although the precise mechanism of action has not been established, it is unlikely that a single mechanism of action is responsible for the therapeutic and adverse effects of lithium. Several mechanisms for the therapeutic effects of lithium have been proposed. Since the chemical properties of lithium are similar to those of sodium, lithium can be substituted for sodium in generating action potentials and in some sodium transport processes across membranes. Lithium also appears to alter neurotransmitters, enhances some actions of serotonin, has variable effects on norepinephrine, augments the synthesis of acetylcholine and increases norepinephrine and dopamine turnover. Lithium also alters brain inositol phosphate levels, affecting second messenger responses for α-adrenergic and muscarinic transmission. A decrease in functioning brain protein kinases has also been identified as a consistent effect of lithium. Lithium also interacts with nuclear regulatory factors that affect gene expression (Baldessarini and Tarazi, 2001).

The potential for lithium to cause toxicity has been of significant concern due to its use on a maintenance basis for a lifelong disorder; thus, a large body of clinical literature on lithium-induced toxicity exists, including several reviews (Gitlin, 1999; Berk and Berk, 2003; Markowitz et al., 2000; Moore, 1995; McIntyre et al., 2001; Awad et al., 2002; Presne et al., 2003; Jefferson, 1998). Effects that are associated with therapeutic use of lithium include: neurological and psychiatric effects (tremor, choreoathetosis, motor hyperactivity, ataxia, aphasia, fatigue, cognitive impairment); decreased thyroid function; hyperparathyroidism; renal effects (nephrogenic diabetes insipidus, nephritis, chronic progressive renal disease); edema (related to sodium retention); cardiovascular effects (T wave flattening); acniform skin eruptions;

benign leukocytosis; and gastrointestinal effects (nausea, vomiting, abdominal pain, diarrhea). Since all therapeutic serum concentrations are associated with adverse effects, long-term treatment strategies for individual patients must balance the beneficial effects of lithium therapy with the risks and severity of toxicity. Although available data are not sufficient to define dose-response relationships, it is generally accepted that severity of adverse effects is related to serum lithium levels.

Adverse renal effects associated with lithium therapy have received extensive focus due to their serious nature and frequency of occurrence. Thus, lithium-induced renal toxicity has been the subject of numerous clinical and animal studies. The most common adverse renal effect is nephrogenic diabetes insipidus (NDI), which occurs over the range of therapeutic serum lithium concentrations (e.g., 0.6-1.4 mmol Li/L). The development of NDI involves lithium-induced down-regulation of the vasopressin-regulated water channel aquaporin-2, expressed on the apical plasma membrane of principal cells of the collecting duct (Markowitz et al., 2000). The consequence of this effect is to reduce the capacity of the kidneys to preserve free water, resulting in impaired renal concentrating ability and the production of excessively dilute urine. Clinically, this manifests as polyuria, with secondary thirst, and volume depletion. Although other mechanisms may also contribute to polyuria, interference with vasopressin-induced antidiuresis is considered the most important cause (Gitlin, 1999). It has been estimated that renal concentrating ability is impaired in at least 50% of patients undergoing lithium treatment, with polyuria and polydipsia in approximately 20% of patients (Presne et al., 2003; Gitlin, 1999; McIntyre et al., 2001). In a review of data from several studies published between 1979 and 1986, impairment of concentrating ability was seen in at least 54% of 1105 patients on chronic lithium therapy, with polyuria observed at serum lithium concentrations ranging from 0.6 to 1.2 mmol/L (Boton et al., 1987). Thus, periodic measurement of serum creatinine, creatinine clearance, 24-hour urine volume and urine protein has become integral to the management patients on long-term lithium therapy (Jefferson, 1998).

NDI appears to be reversible early in treatment, but may be progressive during the first decade, leading to irreversible damage over time (Gitlin, 1999). A small percentage of patients show progressive renal failure indicated by a pronounced decrease in glomerular filtration rate (GFR) and renal insufficiency, with little or no proteinuria (Markowitz et al., 2000). Severe decreases in GFR have resulted in the need for maintenance hemodiaylsis, typically after 10 or more years of lithium therapy. Results of renal biopsy on patients with chronic renal effects showed interstitial fibrosis, tubular atrophy, focal sclerosis, acquired renal cystic disease and cytoplasmic swelling with glycogen deposits in the distal convoluted tubules and collecting ducts (Markowitz et al., 2000; Gitlin, 1999). Confounding factors (other medical disorders such as hypertension, heart disease) may contribute to susceptibility and severity of irreversible damage (Gitlin, 1999).

Studies on Adverse Effects in Patients Treated with Lithium: Renal Effects – The results of retrospective and prospective studies and findings of case reports summarized below focus on adverse effects observed in patients maintained on chronic lithium therapy for the treatment of affective mood disorders. As expected based on the established pharmacological profile of therapeutic lithium, decreased renal concentrating ability is the most frequently reported adverse effect, although neurological, dermal, cardiovascular and endocrine effects are

also observed. Interpretation of results from clinical studies is difficult due to many factors, including the lack of baseline data prior to lithium use, absence of control groups, presence of pre-existing renal and other diseases and use of concomitant medications. Furthermore, since clinicians rely upon serum lithium concentrations, rather than daily doses, to evaluate the dose-response relationships between lithium treatment, efficacy and adverse effects, daily lithium doses often are not reported nor were the results of male vs. female dosing reported separately. However, the clinical literature provides consistent evidence that the kidney is a primary target organ for lithium in men and women, and supports that adverse renal effects occur over the range of desired therapeutic serum concentrations (0.6-1.4 mmol Li/L).

In a prospective study, a cohort of 373 patients who started receiving lithium therapy at various times between 1979 and 1987 were given pre-treatment examinations to establish baseline levels for renal parameters (Schou and Vestergaard, 1988; Vestergaard and Schou, 1988). Patients were examined once before and on the average 3.3 times during lithium therapy. Patients who had been treated with lithium prior to entry into the cohort and patients taking neuroleptic agents during lithium therapy were excluded from analysis. On examination days, urine was collected every 24 hours and data were disregarded if less than 75% of the daily lithium dose was recovered. The desamino-8-D-arginine vasopressin (DDAVP) test was used to determine renal concentrating ability. The mean lithium dose was 23.2 mmol Li/day and the mean lithium serum concentration was 0.68 mmol/L. Because of a high drop-out rate (for various reasons), especially among men, and because the dosing durations of the full cohort ranged from 5 months to 7 years, data from a subcohort of 39 patients who received lithium therapy continuously for 4 years were compared with the data for the whole cohort to guard against errors due to selective sample attrition. The ratio of men to women in the whole cohort and in the subcohort remained constant.

Patients in the whole cohort developed a moderate rise in urine volume and a moderate fall in renal concentrating ability (Schou and Vestergaard, 1988; Vestergaard and Schou, 1988). Urine volume increased by 7% (not statistically significant) for the whole cohort and 23% for the subcohort ($p=0.05$). For the whole cohort, urine volume was positively correlated with lithium dosage ($r=0.29$, $p<0.001$). Renal concentrating ability fell by 7% ($p<0.01$) for the whole cohort and by 10% for the 4-year subcohort ($p<0.01$). Changes in renal concentrating ability took place within the first 1-2 years of lithium therapy for members of the whole cohort, with no additional changes in renal function when treatment duration was extended more than 2 years. There was no correlation between concentrating ability and lithium dosage in the whole cohort. Glomerular function, as determined by measurement of serum creatinine concentrations and urine creatinine concentrations, was not affected by lithium therapy in the whole cohort. In addition, there was no change in the incidence of proteinuria associated with lithium treatment. Complaints of increased thirst, frequent urination and nocturia were made more often during lithium therapy than before lithium therapy in both the cohort and the subcohort. Therefore, the results from the subcohort support the results from the entire cohort. Assuming that the average body weight was 70 kg, patients were exposed to 2.32 mg Li/kg-day. This study identifies a lowest-observed-adverse-effect level (LOAEL) of 2.32 mg Li/kg-day for increased urine volume and decreased urine concentrating ability.

A group of 53 patients (20 men and 33 women) was examined prior to starting long-term lithium therapy and again after 4 and 12 months on lithium (Smigan et al., 1984). Twenty-five patients of this cohort had previously received lithium, but treatment had been withdrawn for 27 months before the start of the present treatment. Over the course of the study, 13-28% of the 53 patients received neuroleptics. Lithium carbonate dosages were not provided, but serum lithium levels were maintained at approximately 0.6 mmol/L. A clinically significant change in renal concentrating ability (defined as having a urine osmolality below 600 mOsm/kg water) was observed in a small group (n=6) of patients after 12 months on lithium treatment. Of these six patients, five had been treated previously with lithium and had some signs of impaired renal concentrating ability at the start of the present treatment. Multiple regression analysis determined that concurrent treatment with neuroleptics did not contribute to the decline of renal function.

Polyuria and/or decreased urine concentrating ability were found among 112 women and 125 men (average weight, 76.2 kg) exposed to 12-57 mmol/day of lithium (mean dose of 32.6 mmol/day of lithium or 3.0 mg Li/kg-day) for 0.5-17 years (mean duration, 5.2 years) in a retrospective study by Vestergaard et al. (1979). Serum lithium concentration ranged from 0.2 to 2.0 mmol/L. Baseline renal function prior to lithium therapy was not assessed, since all patients were on maintenance lithium therapy prior to the start of the study. The majority of patients were also receiving concomitant therapy with other medications, such as neuroleptics and/or antidepressants, and 37% the patients received concomitant therapy with hypnotics or anxiolytics. In a follow-up study, 184 of the original 237 patients were re-examined 2 years later (Vestergaard and Amdisen, 1981). The 184 patients were divided into two groups; those patients who continued with lithium (147) and those who discontinued (37). Lithium-treated patients were compared with 68 manic-depressive patients that were about to receive lithium. Glomerular function did not change over the 2-year period. In patients who had discontinued lithium treatment, there was an improvement in renal concentrating ability when compared with the patients who continued with lithium therapy. However, maximal urine osmolality did not reach the level found in the control (pre-lithium treatment) group, although the urine volume approached levels in the control group. There was a further increase in urine volume and decreased in urine osmolarity for those patients that continued with lithium therapy.

In a study involving 116 men and 152 women who took an average of 1322 mg/day of lithium carbonate (3.57 mg Li/kg-day) for an average period of 37.6 months, maximum concentrating ability was lower in all patients receiving lithium than in 59 control patients not receiving lithium (Gelenberg et al., 1987). However, differences did not achieve statistical significance. A major limitation of this study is that baseline data were not available.

Results of a biopsy study in patients receiving lithium maintenance therapy provide evidence of lithium-induced histopathological changes to the kidney (Hestbech et al., 1977). Fourteen manic-depressive patients received an average of 42 mmol Li/day as lithium carbonate (4.2 mg Li/kg-day) for 1.5-15 years. Serum lithium concentrations ranged from 0.6 to 1.3 mmol/L. Thirteen age-matched patients without renal disease served as the source of kidney biopsy specimens for control observations. Impaired urine concentrating ability and polyuria was observed in lithium patients. Histopathological examination of the kidney biopsy samples revealed a pronounced degree of focal nephron atrophy and/or interstitial fibrosis in 13 of the

14 patients examined. Semiquantitative assessment of renal lesions revealed significantly greater degrees of focal cortical fibrosis, diffuse medullary fibrosis, mononuclear cell infiltrates and distal tubular dilatation in the patients than in the controls. Quantitative assessment revealed significantly greater percentages of totally sclerotic glomeruli, fibrous cortical tissue and unidentifiable and atrophic renal tubules in patients than in controls.

Hansen et al. (1979) also reported impaired renal concentrating ability in 14 patients (7 men and 7 women) treated with 36 mmol/day of lithium (3.6 mg Li/kg-day) for 1.3 to 12 years. Serum lithium concentrations ranged from 1.75 to 4.50 mmol/L. Results of renal biopsy showed interstitial fibrosis and tubular atrophy. Baseline levels were not available and controls were not used. There was a significant negative correlation between the degree of tubular atrophy and renal concentrating ability.

Walker et al. (1982) examined renal function and biopsy samples in 47 patients (18 men and 29 women) who were receiving an average of 1250 mg/day of lithium carbonate (3.38 mg Li/kg-day) for an average of 5 years. The median serum lithium concentration was 0.84 mmol/L. Thirty-two patients not receiving lithium therapy were used as the controls. Decreased urine concentrating ability and impaired urinary acidification, indicative of distal nephron dysfunction, were observed in patients receiving lithium relative to controls. Lithium-treated patients also exhibited decreased glomerular filtration rate, as measured by significantly increased serum creatinine, increased β_2-microglobulins, and decreased Cr-EDTA clearance, compared to controls. Histological examination of kidney biopsy samples did not reveal abnormalities.

Hansen and Amdisen (1978) reported effects on the kidneys in a case study of 23 patients who were exposed to therapeutic doses of 24-56 mmol Li/day (2.4-5.6 mg Li/kg-day) for 6.1-8.5 years. Patients were hospitalized due to severe lithium intoxication. Pre-exposure baseline levels were not available. Impaired renal concentrating ability was a consistent finding. Abnormal electroencephalography (EEG) was also reported. There was no relationship between the severity of symptoms of lithium intoxication and the serum lithium concentration on admission to the hospital. Many of the patients (22 out of 23) included in the Hansen and Amdisen (1978) study experienced frank adverse effects, including renal insufficiency in 17 patients, mental and neurological symptoms (decreased alertness or slight apathy) in18 patients, muscular rigidity and/or muscular fasciculations in 14 patients, slight ataxia in 6 patients, and stupor and latent convulsive movements in 14 patients. Severely abnormal electroencephalograms were observed in 19 patients. Two patients died and two patients developed persisting neurological sequelae.

Studies on Adverse Effects in Patients Treated with Lithium: Other (Non-Renal) Adverse Effects – Neurological effects, including tremors, are commonly reported in patients treated with lithium. Neurological effects of lithium were evaluated in 28 patients (15 men and 13 women) with bipolar affective disorder receiving 1012 mg/day of lithium carbonate (2.74 mg/kg-day) for 4.1 years. The mean serum lithium level was 0.68 mmol/L. Although patients did not develop overt neurological effects, nerve conduction velocities were prolonged (Chang et al., 1990). Electrodiagnostic tests revealed a slowing of motor and sensory nerve conduction velocities and prolonged central neural conduction times obtained from somatosensory and brainstem auditory evoked potentials that correlated with serum lithium levels. Another patient

developed polyneuropathy after being exposed to 1.62 mg Li/kg-day for an unspecified period (years) (Tomasina et al., 1990). The patient had a sensorimotor peripheral neuropathy with mostly axonal degeneration. The patient improved after the lithium therapy was discontinued. Levine and Puchalski (1990) have also described two cases of pseudotumor cerebri syndrome in patients that were exposed to 900-1200 mg/day of lithium carbonate (2.43-3.24 mg Li/kg-day) for 4-8 years. The serum lithium concentration did not exceed 1.0 mmol/L. This syndrome is characterized by chronic headaches, bilateral papilledema and increased intracranial pressure in the absence of any localized neurological signs or symptoms.

Hagino et al. (1995) observed adverse effects on 20 children aged four through six exposed to oral lithium for the treatment of aggressive and/or mood-disordered children. All children were hospitalized during the course of the study as part of the medical intervention program. Daily lithium doses were adjusted to maintain serum lithium concentrations between 0.6 and 1.2 mmol/L and ranged from 12.2 to 48.9 mg/kg-day. Patients remained on lithium therapy for up to 37 days. Adverse effects to the central nervous system (tremor, drowsiness, ataxia, confusion) were the most commonly observed effects, reported in approximately 60% of patients. Other effects observed included gastrointestinal effects (nausea, vomiting, abdominal discomfort) in 25% of patients, renal effects (polyuria) in 10% of patients and blurred vision in 10% of patients. No adverse effects were observed in the cardiovascular, pulmonary, autonomic, hematological or integumental systems. The potential contribution of concomitant medications was not ruled out by the study authors. Sixteen of the 20 children also received one or more psychoactive medications and six children received antibiotics for infections.

Adverse effects on thyroid function, primarily asymptomatic hypothyroidism, have been reported in patients treated with lithium. Thyroid effects may be secondary to altered renal clearance of iodine, rather than to direct effects of lithium on the thyroid (Moore, 1995). A retrospective study was conducted involving 129 patients (46 men, 83 women) who were on lithium therapy for 2-180 months and 21 patients who served as controls (Bocchetta et al., 1991). Most of the patients receiving lithium had previously received or were receiving medication (antipsychotics and antidepressants) other than lithium. Serum lithium concentrations ranged between 0.5 and 1.0 mmol/L. Palpable and/or visible goiter was found in 51% of the patients receiving lithium ($p<0.01$), compared with 9.5% occurrence in the control group. Based on elevated thyroid stimulating hormone (TSH) levels, subclinical hypothyroidism was diagnosed in 19% of the patients on lithium compared with 9.5% in the control group. There were no differences in thyroid function tests between patients receiving lithium alone or receiving additional medication. The researchers noted that lithium-induced subclinical hypothyroidism may be transient and recommended that repeated determinations of TSH is required. Joffe et al. (1988) reported that 20% of the 42 patients receiving lithium carbonate therapy for 3 months required thyroid replacement or had evidence of subclinical hypothyroidism. Cowdry et al. (1983) also reported that 12 of 24 patients who were on lithium therapy for 12 months developed hypothyroidism. Only those patients with a median serum lithium concentration of 0.6 mmol/L were used in the study. When 22 women received lithium therapeutically for more than 2 years, there was evidence of subclinical hypothyroidism in 32% of these patients (Bartalena et al., 1990).

Hyperparathyroidism with progressive hypercalcemia was reported to occur in a patient who received lithium for 6 years (Graze, 1981). The daily dosage was not reported by the author. The patient had progressive hypercalcemia for the duration of the therapy and a parathyroid adenoma.

Raoof et al. (1989) demonstrated a concentration-dependent inhibition by lithium chloride on human sperm motility *in vitro* at semen concentrations that would be expected from therapeutic doses of lithium. However, Raboch et al. (1981) found no abnormality in sperm count, motility or morphology in semen samples obtained from 14 patients that were using lithium for an average of 4.1 years. The mean serum lithium level was 0.64 mmol/L and the mean lithium level in the semen was 1.48 mmol/L.

A group of 16 men and 4 women were treated with 1008 mg/day of lithium carbonate (2.72 mg Li/kg-day) as either a once-daily dose or as a divided twice-daily dose for an average of 4.4 years (Abraham et al., 1992). An elevated white cell count, increased serum phosphate, and elevated serum ionized calcium were observed in the study group receiving the once-daily lithium treatment. These effects were not seen in the study group that received lithium twice a day. No evidence of polyuria was observed in either group and no significant differences were observed between the two treatment regimens with respect to mental status, serum lithium or electrocardiograms.

Adverse effects on cardiac conductivity, including sinoatrial block, sinus bradycardia and junctional escape rhythm, have been reported in patients taking therapeutic lithium (Moore, 1995). A patient who received 600 mg/day of lithium carbonate (1.62 mg Li/kg-day) for 4 months (serum lithium concentration 1.3-2.0 mmol/L) developed symptomatic sinus node dysfunction, which disappeared after discontinuation of lithium therapy (Riccioni et al., 1983). Roose et al. (1979) also reported cardiac sinus node dysfunction during lithium treatment in several patients exposed to at least 8.6 mg/kg-day of lithium for 10 years.

Studies on Developmental Effects of Lithium Treatment During Pregnancy – The potential for lithium-induced developmental effects was the subject of an assessment conducted by Moore and an Institute for Evaluating Health Risks (IEHR) Expert Scientific Committee (Moore, 1995). Data from 139 references, including registries, prospective studies and case histories were reviewed. This assessment determined that sufficient evidence is available to conclude that therapeutic use of lithium causes developmental effects in offspring when maternal serum lithium concentrations are within the therapeutic range. Review of developmental effects reported in birth registries revealed reports of cardiovascular defects associated with lithium treatment. Reports of Ebstein's anomaly (a structural defect in which there is a downward displacement of the trisucpid valve into the right ventricle, and valvular redundancy with adherence of some cusps to the right ventricular wall; affected individuals may have right ventricular failure or conduction abnormalities), in particular, were in "substantial excess among all malformations." Although the magnitude of the increase could not be determined from birth registry reports, data indicate that first-trimester lithium exposure increases the risk of cardiac malformations. Other studies reviewed by Moore (1995) also report an association between maternal lithium treatment and cardiovascular defects in offspring. The literature reviewed also suggests a possible association between maternal lithium treatment and neonatal mortality.

There are also reports that newborn infants of mothers on lithium therapy may exhibit symptoms of acute lithium toxicity such as cyanosis, hypotonia and cardiac toxicity. However, the available data regarding developmental effects in humans are limited by insufficient dose-response information.

Jacobson et al. (1992) prospectively recruited and followed 148 women using lithium during the first trimester of pregnancy. Each study patient was matched with a woman (control) of similar age (within 2 years). The mean lithium dose was 927 mg/day of lithium carbonate (2.5 mg Li/kg-day); the authors did not report serum lithium concentrations. No significant differences between the exposed group and the controls were observed for congenital defects (3% in lithium patients and 2% in controls) and spontaneous abortions (9% in lithium patients and 8% in controls). Kallen and Tandberg (1983) identified a cohort of 350 mothers who were treated with lithium during their pregnancy. The authors reported that the total delivery outcome was poorer than expected, with high perinatal death and malformation rates compared to the national average expected rates in Sweden. Congenital heart defects occurred in 6 cases compared with the national expected number of 2.1 cases ($p<0.05$). However, the sample size was relatively small and the difference between delivery outcome in women on lithium and in women on other psychotropic drugs was not statistically significant. Weinstein and Goldfield (1975) reviewed 143 cases of lithium use during pregnancy collected by the Register of Lithium Babies. There were 13 malformed infants (9.1%) among the 143 in the register. Of these 13 malformed infants, 11 were born with significant malformations of the cardiovascular system. As was the case with the previous study, the daily lithium dosage was not reported and at least 6 of the 13 mothers who delivered malformed babies were exposed to other medications in addition to lithium. Krause et al. (1990) reported a case of severe polyhydramnios that developed from the 26th week of gestation. Except for weeks 6-13 of gestation, the mother was maintained on lithium (serum level, 0.7 mmol/L) prior to the diagnosis. Ang et al. (1990) described a similar case report in a woman who was exposed to lithium during pregnancy. The infant displayed symptoms of lithium toxicity, including polyuria.

Studies on the Carcinogenic Potential of Therapeutic Lithium – Controlled studies on the potential of therapeutic lithium to induce cancer have not been reported. Although a few case studies have reported associations between lithium therapy and recurrence of cancer, data are inadequate to establish any association between lithium and the development or recurrence of cancer in humans. Furthermore, given that the widespread clinical use of lithium as a long-term maintenance treatment in patients with affective mood disorders has not revealed an increased incidence or recurrence of cancer, it is unlikely that lithium is carcinogenic in humans. Nonetheless, the few studies examining potential carcinogenic effects of lithium are briefly reviewed below.

Several case reports that suggest an association of lithium-induced leukocytosis with induction or reinduction of acute and chronic leukemia. Orr and McKerna (1979) reported the recurrence of acute monocytic leukemia in a 64-year-old woman who was previously in remission. This patient received 600 mg/day of lithium carbonate for 7 weeks before the leukemic relapse occurred. Nielsen (1980) reported the development of acute myeloid leukemia in one male and one female patient administered lithium for a duration of 1 and 12 years, respectively. Jim (1980) reported the occurrence of chronic monocytic leukemia in a patient

who received 900 mg/day of lithium carbonate for 11 months prior to the diagnosis of leukemia. A 37-year-old woman developed chronic granulocytic leukemia after receiving 600 mg of lithium carbonate 3 times/day for 5 years (Schottlander et al., 1980).

Contrary to anecdotal reports that attempt to associate an increased risk of leukemia with lithium intake, the limited epidemiological information suggests no increased risk. Resek and Olivieri (1983) examined the relationship between leukemia and chronic lithium therapy during 1971-1980 by examining hospital records of 187 leukemia patients to determine whether these patients were receiving lithium medication prior to the their illness. Only 7% of these patients had received psychiatric services and in all cases, these patients were not treated with lithium. The authors reported that there was no association between lithium therapy and leukemia. In a 14-year ecological study, one human population in El Paso exposed to lithium via drinking water (66 μg/L) was compared with another human population in Dallas-Fort Worth that was not exposed to lithium in drinking water (Frenkel and Herbert, 1974). There was no difference in the incidence of chronic or acute granulocytic leukemia in the two populations.

Only two separate cases have been reported in the literature of the possible association of lithium with cancers other than leukemia. Brownlie et al. (1980) reported the occurrence of papillary cell carcinoma of the thyroid in a 55-year-old woman after 3.5 years of lithium therapy. McHenry et al. (1990) also reported three cases of thyroid carcinoma occurring in association with chronic (9 years) lithium therapy.

Studies of Adverse Effects of Lithium in Healthy Volunteers – The effect of lithium therapy on short- and long-term memory was assessed in healthy volunteers exposed to daily oral lithium for 3 weeks (Stip et al., 2000). Groups of 15 healthy men and women were randomized into placebo or lithium treatment groups. Subjects in the lithium group were administered lithium twice daily at doses ranging from 1050 to 1950 mg/day (197 to 366 mg Li/day) in order to achieve a mean serum lithium concentration of 0.8 mmol/L. The form of the lithium was unstated but the dose is consistent with lithium carbonate. Actual serum lithium concentrations were not reported. Cognitive performance (attention and memory) was assessed in each subject at 3 times during the study: baseline, after 3 weeks of treatment and 2 weeks after discontinuation of treatment. After 3 weeks of treatment, performance scores for short-term memory tasks (assessed using an auditory digit span) for subjects taking lithium were significantly lower ($p<0.03$) compared to placebo. Results of long-term memory assessments (using recall tests) showed adverse effects in lithium-treated subjects compared to controls. Performance on short- and long-term memory tests improved 2 weeks after discontinuation of treatment. Results indicate that lithium produces effects in the central nervous system in healthy subjects at exposure levels corresponding to the target therapeutic serum concentrations. The mean dose, 1569 mg/day (295 mg Li/day) was a LOAEL in this study.

Effects on the hemopoeitic system and clotting have also been reported in healthy volunteers exposed to lithium (Stein et al., 1981). Groups of at least five non-psychiatric volunteers received 900 mg/day lithium carbonate (2.43 mg Li/kg-day). Granulocyte count, expressed as a percent of baseline, was significantly increased by 25% ($p<0.05$), 32% ($p<0.001$), and 42% ($p<0.001$) after 1, 2 and 3 weeks of exposure. Volunteers administered 0, 300, 600, 900, 1200 or 1500 mg/day of lithium carbonate (0, 0.8, 1.62, 2.43, 3.24 or 4.05 mg Li/kg-day)

orally for 1 week developed increased granulocytosis at doses ≥ 2.43 mg Li/kg-day. Granulocyte count was increased by 26, 55 and 43% of baseline values in the 2.43, 3.24 and 4.05 mg Li/kg-day groups, respectively. Decreased bleeding times were also observed in the 3.24 and 4.05 mg Li/kg-day dose groups, although there was no apparent treatment effect on platelet count. Serum lithium concentrations were not reported.

The use of lithium as a therapeutic agent to reverse chemotherapy-induced neutropenia and thrombocytopenia has been explored in humans, with studies providing conflicting results. Richmon et al. (1984) reported an increase in neutrophil and thrombocyte production in five cancer patients who received 900 mg/day of lithium carbonate (169 mg Li/day) for an unspecified duration. Twenty-two patients with oligoblastic leukemia receiving 900 mg/day of lithium carbonate for an unspecified time remained cytopenic without evidence of lithium-induced bone marrow proliferation (Barlogie et al., 1984). In another study, Friedenberg and Marx (1980) reported that lithium increased the granulocyte count in eight healthy volunteers who had received 900 mg/day of lithium carbonate for 1 week. Despite the observed increase in granulocyte number, there was a reduction in bactericidal capacity (function) of granulocytes in these individuals.

Inhalation Exposure

No studies on the effects of inhaled lithium in humans were identified.

Animal Studies

Oral Exposure

Subchronic and chronic oral exposure studies evaluating comprehensive toxicity endpoints in laboratory animals are not available. Few animal studies have investigated the adverse effects of chronic oral exposure to lithium. The primary purpose of animal studies has been to evaluate specific adverse effects associated with the therapeutic serum lithium concentration range, with most studies focusing on lithium-induced renal toxicity. The available data in animals provide supporting evidence that subchronic and chronic oral exposure to lithium induces similar adverse effects as those associated with the therapeutic use of lithium in patients. However, insufficient data are available to determine dose-response relationships for adverse effects.

Cancer Bioassays – No long-term animal bioassays examining the carcinogenicity of lithium were identified. An abstract by Prolov and Pliss (1991) reported that lithium carbonate promoted bladder carcinogenesis in rats previously exposed to N-buty-N-(4-hydroxybutyl)-nitrosamine; no additional publications of this finding were identified. Although Hori and Oka (1979) stimulated cell multiplication of mammary gland explants with lithium in C3H/HeN virgin female mice, Ziche et al. (1980) were unable to demonstrate any growth promoting effect of lithium on primary carcinomas induced by two chemical carcinogens (7,12-dimethylbenz[α]anthracene and N-nitrosomethylurea) in Sprague-Dawley and Buffalo/N female rats.

Adverse Renal Effects – Chronic renal failure was induced in male and female Wistar rats fed diets containing 0 or 40 mmol lithium chloride/kg diet (0 or 3.58 mg Li/kg-day) from birth for 55 to 56 weeks (Christensen and Ottosen, 1986). Plasma lithium concentration ranged from 0.6 to 0.7 mmol/L after 16 weeks and from 1.0 to 1.1 mmol/L after 48 weeks of treatment. Mortality was 51% in lithium-treated rats compared to only 6% in control rats. Mean plasma urea concentration was elevated by 74% after 16 weeks and 175% after 48 weeks, compared to controls. After 55 weeks of treatment, inulin clearance was reduced by 62% and lithium clearance was reduced by 39% compared to controls. Lithium-treated rats also had polyuria and diminished renal concentrating ability (assessed by failure to respond to exogenous vasopressin). No treatment-related effects on systolic or diastolic blood pressure were observed. Morphological examination of the kidneys of lithium-treated rats revealed large cortical cysts, dilated distal tubules and collecting ducts, and widespread interstitial fibrosis. Glomerular volume and proximal tubular mass were significantly reduced. Comprehensive toxicity endpoints were not examined in this study. A LOAEL of 3.58 mg Li/kg-day for adverse renal effects was identified; a NOAEL was not established.

Two groups of six male Wistar SPF rats were exposed to a diet containing 0 or 40 mmol LiCl/kg diet (0 or 4.5 mg Li/kg-day) for 3 weeks and then 0 or 60 mmol LiCl/kg (0 or 6.7 mg Li/kg-day) for an additional 18 weeks (Christensen et al., 1982). The time-weighted average daily dose was 6.4 mg Li/kg-day, with mean serum lithium concentrations of 0.7-0.8 mmol/L. Rats exposed to lithium developed polyuria and lowered renal concentrating ability after 2 weeks of exposure to diets containing lithium. Focal microscopic changes in distal convoluted tubules and collecting ducts were also observed. Focal basal vacuolization of the cytoplasm was observed after 2-4 weeks of lithium exposure. After 8 weeks of treatment, all rats had severe nuclear polymorphism, nuclear hyperchromasia and cellular polymorphism with tubular giant cells. In addition to these cellular changes, dilatations of the tubular lumen and focal atrophy of the tubular cells were observed in rats exposed to lithium for 21 weeks. Renal concentrating ability was significantly decreased after 2 weeks of dietary exposure. After lithium was withdrawn for 8 weeks, structural changes persisted, but concentrating ability was normalized. Based on their experimental findings, the authors concluded that the use of urinary concentrating ability as an index of lithium-induced structural damage may underestimate lithium-induced effects on the kidney. A LOAEL of 4.5 mg Li/kg-day for adverse renal effects observed after 2 weeks of treatment was identified; a NOAEL was not established.

Polyuria and vasopressin-resistant diabetes insipidus developed within 3 weeks of exposure to dietary lithium (Kling et al., 1984). Two groups of 12 male Wistar rats were exposed to 0 or 90 mmol/kg diet of lithium carbonate (11.6 mg Li/kg-day) for 126 days. Serum lithium concentration was maintained at 0.8 mmol/L. Early lesions were associated with the cortical collecting tubules and distal tubules, extending into the medullary collecting tubules by week 3 of treatment. There were alterations in nuclear size and shape and cytoplasmic basophilia of tubular cells, focal dilation and thinning of the tubular epithelium, and occasional sloughing of cells into the tubular lumen. In this study, early tubular lesions correlated with the polyuria. However, polyuria remained constant while morphological changes deteriorated for several more weeks of lithium exposure. A LOAEL of 11.6 mg Li/kg-day for adverse renal effects was identified; a NOAEL was not established.

Two groups of seven male Wistar rats were exposed to a control diets or a diet containing lithium for 112 days (Marcussen et al., 1969). Due to poor reporting of methods and results, the concentration of lithium in the diet or daily dose of lithium could not be determined. Rats exposed to lithium developed uremia and reduced body weight. All kidneys were polycystic in the cortical areas, and distal tubules and cortical collecting ducts were dilated. Severe fibrosis was observed in the interstitial space. Tubular glomeruli (67%) and some hypertrophic glomeruli were also observed. The hypertrophic glomeruli did not compensate adequately for other impaired glomerular function, as indicated by an overall decrease in GFR.

Other (Non-Renal) Adverse Effects – Reductions in body weights were observed in rats exposed to dietary lithium (Ehlers and Koob, 1985). Twenty-nine male Wistar rats were exposed to 0, 30 or 40 mmol Li/kg diet (0, 19 or 26 mg Li/kg-day) for 56 days. In the 40 mmol Li/kg diet group, body weight was reduced by 37% ($p<0.001$). A significant increase ($p<0.05$) in brain theta wave activity in the 6-8 Hz range in all lithium-treated animals was also observed. A LOAEL of 19 mg Li/kg-day for adverse effects to the central nervous system was identified; a NOAEL was not established.

The effect of dietary lithium on thyroid function was examined in two groups of 10 male Wistar rats exposed to 0 or 1100 mg lithium carbonate/kg diet (0 or 20 mg Li/kg-day) for 120 days (Dhawan et al., 1985). Serum lithium levels ranged from 0.44 to 0.65 mmol/L. There was a significant decrease ($p<0.01$) in circulating levels of T_4 and T_3 after 1 month of exposure to lithium. There was also a marked decrease ($p<0.001$) in thyroid hormone levels after 4 months of lithium treatment. A LOAEL of 20 mg Li/kg-day for adverse thyroid effects was identified; a NOAEL was not established. Etling et al. (1987) investigated the effect of lithium on thyroid hormone levels in rats exposed to 0, 300 or 600 mg lithium carbonate/L in drinking water (0, 12.8 or 25.6 mg Li/kg-day) for 5 weeks. Decreases in serum T_3 and T_4 were observed only in the 600 mg/L group. A NOAEL and LOAEL of 12.8 and 25.6 mg Li/kg-day, respectively, for adverse thyroid effects were identified.

The effect of 90-day dietary exposure to lithium on male reproductive organs was evaluated by Thakur et al. (2003). Groups of 20 sexually mature Wistar rats were exposed to lithium carbonate at dietary concentrations of 0, 500, 800 or 1100 mg/kg diet for 90 days (equivalent to 0, 6.6, 10.6 or 14.2 mg Li/kg body weight-day). Serum lithium concentrations were not reported. Assessments included weight of reproductive organs, histopathology of testis, epididymis, seminal vesicle, and prostate, testicular interstitial fluid volume, testosterone level, sperm morphology and fertility index. Weights of the testes and epididymis, sperm number from cauda epididymis, daily sperm production, serum testosterone and interstitial fluid volume were significantly reduced in the mid- and high-dose groups, compared to controls. Seminal vesicle and prostate secretions were completely blocked in the mid- and high-dose groups. The percentage of abnormal sperm was significantly increased in all lithium treatment groups. Histopathological assessments revealed degeneration of spermatogenic cells and vacuolization of Sertoli cell cytoplasm in the high-dose treatment groups. A LOAEL of 6.6 mg Li/kg body weight-day for increased percentage of abnormal sperm was identified; a NOAEL was not established.

Sharma and Iqbal (2005) evaluated the effects of oral exposure of male Wistar rats to lithium nitrate for 7 weeks. Groups of 12 rats were exposed to 0 or 20 mg Li/kg body weight by gavage on alternate days (10 mg Li/kg-day) for 7 weeks and examined for effects on blood chemistry and hematology at the end of the treatment period. Serum lithium concentration was not reported. Numerous blood chemistry and hematology parameters were significantly different from controls: decreased hemoglobin and erythrocyte count, elevated white cell count, elevated erythrocyte sedimentation rate, elevated glucose, decreased protein and elevated blood urea nitrogen (BUN), calcium and phosphorous. Histopathological effects observed in the kidney included ruptured epithelial lining of the proximal and distal tubules of the medulla, renal tubular necrosis, thickened capsular wall of the glomerulus and cytoplasmic vacuolization in the corticomedullary region. Comprehensive toxicity endpoints were not examined in this study. A LOAEL of 10 mg Li/kg body weight for hematological and renal effects was identified; a NOAEL was not established.

Developmental Effects – The review by Moore and an IEHR Expert Scientific Committee (Moore, 1995) on lithium-induced developmental effects included available data from studies in animals using a variety of experimental designs. The data in animals are of limited usefulness to providing a comprehensive picture of lithium-induced developmental effects because of limitations of available studies. Issues with some of the studies include small number of animals, inability to ascertain litter incidence, inadequate reporting, administration of only a single dose, and failure to report or describe chemical characteristics of test materials. Despite these limitations, sufficient data are available to suggest that prenatal developmental toxicity can occur in studies with rats and mice in which lithium is administered during pregnancy and fetuses are examined just before birth. Doses associated with adverse developmental effects ranged from 2.71 to 12.67 mmol/kg body weight-day. Evidence of maternal toxicity was often present. Specific cardiac developmental effects have not been reported in animal studies; however, it does not appear that rigorous assessments of cardiac morphology have been conducted. Results of selected animal developmental studies are briefly summarized below.

Hoberman et al. (1990) evaluated the developmental effects of lithium hypochlorite administered by gavage once daily to groups of 25 Sprague-Dawley rats on days 6 through 15 of gestation at dosages 0 (vehicle), 10, 50, 100 or 500 mg/kg-day (0, 0.4, 2.1, 4.2 or 21 mg Li/kg-day). Six of the 25 rats in the 500 mg/kg-day group died between days 12 and 20 of gestation. Decreased fetal body weight, wavy ribs and delayed ossification of the thoracic vertebrae (bifid centra), forepaw and hindpaw phalanges, and metatarsal and metacarpal bones were observed in the offspring of the highest exposure group. Maternal NOAEL and the developmental NOAEL for lithium hypochlorite were determined by the authors to be 100 mg/kg-day of lithium hypochlorite (4.2 mg Li/kg-day). The LOAEL for developmental toxicity is 500 mg/kg-day (21 mg Li/kg-day) and the maternal frank effect level (FEL) is 500 mg/kg-day (21 mg/kg-day).

Twenty albino Sprague-Dawley rats were exposed to 0 or 100 mg/kg-day (0 or 18.8 mg Li/kg-day) lithium carbonate on days 16 through 20 of gestation (Fritz, 1988). Signs of maternal toxicity, including reduced weight gain and feed consumption, polyuria and polydipsia, were observed. Enlarged renal pelvises were observed in 50% of the fetuses in the lithium-exposed

group. Exposure of rats to a lower daily dose of lithium carbonate (11.3 mg Li/kg-day) on gestational days 16 through 20 induced similar maternal effects and some prenatal mortality; however, no signs of impaired renal development for the young offspring that survived were observed. A LOAEL of 11.3 mg Li/kg body weight for maternal effects was identified; a NOAEL was not established. For fetal effects, NOAEL and LOAEL values were identified as 11.3 and 18.8 mg Li/kg-day, respectively.

Developmental toxicity was evaluated in the offspring of 44 pregnant Wistar rats exposed to 0, 50 or 100 mg/kg-day of lithium carbonate (equivalent to 0, 9.5 or 19 mg Li/kg-day) by oral gavage on gestational days 6 through 15 (Marethe and Thomas, 1986). Reduction in the number of implantations, number of live fetuses and fetal body weights and a higher number of resorptions were reported in the 100 mg/kg-day group. A developmental NOAEL for lithium carbonate is 50 mg/kg-day (9.5 mg Li/kg-day) and a LOAEL of 100 mg/kg-day (19 mg Li/kg-day) was determined.

Statistically significant reductions in total body weights of the fetus and the fetal length were observed in the offspring of albino rats exposed to 7 mg/kg-day of lithium carbonate (1.3 mg Li/kg-day) for the first 10 days of gestation (Sharma and Rawat, 1986). The authors did not report the number of animals used in the study, but rather the number of abnormal developmental observations and expressed these as a percentage of control. The authors also reported a high incidence of cleft palate abnormalities (46%), fetal brain liquification (46%), hepatomegaly (46%) and non-ossification of upper and lower digits (30 and 37%, respectively). Lower incidences of cardiomegaly (3%) and hydronephrosis (3%) were observed. A LOAEL of 1.3 mg Li/kg body weight for adverse developmental effects was identified; a NOAEL was not established.

Groups of 12 female Sprague-Dawley rats were fed 0 or 1000 ppm lithium carbonate (0 or 18.5 mg Li/kg-day) in the diet throughout gestation (Ibrahim and Canolty, 1990). Following parturition, the dams were exposed to the same concentration of lithium and were also allowed to nurse the pups for an additional 21 days. Dietary lithium resulted in decreased growth in both the dams and the offspring, as well as increased mortality of the offspring. Litter size was decreased 25% and mean pup weight was decreased by 10%. The highest mortality was observed in the group of pups that were exposed to lithium during both gestation and lactation. Gross malformations were not observed in the newborn animals. A LOAEL of 18.5 mg Li/kg body weight for adverse developmental effects was identified; a NOAEL was not established.

Groups of three to six mice (HmM/ICR strain) were exposed to 0, 200 or 465 mg/kg-day of lithium carbonate (0, 37.8 or 87.9 mg Li/kg-day) on gestational days 6 through 15 (Szabo, 1970). The human equivalent dose for mice, based on lithium plasma levels of 0.6-1.6 mmol/L, was calculated by the authors as 465 mg/kg-day. The highest dose, 465 mg/kg-day, caused an increased incidence of maternal (37%) and fetal (32%) deaths. Nineteen percent of the surviving fetuses had cleft palate. The 200 mg/kg-day dose (37 mg Li/kg-day) did not cause maternal or fetal deaths; the incidence of cleft palate in fetuses was 0.4%, which was not statistically significantly elevated relative to controls. Cleft palate was not observed in any of the 181 fetuses in the control group. NOAEL and LOAEL values for maternal effects and developmental effects were identified as 37.8 and 87.9 mg Li/kg-day, respectively.

Inhalation Exposure

Only three studies that evaluated the effect of inhaled lithium in animals were identified (Johansson et al., 1988; Greenspan et al., 1986; Rebar et al., 1986). Greenspan et al. (1986) exposed groups of eight male and eight female F344/Lov rats to an aerosol mixture containing lithium carbonate (80%) and lithium hydroxide (20%) at concentrations of 0, 620, 1400, 2300 or 2600 mg Li/m^3 once for 4 hours (Greenspan et al., 1986). The 14-day LC_{50} values were estimated to be 1700 mg Li/m^3 for the males and 2000 mg Li/m^3 for the females from the single exposure. No clinical signs of toxicity were observed in animals exposed to 620 mg Li/m^3. At concentrations $\geq$ 1400 mg/m^3, signs of acute effects on the respiratory system included respiratory distress and bronchospasms. At necropsy, severe congestion of the lungs was observed in 14 out of the 16 animals in the two highest exposure groups. Three of 16 animals at 1400 mg Li/m^3 showed congestion in the lungs. Histopathologic lesions in the respiratory tract were seen in the $\geq$ 1400 mg/m^3 groups, and were found only in animals dying within 12 days of exposure. Lesions were characterized as necrotizing rhinitis, necrotizing laryngitis and secondary suppurative bronchiolitis and bronchopneumonia. There was congestion of the thymus and tracheobronchial lymph nodes in almost half of the animals exposed to lithium aerosols. Similar observations were reported by Rebar et al. (1986) in groups of eight male and eight female rats that were exposed to an aerosol mixture of lithium monoxide and lithium hydroxide at concentrations of 0, 570, 840, 1200 or 1500 mg Li/m^3 for 4 hours. The 14-day LC_{50} value was 940 mg/m^3. In this same study, the 14-day LC_{50} value for a 4-hour exposure to an aerosol containing only lithium hydroxide was 960 mg Li/m^3. Clinical signs and pathological changes were similar to those described in the Greenspan et al. (1986) study. Exposure to the lithium hydroxide/lithium monoxide aerosol mixture at concentrations of 570 Li mg/m^3 and greater resulted in upper respiratory tract and pulmonary lesions.

No adverse respiratory effect were observed in groups of eight male rabbits exposed to aerosols of 0, 0.1 or 0.32 mg Li/m^3 as lithium chloride for 6 hours/day, 5 days/week for 4-8 weeks (Johansson et al., 1988). Inhalation of lithium chloride produced no adverse effects on lung morphology or phospholipid content. The number of alveolar macrophages in lithium-treated animals was not different compared to controls.

Supporting Studies

Toxicokinetic

The clinical pharmacokinetics of lithium has been extensively studied. Reviews and clinical pharmacology text books provide summaries of the pharmacokinetic profile of therapeutic lithium (Ward et al., 1994; Baldessarini and Tarazi, 2001; Potter and Hollister, 2001). The bioavailability of oral lithium preparations ranges from 80 to 100%, although it is generally accepted that the oral bioavailability of lithium is 100%. Peak plasma concentrations are typically reached within 2 hours of administration of lithium. Lithium does not bind to plasma proteins. The volume of distribution of lithium is calculated as 0.66 L/kg. Although lithium is distributed into total body water, lithium distribution is not uniform in all tissue compartments. As an element, lithium is not metabolized and is excreted intact, primarily by the kidney; elimination through sweat, saliva and feces is negligible. Approximately 80% of the filtered load

of lithium is reabsorbed by the kidney and elimination correlates with renal function. Excretion follows first-order kinetics, with an average half-life of 22 hours and an average clearance of 0.35 mL/min-kg (0.5 L/day-kg).

Genotoxicity

Moore (1995) reviewed genotoxicity data as part of their assessment of the developmental effects of lithium and concluded that there is no evidence demonstrating genetic toxicity of lithium in bacterial or *in vitro* mammalian test systems. Garson et al. (1981) reported no increase in the occurrence of chromosome breaks in 23 human subjects under continuous lithium treatment for a period of 1-8 years when compared with 19 healthy age-matched controls. Lithium hypochlorite was not mutagenic in the Ames test nor did it induce DNA damage in the unscheduled DNA synthesis assay using rat primary hepatocytes or increase chromosome aberrations when tested orally in rats at maximally tolerated doses (Weiner et al., 1990).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfD VALUES FOR LITHIUM

The use of lithium as a long-term maintenance therapy in the treatment of bipolar affective disorders has led to an extensive body of literature on the adverse effects associated with oral lithium therapy. Adverse effects, which are observed in several organs and systems, are associated with the entire target therapeutic serum lithium concentration range, leading to treatment strategies based on a risk-benefit assessment for individual patients. The available clinical data identify the lower bound of the therapeutic serum lithium concentration range (0.6 mmol/L) as a LOAEL; the clinical literature does not identify a NOAEL for adverse effects associated with therapeutic lithium. Data reported in humans studies are not sufficient to define the relationship between serum lithium concentrations and the development or severity of adverse effects, although it is generally accepted that the severity of adverse effects is related to serum lithium levels. Given the lack of adequate dose-response data, a single critical effect cannot be identified for lithium. Occupational and environmental oral exposure studies in humans are not available.

Adverse renal effects associated with lithium therapy have received extensive focus due to their serious nature and frequency of occurrence. The most common adverse renal effect is impaired renal concentrating ability and the production of excessively dilute urine. Clinically, this manifests as polyuria, with secondary thirst, and volume depletion. The onset of impaired renal concentrating capacity typically is within the first 2 years of treatment. Although altered renal function appears to be reversible early in treatment, it may be progressive during the first decade of lithium treatment, leading to irreversible damage over time.

Lithium therapy produces side effects in a number of organs and systems other than the kidney. The most frequent neurologic side effects are lethargy, fatigue, weakness, tremor and cognitive impairment. Endocrine glands such as the thyroid and parathyroid can also be affected. Although serious cardiovascular effects are rare, they do occur, the most common

being changes in the EKG. Gastrointestinal side effects include nausea, vomiting, diarrhea and abdominal cramping. The most frequently observed hematological reaction is a benign leukocytosis. Developmental effects, primarily involving the heart, undoubtedly represent the most serious type of unwanted effects.

The available animal data provide supportive evidence that lithium produces adverse effects in several organs and systems at exposure levels that result in serum lithium concentrations in same range as that targeted for therapeutic use in humans. However, available studies do not evaluate comprehensive toxicity endpoints or identify a NOAEL for adverse effects. Thus, although results of toxicity studies in animals are consistent with the adverse effects profile in humans exposed to therapeutic lithium, data are not suitable as the basis for the provisional subchronic and chronic RfD.

The lower bound of the therapeutic serum lithium concentration range of 0.6 mmol/L is selected as the basis for derivation for the provisional RfD and subchronic RfD (p-RfD; p-sRfD). Given that the adverse effects profile of therapeutic lithium is similar for patients on short- and long-term lithium therapy, an RfD based on the LOAEL of 0.6 mmol Li/L is applicable for subchronic and chronic exposures. Based on the pharmacokinetic considerations detailed below, to achieve a serum lithium concentration of 0.6 mmol Li/L, the daily ingestion of lithium by a 70-kg individual is calculated as approximately 1.8 mg Li/kg-day.

At steady state,

$$D = \frac{C_P \cdot Cl}{f}$$

where D is the dose (mg/kg-day), C_p is the plasma concentration (mg/L), Cl is the plasma clearance (L/kg-day) and f is the fraction of the dose absorbed. Assuming values of 0.5 L/kg-day for Cl and 1 for f (Baldessarini and Tarazi, 2001), a steady-state plasma concentration of 0.6 mmol/L (4.2 mg Li/L) corresponds to a daily dose of 2.1 mg Li/kg-day.

The provisional subchronic and chronic RfD for lithium was derived from the LOAEL of 2.1 mg/kg-day for adverse effects in several organs and systems. Dividing the LOAEL of 2.1 mg/kg-day by an uncertainty factor of 1000 yields a subchronic and chronic p-**RfD of 0.002 mg/kg-day or 2 µg/kg-day**.

p-RfD = LOAEL ÷ UF
= 2.1 mg/kg-day ÷ 1000
= 0.002 mg/kg-day or 2 µg/kg-day

The composite uncertainty (UF) of 1000 includes a factor of 10 to extrapolate from a LOAEL to a NOAEL, a factor of 10 to protect susceptible individuals and a factor of 10 to account for database insufficiencies as follows:

- A default 10-fold UF for extrapolation from a LOAEL to a NOAEL was used because the lower bound of the therapeutic serum lithium range is associated with the development of adverse effects in several organs and systems; a NOAEL for adverse effects of therapeutic lithium has not been established in the clinical or animal literature.
- A default 10-fold UF was used to account for potentially susceptible individuals in the absence of quantitative information on the variability of response to lithium in the human population. Since lithium adversely affects several organs and systems, numerous pre-existing disease states (e.g., renal disease, cardiovascular disease, endocrine disease) may increase susceptibility to lithium.
- A UF of 10 was applied for database uncertainties. The renal effects of lithium have been extensively studied in humans and animals. However, much less information is available on the effects of lithium in other systems, including the cardiovascular, neurological and endocrine systems, and subchronic and chronic exposure studies in animals assessing comprehensive endpoints are not available. Furthermore, although lithium appears to produce developmental effects in humans, the database lacks well-controlled epidemiology studies and multi-generation reproduction studies in animals.

A wide range of estimates for daily dietary intake of lithium has been reported. Several authors report estimates for the average daily dietary intake of lithium, ranging from 0.24 to 1.5 μg/kg-day (Noel et al., 2003; Clarke et al., 1987; Hamilton and Minski, 1973; Evans et al., 1985; Clark and Gibson, 1988). A much higher estimate for daily intake from food and municipal drinking water ranging from 33 to 80 μg Li/kg-day was reported by Moore (1995). The source of the discrepancy between these estimates is unknown. The p-RfD of 2 μg/kg-day is above most estimates of daily dietary intake, but below the range estimated by Moore (1995).

Confidence in the LOAEL value is low-to-medium. Since the clinical literature has focused on the therapeutic treatment of patients, information on effects observed below the minimally effective dose is lacking. Confidence in the database is also low-to-medium. Although there is an extensive database demonstrating the adverse effects of chronic exposure to therapeutic lithium, information regarding the dose-response relationship of lithium to the development of adverse effects is lacking. Thus, the relative sensitivity of the different target organs cannot be identified based on human data. Furthermore, since most animals studies have been designed to evaluate specific adverse effects associated with the therapeutic serum lithium concentration range, NOAEL and LOAEL values have not been established in animals studied for comprehensive toxicity endpoints. The database also lacks well-controlled epidemiology studies and multi-generation reproduction studies in animals, even though there is evidence of developmental effects in lithium patients. Low-to-medium confidence in the p-RfD is the result.

FEASIBILITY FOR DERIVING A PROVISIONAL SUBCHRONIC RfC FOR LITHIUM

No studies investigating the effects of acute, subchronic or chronic inhalation exposure to lithium in humans were identified. The available studies in animals did not evaluate comprehensive histopathological, biochemical and clinical endpoints of inhalation exposure.

Thus, due to the lack of data, derivation of a provisional subchronic or chronic RfC for lithium is precluded.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR LITHIUM

Weight of Evidence Descriptor

Cancer studies in humans and cancer bioassays in animals exposed to lithium by the oral or inhaled routes were not found. Results of *in vitro* and *in vivo* studies in bacterial and mammalian systems indicate that lithium is not genotoxic. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the hazard descriptor, "data are inadequate for an assessment of human carcinogenic potential," is appropriate for lithium.

Quantitative Estimates of Carcinogenic Risk

Due to the lack of data, derivation of an oral cancer slope factor and an inhalation cancer unit risk are precluded.

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Attachment

1I

10

Molybdenum; CASRN 7439-98-7

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Molybdenum

File First On-Line 11/01/1992

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	11/01/1992
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	not evaluated	

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Molybdenum
CASRN — 7439-98-7
Last Revised — 11/01/1992

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Increased uric acid levels **Human 6-year to Lifetime Dietary Exposure Study** **Koval'skiy et al., 1961**	NOAEL: None LOAEL: 0.14 mg/kg-day	30	1	5E-3 mg/kg-day

*Conversion Factors: Dose determined from study: molybdenum (Mo) concentration in diet is 10-15 mg/day. Assumed body weight of adult male is 70 kg; 10 mg molybdenum/70-kg body weight = 0.14 mg/kg-day.

I.A.2. Principal and Supporting Studies (Oral RfD)

Koval'skiy, V.V., G.A. Yarovaya and D.M. Shmavonyan. 1961. Changes of purine metabolism in man and animals under conditions of molybdenum biogeochemical provinces. Zh. Obshch. Biol. 22:179-191. (Russian trans.)

In a cross-sectional epidemiology study in a Morich geoprovince of Armenia, Koval'skiy et al. (1961) correlated the dietary intake of molybdenum with serum uric acid levels, several biochemical endpoints, and with a gout- like sickness affecting the adult population in two settlements, Ankava village and a smaller adjoining settlement. Ankava village is a large settlement over 100 years old, while the adjoining settlement (the control) is smaller and was established in the 6-year period prior to the study. This particular region was selected for two reasons: high molybdenum content in the soil and plants (38 and 190 times that of the control area) and low content of copper (Cu). Based on these figures and dietary estimates, the average adult person in the Ankava settlement received 10-15 mg of molybdenum and 5-10 mg of copper. This intake corresponds to molybdenum doses of 0.14- 0.21 mg/kg-day for a 70-kg adult.

These values compare with control area values of 1-2 mg of molybdenum and 10-15 mg of copper. Three hundred villagers (184 of whom were age 18 or older) from Ankava and 100 villagers (78 adults) from the adjoining settlement underwent medical examinations. Only limited data on length of residency were reported. The results from the medical exam indicated that 57 Ankava adults (31% of the adult population) and 14 adults of the new settlement (17.9% of the adult population) had gout-like symptoms as compared with 1-4% as an overall average rate. This condition was characterized by pain, swelling, inflammation and deformities of the joints, and, in all cases, an increase in the uric acid content of the blood. In a number of cases (exact number not reported), this condition was accompanied by illnesses of the GI tract, liver, and kidneys. Fifty-two adults from Ankava and five from the adjoining settlement (controls) underwent a more detailed examination in which blood copper, molybdenum, uric acid, and xanthine oxidase concentrations in blood and molybdenum, copper, and uric acid concentrations in urine were measured. The average uric acid content in blood of the 52 Ankara adults was 6.2 mg as compared with 3.8 mg, the average of the five controls. Above normal blood uric acid content (>5.5 mg) was found in 29 of the 52 adults examined; at least 17 of these 29 had gout-like symptoms. When the 52 inhabitants were segregated as to whether they were sick with gout symptoms or not, the average concentration of uric acid in blood increased to 8.1 mg (n=17) for those sick and to 5.3 mg (n=35) for those healthy. Both serum molybdenum and serum xanthine oxidase (a molybdenum-containing enzyme that converts purines to uric acid) activity were positively correlated with serum uric acid levels. Increasing urinary excretion of copper was inversely related to increasing serum levels of molybdenum. Among the group of 52 adults from Ankara, blood uric acid levels increased with increasing residency time in the region; they increased from 3.75 mg for up to 1 year, to 6.4 mg after 1-5 years, and to 6.8 mg for 5 years or more. Based on these results, a molybdenum intake of 0.14 mg/kg-day may result in serum uric acid levels elevated above the average range of the adult population (2-6 mg; White et al., 1973). This level is designated as a LOAEL.

The effect of dietary molybdenum on uric acid and copper excretion was also observed in experiments with four adult men given diets based on sorghum varieties differing widely in molybdenum content for 10 days (Deosthale and Gopalan, 1974). The urinary excretion of uric acid was unaltered at molybdenum intake levels up to 1540 ug/day (approximately 0.022 mg/kg-day). The urinary excretion of copper increased in direct proportion to dietary molybdenum intake; molybdenum intakes of 0.002 or 0.022 mg/kg-day resulted in the urinary excretion of copper at 24 or 77 ug/day, respectively. Normal urinary copper excretion is less than 40 ug/day.

The effects of human ingestion of molybdenum in drinking water were investigated in two Colorado cities over a 2-year period (U.S. EPA, 1979). Urinary levels of molybdenum and copper and serum levels of ceruloplasmin and uric acid were compared in individuals consuming city drinking water over a 2-year period. The low-molybdenum group consisted of 42 individuals from Denver, Colorado where the molybdenum concentration in the drinking water ranged from

2 to 50 ug/L. The high-molybdenum group consisted of 13 college students from Golden, Colorado where the drinking water molybdenum concentrations were equal to or greater than 200 ug/L.

Among subjects consuming water containing up to 50 ug molybdenum/L, plasma molybdenum levels were within the normal range. No adverse health effects were observed. While higher daily urinary molybdenum was associated with higher molybdenum intake, no adverse biochemical or systemic effects were noted. The Denver subjects had a mean urinary molybdenum value of 87 +/- 18 ug/day as compared with a value of 187 +/- 34 ug/day for the Golden subjects. Higher mean serum ceruloplasmin (40.31 mg/100 mL vs. 30.41 mg/100 mL) and lower mean serum uric acid (4.35 mg/100 mL vs. 5.34 mg/100 mL) were also associated with the higher molybdenum intake. The average dietary intake of molybdenum was 180 ug/day (estimated from foods purchased at Denver area grocery stores) (Tsongas et al., 1980). When the dietary molybdenum was added to the molybdenum from the drinking water, the NOAEL for the Denver subjects was 4 ug/kg-day and 8 ug/kg-day for Golden subjects, assuming a 2-L/day water consumption and a 70-kg body weight.

When these three studies are viewed collectively, the increased serum ceruloplasmin and urinary excretion of copper observed in human studies indicates that high levels of ingested molybdenum may be associated with potential mineral imbalance. Excretion of sufficient quantities of this element may put individuals at risk for the hypochromic microcytic anemia associated with a dietary copper deficiency. Although increased copper excretion and elevated serum ceruloplasmin are not definitive adverse effects, and as presented here are associated with no frank adverse effects in a human population, the potential for mineral imbalance must be weighed in developing an RfD. Laboratory animal studies discussed below demonstrate that the effects of molybdenum on growth and melanin synthesis are more pronounced under situations where dietary copper intake is low. For this reason, the RfD was derived with the Estimated Safe and Adequate Daily Intake (ESAADI) in mind. It is important to note that the average level of copper intake in the American population from 1982 to 1986 was less than the lower limit of the ESAADI recommendation for all age and sex groups studied in the Food and Drug Administration (FDA) Total Diet Study (Pennington et al., 1989).

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 3 is used for protection of sensitive human populations and a factor of 10 for the use of a LOAEL, rather than a NOAEL, from a long-term study in a human population. A full factor of 10 is not used for the protection of sensitive human populations because the study was conducted in a relatively large human population. The database does not contain studies on reproductive and developmental toxicity. However, an additional uncertainty

factor for these deficiencies is not considered necessary because the RfD is only slightly above the ESAADI which was derived from the molybdenum content of the average U.S. diet.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

Molybdenum is an essential dietary nutrient which is a constituent of several mammalian enzymes including xanthine oxidase, sulfite oxidase and aldehyde oxidase (NRC, 1989). The Food and Nutrition Board of the Subcommittee on the Tenth Edition of the RDAs has established ESAADI values for molybdenum of 15-40 ug/day (2.5-4.45 ug/kg-day) for infants, 25-150 ug/day (1.95-5.36 ug/kg-day) for children, and 75-250 ug/day (1.5-3.6 ug/kg-day) for adolescents and adults (NRC, 1989). These values were derived from the reported molybdenum intake of adults and older children with average American diets (ug/kg-day values are derived from the Second National Health and Nutrition Examination Survey (NHANES II). Values for infants and children were extrapolated from the adult values on the basis of body weight. The dietary intake range reported by Tsongas (1980) from foods purchased in the Denver area was 120-240 ug/day with a mean of 180 ug/day. In the 1984 FDA Total Diet Study, the molybdenum intakes of older children and adults ranged from 74-126 ug/day (Pennington and Jones, 1987). Food for this assay was purchased from grocery stores in several northeastern locations. The data from these dietary surveys support the ESAADI recommendations.

Miller et al. (1956) administered diets to groups of Holtzman rats (21 days old; 4/dosage group). The basal diet (which contained 4 mg copper/kg and 0.2 mg molybdenum/kg) was supplemented with hydrogen molybdate at 75 and 300 ppm (approximately 7.5 and 30 mg molybdenum/kg/day, respectively). Some of the groups also received 2200 ppm sulfate (as a 1:1 mixture of sodium sulfate and potassium sulfate) for 6 weeks. Molybdenum alone exerted a significant (p value not reported) growth inhibition at the 75- and 300-ppm levels (50% and 78% reduction in weight gain, respectively). The addition of sulfate reversed this inhibition at molybdenum levels of 75 ppm and reduced it at 300 ppm. The addition of molybdenum alone increased liver copper and molybdenum concentrations. These increases were reduced by sulfate supplementation. An enlargement of the femoro-tibial joint and a thickening of the epiphysis of the femur and tibia were observed in the rats receiving 75 and 300 ppm molybdenum without sulfate and in the rats receiving 2200 ppm molybdenum with sulfate. Histological examination of the femurs indicated a chondrodystrophy of the epiphyseal cartilage. The femurs in the groups receiving lower molybdenum levels were normal. This study suggested a LOAEL of 7.5 mg molybdenum/kg/day based on body weight loss and bone deformities.

Jeter and Davis (1954) tested the effects of dietary molybdenum and copper on Long-Evans rats (4 or 8 pairs/group). The rats received either the basal diet (1.78 mg copper/kg as CuSO4ù5H2O

and <1 mg molybdenum/kg as NaMoO4ù2H2O) or the basal diet supplemented with molybdenum at approximately 2, 8 or 14 mg/kg, ad libitum daily for 13 weeks. Each diet contained 0.5 mg copper/kg. Two groups of animals also received <1 or 8 mg molybdenum/kg with 2 mg copper/kg. The weight gain of male rats given 2, 8 or 14 mg molybdenum/kg/day at the lower copper level (0.5 mg copper/kg/day) was retarded, while that of females was retarded only at the two higher molybdenum levels. Hemoglobin concentrations were not affected by any diet. Achromotrichia (depigmentation of the hair) followed by varying degrees of alopecia (balding) was observed in some but not all rats in the groups receiving 8 or 15 mg/kg-day of molybdenum. Depigmentation was occasionally observed in rats receiving approximately 2 mg molybdenum/kg/day. The change in hair coloration may be explained by the fact that a copper-containing, mixed function oxidase catalyzes the initial reaction in the synthesis of the melanin hair pigments. The 2-3 mg molybdenum/kg/day dose represents a LOAEL in this study.

The effect of excess dietary molybdenum (added as sodium molybdate) was tested in guinea pigs of unspecified strain (Arthur, 1965). In the first experiment, groups of five guinea pigs were maintained for 8 weeks on diets with varying molybdenum content. The basal diet contained 8.9 mg copper/kg, 0.3 mg molybdenum/kg, and 0.25% sulfate. Molybdenum was increased to 8000 mg molybdenum/kg in increments of 1000 mg molybdenum/kg. Assuming a body weight of 0.75 kg and food consumption of 30 g/day for guinea pigs, a dietary level of 8000 mg molybdenum/kg corresponds to 320 mg molybdenum/kg/day. Weight gains decreased as molybdenum was increased from 40-160 mg molybdenum/kg/day, and weight loss occurred above 160 mg molybdenum/kg/day. The color of the hair of the black guinea pigs changed to gray when the dose was higher than 40 mg molybdenum/kg/day. Some fatalities were reported at 200 mg molybdenum/kg/day, and approximately 75% of the animals receiving 240-320 mg molybdenum/kg/day died.

In the second part of the Arthur study (1965), the levels of copper and molybdenum were both varied with either 0, 10 or 20 mg copper/kg and 0 or 2000 mg molybdenum/kg added to the diet. All of the animals at dietary levels of 2000 mg/kg added molybdenum (80 mg molybdenum/kg/day) and either 0 or 0.4 mg copper/kg/day developed gray hair. The inclusion of 0.8 mg copper/kg/day, however, reversed this effect. All animals receiving added molybdenum accumulated molybdenum in the liver. The animals on 80 mg molybdenum/kg/day had the smallest weight gain. The failure to gain weight was only partially alleviated by the addition of copper.

In the third part of the study, three weanling guinea pigs were supplied a low-copper basal diet (5.6 mg copper/kg and 1.8 mg molybdenum/kg) with dietary additions of 0, 200, 500, 1000 or 2000 mg molybdenum (equivalent to 8, 20, 40 or 80 mg/kg-day) for 8 weeks (Arthur, 1965). Molybdenum in the blood, liver and kidneys increased with dietary molybdenum levels. An increase in copper was observed in the blood and kidneys with increasing molybdenum intake.

At 40 and 80 mg molybdenum/kg/day, liver copper concentrations decreased. Guinea pigs appeared to be somewhat less sensitive than rats or rabbits to molybdenum toxicity. The level of 40 mg molybdenum/kg/day represents a LOAEL in this study based on loss of copper.

I.A.5. Confidence in the Oral RfD

Study — Medium
Database — Medium
RfD — Medium

The level of confidence in the oral RfD for molybdenum is medium. It is based on the results of a study that examined only gross physical effects of a gout-like disease and examined some blood chemistry parameters normally associated with gout. An exhaustive analysis of blood chemistry and individual dietary habits was not done. Therefore, the results are clearly generalized for a large population. Studies in human and animals suggest that molybdenum has an adverse effect on copper homeostasis, making the changes in serum ceruloplasmin a matter of possible concern. A study that monitored a broader spectrum of hematological or clinical chemistry parameters, especially those related to copper distribution and copper metalloenzyme function, would have helped to characterize the copper-molybdenum interaction, which appears critical to the development of gout-like symptoms at very high levels of molybdenum. The proposed RfD satisfies molybdenum nutrient requirements for all healthy members of the population, based on a comparison with the ESAADI. Dietary studies conducted by Tsongas et al. (1980) and Pennington and Jones (1987) indicate that people in the U.S. are receiving between 76 and 240 ug/day (1.1-3.4 ug/kg-day, based on a 70-kg adult) in their diets. Much of these data served as the basis for the ESAADI.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 1990

The Drinking Water Health Advisory for Molybdenum has received Agency Review.

Other EPA Documentation — None

Agency Work Group Review — 09/21/1989, 08/15/1991, 09/11/1991, 11/06/1991, 12/12/1991

Verification Date — 11/06/1991

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for Molybdenum

conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Molybdenum
CASRN — 7439-98-7

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Molybdenum
CASRN — 7439-98-7

This substance/agent has not undergone a complete evaluation and determination under US EPA's IRIS program for evidence of human carcinogenic potential.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — Molybdenum
CASRN — 7439-98-7

VI.A. Oral RfD References

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U.S. EPA. 1979. Human health effects of molybdenum in drinking water. Cincinnati, OH. EPA-600A-79-006.

U.S. EPA. 1990. Drinking Water Health Advisory for Molybdenum. Prepared by the Office of Water. (Draft)

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VI.B. Inhalation RfC References

None

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — Molybdenum
CASRN — 7439-98-7

Date	Section	Description
11/01/1992	I.A.	Oral RfD summary on-line
10/28/2003	I.A.6.	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Molybdenum
CASRN — 7439-98-7
Last Revised — 11/01/1992

- 7439-98-7
- Molybdenum
- HSDB 5032
- MCHVL
- TSM1

Attachment

1I

11

TOXICOLOGICAL PROFILE FOR NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

August 2005

DISCLAIMER

The use of company or product name(s) is for identification only and does not imply endorsement by the Agency for Toxic Substances and Disease Registry.

UPDATE STATEMENT

A Toxicological Profile for Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene, Draft for Public Comment was released in September 2003. This edition supersedes any previously released draft or final profile.

Toxicological profiles are revised and republished as necessary. For information regarding the update status of previously released profiles, contact ATSDR at:

Agency for Toxic Substances and Disease Registry
Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE
Mailstop F-32
Atlanta, Georgia 30333

FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

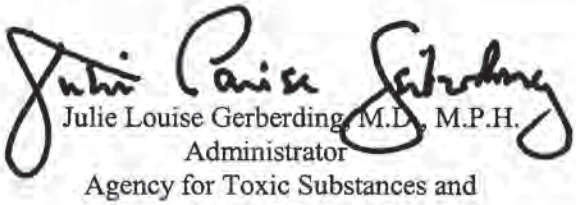
(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



Julie Louise Gerberding, M.D., M.P.H.
Administrator
Agency for Toxic Substances and
Disease Registry

*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on November 7, 2003 (68 FR 63098) . For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); November 17, 1997 (62 FR 61332); October 21, 1999(64 FR 56792) and October 25, 2001 (66 FR 54014). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Relevance to Public Health: The Relevance to Public Health Section evaluates, interprets, and assesses the significance of toxicity data to human health.

Chapter 3: Health Effects: Specific health effects of a given hazardous compound are reported by type of health effect (death, systemic, immunologic, reproductive), by route of exposure, and by length of exposure (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6	**How Can (Chemical X) Affect Children?**
Section 1.7	**How Can Families Reduce the Risk of Exposure to (Chemical X)?**
Section 3.7	**Children's Susceptibility**
Section 6.6	**Exposures of Children**

Other Sections of Interest:

Section 3.8	**Biomarkers of Exposure and Effect**
Section 3.11	**Methods for Reducing Toxic Effects**

ATSDR Information Center

Phone:	1-888-42-ATSDR or (404) 498-0110	***Fax***:	(770) 488-4178
E-mail:	atsdric@cdc.gov	***Internet***:	http://www.atsdr.cdc.gov

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental*

Hazards; Skin Lesions and Environmental Exposures; *Cholinesterase-Inhibiting Pesticide Toxicity*; and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific Minimal Risk Levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

4. Green Border Review. Green Border review assures the consistency with ATSDR policy.

PEER REVIEW

A peer review panel was assembled for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. The panel consisted of the following members:

1. Martin Alexander, Ph.D., Cornell University, Ithaca, New York;

2. Susan Borghoff, Ph.D., DABT, CIIT Centers for Health Research, Research Triangle Park, North Carolina; and

3. G.A. Shakeel Ansari, Ph.D., The University of Texas Medical Branch, Galveston, Texas.

These experts collectively have knowledge of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene and the effects of exposure to these chemicals.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites are then placed on the National Priorities List (NPL) and are targeted for long-term federal clean-up activities. Naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene have been found in at least 654, 36, and 412, respectively, of the 1,662 current or former NPL sites. Although the total number of NPL sites evaluated for these substances is not known, the possibility exists that the number of sites at which naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are found may increase in the future as more sites are evaluated. This information is important because these sites may be sources of exposure and exposure to these substances may harm you.

When a substance is released either from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. Such a release does not always lead to exposure. You can be exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance, or by skin contact.

If you are exposed to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene, many factors will determine whether you will be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with them. You must also consider any other chemicals you are exposed to and your age, sex, diet, family traits, lifestyle, and state of health.

1.1 WHAT ARE NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYL-NAPHTHALENE?

Naphthalene is a white solid that evaporates easily. It is also called mothballs, moth flakes, white tar, and tar camphor. When mixed with air, naphthalene vapors easily burn. Fossil fuels, such as petroleum and coal, naturally contain naphthalene. Burning tobacco or wood produces

naphthalene. The major commercial use of naphthalene is to make other chemicals used in making polyvinyl chloride (PVC) plastics. The major consumer products made from naphthalene are moth repellents, in the form of mothballs or crystals, and toilet deodorant blocks. It is also used for making dyes, resins, leather tanning agents, and the insecticide carbaryl.

Naphthalene has a strong but not unpleasant smell. Its taste is unknown, but it must not be unpleasant since children have eaten mothballs and deodorant blocks. You can smell naphthalene in the air at a concentration of 84 parts naphthalene per one billion parts (ppb) of air. You can smell it in water when 21 ppb are present.

1-Methylnaphthalene is a naphthalene-related compound that is also called alpha methyl-naphthalene. It is a clear liquid. Its taste and odor have not been described, but you can smell it in water when only 7.5 ppb are present.

Another naphthalene-related compound, 2-methylnaphthalene, is also called beta methyl-naphthalene. It is a solid like naphthalene. The taste and odor of 2-methylnaphthalene have not been described. Its presence can be detected at a concentration of 10 ppb in air and 10 ppb in water.

1-Methylnaphthalene and 2-methylnaphthalene are used to make other chemicals such as dyes, and resins. 2-Methylnaphthalene is also used to make vitamin K. All three chemicals are present in cigarette smoke, wood smoke, tar, asphalt, and at some hazardous waste sites.

See Chapters 4, 5, and 6 for more information on the properties and uses of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene.

1.2 WHAT HAPPENS TO NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE WHEN THEY ENTER THE ENVIRONMENT?

Naphthalene enters the environment from industrial uses, from its use as a moth repellent, from the burning of wood or tobacco, and from accidental spills. Naphthalene at hazardous waste

sites and landfills can dissolve in water and be present in drinking water. Naphthalene can become weakly attached to soil or pass through the soil particles into underground water.

Most of the naphthalene entering the environment is from the burning of woods and fossil fuels in the home. The second greatest release of naphthalene is through the use of moth repellents. Only about 10% of the naphthalene entering the environment is from coal production and distillation. Less than 1% of the naphthalene released to the atmosphere can be attributed to the losses from naphthalene production. Cigarette smoking also releases small amounts of naphthalene into the air.

Naphthalene evaporates easily. That is why you can smell mothballs. In the air, moisture and sunlight make it break down, often within 1 day. Naphthalene can change to 1-naphthol or 2-naphthol. These chemicals have some of the toxic properties of naphthalene. Some naphthalene will dissolve in water in rivers, lakes, or wells. Naphthalene in water is destroyed by bacteria or evaporates into the air. Most naphthalene will be gone from water in rivers or lakes within 2 weeks.

Naphthalene binds weakly to soils and sediments. It easily passes through sandy soils to reach underground water. In soil, some microorganisms break down naphthalene. When near the surface of the soil, naphthalene will evaporate into air. Microorganisms present in the soil will break down most of the naphthalene in 1–3 months.

Naphthalene does not accumulate in the flesh of animals and fish that you might eat. If dairy cows are exposed to naphthalene, some naphthalene will be in their milk; if laying hens are exposed, some naphthalene will be in their eggs. Naphthalene and the methylnaphthalenes have been found in very small amounts in some samples of fish and shellfish from polluted waters.

Scientists know very little about what happens to 1-methylnaphthalene and 2-methylnaphthalene in the environment. These compounds are similar to naphthalene and should act like it in air, water, or soil.

See Chapters 5 and 6 for more information on what happens to naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene in the environment.

1.3 HOW MIGHT I BE EXPOSED TO NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE?

You are most likely to be exposed to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene from the air. Outdoor air contains low amounts of these chemicals. Burning of wood or fossil fuels and industrial discharges adds these chemicals to the environment. Automobile exhaust contributes naphthalene among other chemicals to air pollution in the cities. Typical air concentrations for naphthalene are low, 0.2 ppb or less. Studies of outdoor air reported concentrations of 0.09 ppb 1-methylnaphthalene and 0.011 ppb 2-methylnaphthalene. In homes or businesses where cigarettes are smoked, wood is burned, or moth repellents are used, the levels of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in the air are higher. Studies of indoor air typically report that average indoor air concentrations of these contaminants are less than 1 ppb.

You are not likely to be exposed to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene by eating foods or drinking beverages. These materials are unlikely to come in contact with naphthalene or methylnaphthalenes during production or processing. Naphthalene and the methylnaphthalenes are also unlikely to be present in tap water.

If you live near a hazardous waste site and have a well used for drinking water, you might be exposed to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. For this to happen, the chemicals must pass through the soil and dissolve in the underground water that supplies your well. Children might also contact these chemicals by playing in or eating the dirt near a waste site.

Work using or making moth repellents, coal tar products, dyes, or inks could expose you to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in the air. Working in the wood-preserving, leather tanning, or asphalt industries could expose you to naphthalene.

Using moth repellents containing naphthalene in your home will expose you to naphthalene vapors. Your skin can come in contact with naphthalene via the use of naphthalene-treated clothing, blankets, or coverlets. You can breathe in the naphthalene vapors that are present in clothes and linen stored with moth-balls. Smoke from cigarettes can also expose you to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. The highest airborne naphthalene concentrations in indoor air occur in the homes of cigarette smokers.

See Chapter 6 for more information on how you might be exposed to naphthalene, 1-methyl-naphthalene, or 2-methylnaphthalene.

1.4 HOW CAN NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYL-NAPHTHALENE ENTER AND LEAVE MY BODY?

Naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene can enter your body if you breathe air that contains these chemicals, if you smoke, if you eat mothballs, if you drink water that contains these chemicals, or if they touch your skin. These chemicals are most likely to enter your body through the air you breath into your lungs. Naphthalene can also enter your body through your skin when you handle mothballs, particularly if you have used an oil-based skin lotion. You can also breathe in naphthalene vapors from clothes that have been stored in mothballs.

Once naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene enter your body, small amounts will dissolve in your blood. Your blood carries them to your liver and other organs. These organs change them so that they pass through your body, mainly into your urine. Some naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene, and their breakdown products can be present in your stool. Naphthalene also has been found in some samples of fatty tissue and breast milk taken from the general U.S. population, but the concentrations of naphthalene were low. Most naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene that enters your body is expected to leave quickly within 1–3 days.

See Chapter 3 for more information on how naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene enter and leave your body.

1.5 HOW CAN NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYL-NAPHTHALENE AFFECT MY HEALTH?

Scientists use many tests to protect the public from harmful effects of toxic chemicals and to find ways for treating persons who have been harmed.

One way to learn whether a chemical will harm people is to determine how the body absorbs, uses, and releases the chemical. For some chemicals, animal testing may be necessary. Animal testing may also help identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method for getting information needed to make wise decisions that protect public health. Scientists have the responsibility to treat research animals with care and compassion. Scientists must comply with strict animal care guidelines because laws today protect the welfare of research animals.

Exposure to a large amount of naphthalene may damage or destroy some of your red blood cells. This could cause you to have too few red blood cells until your body replaces the destroyed cells. This problem is called hemolytic anemia. People, particularly children, have developed this problem after eating naphthalene-containing mothballs or deodorant blocks. Anemia has also occurred in infants wearing diapers that have been stored in mothballs. If your ancestors were from Africa or Mediterranean countries, naphthalene may be more dangerous to you than to people of other origins. These populations have a higher incidence of problems with an enzyme that usually protects red blood cells from damage created by oxygen in the air.

Some of the symptoms that occur with hemolytic anemia are fatigue, lack of appetite, restlessness, and a pale appearance to your skin. Exposure to a large amount of naphthalene, such as by eating mothballs, may cause nausea, vomiting, diarrhea, blood in the urine, and a yellow color to the skin. If you have these symptoms, you should see a doctor quickly.

Anemia is a common condition in pregnancy that can be due to causes other than naphthalene exposure. However, if you are a pregnant woman and are anemic due to naphthalene exposure, then it is possible that your unborn child may be anemic as well. Naphthalene can move from your blood to your baby's blood. Once your baby is born, naphthalene may also be carried from your body to your baby's body through your milk. It is not completely clear if naphthalene causes reproductive effects in animals; most evidence says that it does not.

Laboratory rabbits, guinea pigs, mice, and rats sometimes develop cataracts (cloudiness) in their eyes after swallowing naphthalene at high dose levels. It is not certain whether cataracts also develop in humans exposed to naphthalene, but the possibility exists.

When mice or rats breathed in naphthalene vapors daily throughout their lives (2 years), cells in the lining of their noses or lungs were damaged. Some exposed female mice also developed lung tumors. Some exposed male and female rats developed nose tumors. When mice or rats were fed naphthalene in their food for 13 weeks, no tumors or other tissue changes were found. The only effect found was decreased body weight in rats that were fed naphthalene.

Based on these results from animal studies, the U.S. Department of Health and Human Services concluded that naphthalene is reasonably anticipated to be a human carcinogen. The International Agency for Research on Cancer (IARC) concluded that naphthalene is possibly carcinogenic to humans, because there is enough evidence that naphthalene causes cancer in animals, but not enough evidence about such an effect in humans. Under the EPA 1986 cancer guidelines, naphthalene was assigned to Group C – possible human carcinogen.

When mice were fed food containing 1-methylnaphthalene or 2-methylnaphthalene for most of their lives (81 weeks), the gas-exchange part of the lungs of some mice became filled with an abnormal material. This type of lung injury is called pulmonary alveolar proteinosis. A few mice also had lung tumors, but the numbers of mice with lung tumors were not enough to conclude that 1-methylnaphthalene or 2-methylnaphthalene caused the tumors. Pulmonary alveolar proteinosis has been seen in some people, but the cause of this uncommon lung disease in humans is unknown.

See Chapter 3 for more information on the effects of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene on your health.

1.6 HOW CAN NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYL-NAPHTHALENE AFFECT CHILDREN?

This section discusses potential health effects in humans from exposures during the period from conception to maturity at 18 years of age.

Hospitals have reported many cases of hemolytic anemia in children, including newborns and infants, who either ate naphthalene mothballs or deodorant cakes or who were in close contact with clothing or blankets stored in naphthalene mothballs. Newborns or infants are thought to be especially susceptible to this effect on the blood, because their bodies are less able to get rid of naphthalene than adults.

Newborn mice appear to be more susceptible to lung injury than adult mice, when they are injected with naphthalene. These results suggest that children may be more susceptible to lung injury from naphthalene than adults. Scientists do not know if lung injury from breathing in naphthalene in childhood may lead to lung disease later in life.

There are no reports that prenatal or postnatal exposure to naphthalene has caused developmental problems in human offspring. When pregnant mice, rats, or rabbits were fed naphthalene during their pregnancy, the development of their offspring was normal. Normal offspring development occurred even when the amounts of naphthalene given were large enough to prevent the pregnant animals from gaining their normal amount of weight.

There are no studies in humans or animals indicating whether or not children are more susceptible to health effects from 1-methylnaphthalene or 2-methylnaphthalene.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE?

If your doctor finds that you have been exposed to substantial amounts of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, ask whether your children might also have been exposed. Your doctor might need to ask your state health department to investigate.

The most important way that families can reduce the risk of exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene is to avoid smoking tobacco, generating smoke during cooking, or using fireplaces or heating appliances in their homes. If families use naphthalene-containing moth repellants, the material should be enclosed in containers that prevent vapors from escaping. The containers should not be accessible to young children. Blankets and clothing stored with naphthalene moth repellents should be aired outdoors to remove naphthalene odors and washed before they are used. To further minimize the risk of exposure to naphthalene, families should inform themselves of the contents of air deodorizers that are used in their homes, and refrain from using deodorizers with naphthalene.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE?

Several tests determine whether you have been exposed to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. These tests include measuring naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, or their breakdown products in samples of urine, stool, blood, maternal milk, or body fat. These tests require special equipment, which is not routinely available in a doctor's office. Body fluids, urine, stool samples, or tissue samples can be sent to a special laboratory for the tests. These tests cannot determine exactly how much naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene you were exposed to or predict whether harmful effects will occur. If the samples are collected within a day or two of exposure, then the tests can show if you were exposed to a large or small amount of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

See Chapters 3 and 7 for more information on tests for exposure to naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations *can* be enforced by law. The EPA, the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA) are some federal agencies that develop regulations for toxic substances. Recommendations provide valuable guidelines to protect public health, but *cannot* be enforced by law. The Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH) are two federal organizations that develop recommendations for toxic substances.

Regulations and recommendations can be expressed as "not-to-exceed" levels, that is, levels of a toxic substance in air, water, soil, or food that do not exceed a critical value that is usually based on levels that affect animals; they are then adjusted to levels that will help protect humans. Sometimes these not-to-exceed levels differ among federal organizations because they used different exposure times (an 8-hour workday or a 24-hour day), different animal studies, or other factors.

Recommendations and regulations are also updated periodically as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene include the following:

The federal government has developed regulations and advisories to protect individuals from the possible health effects of naphthalene in the environment. OSHA set a limit of 10 parts per million (ppm) for the level of naphthalene in workplace air over an 8-hour workday. NIOSH set a limit of 500 ppm for the level of naphthalene in workplace air expected to be immediately

dangerous to life or health. Exposure to workplace air concentrations above this limit for more than 30 minutes would be expected to impair a worker's ability to escape the contaminated workplace.

EPA recommends that children not drink water with over 0.5 ppm naphthalene for more than 10 days or over 0.4 ppm for any longer than 7 years. Adults should not drink water with more than 1 ppm for more than 7 years. For water consumed over a lifetime (70 years), EPA suggests that it contain no more than 0.1 ppm naphthalene.

Industrial releases of naphthalene into the environment of more than 100 pounds must be reported to EPA.

There are no regulations or advisories for 1-methylnaphthalene or 2-methylnaphthalene.

See Chapter 8 for more information on government regulations for naphthalene.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department, or contact ATSDR at the address and phone number below.

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses that result from exposure to hazardous substances.

Toxicological profiles are also available on-line at www.atsdr.cdc.gov and on CD-ROM. You may request a copy of the ATSDR ToxProfiles™ CD-ROM by calling the toll-free information and technical assistance number at 1-888-42ATSDR (1-888-422-8737), by e-mail at atsdric@cdc.gov, or by writing to:

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE
Mailstop F-32
Atlanta, GA 30333
Fax: 1-770-488-4178

Organizations for-profit may request copies of final Toxicological Profiles from the following:

National Technical Information Service (NTIS)
5285 Port Royal Road
Springfield, VA 22161
Phone: 1-800-553-6847 or 1-703-605-6000
Web site: http://www.ntis.gov/

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO NAPHTHALENE, 1-METHYLNAPHTHALENE, AND 2-METHYLNAPHTHALENE IN THE UNITED STATES

Naphthalene and methylnaphthalenes occur naturally in fossil fuels such as petroleum and coal, and are produced when organic materials (e.g., fossil fuels, wood, tobacco) are burned. Naphthalene is also produced commercially from either coal tar or petroleum. In 2000, estimates of commercial production of naphthalene in Japan, Western Europe, and the United States were 179, 205, and 107 thousand tonnes. Commercially-produced naphthalene is predominately used in the production of phthalic anhydride, which is used as an intermediate for polyvinyl chloride plasticizers such as di(2-ethylhexyl) phthalate. In 1999, this use of naphthalene accounted for 73 and 60% of commercial demand for naphthalene in Japan and the United States, respectively. Other uses of naphthalene include production of naphthalene sulfonates (used in concrete additives and synthetic tanning agents), pesticides (e.g., carbaryl insecticides and moth repellents), and dye intermediates.

Naphthalene is frequently present in industrial and automobile emissions and effluents and in various media in the general environment due to its natural occurrence in coal and petroleum products and emissions, its use as an intermediate in the production of plasticizers, resins, and insecticides, and its use in a variety of consumer products such as moth repellants. In 2002, environmental releases of naphthalene reported under the EPA Toxics Release Inventory (TRI) program were about 2.07 million pounds in air emissions, 0.03 million pounds in surface water discharges, 0.23 million pounds in underground injection discharges, and 0.37 million pounds in releases to land. These figures reflect estimates that most naphthalene entering the environment is discharged to the air, with the largest releases associated with the combustion of plant material and fossil fuels and volatilization from naphthalene-containing consumer products.

Monitoring studies of outdoor ambient air levels of naphthalene have reported concentrations in the range of about 0.4–170 $\mu g/m^3$, with a median naphthalene concentration of 0.94 $\mu g/m^3$ (0.0002 ppm) reported for urban/suburban air samples collected from 11 U.S. cities. The highest outdoor air concentrations have been found in the immediate vicinity of certain industrial sources and hazardous waste sites. For example, average concentrations of naphthalene in ambient air at five hazardous waste sites and one landfill in New Jersey ranged from 0.42 to 4.6 $\mu g/m^3$ (0.00008–0.0009 ppm). In indoor air, emissions

from cooking, tobacco smoking, or moth repellants are expected to be the predominant sources of naphthalene. Indoor air concentrations of naphthalene in homes with smoking residents and homes without smoking residents were reported to be 2.2 μg/m^3 (0.0004 ppm) and 1.0 μg/m^3 (0.0002 ppm), respectively. A study of indoor and outdoor air in 24 low-income homes in North Carolina found naphthalene levels ranging from 0.33–9.7 μg/m^3 and 0.57–1.82 μg/m^3 respectively. Methylnaphthalenes have also been detected in ambient outdoor and indoor air. For example, average concentrations of 1-methylnaphthalene and 2-methylnaphthalene in ambient outdoor air samples were reported to be 0.51 and 0.065 μg/m^3, respectively, whereas 2-methylnaphthalene in indoor air samples showed an average concentration of 1.5 μg/m^3 (0.0003 ppm). Based on a median concentration of 0.95 μg/m^3 (0.0002 ppm) naphthalene in urban and suburban air samples and an inhalation rate of 20 m^3/day, the average daily intake of naphthalene from ambient air is estimated at 19 μg/day, or 0.3 μg/kg/day assuming 70-kg body weight.

Levels of naphthalene (and methylnaphthalenes), when detected in water, sediments, and soil tend to be low: usually <10 μg/L in surface water or groundwater, <500 μg/kg in sediments, and 0–3 μg/kg in untreated agricultural soils. However, in the immediate vicinity of point sources of release, such as chemical waste sites, concentrations can be higher. For example, concentrations of 6.1 and 2.9 mg/kg were reported for naphthalene and methylnaphthalene, respectively, in soil samples contaminated with coal tar.

2.2 SUMMARY OF HEALTH EFFECTS

Reports that establish associations between naphthalene exposure and health effects in humans are restricted to numerous reports of hemolytic anemia or cataracts following acute exposure or occupational exposure to naphthalene, either by ingestion or by inhalation of naphthalene vapors, but these reports have not identified exposure levels associated with these effects. A relationship appears to exist between an inherited deficiency in the enzyme, glucose 6-phosphate dehydrogenase (G6PD), and susceptibility to naphthalene-induced hemolysis. Newborn infants also appear to be susceptible to naphthalene-induced hemolysis presumably due to a decreased ability to conjugate and excrete naphthalene metabolites. The only studies of cancer in humans exposed to naphthalene are two case series reports of cancer; one report of four laryngeal cancer cases (all of whom were smokers) among workers in a naphthalene purification plant in East Germany, and another report of 23 cases of colorectal carcinoma admitted to a hospital in Nigeria. NTP, EPA, and IARC concur that these studies provide inadequate evidence of naphthalene

carcinogenicity in humans. No cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available.

Epidemiology studies, case reports, or controlled-exposure studies examining the potential health effects of human exposure to 1-methylnaphthalene or 2-methylnaphthalene by any route of exposure are not available.

Results from animal studies exposed to naphthalene by oral administration, by inhalation exposure, or by parenteral administration identify several health effects of potential concern for humans, including maternal toxicity during pregnancy with acute oral exposure, decreased body weight (without lesions developing in any tissues or organs) with intermediate oral exposure, and increased incidence of nonneoplastic and neoplastic lesions in the nose (in rats and mice) and the lung (in mice only) with chronic inhalation exposure.

Hemolytic and Ocular Effects of Naphthalene in Animals. Rats and mice do not appear to be susceptible to the hemolytic effects of naphthalene as hematological end points have not been affected in acute or intermediate duration oral studies or in acute 14-day inhalation studies. There is one report of hemolytic anemia in a few dogs orally exposed to naphthalene, but the data are inadequate to describe dose-response relationships that can be reliably extrapolated to human exposure scenarios. Naphthalene-induced cataracts or lens opacities are well studied in rats and rabbits and appear to occur at acute- or intermediate-duration oral exposure levels >500 mg/kg/day. Naphthalene-induced cataracts were not found with intermediate-duration (i.e., 13 weeks) oral exposure at lower dose levels up to 200 mg/kg/day in mice or 400 mg/kg/day in rats.

Maternal and Developmental Toxicity of Naphthalene in Animals. Acute oral exposure of pregnant rats to naphthalene doses of 150 or 450 mg/kg/day (but not 50 mg/kg/day) during gestation has produced maternal toxicity including clinical signs (lethargy and prone position) and severe decreases in body weight gain, but clear effects on the developing fetus have not been found at maternal oral doses as high as 450 mg/kg/day in rats, 300 mg/kg/day in mice, or 120 or 400 mg/kg/day in rabbits. Reduced numbers of mouse pups per litter were observed when naphthalene (300 mg/kg/day) in corn oil was orally administered to pregnant mice; however, no fetotoxic effects were seen when pregnant rabbits were orally administered naphthalene at even higher doses (400 mg/kg/day) but delivered in methylcellulose rather than in an oil vehicle. It is unclear if these differences are due to species differences in sensitivity or to

the vehicle used to deliver naphthalene. The finding of maternal toxicity in orally exposed pregnant rats serves as the basis of the acute oral MRL for naphthalene (see Section 2.3). Dermal or inhalation developmental toxicity studies in animals are not available.

Body Weight Effects of Naphthalene in Animals. Comprehensive intermediate-duration (13 weeks) oral toxicity studies found no evidence for naphthalene-induced lesions in any tissue or organs in male or female Fischer 344 rats exposed to doses as high as 400 mg/kg/day or in male or female B6C3F1 mice exposed to doses as high as 200 mg/kg/day. The only biologically significant effects found in these studies were decreases in rat terminal body weights compared with controls at dose levels of 200 mg/kg/day (12% decrease in male rats) and 400 mg/kg/day (28 and 23% decreases in male and female rats, respectively). No effect on food consumption was observed in exposed rats. Exposed male mice had higher body weights than controls, and exposed female mice had lower body weights than controls, but mean body weights were not decreased by more than 5%. In another intermediate-duration oral study with CD-1 mice that focused on a battery of immunologic tests (but did not include comprehensive histopathologic examination of tissues), no biologically significant effects were found except for decreases in weights of several organs (brain, liver, and spleen) in mice exposed to 133 mg/kg/day, but not to 53 or 5.3 mg/kg/day. The lack of naphthalene-induced lesions in these organs in the NTP studies suggests that the brain, liver, and spleen are not sensitive targets of naphthalene following intermediate oral exposure. Body weight changes in rats were the most sensitive, biologically relevant effects observed in the available toxicity studies in animals orally exposed for intermediate durations. These effects were considered in deriving the intermediate-duration oral MRL for naphthalene (see Section 2.3). Chronic-duration oral toxicity studies with naphthalene in animals are not available.

Cancer and Respiratory Effects of Naphthalene in Animals. Chronic inhalation studies found increased incidences of nonneoplastic and neoplastic lesions in the nose of rats, nonneoplastic lesions in the nose of mice, and neoplastic and nonneoplastic lesions in the lungs of mice. In mice of both sexes, chronic inhalation of 10 or 30 ppm naphthalene induced inflammation of the nose and lung, metaplasia of the olfactory epithelium, and hyperplasia of the nasal respiratory epithelium. In female mice (but not male mice), exposure to 30 ppm (but not 10 ppm) increased the incidence of benign lung tumors (alveolar/bronchiolar adenomas) compared with controls. One other female mouse exposed to 30 ppm showed a malignant lung tumor (alveolar/bronchiolar carcinoma). In rats of both sexes, inhalation of 10, 30, or 60 ppm naphthalene induced nonneoplastic and neoplastic lesions only in the nasal cavity. Nonneoplastic nasal lesions included (1) hyperplasia, atrophy, chronic inflammation, and hyaline degeneration of the olfactory epithelium and (2) hyperplasia, metaplasia or degeneration of the respiratory epithelium or

glands. Neoplastic lesions associated with naphthalene exposure in rats were olfactory epithelial neuroblastoma (a rare malignant tumor) and respiratory epithelial adenoma. The chronic inhalation MRL for naphthalene is based on the LOAEL of 10 ppm for nonneoplastic lesions in the olfactory epithelium and respiratory epithelium of the nose of rats (see Section 2.3).

The mechanisms by which naphthalene causes nonneoplastic or neoplastic lesions in the respiratory tract of rodents are incompletely understood, but are thought to involve reactive metabolites of naphthalene, including 1,2-naphthalene oxide, 1,2-naphthoquinone, 1,4-naphthoquinone, and possibly 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene(see Sections 3.4.3. and 3.5).

Comparison of species susceptibility to naphthalene-induced nonneoplastic lung damage suggests that mice are much more sensitive than rats (e.g., nonneoplastic or neoplastic lung lesions were not found in chronically exposed rats in the NTP study) and that differences in rates and stereoselectivity of naphthalene metabolism to epoxide intermediates may be involved in this species difference. Acute (4-hour) inhalation exposure of mice to naphthalene concentrations as low as 2–10 ppm induced lung injury, whereas rats exposed to naphthalene concentrations as high as 110 ppm showed no signs of lung injury. Some evidence has been reported that rates and stereoselectivity of naphthalene metabolism in primate lung tissue may be more like rats than mice. In *in vitro* studies with microsomes from lymphoblastoid cells, which expressed recombinant human CYP2F1, metabolism of naphthalene to epoxide intermediates was demonstrated, but the predominant enantiomeric form produced (1*S*,2*R*-oxide) was different from the form (1*R*,2*S*-oxide) produced by mouse CYP2F2. Although these observations on epoxide formation may suggest that mice may be more sensitive than humans to acute naphthalene lung toxicity from epoxide intermediates, the possible role of other potentially reactive metabolites of naphthalene (e.g., the naphthoquinone metabolites) is unknown with chronic exposure scenarios. To date, mechanistic understanding of species differences in naphthalene bioactivation in the lung is too incomplete to definitively rule out the possible human relevance of naphthalene-induced lung lesions in mice (see Section 3.5).

In contrast, the olfactory epithelium and respiratory epithelium of the nose of rats and mice do not appear to differ in sensitivity to naphthalene nonneoplastic toxicity from chronic inhalation exposure. Nonneoplastic nasal lesions were found in nearly all exposed animals of both species at the lowest exposure level, 10 ppm, in both chronic studies. CYP monooxygenases, which might be involved in naphthalene metabolism and bioactivation, have been demonstrated to exist in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans. Studies designed to specifically characterize

metabolism of naphthalene in nasal tissue, however, have not been conducted, with the exception of a single study, which examined *in vitro* rates of metabolism of naphthalene to naphthalene oxides in postmitochondrial supernatants from mouse, rat, and hamster olfactory tissue. Metabolic rates (units of nmol/min/mg protein) showed the following order: mouse (87.1) > rat (43.5) > hamster (3.9). This order did not correspond with species differences in sensitivity to single intraperitoneal injections of naphthalene in a companion study. The lowest dose levels producing substantial necrosis and exfoliation in olfactory epithelium were 200 mg/kg in rats and 400 mg/kg in mice and hamsters. To date, mechanistic understanding of species differences in naphthalene bioactivation in the respiratory tissues is too incomplete to definitively rule out the possible human relevance of naphthalene-induced nasal lesions in rodents (nonneoplastic lesions in rats and mice and neoplastic lesions in rats; see Section 3.5).

It is unknown whether the naphthalene-induced neoplastic lesions found in mice (lung adenomas) and rats (nose respiratory epithelial adenomas and olfactory epithelial neuroblastomas) are produced via a genotoxic mode of action or a nongenotoxic mode requiring tissue damage and regenerative responses as precursor events. Results from genotoxicity tests for naphthalene have been predominately (but not completely) negative (see Section 3.3), and the general sites of neoplastic lesions, the nose in rats and the lungs in mice, show some correspondence (but not complete) with the general sites of nonneoplastic lesions. However, mechanistic understanding of naphthalene's carcinogenic mode of action is too incomplete to rule out the possibility of a genotoxic mode of action. Key issues that remain unexplained or unstudied include:

(1) the possible significance of the few positive genotoxicity results that have been obtained, including: reverse mutations in *Salmonella typhimurium* by 1,2-naphthoquinone; *in vitro* formation of N-7 guanine adducts of DNA by 1,2-naphtoquinone; reverse mutations for luminescence in the marine bacteria, *Vibrio fischeri*, by naphthalene; induction of sister chromatid exchanges in Chinese hamster ovary cells by naphthalene and in human mononuclear leukocytes by 1,2- or 1,4-naphthoquinone; induction of chromosomal aberrations in Chinese hamster ovaries and preimplantation mouse embryos by naphthalene; induction of somatic mutations and recombination in *Drosophila melanogaster* by naphthalene; and weak (about 2-fold) induction of micronuclei in red blood cells from *Pleurodeles waltl* larvae by naphthalene.

(2) the lack of a mechanistic explanation of why nearly all rats and mice develop nasal nonneoplastic lesions following chronic exposure to naphthalene at concentrations ≥10 ppm, but only some rats develop nasal tumors;

(3) the lack of a mechanistic explanation of why both male and female mice exposed to naphthalene show similar incidences of chronic lung inflammation following chronic exposure to 10 or 30 ppm, but only female mice showed statistically significant increased incidence of lung tumors;

(4) the lack of *in vivo* genotoxicity assays involving target tissues of naphthalene carcinogenicity (nose and lung); and

(5) the lack of information on the possible threshold exposure levels for nonneoplastic nasal lesions in rats and mice at air concentrations <10 ppm.

The National Toxicology Program *11th Report on Carcinogens* includes naphthalene in its list of chemicals *reasonably anticipated to be human carcinogen.*

International Agency for Research on Cancer concluded that naphthalene is *possibly carcinogenic to humans* (Group 2B) based on specific evaluations that there is inadequate evidence in humans and sufficient evidence in animals for the carcinogenicity of naphthalene. IARC considered the findings for nasal tumors in male and female rats and lung tumors in female mice in the NTP bioassays as sufficient evidence, noting that both nasal tumor types (olfactory epithelial neuroblastomas and respiratory epithelial adenomas) are rare in untreated rats.

EPA last assessed the carcinogenicity of naphthalene before the availability of the results from the chronic rat bioassay. In the EPA (1998c) *Toxicological Review on Naphthalene*, it was concluded that there was inadequate evidence in humans and limited evidence in animals of naphthalene carcinogenicity (increased incidence of lung tumors in female mice). Under the EPA 1986e cancer guidelines, naphthalene was assigned to Group C—*possible human carcinogen*. Under the EPA 1996a proposed cancer guidelines, it was judged that the human carcinogenic potential of naphthalene via the oral or inhalation routes “cannot be determined”, but it was noted that there was suggestive evidence of potential human carcinogenicity based on increased lung tumors in female mice. Currently, the EPA Integrated Risk Information System (IRIS) Office is reassessing the inhalation carcinogenicity of naphthalene.

Cancer and Respiratory Effects of 1- and 2-Methylaphthalene in Animals. Increased incidences of pulmonary alveolar proteinosis have been observed in mice of both sexes exposed to 1-methyl-

naphthalene in the diet for 81 weeks at approximate dose levels of 72–75 and 140–144 mg/kg/day and 2-methylnaphthalene in the diet at doses of 50–54 and 108–114 mg/kg/day. Histologic examination of major tissues and organs in these studies showed no other exposure-related nonneoplastic or neoplastic lesions at other sites (including the bronchiolar regions of the lung). Mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene (a mixture of 1- and 2-methylnaphthalene) for 30–61 weeks also showed increased incidence of pulmonary alveolar proteinosis. The chronic studies with mice exposed to 1- or 2-methylnaphthalene in the diet provide the basis for the chronic oral minimal risk levels (MRLs) for these substances (see Section 2.3).

Pulmonary alveolar proteinosis is characterized by an accumulation in the alveolar lumen of foamy cells, cholesterol crystals, and proteinaceous materials rich in lipids. The condition is rare in humans and has not been associated with human exposure to 2-methylnaphthalene or 1-methylnaphthalene. Human subjects with this condition can display pulmonary function deficits. The absence of pulmonary alveolar proteinosis in a 13-week range-finding study that exposed B6C3F1 mice to dietary doses as high as 2,500 mg/kg/day suggests that the development of this lesion requires chronic-duration exposure.

The mechanisms by which 1- or 2-methylnaphthalene may cause pulmonary alveolar proteinosis are poorly understood, but light and electron microscopic observations of lung tissues from mice repeatedly exposed to dermal doses of methylnaphthalene indicate that type II pneumocytes are a specific cellular target. It has been hypothesized that, in response to 1- or 2-methylnaphthalene, type II pneumocytes produce increased amounts of lamellar bodies due to hyperplasia and hypertrophy, and eventually transform into balloon cells. The rupture of balloon cells is hypothesized to lead to the accumulation of proteinaceous materials rich in lipids in the alveolar lumen. It is unknown whether the methyl-naphthalenes themselves or their metabolites are responsible for the development of pulmonary alveolar proteinosis.

The chronic dietary studies with 1- or 2-methylnaphthalene provide limited evidence for the carcinogenicity of these chemicals. In the 1-methylnaphthalene study, respective incidences of mice with lung adenomas or carcinomas were 5/50, 2/50, and 5/50 for control through high-dose females, and 2/49, 13/50, and 15/50 for males. With 2-methylnaphthalene, incidences for lung adenomas or carcinomas were 5/50, 4/49, and 6/48 for females and 2/49, 10/49, and 6/49 for males. The tumorigenic response was predominantly benign and was only consistently seen in male mice exposed to 1-methylnaphthalene. The available data on the methylnaphthalenes appear inadequate to determine their carcinogenicity potential in

humans, given the lack of any human studies on the potential carcinogenicity of the methylnaphthalenes and the limited evidence of carcinogenicity in animals.

The NTP *11th Report on Carcinogens* does not include 1-methylnaphthalene or 2-methylnaphthalene on its list of chemicals *known to be human carcinogens* or *reasonably anticipated to be human carcinogens.* IARC has not assessed the carcinogenicity potential of the methylnaphthalenes. The EPA concluded that the available data for 2-methylnaphthalene are *inadequate to assess human carcinogenic potential*, noting that there are no human data and the available evidence of 2-methylnaphthalene in animals is limited and insufficient to determine that 2-methylnaphthalene is carcinogenic to humans.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 0.0007 ppm was derived for chronic inhalation exposure to naphthalene.

The MRL was derived from two chronic inhalation toxicity and carcinogenicity studies with mice (NTP 1992a) and rats (Abdo et al. 2001; NTP 2000). In one study, groups of 75 B6C3F1 mice of each sex were exposed by inhalation at concentrations of 0, 10, or 30 ppm, 6 hours/day, 5 days/week for 104 weeks. In the other study, groups of 49 male and 49 female F344/N rats were exposed to naphthalene at concentrations of 0, 10, 30, or 60 ppm, 6 hours/day, 5 days/week for 105 weeks. The lowest exposure level in both studies, 10 ppm, was a lowest-observed-adverse-effect level (LOAEL) in both sexes of both species for nonneoplastic lesions in nasal olfactory epithelium (metaplasia in mice, and hyperplasia, atrophy, and chronic inflammation in rats) and respiratory epithelium (hyperplasia in mice, and hyperplasia, metaplasia, hyaline degeneration, or gland hyperplasia in rats). At 10 ppm, nearly all of the animals showed nasal lesions. Exposed rats also showed increased incidences of nasal tumors (respiratory epithelial adenomas and olfactory epithelial neuroblastomas), but mice did not develop nose tumors. Exposed mice also showed an increased incidence of chronic lung inflammation at both exposure levels and an increased incidence of lung tumors in females exposed to 30 ppm. Lung lesions did not occur in exposed rats.

Following EPA (1994b) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, equations for a category 1 gas producing nasal effects were used to derive human equivalent concentrations of 0.2 ppm based on the rat data and 0.3 ppm based on the mouse data (see Appendix B). Using public health protection reasoning, the $LOAEL_{HEC}$ based on the rat data, 0.2 ppm, was selected as the point of departure for the chronic inhalation MRL, which was divided by a total uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability) to derive the MRL of 0.0007 ppm ($3x10^{-3}$ mg/m^3).

No appropriate data were located on effects of acute- and intermediate-duration inhalation exposure in humans or animals that could be used to derive acute and intermediate MRLs for inhalation exposure to naphthalene.

No appropriate data were located for deriving inhalation MRLs for 1-methylnaphthalene or 2-methyl-naphthalene.

Oral MRLs

- An MRL of 0.6 mg/kg/day was derived for acute oral exposure to naphthalene.

A rat developmental toxicity study involving exposure of Sprague-Dawley rats to gavage doses of 50, 150, or 450 mg/kg/day naphthalene on gestation days 6-15 was selected as the basis of the acute oral MRL (NTP 1991a). The only maternal or fetal effects observed at the lowest dose level were slow respiration, lethargy, or prone body posture in most dams following dose administration on the first and second day of dosing. These effects did not occur on subsequent days of dosing at this dose level. Because of the transient nature of these observations and the lack of any other effect, 50 mg/kg/day was judged to be a minimal lowest-observed-adverse-effect level (LOAEL) for clinical signs of toxicity. At 150 and 450 mg/kg/day, clinical signs of toxicity were more persistent and were accompanied with severe decreases in body weight gain during the exposure period (31 and 53%, respectively, compared with controls). No exposure-related fetal effects were found in any of the exposure groups compared with the controls in this study.

The MRL was calculated from the minimal LOAEL of 50 mg/kg/day using an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability) to derive the MRL of 0.6 mg/kg/day (see Appendix A). An uncertainty factor of 3 was used for human variability because the critical effect is based on effects in a sensitive animal subpopulation. Pregnant rats appear to be more sensitive for the effects observed (clinical signs of toxicity in response to gavage exposure and decreased body weight gain) than nonpregnant rats. In 13-week gavage studies with nonpregnant rats (NTP 1980b), similar persistent clinical signs were not observed following administration of doses as high as 200 mg/kg/day, but were observed at 400 mg/kg/day. In nonpregnant rats exposed for 13 weeks, significant body weight decreases occurred at 200 mg/kg/day throughout exposure, but not at 100 mg/kg/day (NTP 1980b) or in nonpregnant mice exposed for 13 weeks to 133 mg/kg/day (Shopp et al. 1984) or 200 mg/kg/day (NTP 1980a). Mice in the NTP (1980a) study showed transient signs of toxicity (lethargy, rough hair coats, and decreased food consumption), but these only occurred between weeks 3 and 5 in the 200-mg/kg/day group.

- The acute-duration oral MRL of 0.6 mg/kg/day is adopted as the intermediate-duration oral MRL for naphthalene.

There are three intermediate-duration oral toxicity studies in laboratory animals that were considered for deriving the intermediate-duration oral MRL for naphthalene. A 13-week comprehensive oral toxicity study in Fischer 344 rats found no adverse exposure-related effects other than decreased body weight (NTP 1980b). This study identified 100 mg/kg/day as a no-observed-adverse-effect level (NOAEL) and 200 mg/kg/day as a LOAEL for decreased body weight in male and female rats. Another 13-week

comprehensive oral toxicity study in B6C3F1 mice found no adverse effects in mice exposed to doses as high as 200 mg/kg/day (NTP 1980a). Another 90-day gavage study in CD-1 mice focused on immune system variables and other toxicity variables (e.g., body weight, organ weight, haematological parameters) and identified 133 mg/kg/day as a LOAEL and 53 mg/kg/day as a NOAEL for weight decreases in several organs (brain, liver, and spleen), but found no biologically significant exposure-related changes in other end points evaluated (Shopp et al. 1984). This study, however, did not include histopathological examination of tissues.

The findings from the three intermediate-duration oral toxicity studies do not collectively identify a clear, biologically significant target of toxicity other than body weight changes in rats (see Appendix A for comprehensive descriptions of the design and results of these studies). Consideration was given to basing the MRL on the NOAEL of 53 mg/kg/day and LOAEL of 133 mg/kg/day for decreases in absolute weight of brain, liver, and spleen, and in relative weight of spleen, in female mice (Shopp et al. 1984). However, the biological significance of these effects is uncertain because (1) the effects were only observed in females, and (2) histological effects in the affected organs were not observed in the other 13-week oral studies with rats and mice.

As discussed in Appendix A, a potential intermediate-duration MRL of 0.7 mg/kg/day was derived based on the duration-adjusted NOAEL of 71 mg/kg/day for decreased body weight in male and female rats exposed by gavage to naphthalene 5 days/week for 13 weeks (NTP 1980b) and a total uncertainty factor of 100 (10 for extrapolating from rats to humans and 10 for human variability). Because the value of 0.7 mg/kg/day is slightly larger than the acute-duration oral MRL of 0.6 mg/kg/day, the acute MRL is expected to be protective for intermediate-duration exposure scenarios and was adopted as the intermediate-duration oral MRL.

No appropriate studies were located for deriving an MRL for chronic oral exposure to naphthalene. One chronic study was located that examined the toxicity of naphthalene in rats (Schmahl 1955). No treatment-related effects were reported at a dose level of 41 mg/kg/day for 700 days. The study was not suitable as the basis for deriving a chronic MRL because only one dose level was evaluated, histopathological examination was limited, and dosing was not precisely controlled.

- An MRL of 0.07 mg/kg/day was derived for chronic oral exposure to 1-methylnaphthalene.

The MRL for 1-methylnaphthalene was derived from an 81-week study in groups of 50 male and 50 female mice using diets containing 0, 71.6 (males), 75.1 (females), 140.2 (males), or 143.7 (females) mg/kg/day (Murata et al. 1993). Food intake, clinical signs, and body weight were determined throughout the study. At the end of 81 weeks, peripheral blood samples were collected and the animals were sacrificed. Organ weights were determined and the tissues examined histologically; tumors were identified and characterized. Hematological parameters and biochemical indices were evaluated in the blood samples.

Male and female mice in both exposure groups showed increased incidences of pulmonary alveolar proteinosis. In males, there was also a significant increase in pulmonary adenomas. The alveolar nodules were filled with an amorphous acidophilic material, cholesterol crystals, and foamy cells. They were not accompanied by inflammation, edema, or fibrosis. The LOAEL of 71.6 mg/kg/day for pulmonary alveolar proteinosis in female mice was used for the derivation of the MRL (see Appendix A), employing an uncertainty factor of 1,000 (10 for using a LOAEL, 10 for extrapolating from animals to humans, and 10 for human variability).

- An MRL of 0.04 mg/kg/day was derived for chronic oral exposure to 2-methylnaphthalene.

The chronic MRL is based on a study in which groups of 50 male and 50 female B6C3F1 mice were exposed to dietary levels of 0, 0.075, or 0.15% 2-methylnaphthalene (Murata et al. 1997). Average intakes were reported as 0, 54.3, or 113.8 mg/kg/day for males and 0, 50.3, or 107.6 mg/kg/day for females. Survival and food consumption were not affected by exposure. Mean final body weights were decreased by 7.5 and 4.5% in high-dose males and females, respectively; these changes are not considered to be biologically significant. Histopathology only found exposure-related changes in the lung. Tissues examined were brain, heart, kidney, liver, lung, pancreas, salivary glands, spleen, testis, adrenals, bone, eye, Harderian glands, mammary gland, ovary, seminal vesicle, skeletal muscle, skin, small and large intestine, spinal cord, stomach, trachea, uterus, and vagina. No evidence of bronchiolar Clara cell necrosis or sloughing was found. Females showed statistically significantly decreased differential counts of stab and segmented form neutrophils and increased lymphocytes compared to controls, but the biological significance of these changes is not clear due to a lack of reporting of the data (i.e., the report did not specify the response magnitudes or the dose levels at which they occurred). Incidences for mice with pulmonary alveolar proteinosis were (control through high-dose groups): 5/50, 27/49, and 22/49 for females, and 4/49, 21/49, and 23/49 for males. Incidences for mice with lung adenomas were: 4/50, 4/49, and 5/48 in females, and 2/49, 9/49, and 5/49 in males. Only the lung adenoma incidence in the male

54.3-mg/kg/day groups was significantly different from the control incidence. Combined incidences for lung adenomas or adenocarcinomas were: 5/50, 4/49, and 6/48 for females, and 2/49, 10/49, and 6/49 for males.

Support for pulmonary alveolar proteinosis as the critical effect for the chronic oral MRL for 2-methylnaphthalene comes from chronic duration studies with the isomer, 1-methylnaphthalene, and methylnaphthalene (a mixture of 1- and 2-methylnaphthalene). Increased incidence of pulmonary alveolar proteinosis was reported in B6C3F1 mice exposed to 1-methylnaphthalene in the diet for 81 weeks at dose levels as low as 71.6 mg/kg/day (Murata et al. 1993), and in mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene for 30–61 weeks (a mixture of 1- and 2-methylnaphthalene) (Emi and Konishi 1985; Murata et al. 1992).

The lower 95% confidence limit on a benchmark dose associated with 5% extra risk for pulmonary alveolar proteinosis in male mice (4 mg/kg/day) was selected as the point of departure for deriving the chronic-duration oral MRL for 2-methylnaphthalene (see Appendix A). A benchmark response of 5% extra risk was selected over a default value of 10% extra risk in order to provide protection for children who may develop pulmonary alveolar proteinosis. This selection is supported by reports that children with pulmonary alveolar proteinosis (albeit of unknown etiology) experience more severe symptoms of respiratory dysfunction than do adults (EPA 2003r; Mazzone et al. 2001). The point of departure was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive the chronic oral MRL of 0.04 mg/kg/day for 2-methylnaphthalene.

No appropriate studies were located for deriving acute or intermediate-duration oral MRLs for 1-methylnaphthalene or 2-methylnaphthalene.

3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between

"less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Levels of exposure associated with carcinogenic effects (Cancer Effect Levels, CELs) of naphthalene, are indicated in Tables 3-1 and 3-2 and Figures 3-1 and 3-2.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

3.2.1.1 Death

Two Greek infants died as a consequence of acute hemolysis that resulted from exposure to naphthalene-treated materials (clothing, diapers, blankets, rugs, etc.). Both infants exhibited a severe form of jaundice (kernicterus), which often causes brain damage (Valaes et al. 1963). Exposure levels experienced by these children are unknown. One infant suffered from a glucose-6-phosphate dehydrogenase (G6PD) deficiency. The other infant was apparently heterozygous for this trait. Individuals with a G6PD genetic defect are prone to hemolysis after exposure to a variety of chemical oxidizing agents including nitrates, nitrites, aniline, phenols (Dean et al. 1992), and naphthalene.

No studies were located that documented lethal effects in humans after inhalation exposure to 1-methyl-naphthalene or 2-methylnaphthalene.

Exposure to 78 ppm naphthalene for 4 hours did not cause any deaths in rats. In addition, no definitive adverse clinical signs were observed during the 14 days after exposure, and no gross pathologic lesions were observed at necropsy (Fait and Nachreiner 1985). A high background mortality in the male control group precluded drawing conclusions regarding the effects of lifetime exposures to 10 and 30 ppm naphthalene (6 hours/day, 5 days/week) on lifetime mortality; no apparent effects on mortality occurred in the females (NTP 1992a). Similarly, exposure of male and female rats to 10, 30, or 60 ppm naphthalene (6 hours/day, 5 days/week) for 2 years did not affect survival, compared to controls (Abdo et al. 2001; NTP 2000).

No studies were located that documented lethal effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.1.2 Systemic Effects

No studies were located that documented dermal effects in humans or animals after inhalation exposure to naphthalene. Most of the human data come from occupational and domestic settings where mothballs were the source of the naphthalene vapors. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-1 and plotted in Figure 3-1. No studies were located that documented systemic effects in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene. In animals, one study evaluated hematological end points in dogs following acute inhalation exposure to undetermined air concentrations of 1-methylnaphthalene or 2-methylnaphthalene (Lorber 1972). This study, however, did not identify reliable NOAEL or LOAEL values, and the results are not included in Table 3-1 or Figure 3-1.

Respiratory Effects. No studies were located that documented respiratory effects in humans after inhalation exposure to naphthalene.

The nose is the most sensitive toxicity target in rats and mice following chronic inhalation exposure to naphthalene. Chronic inhalation exposure resulted in increased incidences of nonneoplastic and neoplastic lesions in the nose of rats (Abdo et al. 2001; Long et al. 2003; NTP 2000), nonneoplastic lesions in the nose of mice (NTP 1992a), and neoplastic and nonneoplastic lesions in the lungs of mice (NTP 1992a). No exposure-related lesions were found in other tissues or organs in these studies, which included comprehensive histopathological examinations of major tissues and organs. Nearly all mice of

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	ACUTE EXPOSURE						
	Systemic						
1	Rat (Sprague-Dawley)	4 h	Resp	100			West et al. 2001 NAP
2	Mouse B6C3F1	14 d 5 d/wk 6 hr/d	Hemato	30			NTP 1992a NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)		LOAEL Serious (ppm)		Reference Chemical Form
3	Mouse (Swiss-Webster)	4 h	Resp	2	10	(Clara cell necrosis and decreased Clara cell mass [volume/surface area] in proximal airways)	75	(Proximal and terminal epithelium devoid of Clara cells)	West et al. 2001 NAP
	Neurological								
4	Rat (Wistar)	4 h		26	44	(increased latency of paw lick response to being placed on a hot surface [decreased pain sensitivity]; no change in rotarod performance)			Korsak et al. 1998 1-MN
5	Rat (Wistar)	4 h		39	61	(increased latency of paw lick response to being placed on a hot surface [decreased pain sensitivity]; no change in rotarod performance)			Korsak et al. 1998 2-MN

[a]

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Systemic						
6	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor	Resp		10[b] (inflammation of the nose; olfactory epithelium: atypical hyperplasia, atrophy, degeneration; nasal respiratory epithelium: hyperplasia, squamous metaplasia, degeneration; Bowman's glands: hyperplasia)		NTP 2000 (Abdo et al. 2001) NAP
			Cardio	10			
			Gastro	10			
			Musc/skel	10			
			Hepatic	10			
			Renal	10			
			Endocr	10			
			Ocular	10			
			Bd Wt	10			

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
7	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d	Resp		10 (inflammation of the nose and lung, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium)		NTP 1992a NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
8	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d	Cardio	30			NTP 1992a NAP
			Gastro	30			
			Hepatic	30			
			Renal	30			
			Dermal	30			
	Neurological						
9	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor		60			NTP 2000 (Abdo et al. 2001) NAP

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference Chemical Form
10	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d		30			NTP 1992a NAP
	Reproductive						
11	Rat (Fischer- 344)	105 wk 5 d/wk 6 hr/d vapor		60			NTP 2000 (Abdo et al. 2001) NAP

[a]

Table 3-1 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Inhalation (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)		Reference Chemical Form
12	Mouse B6C3F1	104 wk 5d/wk 6 hr/d		30				NTP 1992a NAP
	Cancer							
13	Rat (Fischer- 344)	105 wk 5d/wk 6hr/d vapor				10	(CEL: nasal respiratory epithelial adenomas in males & in females at higher concentrations; olfactory epithelial neuroblastomas in both sexes at higher concentrations)	NTP 2000 (Abdo et al. 2001) NAP
14	Mouse B6C3F1	104 wk 5 d/wk 6 hr/d				30	(CEL: pulmonary alveolar adenomas in females)	NTP 1992a NAP

a The number corresponds to the entries in Figure 3-1.

b Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.0007 ppm; based on a human equivalent concentration LOAEL of 0.2 ppm which was divided by an uncertainty factor of 300 (10 for the use of LOAEL, 3 for extrapolating from rodents to humans with interspecies dosimetric adjustment, and 10 for human variability).

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

Figure 3-1. Levels of Significant Exposure to Naphthalene (NAP) Or Methylnaphthalene (1-MN Or 2-MN)- Inhalation

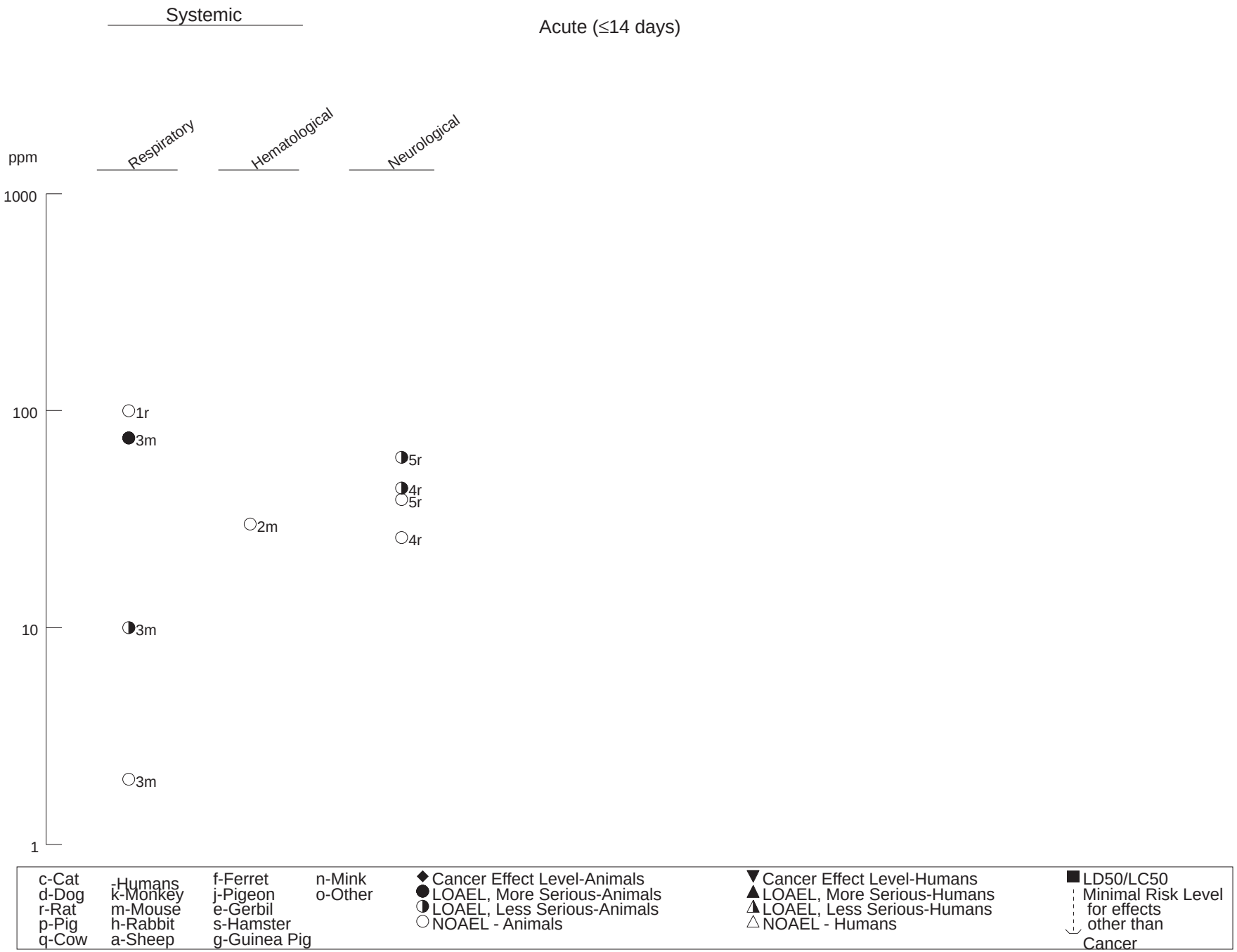
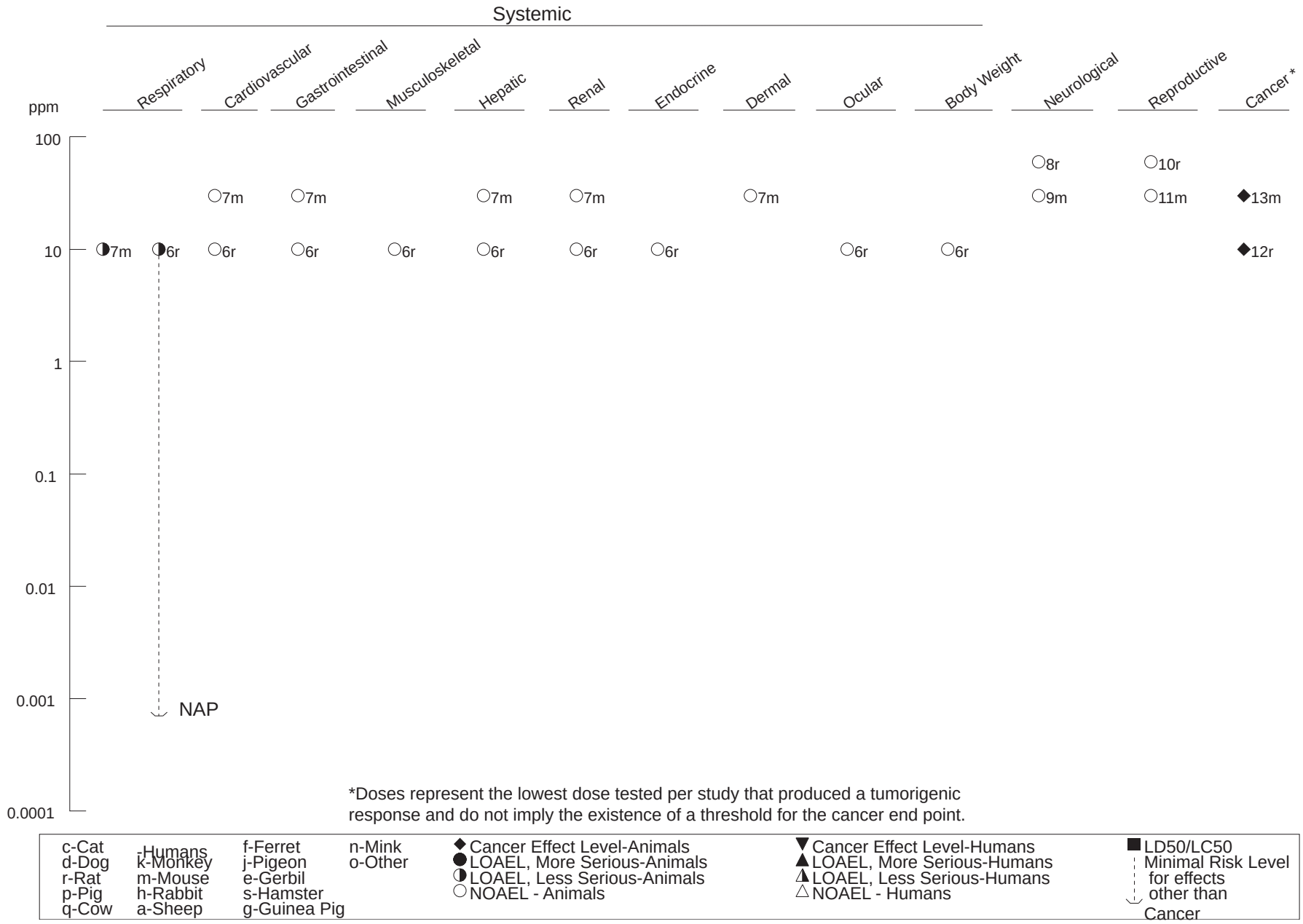


Figure 3-1. Levels of Significant Exposure to Naphthalene (NAP) Or Methylnaphthalene (1-MN Or 2-MN) - Inhalation (*Continued*)

Chronic (≥365 days)



both sexes (>95%) exposed to naphthalene vapors for 2 years (10 or 30 ppm) showed chronic inflammation and metaplasia of the olfactory epithelium and hyperplasia of the nasal respiratory epithelium (NTP 1992a). Chronic lung inflammation was also observed in exposed mice, but at lower incidences than incidences for nasal lesions. Incidences for chronic lung inflammation were 0/70, 21/69, and 56/135 for male mice and 3/69, 13/65, and 52/135 for female mice exposed to 0, 10, or 30 ppm. In addition, exposure to 30 ppm (but not 10 ppm) increased the incidence of benign lung tumors (alveolar/bronchiolar adenomas) in female mice, compared with controls. One other female mouse exposed to 30 ppm showed a malignant lung tumor (alveolar/bronchiolar carcinoma). In rats of both sexes, inhalation of 10, 30, or 60 ppm naphthalene induced nonneoplastic and neoplastic lesions only in the nasal cavity (Abdo et al. 2001; NTP 2000). Nearly all rats in each exposure group (>95%) showed nonneoplastic nasal lesions. Nonneoplastic nasal lesions in exposed rats included (1) hyperplasia, atrophy, chronic inflammation, and hyaline degeneration of the olfactory epithelium and (2) hyperplasia, metaplasia, or degeneration of the respiratory epithelium or glands. Neoplastic lesions associated with naphthalene exposure in rats were olfactory epithelial neuroblastoma (a rare malignant tumor) and respiratory epithelial adenoma.

The chronic inhalation MRL for naphthalene is based on the LOAEL of 10 ppm for nonneoplastic lesions in the olfactory epithelium and respiratory epithelium of the nose of rats (NTP 2000; see Table 3-1, Figure 3-1, Appendix A, and Section 2.3). To derive the chronic MRL, the rat LOAEL was converted to a human equivalent concentration of 0.2 ppm for continuous exposure using EPA (1994b) equations for a category 1 gas producing nasal effects and divided by an uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability). Naphthalene-induced damage to the nasal tissue is thought to be due to reactive metabolites formed in the nasal tissues (Buckpitt et al. 2002). Sections 3.4.3 and 3.5 discuss current mechanistic hypotheses in more detail.

Acute (4-hour) inhalation exposure to naphthalene induced necrosis of Clara cells in the epithelium of the proximal airways of the lungs of mice at exposure levels as low as 10 ppm, but did not affect lung tissue in rats at concentrations as high as 100 ppm (West et al. 2001). These results, and those from the chronic inhalation studies, show that mice are more susceptible than rats to lung damage from inhaled naphthalene. However, there are no studies that have examined nasal tissues for the development of lesions following acute inhalation exposure. No acute inhalation MRL was derived for naphthalene, due to the lack of such data and the results of the chronic studies indicating that nasal tissues are the critical toxicity targets of inhaled naphthalene in both rats and mice.

A change to mouth breathing occurred in rats during exposure to 78 ppm naphthalene, but no other effects on respiration were noted (Fait and Nachreiner 1985).

Cardiovascular Effects. No studies were located that documented cardiovascular effects in humans after inhalation exposure to naphthalene.

No histological changes were seen in the hearts of mice (30 ppm) or rats (60 ppm) that were exposed to naphthalene for 2 years (Abdo et al. 2001; NTP 1992a, 2000).

Gastrointestinal Effects. Nausea, vomiting, and abdominal pain were reported in eight adults and one child exposed to naphthalene vapors from large numbers of mothballs (300–500) scattered throughout their homes for odor and pest control (Linick 1983). Air samples collected in one home contained naphthalene at 20 ppb; concentrations could have been higher when the mothballs were fresh. Gastrointestinal symptoms disappeared after the mothballs were removed. Few location-specific background data to support this air concentration were reported.

There were no histopathological changes in the stomach or intestines of mice (30 ppm) or rats (60 ppm) exposed to naphthalene for 2 years (Abdo et al. 2001; NTP 1992a, 2000).

Hematological Effects. Hemolytic anemia is the most frequently reported manifestation of naphthalene exposure in humans. Acute hemolytic anemia was observed in 21 infants exposed to naphthalene via mothball-treated blankets, woolen clothes, or materials in the infants' rooms (Valaes et al. 1963). Ten of these children had a G6PD genetic defect that increased their sensitivity to hemolysis from a variety of chemicals, including naphthalene. Clinical observations included high serum bilirubin values, methemoglobin, Heinz bodies, and fragmented red blood cells. Inhalation appeared to be the primary route of exposure because in all children but two, the naphthalene-treated material was not worn next to the skin. One of the exceptions was an infant who wore diapers that had been stored in naphthalene.

Anemia was reported in nine individuals exposed to large numbers of mothballs distributed throughout their homes (Linick 1983). The nature of the anemia and specific levels of naphthalene exposure were not identified. In one home, the naphthalene concentration was determined to be 20 ppb at the time of testing, but could have been higher when the mothballs were first distributed.

In another study, a woman who was exposed to reportedly high (but unmeasured) concentrations of a combination of naphthalene and paradichlorobenzene for several weeks in a hot, poorly ventilated work area developed aplastic anemia (Harden and Baetjer 1978). It is difficult to determine the contribution of naphthalene to the aplastic anemia since there was simultaneous exposure to paradichlorobenzene.

In animals, no treatment-related effects on hematologic parameters (hematocrit, hemoglobin concentration, erythrocyte counts, mean cell volume, reticulocytes, and leucocytes) were observed among mice exposed to 10 and 30 ppm naphthalene for 14 days (NTP 1992a). Due to high mortality in the control males, hematology measurements were not continued beyond 14 days.

The effects of 1-methylnaphthalene (pure and practical grade) and 2-methylnaphthalene (pure and practical grade) on the hematocrit values, total and differential white blood cell counts, and reticulocyte counts were determined in intact and splenectomized dogs. Each compound was dispersed in the atmosphere in a refined kerosene base using a fogger. Exposures occurred on four consecutive mornings (Lorber 1972). Based on the information presented, it was not possible to determine the exposure concentration.

Pure 1-methylnaphthalene increased the reticulocyte counts in the splenectomized dogs but not the intact dogs. Reticulocyte values remained elevated for 10 days after the fogging ceased. Practical grade 1-methylnaphthalene increased leukocyte counts in intact and splenectomized dogs and neutrophil counts in intact dogs, but pure 1-methylnaphthalene had no effect on these parameters. 2-Methylnaphthalene had no effect on any of the parameters monitored (Lorber 1972).

Neither 1-methylnaphthalene nor 2-methylnaphthalene had an effect on hematocrit values, suggesting that these compounds do not cause hemolysis under the conditions of the study. Since the increased reticulocyte counts were seen only in splenectomized dogs, it is difficult to interpret whether or not this change signifies increased hematopoiesis in response to 1-methylnaphthalene exposure (Lorber 1972).

Musculoskeletal Effects. No studies were located that documented musculoskeletal effects in humans after inhalation exposure to naphthalene.

Histological examination of the femur did not reveal compound-related effects in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Hepatic Effects. Jaundice has been reported in infants and adults after exposure to naphthalene (Linick 1983; Valaes et al. 1963). However, the jaundice is a consequence of hemolysis rather than a direct effect of naphthalene on the liver. Infant exposures lasted 1–7 days (Valaes et al. 1963); adult exposure durations were not provided (Linick 1983). Dose was not determined in either instance, although a concentration of 20 ppb was measured in the home of one affected individual (Linick 1983).

In animals, no treatment-related gross or histopathological lesions of the liver were reported in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Renal Effects. Renal disease was reported in nine individuals (details not specified) exposed to large numbers of mothballs in their homes, but symptoms were not described and dose could not be determined (Linick 1983).

In animals, no treatment-related gross or histopathological lesions of the kidneys were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively.

Ocular Effects. Twenty-one workers exposed to naphthalene for up to 5 years in a plant that manufactured dye intermediates were examined for eye problems (Ghetti and Mariani 1956). During the period of exposure, plant conditions were primitive, involving heating of naphthalene in open vats and considerable worker contact with the naphthalene. Eight of the 21 workers developed multiple pin-point lens opacities that had no correlation with the age of the workers. These effects were not overtly noticeable and apparently had no effect on vision. They were judged to be a consequence of naphthalene exposure on the basis of their location in the crystalline lens and the fact that occurrence did not correlate with age. Exposure involved long-term inhalation of vapors and direct contact of vapors with the eyes and skin.

Retinal bleeding and the beginnings of a cataract were identified in a worker from a naphthalene storage area who was most likely exposed to naphthalene through inhalation and dermal/ocular contact (van der Hoeve 1906). The duration of exposure prior to seeking medical attention for eye irritation and problems with vision was not identified.

In animals, no treatment-related gross or histopathological lesions of the eyes were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. However, during a 4-hour exposure of rats to a concentration of 78 ppm, irritation to the eyes was evidenced through lacrimation (Fait and Nachreiner 1985).

3.2.1.3 Immunological and Lymphoreticular Effects

No studies were located that examined immunological or lymphoreticular end points in humans or animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.2.1.4 Neurological Effects

Infants are prone to permanent neurological damage (kernicterus) as a consequence of the jaundice that results from naphthalene-induced hemolysis. Bilirubin is absorbed by vulnerable brain cells and this leads to convulsions and sometimes death. Survivors often suffer from motor disturbances and mental retardation (McMurray 1977). Kernicterus was diagnosed in 8 of 21 Greek infants that experienced hemolysis as a result of naphthalene exposure (Valaes et al. 1963). Two of the eight died. One of the infants that died had no G6PD enzyme activity and the other had intermediate activity. Two of the infants were normal with regard to the G6PD trait. Of the remaining infants, three had no G6PD activity and the fourth had intermediate activity. Brain damage seldom occurs in adults as a consequence of jaundice (McMurray 1977).

Nausea, headache, malaise, and confusion were reported in several individuals (children and adults) exposed to large numbers of mothballs in their homes (Linick 1983). Actual levels and duration of exposure were unknown, although a concentration of 20 ppb was measured in one of the affected residences.

In animals, no treatment-related gross or histopathological lesions of the brain were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. Clinical observations (made twice daily in these studies) revealed no gross behavioral changes except that exposed mice tended to huddle together in cage corners during exposure periods.

No studies were located that documented neurological effects in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

In male Wistar rats, decreased sensitivity to pain occurred after 4-hour inhalation exposures to 253 or 407 mg/m^3 1-methylnaphthalene (44 or 70 ppm), or 352 or 525 mg/m^3 2-methylnaphthalene (61 or 90 ppm), but not after exposure to 152 mg/m^3 (26 ppm) 1-methylnaphthalene or 229 mg/m^3 (39 ppm) 2-methylnaphthalene (Korsak et al. 1998). Decreased sensitivity to pain was measured as a decreased time to begin licking of the paws after being placed on a hot plate at 54.5 °C. The ability of exposed rats to balance on a rotating rod (rotarod performance), however, was not affected by any of these exposure conditions (Korsak et al. 1998). NOAEL and LOAEL values for decreased pain sensitivity from this study are included in Table 3-1 and Figure 3-1.

3.2.1.5 Reproductive Effects

No studies were located that documented reproductive effects in humans after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In animals, histological examination did not reveal damage to male or female reproductive organs in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to 30 or 60 ppm, respectively.

No studies were located that documented reproductive effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.1.6 Developmental Effects

No studies were located that examined developmental end points in humans or animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.2.1.7 Cancer

No studies were located that documented carcinogenic effects in humans after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In animals, inhalation exposure to naphthalene (6 hours/day) has been associated with: (1) increased incidences of F344/N rats of both sexes with nasal tumors following 2 years of exposure (Abdo et al. 2001; NTP 2000); (2) increased incidences of female B6C3F1 mice, but not male mice, with lung tumors following 2 years of exposure (NTP 1992a); and (3) increased number of tumors per tumor-bearing A/J strain mice following 6 months of exposure (Adkins et al. 1986).

In F344/N rats, incidences of nasal respiratory epithelial adenomas were statistically significantly elevated, compared with controls, in males exposed to 0, 10, 30, or 60 ppm naphthalene (0/49, 6/49, 8/48, or 15/48), but not in females (0/49, 0/49, 4/49, 2/49) (Abdo et al. 2001; NTP 2000). Incidences for olfactory epithelial neuroblastoma were 0/49, 0/49, 4/48, and 3/48 in male rats, and 0/49, 2/49, 4/48, and 12/49 in female rats. Both tumor types are rare in NTP control F344/N rats (NTP 2000). For example, neither tumor type was observed in 299 control male rats given NTP-2000 feed or 1,048 control male rats given NIH-07 feed. NTP (2000) concluded that there was clear evidence of carcinogenic activity of naphthalene in male and female F344/N rats based on increased incidences of respiratory epithelial adenoma and olfactory epithelial neuroblastoma of the nose. Nearly all rats in all exposure groups showed nonneoplastic nasal lesions in both olfactory and respiratory epithelia, including atypical hyperplasia in olfactory epithelium, hyaline degeneration in olfactory and respiratory epithelia, and Bowman’s gland hyperplasia.

In B6C3F1 mice, statistically significant increased incidence of alveolar/bronchiolar adenomas and carcinoma was found in 30-ppm females, but not in 10-ppm females or in males (females: 5/69, 2/65, 29/135; males: 7/70, 17/69, and 31/135) (NTP 1992a). Although Fisher Exact tests indicated that incidences in both exposed male groups and the high-dose female group were significantly increased compared with control groups, logistic regression analysis, which modeled tumor incidence as a function of dose and exposure time, indicated that only the incidence in the 30-ppm female group was elevated compared with controls. The response was predominantly benign; only one female mouse in the 30-ppm group developed a carcinoma. Exposed mice of both sexes also showed increased incidences of chronic lung inflammation (males: 0/70, 21/69, 56/135; females: 3/69, 13/65, 52/135). Nonneoplastic nasal lesions were found in nearly all exposed mice, but no nasal tumors developed. On the basis of this analysis, NTP (1992a) determined that there was some evidence of naphthalene carcinogenicity in female mice, but no evidence of carcinogenicity in male mice in this study.

In a 6-month study, there was a statistically significant increase in the number of tumors per tumor-bearing mouse, but not in the number of mice with pulmonary adenomas after exposure to 10 or 30 ppm

naphthalene vapors (Adkins et al. 1986). However, the incidence of adenomas in the control group for this experiment was significantly lower than the pooled incidence observed in the control groups of eight concurrently conducted 6-month studies, and the difference in tumor incidence was not significantly greater than that of the historic controls.

No studies were located that documented carcinogenic effects in animals after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.2 Oral Exposure

3.2.2.1 Death

Death has been documented in humans who intentionally ingested naphthalene. A 17-year-old male died 5 days after the ingestion of an unknown quantity of naphthalene mothballs. Death was preceded by vomiting, evidence of gastrointestinal bleeding, blood-tinged urine, and coma (Gupta et al. 1979). A 30-year-old female died following similar sequelae 5 days after reportedly swallowing 40 mothballs (25 were recovered intact from the stomach upon autopsy) (Kurz 1987). No studies were located that documented lethal effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Several animal studies have been conducted to estimate lethal doses of naphthalene. Mice appear to be more sensitive than rats or rabbits. The LD_{50} values in male and female mice were 533 and 710 mg/kg, respectively (Shopp et al. 1984). An LD_{50} of 354 mg/kg was estimated in female mice treated with naphthalene once daily by gavage for 8 consecutive days (Plasterer et al. 1985). The dose response curve appeared to be very steep because no deaths occurred at 250 mg/kg/day, but all animals died with a dose of 500 mg/kg/day. At the 300 mg/kg/day dose, mortality was approximately 15%. In a different study with a 14-day dosing period, 10% of the males and 5% of the females died at a dose of 267 mg/kg/day, but none were affected by doses of 27 and 53 mg/kg/day (Shopp et al. 1984).

The oral LD_{50} values in male and female rats were 2,200 and 2,400 mg/kg, respectively, in one study (Gaines 1969), and 2,600 in a second study that did not differentiate by sex (Papciak and Mallory 1990). Male rats tolerated daily doses of 1,000 mg/kg without lethality, even after 18 days of administration (Yamauchi et al. 1986). In an increasing dose study, Germansky and Jamall (1988) treated male rats with naphthalene at doses beginning at 100 mg/kg/day and raised the dose weekly to a final level of 750 mg/kg/day over 6 weeks. Doses were then kept constant for an additional 3 weeks. The animals

tolerated 750 mg/kg/day with no mortalities. No increase in mortality was observed in rats administered naphthalene at 41 mg/kg/day in a 2-year feeding study (Schmahl 1955).

Although few data are available, rabbits appear to tolerate naphthalene in doses similar to those administered to rats. Two different rabbit strains were administered 1,000 mg/kg twice per week for 12 weeks without lethality (Rossa and Pau 1988).

Male and female mice survived oral exposure to doses of 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). No studies were located that documented lethal effects in animals after ingestion of 1-methylnaphthalene.

All LOAEL values for lethality in each species after acute exposure to naphthalene are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.2 Systemic Effects

No studies were located that documented musculoskeletal or dermal effects in humans or animals after oral exposure to naphthalene; data were available for all other systems. The highest NOAEL values and all LOAEL values from each reliable study for systemic effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

No studies were located that documented systemic effects in humans after oral exposure to 1-methyl-naphthalene or 2-methylnaphthalene. In animals, data are restricted to two studies with B6C3F1 mice exposed to 1-methylnaphthalene (Murata et al. 1993) or 2-methylnaphthalene (Murata et al. 1997) in the diet for 81 weeks. The highest chronic NOAEL values and the lowest LOAEL value for systemic effects in mice are recorded in Table 3-2 and plotted in Figure 3-2.

Respiratory Effects. No reports have been located to indicate that there are direct effects of oral exposure to naphthalene on the respiratory system in humans. In situations where respiratory effects such as hypoxia or pulmonary edema were noted, the respiratory effects appear to be secondary to hemolysis and the events leading to general multiple organ failure (Gupta et al. 1979; Kurz 1987). On hospital admission, one male infant was described as experiencing labored breathing after presumably chewing a

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
ACUTE EXPOSURE							
Death							
1	Rat Sherman	once (GO)				2200 (LD50 - male) 2400 (LD50 - female)	Gaines 1969 NAP
2	Rat Sprague-Dawley	once (GO)				2600 LD50	Papciak and Mallory 1990 NAP
3	Mouse CD-1	8 d 1x/d (GO)				300 (5/33 died)	Plasterer et al. 1985 NAP
4	Mouse CD-1	once (GO)				710 (LD50) 533 (LD50)	Shopp et al. 1984 NAP
5	Mouse CD-1	14 d 1x/d (GO)				267 (10/96 male, 3/60 female)	Shopp et al. 1984 NAP
Systemic							
6	Human	once	Gastro		109 (adbominal pain)		Gidron and Leurer 1956 NAP
			Hemato			109 (hemolytic anemia)	
			Other			109 (106 degree F fever)	
7	Rat Sprague-Dawley	9 d Gd 6-15 (GO)	Bd Wt	50		150 (31% decrease in maternal body weight gain)	NTP 1991a NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)		LOAEL Serious (mg/kg)		Reference Chemical Form
8	Rat Sprague-Dawley	once (GO)	Resp		1000	lung lesions			Papciak and Mallory 1990 NAP
9	Rat Sprague-Dawley	once (GO)	Gastro		1000	stomach lesions			Papciak and Mallory 1990 NAP
10	Rat NS	10 d 1x/d (G)	Hepatic		1000	(39% increase in liver weight; increased lipid peroxidation, aniline hydroxylase activity)			Rao and Pandya 1981 NAP
			Renal	1000					
			Ocular	1000					
11	Mouse CD-1	14 d 1x/d (GO)	Resp	267 M 53 F	267 F	(increase in lung weight)			Shopp et al. 1984 NAP
			Hemato	267					
			Hepatic	267					
			Renal	267					
			Bd Wt	53	267	(6% (female) or 13% (male) decreased final body weight)			
12	Dog NS	once (F)	Hemato				1525	(hemolysis)	Zuelzer and Apt 1949 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
13	Rabbit NS	5 d (F)	Hepatic	2000					Srivastava and Nath 1969 NAP
			Ocular				2000	(cataracts)	
14	Rabbit NS	10 d 1x/d (GO)	Ocular				1000	(lens opacities, decreased ascorbic acid in aqueous humor)	van Heyningen and Pirie 1967 NAP
Immuno/ Lymphoret									
15	Mouse CD-1	14 d 1x/d (GO)		53	267	(30% decrease in thymus weight in males; 18% decrease in spleen weight in females)			Shopp et al. 1984 NAP
Neurological									
16	Rat Sprague-Dawley	9d Gd 6-15 (GO)			50[b]	(transient clinical signs of toxicity in dams; at higher exposure levels, signs were more persistent and accompanied by decreases in body weight gain)			NTP 1991a NAP
17	Mouse CD-1	14 d (GO)		267					Shopp et al. 1984 NAP
Reproductive									
18	Rat Sprague-Dawley	9 d Gd 6-19 (GO)		450					NTP 1991a NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
19	Mouse CD-1	8d Gd 7-14 (GO)					300	(>10% maternal mortality)	Plasterer et al. 1985 NAP
20	Rabbit New Zealand white	14 d Gd 6-19 (GO)		120					NTP 1992b NAP
Developmental									
21	Rat Sprague-Dawley	9 d Gd 6-15 (GO)					150	(decreased maternal weight gain >20%; no fetotoxic or teratogenic effects at 150 or 450 mg/kg/day)	NTP 1991a NAP
				50					
22	Mouse CD-1	8d Gd 7-14 (GO)		300					Plasterer et al. 1985 NAP
23	Rabbit New Zealand white	14 d Gd 6-19 (GO)		120					NTP 1992b NAP
24	Rabbit New Zealand white	13 d 1x/d Gd 6-18 (G)		40	200	(maternal dyspnea, cyanosis, body drop, hypoactivity with no pathological aberrations)			PRI 1985, 1986 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
INTERMEDIATE EXPOSURE							
Systemic							
25	Rat blue spruce	9 wk 3.5d/wk (GO)	Resp	169			Germansky and Jamall 1988 NAP
			Hepatic		169 (elevated lipid peroxides)		
			Bd Wt			169 (20% decreased body weight gain)	
26	Rat Brown-Norway	4 wk 3.5d/wk (GO)	Ocular			500 (lens opacity)	Kojima 1992 NAP
27	Rat Sprague-Dawley Brown-Norway	6 wk	Ocular			500 (cataract formation)	Murano et al. 1993 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
28	Rat Fischer 344	13 wk 5x/wk (GO)	Resp	400					NTP 1980b NAP
			Cardio	400					
			Gastro		400	(intermittent diarrhea)			
			Hemato	400					
			Hepatic	400					
			Renal	200 M 400 F	400 M	(10% had cortical tubular degeneration)			
			Ocular	400					
			Bd Wt	100	200	(decreased terminal body weight: 12% male & 6% female)	400		
29	Rat black-hooded	79 d (GO)	Ocular				5000	(lens opacity)	Rathbun et al. 1990 NAP
30	Rat Brown-Norway	102 d NS (GO)	Ocular				700	(lens opacity)	Tao et al. 1991 NAP
31	Rat 5 strains	4-6 wk (GO)	Ocular				1000	(lens opacity)	Xu et al. 1992b NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
32	Rat Wistar	18 d 1x/d (G)	Hepatic		1000 (elevated lipid peroxides)		Yamauchi et al. 1986 NAP
			Ocular			1000 (cataracts)	
33	Mouse B6C3F1	13 wk 5x/wk 1x/d (GO)	Resp	200			NTP 1980a NAP
			Cardio	200			
			Gastro	200			
			Hemato	200			
			Hepatic	200			
			Renal	200			
			Ocular	200			
			Bd Wt	200			

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
34	Mouse CD-1	90 d 7d/wk 1x/d (GO)	Resp	133					Shopp et al. 1984 NAP
			Hemato	133					
			Hepatic	133					
			Renal	133					
			Bd Wt	133					
			Other	53	133	(decreases in absolute weights of brain (9%), liver (18%), and spleen (28%) and relative weight of spleen (24%) in females only)			
35	Rabbit NS	5 wk	Ocular				500	(destruction of retinal photoreceptors and vascularization of the retinal area)	Orzalesi et al. 1994 NAP
36	Rabbit Chinchilla Bastard New Zealand white	12 wk 2d/wk 1x/d (GO)	Ocular				1000	(cataracts)	Rossa and Pau 1988 NAP
37	Rabbit NS	4 wk 1x/d (GO)	Ocular		1000	(increased ascorbic acid in lens)			van Heyningen 1970 NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
38	Rabbit NS	4 wk 1x/d (GO)	Ocular			1000 (lens opacities, retinal damage)	van Heyningen and Pirie 1967 NAP
Immuno/ Lymphoret							
39	Rat Fischer 344	13 wk 5d/wk 1x/d (GO)			400 (lymphoid depletion of thymus in 2/10 females)		NTP 1980b NAP
				200			
40	Mouse CD-1	90 d (GO)		133			Shopp et al. 1984 NAP
Neurological							
41	Rat Fischer 344	13 wk 5x/wk (GO)			400 (hunched posture and lethargy)		NTP 1980b NAP
42	Mouse B6C3F1	13 wk 5d/wk 1x/d (GO)		200			NTP 1980a NAP
43	Mouse CD-1	90 d (GO)		133			Shopp et al. 1984 NAP
Reproductive							
44	Rat Fischer 344	13 wk 5x/wk (GO)		400			NTP 1980b NAP

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
45	Mouse B6C3F1	13 wk 5d/wk 1x/d (GO)		200			NTP 1980a NAP
CHRONIC EXPOSURE							
Systemic							
46	Mouse B6C3F1	81 wk (F)	Resp		71.6[c] (increased incidence of pulmonary alveolar proteinosis in males and females)		Murata et al. 1993 1-MN
			Cardio	143.7			
			Gastro	143.7			
			Hemato	143.7			
			Hepatic	143.7			
			Renal	143.7			
			Endocr	143.7			
			Bd Wt	143.7			

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
47	Mouse (B6C3F1)	81 wk (F)	Resp		50.3[d] (increased incidence of pulmonary alveolar proteinosis in males and females)		Murata et al. 1997 2-MN
			Cardio	113.8			
			Gastro	113.8			
			Hemato	113.8			
			Musc/skel	113.8			
			Hepatic	113.8			
			Renal	113.8			
			Dermal	113.8			
			Ocular	113.8			
			Bd Wt	113.8			
Immuno/ Lymphoret							
48	Mouse B6C3F1	81 wk 1x/d		143.7			Murata et al. 1993 1-MN
		(F)					
49	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Neurological							
50	Mouse B6C3F1	81 wk 1x/d (F)		143.7			Murata et al. 1993 1-MN
51	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN
Reproductive							
52	Mouse B6C3F1	81 wk 1x/d (F)		143.7 F			Murata et al. 1993 1-MN
53	Mouse (B6C3F1)	81 wk (F)		113.8			Murata et al. 1997 2-MN

Table 3-2 Levels of Significant Exposure to Naphthalene (nap) Or Methylnaphthalene (1-mn Or 2-mn) - Oral (continued)

Key to Figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
Cancer								
54	Mouse B6C3F1	81 wk 1x/d (F)				71.6	(CEL: increased incidence of lung adenomas in males only)	Murata et al. 1993 1-MN
55	Mouse (B6C3F1)	81 wk (F)				54.3	(CEL: increased incidence of lung adenomas in males only; not at higher exposure level in males or in females at either exposure level)	Murata et al. 1997 2-MN

a The number corresponds to the entries in Figure 3-2

b Used to derive an acute-duration Minimal Risk Level (MRL) of 0.6mg/kg/day; based on a minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity in pregnant rats, which was divided by an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). Based on an analysis of results from the three available intermediate-duration oral toxicity studies in animals (NTP 1980a,b; Shopp et al. 1984), the acute-duration MRL is expected to be applicable to and protective for intermediate-duration exposure scenarios (see Section 2.3 and Appendix A).

c Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.07 mg/kg/day for 1-MN; based on a LOAEL of 71.6 mg/kg/day which was divided by an uncertainty factor of 1000 (10 for use the use of a LOAEL, 10 for extrapolation from animals to humans; and 10 for human variability)

d Used to derive a chronic-duration Minimal Risk Level (MRL) of 0.04 mg/kg/day for 2-MN; based on a BMDL (LED05) of 4 mg/kg/day which was divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans, and 10 for human variability).

Bd Wt = body weight; BMDL (LED05) = lower 95% confidence limit on a dose associated with 5% extra risk; BUN = blood urea nitrogen; Cardio = cardiovascular; d = day(s); Endocr = endocrine; F = females; (F) = feed; (G) = gavage; Gastro = gastrointestinal; Gd = gestation day(s); (G) = gavage in oil; Hemato = hematological; hr = hour(s); Immuno = immunological; LD50 = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; M = males; NOAEL = no-observed-adverse-effect level; NS = not specified; Resp = respiratory; wk = week(s); x = time(s); 1-Mn = 1-methylnaphthalene; 2-Mn = 2-methylnaphthalene.

Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral

Acute (≤14 days)

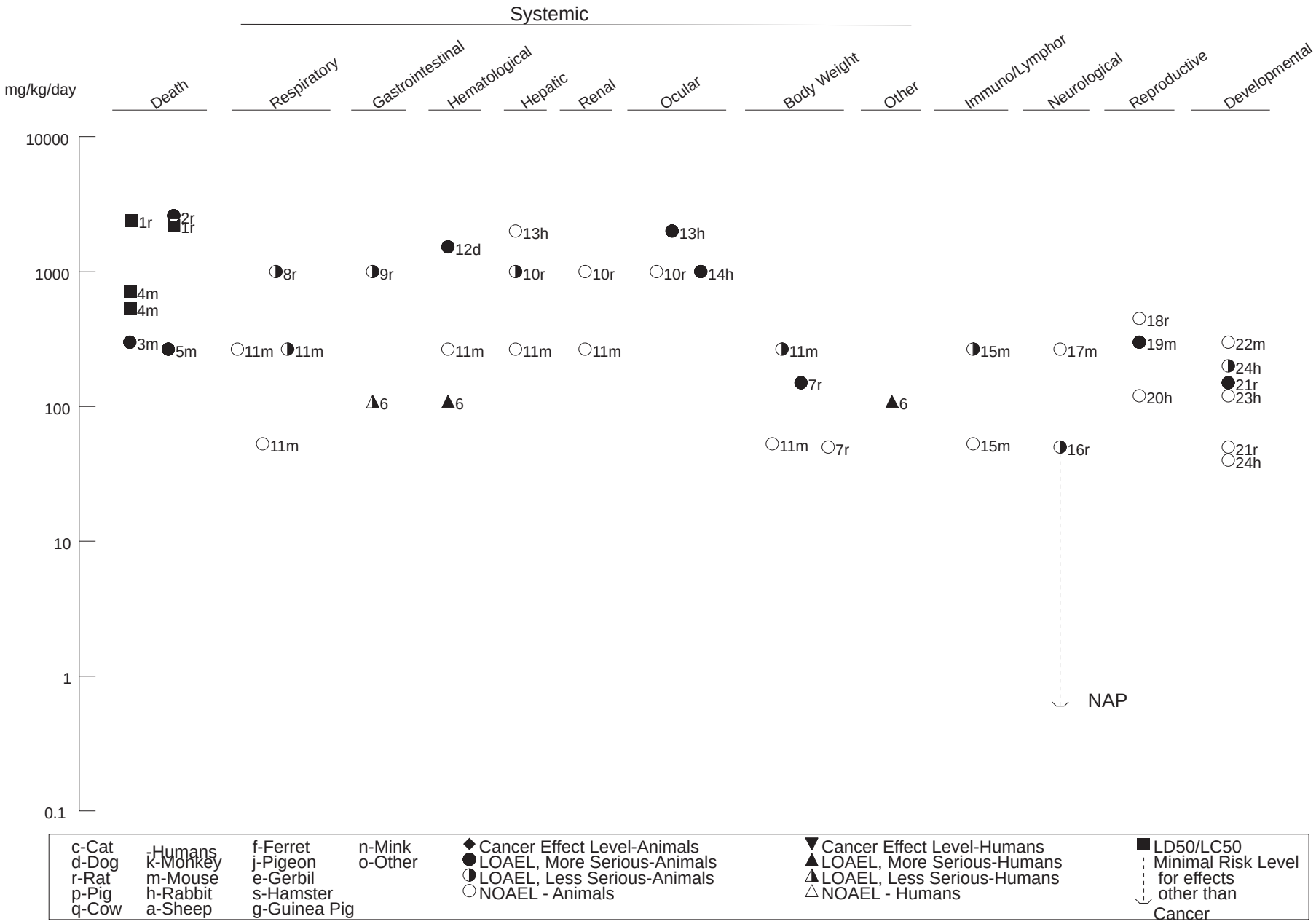


Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral (*Continued*)

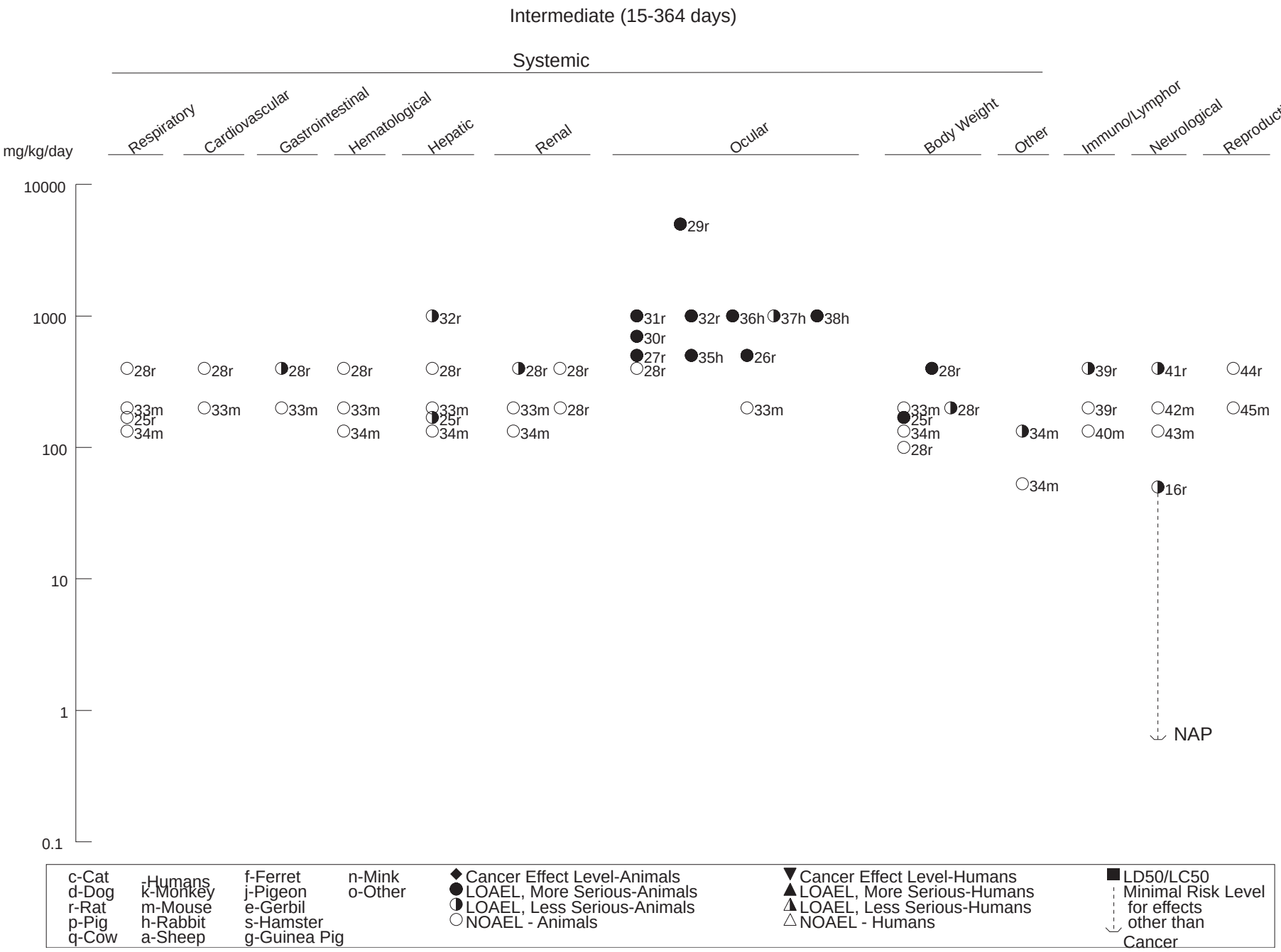
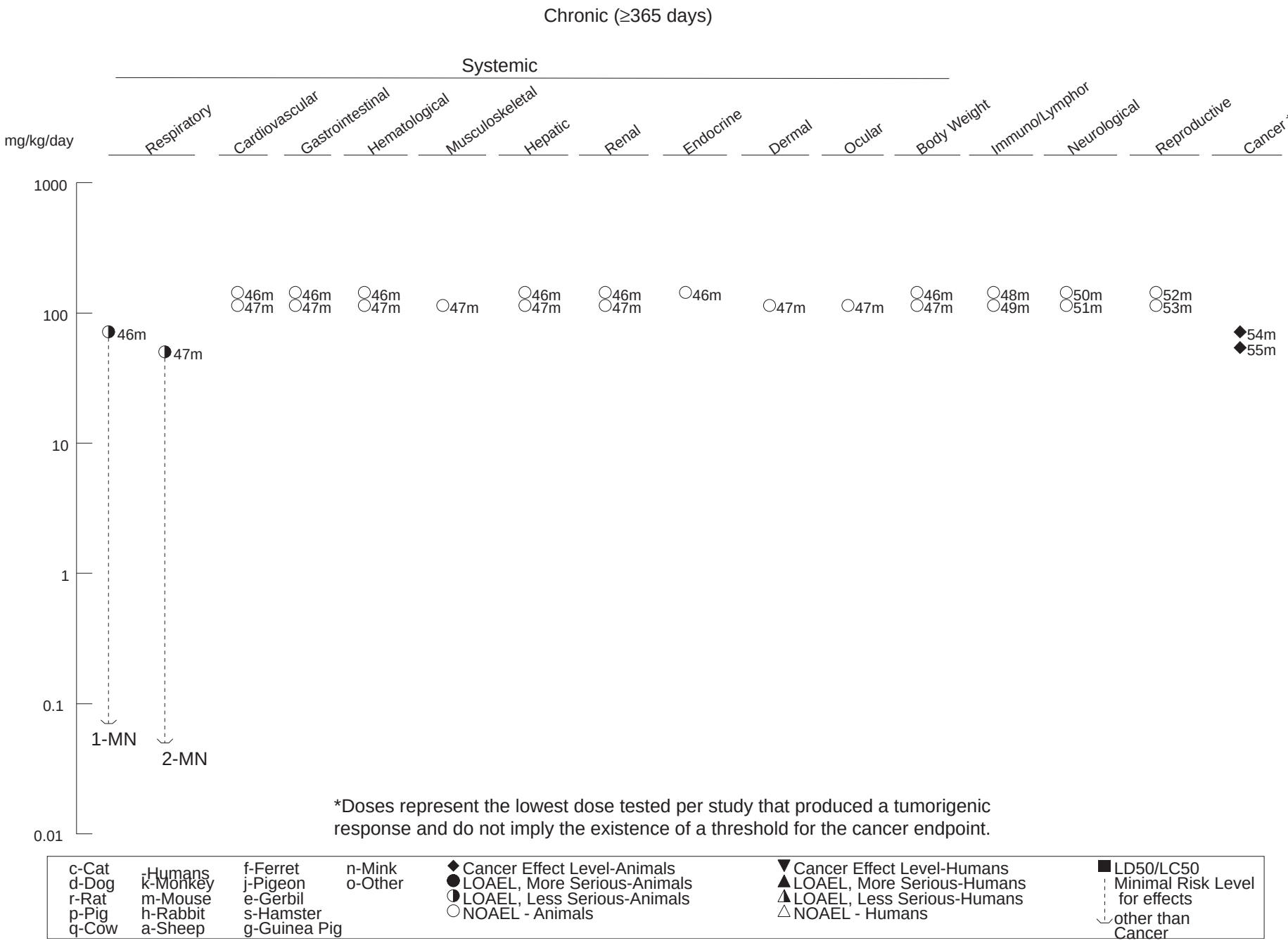


Figure 3-2. Levels of Significant Exposure to Naphthalene (NAP) or Methylnaphthalene (1-MN or 2-MN) - Oral (*Continued*)



naphthalene-containing diaper pail deodorant block (Haggerty 1956). This may have been a reflection of the reduced oxygen carrying capacity of the blood due to hemolysis.

Lesions of the lungs were seen in rats that died after being given a single large dose of naphthalene (1,000–4,000 mg/kg) during an LD_{50} study (Papciak and Mallory 1990). On the other hand, no significant respiratory toxicity was seen in rats following oral administration of naphthalene at time-weighted average doses of 169 mg/kg/day for 9 weeks (Germansky and Jamall 1988). Dosages were increased from 100 to 750 mg/kg/day over a 6-week period and held constant at 750 mg/kg/day for the last 3 weeks of the 9-week exposure period.

Lung weights were increased in female mice administered naphthalene at 267 mg/kg/day for 14 days; however, these effects were not seen in either sex at 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the lungs were noted in mice at doses up to 200 mg/kg/day (NTP 1980a) or in rats at doses of 400 mg/kg/day (NTP 1980b) after 13 weeks of exposure.

There was a significantly increased incidence of pulmonary alveolar proteinosis in male and female B6C3F1 mice fed diets containing 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The lesions contained acidophilic amorphous material, foam cells, and cholesterol crystals. There was no apparent inflammation, edema, or fibrosis of the tissues. Average administered doses were 0, 71.6, or 140.2 mg/kg/day for males and 0, 75.1, or 143.7 mg/kg/day for females. Respective incidences for pulmonary alveolar proteinosis in the control, low-, and high-dose groups were 4/49, 23/50, and 19/49 for males and 5/50, 23/50, and 17/49 for females. Histopathological examination of major organs and tissues only found exposure-related lesions in the lung. This effect was used as the basis of the chronic-duration oral MRL for 1-methylnaphthalene.

Pulmonary alveolar proteinosis is characterized by the accumulation of surfactant material in the alveolar lumen, and has been hypothesized to be caused by either excessive secretion of surfactant by type II pneumocytes, or disruption of surfactant clearance by macrophages (Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997). Electron microscopic examination of lungs of mice exposed dermally to a mixture of 1-methylnaphthalene and 2-methylnaphthalene showed that alveolar spaces were filled with numerous myelinoid structures resembling lamellar bodies of type II pneumocytes (Murata et al. 1992).

In a companion study, pulmonary alveolar proteinosis was the only exposure-related lesion found in B6C3F1 mice of both sexes exposed to 2-methylnaphthalene in the diet at doses as low as 50.3 mg/kg/day

(Murata et al. 1997). Average administered doses were 0, 54.3, or 113.8 mg/kg/day for males and 0, 50.3, or 107.6 mg/kg/day for females. Respective incidences for pulmonary alveolar proteinosis in the control, low-, and high-dose groups were 4/49, 21/49, and 23/49 for males and 5/50, 27/49, and 22/49 for females. This effect was used as the basis of the chronic-duration oral MRL for 2-methylnaphthalene.

Cardiovascular Effects. No studies were located that demonstrate any direct effects of naphthalene ingestion on the cardiovascular system. In those reports where cardiovascular effects such as increased heart rate and decreased blood pressure were noted in humans, the cardiovascular effects appeared to be secondary to the hemolytic effects and the events leading to general multiple organ failure (Gupta et al. 1979; Kurz 1987).

No gross or histopathological lesions of the heart were noted in mice at doses up to 200 mg/kg/day (NTP 1980a) or in rats at doses of 400 mg/kg/day (NTP 1980b) after 13 weeks of exposure.

Heart weights were significantly decreased (6–7%) in male and female mice that were fed 1-methyl-naphthalene for 81 weeks in their diet. However, the changes in heart weight were not dose-related and there were no accompanying tissue abnormalities (Murata et al. 1993). Histopathological examination revealed no lesions in the hearts of mice fed 1-methylnaphthalene at doses as high as 143.7 mg/kg/day (Murata et al. 1993) or 2-methylnaphthalene at doses as high as 113.8 mg/kg/day (Murata et al. 1997).

Gastrointestinal Effects. Gastrointestinal disorders are common following naphthalene ingestion by humans. These effects have been attributed to the irritant properties of naphthalene (Kurz 1987). Nausea, vomiting, abdominal pain, and diarrhea (occasionally containing blood) have been reported (Bregman 1954; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985). While the presence of blood in the stool is indicative of intestinal bleeding, only a few areas of mucosal hemorrhage were noted in postmortem examination of the intestines (Kurz 1987). These areas were restricted to the small bowel and colon. No frank erosions or perforations were noted anywhere in the gastrointestinal tract.

A single dose of 1,000–4,000 mg/kg was associated with stomach lesions and discoloration of the intestines in rats that died during an LD_{50} study. The survivors were not affected (Papciak and Mallory 1990). No gross or histopathological lesions of the stomach, small intestine, and colon were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after

13 weeks of exposure (NTP 1980b). There was some intermittent diarrhea in the rats, but this may not have been treatment related.

No histopathological lesions were seen in the stomach or intestines of mice fed 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene for 81 weeks (Murata et al. 1997).

Hematological Effects. The most commonly reported hematologic effect in humans following the ingestion of naphthalene is hemolytic anemia (Dawson et al. 1958; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Mackell et al. 1951; Melzer-Lange and Walsh-Kelly 1989; Ojwang et al. 1985; Shannon and Buchanan 1982). Changes observed in hematology and blood chemistry are consistent with this effect: hemolysis, decreased hemoglobin and hematocrit values, increased reticulocyte counts, serum bilirubin levels, and Heinz bodies. This was caused by hemolysis. Most of the reported case studies provide no information on dose. However, in one case report, a 16-year-old girl swallowed 6 g of naphthalene before exhibiting hemolytic anemia (Gidron and Leurer 1956). This is a dose of 109 mg/kg (assuming a 55-kg body weight). The hematological condition of this individual, who was an immigrant from Kurdistan, was not provided.

As mentioned previously, there is an association between G6PD deficiency and the hemolytic effects of naphthalene (Dawson et al. 1958; Melzer-Lange and Walsh-Kelly 1989; Shannon and Buchanan 1982). Individuals with a genetic defect for this enzyme show an increased susceptibility to hemolysis from naphthalene exposure.

Few hematologic changes have been reported in animals. Standard laboratory animals do not appear to be sensitive to the hemolytic effects of naphthalene. In CD-1 mice, naphthalene at doses up to 267 mg/kg/day for 14 days or up to 133 mg/kg/day for 90 days did not result in hemolytic anemia (Shopp et al. 1984). However there was an increase in eosinophils in the 14- and 90-day studies. There was an increase in prothrombin time at 14 days. The clinical significance of these observations is not clear; the effects are not considered to be adverse.

There were no pronounced changes in red cell related hematological parameters in mice following 13-week exposures to doses of up to 200 mg/kg/day (NTP 1980a) and up to 400 mg/kg/day in rats (NTP 1980b). In male mice exposed to 200 mg/kg/day for 13 weeks, there was a decrease in segmented neutrophils and an increase in lymphocytes, but in male rats given 400 mg/kg/day, there were increased

neutrophils and decreased lymphocytes. These effects are not considered to be biologically significant or adverse.

Hemolytic anemia was reported by Zuelzer and Apt (1949) in a dog receiving a single 1,525 mg/kg dose of naphthalene in food and in another dog receiving approximately 263 mg/kg/day for 7 days in food. Dogs are more susceptible to chemically induced hemolysis than are rats and mice.

Exposure to 75.1 or 143.7 mg/kg/day 1-methylnaphthalene for 81 weeks was associated with a slight but statistically significant increase in the hemoglobin concentration, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration in female mice (Murata et al. 1993). Corresponding changes were not observed in male mice given comparable doses of 1-methylnaphthalene, or in male or female mice exposed to 2-methylnaphthalene doses as high as 113.8 mg/kg/day (Murata et al. 1997). Consistent exposure-related changes were not found in differential white blood cell counts or several serum biochemical parameters in male and female mice exposed to 1-methylnaphthalene or 2-methyl-naphthalene in these studies. The results from these studies do not provide consistent evidence that hematological parameters are consistent toxicity targets of chronic oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Hepatic Effects. Evidence of hepatotoxicity following oral exposure to naphthalene has been reported in humans, based on elevated plasma levels of hepatic enzymes (such as aspartate aminotransferase and lactic acid dehydrogenase) (Kurz 1987; Ojwang et al. 1985) and liver enlargement (Gupta et al. 1979; MacGregor 1954). The relationship between liver enlargement and potential naphthalene-induced hemolysis is unknown.

There is limited evidence of hepatic effects in laboratory animals, but the liver does not appear to be a critical toxicity target of orally administered naphthalene. A 39% increase in liver weight, a modest elevation in activity of aniline hydroxylase, and evidence of lipid peroxidation were observed in male rats treated with naphthalene at 1,000 mg/kg/day for 10 days (Rao and Pandya 1981). Male rats demonstrated an elevation in hepatic lipid peroxides at naphthalene doses of 1,000 mg/kg/day for 18 days (Yamauchi et al. 1986). In rats administered increasing doses of naphthalene up to 750 mg/kg/day (time-weighted average of 169 mg/kg/day), hepatic lipid peroxides were doubled at the end of 9 weeks of treatment (Germansky and Jamall 1988).

No effects on liver weight were observed in male or female mice receiving naphthalene at doses up to 267 mg/kg/day for 14 days or male mice receiving 133 mg/kg/day for 90 days (Shopp et al. 1984). Absolute liver weight was statistically significantly decreased, compared with the control value (by about 18%), in female mice receiving 133 mg/kg/day naphthalene for 90 days, but the biological significance of this change is unclear. Relative liver weight in exposed females was not changed to a statistically significant degree, and several serum biochemical end points indicative of liver damage (e.g., lactate dehydrogenase, SGPT, SGOT, and alkaline phosphatase) were unaffected in male and female mice exposed to doses up to 133 mg/kg/day for 90 days (Shopp et al. 1984). No other consistent biologically relevant exposure-related changes in serum chemistry end points were found. Activities of two hepatic microsomal mixed function oxidases (aniline hydroxylase, aminopyrine N-demethylase) were unchanged in exposed mice, although hepatic activities of benzo[a]pyrene hydroxylase were statistically significantly decreased in exposed mice (Shopp et al. 1984). The biological significance of this change is unclear. Supporting the concept that the liver is not a critical toxicity target of oral exposure to naphthalene, no gross or histopathological lesions of the liver were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b).

There were no changes in liver weights or tissue histopathology in male or female mice that consumed 71.6–143.7 mg/kg/day 1-methylnaphthalene in the diet for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Renal Effects. Renal toxicity has been reported in case studies of humans who ingested naphthalene. Frequent findings include the elevation of creatinine and blood urea nitrogen and the presence of proteinuria and hemoglobinuria (Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985; Zuelzer and Apt 1949). The presence of blood in the urine and increased concentrations of urobilinogen are a consequence of acute hemolysis and do not reflect any direct action of naphthalene on the kidney. Oliguria (Kurz 1987) and anuria (Gupta et al. 1979) were noted in two case reports, although urine output was normal in a third (Ojwang et al. 1985). Painful urination with swelling of the urethral orifice was also associated with medicinal naphthalene ingestion (Lezenius 1902). Proximal tubule damage and general tubular necrosis were found in postmortem examinations of two individuals who died following naphthalene ingestion (Gupta et al. 1979; Kurz 1987).

Renal effects were not consistently observed in animals exposed orally to naphthalene. Following 10 days of exposure of rats to naphthalene at 1,000 mg/kg/day, no changes were noted in kidney weight, lipid peroxidation, or in the activity of alkaline phosphatase and aniline hydroxylase (Rao and Pandya

1981). No changes were observed in the kidney weights of mice administered naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the kidney were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 200 mg/kg/day after 13 weeks of exposure (NTP 1980b). In the male rats, 10% showed cortical tubular degeneration that may have been compound-related at a dose of 400 mg/kg/day (NTP 1980b).

Relative kidney weights were increased slightly in male mice fed diets containing 71.6 or 140.2 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The females were not affected, and there were no histopathological lesions in the males or females. There were no changes in kidney weights or tissue histopathology in male or female mice consuming 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Ocular Effects. In an early report of naphthalene toxicity, a 36-year-old pharmacist who ingested an unspecified amount of unpurified naphthalene in a castor oil emulsion over a 13-hour period as treatment of an intestinal disorder became nearly blind 8 or 9 hours later (Lezenius 1902). A medical examination the following month revealed constricted visual fields associated with optic atrophy and bilateral zonular cataracts. At 1.5 meters, the patient's vision was limited to finger counting.

Several animal studies have demonstrated ocular changes following oral naphthalene exposure. Within 1 week following exposure to naphthalene (500 or 1,000 mg/kg/day), lens densities were increased in rats and cataracts developed within 4 weeks (Kojima 1992; Murano et al. 1993; Yamauchi et al. 1986). Eight rabbits (strain not identified) developed cataracts during oral administration of naphthalene at 2,000 mg/kg/day for 5 days (Srivastava and Nath 1969). Cataracts began to develop by the first day after a single 1,000 mg/kg naphthalene dose in three Chinchilla Bastard rabbits (Rossa and Pau 1988). In the solitary New Zealand white rabbit tested, cataracts began to develop after administration of four 1,000 mg/kg doses (dosing 2 times/week) and maximized after 12 weeks (Rossa and Pau 1988).

When naphthalene was administered orally at 1,000 mg/kg/day for up to 28 days, cataracts developed in 10 of 16 Dutch (pigmented) rabbits and in 11 of 12 albino rabbits (Van Heyningen and Pirie 1976). Lens changes were seen as early as day 2 of exposure. The authors noted that albino strains were more likely to develop cataracts over a 4-week course of treatment at 1,000 mg/kg/day than pigmented strains such as the Dutch rabbit.

In contrast, administration of a time-weighted-average 500-mg/kg/day dose of naphthalene in corn oil by gavage for 6 weeks resulted in more rapid development of cataracts in pigmented Brown-Norway rats than in nonpigmented Sprague-Dawley rats (Murano et al. 1993). Cataracts developed in three distinct phases. In the first phase, water clefts formed in the anterior subcapsular region of the eye. The second stage was the development of a semicircular opaque area in the lens, and the last stage was the appearance of a wedge-shaped opacity that could be seen with retroillumination and a wide, zonular-ring opacity that was seen with slit imaging. Each stage occurred about 1 week earlier in the Brown-Norway rats than in the Sprague Dawley rats. The first stage began 1 week after treatment was initiated in the Brown-Norway rats, and stage three cataracts were seen in all animals by the end of the 6 weeks. Progressive development of lens opacities was also reported in rats that were exposed to 700 or 5,000 mg/kg/day naphthalene by gavage for 79–102 days (Rathburn et al. 1990; Tao et al. 1991).

Damage to the eyes with continued exposure to naphthalene is not limited to lens opacification (Orzalesi et al. 1994). Retinal damage was noted in pigmented rabbits given time-weighted-average doses of 500 mg/kg/day naphthalene in corn oil by gavage for 5 weeks. The first changes to the retina occurred at about 3 weeks with degeneration of the photoreceptors. There was a subsequent increase in the retinal pigment epithelium as these cells phagocytized the debris from the photoreceptors. By the end of 6 weeks, the photoreceptor layer had almost entirely disappeared and was replaced with fibroglial tissue. As damage progressed, there was dense subretinal neovascularization of the area.

A number of biochemical changes were seen in the eyes after acute- and intermediate-duration naphthalene exposures. After 1 week of treatment with 1,000 mg/kg/day, glutathione levels in the lens were decreased in rats (Xu et al. 1992b; Yamauchi et al. 1986). After 30 days of treatment with doses of 5,000 mg/kg/day, total glutathione levels were reduced by 20% (Rathbun et al. 1990), and there was a 22% reduction at 60 days with a dose of 700 mg/kg/day (Tao et al. 1991). At 60 days, glutathione peroxidase activity in the lens was decreased by up to 45% and there was a 20–30% decrease in glutathione reductase activity (Rathbun et al. 1990). Comparable decreases in the activities of both enzymes were seen at 102 days with lower naphthalene doses (Tao et al. 1991). No changes were observed in the activity of glutathione synthetase or gamma-glutamyl cysteine synthetase (Rathbun et al. 1990). After 4 weeks of compound treatment (500 mg/kg/day), the activities of aldose reductase, sorbitol dehydrogenase, lactic dehydrogenase, and glutathione reductase were lower than in controls (Kojima 1992). No changes in ocular lipid peroxides were reported when male Blue Spruce pigmented rats were administered incremental doses of naphthalene that peaked at 750 mg/kg/day for 9 weeks (Germansky

and Jamall 1988). Lens and capsule LDH activities were greatly reduced in rabbits while o-diphenyl oxidase activity was elevated with a dose of 2,000 mg/kg/day for 5 days (Srivastava and Nath 1969).

In 13-week studies, histopathologic examination revealed no ocular lesions in F344/N rats or B6C3F1 mice exposed to doses as high as 400 or 200 mg/kg/day, respectively (NTP 1980a, 1980b). In a 2-year rat feeding study, no eye damage was seen at a naphthalene dosage of 41 mg/kg/day (Schmahl 1955). The details of the eye examination were not provided.

There were no changes in eye tissue histopathology in male or female mice that consumed 71.6–143.7 mg/kg/day 1-methylnaphthalene in the diet for 81 weeks (Murata et al. 1993) or 50.3–113.8 mg/kg/day 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1997).

Body Weight Effects. No studies were located that documented effects on body weight in humans after oral exposure to naphthalene.

In pregnant Sprague-Dawley rats exposed to 50, 150, or 450 mg/kg/day on gestation days 6–15, body weight gains were depressed by 31 and 53% at 150 and 450 mg/kg/day, respectively, but were unaffected at 50 mg/kg/day. The decreased body weight gains were accompanied by persistent clinical signs of toxicity (slow respiration, lethargy, or prone position) at the 150 and 450 mg/kg/day dose levels, but these signs were only apparent at the 50-mg/kg/day level during the first 2 days of dosing. The minimal LOAEL of 50 mg/kg/day for transient clinical signs and the LOAEL of 150 mg/kg/day for clinical signs associated with decreased body weight gains in pregnant rats are the basis of the acute oral MRL for naphthalene (see Section 2.3 and Appendix A).

In animals, body weight effects appear to be the critical effect associated with intermediate-duration oral exposure to naphthalene. After 13 weeks of exposure to naphthalene, mean terminal body weights in F344/N rats exposed to gavage doses ≥200 mg/kg/day were decreased by more than 10% relative to control values (NTP 1980b). Body weights were decreased by 12 and 28% in 200- and 400-mg/kg/day male rats, and by 23% in 400-mg/kg/day female rats. Food consumption was not affected by exposure. In B6C3F1 mice exposed to naphthalene doses up to 200 mg/kg/day for 13 weeks, exposed males gained more weight than controls during exposure, whereas exposed females gained less weight than controls (NTP 1980a). However, terminal body weights in exposed female mice were within 95% of control values, indicating that the naphthalene-induced changes were not biologically significant. In male and female CD-1 mice exposed to doses as high as 133 mg/kg/day for 90 days, average terminal body weight

in exposed groups were within 90% of control values (Shopp et al. 1984). Mice exposed to 267 mg/kg/day naphthalene for 14 days showed a decreased body weight gain; terminal body weights were decreased by 6% in females and 13% in males compared with control values (Shopp et al. 1984).

As discussed in Section 2.3 and Appendix A, the NOAEL of 100 mg/kg/day and the LOAEL of 200 mg/kg/day for decreased body weights in rats exposed by gavage to naphthalene 5 days/week for 13 weeks (NTP 1980b) provide the best available basis for MRL derivation among the findings from the studies in animals orally exposed to naphthalene for intermediate-durations. However, because an intermediate-duration oral MRL based on these data is slightly larger than the acute-duration oral MRL for naphthalene, the acute MRL was adopted as the intermediate-duration oral MRL for naphthalene (as indicated in Figure 3-2 and discussed in Section 2.3).

There was no significant difference between body weights of mice that were given up to 143.7 mg/kg/day 1-methylnaphthalene in their diets and those of the control animals throughout an 81-week exposure period (Murata et al. 1993). In mice exposed to 2-methylnaphthalene doses as high as 113.8 mg/kg/day in the diet for up to 81 weeks, average body weights were within 10% of control values (Murata et al. 1997).

Other Systemic Effects. Several humans who consumed naphthalene experienced elevated body temperatures which may have been related to their hemolytic crisis (Chusid and Fried 1955; Gidron and Leurer 1956; Haggerty 1956; Kurz 1987; MacGregor 1954; Ojwang et al. 1985). However, in some situations, bacterial infections rather than hemolysis may have been the cause of the fever (Kurz 1987; Melzer-Lange and Walsh-Kelly 1989; Ojwang et al. 1985; Zuelzer and Apt 1949).

No studies were located that documented other systemic effects in animals after oral exposure to naphthalene.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located that documented immunological or lymphoreticular effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. However, an enlarged spleen is a frequent consequence of hemolysis and was noted in the postmortem examination of one human subject who died after ingesting a large quantity of naphthalene (Kurz 1987).

Mice treated with naphthalene at oral doses as high as 267 mg/kg/day for 14 days showed no effects on humoral immune responses, delayed hypersensitivity responses, bone marrow stem cell number, or bone marrow DNA synthesis (Shopp et al. 1984). Mitogenic responses to concanavalin A (but not to lipopolysaccharide) were reduced in high dose females only. None of these effects were noted at doses of 27 or 53 mg/kg/day. At naphthalene doses of 133 mg/kg/day for 13 weeks, naphthalene had no effect on immune function (Shopp et al. 1984). After 14 days, thymus weights were reduced approximately 30% in male mice, but no differences were seen with a dose of 133 mg/kg/day at 13 weeks (Shopp et al. 1984). There was lymphoid depletion of the thymus in 2 of 10 female rats exposed to 400 mg/kg/day naphthalene for 13 weeks (NTP 1980b).

Spleen weights were reduced approximately 20% in female mice exposed to 267 mg/kg/day naphthalene for 14 days and 25% in females exposed to 133 mg/kg/day for 13 weeks (Shopp et al. 1984).

Monocyte concentrations were significantly elevated in male and female mice exposed to 71.6–143.7 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993). The increase in monocyte counts appeared to be dose related. The authors hypothesized that these changes may have been a physiological response to the pulmonary alveolar proteinosis seen in the exposed animals. There were no changes in spleen or thymus weights and the histopathology of these tissues was normal. With 81 weeks of exposure of male and female B6C3F1 mice to 2-methylnaphthalene, neutrophils were reported to be decreased, and lymphocytes increased, compared with control values, but neither the magnitude of these changes, or the dose groups in which they occurred, were specified in the study report (Murata et al. 1997). As with 1-methylnaphthalene, histologic examination revealed no exposure-related lesions in the spleen or thymus.

The highest NOAEL values and all LOAEL values from each reliable naphthalene study for immunological/lymphoreticular effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. The highest NOAEL values from the 1-methylnaphthalene and 2-methyl-naphthalene studies for immunological/lymphoreticular effects are also recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.4 Neurological Effects

The neurologic symptoms of naphthalene ingestion reported in human case studies include confusion (Ojwang et al. 1985), altered sensorium (Gupta et al. 1979), listlessness and lethargy (Bregman 1954; Chusid and Fried 1955; Kurz 1987; MacGregor 1954; Zuelzer and Apt 1949), and vertigo (Gidron and Leurer 1956). Muscle twitching, convulsions (Kurz 1987; Zuelzer and Apt 1949), decreased responses to painful stimuli, and coma occurred prior to death in individuals who ingested naphthalene (Gupta et al. 1979; Kurz 1987). At autopsy, the brain has appeared edematous (Gupta et al. 1979; Kurz 1987), with separation of neural fibers and swelling of myelin sheaths being noted histologically (Gupta et al. 1979). The neurologic symptomatology could result from the cerebral edema, which was probably secondary to acute hemolysis.

No studies were located that documented neurological effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Dose-related clinical signs of toxicity were apparent in female Sprague-Dawley rats exposed to doses of 50, 150, or 450 mg/kg/day naphthalene for 10 days during organogenesis. Slow respiration and lethargy were observed in a large percentage of the exposed animals. Some rats were dazed, had periods of apnea, or were unable to move after exposure. In the lowest dose group, 73% of the animals were affected on the first day of dosing. In the two higher dose groups, over 90% of the rats were affected (NTP 1991a).

The animals in the 50-mg/kg/day group acclimatized quickly. Symptoms were only apparent during the first 2 days of dosing. Clinical signs of toxicity persisted for longer periods in the higher dose groups, and were accompanied by decreased body weight gains (31 and 53% decreased at 150 and 450 mg/kg/day, respectively compared with control). It is not known if the observed clinical signs were due to treatment-related effects on the nervous system or were the indirect consequence of severe systemic toxicity, as indicated by the dramatic decreases in body weight gain. Comparable effects were not observed in F344/N rats exposed to doses of up to 400 mg/kg/day for 13 weeks or in B6C3F1 mice at doses of up to 200 mg/kg/day (NTP 1980a, 1980b). These results suggest that pregnant animals may be more susceptible to the effects of naphthalene than non-pregnant animals. The minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity and the LOAEL of 150 mg/kg/day for more persistent signs of toxicity accompanied with depressed weight gain in pregnant rats exposed on gestation days 6–15 are the basis of the acute oral MRL for naphthalene (see Section 2.3 and Appendix A).

There were no changes in the brain weights in mice exposed to naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the brain were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b). Transient clinical signs of neurotoxicity were observed in rats following daily gavage administration of 400 mg/kg, but not 200 mg/kg, doses (NTP 1980b). In mice, transient lethargy was observed following dose administration only between weeks 3 and 5 in the highest dose group, 200 mg/kg/day (NTP 1980a).

Absolute brain weight was significantly increased in male mice fed diets containing 71.6 or 140.2 mg/kg/day 1-methylnaphthalene for 81 weeks (Murata et al. 1993), or 54.3 or 113.8 mg/kg/day 2-methylnaphthalene for 81 weeks (Murata et al. 1997). The increases in brain weights were not dose related and there were no histopathological abnormalities of the brain. There were no differences in brain weights or histopathology in the female mice given comparable doses (Murata et al. 1993, 1997).

No studies were located that documented neurological effects in animals after oral exposure to 2-methyl-naphthalene.

The highest NOAEL values and all LOAEL values from each reliable study for neurological effects for naphthalene exposure in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2. The highest NOAEL values for neurological effects in the intermediate-duration 1-methyl-naphthalene and 2-methylnaphthalene mouse studies are also recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.5 Reproductive Effects

No studies were located that documented reproductive effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

Oral exposures of pregnant rabbits to naphthalene at dosages up to 400 mg/kg/day (gestational days 6–18), using methylcellulose as the vehicle, resulted in no apparent adverse reproductive effects (PRI 1986). When administered in corn oil to pregnant mice, however, a dosage of 300 mg/kg/day (gestational days 7–14) resulted in a decrease in the number of live pups per litter (Plasterer et al. 1985). It is not clear whether the observed differences in response are attributable to species differences or a possible increase

in the absorption of naphthalene when it is administered in corn oil compared with administration as a suspension in methyl cellulose.

Transient signs of toxicity were present in female rats exposed to doses of 50, 150, or 450 mg/kg/day on gestational days 6–15 (NTP 1991a). Effects on maternal weight gain were noted in the mid- and high-dose groups but not in the lowest dose group. The mid-dose group had a 31% decrease in weight gain while the high-dose group had a 53% weight gain decrease.

No treatment-related effects were reported on testicular weights of mice administered naphthalene at doses up to 267 mg/kg/day for 14 days or 133 mg/kg/day for 90 days (Shopp et al. 1984). No gross or histopathological lesions of the testes were noted in mice at doses of up to 200 mg/kg/day (NTP 1980a) or in rats at doses of up to 400 mg/kg/day after 13 weeks of exposure (NTP 1980b).

No gross or histopathological lesions of the testis, seminal vesicles, ovaries, uterus, or vagina were observed in mice exposed to 1-methylnaphthalene doses as high as 143.7 mg/kg/day (Murata et al. 1993) or 2-methylnaphthalene doses as high as 113.8 mg/kg/day (Murata et al. 1997).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.6 Developmental Effects

In humans, transplacental exposure of the fetus to naphthalene that had been ingested by the mother resulted in neonatal (and presumably fetal) hemolytic anemia (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958). No estimates of dose or duration were available, although in one case naphthalene consumption was described as being most pronounced during the last trimester (Zinkham and Childs 1958).

No studies were located that documented developmental effects in humans after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

No congenital abnormalities were observed after oral administration of naphthalene at 300 mg/kg/day to pregnant mice on days 7–14 of gestation (Plasterer et al. 1985), or at doses up to 400 mg/kg/day to

pregnant rabbits on days 6–18 of gestation (PRI 1986). Similarly, naphthalene was not teratogenic in rats at doses up to 450 mg/kg/day during gestation days 6–15 (NTP 1991a). However, there was a slight, but dose-related, increase in fused sternebrae in female pups of rabbits administered doses of 20–120 mg/kg/day on days 6–19 of gestation (NTP 1992b). These effects were seen in 2 of 21 litters at 80 mg/kg/day and 3 of 20 litters at 120 mg/kg/day. No other developmental effects were noted in this study.

No studies were located that evaluated developmental end points in animals after oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

The highest NOAEL values and all LOAEL values from each reliable study for developmental effects in each species and duration category are recorded in Table 3-2 and plotted in Figure 3-2.

3.2.2.7 Cancer

No studies were located that documented carcinogenic effects in humans after oral exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

In a 2-year feeding study of rats receiving naphthalene at about 41 mg/kg/day, no tumors were reported (Schmahl 1955). Specific details pertaining to the tissues examined were not provided.

The chronic dietary studies with 1-methylnaphthalene or 2-methylnaphthalene provide limited evidence for the carcinogenicity of these chemicals. Long-term exposure (81 weeks) of mice to 71.6 or 140.2 mg/kg/day 1-methylnaphthalene in the diet was associated with statistically significant increases in bronchiolar/alveolar adenomas in males, but not in females (Murata et al. 1993). Incidences for mice with lung adenomas were 2/49, 13/50, and 12/50 for control through high-dose male mice, and 4/50, 2/50, and 4/49 for female mice. Combined incidence for mice with lung adenomas or adenocarcinomas were 2/49, 13/50, and 15/50 for male mice, and 5/50, 2/50, and 5/50 for female mice. In mice exposed to 2-methylnaphthalene in the diet for 81 weeks, incidences for mice with lung adenomas were 2/49, 9/49, and 5/49 in males groups that received 0, 54.3, or 113.8 mg/kg/day, and 4/50, 4/49, and 5/48 in female groups that received comparable doses (Murata et al. 1997). Only the incidence in the 54.3-mg/kg/day group was elevated to a statistically significant degree.

3.2.3 Dermal Exposure

3.2.3.1 Death

Two cases of hemolytic anemia were observed in infants exposed to naphthalene-treated diapers (Schafer 1951; Valaes et al. 1963). One case was fatal. Jaundice, methemoglobinemia, hemolysis, and cyanosis were noted. In the fatal case the symptoms persisted, even after the naphthalene-containing diapers were no longer used (Schafer 1951). The author suggested that use of baby oil on the infant's skin might have facilitated the naphthalene absorption.

No treatment-related deaths occurred within the 14-day observation period when naphthalene was applied at 2,500 mg/kg to the skin of male and female rats or when doses of up to 1,000 mg/kg/day were applied to the skin for 6 hours/day, 5 days/week for 13 weeks (Frantz et al. 1986; Gaines 1969). There were also no deaths in New Zealand White rabbits after application of 2,000 mg/kg naphthalene to intact and abraded shaved areas of skin in an LD_{50} study (Papciak and Mallory 1990).

No studies were located that documented lethal effects in humans or animals after dermal exposure to 1-methylnaphthalene or 2-methylnaphthalene.

3.2.3.2 Systemic Effects

No studies were located that documented musculoskeletal effects in humans or animals after dermal exposure to naphthalene. The highest NOAEL and all LOAEL values for dermal exposure to naphthalene are recorded in Table 3-3. Data for systemic effects in humans or animals from dermal exposure to 1-methylnaphthalene or 2-methylnaphthlene are restricted to two studies that only examined the lung for lesions following repeated dermal exposure to methylnaphthalene, a mixture of 1-methylnaphthalene and 2-methylnaphthalene (Emi and Konishi 1985; Murata et al. 1992).

Respiratory Effects. No studies were located that documented respiratory effects in humans after dermal exposure to naphthalene.

No histological changes of the lungs were noted in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
ACUTE EXPOSURE						
Systemic						
Rabbit New Zealand White	once 24hr contact	Dermal		2000 mg/kg	(skin irritation, edema, fissuring)	Papciak and Mallory 1990 NAP
Rabbit	once	Dermal		125 mg/kg	(reversible erythema)	PRI 1985a NAP
Immuno/ Lymphoret						
Gn Pig	3 wk 1x/wk		1000 mg/kg			PRI 1985c NAP

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL		Reference Chemical Form
				Less Serious	Serious	
INTERMEDIATE EXPOSURE						
Systemic						
Rat	90 d 5d/wk 6 hr/d	Resp	1000 mg/kg/day			Frantz et al. 1986 NAP
		Cardio	1000 mg/kg/day			
		Gastro	1000 mg/kg/day			
		Hemato	1000 mg/kg/day			
		Hepatic	1000 mg/kg/day			
		Renal	1000 mg/kg/day			
		Dermal	300 mg/kg/day	1000 mg/kg/day	(increased incidence of excoriated skin and papules)	
Mouse (B6C3F1)	30 wk 2x/wk	Resp		119 mg/kg	(100% incidence of mice with pulmonary alveolar proteinosis)	Murata et al. 1992 1-MN+2-MN

Table 3-3 Levels of Significant Exposure to Naphthalene (Nap) Or Methylnaphthalene (1-Mn Or 2-Mn) - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL	LOAEL			Reference Chemical Form
				Less Serious	Serious		
CHRONIC EXPOSURE							
Systemic							
Mouse (B6C3F1)	61 wk 2x/wk	Resp	30 mg/kg		119 mg/kg	(31/32 mice had pulmonary alveolar proteinosis; an unspecified number died)	Emi and Konishi 1985 1-MN+2-MN

Cardio = cardiovascular; d = day(s); Gastro = gastrointestinal; Gn Pig = Guinea pig; Hemato = hematological; hr = hour(s); LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); x = time(s). 1-Mn = 1-methylnaphthalene; 2-Mn = 2-methylnaphthalene.

Pulmonary alveolar proteinosis was noted in nearly all female B6C3F1 mice given dermal doses of methylnaphthalene (a mixture of 1-methylnaphthalene and 2-methylnaphthalene) twice a week at a dose level of 119 mg/kg for 30 weeks (Murata et al. 1992) or 61 weeks (Emi and Konishi 1985). Endogenous lipid pneumonia was the term used to describe this lesion in the earlier study. With the longer-duration exposure to 119 mg/kg methylnaphthalene, an unspecified number of mice died early. Pulmonary alveolar proteinosis developed in 3/11 female mice treated twice weekly with dermal doses of 30 mg/kg for 61 weeks, compared with 0/4 controls (Emi and Konishi 1985).

Cardiovascular Effects. No studies were located that documented cardiovascular effects in humans after dermal exposure to naphthalene.

No differences in organ weight or histological changes of the heart were noted in rats dermally treated with 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Gastrointestinal Effects. No studies were located that documented gastrointestinal effects in humans after dermal exposure to naphthalene.

No histological changes of the esophagus, stomach, or intestines were noted in rats dermally treated with 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Hematological Effects. Hemolytic anemia was reported in infants dermally exposed to diapers or other clothing treated with naphthalene mothballs (Dawson et al. 1958; Schafer 1951; Valaes et al. 1963). Jaundice, fragmentation of erythrocytes, Heinz bodies, methemoglobinemia, and reticulocytosis were observed. Several of the infants had G6PD deficiencies. Individuals with this genetic disorder are particularly susceptible to hemolysis from chemical agents. The application of oil to the skin may have aided absorption of naphthalene, as shown by the increasing severity of symptoms (jaundice and cyanosis) even after the use of the naphthalene-containing diapers ceased (Schafer 1951).

There were no changes in hemoglobin, hematocrit, red blood cell count, leukocyte count, or platelet count at 4 and 13 weeks in rats treated with doses of up to 1,000 mg/kg/day applied to the skin (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986).

Hepatic Effects. The liver was enlarged in two infants who experienced acute hemolysis after dermal exposure to naphthalene (Dawson et al. 1958; Schafer 1951). The relationship between liver enlargement and potential naphthalene-induced hemolysis is unknown.

There were no differences in liver weights or histological damage to the liver in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986). In addition, the levels of aspartate amino transferase, alanine amino transferase, urea nitrogen, and bilirubin were not elevated in the exposed rats as compared to the controls.

Renal Effects. No studies were located that documented renal effects in humans after dermal exposure to naphthalene.

There were no differences in kidney weights or histological damage to the liver in rats dermally treated with doses of up to 1,000 mg/kg/day naphthalene (6 hours/day, 5 days/week) for 13 weeks (Frantz et al. 1986). In addition, the results of urinalysis conducted at 4 and 13 weeks on the treated rats were not different from the control results, indicating that there was no impairment of kidney function.

Dermal Effects. No studies were located that documented dermal effects in humans after dermal exposure to naphthalene.

A study in rabbits has shown that naphthalene is a mild dermal irritant, causing erythema and fissuring, when directly applied to the shaved, abraded, or nonabraded skin under a dressing; healing occurred within 6–7 days (Papciak and Mallory 1990; PRI 1985a). In rats that were dermally treated for 6 hours/day, 5 days/week, for 13 weeks with 1,000 mg/kg/day naphthalene, there was an increased incidence of excoriated skin lesions and papules (Frantz et al. 1986). However, similar lesions were seen in the controls and lower dose group animals. At the high dose, naphthalene appeared to exacerbate the severity of the lesions. Acute and chronic exposures of animal skin to naphthalene appear to cause dermal irritation.

Ocular Effects. Two case studies were reported in which humans experienced eye irritation and conjunctivitis as a result of naphthalene exposure (van der Hoeve 1906). In one case, a worker accidentally got naphthalene powder in his left eye. The exact amount was unknown, but was described by the worker as large. Despite immediate cleansing of the eye, the subject experienced conjunctivitis and pain shortly after exposure. Symptoms of irritation subsided, but then reappeared 6 weeks later. At

that time, the subject noticed decreased vision in his left eye. When examined by a doctor, the eye had retinal lesions (one fresh and others seemingly older); the entire retina appeared clouded. The subject's vision in the left eye was poorer than in the right. Five years earlier, vision was the same in both eyes.

In the second case study, an adult male who worked in a storage area where naphthalene was used as a pesticide complained of ocular pain, conjunctivitis, and impaired vision (van der Hoeve 1906). Neither the duration nor the mode of exposure was described. The subject most likely was exposed to naphthalene vapors. When examined by a doctor, the subject was found to have retinal bleeding and the beginning of a cataract.

Dermal and ocular contact with naphthalene vapors accompanied by inhalation may have contributed to the development of multiple lens opacities in 8 of 21 workers involved with a dye manufacturing process that used naphthalene as a raw material (Ghetti and Mariani 1956). Workers, who were employed at the plant for up to 5 years, melted naphthalene in open vats, resulting in high atmospheric vapor concentrations.

Mild ocular irritation was observed in the nonrinsed eyes of rabbits after instillation of naphthalene at 0.1 mg/eye (Papciak and Mallory 1990; PRI 1985b). Observed effects were reversible within 7 days after exposure. When the eyes were rinsed with water immediately after exposure, there were no signs of irritation (Papciak and Mallory 1990). Oral administration of naphthalene in rats resulted in cataract formation beginning at the posterior outer cortex, suggesting that this region is the most sensitive part of the lens (Kojima 1992). The lenses of pigmented Brown-Norway rats had changes, such as water cleft formation, during the first week that 10 mg/kg/day naphthalene was orally administered every other day (Murano et al. 1993). These rats were more sensitive to cataract formation than albino Sprague-Dawley rats, presumably because they more effectively metabolized naphthalene to the toxic compound naphthoquinone (Murano et al. 1993).

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located that documented immunological or lymphoreticular effects in humans after dermal exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. An enlarged spleen was noted in two human subjects dermally exposed to unspecified doses of naphthalene (Dawson et al. 1958;

Schafer 1951). However, spleen enlargement is a result of hemolysis rather than a direct effect of naphthalene on the spleen.

In animals, dermal application of pure naphthalene (1,000 mg/kg) 1 time/week for 3 weeks did not result in delayed hypersensitivity reactions in guinea pigs (Papciak and Mallory 1990; PRI 1985c).

No studies were located that documented immunological or lymphoreticular effects in animals after dermal exposure to 1-methylnaphthalene or 2-methylnaphthalene.

A NOAEL for immunological/lymphoreticular effects following dermal exposure to naphthalene is recorded in Table 3-3.

No studies were located that documented the following health effects in humans or animals after dermal exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene:

3.2.3.4 Neurological Effects

3.2.3.5 Reproductive Effects

3.2.3.6 Developmental Effects

3.2.3.7 Cancer

3.3 GENOTOXICITY

No studies of genotoxic effects in humans exposed to naphthalene were located.

Table 3-4 summarizes results for naphthalene and its metabolites in bacterial mutation assays; *in vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays; and *in vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays.

Bacterial Gene Mutation Assays for Naphthalene. Naphthalene was not mutagenic in *Salmonella typhimurium* assays in the presence or absence of rat liver metabolic preparations (Bos et al. 1988; Connor et al. 1985; Florin et al. 1980; Gatehouse 1980; Godek et al. 1985; Kaden et al. 1979; McCann et al. 1975; Mortelmans et al. 1986; Nakamura et al. 1987; Narbonne et al. 1987; NTP 1992a; Sakai et al. 1985). The metabolites, 1-naphthol and 1,4-naphthoquine, were not mutagenic in several *S. typhimurium*

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
Bacterial gene mutation assays					
Reverse mutation	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100	100 µg/plate ±S9 activation	100	Negative	McCann et al. 1975
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3–100 µg/plate ±S9 activation	100	Negative	Mortelmans et al. 1986
	S. typhimurium TA1535, TA1537, TA98, TA100	0.3–100 µg/plate ±S9 activation	100	Negative	NTP 1992a
	S. typhimurium TA1537, TA1538	10–200 µg/plate ±S9 activation	100	Negative, toxic above 100 µg/plate	Gatehouse 1980
	S. typhimurium TA98, TA100	10–50 µg/plate ±S9 activation	50	Negative	Bos et al. 1988
	S. typhimurium TA1535, TA1537, TA98, TA100	0.03–30 µmol/plate ±S9 activation	3	Negative, toxic above 3 µmol/plate	Florin et al. 1980
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ±S9 activation	250	Negative	Sakai et al. 1995
	S. typhimurium TA1535, TA1537, TA1538, TA98, TA100	3–300 µg/plate ±S9 activation	300	Negative, toxic above 300 µg/plate	Godek 1985
	S. typhimurium TM677	1–2 mM ±S9 activation	2	Negative	Kaden et al. 1979
	S. typhimurium TA98, TA1535	5–1,000 µg/plate ±S9 activation	1,000	Negative	Narbonne et al. 1987
	S. typhimurium UTH8413, UTH8414, TA98, TA100	100–2,000 µg/plate ±S9 activation	2,000	Negative	Conner et al. 1985
	S. typhimurium TA1535, TA1537, TA98, TA100	1,000 µg/plate ±S9 activation	1,000	Negative (1-naphthol)	McCann et al. 1975
	S. typhimurium TA98, TA1535	5–1,000 µg/plate ±S9 activation	1,000	Negative (1-naphthol)	Narbonne et al. 1987
	S. typhimurium TA1535, TA1537, TA98, TA100	250 µg/plate ±S9 activation	250	Negative (1,4-naphthoquinone)	Sakai et al. 1995

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
Bacterial gene mutation assays (continued)					
	S. typhimurium TA97a, TA98, TA100, TA104	0–100 nmol/plate ±S9 activation	17.5	Positive (1,2-naphtho-quinone), 1.8- to 3.4-fold increase without S9; +S9 results similar to -S9 results	Flowers-Geary et al. 1996
SOS response	*S. typhimurium* TA1535/p5K1002 (uMuC-lacZ)	83 µg/mL ±S9 activation	83	Negative	Nakamura et al. 1987
	Escherichia coli K12 inductest (λ lysogen GY5027; uvrB-, envA-)	2,000 µg/plate ±S9 activation	2,000	Negative	Mamber et al. 1984
SOS chromotest	*E. coli* PQ37 (sfiA::lacZ fusion)	0.156–10.0 µg/assay ±S9 activation	10	Negative	Mersch-Sundermann et al. 1993
Pol A- or Rec assay	*E. coli* WP2/WP10 (uvrA-, recA-)	2,000 µg/mL ±S9 activation	2,000	Negative	Mamber et al. 1983
	E. coli WP2/WP67 (uvrA-, pol A-)	Dose not specified ±S9 activation	NS	Negative	Mamber et al. 1983
Pol A- or Rec assay	*E. coli* WP2/WP3478 (pol A-)	Dose not specified ±S9 activation	NS	Negative	Mamber et al. 1983
Mutatox (reversion to luminescence)	*Vibrio fischeri* M169	Up to 5,000 µg/tube ±S9 activation	0.203 0.625	Negative without S9 activation Positive with S9 activation	Arfsten et al. 1994
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
Mutation at hprt and tk loci	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative	Sasaki et al. 1997
	Human B-lymphoblastoid cell line MCL-5	40 µg/mL	40	Negative (1,4-naphthoquinone)	Sasaki et al. 1997
Chromosomal aberrations	Chinese hamster ovary cells	15–75 µg/mL ±S9 activation	30 75	Positive with S9 activation Negative without S9 activation	NTP 1992a
Chromosomal aberrations	Preimplantation whole mouse embryos	0.16 mM ±S9 activation	0.16	Positive, more pronounced with S9 activation	Gollahon et al. 1990 [abstract only]

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
***In vitro* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
Sister chromatid exchange	Human mononuclear leukocytes	100 µM ± human liver microsomes	100	Negative	Tingle et al. 1993; Wilson et al. 1995
Sister chromatid exchange	Human mononuclear leukocytes	0–100 µM ± human liver microsomes	10	Positive (1,2- and 1,4-naphthoquinone) Negative (naphthalene 1,2-epoxide)	Wilson et al. 1996
Sister chromatid exchange	Chinese hamster ovary cells	9–90 µg/mL ±S9 activation	27	Positive with S9 in the second of two trials and without S9 in both trials	NTP 1992a
Alkaline elution (*in vitro*)	Rat hepatocytes	3 mM, 3-hour exposure	3 mM	Negative for increased incidence of DNA single-strand breaks	Sina et al. 1983
Unscheduled DNA synthesis (*in vitro*)	Rat primary hepatocytes	0.16–5,000 µg/mL	16	Negative, toxic above 16 µg/mL	Barfknecht et al. 1985
	Rat primary hepatocytes	0.5–1,000 nM/mL	1,000	Negative (1-naphthol, 2-naphthol)	Probst et al. 1981
Cell transformation	Fischer rat embryo cells (F1706P96)	0.1, 0.5 µg/mL	0.5	Negative	Freeman et al. 1973
	Syrian baby hamster kidney cells (BHK-21C13)	0.08–250 µg/mL +S9	250	Negative	Purchase et al. 1978
	Mouse (BALB/c) whole mammary gland cultures	0.001–1.0 µg/gland	0.1	Negative, cytotoxic above 0.1 µg/gland	Tonelli et al. 1979
	Mouse BALB/c 3T3 cell culture	15–150 µg/mL	150	Negative, toxic at highest dose	Rundell et al. 1983
	Human diploid fibroblasts (WI-38)	0.08–250 µg/mL +S9	250	Negative	Purchase et al. 1978
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays**					
Somatic mutation, recombination	*Drosophila melanogaster*	1, 5, 10 mM (feeding larvae)	5	Positive, loss of heterozygosity of two recessive wing genes (about 2-fold increase in number of wing spots)	Delgado-Rodriguez et al. 1995
Micronuclei induction	Male ICR Swiss mice: bone marrow cells	50, 250, and 500 mg/kg gavage	500	Negative	Harper et al. 1984

Table 3-4. Results of Genotoxicity Testing of Naphthalene or Metabolites[a]

Assay	Test system	Dose/ concentration	HID or LED	Result	Reference
***In vivo* eukaryotic gene mutation, cytogenetic, or DNA damage assays (continued)**					
	Male and female CD-1 mice: bone marrow cells	250 mg/kg intraperitoneal	250	Negative	Sorg 1985
Micronuclei induction	Salamander larvae (*Pleurodeles waltl*): erythrocytes	0.125–0.5 ppm in the tank water	0.25	Positive at 0.5 ppm, weakly positive at 0.25 ppm	Djomo et al. 1995
Alkaline elution (*in vivo*)	DNA from hepatocytes of female rats given single oral doses	359 mg/kg oral	359	Negative for DNA single-strand breaks	Kitchin et al. 1992, 1994
Unscheduled DNA synthesis (*in vivo*)	Hepatocytes from rats given single oral doses	600, 1,000, and 1,600 mg/kg gavage	1,600	Negative	RTC 1999
DNA fragmentation	DNA fragmentation in liver or brain tissue from mice given single doses	0, 3, 32, and 158 mg/kg (0.01, 0.1, 0.5 of LD_{50}=316 mg/kg)	32	Positive (1.0- to 1.5-fold and 1.8- to 2.2-fold increase in DNA fragmentation at 32 and 158 mg/kg, respectively)	Bagchi et al. 2002
DNA fragmentation	DNA fragmentation in liver or brain tissue from rats given daily doses for up to 120 days	0, and 110 mg/kg in corn oil	110	Positive (1.9- to 2.5-fold maximal increases in DNA fragmentation in brain and liver tissue)	Bagchi et al. 1998a
DNA fragmentation	DNA fragmentation in liver or brain tissue from p53-deficient and standard mice given single oral doses	0, 3, 32, and 158 mg/kg (0.01, 0.1, and 0.5 of LD_{50}=316 mg/kg)	158 (std) 3 (-p53)	Positive (1.8- to 3.9-fold increases in DNA fragmentation in brain and liver tissue; p53-deficient (tumor suppressor gene) strain was more sensitive	Bagchi et al. 2000
Neoplastic transformation (*in vivo*)	F344 partially hepatectomized rats (sex not specified)	100 mg/kg gavage (in corn oil)	100	Negative for gamma-glutamyl trans-peptidase foci	Tsuda et al. 1980

[a]Metabolites are noted in result column.

DNA = dioxyribonucleic acid; HID = highest ineffective dose for negative tests; LED = lowest effective dose for positive tests; NS = not specified; SOS = an emergency system to repair single strand DNA breaks; std = standard deviation

strains in the presence or absence of metabolic activation (McCann et al. 1975; Narbonne et al. 1987; Sakai et al. 1985). Naphthalene was not mutagenic, with or without metabolic activation, in the Pol A- or Rec assays in several *Escherichia coli* strains (Mamber et al. 1983). Naphthalene did not damage DNA (as assayed by the induction of the SOS-repair system) in *E. coli* PQ37 (Mersch-Sundermann et al. 1993), in *E. coli* K12 (Mamber et al. 1984), or in *S. typhimurium* TA1535/p5K1002 (Nakamura et al. 1987).

1,2-Naphthoquinone induced reverse mutations in several *S. typhimurium* strains without a metabolic activation system (Flowers-Geary et al. 1996), and naphthalene, in the presence of rat liver metabolic activation, induced reverse mutations in the marine bacterium *Vibrio fischeri* (Arfsten et al. 1994).

In Vitro Eukaryotic Gene Mutation, Cytogenetic, or DNA Damage Assays for Naphthalene. *In vitro* eukaryotic gene mutation assays are restricted to a single report that naphthalene and 1,4-naphthoquinone (1,2-naphthoquinone was not tested) did not induce mutations at the hprt and tk loci in human lymphoblastoid cells (Sasaki et al. 1997). However, naphthalene (in the presence of rat liver metabolic activation) induced chromosomal aberrations in Chinese hamster ovary cells (NTP 1992a) and preimplantation whole mouse embryos (Gollahon et al. 1990). Naphthalene also induced sister chromatid exchanges (in the presence or absence of rat liver metabolic activation) in Chinese hamster ovary cells (NTP 1992a), but did not do so in human mononuclear leukocytes in the presence or absence of human liver microsomes (Tingle et al. 1993; Wilson et al. 1995). In contrast, 1,2-naphthoquinone and 1,4-naphthoquinone (but not 1,2-naphthalene oxide), in the absence of metabolic activation, induced sister chromatid exchanges in human leukocytes at concentrations (10 and about 50 μM) that depleted cellular glutathione levels and induced about 35-45% cell death (Wilson et al. 1996). Naphthalene did not induce cell transformations in several mammalian cell types (see Table 3-4) or DNA single-strand breaks (Sina et al. 1983) or unscheduled DNA synthesis (Barfknecht et al. 1985; Probst et al. 1981) in rat hepatocytes.

In cell-free test systems (not included in Table 3-4), 1,2-naphthoquinone formed N7 adducts with deoxyguanosine (McCoull et al. 1999) and caused DNA strand scission in the presence of NADPH and copper via reactive oxygen species from a Cu(II)/Cu(I) oxidation/reduction cycle (Flowers et al. 1997).

In Vivo Eukaryotic Gene Mutation, Cytogenetic, or DNA Damage Assays for Naphthalene. Naphthalene was mutagenic in *Drosophila melanogaster* (Delgado-Rodriquez et al. 1995), but no *in vivo* mutagenicity tests of naphthalene or its metabolites are available in mammalian systems (Table 3-4). Naphthalene induced micronuclei in erythrocytes of salamander (*Pleurodeles waltl*) larvae exposed to concentrations of 0.5 mM, but did not induce micronuclei in bone marrow of mice given single oral doses

(50, 250, or 500 mg/kg) or intraperitoneal doses (250 mg/kg) (Harper et al. 1984; Sorg 1985). Naphthalene did not cause increased single-stranded DNA breaks in hepatocytes of rats given single oral doses of 359 mg/kg (Kitchin et al. 1992, 1994), unscheduled DNA synthesis in hepatocytes from rats given single doses as high as 1,600 mg/kg (RTC 1999), or transformation foci (γ-glutamyl transpeptidase-positive) in livers of F344 partially hepatectomized rats given single 100 mg/kg doses, but did cause DNA fragmentation in brain and liver tissue from mice given single doses of 32 or 158 mg/kg (Bagchi et al. 2000, 2002) and rats exposed to 110 mg/kg/day for up to 120 days (Bagchi et al. 1998a). In the DNA fragmentation assays, the effect was accompanied by increased lipid peroxidation in the same tissues. It is unclear whether the apparent DNA damage in these assays was due to direct effects of naphthalene metabolites or reactive oxygen species or was secondary to cell death induced at an extranuclear site.

No studies were located that examined possible genotoxic effects of naphthalene or its metabolites in sensitive target tissues of naphthalene in rodents (lung and nasal epithelial tissue).

Genotoxicity Assays for 1-Methylnaphthalene and 2-Methylnaphthalene. No studies were located that documented genotoxic effects of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals by any route of exposure. Data are limited to one *in vitro* study where 1-methylnaphthalene and 2-methyl-naphthalene failed to induce chromosomal aberrations or sister chromatid exchanges in human peripheral lymphocytes (Kulka et al. 1988). In an *in vitro* microbial assay employing *S. typhimurium*, mutagenic activity was not detected with either compound, with either the presence or absence of microsomal activation (Florin et al. 1980). These studies are presented in Table 3-5.

3.4 TOXICOKINETICS

Little information is available that documented the toxicokinetics of naphthalene in humans by any route of exposure. No information on the toxicokinetics of 1-methylnaphthalene or 2-methylnaphthalene in humans was located. The available animal data pertaining to naphthalene are described in the following sections. The relevance of this information to the toxicokinetics of naphthalene in exposed humans, however, is not known.

No toxicokinetic data on 1-methylnaphthalene-exposed animals were located. Animal data pertaining to 2-methylnaphthalene were limited.

Table 3-5. Genotoxicity of 1-Methylnaphthalene and 2-Methylnaphthalene *In Vitro*

Species (test system)	End point	Results		Reference
		With activation	Without activation	
1-Methylnaphthalene				
Prokaryotic organisms:				
Salmonella typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Florin et al. 1980
Mammalian cells:				
Human lymphocytes	Chromosomal aberration, sister chromatid exchange	–	–	Kulka et al. 1988
2-Methylnaphthalene				
Prokaryotic organisms:				
S. typhimurium (TA98, TA100, TA1535, TA1537)	Gene mutation	–	–	Florin et al. 1980
Mammalian cells:				
Human lymphocytes	Chromosomal aberration, sister chromatid exchange	–	–	Kulka et al. 1988

– = negative result

3.4.1 Absorption

Based on the presence of adverse effects following exposure, humans and animals can absorb naphthalene by pulmonary, gastrointestinal, and cutaneous routes. However, the rate and extent of naphthalene absorption are unknown in many instances.

3.4.1.1 Inhalation Exposure

Clinical reports suggest that prolonged exposure to naphthalene vapors can cause adverse health effects in humans (Harden and Baetjer 1978; Linick 1983; Valaes et al. 1963). Unfortunately, the rate and extent of naphthalene absorption were not determined in these studies. Presumably naphthalene moves across the alveolar membrane by passive diffusion through the lipophilic matrix.

No animal data that documented the absorption of naphthalene after inhalation were located. The only data observed in animal studies involved localized effects in the lungs and nasal passages. Thus, it is not possible to conclude that they were the consequence of absorbed naphthalene. However, absorption can be presumed to occur based on the human data.

No information has been located that documented the absorption of 1-methylnaphthalene or 2-methyl-naphthalene in humans or animals after inhalation exposure.

3.4.1.2 Oral Exposure

Several case reports indicate that naphthalene ingested by humans can be absorbed in quantities sufficient to elicit toxicity (Bregman 1954; Chusid and Fried 1955; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; MacGregor 1954; Mackell et al. 1951; Ojwang et al. 1985; Santhanakrishnan et al. 1973; Shannon and Buchanan 1982; Zuelzer and Apt 1949). However, no studies have been located that report the rate or extent of absorption. Absorption of naphthalene presumably occurs by passive diffusion through the lipophilic matrix of the intestinal membrane.

In one patient who died as a result of naphthalene ingestion, 25 mothballs were found in the stomach 5 days after her death (Kurz 1987). A single naphthalene mothball reportedly weighs between 0.5 and 5 g

depending on its size (Ambre et al. 1986; Siegel and Wason 1986). The gastric contents of a person who mistakenly ingested naphthalene flakes still smelled strongly of naphthalene at least 2 days following ingestion (Ojwang et al. 1985). These findings suggest that dissolved naphthalene is transported slowly into the intestines. Uptake from the intestines is governed by the partition coefficient between the materials in the intestinal lumen and the membrane lipids. Ingestion of mothballs or other forms of particulate naphthalene will lead to continued absorption over a period of several days as the solid dissolves. Unfortunately, none of the human data permit a quantitative evaluation of absorption coefficients or rates.

No information that documented the absorption of naphthalene after oral administration to animals has been located. The occurrence of ocular effects in rats and rabbits indicates that gastrointestinal absorption does occur (Kojima 1992; Murano et al. 1993; Srivastava and Nath 1969).

No information was located that documented the absorption of 1-methylnaphthalene in humans or animals after oral administration. Systemic effects observed after the ingestion of 1-methylnaphthalene demonstrate that intestinal absorption does occur in rats (Murano et al. 1993).

No information has been located that documented absorption in humans after oral exposure to 2-methyl-naphthalene. Small doses of 2-methylnaphthalene appear to be rapidly absorbed from the gastrointestinal tract in guinea pigs. At least 80% of a 10 mg/kg oral dose of 2-methylnaphthalene was absorbed within 24 hours based on recovery of the radiolabel in the urine (Teshima et al. 1983).

3.4.1.3 Dermal Exposure

Several cases of naphthalene toxicity in neonates have been reported in which the proposed route of exposure was dermal (Dawson et al. 1958; Schafer 1951). Each case involved the use of diapers which had been stored in contact with naphthalene (mothballs or naphthalene flakes). The authors proposed that the naphthalene was absorbed through the skin, causing hemolytic anemia. It was suggested that this absorption may have been enhanced by the presence of oils which had been applied to the babies' skin (Schafer 1951). Inhalation of vapors from the treated diapers probably contributed to the total exposure.

^{14}C-Naphthalene was rapidly absorbed when the neat material (43 µg) was applied for a 48-hour period under a sealed glass cap to shaved 13-cm^2 areas of rat skin. Half of the sample (3.3 µg/cm^3) was absorbed

in 2.1 hours (Turkall et al. 1994). When the naphthalene was mixed with either a sandy soil or a clay soil prior to contact with the skin, the presence of the soil slowed the absorption (Turkall et al. 1994). The absorption half-time from the clay and sandy soil samples were 2.8 and 4.6 hours, respectively. The rate of absorption did not influence the total amount of naphthalene absorbed in 48 hours since the areas under the plasma concentration curve did not differ significantly with any of the three exposure scenarios (0.42–0.63%/mL hour). The authors proposed that naphthalene was absorbed more slowly from the sandy soil than the clay soil because the sandy soil had a higher organic carbon content (Turkall et al. 1994). The sandy soil contained 4.4% organic matter and the clay soil 1.6% organic matter.

No studies were located that examined the absorption of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals after dermal administration.

3.4.2 Distribution

There are limited data concerning the distribution of naphthalene in human tissues. Naphthalene was present in 40% of the adipose tissue samples that were analyzed as part of the National Human Adipose Tissue Survey (EPA 1986g). The maximum concentration observed was 63 ng/g. Naphthalene was also detected in human milk samples (concentration not reported) (Pellizzari et al. 1982). The sources of naphthalene in these milk and body fat samples are not known.

Information is available for the distribution of naphthalene in swine after oral exposure, the distribution of naphthalene in rats after dermal exposure, and the distribution of 2-methylnaphthalene in guinea pigs after oral exposure. No data were located for the inhalation exposure routes and no data were identified on the distribution of 1-methylnaphthalene by any route of exposure.

3.4.2.1 Inhalation Exposure

No studies were located that examined the distribution of naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene in humans or animals after inhalation exposure.

3.4.2.2 Oral Exposure

Naphthalene can cross the human placenta in concentrations high enough to cause red cell hemolysis and lead to anemia in newborn infants of mothers who consumed naphthalene during pregnancy (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958).

The distribution of naphthalene and its metabolites in young pigs given a single dose of 0.123 mg/kg (4.8 Ci/kg) ^{14}C-labeled naphthalene was monitored at 24 and 72 hours (Eisele 1985). At 24 hours, the highest percentage of the label (3.48±2.16% dose/mg tissue) was in the adipose tissue. The kidneys had the next highest concentration of label (0.96% dose/mg tissue), followed by the liver (0.26±0.06% dose/mg tissue) and lungs (0.16% dose/mg tissue). The heart contained 0.09±0.04% dose/mg tissue and the spleen contained 0.07±0.01% dose/mg tissue. At 72 hours, the amount of label in the fat had fallen to 2.18±1.16% dose/mg tissue, that in the liver to 0.34±0.24% dose/mg tissue, and the kidneys and lungs contained the same concentration (0.26% dose/mg tissue).

Pigs were also given oral doses of 0.006 mg/kg/day (0.22 Ci/kg/day) ^{14}C-labeled naphthalene for 31 days (Eisele 1985). With repeated administration of the radiolabel, the tissue distribution differed considerably from that observed with a single dose of the compound. The highest concentration of label was in the lungs (0.15% dose/mg tissue), followed by the liver and heart (0.11% dose/mg tissue). There was very little label in the fat tissue (0.03% dose/mg tissue). The spleen had 0.09±0.05% dose/mg tissue and the kidney had 0.09% dose/mg tissue.

In one dairy cow, naphthalene distributed to milk with both single and repeated doses of ^{14}C-labeled naphthalene. The label was distributed between the milk and the milk fat (Eisele 1985). When the cow was given naphthalene for a 31-day period, the amount of label found in the milk remained relatively constant throughout the exposure period. The amount in the milk fat was lower for the first 7 days than it was for the remainder of the exposure.

The tissue distribution of 2-methylnaphthalene was measured in guinea pigs 3, 6, 24, and 48 hours after oral administration of tritium-labeled 2-methylnaphthalene (10 mg/kg; 59 µCi/kg) (Teshima et al. 1983). The highest concentration of label was present in the gallbladder with 20.17 µg at 3 hours and 15.72 µg at 6 hours. (All concentrations are expressed in µg equivalents of ^{3}H/g wet tissue.) At 24 hours, the value fell to 0.43 µg and at 48 hours, to 0.04 µg. The presence of label in the gallbladder presumably reflects

the excretion of hepatic metabolites in the bile. The values for the kidney were 5.64 µg at 3 hours, 7.62 µg at 6 hours, 0.29 µg at 24 hours, and 0.09 µg at 48 hours.

Radiolabelled compound was detected in the liver immediately after exposure (Teshima et al. 1983). When converted to units of mass, hepatic concentrations were 1.71 µg at 3 hours and 2.66 µg at 6 hours, falling to 0.18 µg at 24 hours. Lung concentrations were similar to those for blood at all time points. The amount in blood at 3 hours was 0.75 µg and that for the lungs was 0.69 µg; at 6 hours, the blood had a concentration of 0.71 µg and the lung had 0.76 µg. The half-life of 2-methylnaphthalene in the blood was 10.4 hours. The decay of naphthalene in the other tissues examined was described as biphasic.

3.4.2.3 Dermal Exposure

No information was located that documented the distribution of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene in humans after dermal exposure.

In rats, radiolabel from naphthalene distributed to the ileum, duodenum, and kidney (0.01–0.02% of initial dose) when tissues were analyzed 48 hours after naphthalene contact with the skin (Turkall et al. 1994). The largest concentration was found at the site of application (0.56% of initial dose). A total of 20 tissues were evaluated; the percentage of label in all other tissues was minimal.

No information that documented the distribution of 1-methylnaphthalene or 2-methylnaphthalene in dermally exposed animals was located.

3.4.2.4 Other Routes of Exposure

After intraperitoneal administration in mice, ^{14}C-labeled 2-methylnaphthalene distribution was measured in the fat, kidney, liver, and lung for 24 hours (Griffin et al. 1982). The amount of label in the fat peaked 3 hours after exposure and remained higher than the amount of label in other tissues at 8 hours. The liver, kidney, and lung followed the fat in order of decreasing concentration. The maximum concentration in the fat was 13 nmol equivalents/mg wet weight. The maximum value for the liver was 3.5 nmol equivalents/mg wet weight at 1 hour. Maximum values were about 1.75 nmol equivalents/mg wet weight for the kidneys at 2 hours and 0.8 nmol equivalents/mg wet weight for the lungs at 4 hours.

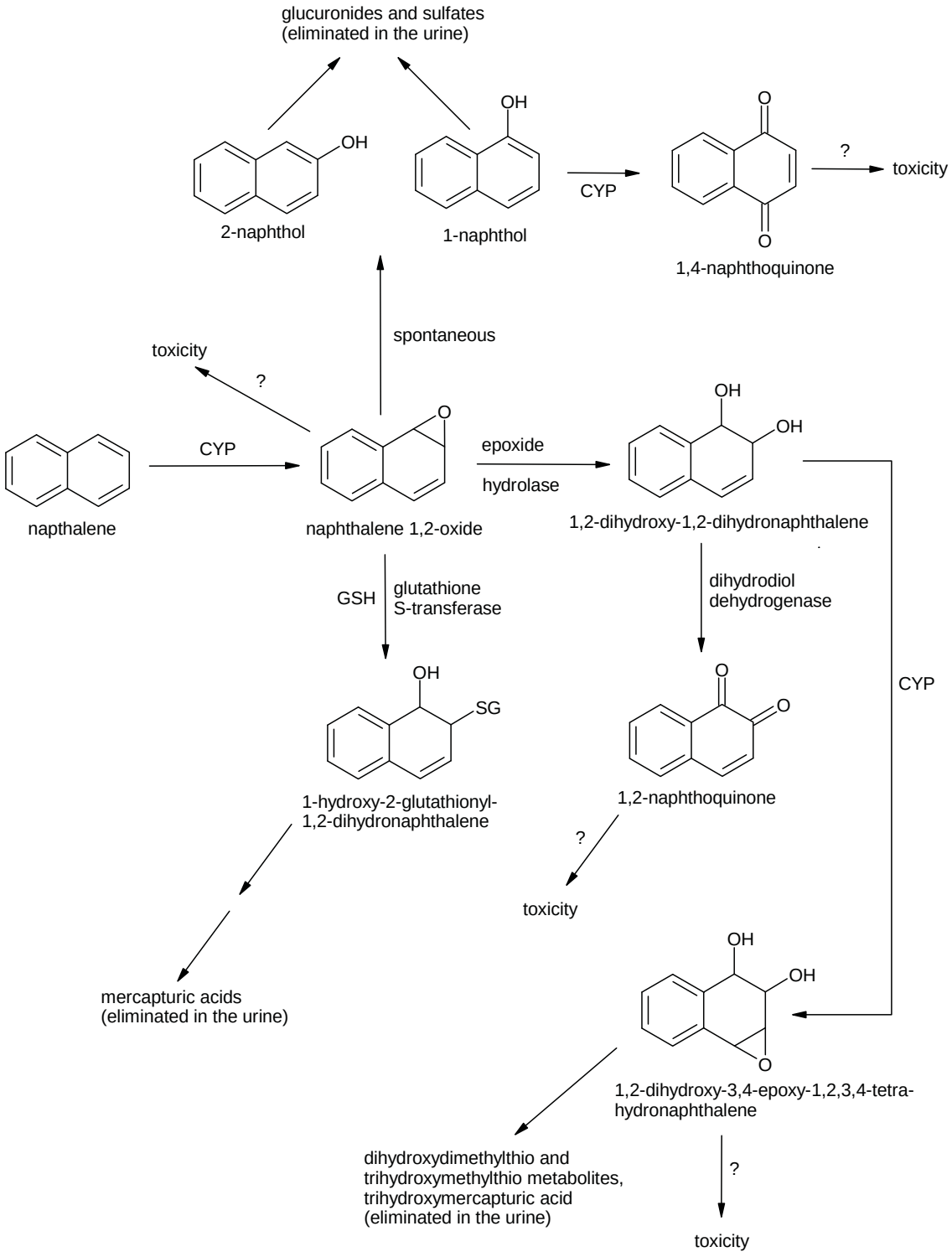
3.4.3 Metabolism

The metabolism of naphthalene in mammalian systems has been studied extensively and is depicted in Figure 3-3. The metabolic scheme in Figure 3-3 illustrates that there are multiple reactive metabolites formed from naphthalene: 1,2-naphthalene oxide, 1,2-naphthoquinone, 1,4-naphthoquinone, and 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene. This section presents an overview of the metabolic scheme and the evidence for the involvement of the 1,2-epoxide and the naphthoquinones in naphthalene toxicity. The fourth metabolite listed above is expected to be reactive, but its potential role in naphthalene toxicity has not been investigated. A recent review of the metabolism and bioactivation of naphthalene has been published by Buckpitt et al. (2002).

The first step in naphthalene metabolism is catalyzed by cytochrome P-450 (CYP) oxygenases and produces a reactive electrophilic arene epoxide intermediate, 1,2-naphthalene oxide. In mammalian systems, several CYP isozymes have been demonstrated to metabolize naphthalene, including 1A1, 1A2, 1B1, 3A7, 3A5 (Juchau et al. 1998), 2E1 (Wilson et al. 1996), 2F2 (Buckpitt et al. 1995; Shultz et al. 1999), and 2B4 (Van Winkle et al. 1996). The epoxide can spontaneously rearrange to form naphthols (predominantly 1-naphthol) and subsequently conjugate with glucuronic acid or sulfate to form conjugates, which are excreted in urine.

Alternatively, the 1,2-epoxide can react with tissue macromolecules. This reaction is thought to be involved in several aspects of naphthalene toxicity, especially injury to Clara cells (ciliated cells in the epithelium of proximal and distal airways of the lung) from acute exposure to naphthalene (Buckpitt et al. 2002; Zheng et al. 1997). In pH 7.4 buffer, the epoxide has been shown to have a half-life of approximately 2–3 minutes, which is extended by the presence of albumins to about 11 minutes (Buckpitt et al. 2002; Kanekal et al. 1991). Mice are markedly more susceptible than rats to acute naphthalene-induced Clara cell injury (Buckpitt et al. 1992; West et al. 2001). The susceptibility difference apparently extends to chronic exposure scenarios. Mice exposed by inhalation to 10 or 30 ppm naphthalene for 2 years showed lung inflammation, but rats exposed to concentrations up to 60 ppm showed no lung inflammation (Abdo et al. 2001; NTP 1992a, 2000). The species difference in lung susceptibility has been correlated with higher rates of formation of a specific enantiomeric epoxide (1*R*,2*S*-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats (Buckpitt et al. 1992, 1995). Rat, hamster, and monkey lung microsomes preferentially formed the 1*S*,2*R*-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes (Buckpitt et al. 1992). Microsomes from human lymphoblastoid cells expressing recombinant human

Figure 3-3. Scheme for Naphthalene Metabolism and Formation of Multiple Reactive Metabolites, That May Be Involved in Naphthalene Toxicity*



CYP = cytochrome P450 enzyme(s); GSH = reduced glutathione; SG = glutathione

*Adapted from Buckpitt et al. (2002) and Waidyanatha et al. (2002)

CYP2F1 also showed preferential formation of the 1*S*,2*R*-naphthalene oxide enantiomer, providing some evidence that human transformation of naphthalene to reactive epoxides in lung tissue may be more like rats than mice (Lanza et al. 1999).

In contrast to the lung, species differences in susceptibility at another sensitive target of naphthalene, the olfactory and respiratory epithelia of the nose, do not correlate with differences in rates of transformation to 1,2-epoxide derivatives in extracts of olfactory tissue (Buckpitt et al. 1992; Plopper et al. 1992a). Metabolic rates (units of nmol naphthalene converted to epoxide derivatives/minute/mg protein) in olfactory tissue extracts showed the following order: mouse (87.1) > rat (43.5) > hamster (3.9). However, rats were more susceptible to naphthalene-induced cell injury than mice or hamsters. The lowest single intraperitoneal doses producing necrosis and exfoliation in olfactory epithelium were 200 mg/kg in rats and 400 mg/kg in mice and hamsters. These observations suggest that the reasons for species differences in susceptibility to naphthalene toxicity are complex and do not solely involve the formation of the 1,2-epoxide metabolites. Although CYP monooxygenases, which might be involved in naphthalene metabolism and bioactivation, have been demonstrated to exist in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Thornton-Manning and Dahl 1997), studies designed to specifically characterize metabolism of naphthalene in nasal tissue are restricted to those by Buckpitt et al. (1992) and Plopper et al. (1992a).

In addition to being converted to the naphthols, the 1,2-epoxide can be conjugated with glutathione via glutathione-S-transferase catalysis. Figure 3-3 shows one such conjugate, 1-hydroxy-2-glutathionyl-1,2-dihydronaphthalene. The glutathionyl conjugates are converted in several steps to mercapturic acids, which are excreted in the urine. The conjugation of the epoxide is thought of as a detoxication mechanism, as evidenced by studies showing that glutathione depletion increased the degree of acute naphthalene-induced Clara cell injury in mice (Warren et al. 1982; West et al. 2000a). In addition, elevated activities of γ-glutamylcysteine synthetase, the enzyme catalyzing the rate limiting step in glutathione synthesis, were observed in dissected airways from mice that developed tolerance to acute naphthalene Clara cell cytotoxicity (West et al. 2000a).

The 1,2-epoxide can also be enzymatically hydrated by epoxide hydrolase to form 1,2-dihydroxy-1,2-dihydronaphthalene (Figure 3-3). This 1,2-dihydrodiol derivative was the major stable metabolite of naphthalene produced by human liver microsomes, whereas the major stable metabolite formed by mouse liver microsomes was 1-naphthol (Tingle et al. 1993). In the presence of an inhibitor of epoxide hydrolase (trichloropropene oxide), the major stable metabolite with human liver microsomes was

1-naphthol. How this species difference in liver metabolism may relate to the human relevance of toxicity of inhaled naphthalene in sensitive target tissues in the nose and lung of mice is unknown.

The 1,2-dihydrodiol can be catalytically transformed by dihydrodiol dehydrogenase to 1,2-naphthoquinone (also known as naphthalene-1,2-dione). 1,2-Naphthoquinone is both reactive itself and capable of producing reactive oxygen species through redox cycling (Flowers et al. 1997) and has been shown to be mutagenic in several strains of *S. typhimurium* (Flowers-Geary et al. 1996). In isolated Clara cells incubated with 0.5 mM naphthalene, 1,2-naphthoquinone was the major naphthalene derivative covalently bound to proteins, although covalent binding with the 1,2-epoxide was also observed (Zheng et al. 1997). The formation of the other naphthoquinone, 1,4-naphthoquinone, from 1-naphthol, presumably via a CYP monooxygenase, has been proposed based on the finding that, following incubations of liver microsomes with 1-naphthol, ethylene diamine, a compound that reacts readily with 1,2-naphthoquinone, did not trap reactive metabolites (D'Arcy Doherty et al. 1984). Cysteinyl adducts of both 1,2-naphthoquinone and 1,4-naphthoquinone (and of 1,2-naphthalene oxide) with hemoglobin and albumin have been detected in blood of rats given single oral doses of naphthalene ranging from 100 to 800 mg/kg (Troester et al. 2002; Waidyanatha et al. 2002). Levels of 1,2-naphthalene oxide adducts were greater than levels of 1,2-naphthoquinone adducts, which were greater than levels of 1,4-naphthoquinone adducts (Troester et al. 2002; Waidyanatha et al. 2002). In *in vitro* studies with whole human blood samples, 1,2- or 1,4-naphthoquinone induced increased frequencies of sister chromatid exchanges at concentrations ≥10 µM, whereas naphthalene 1,2-epoxide did not at concentrations up to 100 µM (Wilson et al. 1996). Similarly, incubation of human mononuclear leukocytes with 1,2-naphthoquinone or 1,4-naphthoquinone caused significant depletion of cellular glutathione levels and significant cytotoxicity at concentrations between 1 and 100 µM, whereas naphthalene 1,2-epoxide did not display these toxic actions in this concentration range (Wilson et al. 1996).

1,2-Naphthoquinone formed in lens tissue is thought to be involved in naphthalene-induced cataracts in rats and rabbits. The enzyme involved in the transformation of the 1,2-dihydrodiol to 1,2-naphthoquinone in lens tissue is thought to be aldose reductase (this enzyme is not specified in Figure 3-3). Support for this hypothesis includes findings that aldose reductase inhibitors prevent cataract formation in naphthalene-fed rats (Tao et al. 1991; Xu et al. 1992a), dihydrodiol dehydrogenase is apparently absent in rat lens (Greene et al. 2000), and aldose reductase appears to be the only enzyme in rat lens that can transform 1,2-dihydroxy-1,2-dihydronaphthalene to 1,2-naphthoquinone (Sugiyama et al. 1999).

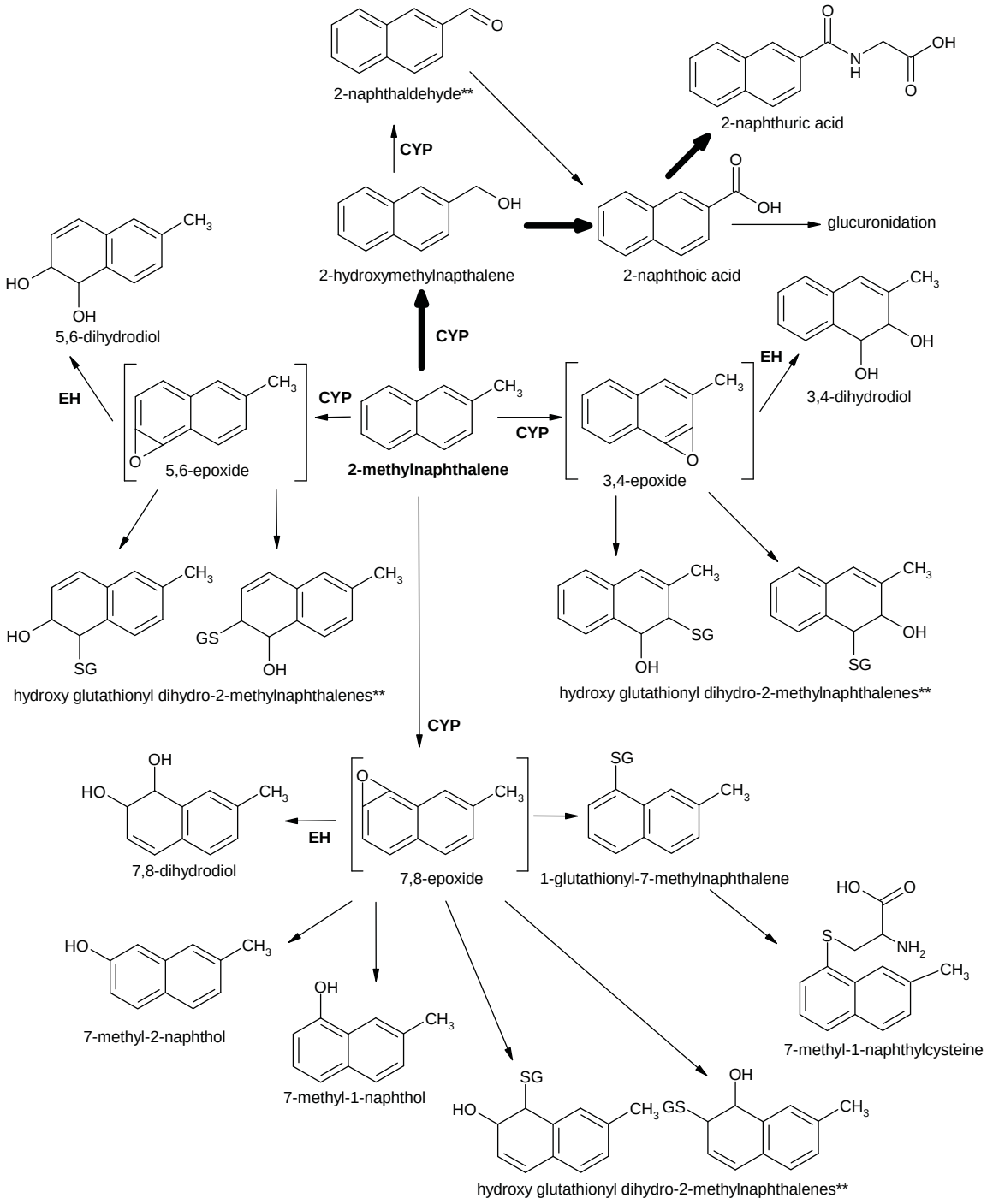
Support for the *in vivo* formation of another potentially reactive metabolite, 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, comes from the identification of several urinary metabolites, including a number of trihydroxytetrahydromethylthio derivatives (Horning et al. 1980) and a trihydroxytetrahydro-mercapturic acid (Pakenham et al. 2002). These urinary metabolites, however, are minor, and the importance of their common proposed precursor in naphthalene toxicity is unstudied to date. Figure 3-3 proposes an oxidative transformation of dihydrodiol derivative to the tetrahydrodiol epoxide derivative via CYP catalysis.

The methyl substituent of 1-methylnaphthalene and 2-methylnaphthalene presents the opportunity for side chain oxidation reactions in addition to the ring oxidation, which is the sole initial step in naphthalene metabolism. A proposed metabolic scheme for 2-methylnaphthalene is shown in Figure 3-4. Oxidation at the methyl group (the predominant path), or at several competitive positions on the rings, is catalyzed by CYP monooxygenases (Figure 3-4). No information was located that documented the metabolism of 1-methylnaphthalene. It may be similar to that for 2-methylnaphthalene with oxidation of the side chain and the ring.

In rats and mice, about 50–80% of 2-methylnaphthalene is oxidized at the 2-methyl group to produce 2-hydroxymethylnaphthalene (Breger et al. 1983; Teshima et al. 1983). This 2-hydroxymethyl-naphthalene metabolite is further oxidized to 2-naphthoic acid (Grimes and Young 1956; Melancon et al. 1982; Teshima et al. 1983), and this step proceeds either directly or through the intermediate, 2-naphthaldehyde (Figure 3-4). Detection of 2-naphthaldehyde has only been reported following *in vitro* incubation of 2-methylnaphthalene with recombinant mouse CYP2F2 (Shultz et al. 2001). 2-Naphthoic acid may be conjugated with either glycine or glucuronic acid (Figure 3-4). The glycine conjugate of 2-naphthoic acid forms 2-naphthuric acid, which is the most prevalent urinary metabolite of 2-methyl-naphthalene detected in exposed animals (Grimes and Young 1956; Melancon et al. 1982; Teshima et al. 1983).

Ring epoxidation at the 7,8-, 3,4-, or 5,6- positions occurs in approximately 15–20% of 2-methyl-naphthalene (Breger et al. 1983; Melancon et al. 1985). These epoxidation reactions are catalyzed by CYP isozymes that include CYP1A and CYP1B. These epoxides are proposed intermediates based on experimentally-observed metabolites, but have not been individually isolated (Figure 3-4). These epoxides may be further oxidized by epoxide hydrolase to produce dihydrodiols (the 7,8-dihydrodiol, 3,4-dihydrodiol, or 5,6-dihydrodiol of 2-methylnaphthalene) or may be conjugated with glutathione (Griffin et al. 1982; Melancon et al. 1985) by glutathione S-transferase catalysis or can proceed

Figure 3-4. Metabolism of 2-Methylnaphthalene*



[ ] = putative metabolite; CYP = cytochrome P450 enzyme(s); EH = epoxide hydrolase; GS = glutathione

*Adapted from Buckpitt and Franklin (1989), EPA (2003r); Shultz et al. (2001), and Teshima et al. (1983)
**Metabolites identified *in vitro* only

spontaneously. The hydroxy glutathionyl dihydro-2-methylnaphthalenes (Figure 3-4) have been detected after incubation of 2-methylnaphthalene with hepatic microsomes from Swiss-Webster mice or with isolated recombinant mouse CYP2F2 enzyme and glutathione S-transferase (Shultz et al. 2001). Figure 3-4 indicates six hydroxy glutathionyl 2-methylnaphthalenes; two are formed for each of the epoxide intermediates (3,4-, 5,6-, and 7,8-epoxides), and each can exist in two enantiomeric forms not shown in Figure 3-4 (Shultz et al. 2001).

Three other minor metabolites formed via the 7,8-epoxide pathway are shown in Figure 3-4. Urinary 1-glutathionyl-7-methylnaphthalene was identified in guinea pigs and by *in vitro* experiments with guinea pig microsomes (Teshima et al. 1983). 7-Methyl-1-naphthol and 7-methyl-2-naphthol were identified in the urine of rats, mice, guinea pigs, and rabbits following oral exposure (Grimes and Young 1956).

In rats administered subcutaneous injections of 2-methylnaphthalene (0.3 mg/kg 2-methyl-[8-^{14}C]-naphthalene), 2-naphthoic acid, and naphthoic acid conjugates were identified in the urine (Melancon et al. 1982). The naphthoic acid and various conjugates of the acid were estimated to account for 36–43% of the radiolabel in collected urine. Most of this (30–35% of radiolabel in urine) was found as a glycine conjugate. The urine contained 3–5% unreacted 2-methylnaphthalene; free dihydrodiols accounted for 6–8% of the label. Unidentified highly polar metabolites comprised another 36–45% of the excreted label. At least three diol derivatives of 2-methylnaphthalene were produced by hepatic microsomes from mice (Griffin et al. 1982) suggesting that the ring oxidation reactions of 2-methylnaphthalene are similar to those for naphthalene. Rat liver microsomes also produced 2-hydroxymethylnaphthalene and three diols from 2-methylnaphthalene (Breger et al. 1981, 1983; Melancon et al. 1985). The three diols were identified as 3,4-dihydrodiol, 5,6-dihydrodiol, and 7,8-dihydrodiol (Breger et al. 1983).

Metabolites isolated in the urine of guinea pigs after oral dosing with tritium labeled 2-methylnaphthalene (10 mg/kg) were 2-naphthoic acid and its glycine and glucuronic acid conjugates (Teshima et al. 1983). These metabolites accounted for 76% of the label in collected urine. Glucuronic acid and sulfate conjugates of 7-methyl-1-naphthol along with *S*-(7-methyl-1-naphthyl)cysteine accounted for 18% of the excreted label. No diol metabolites were identified.

Glutathione conjugation appears to be an important detoxication pathway for 2-methylnaphthalene. Pretreatment of male C57BL/6J mice with 625 mg/kg of diethylmaleate (a depletor of glutathione) 1 hour prior to intraperitoneal administration of 400 mg/kg of 2-methylnaphthalene resulted in mortality in 4/5 mice, whereas treatment without glutathione depletion was not fatal (Griffin et al. 1982). Bronchiolar

necrosis was not observed in male ddY mice given single intraperitoneal injections of 200 mg/kg of 2-methylnaphthalene; pretreatment with the glutathione depletor diethylmaleate (600 µL/kg) 1 hour prior to injections caused "extensive sloughing and exfoliation of bronchiolar epithelial cells" in all animals (5/5) (Honda et al. 1990). In contrast, pretreatment of male DBA/2J mice (5/group) with 625 mg/kg of diethylmaleate did not increase the severity of pulmonary necrosis induced by 400 mg/kg of 2-methylnaphthalene (Griffin et al. 1983). The observed differences among mouse strains in response to depletion of glutathione remain unexplained. Other experiments (without pretreatment) observed decreased tissue or intracellular levels of glutathione in response to exposure to high acute doses of 2-methylnaphthalene, demonstrative of glutathione conjugation (Griffin et al. 1982, 1983; Honda et al. 1990). Similarly, depletion of glutathione (by 35% compared to controls) was detected in primary cultures of female Sprague-Dawley rat hepatocytes treated with 1,000 µM of 2-methylnaphthalene (Zhao and Ramos 1998).

3.4.4 Elimination and Excretion

3.4.4.1 Inhalation Exposure

Little information is available pertaining to the excretion of naphthalene in humans after inhalation exposure to naphthalene. Workers employed in the distillation of naphthalene oil and at a coke plant had peak levels of urinary 1-naphthol 1 hour after finishing a shift. Of three workers and a nonoccupationally exposed group, naphthalene oil distribution plant workers had the highest concentrations of urinary 1-naphthol, with a mean excretion rate of 0.57% mg/hour. Investigators calculated the half-life for the urinary excretion of 1-naphthol as approximately 4 hours (Bieniek 1994). This urinary metabolite may indicate both exposure to naphthalene and low concentrations of 1-naphthol during naphthalene oil distillation (Bieniek 1994). No studies were located that documented excretion in humans after inhalation exposure to 1-methylnaphthalene or 2-methylnaphthalene.

No studies were located that documented excretion in animals after inhalation exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.4.4.2 Oral Exposure

Little information is available pertaining to the excretion of orally ingested naphthalene by humans. The urine of one patient was tested for naphthalene and its derivatives. Naphthol was found at the time of hospital admission (4 days post-ingestion). Smaller quantities were present 1 day later, but naphthalene

was not detected in later specimens (Zuelzer and Apt 1949). In another instance, the urine of an 18-month-old child was found to contain 1-naphthol, 2-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone (but no naphthalene) 9 days after exposure (Mackell et al. 1951). With the exception of the 1,4-naphthoquinone, these metabolites were still detectable on day 13, but not on day 17. These data indicate that urinary excretion of metabolites may be prolonged following exposure. It is important to note, however, that delayed dissolution and absorption from the gastrointestinal tract may also be a contributing factor. Unabsorbed naphthalene was visible in the fecal matter after ingestion of naphthalene flakes or mothballs in several individuals (Zuelzer and Apt 1949).

In nonhuman primate studies, Rhesus monkeys given naphthalene at oral doses up to 200 mg/kg did not excrete naphthalene as thioethers in urine or feces (Rozman et al. 1982). In a similar study, chimpanzees orally administered naphthalene at 200 mg/kg did not excrete naphthalene as thioethers in urine (Summer et al. 1979). These data suggest that glutathione conjugation of naphthalene may not occur to any great extent in nonhuman primates. Data from two chimpanzees indicate that most of the naphthalene excreted in this species is excreted as glucuronic acid and sulfate conjugates (Summer et al. 1979).

In rats administered radiolabelled naphthalene, the amount of label recovered in 24 hours was 77–93% in urine and 6–7% in feces (Bakke et al. 1985). There was a dose-dependent increase in urinary thioether excretion following gavage doses of naphthalene at 30, 75, and 200 mg/kg within 24 hours (Summer et al. 1979). The levels of thioethers excreted accounted for approximately 39, 32, and 26% of the three dose levels tested.

No information was located that documented excretion in humans after oral exposure to 2-methylnaphthalene. In guinea pigs, 80% of a 10 mg/kg tritium-labeled dose was excreted in the urine within 24 hours and about 10% was recovered in the feces (Teshima et al. 1983). Most of the excreted material (76%) was found as 2-naphthoic acid or its conjugates. About 18% of the recovered label was found as conjugates of 7-methyl-1-naphthol.

No studies were located that examined excretion in humans or animals after oral exposure to 1-methylnaphthalene.

3.4.4.3 Dermal Exposure

No reports have been located which discuss the excretion of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene in humans following dermal exposure.

The dermal exposure of rats to ^{14}C-labeled naphthalene was evaluated over a 48-hour period (Turkall et al. 1994). Naphthalene (43 μg) samples were applied to shaved 13-cm^2 areas on the skin under a sealed plastic cap. Neat naphthalene or naphthalene adsorbed to the surface of sandy soil or clay soil was tested. In all three cases, excretion of the label was primarily through the urine (70–87%). With the pure naphthalene and naphthalene adsorbed to clay soil, the exhaled air accounted for 6–14% of the administered label. Exhaled air contained only 0.9% of the label in the sandy soil group. This finding was presumably related to the slower adsorption of naphthalene from the sandy soil and its more rapid metabolism to nonvolatile metabolites. Less than 0.02% of the label was exhaled as carbon dioxide in all groups. The feces contained 2–4% of the label.

The primary metabolites in the urine after dermal application of naphthalene were 2,7-dihydroxy-naphthalene, 1,2-dihydroxynaphthalene, and 1,2-naphthoquinone (Turkall et al. 1994). The ratio of these metabolites for pure naphthalene and naphthalene adsorbed to clay soil were roughly 3:2:1. For the sandy soil, the corresponding ratio was 3:2:1.5. Small amounts of 1-naphthol and 2-naphthol were also excreted. In all cases, the amount of urinary free naphthalene was less than 0.4% of the administered label.

No studies were located that documented excretion in animals after dermal exposure to 1-methyl-naphthalene or 2-methylnaphthalene.

3.4.4.4 Other Routes of Exposure

In mouse studies using the intraperitoneal or subcutaneous exposure routes, several naphthalene metabolites were excreted in the urine. After intraperitoneal administration of 100 mg/kg naphthalene, conjugates accounted for 80–95% of the urinary metabolites (Horning et al. 1980; Stillwell et al. 1982). Much of the conjugated material was present as thioethers (glutathione conjugates and their derivatives). The major oxidation products of naphthalene metabolism were 1-naphthol and trans-1,2-di-hydro-1,2-naphthalenediol.

Following subcutaneous administration of 0.3 mg/kg ^{14}C-labeled 2-methylnaphthalene, 55% was found in the urine of rats (Melancon et al. 1982). Naphthoic acid and its glycine conjugate were identified. Three other metabolites were tentatively identified as isomeric diols.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen and Krishnan 1994; Andersen et al. 1987). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parameterization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations

provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

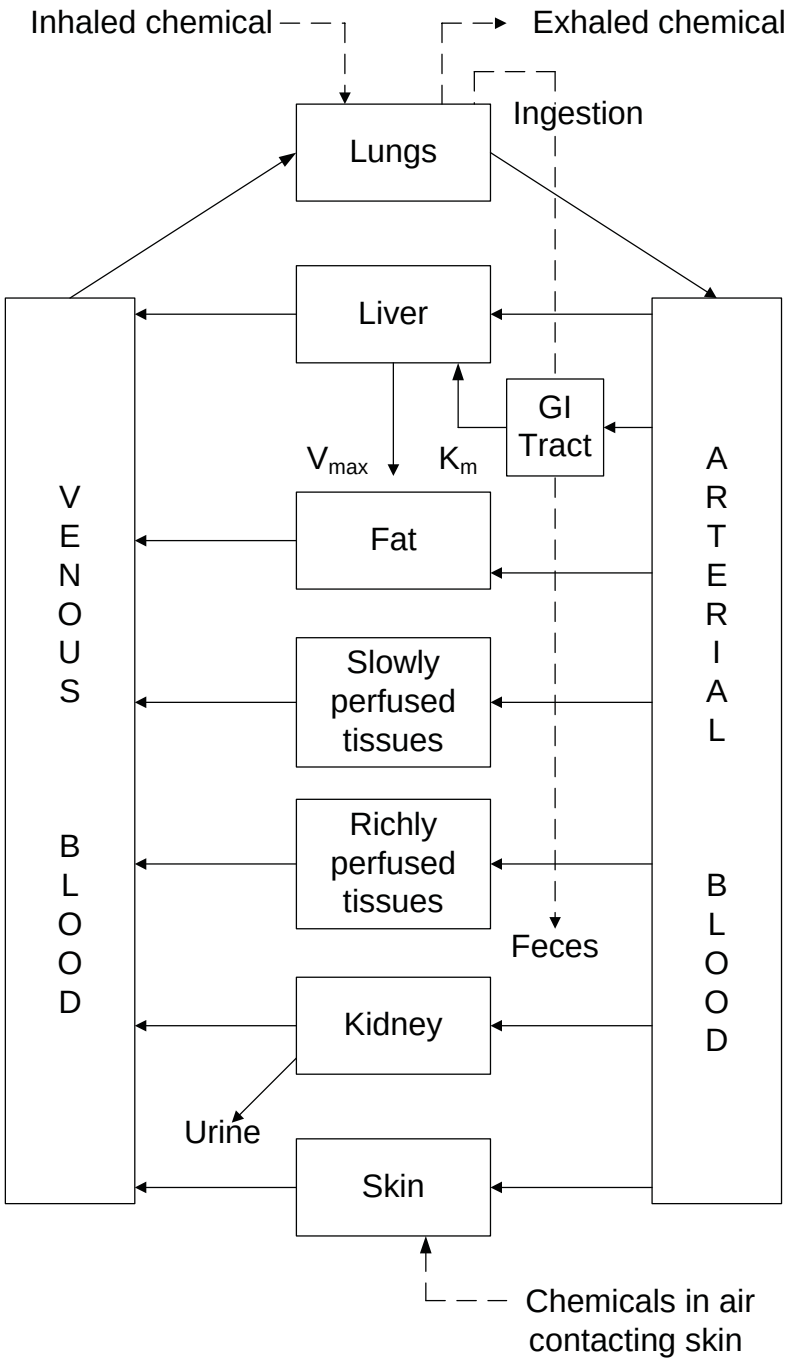
The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) are adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-5 shows a conceptualized representation of a PBPK model.

If PBPK models for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

This section will discuss the structure and application of the most recent PBPK models for naphthalene that were developed with *in vivo* data for the time-course of naphthalene in blood in rats and mice following inhalation exposure or intravenous administration (Willems et al. 2001). The inhalation data were used to select best-fitting models with the fewest assumptions possible and to optimize model parameters. The intravenous data were used to examine the validity of the final models. These models are refinements of earlier PBPK models for naphthalene in rats and mice, which were developed using parameters estimated from *in vitro* data (Ghanem and Shuler 2000; Quick and Shuler 1999; Sweeney et al. 1996). The most recent models have been used to attempt to explain why naphthalene-induced lung tumors in female B6C3F1 mice, but did not induce lung tumors in F344/N rats in chronic inhalation studies (Abdo et al. 2001; NTP 1992a, 2000). The use of these models to extrapolate dosimetry from rodents to humans is not possible until appropriate validated human physiologically based toxicokinetic (PBTK) models for naphthalene are developed.

Figure 3-5. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

The models do not include nasal compartments that metabolize naphthalene, because no data were available on nasal deposition and epithelial absorption of naphthalene (Willems et al. 2001). Without such data, reliable models for nasal deposition, tissue dosimetry, and nasal-tissue metabolism cannot be developed for naphthalene (models similar to those developed for other nasal toxicants such as acrylic acid [Frederick et al. 2001]). The existence of validated PBTK models with metabolizing nasal compartments would be useful to help to explain why male and female rats develop nasal tumors with chronic inhalation exposure to naphthalene, but mice do not, even though both species develop nonneoplastic lesions in the nasal tissues in which tumors developed in rats (Abdo et al. 2001; NTP 1992a, 2000). In addition, development of human models incorporating anatomical and physiological characteristics of nasal tissue will be useful to decrease uncertainty in extrapolating dose-response relationships for nasal effects in rodents to humans.

The final best-fitting models for rats and mice are comprised of two parts: (1) a diffusion-limited naphthalene submodel with compartments for arterial and venous blood, alveolar space, and tissue and capillary spaces for the lung, liver, kidney, fat, and other organs (with naphthalene metabolism occurring in the liver and lung by the same CYP isozyme with one set of Michaelis-Menten metabolic rate constants); and (2) a flow-limited 1,2- naphthalene oxide submodel describing metabolism and distribution of naphthalene oxide in the same compartments as in the naphthalene submodel (but without tissue capillary spaces) (Willems et al. 2001). Physiological parameters in both submodels (e.g., cardiac output, ventilation rates, tissue volumes, tissue capillary volumes, tissue blood flows) were taken from the literature and scaled to body weights of rats in the NTP (2000) bioassay and reference values for mice. Partition coefficients between the various compartments were calculated from octanol-water partition coefficients. Metabolic rate constants (Vmax and Km) and permeability constants (blood:fat and blood:other tissues) for naphthalene were estimated by fitting the models to naphthalene blood time-course data from the inhalation studies. The naphthalene oxide submodel was essentially the same as that developed by Quick and Shuler (1999) with *in vitro* data, with the exception that it contained a subroutine for reduced glutathione synthesis involving γ-glutamylcysteine synthetase modeled with Michaelis-Menten rate constants and noncompetitive inhibition by reduced glutathione. The metabolic fate of naphthalene oxide in the lung and liver was restricted to dihydrodiol formation via epoxide hydrolase and conjugation to glutathione via glutathione-*S*-transferase. The model did not include spontaneous conversion of naphthalene oxide to 1-naphthol or metabolic transformations to the naphthoquinones. Because no *in vivo* data were available on naphthalene oxide distribution or metabolism, the model predictions for naphthalene oxide tissue dosimetry could not be verified.

Under exposure conditions used in the rat (0, 10, 30, or 60 ppm) and mouse (0, 10, or 30 ppm) NTP (1992a, 2000) chronic inhalation bioassays with naphthalene (6 hours/day), the models predicted that: (1) steady-state lung concentrations of the parent compound, naphthalene, were not very different in rats and mice at equivalent exposure concentrations; (2) cumulative daily naphthalene metabolism in the lung was greater in the mouse than in the rat (by about 1.5- to 2.5-fold) at equivalent exposure concentrations; (3) cumulative daily naphthalene metabolism in the lung (64.9 mg/kg) and estimated maximal lung concentrations of naphthalene oxide (about 12 nmol/mL) for 30-ppm female mice, some of which developed lung tumors, were greater than respective values of 45.9 mg/kg and about 8 nmol/mL in 60-ppm female rats, which did not develop lung tumors; and (4) cumulative daily naphthalene metabolism in the lung was only slightly greater in 30-ppm female mice (64.9 mg/kg) than in the 30-ppm male mice (60.7 mg/kg), which did show statistically significant increased incidence of lung tumors (comparisons of lung concentrations of naphthalene oxide in female and male mice were not reported).

The model simulations are consistent with the hypothesis that the difference in lung tumor response between mice and rats may be due to a combination of greater maximal levels of naphthalene oxide or other metabolites in the mouse lung and, perhaps, a greater susceptibility of the mouse lung to epoxide-induced carcinogenesis. Results with other chemicals, such as ethylene oxide, suggest that the mouse lung may be more susceptible to epoxides than the rat lung (Willems et al. 2001). Differences in predicted cumulative lung metabolism of naphthalene in 30-ppm female mice and 30-ppm male mice were smaller than the difference noted between 30-ppm female mice and 60-ppm female rats; thus the model simulations do not explain the apparent gender difference in tumor response of the mouse lung. The formation of naphthoquinone metabolites was not included in the model. Thus, the model simulations do not provide a basis for identifying which metabolite is responsible for the nonneoplastic and neoplastic responses to naphthalene in the female mouse lung.

3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanisms by which naphthalene, 1-methyl-naphthalene, or 2-methylnaphthalene are absorbed from the guts, lungs, or skin. Although absorption of these compounds at these sites has been demonstrated, it is unknown if the transport is passive, active, or carried out by a facilitated diffusion mechanism. The relatively small molecular weights and lipophilicity of these compounds indicate that passive diffusion across cell membranes is a possible mechanistic path.

There is some evidence that different vehicles may influence the rate and extent of gastrointestinal or dermal absorption. Naphthalene adsorbed to organic-rich soils was absorbed across the skin more slowly than naphthalene from organic-poor soils (Turkall et al. 1994).

Distribution. As discussed in more detail in Section 3.4.2, there are limited data on the distribution of naphthalene and 2-methylnaphthalene in animals following oral or parenteral administration, but there are no data for these compounds following inhalation exposure or for 1-methylnaphthalene by any exposure route. The available data are inadequate to characterize the mechanisms by which 2-methylnaphthalene may be transported following oral exposure to the lung, the site of toxic action with acute or chronic exposure. No data are available on differences in deposition and absorption of inhaled naphthalene in nasal epithelial tissue, two of which (olfactory epithelium and respiratory epithelium) are key toxicity targets in rats and mice following chronic inhalation exposure to naphthalene.

Metabolism. As discussed in more detail in Section 3.4.3, results from *in vitro* and *in vivo* metabolic studies in mammalian systems indicate that naphthalene metabolism is complex, with multiple competing pathways leading to the formation of several reactive metabolites (e.g., 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone) and an array of conjugated and nonconjugated metabolites that are excreted predominantly in the urine. Conjugation of the reactive metabolites is viewed as a detoxifying mechanism for the reactive metabolites. With oral exposure, the liver is expected to be the principal site of metabolism, but metabolism of naphthalene at other tissue sites, including the nasal olfactory epithelium, Clara cells in pulmonary epithelial tissue, and eye tissue, has been demonstrated. A first-pass metabolic effect due to liver metabolism is expected with oral exposure, but the degree to which a first-pass effect due to respiratory tissue metabolism occurs with inhalation exposure to naphthalene has not been studied quantitatively.

Section 3.4.3 also discusses in more detail the complexity of 2-methylnaphthalene metabolism, which, in contrast to naphthalene, involves several competing initial steps: oxidation of the methyl side group and oxidation at several positions on the rings. Oxidation of the methyl side group is the principal metabolic pathway, representing about 50–80% of administered doses in animal studies. An array of conjugated and nonconjugated metabolites that are principally excreted in the urine have been identified in animal studies. Although conjugation of metabolites (principally with glutathione) appears to be a detoxication mechanism with acute exposure in animal studies, the involvement of reactive metabolites in the development of pulmonary alveolar proteinosis from chronic exposure to 2-methylnaphthalene is

uncertain (see Section 3.5.2). No studies were located on the metabolism of 1-methylnaphthalene in humans or animals, but it is expected to be similar to 2-methylnaphthalene metabolism based on its similar chemical, physical, and toxicological properties.

Excretion. As discussed in more detail in Section 3.4.4, results from animal studies involving oral or parenteral exposure indicate that naphthalene and 2-methylnaphthalene are principally excreted as metabolites in urine. Excretion in the feces represents a minor excretion pathway for these chemicals, and the possibility of excretion via exhalation of unmetabolized parent compounds has not been examined in available studies. Data for 1-methylnaphthalene were not located, but excretion is likely to be similar to 2-methylnaphthalene given the similarity in chemical and physical properties of these chemicals.

3.5.2 Mechanisms of Toxicity

Some information on the mechanism of toxicity is available for three of the health effects associated with naphthalene exposure: hemolysis, the development of lens opacities (cataracts), and nonneoplastic and neoplastic respiratory tract lesions. Mechanistic hypotheses for these naphthalene-induced effects are discussed below, followed by a discussion of the limited mechanistic information on 1-methyl-naphthalene- and 2-methylnaphthalene-induced pulmonary alveolar proteinosis.

Naphthalene-induced Hemolysis. Humans experience red-cell hemolysis after naphthalene exposure by the inhalation, oral, and dermal routes. In general, animal species are less susceptible than humans. There are no reports of naphthalene-induced hemolysis in either rats or mice; however, hemolysis has been observed in dogs.

Chemically induced red blood cell hemolysis is caused by a breakdown of the system that protects the erythrocyte biomolecules from oxidation. In the erythrocyte, glutathione peroxidase rather than catalase is the major antioxidant enzyme. Glutathione peroxidase (Gpx) is a selenium containing metalloprotein that utilizes reduced glutathione as a cofactor. Oxidized glutathione is reduced by glutathione reductase, a nicotinamide adenine dinucleotide phosphate (NADPH)-requiring enzyme.

The primary source of erythrocyte NADPH is glucose-6-phosphate oxidation by the enzyme G6PD. Individuals who suffer from a genetic defect resulting in a modified enzyme structure (a recessive trait) have a reduced capacity to produce NADPH. Accordingly, they are more susceptible to red cell

hemolysis than individuals without this defect (Gosselin et al. 1984). There is some evidence that heterozygotes may also have an increased susceptibility to red cell hemolysis (Dawson et al. 1958).

When the red blood cell is exposed to oxidizing agents, heme iron is oxidized to the ferric state, producing methemoglobin. This in turn leads to Heinz body formation. It is believed that free radical oxygen modifies membrane lipids leading to increased membrane fragility and lysis. Destruction of the red blood cells decreases erythrocyte counts and stimulates hematopoiesis (leading to increased numbers of reticulocytes). The oxygen carrying capacity of the blood is reduced. Cell lysis releases heme and protein into the blood. Heme breakdown produces bilirubin and biliverdin, causing jaundice. Both erythrocytes and heme breakdown products (urobilinogen) spill into the urine.

Several suggestions can be made regarding the impact of naphthalene on this sequence of events. Since naphthalene is conjugated with glutathione for excretion, it can reduce the supplies of glutathione available for glutathione peroxidase and increase the vulnerability of the cell to oxidation. It is also possible that a naphthalene metabolite may act as an inhibitor for either glutathione peroxidase or glutathione reductase. Glutathione reductase activity was reduced in children who experienced hemolysis following dermal exposure to naphthalene and in related family members (Dawson et al. 1958). Both glutathione peroxidase and glutathione reductase activity were decreased in the lens of rats orally exposed to naphthalene (Rathbun et al. 1990; Tao et al. 1991).

Each of the hypotheses discussed above would serve to increase the sensitivity of any naphthalene-exposed subject to an external oxidizing agent. However, given the severity of the hemolysis that follows naphthalene exposure, it is probable that naphthalene or a naphthalene metabolite also acts as an oxidizing agent in the erythrocyte. Unfortunately, data could not be identified which would correlate the production of any particular metabolite with initiation of red cell peroxidation.

Naphthalene-induced Cataracts. Although there are reports that inhalation, oral, and dermal naphthalene exposure in humans can lead to lens opacities (Grant 1986), the case studies or industrial exposure reports that link naphthalene to cataracts in humans have not been verified by well-conducted epidemiological studies of individuals exposed to naphthalene vapors on a chronic basis. In addition, impurities present in the naphthalene may have contributed to the cataract development in all recorded human cases. Conversely, there are data from a number of well-conducted studies which demonstrate that naphthalene can induce cataracts in animals.

Much of the animal data regarding ocular effects suggest that the toxicity of naphthalene is mediated by the *in situ* formation of 1,2-naphthalenediol in the lens. It has been proposed that metabolism of naphthalene starts in the liver, yielding epoxide metabolites that are subsequently converted to stable hydroxy compounds that circulate to the lens (Van Heyningen and Pirie 1967). The 1,2-naphthalenediol metabolite is subsequently oxidized to 1,2-naphthaquinone and hydrogen peroxide. The quinone metabolite binds to constituents of the lens (protein, amino acids, and glutathione), disrupting its integrity and transparency (Rees and Pirie 1967; Uyama et al. 1955; Van Heyningen and Pirie 1967; Van Heyningen 1976, 1979; Wells et al. 1989).

Intraperitoneal administration of naphthalene (125–1,000 mg/kg), 1-naphthol (56–562 mg/kg), 1,2-naphthoquinone (5–250 mg/kg), and 1,4-naphthoquinone (5–250 mg/kg) caused a dose-related increase in cataracts in C57BL/6 mice, but administration of 2-naphthol (56–456 mg/kg) did not (Wells et al. 1989). The cataractogenic potency of the naphthoquinones was about 10 times that of naphthalene. The cataractogenic potency of 1-naphthol was intermediate to that of naphthalene and the naphthoquinones. The potency of naphthalene was increased by pretreatment with cytochrome P-450 inducers and a glutathione-depleting agent. It was inhibited by pretreatment with a cytochrome P-450 inhibitor. This suggests that the unconjugated oxidized naphthoquinone metabolites are a necessary prerequisite for cataract formation. There are differences in species and strain susceptibility to cataract formation that theoretically relate to the animals' ability to form these metabolites. Naphthalene, 1-naphthol, 1,2-naphthoquinone, and 1,4-naphthoquinone did not form cataracts in DBA/2 mice suggesting the difference between strains is not simply due to metabolite exposure (Wells et al. 1989).

Because hydrogen peroxide is also formed following the oxidation of 1,2-dihyroxynaphthalene, peroxides may play a role in naphthalene-induced ocular damage. Increased levels of ocular lipid peroxides were noted in rats given incremental doses of naphthalene which increased from 100 to 750 mg/kg/day during a 9 week period (Germansky and Jamall 1988). The antioxidants caffeic acid (527 mg/kg) and vitamin E (250 mg/kg), which have free radical protection properties, and the free radical spin trapping agent α-phenyl-N-t-butylnitrone (PBN) (518 mg/kg) diminished the incidence of cataracts in animals given 750 mg/kg naphthalene (Wells et al. 1989). There were no cataracts in the rats given only PBN.

Support for this mechanism of cataract formation was provided by a gavage study in which five rat strains (pigmented and albino) were given 500 mg/kg/day naphthalene for 3 days and 1,000 mg/kg/day for the remainder of the 28-day treatment period (Xu et al. 1992b). After 3 weeks, there was a decrease in reduced glutathione (GSH) in the lens, an increase in protein-glutathione mixed disulfides, and an

increase in high molecular weight insoluble proteins (Xu et al. 1992a, 1992b). The only metabolite detected in the aqueous humor of the lens was 1,2-dihydro-1,2-naphthalenediol. The authors hypothesized that 1,2-dihydro-1,2-naphthalenediol was oxidized to 1,2-naphthalenediol and then to 1,2-naphthoquinone. The 1,2-naphthoquinone is believed to be responsible for the chemical changes in the eyes either through crosslinking reactions or by generating free radicals (Xu et al. 1992a). All of the rats developed cataracts.

The complete mechanism for this sequence of reactions is not clear. In *in vitro* studies of cataract formation, 1,2-dihydro-1,2,naphthalenediol was the only metabolite that resulted in cataracts that were morphologically the same as those generated *in vivo* (Xu et al. 1992a). Although 1,2-naphthalenediol and naphthoquinone also formed cataracts in lens culture studies, the opacities were located in the outer layer of the cortex rather than inside the lens. Also, the permeability of the cultured lens to the metabolites in the media may have contributed to the differences in lesion location.

When the aldose reductase inhibitor, AL01576, was given to rats along with the same naphthalene doses, no cataracts developed (Xu et al. 1992a, 1992b). Aldose reductase is an enzyme found in the lens, liver, and peripheral neurons that reduces aldehyde sugars such as glucose to their corresponding alcohols (McGilvery 1983). It is believed to oxidize 1,2-naphthalenediol to 1,2-naphthoquinone; therefore, when this reaction is inhibited, the quinone hypothetically does not form and there is no eye damage (Xu et al. 1992a). Support for this hypothesis includes observations that aldose reductase inhibitors inhibit cataract formation in naphthalene-exposed rats (Tao et al. 1991; Xu et al. 1992a), dihydrodiol dehydrogenase is apparently absent in rat lens (Greene et al. 2000), and aldose reductase appears to be the only enzyme in rat lens that can transform 1,2-dihydroxy-1,2-dihydronaphthalene to 1,2-naphthoquinone (Sugiyama et al. 1999).

Naphthalene-induced Nonneoplastic and Neoplastic Respiratory Tract Lesions. The mechanisms by which naphthalene affects mouse lung epithelial tissue and mouse and rat nasal epithelial tissue are thought to involve metabolic intermediates that can react with tissue macromolecules: 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone (Buckpitt et al. 2002). The innate reactivity of 1,2-naphthalene oxide is demonstrated by a half-life of approximately 2–3 minutes in buffer at pH 7.4; the half-life is extended by the presence of albumins to about 11 minutes (Buckpitt et al. 2002; Kanekal et al. 1991). The reactivity of 1,2-naphthoquinone has been demonstrated by its ability to form N7-adducts with deoxyguanosine under acidic conditions (McCoull et al. 1999). A second mode by which 1,2-naphthoquinone may damage tissue macromolecules involves redox cycling of the ortho-quinone

moiety and the subsequent generation of reactive oxygen species, which can lead to lipid peroxidation, consumption of reducing equivalents, oxidation of DNA, or DNA strand breaks (Bolton et al. 2000). 1,2-Naphthoquinone caused hydroxyl radical formation and DNA strand scission in buffered solutions in the presence of NADPH and $CuCl_2$ (Flowers et al. 1997), was directly mutagenic in *S. typhimurium* (Flowers-Geary et al. 1996), and directly induced sister chromatid exchanges in human mononuclear leukocytes (Wilson et al. 1996). The comparative importance of these reactive metabolic intermediates of naphthalene in producing nonneoplastic and neoplastic lesions in lung or nasal epithelial tissue is unknown, although the difference between mice and rats in susceptibility to naphthalene-induced lung damage has been associated with greater rates of naphthalene transformation to epoxides and the formation of a different enantiomeric form of 1,2-naphthalene oxide in mice compared with rats.

A fourth reactive metabolic intermediate, 1,2-dihyroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene, has been proposed based on molecular structure characterizations of some urinary metabolites (Horning et al. 1980; Pakenham et al. 2002), but these metabolites represent minor metabolic fates of naphthalene and the potential importance of their proposed precursor in naphthalene toxicity is unstudied to date.

Mice are markedly more susceptible than rats to acute naphthalene-induced Clara cell injury (Buckpitt et al. 1992; West et al. 2001), as well as to lung inflammation and tumor development from chronic inhalation exposure (Abdo et al. 2001; NTP 1992a, 2000). The species difference in lung susceptibility has been correlated with higher rates of formation of a specific enantiomeric epoxide (1*R*,2*S*-naphthalene oxide) in lung microsomes and isolated dissected airways of mice compared with rats (Buckpitt et al. 1992, 1995). Rat, hamster, and monkey lung microsomes preferentially formed the 1*S*,2*R*-naphthalene oxide enantiomer and showed lower rates of formation of epoxides than mouse lung microsomes (Buckpitt et al. 1992). Microsomes from human lymphoblastoid cells expressing recombinant human CYP2F1 also showed preferential formation of the 1*S*,2*R*-naphthalene oxide enantiomer, providing some evidence that human transformation of naphthalene to reactive epoxides in lung tissue may be more like rats than mice (Lanza et al. 1999).

Although these observations on epoxide formation suggest that naphthalene may be metabolized to epoxide intermediates at faster rates and with different stereoselectivity in the mouse lung than in the human lung, the toxicologic significance of this species difference is uncertain. The uncertainty arises due to the possibility (and potential toxicological importance) of species differences in several steps in downstream metabolism including glutathione conjugation of the epoxide, transformation to the dihydrodiol via epoxide hydrolase, and transformations to 1,2- or 1,4-naphthoquinone. For example,

human liver microsomes have been reported to be more proficient at converting naphthalene to the dihydrodiol metabolite than rat and mouse liver microsomes (Kitteringham et al. 1996). These results suggest that epoxide hydrolase activities may be higher in humans than mice (although they do not necessarily reflect activities in the pertinent naphthalene target tissues) and that this may decrease the potential for epoxide-induced tissue damage in humans relative to mice (see Figure 3-3). However, this difference may cause relatively greater formation of 1,2-naphthoquinone (from the dihydrodiol via dihydrodiol dehydrogenase) in human tissue than in mouse tissue. While the toxicologic significance of such a difference is uncertain, it is possible that humans may be more susceptible than mice, due to the possible involvement of 1,2-naphthoquinone in naphthalene-induced lung injury as suggested by a report that 1,2-naphthoquinone was the predominant naphthalene metabolite covalently bound to proteins obtained from freshly isolated mouse Clara cells incubated for 1 hour with 0.5 mM naphthalene (Zheng et al. 1997). To date, mechanistic understanding of species differences in naphthalene bioactivation in the lung is too incomplete to definitively identify which naphthalene metabolite is responsible for the development of nonneoplastic or neoplastic lung lesions, or to rule out the possible human relevance of naphthalene-induced lung lesions in mice.

Species differences in susceptibility to naphthalene-induced nonneoplastic and neoplastic lesions in the olfactory and respiratory epithelia of the nose have not been correlated with differences in rates of transformation to 1,2-epoxide derivatives in extracts of olfactory tissue (Buckpitt et al. 1992; Plopper et al. 1992a). Rates of epoxide formation showed the order, mouse > rat > hamster, but rats were the most susceptible to acute nasal injury from naphthalene, showing olfactory epithelial necrosis and exfoliation following single intraperitoneal doses as low as 200 mg/kg naphthalene, compared with 400 mg/kg in mice and hamsters (Plopper et al. 1992a). These observations suggest that the reasons for species differences in susceptibility to naphthalene nasal toxicity are complex and do not solely involve differences in the formation of the 1,2-epoxide metabolic intermediates.

Involvement of the naphthoquinone metabolites is possible, but studies comparing species in their ability to form or accumulate reacted derivatives of naphthoquinones (or 1,2-naphthalene oxide) in nasal tissues (i.e., protein adducts) are not available. In blood of rats following gavage administration of single oral doses of naphthalene (100–800 mg/kg), levels of hemoglobin and albumin adducts with 1,2-naphthalene oxide were greater than levels of adducts of 1,2- and 1,4-naphthoquinone (Troester et al. 2002; Waidyanatha et al. 2002). These findings suggest that levels of the epoxide in the rats' blood were greater than levels of the naphthoquinones, but do not provide information on the relative amounts of these reactive metabolites in the target tissue, the nose.

Current information is inadequate to (1) identify which metabolite(s) are responsible for nonneoplastic or neoplastic nasal lesions that develop in rodents following chronic inhalation exposure, (2) explain why nasal tumors develop in rats but not in mice, or (3) rule out the possible human relevance of naphthalene-induced nasal lesions in rats or mice.

Evidence to support a nongenotoxic mode of action in naphthalene carcinogenicity involving sustained cell proliferation following repeated naphthalene-induced tissue damage includes the negative results in the genotoxicity database (see Section 3.3) suggesting that naphthalene and its metabolites (with the likely exception of 1,2-naphthoquinone) are not mutagens, and the findings that naphthalene-induced tumors in mice and rats occur in the same general tissues as those displaying nonneoplastic lesions. Evidence to support a genotoxic mode of action includes the consistently positive results for genotoxic action by 1,2-naphthoquinone and the limited and scattered positive results for genotoxic action by naphthalene in the presence of metabolic activation. Current evidence is not adequate to rule out the possibility of naphthalene genotoxic action or to determine pertinent threshold levels for genotoxic action, due to the absence of studies examining genotoxic end points in naphthalene target tissues, the nose and lung. As suggested by Moore and Harrington-Brock (2000), answering critical questions in human cancer risk assessment involves an understanding of the mode(s) of action of tumor induction in the target tissue(s) at environmentally-relevant concentrations. Such understanding can come from experiments examining genotoxic endpoints in target tissues. These data are not available for naphthalene.

In summary, the available evidence regarding the mechanism(s) by which naphthalene produces neoplastic and nonneoplastic lesions in the respiratory tract of rodents suggests the involvement of reactive metabolites. The identity of this metabolite(s), and evidence of its presence in known target tissues, remains unknown. The finding that mice are more susceptible than rats to naphthalene-induced lung toxicity may correlate with the *in vivo* generation of this reactive intermediate in target tissues. Whether the mechanism by which naphthalene produces neoplastic and nonneoplastic changes in the respiratory tract of rodents involves genotoxicity remains unknown.

1-Methylnaphthalene or 2-Methylnaphthalene-induced Pulmonary Alveolar Proteinosis. Exposure of mice to 1-methylnaphthalene or 2-methylnaphthalene in the diet for 81 weeks induced increased incidences of pulmonary alveolar proteinosis (Murata et al. 1993, 1997). The absence of nonneoplastic lesions in other lung regions or in other tissues indicates that the alveolar region of the lung is a critical and specific toxicity target of chronic oral exposure to 1-methylnaphthalene or 2-methylnaphthalene.

Increased incidences of pulmonary alveolar proteinosis have also been observed in mice exposed to dermal doses of methylnaphthalene (a 2:1 mixture of 2-methylnaphthalene and 1-methylnaphthalene) applied twice weekly for 20–61 weeks (Emi and Konishi 1985; Murata et al. 1992).

There is evidence to suggest that type II pneumocytes are specific cellular targets of the methylnaphthalenes. Pulmonary hyperplasia and hypertrophy of type II pneumocytes in alveolar regions with proteinosis was observed by light microscopy in mice that were repeatedly exposed to dermal doses of methylnaphthalene (119 mg/kg methylnaphthalene twice a week for 30 weeks [Murata et al. 1992]). In this same study, electron microscopic examination showed that alveolar spaces were filled with numerous myelinoid structures that resembled lamellar bodies of type II pneumocytes. This extracellular material was associated with mononucleated giant cells (called balloon cells) containing numerous myelinoid structures, lipid droplets, and electron dense ascicular crystals. The authors hypothesized that, in response to 1-methylnaphthalene or 2-methylnaphthalene, type II pneumocytes produce increased amounts of lamellar bodies due to hyperplasia and hypertrophy, and eventually transform into balloon cells. Balloon cell rupture has been hypothesized to lead to the accumulation of the myelinoid structures in the alveolar lumen. Ultrastructural studies of the pathogenesis of pulmonary alveolar proteinosis from chronic exposure to 2-methylnaphthalene or 1-methylnaphthalene alone were not available. However, the lesions detected by light microscopy following chronic oral exposure to 2-methylnaphthalene or 1-methylnaphthalene alone were very similar to the lesions detected following chronic dermal exposure to the mixture. These similarities suggest that the mechanistic hypotheses prompted by observations for the mixture are relevant to the individual methylnaphthalenes.

The mechanism of targeting type II pneumocytes is consistent with what is generally known regarding the etiology of pulmonary alveolar proteinosis in humans. The disease in humans, characterized by the accumulation of surfactant material in the alveolar lumen, has been hypothesized to be caused by either excessive secretion of surfactant by type II pneumocytes, or disruption of surfactant clearance by macrophages (Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997). The condition in humans has been associated with pulmonary dysfunction, characterized by decreased functional lung volume, reduced diffusing capacity, and symptoms such as dyspnea and cough. Pulmonary alveolar proteinosis has not been associated with airflow obstruction (EPA 2003; Lee et al. 1997; Mazzone et al. 2001; Wang et al. 1997).

The development of pulmonary alveolar proteinosis in mice appears to require prolonged oral exposure to 2-methylnaphthalene (or 1-methylnaphthalene). Exposure to a dietary concentration of 0.075% 2-methyl-

naphthalene for 81 weeks induced increased incidences of the lesion, but 13-week exposure to concentrations as high as 1.33% 2-methylnaphthalene did not (Murata et al. 1997). No further studies of the temporal development of methylnaphthalene-induced pulmonary alveolar proteinosis are available.

It is unknown whether the parent compounds or metabolites are responsible for the development of methylnaphthalene-induced pulmonary alveolar proteinosis. Type II pneumocytes are enriched in CYP monooxygenases (Castranova et al. 1988), which are involved in metabolizing 2-methylnaphthalene, and it is possible that metabolites may play a role in the pathogenesis of pulmonary alveolar proteinosis. Studies designed to test this hypothesis, however, have not been conducted.

In contrast to chronic oral exposure, which targets alveolar type II pneumocytes, acute intraperitoneal injection of 2-methylnaphthalene into mice targets bronchiolar Clara cells, inducing Clara cell abnormalities, focal or complete sloughing of Clara cells, or complete sloughing of the entire bronchiolar lining (Buckpitt et al. 1986; Griffin et al. 1981, 1982, 1983; Honda et al. 1990; Rasmussen et al. 1986). Mechanistic studies have not provided clear evidence that metabolites are involved in this response to acute exposure to 2-methylnaphthalene. For example, pretreatment of male C57BL/6J mice with phenobarbital (an inducer of CYP2B; 75 mg/kg, 4 days prior) or 3-methylcholanthrene (an inducer of CYP1A; 80 mg/kg, 2 days prior) prior to injection with 400 mg/kg 2-methylnaphthalene reduced the severity of bronchiolar necrosis in all mice compared to those injected without pretreatment (Griffin et al. 1982). However, CYP inhibitors, such as piperonyl butoxide (a mixed monooxygenase inhibitor; 1,000 mg/kg, 30 minutes prior) and SKF 525-A (an inhibitor of CYP1B; 25 mg/kg, 30 minutes prior), had no effect on the severity of the lung lesions. The mechanism of acute Clara cell toxicity of 2-methyl-naphthalene may be similar to that of naphthalene, which involves CYP-mediated metabolism via ring epoxidation to reactive species such as the 1,2-naphthalene oxide and 1,2-naphthoquinone (Cho et al. 1995; Greene et al. 2000; Lakritz et al. 1996; Van Winkle et al. 1999). This hypothesis is supported by the finding that 2-methylnaphthalene is less acutely toxic than naphthalene (Buckpitt and Franklin 1989; Cho et al. 1995) and that only a small fraction of 2-methylnaphthalene (15-20%) undergoes metabolic ring epoxidation (Breger et al. 1983; Melancon et al. 1985). Information on the mechanism of the acute response of Clara cells is not expected to be directly related to the pathogenesis of pulmonary alveolar proteinosis from chronic oral or dermal exposure to 2-methylnaphthalene, because in mice chronically exposed to 2-methylnaphthalene or 1-methylnaphthalene for 81 weeks, no evidence for exposure-related bronchiolar Clara cell lesions was found (Murata et al. 1993, 1997). This finding is not surprising, as Clara cells have been shown to develop resistance to the acute toxicity of naphthalene (Lakritz et al.

1996). The possible development of Clara cell resistance to the acute toxicity of 2-methylnaphthalene, however, has not been studied.

Data are limited to support the hypothesis that rats are less sensitive than mice to the lung damage caused by acute exposure to 2-methylnaphthalene. Wistar rats given intraperitoneal doses of 142 mg/kg 2-methylnaphthalene did not develop lung lesions (Dinsdale and Verschoyle 1987). However, bronchiolar necrosis was induced in Swiss-Webster mice injected with the same dose (Rasmussen et al. 1986) and in C57BL/6J and DBA/2J mice injected with 100 mg/kg 2-methylnaphthalene (Griffin et al. 1981, 1982, 1983). No data are available for interspecies comparisons of the chronic toxicity of 1-methynaphthalene or 2-methylnaphthalene.

3.5.3 Animal-to-Human Extrapolations

Naphthalene-induced lesions in nasal epithelia of mice and rats appear to be the critical nonneoplastic effect (i.e., the effect occurring at the lowest exposure level) associated with inhalation exposure to naphthalene. As discussed in Section 3.5.2, studies with microsomes from human and animal cells indicate that there are species differences in specific steps of naphthalene metabolism (Buckpitt et al. 1992; Kitteringham et al. 1996; Lanza et al. 1999), but mechanistic understanding of these differences is too incomplete to effectively argue that they rule out the possible human relevance of naphthalene-induced lung lesions in mice or nasal lesions in rats or mice. Rodents and humans also display distinct differences in nasal anatomy and respiratory physiology that may cause different deposited doses, and subsequently different responses, in human nasal tissue relative to rats or mice. However, the anatomical and physiological differences alone are insufficient to rule out the possible human relevance of naphthalene-induced nasal lesions in rats or mice. For example, rat and human hybrid computational fluid dynamics and PBPK models, developed for acrylic acid, another rodent nasal toxicant, predicted that tissue concentrations of acrylic acid in human and rat nasal tissues would be similar when exposure conditions were the same (Frederick et al. 2001). Current PBPK models for naphthalene do not include nasal compartments that metabolize naphthalene, because no data were available on nasal deposition and epithelial absorption of naphthalene (Willems et al. 2001). In the absence of this type of data or a pertinent validated human PBPK model, it is reasonable to assume that naphthalene-induced nonneoplastic and neoplastic lesions observed in nasal tissues of rats and mice are relevant to humans. Development of rat, mouse, and human hybrid computational fluid dynamics and PBPK models that include metabolizing nasal compartments and the application of the models to extrapolating rat or mouse

nasal doses to humans will likely decrease uncertainty in extrapolating naphthalene health hazards from rodents to humans.

In animals orally exposed to naphthalene, the critical effects appear to be decreased weight gain and clinical signs of toxicity in pregnant rats with acute exposure and decreased body weight in rats with intermediate-duration exposure. Mechanisms associated with these effects are unstudied. Reliable data to preclude the relevance of these effects to humans were not located.

Pulmonary alveolar proteinosis induced in mice following chronic oral exposure to 1-methylnaphthalene or 2-methylnaphthalene is assumed to be relevant to humans, in the absence of data to indicate otherwise. Pulmonary alveolar proteinosis is a condition that has been described in humans, although reports noting associations with human exposure to 1-methylnaphthalene or 2-methylnaphthalene were not located.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Clement (1992), was also used in 1996 when Congress mandated the EPA to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), and in 1998, the EDSTAC completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and

descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

No studies were located regarding endocrine disruption in human or animals after exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

No *in vitro* studies were located regarding endocrine disruption of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene.

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6, Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage

may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

Newborns and infants are thought to be more susceptible to adverse health effects from naphthalene (e.g., hemolytic anemia from acute exposure) because hepatic enzyme systems involved in conjugation and excretion of naphthalene metabolites are not well developed shortly after birth (EPA 1987a). No studies were located, however, that specifically examined the influence of age on naphthalene toxicokinetic capabilities in humans.

Although the occurrence of hemolytic anemia in neonates of anemic, naphthalene-exposed mothers demonstrates that naphthalene and/or its metabolites can cross the placental barrier (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958), oral-exposure developmental toxicity studies in animals do not provide evidence that naphthalene was fetotoxic or impaired fetal development, even at maternally toxic dose levels as high as 450 mg/kg/day (NTP 1991a; Plasterer et al. 1985; PRI 1986).

Naphthalene has been detected in human milk samples (concentration not reported) (Pellizzari et al. 1982), but no studies were located that have specifically examined the rate or extent of naphthalene distribution to breast milk in exposed humans or animals.

Children with genetically determined glucose-6-phosphate dehydrogenase (G6PD) deficiency are expected to be especially susceptible to the hemolytic action of naphthalene (Owa 1989; Owa et al. 1993; Santucci and Shah 2000; Valaes et al. 1963). In support of this hypothesis, in 21 cases of hemolytic anemia in Greek infants exposed to naphthalene, 10 of the children had a genetically determined deficiency in G6PD (Valaes et al. 1963). In a 10-year chart review of 24 African-American children hospitalized with acute hemolytic anemia, 14 were noted to have been exposed to naphthalene-containing moth repellants (Santucci and Shah 2000). Deficiency in G6PD makes red blood cells more susceptible to oxidative damage from a wide range of causes including naphthalene exposure. Relatively high rates of genetically determined G6PD deficiency have been reported in males of certain subpopulations of Asian, Arabic, Caucasian, African, and African-American ancestry (EPA 1987a).

The limited mobility of infants when they are wearing naphthalene-treated clothing or when they are near other naphthalene-treated articles (e.g., blankets treated with naphthalene-containing moth repellants) may maximize exposure due to the development of a microenvironment with a high level of naphthalene vapor in the space around the infant. The tendency for infants and small children to place small objects, such as mothballs, in their mouths also increases their risk.

An association between elevated maternal exposure to naphthalene and increased maternal cord-blood levels of one of four T cell types, IL-4, has recently been reported (Lehmann et al. 2002). The study looked for possible associations between maternal indoor exposure to 28 volatile organic chemicals (including naphthalene) and putative immune status at birth assessed by cord-blood levels of cytokine-producing T cells [interleukin-4 (IL-4), interleukin-2 (IL-2), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α)]. Levels of 28 volatile organic chemicals in air samples, collected during a 4-week postnatal period in bedrooms of 85 newborn children, were measured as surrogate indices of maternal

indoor exposure. A logistic regression analysis found an elevated odds ratio (OR=2.9; 95% CI 1.0–8.2) for elevated naphthalene air concentrations (>75th percentile) and elevated percentage of IL-4-producing T cells in cord blood. The analysis adjusted for possible confounding factors of family allergic (i.e., atopic) history and maternal smoking during pregnancy. Several other statistically significant associations were found for changes in levels of different types of T cells and air levels of other chemicals, including methylcyclopentane, trichloroethylene, and tetrachloroethylene. The significance of the observed variations in cord blood T cell levels to the immune status of the newborn children is unknown. The findings from this study are inadequate to determine if maternal exposure to naphthalene may influence the immune status of newborn children.

Studies that have examined age-related effects of toxicokinetic variables specifically related to naphthalene are restricted to a study with results indicating that neonatal mice may be more susceptible than adult mice to lung injury from single intraperitoneal doses of 25, 50, or 100 mg/kg naphthalene (Fanucchi et al. 1997). Epithelial damage in terminal bronchioles (principally in the Clara cells) was observed in 7-day-old mice exposed to 25 mg/kg, but was absent in adult mice at the same dose level. In adult mice exposed to 50 mg/kg, injury was only mild and variable (from mouse to mouse) and only became consistent with exposure to 100 mg/kg. Epithelial damage in 14-day-old mice was less severe than the damage in 7-day-old mice. Activities of CYP-mediated naphthalene metabolism in bronchiolar tissues were 2.5 times lower in neonatal mice than in adult mice, suggesting that the difference in susceptibility is not explained by differences in ability to form reactive metabolites alone (e.g., 1,2-naphthalene oxide). Differences between neonates and adults in the balance between formation of reactive naphthalene metabolites and downstream transformations could potentially explain the difference in susceptibility to naphthalene toxicity, but the possibilities for specific, age-related differences in downstream enzyme activities for naphthalene (e.g., epoxide hydrolase, dihydrodiol dehydrogenase) have not been studied to date. Alternatively, toxicodynamic differences may exist between neonatal and adult mice (e.g., different target macromolecules). Based on findings that *in utero* exposure to other chemicals, which are bioactivated by CYP, caused Clara cell tumors in adult offspring, Fanucchi et al. (1997) postulated that naphthalene exposure during the neonatal period, when increased susceptibility to naphthalene-induced cytotoxicity occurs, may lead to loss of regulatory mechanisms resulting in Clara cell proliferation and tumor formation in adult animals, but direct evidence for naphthalene in support of this hypothesis is not available (e.g., demonstration that *in utero* or neonatal naphthalene exposure will cause increased incidence of lung tumors in adult mice).

No direct information was located on the relative susceptibility of children or young animals to 1-methylnaphthalene or 2-methylnaphthalene toxicity, compared with adults. However, clinical experience with humans displaying pulmonary alveolar proteinosis of unknown etiology has indicated that children with this condition experience more severe symptoms and a poor prognosis for survival than do adults (EPA 2003r; Mazzone et al. 2001).

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung

capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10, Populations That Are Unusually Susceptible.

Additional information concerning biomarkers for effects on the immune, renal, and hepatic systems can be found in the CDC/ATSDR Subcommittee Report on Biological Indicators of Organ Damage (CDC/ATSDR 1990), and on the neurological system in the Office of Technology Assessment Report on Identifying and Controlling Poisons of the Nervous System (OTA 1990). Additional details concerning the health effects caused by naphthalene can be found in Section 3.2.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

In cases where humans have swallowed one or more mothballs, it is possible to identify the undissolved naphthalene in the stomach or duodenum by radioluminescence (Woolf et al. 1993). Thus, radiography of the abdominal area is of value in determining if exposure has occurred, especially in children who are often unreliable sources of exposure information. Of the 2,400 cases on naphthalene ingestion reported to 72 Poison Control Centers in the United States, 2,100 involve children less than 6 years old. Radioluminescence has the advantage of differentiating naphthalene-containing solids in the gastrointestinal tract from paradichlorobenzene or other materials used in moth repellants and deodorizers.

Methods are available for the determination of naphthalene in human adipose tissue (EPA 1986g; Liao et al. 1988). In the National Human Adipose Tissue Survey, 40% of the subjects surveyed had measurable levels of naphthalene with concentrations of up to 63 ng/g. Naphthalene and its metabolites can be detected in human and animal urine (Horning et al. 1980; Mackell et al. 1951; Stillwell et al. 1982). Investigators have reported strong correlations between 1-naphthol concentrations in the urine of exposed workers and naphthalene concentrations in the breathing zone air (Bieniek 1994). Peak naphthalene concentrations in the urine occurred immediately after the end of the exposure period and declined

thereafter. In some instances, 1-naphthol concentrations had returned to baseline 8 hours later. Few current data are available relating naphthalene levels in adipose tissue or urine with the human exposure concentrations.

In swine, a good correlation existed between 1-naphthol levels in hydrolyzed urine samples collected in the first and second 24 hours after dosing with as little as 7 µg/kg/day naphthalene (Keimig and Morgan 1986). Thus, 1-naphthol may be an appropriate biomarker for monitoring naphthalene exposures in the occupational setting. Some caution must be exercised in using 1-naphthol as a biomarker of naphthalene exposure in the general population since this metabolite is also excreted after exposure to the common insecticide, carbaryl (Benson and Dorough 1984).

Early work to develop biomarkers of exposure, such as naphthalene mercapturic acid derivatives in urine (Marco et al. 1993) and naphthalene hemoglobin adducts in blood (Cho et al. 1994b), has been extended to develop techniques to measure cysteinyl adducts formed from reactions of hemoglobin and albumin with reactive metabolites of naphthalene (Troester et al. 2002; Waidyanatha et al. 2002). One of the reasons for developing these techniques is that it is difficult to measure reactive metabolites of naphthalene *in vivo*. Using these techniques, hemoglobin and albumin adducts of 1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone were shown to increase with increasing dose in F344 rats given single oral doses of 0, 100, 200, 400, or 800 mg/kg naphthalene (Waidyanatha et al. 2002). The stabilities of the adducts were measured in rats following exposure to naphthalene (Troester et al. 2002). Some were found to be stable and others unstable, although they all were more stable than the reactive metabolites themselves. As such, the adducts are expected to be useful in estimating internal doses of these metabolites.

An analytical method is available to determine levels of 2-methylnaphthalene and its derivatives in rat urine (Melancon et al. 1982). This method would probably also be useful in measuring 2-methyl-naphthalene levels in human urine. Because of the lack of information for 1-methylnaphthalene, it is not possible to identify a biomarker of exposure for this substance.

3.8.2 Biomarkers Used to Characterize Effects Caused by Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Hemolytic anemia has been frequently reported to be a consequence of exposure to naphthalene. However, this effect can also occur without exposure to naphthalene, and may not be useful as a specific biomarker of effect.

Clara cell damage may be identified by the presence of naphthalene/protein adducts in lung lavage fluids (Cho et al. 1994a). Additional research is needed to improve the specificity of this technique as a biomarker of effect.

Because of the lack of information for 1-methylnaphthalene or 2-methylnaphthalene, it is not possible to identify a biomarker of effects for these chemicals.

3.9 INTERACTIONS WITH OTHER CHEMICALS

When either naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene was applied dermally in combination with benzo[a]pyrene (BaP), there was an inhibitory effect on the induction of skin tumors in female mice (Schmeltz et al. 1978). These investigators also reported that a mixture containing naphthalene (0.02%), 2-methylnaphthalene (0.02%) and 10 other methylated and ethylated naphthalenes (each at 0.02%) also appeared to inhibit the development of BaP-induced skin tumors. The authors suggested that it is likely that certain naphthalenes compete with BaP for the same enzyme sites, resulting in alteration of the BaP metabolic pathway and decreased production of the active BaP metabolite. This hypothesis is consistent with the observation that benzo(a)pyrene hydroxylase is inhibited by naphthalene (Shopp et al. 1984). Dermal application of the naphthalene mixture did not induce tumors in the absence of BaP. The results of these studies were not analyzed statistically.

Several studies have been conducted to assess factors that influence the toxicity of naphthalene. For the most part, these studies have evaluated the effects of mixed function oxidase activity (MFO) and alterations in glutathione levels on pulmonary and ocular toxicities. The effects of cyclooxygenase activity, antioxidants, and epoxide hydrolase inhibitors on the cataractogenic effect of naphthalene have also been evaluated. The administration of MFO inhibitors (SKF-525A, metyrapone) and antioxidants (caffeic acid and vitamin E) decreased ocular toxicity in mice (Wells et al. 1989). Use of ALO1576, an inhibitor of the enzyme aldose reductase, prevented cataract formation in both *in vivo* and *in vitro* studies

(Xu et al. 1992a, 1992b). On the other hand, naphthalene-induced cataracts were enhanced by pretreatment with a MFO inducer (phenobarbital) and a glutathione depletor (diethyl maleate) (Wells et al. 1989). Pulmonary damage was decreased by prior treatment with a MFO inhibitor (piperonyl butoxide), but enhanced by prior treatment with a glutathione depletor (diethyl maleate) (Warren et al. 1982). For the most part, these studies support the role for mixed function oxidase activity and glutathione conjugation in naphthalene-induced pulmonary and ocular lesions.

Mixed function oxidase inducers also affect the metabolism of 2-methylnaphthalene. Inducers that influence cytochrome P-450 increase the oxidation of the side chain and the concentration of one dihydrodiol. Induction of cytochrome P-450 increased the production of two other dihydrodiols (Melancon et al. 1985). The production of naphthoic acid in preference to the diols may explain why acute exposure to 2-methylnaphthalene is less toxic to Clara cells than acute exposure to naphthalene.

In general, interactions with environmental contaminants, such as polycyclic aromatic hydrocarbons, should be expected at hazardous waste sites. Most hazardous waste sites (with the notable exception of certain pharmaceutical sites) would not be expected to contain substantial volumes of certain types of contaminants, such as antioxidants or cytochrome P-450 inhibitors.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene than will most persons exposed to the same level of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene, or compromised function of organs affected by naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. Populations who are at greater risk due to their unusually high exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are discussed in Section 6.7, Populations with Potentially High Exposures.

The hemolytic response to naphthalene is enhanced by the presence of inherited erythrocyte G6PD deficiency. Although any human may experience acute hemolysis if exposed to a sufficiently high dose of naphthalene, this enzyme deficiency may cause some persons to be unusually sensitive. The incidence

of the deficiency among Caucasians of European origin is relatively low, while there is a higher incidence among certain groups of Asians and Middle Eastern populations. A study of hemolytic anemia in African-American children with G6PD deficiency by Shannon and Buchanan (1982) suggests that this is a population that may be susceptible to the hemolytic effects of naphthalene exposure. It was also reported that 16% of African-American males are G6PD-deficient (Calabrese 1986). According to Shannon and Buchanan (1982), a syndrome of acute severe hemolysis following exposure to oxidative stress is associated with the Mediterranean variant of the deficiency, whereas the hemolytic anemia seen in African-Americans is generally mild.

Results from a recent study indicate that female mice are more susceptible than male mice to lung injury from acute parenteral exposure to naphthalene (Van Winkle et al. 2002). Male and female Swiss-Webster mice were given intraperitoneal injections of 0 or 200 mg/kg naphthalene in corn oil, and lungs were removed at 1, 2, 3, 6, and 24 hours after treatment. Acute lung injury was determined by (1) high-resolution microscopic assessment of differential permeability to fluorescent nuclear dyes in cells along the long axis of conducting airway trees of microdissected right middle lung lobes and (2) high-resolution histopathology of sections of Karnovsky-fixed left lung lobes. Clara cell injury occurred in the terminal bronchioles of both male and female mice. Clara cell injury in terminal bronchioles, however, occurred earlier, affected cells farther up the airway tree, and showed a different temporal pattern of changes in female mice compared with male mice. Twenty-four hours after injection, Clara cell injury in the lobar bronchus of female mice was evidenced by numerous vacuolated cells, whereas normal bronchiolar epithelium containing Clara and ciliated cells was found in vehicle-control males and females, as well as in exposed male mice. Assessment of *in vitro* naphthalene metabolism in microdissected regions of airways from male and female mice by high performance liquid chromatography (HPLC) analysis indicated that the rate of formation of a dihydrodiol metabolite (1,2-dihydroxy-1,2-dihydronaphthalene) was greater in female tissue than in male tissue. This metabolic difference may be related to the apparent gender difference in susceptibility to acute lung injury from naphthalene. It is unknown whether or not the gender difference in susceptibility to acute lung injury is relevant to nasal or lung lesions formed with chronic-duration exposure to naphthalene.

There are no data that indicate whether there are populations that are unusually susceptible to the toxic effects of 1-methylnaphthalene or 2-methylnaphthalene.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene:

Kurz JM. 1987. Naphthalene poisoning: Critical care nursing techniques. Dimens Crit Care Nurs 6:264-270.

Melzer-Lange M, Walsh-Kelly C. 1989. Naphthalene-induced hemolysis in a black female toddler deficient in glucose-6-phosphate dehydrogenase. Pediatr Emerg Care 5:24-26.

Siegel E, Wason S. 1986. Mothball toxicity. Pediatr Clin North Am 33:369-374.

Stutz DR, Janusz SJ. 1988. Hazardous materials injuries: A handbook for pre-hospital care. Second edition. Beltsville, MD: Bradford Communications Corporation.

3.11.1 Reducing Peak Absorption Following Exposure

If inhalation of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene has occurred, movement to fresh air is recommended. In cases where a small amount (e.g., one mothball, 0.5–3.6 g) of naphthalene has been ingested, measures are implemented to empty the stomach contents. Syrup of Ipecac, which may be used for this purpose, is administered after ingestion to induce vomiting and is most effective if initiated within a 2-hour period after exposure (Siegel and Wason 1986). If large quantities of naphthalene have been ingested, syrup-of-ipecac-induced vomiting is usually followed by gastric aspiration using a large gauge lavaculator (to remove mothballs) (Kurz 1987). This will only be of value if the naphthalene particles are small enough to be aspirated. Measures are usually taken to protect the respiratory tract from aspiration of gastric contents. Activated charcoal can be given to bind dissolved naphthalene in the gastrointestinal tract. Further treatment with a cathartic (e.g., magnesium sulfate) to speed fecal excretion is recommended (Melzer-Lange and Walsh-Kelly 1989). Milk or fatty meals ingested within 2–3 hours after exposure may increase absorption (Siegel and Wason 1986).

In order to reduce absorption of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene through the skin, areas of skin that have come in contact with the compound should be washed with soap and water. Application of oil based lotions should be avoided. If these compounds are splashed into the eyes, irrigation with large amounts of water for 15–30 minutes may be useful to wash away unabsorbed material (Stutz and Janusz 1988).

3.11.2 Reducing Body Burden

Some evidence exists that naphthalene metabolites may be retained in the body in adipose tissue (EPA 1986g). Naphthalene was identified in 40% of the samples evaluated for the Human Adipose Tissue Survey (EPA 1986g). Naphthalene metabolites were detected in urine up to 13 days following exposure (Mackell et al. 1951).

The most frequently documented acute toxic effect of naphthalene in humans is red cell hemolysis. In cases of clinically significant hemolysis, accelerated urinary excretion of naphthol metabolites is recommended to protect the kidney from products of hemolysis (EPA 1989d). In cases of renal failure, hemodialysis may be effective in controlling extracellular fluid (plasma) composition (EPA 1989d). It should be noted that this method is not very effective in removing lipophilic compounds from blood. Ocular effects have also been reported in humans; however, there are no specific treatments for reducing the toxic effects on the eyes. Respiratory effects have been observed in animals but these effects have not been reported in humans. Due to lack of data, it is difficult to speculate regarding the benefits of treatments that enhance elimination of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene and their metabolites as a basis for reducing toxic effects.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

Existing data indicate that lung, nose, and eye toxicity may be mediated by reactive metabolites for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, although the evidence for the involvement of reactive metabolites is greater than the evidence for methylnaphthalenes. More information is needed on the bioactivation of naphthalene and transport mechanisms before methods for blocking those mechanisms can be developed.

Many of the symptoms of acute naphthalene poisoning in humans are a direct consequence of red blood cell hemolysis. Blood transfusions, packed red blood cell transfusions, and exchange transfusions (particularly in infants) can be used to replenish the concentration of red blood cells and diminish the risks of cellular anoxia (Bregman 1954; Chusid and Fried 1955; MacGregor 1954; Mackell et al. 1951). Bicarbonate is also administered to hemolysis patients to increase the alkalinity of the urine and thereby minimize deposition of hemoglobin in the kidney tubules (Chusid and Fried 1955; Gidron and Leurer 1956).

3.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

3.12.1 Existing Information on Health Effects of Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene are summarized in Figures 3-6, 3-7, and 3-8, respectively. The purpose of this figure is to illustrate the existing information concerning the health effects of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in

Figure 3-6. Existing Information on Health Effects of Naphthalene

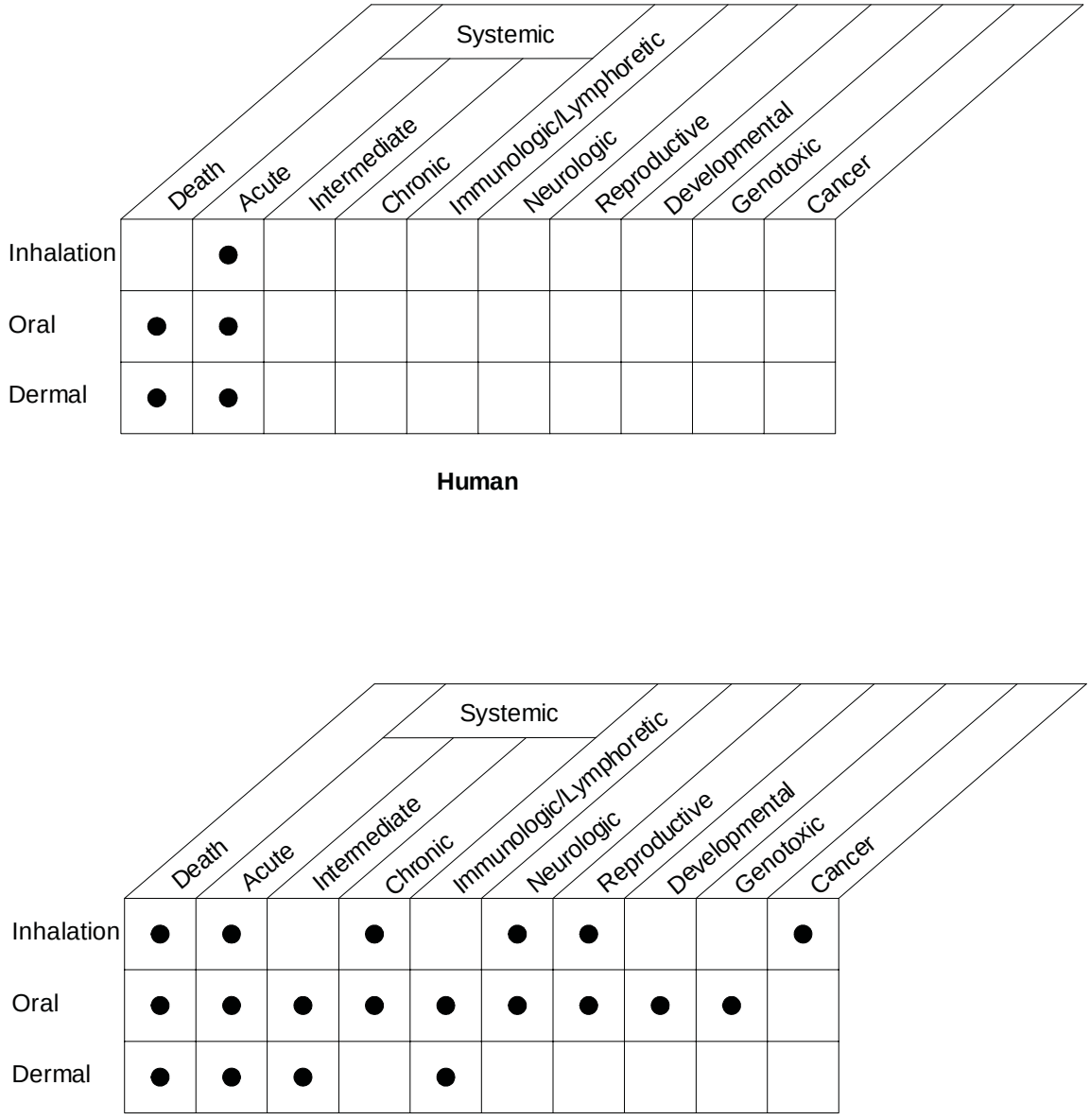


Figure 3-7. Existing Information on Health Effects of 1-Methylnaphthalene

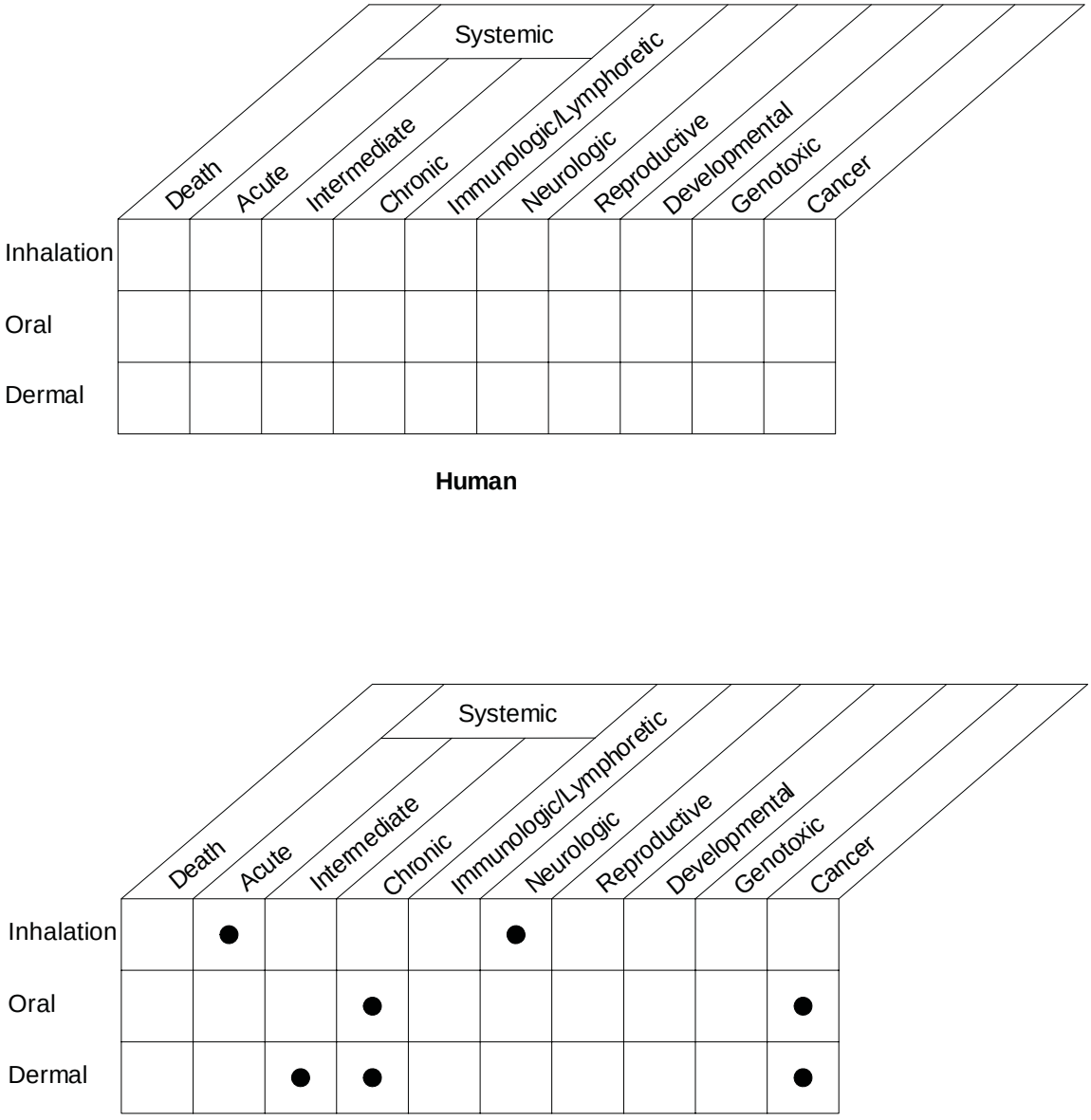
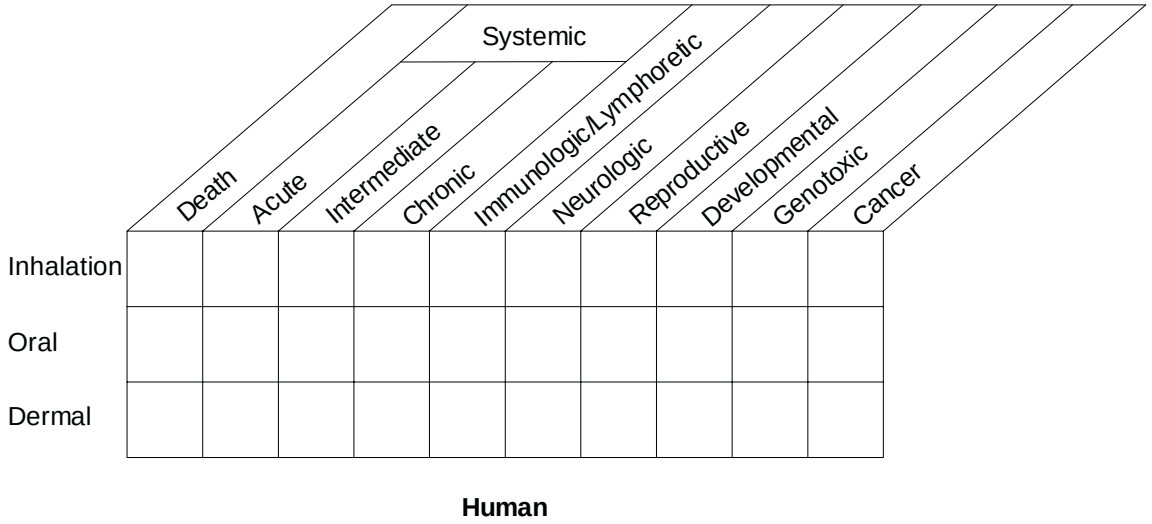
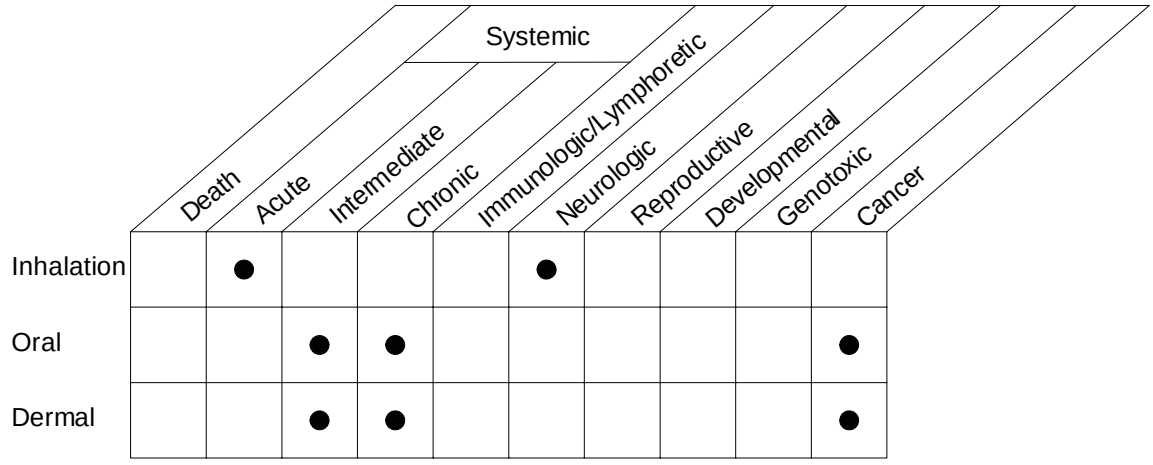


Figure 3-8. Existing Information on Health Effects of 2-Methylnaphthalene



Human



Animal

● Existing Studies

this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (Agency for Toxic Substances and Disease Registry 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Figure 3-6 shows that the database on naphthalene toxicity in humans is not extensive. There are case reports and case series of deaths, acute hemolytic anemia, and ocular effects in humans, but these reports lack quantitative information on exposure levels. Epidemiologic studies designed to examine possible associations between intermediate- or chronic-duration human exposure to naphthalene by any route of exposure and neoplastic or nonneoplastic health effects are not available. Animal data on naphthalene exist in several areas. Oral toxicity data are adequate for deriving acute- and intermediate-duration oral MRLs, but adequate chronic-duration oral toxicity studies in animals are not available. Available toxicology and carcinogenesis studies of chronic inhalation exposure to naphthalene in rats and mice are adequate for deriving a chronic-duration inhalation MRL for naphthalene and assessing the potential carcinogenicity of naphthalene, but available acute- and intermediate-duration inhalation toxicity studies are not adequate for deriving MRLs.

Figures 3-7 and 3-8 show that no information was located on the health effects of 1-methylnaphthalene or 2-methylnaphthalene in humans via inhalation, oral, or dermal exposure. These figures also reflect that data in animals are limited to cancer and toxicity studies of intermediate- and chronic-duration oral exposure of mice to 1-methylnaphthalene or 2-methylnaphthalene, a single poorly reported acute inhalation exposure study of hematologic end points in dogs exposed by inhalation to 1-methylnaphthalene or 2-methylnaphthalene, a study that reported decreased pain sensitivity, but no effects on the ability to balance on a rotating rod, in rats exposed for 4 hours by inhalation to 1-methylnaphthalene or 2-methylnaphthalene, and cancer and toxicity studies of intermediate- and chronic-duration dermal exposure of mice to a mixture of 1-methylnaphthalene and 2-methylnaphthalene.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. A number of reports of human exposure to acute inhalation, oral, or dermal doses of naphthalene have established the erythrocyte as a toxicity target (Dawson et al. 1958; Haggerty 1956; Kurz 1987; Linick 1983; MacGregor 1954; Mackell et al. 1951; Melzer-Lange and

Walsh-Kelly 1989; Ojwang et al. 1985; Schafer 1951; Shannon and Buchanan 1982; Valaes et al. 1963). However, the data from these reports were not useful in predicting toxic or lethal dose levels by any of these routes because the exposure levels were not defined.

The acute oral toxicity of naphthalene has been studied in animals but there are limited data for acute inhalation and dermal exposures.

The most frequently reported adverse effects associated with acute oral exposure are ocular lesions (primarily cataracts). These have been observed in rabbits (Srivastava and Nath 1969; Van Heyningen and Pirie 1967) and rats (Kojima 1992; Murano et al. 1993; Rathburn et al. 1990; Tao et al. 1991; Yamauchi et al. 1986) and occur following exposure to high (>500 mg/kg) doses. Acute oral exposure of pregnant rats to naphthalene doses of 150 or 450 mg/kg/day (but not 50 mg/kg/day) during gestation produced maternal toxicity including clinical signs (lethargy and prone position) and marked decreases in body weight gain (NTP 1991a), but clear effects on the developing fetus have not been found at maternal oral doses as high as 450 mg/kg/day in rats (NTP 1991a), 300 mg/kg/day in mice (Plasterer et al. 1985), or 120 (NTP 1992b) or 400 mg/kg/day (PRI 1985i,1986) in rabbits. Slightly reduced numbers of mouse pups per litter were observed when naphthalene in corn oil was orally administered to pregnant mice (Plasterer et al. 1985); however, no effects were seen when pregnant rabbits were orally administered naphthalene at even higher doses but delivered in methylcellulose rather than in an oil vehicle (PRI 1986). It is unclear if these differences are due to species differences in sensitivity or to possible differences in the effects of the two vehicles on naphthalene absorption. Effects on liver (Rao and Pandya 1981) and lung (Shopp et al. 1984) weights have been reported, but no treatment-related histopathological lesions were observed in these acute oral exposure studies. Lethal doses have been identified in mice (Plasterer et al. 1985; Shopp et al. 1984) and rats (Gaines 1969).

The finding of transient clinical signs of toxicity in orally-exposed pregnant rats (NTP 1991a) serves as the basis of the acute-duration oral MRL for naphthalene. The MRL was calculated from a minimal LOAEL of 50 mg/kg/day using an uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). An uncertainty factor of 3 was used for human variability because the critical effect is based on effects in a sensitive animal subpopulation. Dermal or inhalation developmental toxicity studies in animals are not available. Pregnant rats appear to be more sensitive for the effects observed (clinical signs in response to gavage exposure and decreased body weight gain) than nonpregnant rats. In 13-week gavage studies with nonpregnant rats (NTP 1980b), similar persistent clinical signs were not observed following administration of doses as high as

200 mg/kg/day, but were observed at 400 mg/kg/day. In nonpregnant rats exposed for 13 weeks, significant body weight decreases occurred at 200 mg/kg/day throughout exposure, but not at 100 mg/kg/day (NTP 1980b) or in nonpregnant mice exposed for 13 weeks to 133 mg/kg/day (Shopp et al. 1984) or 200 mg/kg/day (NTP 1980a). Mice in the NTP (1980a) study showed transient signs of toxicity (lethargy, rough hair coats, and decreased food consumption), but these only occurred between weeks 3 and 5 in the 200-mg/kg/day group.

Data are inadequate for deriving an acute-duration inhalation MRL for naphthalene. Data are restricted to a 14-day (6 hours/day, 5 days/week) range-finding study in B6C3F1 mice (NTP 1992a), which only examined hematologic end points and did not histologically examine expected critical toxicity targets (lung and nasal cavity epithelial tissue) (NTP 1992a), and a study (West et al. 2001) with Swiss Webster mice and Sprague-Dawley rats, which involved single 4-hour exposure periods. The more recent study, however, only histologically examined the lung and did not examine nasal tissue. A comprehensive inhalation study involving an acute repeated exposure scenario and examining the other critical target (the nose, based on the findings from chronic mouse and rat bioassays) is not currently available. Results from such a study may be useful for deriving an acute-duration inhalation MRL for naphthalene.

Hemolysis is the best documented effect of acute naphthalene exposures in humans, but it has not been observed in studied strains of rats (F344) or mice (CD-1, B6C3F1). Dose-response data for hemolysis from a susceptible animal species (such as dogs or the Jackson Laboratory hemolytic anemia mouse) may be useful to obtain data that could be used for considering changes to the acute-duration oral MRL. Data from both inhalation and oral exposure protocols would be useful.

No acute-duration studies are available on 1-methylnaphthalene or 2-methylnaphthalene exposure in humans using the inhalation, oral, or dermal routes. Two acute inhalation studies in animals were identified. The first study reported that 1-methylnaphthalene (pure) administered in a kerosene aerosol was associated with increased reticulocyte and lymphocyte counts in splenectomized dogs and practical grade 1-methylnaphthalene was associated with increased leucocyte and neutrophil counts (Lorber 1972). Neither grade of 1-methylnaphthalene had any effect on hematocrit values. None of these parameters were affected when 2-methylnaphthalene aerosols were used. The physiological significance of these findings is not apparent and the exposure levels in the study were not clearly specified. As such, the data are not suitable for use in deriving an MRL for 1-methylnaphthalene or 2-methylnaphthalene. The second study measured decreased sensitivity to pain in rats exposed by inhalation for 4 hours to 1-methyl-naphthalene (44 ppm) or 2-methylnaphthalene (61 ppm), but found no effects on the ability to balance on

a rotating rod at exposure levels as high as 70 ppm 1-methylnaphthalene or 90 ppm 2-methylnaphthalene (Korsak et al. 1998). The biological significance of these findings is uncertain, and, in the absence of corroborative evidence of acute neurotoxicity, the findings are not suitable for deriving acute inhalation MRLs for 1-methylnaphthalene or 2-methylnaphthalene.

Parenteral studies in animals revealed that a single intraperitoneal injection of 2-methylnaphthalene (1,000 mg/kg) was lethal in mice (Griffin et al. 1981). When a glutathione-depleting agent (diethyl maleate) was administered prior to administration of 2-methylnaphthalene, a lower dose of 2-methylnaphthalene (400 mg/kg) was also lethal. A single intraperitoneal injection of 1-methylnaphthalene (426 mg/kg) was not lethal in mice (Griffin et al. 1982). Systemic effects have been reported and were limited to effects on the respiratory system (Rasmussen et al. 1986). Exfoliation of the bronchiolar epithelium in mice was reported following a single intraperitoneal injection of 2-methylnaphthalene (Buckpitt et al. 1986; Griffin et al. 1981, 1983). A single intraperitoneal injection of 2-methylnaphthalene (1,000 mg/kg) did not cause liver or kidney lesions (Griffin et al. 1981, 1983).

Because populations living near hazardous waste sites might be exposed to 1-methylnaphthalene or 2-methylnaphthalene for short periods, comprehensive toxicity studies of acute exposure in animals by the inhalation and oral routes to determine potential target tissues and dose-related effects would be useful in assessing possible health hazards to humans. The studies would be most useful if they included a battery of neurological end points and comprehensive histological examination of nasal and lung tissue.

Intermediate-Duration Exposure. Quantitative data were not provided in any intermediate-duration inhalation case studies of human naphthalene exposure and, in one case, there was simultaneous exposure to paradichlorobenzene (Harden and Baetjer 1978; Linick 1983).

The results from three intermediate-duration oral toxicity studies in animals (two in mice and one in rats) identified body weight changes as the most sensitive biologically significant effect on which to base the intermediate-duration oral MRL for naphthalene. Comprehensive intermediate-duration oral toxicity studies found no evidence for naphthalene-induced lesions in any tissue or organs in male or female Fischer 344 rats exposed to doses up to 400 mg/kg/day (NTP 1980b) or in male or female B6C3F1 mice exposed to doses up to 200 mg/kg/day (NTP 1980a). The only biologically significant effect found in these studies was decreased body weight (>10% decreased compared with control values) in rats at doses of 200 and 400 mg/kg/day. The other intermediate-duration oral study (with CD-1 mice) focused on a battery of immunologic tests, but did not include comprehensive histopathologic examination of tissues

(Shopp et al. 1984). No biologically significant effects were found except for decreases in weights of several organs (brain, liver, and spleen) in mice exposed to 133 mg/kg/day, but not to 53 or 5.3 mg/kg/day. The lack of naphthalene-induced lesions in these organs in the NTP (1980a, 1980b) studies suggests that the brain, liver, and spleen are not sensitive targets of naphthalene following intermediate-duration oral exposure. Statistically significant changes were reported in several hematological parameters, hepatic enzyme activities, and serum chemical parameters (Shopp et al. 1984), but these changes are not considered to be biologically significant or adverse. The acute-duration oral MRL was adopted as the intermediate-duration oral MRL for naphthalene, because a potential intermediate-duration oral MRL (see Section 2.3 and Appendix A) based on the NOAEL for decreased body weight changes in rats exposed by gavage 5 days/week for 13 weeks (NTP 1980b) was slightly larger than the acute MRL value.

No data were suitable for the development on an intermediate-duration inhalation MRL for naphthalene.

Intermediate-duration dermal toxicity data are restricted to an report that dermal exposure of male and female Sprague-Dawley rats (occluded exposure 6 hours/day, 5 days/week) to technical-grade naphthalene at doses up to 1,000 mg/kg/day for 13 weeks did not affect comprehensive ophthalmologic, hematologic, serum chemistry, or urinalysis parameters (Frantz et al. 1986). In addition, exposure did not produce increased incidences of histological lesions in 34 tissues that were examined (however, the nasal cavity was not included). The only exposure-related effect found was an increased incidence of excoriated skin and papules at the site of exposure at the highest dose level (1,000 mg/kg/day).

Intermediate-duration studies on 1-methylnaphthalene or 2-methylnaphthalene exposure in humans or animals using the inhalation, oral, or dermal routes are restricted to a study that found no pulmonary alveolar proteinosis in male or female mice exposed to diets containing up to 1.33% 2-methylnaphthalene for 13 weeks (Murata et al. 1997). The reporting of the experimental protocol and results from this study, however, is too limited to reliably use the results as a basis for an intermediate-duration oral MRL for 2-methylnaphthalene. New intermediate-duration toxicity studies using the inhalation route of exposure may be the most useful to better assess the health hazard of intermediate-duration exposure to naphthalene, based on the findings that the alveolar region of the lung is the most sensitive tissue in mice chronically exposed to 1-methylnaphthalene or 2-methylnaphthalene in the diet (Murata et al. 1993, 1997).

Chronic-Duration Exposure and Cancer. There is one report of cataracts occurring in humans following chronic-duration inhalation exposure to naphthalene (Ghetti and Mariani 1956) but no information on effects from exposures by the oral or dermal routes. The only studies of cancer in humans exposed to naphthalene are two case series reports of cancer; one report of four laryngeal cancer cases (all of whom were smokers) among workers in a naphthalene purification plant in East Germany (Wolf 1976, 1978), and another report of 23 cases of colorectal carcinoma admitted to a hospital in Nigeria (Ajao et al. 1988). NTP (2002b), EPA (2002b), and IARC (2002) concurred that these studies provide inadequate evidence of naphthalene carcinogenicity in humans. No cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available.

There are two comprehensive chronic-duration inhalation toxicology and carcinogenicity studies of naphthalene in animals, one in rats (Abdo et al. 2001; NTP 2000) and one in mice (NTP 1992a). These studies identify respiratory tissues as the most sensitive toxicity targets of chronic-duration exposure to inhaled naphthalene in animals: nonneoplastic and neoplastic lesions in the nose of rats, nonneoplastic lesions in the nose of mice, and nonneoplastic and neoplastic lesions in the lungs of mice. Exposure-related lesions in other tissues were not found in these studies. NTP (2002b) and IARC (2002) concurred that these studies provide sufficient evidence of naphthalene carcinogenicity in animals. The chronic-duration inhalation MRL for naphthalene is based on the LOAEL of 10 ppm for nonneoplastic lesions in the olfactory epithelium and respiratory epithelium of the nose of rats.

No appropriate studies were located for deriving an MRL for chronic-duration oral exposure to naphthalene. One chronic study was located that examined the toxicity of naphthalene in rats (Schmahl 1955). No treatment-related effects were reported at a dose level of 41 mg/kg/day for 700 days. The study was not suitable as the basis for deriving a chronic MRL or for assessing carcinogenicity because only one dose level was evaluated (apparently below the maximum tolerated dose), histopathological examination was limited, and dosing was not precisely controlled.

New chronic oral or dermal toxicity studies would be useful to better determine the possible carcinogenicity and noncancer toxicity of naphthalene via these routes of exposure.

Epidemiology studies, case reports, or controlled-exposure studies examining the potential health effects of human chronic exposure to 1-methylnaphthalene or 2-methylnaphthalene by any route of exposure are not available.

No chronic-duration studies are available on 1-methylnaphthalene or 2-methylnaphthalene exposure in animals using the inhalation routes.

A chronic-duration study of 1-methylnaphthalene in the diet that identified a LOAEL of 71.6 mg/kg/day for the occurrence of pulmonary alveolar proteinosis in mice (Murata et al. 1993) was used as the basis of the oral MRL for 1-methylnaphthalene. A chronic-duration oral study of 2-methylnaphthalene in the diet (Murata et al. 1997) that identified a LOAEL of 50.3 mg/kg/day for pulmonary alveolar proteinosis in mice was the basis of the chronic oral MRL for 2-methylnaphthalene. Support for pulmonary alveolar proteinosis as the critical effect for the chronic oral MRLs for 1-methylnaphthalene and 2-methyl-naphthalene comes from dermal chronic-duration studies with methylnaphthalene (a mixture of 1- and 2-methylnaphthalene), which reported increased incidences of this lesion in mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene for 30–61 weeks (Emi and Konishi 1985; Murata et al. 1992). Increased incidences of lung adenomas were found in several exposed groups in the oral chronic-duration studies, but the evidence for carcinogenicity is considered to be limited. The tumorigenic response was predominantly benign and was only consistently seen in male mice exposed to 1-methylnaphthalene. The available data on the methylnaphthalenes appear inadequate to determine the potential carcinogenicity in humans.

A new chronic-duration oral study in rats or another animal species may help to better assess the potential carcinogenicity and noncancer toxicity of the methylnaphthalenes. Because the lung is the most sensitive toxicity target of the methylnaphthalenes in mice exposed orally or dermally, it is plausible that chronic inhalation exposure may also target the lung. The availability of repeated-exposure inhalation carcinogenicity and toxicity studies would help to better determine this possibility.

Genotoxicity. As discussed in Section 3.3, results in bacterial mutation assays were predominantly negative (see Table 3-4 for citations) with the exceptions that the metabolite, 1,2-naphthoquinone, was mutagenic in *S. typhimurium* without metabolic activation (Flowers-Geary 1996), and naphthalene was mutagenic in *V. fischeri* with metabolic activation (Arfsten et al. 1994).

Results from a limited number of *in vitro* eukaryotic genotoxicity assays are mixed. Negative results were obtained for mutations and sister chromatid exchanges in cultured human cells exposed to naphthalene, for DNA single strand breaks and unscheduled DNA synthesis in rat hepatocytes, and for cell transformation in several types of mammalian cells (see Table 3-3 for citations). Positive results

included increased chromosomal aberrations in Chinese hamster ovary cells and preimplantation whole mouse embryos exposed to naphthalene, and increased sister chromatid exchanges in human mononuclear leukocytes exposed to 1,2- or 1,4-naphthoquinone and in Chinese hamster ovary cells exposed to naphthalene (see Table 3-3 for citations). Other studies in cell-free systems reported that 1,2-naphthoquinone formed N7 adducts with deoxyguanosine (McCoull et al. 1999) and caused DNA strand scission in the presence of NADPH and copper via reactive oxygen species from an oxidation/reduction cycle (Flowers et al. 1997).

In vivo genotoxicity assays with naphthalene are also limited and do not provide consistently negative or positive results for naphthalene genotoxicity. Positive results were obtained for somatic mutations in *D. melanogaster*, micronuclei in salamander larvae erythrocytes, and DNA fragmentation in liver and brain tissue from mice and rats orally exposed to naphthalene (see Table 3-3 for citations). Negative results were obtained for micronuclei formation in bone marrow of mice given oral or intraperitoneal injections of naphthalene, DNA single strand breaks and unscheduled DNA synthesis in hepatocytes of rats given oral doses of naphthalene, and neoplastic transformations in liver cells of partially hepatectomized rats given oral doses of naphthalene (see Table 3-3 for citations).

The available data suggest that genotoxic action by the naphthalene metabolite, 1,2-naphthoquinone, is plausible and that the mutagenic/genotoxic potential of naphthalene and its metabolites may be weak. Assays of possible genotoxic action in sensitive target tissues of naphthalene in rodents (lung and nasal epithelial tissue), however, are not available. New studies examining genotoxic end points in lung and nasal epithelial tissue following inhalation exposure to naphthalene would help to better determine the potential genotoxicity of naphthalene and it metabolites.

For the methylnaphthalenes, data in humans are limited to one study that reported no effects on human chromosomes in tests evaluating the effects of 1-methylnaphthalene or 2-methylnaphthalene on human peripheral lymphocytes *in vitro* (Kulka et al. 1988). 1-Methylnaphthalene and 2-methylnaphthalene were also determined to be nonmutagenic in four strains of *S. typhimurium* (Florin et al. 1980). Additional mutagenicity studies using an *in vivo* approach would be useful to better assess the genotoxicity potentials of 1-methylnaphthalene and 2-methylnaphthalene.

Reproductive Toxicity. No information is available on the reproductive effects of naphthalene in humans, although the occurrence of hemolytic anemia in the neonates of anemic, naphthalene-exposed mothers demonstrates that naphthalene and/or its metabolites can cross the placental barrier (Anziulewicz

et al. 1959; Zinkham and Childs 1957, 1958). Animal studies involving naphthalene exposure during gestation reported no reproductive effects in rabbits administered doses of up to 120 mg/kg/day by gavage or in rats given doses of up to 450 mg/kg/day, although doses of 150 mg/kg/day and greater were maternally toxic to rats. There was a decrease in the number of live mouse pups per litter with a dose of 300 mg/kg/day given during gestation (Plasterer et al. 1985) and *in vitro* studies of naphthalene embryotoxicity in the presence of liver microsomes support the concept that naphthalene metabolites may be harmful to the developing embryo (Iyer et al. 1991). No exposure-related lesions in reproductive tissues were found in intermediate-duration oral exposure studies in rats (NTP 1980b) and mice (NTP 1980a) or in chronic inhalation studies in rats (Abdo et al. 2001; NTP 2000) or mice (NTP 1992a). One- or two-generation reproductive toxicity studies evaluating reproductive performance variables in male and female animals exposed to naphthalene are not available. Results from such studies may help to better determine the potential reproductive toxicity of naphthalene.

No studies are available on the reproductive toxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure, with the exceptions of the reports that 81-week oral exposure to 1-methylnaphthalene or 2-methylnaphthalene did not induce lesions in reproductive tissues of male or female mice (Murata et al. 1993; 1997). One- or two-generation reproductive toxicity studies evaluating reproductive performance variables in male and female animals exposed to 1-methylnaphthalene or 2-methylnaphthalene are not available. Results from such studies may help to better determine the potential reproductive toxicity of the methylnaphthalenes.

Developmental Toxicity. There is no information on the potential developmental effects of naphthalene in humans, although, as mentioned previously, naphthalene and/or its metabolites can cross the placental barrier and cause hemolytic anemia in newborns (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958). Studies of the developmental effects of orally administered naphthalene in rats (NTP 1991a), mice (Plasterer et al. 1985), and rabbits (NTP 1992b; PRI 1985i, 1986) have been negative, except for a slight nonsignificant increase in fused sternebrae in female rabbit pups from a small number of litters at doses of 80 and 120 mg/kg/day (NTP 1992b). No developmental toxicity studies involving inhalation or dermal exposure to naphthalene are available. The availability of such studies would help to better determine the developmental toxicity potential of naphthalene.

No studies are available on the developmental toxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure.

Immunotoxicity. There have been no comprehensive studies of the immunotoxicity of naphthalene in humans exposed by the inhalation, oral, or dermal routes. The animal oral exposure data indicate that naphthalene did not affect humoral or cell-mediated immunity in mice (Shopp et al. 1984). Minor effects on the thymus and spleen were noted in mice and rats (NTP 1980b; Shopp et al. 1984), but in no case were animals of both sexes affected. Because there are few data pertaining to the immunotoxicity of naphthalene, a battery of *in vitro*/*in vivo* screening assays of immune function may be useful to determine whether more detailed and longer-term studies are needed.

No studies are available on the immunotoxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure. However, the reported increase in the level of monocytes in mice following long-term oral exposure to 1-methylnaphthalene (Murata et al. 1993) may deserve additional study. As with naphthalene, a battery of *in vitro*/*in vivo* screening assays of immune function may be useful to determine whether more detailed and longer-term studies are needed.

Neurotoxicity. The direct effects of naphthalene on the central nervous system have not been investigated in either humans or animals. Neurotoxic effects seen in humans exposed to naphthalene via inhalation or oral exposure may be a consequence of the diminished oxygen-carrying capacity of the blood which results from red cell hemolysis (Bregman 1954; Gupta et al. 1979; Kurz 1987; Linick 1983; MacGregor 1954; Ojwang et al. 1985; Zuelzer and Apt 1949). Persistent clinical signs of toxicity (lethargy and prone position) were seen in pregnant rats following gavage administration of naphthalene at dose levels of 150 or 450 mg/kg/day; at 50 mg/kg/day, the signs were only observed during the first 2 days of dose administration (NTP 1991a). Comparable effects were not observed in F344/N rats exposed to doses of up to 400 mg/kg/day for 13 weeks or in B6C3F1 mice at doses of up to 200 mg/kg/day (NTP 1980a, 1980b). With inhalation exposure, no treatment-related gross or histopathological lesions of the brain were observed in mice (NTP 1992a) or rats (Abdo et al. 2001; NTP 2000) exposed for 2 years to naphthalene concentrations as high as 30 or 60 ppm, respectively. Clinical observations revealed no gross behavioral changes indicative of neurological impairment. Additional studies involving batteries of neurological end points following oral and/or inhalation exposure may help to better determine the potential neurotoxicity of naphthalene and explain why pregnant rats appear to be more susceptible to the behavioral effects of acute-duration exposures to naphthalene.

No studies on the neurotoxicity of 1-methylnaphthalene or 2-methylnaphthalene in humans following inhalation, oral, or dermal exposure were located with the exception of a single study that found decreased sensitivity to pain in rats exposed by inhalation for 4 hours to 1-methylnaphthalene (44 ppm) or

2-methylnaphthalene (61 ppm), but no effects on rotarod performance at exposure levels as high as 70 ppm 1-methylnaphthalene or 90 ppm 2-methylnaphthalene (Korsak et al. 1998). The biological significance of these findings is uncertain. Additional studies involving batteries of neurological end points may help to better determine the potential neurotoxicity of the methylnaphthalenes.

Epidemiological and Human Dosimetry Studies. A small number of reports have equivocally suggested that workers exposed to naphthalene for long periods of time may have an elevated risk of cataract development (Ghetti and Mariani 1956; Lezenius 1902). This information, coupled with the cataractogenic effects of naphthalene in orally exposed rats (Kojima 1992; Xu et al. 1992b; Yamauchi et al. 1986) and rabbits (Rossa and Pau 1988; Srivastava and Nath 1969; Van Heyningen and Pirie 1967) in acute- and intermediate-duration studies, suggests that studies of occupationally-exposed workers would help to determine its potential to produce ocular toxicity in humans. The incidence of tumors, anemia, and reproductive problems in this population could be determined at the same time. Available case reports of cancer in naphthalene-exposed humans provide inadequate evidence of naphthalene carcinogenicity. Currently, no cohort mortality or morbidity studies or case-control studies examining possible associations between naphthalene exposure and increased risk of cancer (or other health effects) are available. If human populations that are specifically and repeatedly exposed to naphthalene can be identified, epidemiological studies of these populations may help to better assess the potential chronic-duration toxicity and carcinogenicity of naphthalene.

No epidemiological or human dosimetry studies on the effects of 1-methylnaphthalene or 2-methyl-naphthalene were located. Exposure to these compounds, particularly through dermal contact or inhalation, can occur in workplaces where the compounds are produced or used. Populations living near hazardous waste sites can potentially be exposed by the oral, inhalation, and dermal routes. If an appropriate population can be identified, it may be helpful to conduct epidemiological studies to determine if there are toxic effects (particularly on the lungs) resulting from exposure to these substances.

Biomarkers of Exposure and Effect.

Exposure. There are methods to determine the presence of naphthalene in adipose tissue and these methods have been used in a national monitoring program for the analysis of naphthalene in the adipose tissue of the general population (EPA 1986g). Metabolites of naphthalene, such as naphthols and naphthoquinones, have been detected in the urine of a patient 4 days after ingestion of naphthalene (Zuelzer and Apt 1949), but not in another patient at 17 days after ingestion (Mackell et al. 1951).

1-Naphthol is present in the urine of workers occupationally exposed to naphthalene. Maximum 1-naphthol levels occurred immediately after the end of the work period and in some cases had returned to baseline levels 8 hours later (Bieniek 1994). New techniques have been developed to measure cysteinyl adducts formed from reactions of hemoglobin and albumin with reactive metabolites of naphthalene (Troester et al. 2002; Waidyanatha et al. 2002). The adducts are expected to be useful in estimating internal doses of these metabolites, and with further development, they may become useful biomarkers of exposure.

Effect. There are no known specific biomarkers of effects for naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene. Hemolytic anemia has been frequently associated with human exposure to naphthalene, but may also be the result of exposure to other chemicals. Pulmonary alveolar proteinosis in mice has been associated with chronic oral exposure to 1-methylnaphthalene and 2-methylnaphthalene. The condition has been described in humans, but has not been associated with human exposure to 1-methylnaphthalene or 2-methylnaphthalene. Currently, these effects (hemolytic anemia or pulmonary alveolar proteinosis) do not hold promise as specific biomarkers of effect for naphthalene or methyl-naphthalenes. Identification of specific biomarkers of effect such as particular protein adducts in naphthalene-affected target tissues in animals (e.g., nasal epithelium tissue) may be useful to test whether similar biomarkers of effect may exist in naphthalene-exposed human populations.

Absorption, Distribution, Metabolism, and Excretion. Although human absorption of naphthalene has not been quantitatively characterized, case reports indicate that humans can absorb toxicologically significant amounts of this compound by the oral, inhalation, or dermal routes (Bregman 1954; Chusid and Fried 1955; Dawson et al. 1958; Gidron and Leurer 1956; Gupta et al. 1979; Haggerty 1956; Kurz 1987; Linick 1983; MacGregor 1954; Mackell et al. 1951; Ojwang et al. 1985; Santhanakrishnan et al. 1973; Schafer 1951; Shannon and Buchanan 1982; Valaes et al. 1963; Zuelzer and Apt 1949). Laboratory animals such as rats, mice, and rabbits also absorb the chemical via their skin and gastrointestinal and respiratory tracts (NTP 1992a; Rao and Pandya 1981; Shopp et al. 1984; Srivastava and Nath 1969; Turkall et al. 1994; van Heyningen and Pirie 1967). Naphthalene adsorbed to organic-rich soils is absorbed across the skin more slowly than naphthalene from organic-poor soils (Turkall et al. 1994). The compound apparently partitions between the soil organic carbon and the hydrophobic components of the epidermis and dermis. More information concerning the mechanism of absorption (facilitated versus passive transport) across nasal and pulmonary epithelial membranes, the gastrointestinal tract, and the skin may be helpful in estimating the effect of dose on absorption coefficients and in better determining the effect of the medium of exposure (water, oil, food, etc.) on oral

or dermal absorption. Empirical measurements of permeability coefficients for naphthalene in blood or air with various tissues from various species may be useful to further develop PBPK models for naphthalene.

As discussed in Sections 3.4.3 and 3.5.2, extensive research on the bioactivation and metabolic transformations of naphthalene in mammalian systems has identified several reactive metabolites that are potentially responsible for the nasal, pulmonary, and ocular toxicity of naphthalene (1,2-naphthalene oxide, 1,2-naphthoquinone, and 1,4-naphthoquinone), but the relative importance of these metabolites in affecting these toxicity targets remains uncertain. Because nasal respiratory and olfactory epithelia are the most sensitive targets in rodents following acute or chronic inhalation exposure, better understanding of the deposition, absorption, and metabolism of inhaled naphthalene in different regions of nasal epithelia, and the degree to which species (particularly rodents and primates) differ in these processes, may be useful for decreasing uncertainty in extrapolating human health hazards from data for rodents exposed to naphthalene. *In vivo*, *in vitro*, and modeling research approaches are likely to create better understanding of these processes, which may also provide explanations for observed species differences in response to naphthalene. For example, both rats and mice developed nonneoplastic nasal lesions following chronic inhalation exposure to naphthalene concentrations as low as 10 ppm, but only rats developed nasal tumors (Abdo et al. 2001; NTP 1992a, 2000). Other examples are the findings that *in vitro* rates of epoxide formation from naphthalene in extracts of nasal olfactory tissue showed the order, mouse>rat>hamster, but rats were more susceptible to acute nasal injury from naphthalene than mice or hamsters (Buckpitt et al. 1992; Plopper et al. 1992a). Mechanistic explanations for these differences are not currently available.

The most recently developed PBPK models for naphthalene in mice and rats (Willems et al. 2001) do not include nasal compartments that metabolize naphthalene and do not include the spontaneous conversion of 1,2-naphthalene oxide to 1-naphthol or metabolic transformations to the naphthoquinones. Additional toxicokinetic data are needed to further refine these models to include these potentially important processes. Application of such further refined models, and the development of comparable models for humans, may be useful to decrease uncertainty in extrapolating dose-response relationships for nasal effect in rodents to humans.

No studies were located on the absorption, metabolism, and excretion of 1-methylnaphthalene in humans or animals following inhalation, oral, or dermal exposure. There was one study of 2-methylnaphthalene in guinea pigs (Teshima et al. 1983). Parenteral studies in animals show that 2-methylnaphthalene is

converted to both monohydrated compounds and dihydrodiols (Breger et al. 1981, 1983; Melancon et al. 1982). In addition, 2-naphthoic acid and the glycine or the cysteine conjugates were identified in rats (Melancon et al. 1982) and guinea pigs (Teshima et al. 1983). Studies by relevant exposure routes would further characterize the toxicokinetics of these compounds and may enhance the understanding of the potential risk associated with exposure to these compounds.

Comparative Toxicokinetics. Data suggest that there are strain- and species-specific effects associated with naphthalene toxicity. Laboratory animals, such as rats and mice, do not exhibit red cell hemolysis after exposure to naphthalene, while humans and dogs do (NTP 1980a, 1980b, 1992a; Shopp et al. 1984; Zuelzer and Apt 1949). Mice and rats both develop nonneoplastic nasal lesions after chronic inhalation exposure to naphthalene, but only rats develop nasal tumors, and only mice develop nonneoplastic lung lesions or lung tumors (Abdo et al. 2001; NTP 1992a; 2000). There are differences in susceptibility to the acute pulmonary toxicity of naphthalene among mice, rats, hamsters, and guinea pigs (Buckpitt et al. 2002; Plopper et al. 1992a, 1992b). Differences in the susceptibility of rats and mice, and of different mouse strains, to the cataractogenic properties of naphthalene have also been reported (Wells et al. 1989). These differences may relate to differences in tissue distribution of specific CYP isoenzymes, rates of formation of reactive metabolites, rates of transformation of reactive metabolites to nonreactive metabolites, or partitioning of the parent compound or metabolites within and between tissues. For example, the difference in susceptibility to the acute pulmonary toxicity of naphthalene between mice and rats has been correlated with higher rates of metabolic formation and different stereoselectivity of epoxide metabolites in mice compared with rats (Buckpitt et al. 1992; 1995; 2002). In contrast, differences among rat, mice and hamsters in susceptibility to naphthalene-induced nasal lesions were not correlated with species differences in rates of epoxide formation from naphthalene in extracts of olfactory epithelial tissue (Plopper et al. 1992; see Section 3.5.2). Further evaluation of these differences and comparative studies of distribution and metabolic patterns among species may help to decrease uncertainty in extrapolating estimates of human health hazards from data for animals exposed to naphthalene.

There are no data available concerning the toxicokinetics of 1-methylnaphthalene or 2-methylnaphthalene in humans following inhalation, oral, or dermal exposure. There are no data from studies of 1-methylnaphthalene in animals, but there are limited data for 2-methylnaphthalene (Breger et al. 1983; Griffin et al. 1982; Melancon et al. 1982, 1985; Teshima et al. 1983). New studies that evaluate toxicokinetic parameters in several animal species may be useful to decrease uncertainty in the chronic oral MRLs for

1-methylnaphthalene and 2-methylnaphthalene, which are based on the occurrence of pulmonary alveolar proteinosis in mice.

Methods for Reducing Toxic Effects. Available methods are sufficient for reducing peak absorption of naphthalene following ingestion (Melzer-Lange and Walsh-Kelly 1989; Siegel and Wason 1986; Stutz and Janusz 1988). No antidotal methods are available that would be useful for treatment of naphthalene exposure based on any proposed hypothesis pertaining to the mechanism of action. Additional studies to characterize the metabolic activation of naphthalene and the role of circulating reactive metabolites from nontarget tissues may be useful in developing methods for interfering with the mechanism of action. Further studies to identify ways to reduce or prevent accumulation of toxic metabolites in target tissues may be warranted when mechanisms of naphthalene toxic action are better understood.

There are no compound-specific methods for reducing the toxic effects of 1-methylnaphthalene and 2-methylnaphthalene. Additional information on the toxicokinetics and mechanism of action for these compounds may be beneficial in identifying possible approaches for reducing compound toxicity.

Children's Susceptibility. Data needs relating to both prenatal and childhood exposures, and developmental effects expressed either prenatally or during childhood, are discussed in detail in the Developmental Toxicity subsection above.

As discussed in Section 3.7, cases of naphthalene-induced hemolytic anemia in children have been frequently reported (Owa 1989; Owa et al. 1993; Santucci and Shah 2000; Valaes et al. 1963). Newborns and infants are thought to be more susceptible than older people because hepatic enzymes involved in conjugation and excretion of naphthalene metabolites are not well developed after birth, and children with genetically determined G6PD deficiency are thought to be especially susceptible to chemically-induced hemolytic anemia (EPA 1987a). There are no studies that have specifically examined the influence of age on naphthalene toxicokinetic capabilities in humans. Although the availability of such studies may increase the understanding of the specific physiological basis for the apparent susceptibility of newborns, they are unlikely to be conducted. Experiments examining the most sensitive targets in animals (see below) are likely surrogates.

Although naphthalene and/or its metabolites can cross the placental barrier (Anziulewicz et al. 1959; Zinkham and Childs 1957, 1958), oral-exposure developmental toxicity studies in animals do not provide

evidence that naphthalene was fetotoxic or impaired fetal development, even at maternally toxic dose levels as high as 450 mg/kg/day (NTP 1991a; Plasterer et al. 1985; PRI 1986). Additional developmental toxicity studies in animals with inhalation or dermal exposure would determine if naphthalene exposure by these routes represents a greater developmental hazard than oral exposure.

Neonatal mice (7 days old) appear to be more susceptible than adult mice to lung injury induced by acute intraperitoneal injection of naphthalene (Fanucchi et al. 1997). The mechanistic basis of this difference is currently unknown, but does not appear to be explained by differences in CYP catalytic capabilities to produce epoxide metabolites, since CYP activities were 2.5 time lower in neonates than in adults. Downstream metabolic capabilities, however, were not examined in this study. Comparison of neonatal and adult tissues in these metabolic steps may help to explain this apparent susceptibility of neonatal mice. Based on findings that *in utero* exposure to other CYP-bioactivated chemicals caused Clara cell tumors in adult offspring, Fanucchi et al. (1997) postulated that naphthalene exposure during the neonatal period may lead to loss of regulatory mechanisms resulting in Clara cell proliferation and tumor formation in adult animals. Direct evidence for naphthalene in support of this hypothesis, however, is not available. Additional research may help to determine whether or not *in utero* or neonatal naphthalene exposure will cause increased incidence of lung tumors in adult mice.

Child health data needs relating to exposure are discussed in Section 6.8.1, Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Dr. Alan Buckpitt and colleagues at the University of California, Davis have been conducting studies in several areas related to naphthalene toxicology including (1) identifying specific naphthalene-protein adducts in lungs of mice, rats, and Rhesus macaques and characterizing the time course of their generation and disappearance; (2) identifying cellular and molecular events involved in the development of naphthalene-induced acute lung injury by comparing lung tissue from rodents, Rhesus macaques, and humans; and (3) comparing the cellular distribution and catalytic activities of CYP monooxygenases in lung tissues from various species.

Dr. Charles Plopper and colleagues at the University of California, Davis have been conducting studies comparing acute naphthalene-induced lung injury in neonatal mice and adult mice and the biochemical

effects of *in utero* or neonatal exposure to lung toxicants on the development of bronchiolar repair capabilities. This work is part of an effort to increase understanding of molecular mechanisms involved in lung diseases that may originate in childhood exposures.

Dr. Leena Nylander French and colleagues at the University of North Carolina, Chapel Hill have been conducting studies to test the hypothesis that low levels of exposure to benzene or naphthalene can be detected using samples of keratinized epidermis removed by tape stripping.

Dr. Y. Awasthi and colleagues at the University of Texas, Galveston are studying the roles of glutathione S-transferases in protecting against ocular cytotoxicity and apoptosis caused by several oxidants, including naphthalene. Studies include the use of genetically altered knock-out mice strains, which are deficient in specific types of glutathione-*S*-transferases.

Dr. Barry Stripp and colleagues at the University of Pittsburgh are studying the role of proliferative cells originating from the neuroepithelial body in repair of airway epithelial cell damage in mice exposed to ozone or naphthalene.

Dr. John Markley and colleagues at the University of Wisconsin, Madison are studying the 1-, 2-, and 3-dimensional molecular structures of toluene 4-monooxygenase, an enzyme that catalyzes NADH- and O_2-dependent conversion of toluene to p-cresol, as well as the oxidation of numerous hydrocarbons, including naphthalene.

4. CHEMICAL AND PHYSICAL INFORMATION

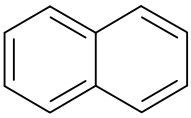
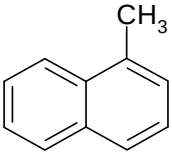
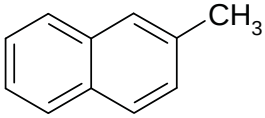
4.1 CHEMICAL IDENTITY

Information regarding the chemical identity of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene is located in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene is located in Table 4-2.

Table 4-1. Chemical Identity of Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Characteristic	Naphthalene	1-Methylnaphthalene	2-Methylnaphthalene	Reference
Synonyms	Tar camphor; albocarbon; naphthene; mothballs; moth-flakes; white tar; and others	Alpha-methyl-naphthalene; naphthalene, 1-methyl; naphthalene, alpha-methyl	Beta-methyl-naphthalene; naphthalene, 2-methyl; naphthalene, beta-methyl	HSDB 2004
Trade name	Caswell No. 5877®	No data	No data	HSDB 2004
Chemical formula	$C_{10}H_8$	$C_{11}H_{10}$	$C_{11}H_{10}$	HSDB 2004
Chemical structure				HSDB 2004
Identification numbers:				
CAS registry	91-20-3	90-12-0	91-57-6	HSDB 2004
NIOSH RTECS	QJ0525000	QJ9630000	QJ9635000	NIOSH 1987
EPA hazardous waste	U165	No data	No data	HSDB 2004
OHM/TADS	7216808	No data	No data	Agency for Toxic Substances and Disease Registry 1995
DOT/UN/NA/IMCO shipping	UN1334, UN2304, IMCO 4.1	No data	No data	HSDB 2004
HSDB	184	5268	5274	HSDB 2004
NCI	C52904	No data	No data	HSDB 2004

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 4-2. Physical and Chemical Properties of Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Property	Naphthalene	1-Methyl-naphthalene	2-Methyl-naphthalene	Reference
Molecular weight	128.19	142.20	142.20	Weast et al. 1985
Color	White	Colorless	No data	Verschueren 1983
Physical state	Solid	Liquid	Solid	Verschueren 1983
Melting point	80.5 °C	-22 °C	34.6 °C	Weast et al. 1985
Boiling point	218 °C	244.6 °C	241 °C	Sax and Lewis 1989; Weast et al. 1985
Density at 20 °C	1.145 g/mL	1.0202 g/mL	1.0058 g/mL	Weast et al. 1985
Odor	Strong (tar or mothballs)	No data	No data	HSDB 2004
Odor threshold:				
Water	0.021 mg/L	0.0075 mg/L	0.01 mg/L	Amoore and Hautala 1983; HSDB 2004; Verschueren 1983
Air	0.44 mg/m^3	No data	0.0581–0.2905 mg/m^3	Amoore and Hautala 1983; Ruth 1986
Solubility:				
Water at 25 °C	31.7 mg/L	25.8 mg/L	24.6 mg/L	EPA 1982e; HSDB 2004
Organic solvents	Soluble in benzene, alcohol, ether, acetone	Soluble in alcohol, ether, benzene	Soluble in alcohol, ether, benzene	Sax and Lewis 1989; Weast et al. 1985
Partition coefficients:				
Log K_{ow}	3.29	3.87	3.86	EPA 1982e; HSDB 1995
Log K_{oc}	2.97	No data	3.39	EPA 1982e; GDCH 1992; Kenaga 1980
Vapor pressure	0.087 mmHg	0.054 mmHg	0.068 mmHg	EPA 1982e; HSDB 1995
Henry's law constant	4.6x10^{-4} atm-m^3/mol	3.6x10^{-4} atm-m^3/mol	4.99x10^{-4} atm-m^3/mol	EPA 1982e; Yaws et al. 1991
Autoignition temperature	567 °C	529 °C	No data	Sax and Lewis 1989
Flashpoint	79 °C (open cup)	No data	No data	Sax and Lewis 1989
Flammability limits	0.9–5.9%	No data	No data	HSDB 2004
Conversion factors	1 ppm=5.24 mg/m^3 1 mg/m^3=0.191 ppm	1 ppm=5.91 mg/m^3 1 mg/m^3=0.17 ppm	1 ppm=5.91 mg/m^3 1 mg/m^3=0.17 ppm	Verschueren 1983
Explosive limits	No data	No data	No data	

5. PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.1 PRODUCTION

Naphthalene may be produced from either coal tar or petroleum. Distillation and fractionation of coal tar is the most common production process. The middle fraction (containing most of the naphthalene) is cooled, crystallizing the naphthalene. The crude naphthalene may be refined by distillation, washing, and sublimation (EPA 1982d; Hughes et al. 1985). 1-Methylnaphthalene and 2-methylnaphthalene are also produced from coal tar by first extracting the heteroaromatics and phenols, then filtering off the crystallized 2-methylnaphthalene and redistilling the filtrate to yield 1-methylnaphthalene (GDCH 1992; Sax and Lewis 1987).

Since 1960, recovery of naphthalene from petroleum by dealkylation of methyl naphthalenes in the presence of hydrogen at high temperature and pressure has become a commercial production process. The naphthalene is then recovered by fractionation, decolorized, and purified by crystallization. Naphthalene produced from petroleum is about 99% pure. In the United States, most naphthalene is produced from petroleum (EPA 1982d; Hughes et al. 1985).

The production volume of naphthalene in the United States decreased significantly from a peak of 900 million pounds (409,000 metric tons) in 1968 to 222 million pounds (101,000 metric tons) in 1994. Production capacity has remained relatively stable in recent years, with estimated capacity for 2004 at 215 million pounds (97,700 metric tons) (Hughes et al. 1985; Mason 1995; SRI 2002).

There are currently two companies in the United States producing naphthalene: Advanced Aromatics, L.P., Baytown, Texas and Koppers Industries, Inc., Follansbee, West Virginia. Koppers Industries, Inc. produces 1-methylnaphthalene; Flint Hills Resources L.P., Corpus Christi, Texas, produces 2-methyl-naphthalene; and Crowley Chemical Company, Inc., Kent, Ohio and Oklahoma City, Oklahoma, produces 1-methylnaphthalene/2-methylnaphthalene (mixed isomers) (SRI 2004). No data on production volume of 1-methylnaphthalene or 2-methylnaphthalene were located.

Table 5-1 lists information on United States companies that reported the manufacture and use of naphthalene in 2002 (TRI02 2004). The Toxics Release Inventory (TRI) data should be used with caution since only certain types of facilities are required to report. TRI is not an exhaustive list. 1-Methyl

Table 5-1. Facilities that Produce, Process, or Use Naphthalene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
AK	9	1,000	9,999,999	1, 3, 4, 5, 7, 8, 9, 12
AL	79	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
AR	36	0	49,999,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14
AZ	12	100	999,999	3, 6, 7, 8, 9, 10, 11, 12
CA	141	0	10,000,000,000	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
CO	21	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13
CT	16	100	49,999,999	2, 3, 6, 7, 8, 9, 10, 11, 12, 13
DE	12	10,000	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13
FL	28	0	9,999,999	1, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
GA	36	0	999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
GU	4	0	9,999,999	9, 12
HI	16	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
IA	32	100	9,999,999	1, 2, 3, 4, 5, 7, 8, 9, 10, 11, 12, 14
ID	2	10,000	999,999	1, 5, 12
IL	96	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
IN	76	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
KS	40	100	499,999,999	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14
KY	53	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
LA	117	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MA	15	1,000	49,999,999	2, 3, 6, 7, 9, 10, 11, 12
MD	20	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 14
ME	7	1,000	49,999,999	2, 3, 8, 9, 11, 12
MI	70	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MN	25	100	49,999,999	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14
MO	35	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
MS	44	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
MT	20	10,000	9,999,999	1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14
NC	34	0	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
ND	8	100	9,999,999	1, 2, 3, 4, 6, 7, 9, 10, 11, 12
NE	9	0	99,999	2, 7, 10, 11, 12
NJ	65	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
NM	12	1,000	9,999,999	1, 3, 4, 7, 8, 9, 12, 13
NV	3	1,000	999,999	2, 3, 4, 9, 12
NY	31	0	49,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13
OH	97	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
OK	46	100	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
OR	21	100	499,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 13, 14
PA	87	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13

Table 5-1. Facilities that Produce, Process, or Use Naphthalene

State[a]	Number of facilities	Minimum amount on site in pounds[b]	Maximum amount on site in pounds[b]	Activities and uses[c]
PR	24	100	99,999,999	1, 2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14
RI	4	1,000	9,999,999	1, 5, 9, 10, 12
SC	30	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13
SD	7	100	999,999	7, 8, 12
TN	48	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
TX	247	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
UT	46	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
VA	35	100	9,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
VI	5	1,000	49,999,999	1, 2, 3, 4, 6, 7, 9, 12
VT	1	100,000	999,999	12
WA	50	0	499,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13
WI	30	0	9,999,999	1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WV	43	0	99,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
WY	33	0	49,999,999	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14

Source: TRI02 2004 (Data are from 2002)

[a]Post office state abbreviations used
[b]Amounts on site reported by facilities in each state
[c]Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses
14. Process Impurity

naphthalene and 2-methylnaphthalene are not included in the list of chemicals for which reporting is required for the TRI.

5.2 IMPORT/EXPORT

In 1978, about 7 million pounds (3,260 metric tons) of naphthalene were imported to the United States and 9 million pounds (3,960 metric tons) were exported from the United States (EPA 1982d). More recently, imports increased to about 8 million pounds (3,600 metric tons), while exports increased to 38 million pounds (17,000 metric tons) in 2002 (USITC 2003). In 1986, 24,400 pounds of 1-methyl-naphthalene were imported in the United States (HSDB 2004). No recent information was located for 1-methylnaphthalene. No information was located on import or export quantities of 2-methylnaphthalene.

5.3 USE

The U.S. consumption of naphthalene was 238 million pounds (108,000 metric tons) in 1996 (Lacson et al. 2000; EPA 2002b). The principal end use for naphthalene is as an intermediate in the production of phthalic anhydride (more than 60% of consumption), which is used as an intermediate in the production of phthalate plasticizers, resins, phthaleins, dyes, pharmaceuticals, insect repellents, and other materials. It is also used in the production of the insecticide carbaryl, synthetic leather-tanning agents and surface active agents (naphthalene sulfonates and derivatives, which are used as dispersants or wetting agents in paint, dye, and paper-coating formulations), and miscellaneous organic chemicals, including dyes and resins. Crystalline naphthalene is also used as a moth repellent. In 1989, about 12 million pounds (5,500 metric tons) of naphthalene were used for this purpose (CEH 1993; HSDB 2004). Crystalline naphthalene has also been used as a solid block deodorizer for diaper pails and toilets (Haggerty 1956). Also, in the early 1900s naphthalene was used in medicine as an antiseptic, expectorant, and anthelmintic (Grant 1986; Lezenius 1902). It was commonly administered for diseases of the gastrointestinal tract and applied externally for treatment of skin disorders (Lezenius 1902).

It is anticipated that consumption of naphthalene for phthalic anhydride and production of naphthalene sulfonates will increase due to increased demand for these products. About 15–16 million pounds (6,800–7,300 metric tons) of naphthalene were expected to be used for moth repellents by 1994 (CEH 1993).

1-Methylnaphthalene is used in the synthesis of 1-methylnaphthoic acid and, to a lesser degree, as a dyeing agent and as a test substance for determining the ignition capability of diesel fuels. 2-Methylnaphthalene is used in vitamin K production by oxidation to 2-methyl-1,4-naphthoquinone, which can then be reacted to yield phytomenadione (vitamin K). It can also be chlorinated and oxidized to form dyes and small amounts in sulfonated form are used as textile aids, wetting agents, and emulators (GDCH 1992).

5.4 DISPOSAL

Naphthalene and waste containing naphthalene are classified as hazardous wastes by EPA. Generators of waste containing this contaminant must conform to EPA regulations for treatment, storage, and disposal (see Chapter 8). Rotary kiln or fluidized bed incineration methods are acceptable disposal methods for these wastes (EPA 1988a, 1989e).

According to the TRI, about 306,345 pounds of naphthalene were transferred off-site, including to publicly owned treatment works (POTW) in 2002 (TRI02 2004). Although data on quantities of naphthalene disposed of by various disposal methods in the past were not located, it was estimated that about 524,000 pounds (238 metric tons) of naphthalene were disposed of on land and 504,000 pounds (229 metric tons) were discharged to POTWs from production and inadvertent sources in 1978 (EPA 1982d).

No information was located on disposal methods or quantities of wastes containing 1-methylnaphthalene or 2-methylnaphthalene. However, these chemicals have been detected at hazardous waste sites (see Section 6.1).

6. POTENTIAL FOR HUMAN EXPOSURE

6.1 OVERVIEW

Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene have been identified in at least 654, 36, and 412, respectively, of the 1,662 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2005). However, the number of sites evaluated for naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene is not known. The frequency of these sites can be seen in Figures 6-1, 6-2, and 6-3, respectively. Of these sites, 654, 36, and 410, respectively, are located within the United States and 0, 0, and 2, respectively, are located in the Virgin Islands (not shown).

Most of the naphthalene entering the environment is discharged to the air. The largest releases result from the combustion of wood and fossil fuels and the off-gassing of naphthalene-containing moth repellents. Smaller amounts of naphthalene are introduced to water as the result of discharges from coal-tar production and distillation processes. The coal-tar industry is also a major source of the small amounts of naphthalene that are directly discharged to land. A large amount of naphthalene (often considerably more than 1,000 mg/kg) is present in soils contaminated with wastes from manufactured-gas plants.

Naphthalene in the atmosphere is subject to a number of degradation processes, including reaction with photochemically produced hydroxyl radicals. Naphthalene has a short half-life in most natural waters and soils because of its tendency to volatilize and biodegrade. As a consequence of these processes, there is little tendency for naphthalene to build up in the environment over time.

The concentration of naphthalene in air tends to be low in rural areas, but is elevated in urban areas. The highest atmospheric concentrations have been found in the immediate vicinity of specific industrial sources and hazardous waste sites. Naphthalene is also a common indoor contaminant in households using naphthalene-containing moth repellents or where tobacco is smoked. Sidestream smoke from one cigarette contained 46, 30, and 32 µg of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, respectively. Levels in water, sediments, and soil tend to be low, except in the immediate vicinity of point sources of release, such as chemical waste sites.

Figure 6-1. Frequency of NPL Sites with Naphthalene Contamination

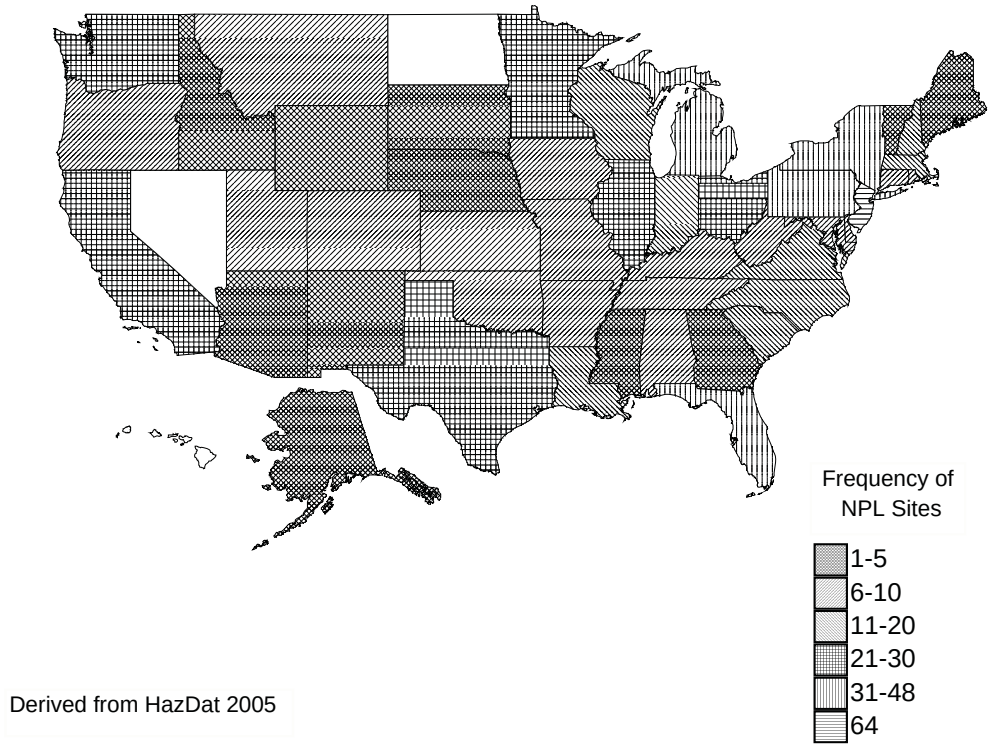


Figure 6-2. Frequency of NPL Sites with 1-Methylnaphthalene Contamination

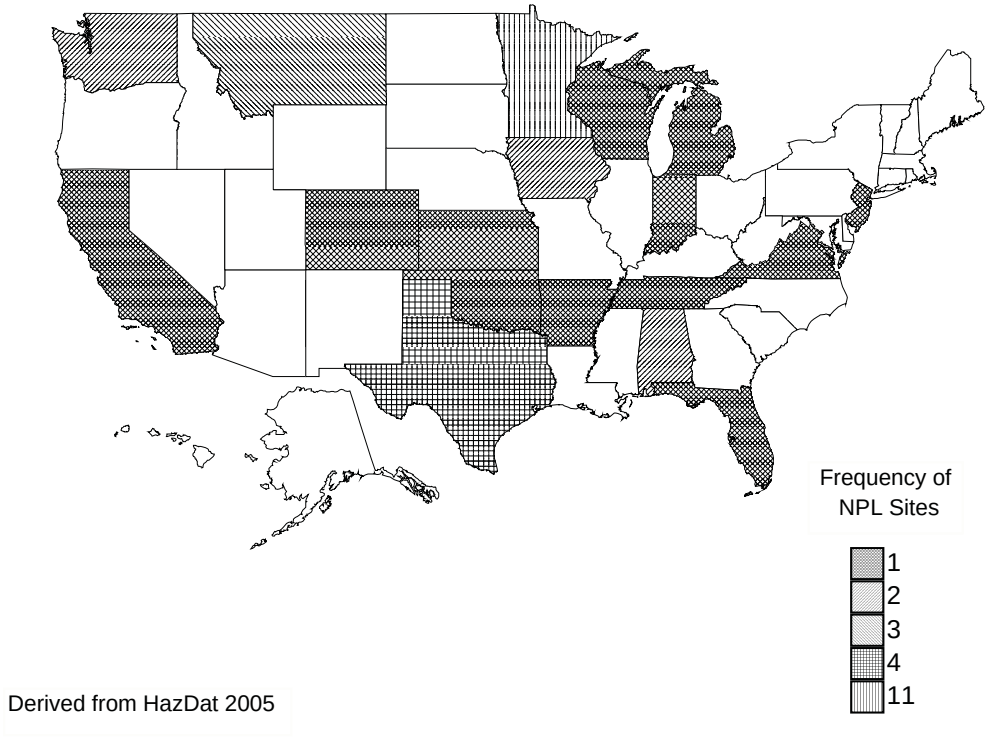
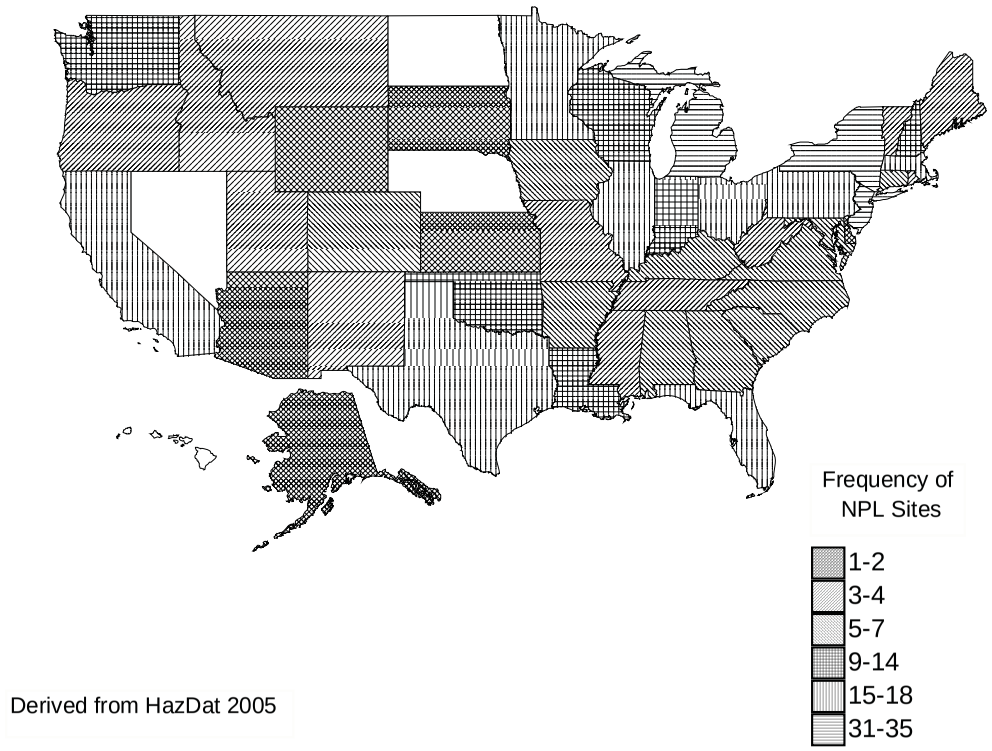


Figure 6-3. Frequency of NPL Sites with 2-Methylnaphthalene Contamination



The most likely pathway by which the general public is exposed to naphthalene is by inhalation due to the release of this substance from combustion fuels, moth repellents, and cigarette smoke. The estimated average per capita daily intake from ambient air is 19 µg. Exposure by other routes is not likely.

High naphthalene exposure levels could occur near industrial sources or chemical waste sites, but the extent of such exposure to individuals can only be evaluated on a site-by-site basis. High naphthalene exposure levels could also occur in certain work environments in industries that produce and use naphthalene such as wood preserving, tanning, coal distillation, and ink and dye production.

Based on limited data, potential human exposure to 1-methylnaphthalene or 2-methylnaphthalene is expected to be mainly by inhalation from ambient air. Exposure to these chemicals from tobacco smoke is likely.

1-Methylnaphthalene and 2-methylnaphthalene have also been detected in the environment, particularly in air. These are released from many of the same natural and industrial sources as naphthalene (combustion of wood and fossil fuels, tobacco smoke, coal distillation), but in smaller quantities.

Naphthalene has been identified in at least 654 of the 1,662 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (HazDat 2005). 1-Methylnaphthalene has been identified in at least 36 of these sites, and 2-methylnaphthalene has been identified in at least 412 of these sites. However, the number of sites evaluated for these chemicals is not known. The frequency of the sites at which naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene have been identified within the United States can be seen in Figures 6-1 through 6-3.

6.2 RELEASES TO THE ENVIRONMENT

The TRI data should be used with caution because only certain types of facilities are required to report (EPA 1997). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the Toxics Release Inventory only if they employ 10 or more full-time employees; if their facility is classified under Standard Industrial Classification (SIC) codes 20–39; and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 1997).

Most of the naphthalene entering environmental media is from combustion, mainly residential wood heating, or from the use of naphthalene in moth repellents. About 10% of environmental releases are attributable to coal production and distillation, while naphthalene production losses contribute <1% of environmental releases (EPA 1982d). Methylnaphthalenes are released from similar sources, including fuel combustion and industrial discharges (GDCH 1992). Smoking tobacco also releases small amounts of naphthalene and methylnaphthalenes into the environment.

6.2.1 Air

Estimated releases of 2.07 million pounds (940.2 metric tons) of naphthalene to the atmosphere from 781 domestic manufacturing and processing facilities in 2002, accounted for about 72% of the estimated total environmental releases from facilities required to report to the TRI (TRI02 2004). These releases are summarized in Table 6-1.

Nearly all naphthalene entering the environment is released directly to the air (92.2%). The largest source of emission (more than 50%) is through inadvertent releases due to residential combustion of wood and fossil fuels (EPA 1982d). Naphthalene emissions from unvented kerosene space heaters have been reported (Traynor et al. 1990).

The second greatest contribution comes from the use of naphthalene as a moth repellent (EPA 1982d). Because it volatilizes appreciably at room temperature, virtually all of the naphthalene contained in moth repellent is emitted to the atmosphere. Thus, in 1989, about 12 million pounds of naphthalene were released to air from moth repellent use (see Section 5.3).

Naphthalene may also enter the atmosphere during coal-tar production and distillation processes, through volatilization processes (aeration) in publicly owned treatment works (POTWs), from the use of naphthalene in the manufacture of phthalic anhydride, during the production of naphthalene, and from tobacco smoke. Methylnaphthalenes may be released to air in stack emissions and from fuel combustion, forest fires, and tobacco smoke (GDCH 1992; HSDB 2004; IARC 1993). 1-Methylnaphthalene and 2-methylnaphthalene were reported in jet exhaust at average concentrations of 421 and 430 $\mu g/m^3$, respectively, and in the gas phase of diesel motor exhaust at 1.57 $\mu g/m^3$ each (GDCH 1992). The smoke of an American unfiltered cigarette contains 2.8 µg of naphthalene, 1.2 µg of 1-methylnaphthalene, and

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Naphthalene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
AK	1	52	0	0	12	0	52	12	64
AL	22	108,624	58	0	221,582	5	295,685	34,585	330,270
AR	8	6,113	7	0	42	250	6,120	292	6,412
AZ	1	14	No data	0	0	0	14	0	14
CA	44	13,482	89	0	17,729	439	28,581	3,158	31,739
CO	7	2,106	0	0	0	0	2,106	0	2,106
CT	4	17,579	0	0	0	0	17,579	0	17,579
DE	2	721	0	0	0	0	721	0	721
FL	15	515,776	5	0	0	0	515,781	0	515,781
GA	13	18,576	0	0	9	0	18,576	9	18,585
GU	1	288	No data	0	0	0	288	0	288
HI	2	623	20	0	50	0	643	50	693
IA	11	13,873	0	0	11	0	13,873	11	13,884
IL	42	161,983	88	0	24,504	2,509	162,071	27,013	189,084
IN	35	166,075	526	26,140	29,037	17	221,609	186	221,795
KS	11	6,607	16	0	2	31,587	6,626	31,587	38,213
KY	20	23,003	840	0	323	0	24,103	63	24,166
LA	51	89,494	1,072	3	4,722	0	92,701	2,590	95,291
MA	7	4,948	0	0	5	262	4,948	267	5,215
MD	11	13,018	0	0	0	0	13,018	0	13,018
ME	3	6,208	No data	0	0	17	6,208	17	6,225
MI	26	104,586	0	0	463	2,040	104,836	2,253	107,089
MN	4	5,369	0	0	6	70	5,371	74	5,445
MO	20	59,389	6	0	8	84	59,400	87	59,487
MS	8	46,209	277	0	0	0	46,487	0	46,487
MT	4	1,192	8	0	17	0	1,216	1	1,217
NC	11	5,273	21	0	2,405	32	6,981	750	7,731
ND	4	1,934	2	0	0	893	1,936	893	2,829
NE	1	1,465	No data	0	9,853	0	1,465	9,853	11,318
NJ	21	17,753	629	0	712	5	18,382	717	19,099
NM	11	10,637	5	5	0	1,199	10,647	1,199	11,846
NV	1	8,075	No data	0	0	0	8,075	0	8,075
NY	19	6,141	0	0	0	500	6,141	500	6,641
OH	47	51,153	37	0	12,718	667	51,198	13,376	64,574
OK	10	28,605	299	0	6,801	48	35,327	426	35,753
OR	3	16,276	0	0	0	3	16,276	3	16,279

Table 6-1. Releases to the Environment from Facilities that Produce, Process, or Use Naphthalene[a]

		Reported amounts released in pounds per year[b]							
							Total release		
State[c]	RF[d]	Air[e]	Water[f]	UI[g]	Land[h]	Other[i]	On-site[j]	Off-site[k]	On- and off-site
PA	55	95,520	643	0	9,646	4,065	96,174	13,700	109,874
PR	12	1,745	0	0	0	0	1,745	0	1,745
SC	11	37,030	22,000	0	0	0	59,030	0	59,030
SD	3	52	No data	0	1	0	52	1	53
TN	6	4,706	No data	0	260	0	4,706	260	4,966
TX	127	282,582	738	204,570	11,548	9,282	470,932	37,788	508,720
UT	8	3,999	5	0	250	0	4,003	250	4,253
VA	11	1,357	8	0	2	0	1,365	2	1,367
VI	3	1,667	0	0	25	0	1,667	25	1,692
VT	1	4	No data	0	0	600	4	600	604
WA	15	4,840	0	0	1,087	0	5,537	390	5,927
WI	9	15,802	39	0	1,929	9	15,840	1,938	17,778
WV	10	85,154	63	0	10,983	110,190	85,221	121,169	206,390
WY	9	676	No data	0	1	250	677	250	927
Total	781	2,068,353	27,502	230,718	366,742	165,023	2,551,993	306,345	2,858,337

Source: TRI02 2004 (Data are from 2002)

[a]The TRI data should be used with caution since only certain types of facilities are required to report. This is not an exhaustive list. Data are rounded to nearest whole number.
[b]Data in TRI are maximum amounts released by each facility.
[c]Post office state abbreviations are used.
[d]Number of reporting facilities.
[e]The sum of fugitive and point source releases are included in releases to air by a given facility.
[f]Surface water discharges, wastewater treatment-(metals only), and publicly owned treatment works (POTWs) (metal and metal compounds).
[g]Class I wells, Class II-V wells, and underground injection.
[h]Resource Conservation and Recovery Act (RCRA) subtitle C landfills; other on-site landfills, land treatment, surface impoundments, other land disposal, other landfills.
[i]Storage only, solidification/stabilization (metals only), other off-site management, transfers to waste broker for disposal, unknown
[j]The sum of all releases of the chemical to air, land, water, and underground injection wells.
[k]Total amount of chemical transferred off-site, including to POTWs.

RF = reporting facilities; UI = underground injection

1.0 µg of 2-methylnaphthalene. Smoke from an equivalently filtered "little cigar" contains 1.2 µg of naphthalene, 0.9 µg of 1-methylnaphthalene, and 0.7 µg of 2-methylnaphthalene (Schmeltz et al. 1976).

As shown in Table 6-1, an estimated total of 2.1 million pounds of naphthalene, amounting to about 72% of the total environmental release under the TRI program, was discharged to the air from manufacturing and processing facilities in the United States in 2002 (TRI02 2004). The TRI data should be used with caution since only certain types of facilities are required to report. TRI is not an exhaustive list.

6.2.2 Water

Estimated releases of 27.5 thousand pounds (21.5 metric tons) of naphthalene to surface water from 781 domestic manufacturing and processing facilities in 2002, accounted for about 1% of the estimated total environmental releases from facilities required to report to the TRI (TRI02 2004). These releases are summarized in Table 6-1.

About 5% of all naphthalene entering the environment is released to water (EPA 1982d). Most of that amount is attributable to coal-tar production and distillation processes. Some naphthalene (about 60%) from these sources is discharged directly to surface waters; the remainder is distributed to POTWs. The effluent and oil-spills from the wood-preserving industry is the only other source of consequence that releases naphthalene into the nation's waterways,

Naphthalene was detected in 1.6% of effluent samples reported on the STORET database from 1980 to 1982 (Staples et al. 1985). Analysis of STORET data for 1978–1981 indicated that the range of detectable naphthalene concentrations in effluents was <1–36,000 µg/L (EPA 1982d).

The detection of naphthalene and methylnaphthalenes in groundwater in the vicinity of industrial facilities and landfills (see Section 6.4.2) (Brown and Donnelly 1988; Rosenfeld and Plumb 1991) indicates that these chemicals are released to water from these sources. Methylnaphthalenes have been detected in effluents from industrial sources (GDCH 1992; HSDB 2004). 1-Methylnaphthalene and 2-methyl-naphthalene were reported in process sewage and production water samples from coal gasification plants at concentrations ranging from 78 to 278 µg/L and from 66 to 960 µg/L, respectively (GDCH 1992).

As shown in Table 6-1, an estimated total of 27,502 pounds of naphthalene, amounting to about 1.0% of the total environmental release, was discharged to surface water from manufacturing and processing facilities in the United States in 2002 (TRI02 2004). An additional 230,718 pounds (8.0% of the total) was discharged by underground injection. The TRI data should be used with caution since only certain types of facilities are required to report.

6.2.3 Soil

Estimated releases of 366 million pounds reported under the TRI program (166.7 metric tons) of naphthalene to soils from 781 domestic manufacturing and processing facilities in 2002, accounted for about 12.8% of the estimated total environmental releases from facilities required to report to the TRI (TRI02 2004). An additional 0.231 million pounds (104.8 metric tons), constituting about 8.0% of the total TRI environmental emissions, were released via underground injection from facilities required to report to the TRI (TRI02 2004). These releases are summarized in Table 6-1.

It is estimated that only about 2.7% of the environmental releases of naphthalene are discharged to land (EPA 1982d). Sources include coal-tar production and minor contributions from naphthalene production, POTW sludge disposal, and the use of organic chemicals that include naphthalene.

The residuals produced in gas production by coal carbonization, carbureted water gas production, or oil gas production at manufactured gas plants (MGPs) included PAHs (naphthalene, anthracene, phenanthrene and benzo[1]pyrene). These residuals were deposited on site in tar wells, sewers, nearby pits, or streams resulting in widespread soil and groundwater contamination (Luthy et al. 1994).

As shown in Table 6-1, an estimated 366,742 pounds of naphthalene, amounting to about 8.1% of the total environmental release, was discharged to land from manufacturing and processing facilities producing and using naphthalene in the United States in 2002 (TRI02 2004). The TRI data should be used with caution since only certain types of facilities are required to report.

No information was located on releases of 1-methylnaphthalene or 2-methylnaphthalene to soil.

6.3 ENVIRONMENTAL FATE

6.3.1 Transport and Partitioning

Naphthalene released to the atmosphere may be transported to surface water and/or soil by wet or dry deposition. Since most airborne naphthalene is in the vapor phase, deposition is expected to be very slow (about 0.04–0.06 cm/sec). It has been estimated that about 2–3% of naphthalene emitted to air is transported to other environmental media, mostly by dry deposition (EPA 1982d).

Naphthalene in surface water may volatilize to the atmosphere. With a vapor pressure of 0.087 mm Hg at 25 °C, solubility in water of 31.7 mg/L at 20 °C, and a Henry's law constant of $4.6x10^{-4}$ (EPA 1982e), it is likely that volatilization will be an important route of naphthalene loss from water. The rate of volatilization also depends upon several environmental conditions, including temperature, wind velocity, and mixing rates of the air and water columns (EPA 1982d). The half-life of naphthalene in the Rhine River was 2.3 days, based on monitoring data (Zoeteman et al. 1980). In an experiment using a mesocosm, that simulated Narragansett Bay, the half-life in water was 12 days during winter, with loss primarily due to volatilization (Wakeham et al. 1983).

Log octanol/water partition coefficients (K_{ow}) for naphthalene range from 3.29 to 3.37 and log organic carbon coefficients (K_{oc}) range from 2.97 to 3.27 (Bahnick and Doucette 1988; EPA 1982e; Howard 1989; Klecka et al. 1990; Thomann and Mueller 1987). These values include both experimentally determined and calculated values. The reported experimentally determined log K_{oc} is 3.11 (Bahnick and Doucette 1988). Based on the magnitude of these values, it is expected that only a small fraction (<10%) of naphthalene in typical surface water would be associated with particulate matter (Thomann and Mueller 1987). Thus, naphthalene discharged to surface waters would remain largely in solution, with smaller quantities being associated with suspended solids and benthic sediments.

Naphthalene is easily volatilized from aerated soils (Park et al. 1990) and is adsorbed to a moderate extent (10%) (Karickhoff 1981; Schwarzenbach and Westall 1981). The extent of sorption depends on the organic carbon content of the soil, with rapid movement expected through sandy soils (Howard 1989). The estimated soil adsorption coefficient for naphthalene in a soil with <0.6% organic carbon is 1.8 (Klecka et al. 1990). Because it adsorbs to aquifer material (Ehrlich et al. 1982), naphthalene's passage through groundwater will be somewhat retarded. Nevertheless, naphthalene frequently appears in effluent drainage from disposal sites (Rittman et al. 1980; Roberts et al. 1980; Schwarzenbach et al. 1983). However, sorption of naphthalene to aquifer materials with low organic carbon content (<0.03%)

may be enhanced by the presence of nonionic low-polarity organics, such as tetrachloroethene, commonly found at hazardous waste sites (Brusseau 1991a).

Bioconcentration factors (BCFs) for naphthalene have been measured and calculated from the K_{ow}, K_{oc}, or water solubility. The values reported for log BCF range from 1.6 to 3 (Banerjee and Baughman 1991; Bysshe 1982; Geyer et al. 1982; Kenaga 1980; Southworth et al. 1978; Veith et al. 1979), indicating moderate bioconcentration in aquatic organisms. Naphthalene is reported to be rapidly eliminated from invertebrates when the organisms are placed in pollutant-free water (Eastmond et al. 1984; Tarshis 1981), and naphthalene is readily metabolized in fish (Howard 1989). Based on the magnitude of the K_{ow}, bioaccumulation in the food chain is not expected to occur (Thomann 1989). However, naphthalene exposure of cows and chickens could lead to the presence of naphthalene in milk and eggs (Eisele 1985).

Limited data were located on transport and partitioning of methylnaphthalenes in the environment. The respective vapor pressures (0.054 and 0.068 mmHg), water solubilities (25.8 and 24.6 mg/L), and Henry's law constants ($3.60x10^{-4}$ and $4.99x10^{-4}$ atm-m^3/mol) for 1-methylnaphthalene and 2-methylnaphthalene are of similar magnitude to these properties for naphthalene (HSDB 2004; Yaws et al. 1991). Thus, it is likely that loss of methylnaphthalenes from ambient water occurs by volatilization. In a mesocosm experiment, that simulated Narragansett Bay, the half-life of 2-methylnaphthalene in water was 13 days in winter, with loss primarily due to volatilization (Wakeham et al. 1983). Based on the magnitude of log K_{ow} for 1-methylnaphthalene and 2-methylnaphthalene (3.87 and 3.86, respectively) (HSDB 2004) and the experimental log K_{oc} for 2-methylnaphthalene (3.93) (Bahnick and Doucette 1988), these chemicals may partition similarly to naphthalene in environmental media and are expected to be slightly mobile to immobile in soils (HSDB 2004). Log BCFs calculated for 2-methylnaphthalene range from 2 to 2.8 (Kenaga 1980) and measured log BCFs for 1-methylnaphthalene and 2-methylnaphthalene in oysters ranged from 2.7 to 4.1 (GDCH 1992). Methylnaphthalenes are also metabolized and excreted rapidly by fish and shellfish when they are removed from polluted waters (Breger et al. 1981; GDCH 1992).

6.3.2 Transformation and Degradation

6.3.2.1 Air

The most important atmospheric removal process for naphthalene is reaction with photochemically produced hydroxyl radicals (Howard 1989). The rate for this reaction is $2.17x10^{-11}$ cm^3/molecule-sec (Atkinson et al. 1987) and the atmospheric half-life for naphthalene based on this reaction is <1 day. The

major products of this reaction are 1- and 2-naphthol and 1- and 2-nitronaphthalene (Atkinson et al. 1987). Naphthalene also reacts with N_2O_5, nitrate radicals, and ozone in the atmosphere (Atkinson et al. 1984, 1987) and photolysis is expected to occur, although no experimental data were located (Howard 1989).

Methylnaphthalenes also react with hydroxyl radicals. The reported rate constants are $5.30x10^{-11}$ and $5.23x10^{-11}$ cm^3/molecule-sec for 1-methylnaphthalene and 2-methylnaphthalene, respectively. Based on an atmospheric hydroxyl radical concentration of $1x10^6/cm^3$, the corresponding atmospheric half-lives are 3.6 and 3.7 hours (GDCH 1992). Reactions of 1-methylnaphthalene and 2-methylnaphthalene with N_2O_5 radicals have half-lives of 24 and 19 days, respectively (GDCH 1992). These chemicals also react with atmospheric ozone.

6.3.2.2 Water

Naphthalene and methylnaphthalenes are degraded in water by photolysis and biological processes. The half-life for photolysis of naphthalene in surface water is estimated to be about 71 hours, but the half-life in deeper water (5 m) is estimated at 550 days (Zepp and Schlotzhauer 1979). The half-lives for photolysis of 1-methylnaphthalene and 2-methylnaphthalene were estimated at 22 and 54 hours, respectively (GDCH 1992).

Biodegradation of naphthalene is sufficiently rapid for it to be a dominant fate process in aquatic systems (Tabak et al. 1981). Data on biodegradation of naphthalene in biodegradability tests and natural systems suggest that biodegradation occurs after a relatively short period of acclimation (rapidly, half-life about 7 days] in oil-polluted water) and the biodegradation rate increases with the naphthalene concentration. The biodegradation occurs slowly (half-lives up to 1,700 days) in unpolluted water (Herbes 1981; Herbes and Schwall 1978; Herbes et al. 1980; Howard 1989; Kappeler and Wuhrmann 1978). Reported biodegradation half-lives range from 3 to 1,700 days in various water systems (Howard 1989). In a static-flask-screening test, naphthalene showed rapid acclimation and 100% loss from the test medium in 7 days (Tabak et al. 1981). In an experiment with Narragansett Bay seawater, the half-life of naphthalene in late summer was reported at 0.8 days, mainly due to biodegradation (Wakeham et al. 1983). The half-life of 2-methylnaphthalene was 0.7 days in the same experiment.

Methylnaphthalenes are biodegraded under aerobic conditions after adaptation. The highest degradation rates were reported in water constantly polluted with petroleum (GDCH 1992).

6.3.2.3 Sediment and Soil

Naphthalene biodegradation rates are about 8–20 times higher in sediment than in the water column above the sediment (Herbes and Schwall 1978). Half-lives reported in sediment include 4.9 hours and >88 days in oil-contaminated and uncontaminated sediment, respectively (Herbes and Schwall 1978), 9 days in sediment near a coal-coking discharge (Herbes 1981), 3, 5, and >2,000 hours in sediments with high, medium, and low PAH levels, respectively (Herbes et al. 1980), and ranging from 2.4 weeks in sediments exposed to petroleum hydrocarbons to 4.4 weeks in sediments from a pristine environment (Howard 1989). Methylnaphthalenes biodegrade more slowly. Reported half-lives in sediments were 46 weeks for 1-methylnaphthalene and ranged from 14 to 50 weeks for 2-methylnaphthalene (GDCH 1992).

In soils, biodegradation potential is important to biological remediation of soil. Studies on biodegradation of PAHs suggest that adsorption to the organic matter significantly reduces the bioavailability for microorganisms, and thus the biodegradability, of PAHs, including naphthalene (Heitzer et al. 1992; Weissenfels et al. 1992). There is considerable variability in reported naphthalene soil half-lives. The estimated half-life of naphthalene reported for a solid waste site was 3.6 months (Howard 1989). In less contaminated soils, more rapid biodegradation is expected to occur (Howard 1989). In soils with 0.2–0.6% organic carbon and 92–94% sand, the half-lives were 11–18 days (Klecka et al. 1990). In another study, sandy loams with 0.5–1% organic carbon had naphthalene half-lives of 2–3 days (Park et al. 1990). Biodegradation is accomplished through the action of aerobic microorganisms and declines precipitously when soil conditions become anaerobic (Klecka et al. 1990). Studies indicate that naphthalene biodegrades to carbon dioxide in aerobic soils, with salicylate as an intermediate product (Heitzer et al. 1992).

Abiotic degradation of naphthalene seldom occurs in soils. In one study only about 10% of the naphthalene added to two soil samples treated with mercuric chloride to kill microorganisms was degraded over a 105- or 196-day period (Park et al. 1990).

In contaminated subsurface soils often found at former MGP sites, naphthalene is present as a component coal tar, a dense nonaqueous-phase liquid (DNAPL). It may exist in the subsurface in the form of trapped pools of organic liquid or as immobilized macroporous ganglia. Slow dissolution of naphthalene and other PAHs from DNAPLs into the aqueous phase causes them to be unavailable to the microorganism, thus resulting in the dissolution of the PAHs being the rate-limiting step in their biodegradation (Thomas et al. 1986). Using phenanthrene as a test substance, Birman and Alexander (1996) showed that the viscosity of the NAPL may reflect a slower diffusion of the aromatic substrate in the more viscous NAPLs and its subsequent slower mass transfer to water. Ghoshal and Luthy (1996) demonstrated that a very large fraction of naphthalene can be biodegraded from an accessible coal-tar-NAPL (free flowing) by microorganisms in bioslurry systems. Metabolically active microflora were detected beneath the water table at a former MGP sites from 2.6 to 30.8 m below the ground surface. The subsurface micorflora appeared to be acclimated to the presence of PAHs and were found to mineralize naphthalene (8–55%) in sediment-water microcosms under aerobic conditions. Naphthalene biodegradation half-lives ranged from 18 to 480 days (Durrant et al. 1994).

Naphthalene remaining in soil for extended periods of time was shown to become less available to bacteria and earthworms (Kelsey and Alexander 1997).

The behavior of 1-methylnaphthalene in sandy loam was very similar to that of naphthalene. 1-Methyl-naphthalene was easily volatilized from aerated soil, and the biodegradation half-life averaged between 1.7 and 2.2 days (Park et al. 1990). No data were identified on the biodegradation of 2-methyl-naphthalene in soil.

6.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is

bioavailable. The analytical methods available for monitoring naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in a variety of environmental media are detailed in Chapter 7.

6.4.1 Air

Naphthalene has been reported in ambient air at several locations in the United States. The average reported concentration for 67 samples was 5.19 μg/m^3, with most (60) of the samples and the highest concentrations at source-dominated locations (EPA 1988g). A median naphthalene level in urban air in 11 U.S. cities of 0.94 μg/m^3 has been reported (Howard 1989). An average naphthalene concentration of 170 μg/m^3 in outdoor air was reported in a residential area of Columbus, Ohio (Chuang et al. 1991), and naphthalene was measured in ambient air in Torrance, California at a concentration of 3.3 μg/m^3 (Propper 1988). Average naphthalene concentrations ranging from 10 to 888 ng/m^3 were measured in several sites in Phoenix and Tucson, Arizona from 1994 to 1996 (Zielinska et al. 1998). A mean naphthalene concentration of 0.129 ng/m^3 was detected in ambient air at the Mississippi Sandhill Crane National Wildlife Refuge, Jackson County from May to September 1991 (White and Hardy 1994).

Average naphthalene concentrations detected in ambient air at five hazardous waste sites and one landfill in New Jersey ranged from 0.42 to 4.6 μg/m^3 (LaRegina et al. 1986).

Naphthalene concentrations in indoor air may be higher than outdoors, with reported average indoor concentrations in various areas of homes ranging from 0.860 to 1,600 μg/m^3 (Chuang et al. 1991; Hung et al. 1992; Wilson et al. 1989). However, based on a careful analysis of Chuang et al. (1991), the reported upper range value may be in error. A more representative upper limit concentration for indoor air may be 32 μg/m^3, recorded in buildings in heavily trafficked urban areas of Taiwan (Hung et al. 1992). Concentrations of naphthalene detected in indoor and outdoor air measured in 24 low-income homes in North Carolina ranged from 0.33 to 9.7 and from 0.57 to1.82 μg/m^3 respectively (Chuang et al. 1999). In homes with smokers, indoor and outdoor air concentrations were measured to be 2.2 and 0.3 μg/m^3, respectively. Comparable values in homes without smokers were 1.0 and 0.1 μg/m^3, respectively (EPA 1991e; IARC 1993). The average reported concentration of naphthalene inside automobiles in commuter traffic is about 4.5 μg/m^3 (Lofgren et al. 1991).

Naphthalene has also been detected in air in industrial facilities. Reported naphthalene vapor levels ranged from 11 to 1,100 μg/m^3 in a coke plant and from 0.72 to 310 μg/m^3 in an aluminum reduction plant

(Bjorseth et al. 1978a, 1978b). Reported particulate levels for the same facilities ranged from nondetected to 4.4 μg/m^3, and from 0.9 to 4 μg/m^3, respectively.

Naphthalene has been detected in the emissions from motor vehicles. Mean concentrations of 104.3, 31.9, and 54.1 μg/m^3 of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, respectively, were measured in the air samples collected from the Caldecott Tunnel located in San Francisco (Zielinska and Fung 1994). Mean concentrations of 1,709, 131, and 162.5 mg/m^3 of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, respectively, were measured in the air samples collected from the Van Nuys Tunnel in Los Angeles (Fraser et al. 1998a). Mean concentration ranges of 0–589.2, 0–188.6, and 0–333.3 μg/m^3 of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, respectively, were measured in the air samples collected from the Fort McHenry Tunnel in Baltimore. Mean concentration ranges of 16.2–68.9, 9.4–20.0, and 21.9–35.7 μg/m^3 of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene, respectively, were measured in the air samples collected from the Tuscarora Tunnel on the Pennsylvania Turnpike (Zielinska et al. 1996). Average concentrations of 137–1714, 92–1,458, and 154–2,129 ng/m^3 of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene, respectively, were detected during various flight related and ground-support activities of C-130H aircraft at an Air National Guard base (Childers et al. 2000).

Shauer et al. (2002) compared the tailpipe emissions of catalyst- and noncatalyst-converter-equipped motor vehicles. Approximately 1,000 μg/km^{-1} of naphthalene and 2-methylnaphthalene and 500 μg/km^{-1} of 1-methylnaphthalene were detected in the catalyst-equipped vehicles. Approximately 50,000 μg/km^{-1} of naphthalene and 2-methylnaphthalene and 30,000 μg/km^{-1} of 1-methylnaphthalene were detected in the noncatalyst converter-equipped vehicles.

1-Methylnaphthalene and 2-methylnaphthalene have been reported in ambient air at average concentrations of 0.51 and 0.065 μg/m^3, respectively (EPA 1988g). Most of the data reported are from source-dominated areas, where the highest concentrations were detected. Methylnaphthalene (isomer not specified) was detected (concentration not reported) in ambient air at a hazardous waste site in New Jersey (LaRegina et al. 1986). 2-Methylnaphthalene was also reported in indoor air at an average concentration of 1.5 μg/m^3 (EPA 1988g).

6.4.2 Water

Naphthalene has been detected in surface water and groundwater in the United States. An analysis of 1980–1982 data from the STORET database indicates that naphthalene was detectable in 7% of 630 ambient water samples (Staples et al. 1985). The median concentration for all samples was <10 µg/L. Analysis of earlier (1978–1980) STORET data for naphthalene showed concentrations in positive samples ranging from 0.005 to 17 µg/L (EPA 1982d). Naphthalene was also detected in 11% of 86 urban runoff samples at concentrations ranging from 0.8 to 2.3 µg/L (Cole et al. 1984). In a study of contaminants of an urban watershed of Chesapeake Bay, naphthalene was detected in the northeast and northwest branches of Anacostia River (an urban watershed of Chesapeake Bay) at a concentration range of 0.18–21.6 ng/L. 2-Methylnaphthalene was also detected at a concentration of 0.57–62.7 ng/L (Foster et al. 2000). The mean concentration of naphthalene found in the water samples taken from 31 freshwater and estuarine sites adjacent to, nearby, or downstream from potential pollutant sources in Florida was 33 mg/L (Miles and Delfino 1999).

Naphthalene was detected in fewer than 5% of the 208 wells sampled from a variety of urban setting across the United States (Koplin et al. 1997). Naphthalene was detected in 3% of the samples taken from urban and rural wells from 1985 to 1995 (Squillace et al. 1999).

Naphthalene is rarely detected in drinking water. Naphthalene was reported in drinking water supplies in one area in the United States at levels up to 1.4 µg/L (EPA 1982d). Low levels of naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene were detected in drinking water samples taken from a chlorine dioxide disinfection pilot plant in Evansville, Indiana. These compounds were identified as organic disinfection byproducts produced by chlorine dioxide treatment (Richardson et al. 1994).

Naphthalene and 2-methylnaphthalene were detected in groundwater at five wood treatment facilities (Rosenfeld and Plumb 1991). Naphthalene was reported in 35% of samples at all five sites at an average concentration of 3,312 µg/L. 2-Methylnaphthalene was reported in 27% of samples at four sites at an average concentration of 563 µg/L. Naphthalene was reported in leachate or groundwater plume from industrial and municipal landfills at concentrations ranging from <10 to 18.69 mg/L and from 0.11 to 19 mg/L, respectively. The methylnaphthalene (isomer not specified) concentration reported at a municipal landfill was 0.033 mg/L (Brown and Donnelly 1988). Naphthalene was detected in the groundwater in 12.7% of the 479 U.S. waste disposal sites (Barbee 1994). Naphthalene was also reported in the leachate of a household hazardous waste disposal in sanitary landfill. The 4-year mean

concentrations of naphthalene ranged from 128.9 to 496.6 µg/L (Kinman et al. 1995). Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene have been detected in groundwater at gas Works Park, Seattle, Washington, in the range of 0.02–12, 0.02–1.1, and 0.03–1.4 mg/L, respectively (Turney and Goerlitz 1990). Gas Works Park is located on the site of a coal and oil gasificaton plant that ceased operation in 1956.

1-Methylnaphthalene and 2-methylnaphthalene were reported in an urban snow-pack in Michigan at concentrations of <0.05–0.177 and <0.05–0.251 µg/L, respectively (Boom and Marsalek 1988).

Naphthalene has been reported at a mean concentration of 6.3 ng/L in seawater in the south Atlantic Ocean (Cripps 1992).

6.4.3 Sediment and Soil

Naphthalene and methylnaphthalenes have been reported at low concentrations in uncontaminated soils and sediments and at higher concentrations near or within sources of contamination. Naphthalene has been reported in untreated agricultural soils at levels ranging from 0 to 3 µg/kg (Wild et al. 1990). Naphthalene was detected in urban soil samples taken from Boston, Massachusetts, Providence, Rhode Island, and Springfield, Massachusetts at a mean concentration of 0.125 mg/kg (Bradley et al. 1994). Reported naphthalene concentrations in contaminated soils included 6.1 µg/g in coal-tar contaminated soil (Yu et al. 1990), 16.7 mg/kg in soil from a former tar-oil refinery (Weissenfels et al. 1992) and up to 66 µg/kg in sludge-treated soils (Wild et al. 1990). Methylnaphthalenes (isomer not specified) were reported at a concentration of 2.9 µg/g in coal-tar contaminated soil (Yu et al. 1990). Hawthrone et al. have reported concentration of naphthalene to be 48 mg/kg in the soil from an unspecified manufactured gas plant in Midwestern United States (Hawthrone et al. 2001). Naphthalene and 2-methylnaphthalene have been detected in groundwater at Gas Works Park, Seattle, Washington, in the range of 0–46 and 0–6.3 mg/L, respectively (Turney and Goerlitz 1990). Gas Works Park is located on the site of a coal and oil gasificaton plant that ceased operation in 1956.

Naphthalene was reported as detectable in 7% of 267 sediment samples entered into the STORET database (1980–1982), with the median concentration for all samples of <500 µg/kg (Staples et al. 1985). Another analysis of STORET data indicated that concentrations in positive sediment samples ranged from 0.02 to 496 µg/kg (EPA 1982d). Naphthalene and methylnaphthalenes (isomers not specified) were

detected in contaminated and noncontaminated estuarine sediments (Brooks et al. 1990). Average concentrations of naphthalene detected in samples taken at 10 and 25 miles from an offshore coastal multiwell drilling platform were 54.7 and 61.9 µg/kg, respectively while concentrations of methylnaphthalenes were 50.4 and 55.3 µg/kg, respectively. Naphthalene and methylnaphthalenes concentrations in nearby noncontaminated estuarine sediments were 2.1 and 1.9 µg/kg, respectively. Naphthalene was detected in 7% of 496 streambed sediment sites across the United States tested for the presence of semivolatile organic compounds. The maximum concentration of naphthalene measured was 4,900 µg/kg dry weight (Lopes and Furlong 2001). Concentration of naphthalene detected decreased from 33 to 2.1 ng/g dry weight with increasing depth (0–148 cm) in the sediment core in Richardson Bay and from 18–4.1 ng/g dry weight with increasing depth (0–239 cm) in the sediment core in San Pablo Bay (Pereira et al. 1999). These bays are located in the San Francisco Bay which is the larges urbanized estuary on the west coast of the United States.

6.4.4 Other Environmental Media

Naphthalene is not generally reported in fish, but has been detected in shellfish in the United States. Naphthalene was not detected in 83 biota samples (median detection limit 2.5 mg/kg) reported from 1980–1982 in the STORET database (Staples et al. 1985). Reported naphthalene concentrations ranged from 5 to 176 ng/g in oysters, from 4 to 10 ng/g in mussels, and from <1 to 10 ng/g in clams from U.S. waters (Bender and Huggett 1989). In shore crabs collected from the San Francisco Bay area, average naphthalene concentrations were 7.4 ng/g (Miles and Roster 1999). Naphthalene constituted 75–80% of total polyaromatic hydrocarbons (PAHs) found in the muscle, liver, and gonads of American plaice and yellow tail flounder caught off the coast of Newfoundland (Hellou and Warren 1996). Naphthalene and methylnaphthalene (isomer not specified) were detected in the muscle (1.5–3.1 ng/g wet weight), kidney (1.4–4.3 ng/g wet weight), liver (1.4–4.7 ng/g wet weight), and blubber (8.3–23.5 ng/g wet weight) of harp seals caught in southern Labrador on the eastern coast of Canada (Zitko et al. 1998). Naphthalene and methylnaphthalenes (isomer not specified) were detected at concentrations of 7.15 and 65.11 µg/kg of salmon tissue, respectively, and at 12.9 and 17.3 µg/kg of mussels, respectively. Both the salmon and mussels were caught in Exxon Valdez spill affected Snug Harbor in the Prince William Sound (Neff and Burns 1996). Methylnaphthalenes have occasionally been detected in fish from polluted waters. 2-Methylnaphthalene was reported at concentrations ranging from 0.4 to 320 µg/g in fish from Ohio waters, but neither isomer of methylnaphthalene was detected in muscle tissue of fish from polluted areas of Puget Sound (GDCH 1992). Methylnaphthalenes were also detected in oysters in Australia at <0.3–

2 µg/g. Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene were detected at mean concentrations of 1.98, 0.96, and 1.98 ng/g, respectively, in farmed salmon and at 2.15, 1.53, and 2.93 ng/g, respectively, in wild salmon from the Pacific coast (Easton et al. 2002).

Naphthalene was detected in 2 of 13,980 samples of foods analyzed in six states (Minyard and Roberts 1991). In a Lower Rio Grande Valley environmental study, naphthalene (median concentration, 2.159 µg/kg body weight) was detected in five of the nine duplicate-diet samples (Berry et al. 1997). Naphthalene (1–7 µg/kg) was also detected in fresh tree-ripened apricots, plums, and their interspecific hybrids (Gomez et al. 1993). Naphthalene concentrations from vegetables grown in an industrial area of Thessaloniki, Greece were measured to be 0.37–15 µg/kg dry weight in cabbage; 8.9–30 µg/kg dry weight in carrots; 6.3–35 µg/kg dry weight in leeks; 4.9–53 µg/kg dry weight in lettuce; and 27–63 µg/kg dry weight in endive (Kipopoulou et al. 1999). Naphthalene was among the volatile organic compounds identified in whole and ground sorghum (Seitz et al. 1999).

Naphthalene levels in sterilized milk drinks contained in low-density polyethylene (LDPE) bottles were shown to be low (0.02 µg/mL) at the time of purchase, increasing to 0.1 µg/mL 30 days later, and averaging 0.25 µg/mL at the expiration date of the milk (Lau et al. 1994). Residual naphthalene present in the LDPE packaging was hypothesized to be the source of naphthalene contamination. A later study by the same authors observed that the level of naphthalene in LDPE milk bottle material had been reduced to 0.1–0.4 µg/g due to the use of new packaging material (Lau et al. 1995).

No information was located that documented methylnaphthalenes in food products.

Naphthalene was detected in the gas phase (5,860 µg/kg of meat cooked) as well as the particle phase (1,440–1,690 µg/kg of cooked meat) in the emissions from the process of charbroiling hamburger meat over a natural gas grill (Schauer et al. 1999a). Naphthalene was detected at a concentration of 227 mg/kg of wood burned from the fireplace combustion of pine wood. 1-Methylnaphthalene was detected at concentrations of 10.6, 6.39, and 4.31 mg/kg of wood burned from the combustion of pine, oak, and eucalyptus wood respectively. 2-Methylnaphthalene was detected at concentrations of 15.0, 9.31, and 5.69 mg/kg of wood burned from combustion of pine, oak, and eucalyptus wood, respectively. Naphthalene was not measured from the oak and eucalyptus fires (Schauer et al. 2001). In another study, the respective median concentrations of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene were determined to be 22.57, 4.14, and 4.76 mg/kg of burned soft wood in the fireplace; 60.86, 12.71, and

15.55 mg/kg for hardwood in the fireplace; and 34.96, 5.23 and 6.32 mg/kg for hardwood burned in a woodstove (McDonald et al. 2000).

Reported levels of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in measured in the smoke from U.S. commercial unfiltered cigarettes were 3, 1, and 1 μg, respectively (Schmeltz et al. 1978). Levels in sidestream smoke were found to be higher; 46, 30, and 32 μg/cigarette, respectively (Schmeltz et al. 1976).

Naphthalene has been detected in ash from municipal refuse and hazardous waste incinerators (EPA 1989g; Shane et al. 1990). Naphthalene was detected in 7 of 8 municipal refuse ash samples at 6–28,000 μg/kg (Shane et al. 1990) and in 5 of 18 hazardous waste incinerator ash samples at 0.17–41 mg/kg (EPA 1989g). Higher concentrations were detected in bottom ash than in fly ash (Shane et al. 1990).

Naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene were among the chemicals detected in Lower Manhattan in the aftermath of the destruction of the World Trade Center, New York City, New York on September 11th, 2001. Concentration of naphthalene ranged from 699 ng/m^{-3} on 9/26–9/27 to 42 ng/m^{-3} on 10/21–10/22. The concentration of 1-methylnaphthalene ranged from 178 to 100 ng/m^{-3} and that of 2-methylnaphthylene ranged from 267 to 165 ng/m^{-3} for the same days (Swartz et al. 2003).

6.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The general population is exposed to naphthalene mainly by inhalation of ambient and indoor air. The use of naphthalene-containing moth repellents and smoke from cigarettes are the main sources of naphthalene in indoor air. Other sources include kerosene heaters. Based on an urban/suburban average air concentration of 0.95 μg/m^3 and an inhalation rate of 20 m^3/day, it has been estimated that the average daily intake from ambient air is 19 μg (Howard 1989). Intake from indoor air may be higher, depending on the presence of indoor sources.

The estimated average daily intake from ambient air may be about 10 μg for 1-methylnaphthalene and 1 μg for 2-methylnaphthalene. These estimates are based on ambient air samples taken from 64 (1-methylnaphthalene) and 17 (2-methylnaphthalene) locations (EPA 1988g) and an assumed human daily intake of 20 m^3. Naphthalene was one of the PAHs detected in an 8-home pilot study that was

conducted in Columbus, Ohio to measure the PAH concentration profiles in house-dust. The average concentration of naphthalene was found to be dependant upon the method of extraction (2.8 mg/m^3 by soxhlet extraction and 1.8 mg/m^3 by sonication extraction) (Chuang et al. 1995). Concentrations of naphthalene detected in the indoor and outdoor air measured in 24 low-income homes in North Carolina ranged from 0.33 to 9.7 and from 0.57 to1.82 μg/m^3, respectively (Chuang et al. 1999). In a study reporting the concentrations of volatile organic compounds (VOCs) in a wide range of environments (i.e., homes, offices, restaurants, pubs, department stores, train and bus stations, heavily trafficked roadside locations, buses, trains and automobiles) in Birmingham, United Kingdom, naphthalene concentrations were found to range from 0.1 μg/m^3 (labs) to 12.1 μg/m^3 (heavily trafficked roadside) (Kim et al. 2001). A mean concentration of naphthalene was found to be 2.3 μg/m^3 in a German environmental survey that monitored 113 adults aged 25–69 years, selected at random, for personal exposure to VOCs including naphthalene (Hoffman et al. 2000). Low levels of naphthalene (average concentration, 0.44 μg/m^3) and 1-methylnaphthalene (average concentration 0.08 μg/m^3) were found in the indoor air of 92 and 81% of single family homes and apartments monitored, respectively (Kostianen 1995). Naphthalene has been detected in the smoke from charbroiling meat (Schauer et al. 1999a) and from the smoke from domestic fireplaces and wood burning stoves (McDonald et al. 2000; Schauer et al. 2001).

Exposure to naphthalene may occur from ingestion of drinking water and/or food, but these exposures are expected to be much less than inhalation exposures for the general population. Estimated exposure from drinking water, assuming a water concentration range of 0.001–2 μg/L, is 0.002–4 μg/day (Howard 1989). Estimates for food were not calculated. In a Lower Rio Grande Valley environmental study, naphthalene (median concentration, 2.159 μg/kg body weight) was detected in five of the nine duplicate-diet samples (Berry et al. 1997). Naphthalene was also detected in fresh tree-ripened apricots, plums, and their interspecific hybrids (Gomez et al. 1993), in vegetables such as cabbage, carrots, leeks, lettuce, and endive (Kipopoulou et al. 1999), and in whole and ground sorghum (Seitz et al. 1999). It has also been found in fish such as American plaice, yellow tail flounder (Hellou and Warren 1996), and salmon (Neff and Burns 1996).

Accidental ingestion of household products containing naphthalene such as mothballs or deodorant blocks frequently occurs in children. In 1990, 2,400 cases of accidental naphthalene ingestion were reported to 72 Poison Control Centers in the United States (Woolf et al. 1993). Nearly 90% of these cases occurred in children under 6 years of age.

Dermal exposure to naphthalene may occur from handling or wearing clothing stored in naphthalene-containing moth repellents. However, no data were located concerning the level of human exposure to naphthalene, 1-methylnaphthalene, or 2-methylnaphthalene via this exposure route. Experimental studies have shown that naphthalene can be dermally absorbed and systemically metabolized in rats (Turkall et al. 1994).

Naphthalene was detected in 40% of human adipose tissue samples at concentrations ranging from <9 to 63 μg/kg in a National Human Adipose Tissue Survey (NHATS) (EPA 1986g). Naphthalene was also detected (concentrations not reported) in six of eight selected breast milk samples from women in four U.S. cities (Pellizzari et al. 1982).

Naphthalene exposure may also occur in the workplace. Bjorseth et al. (1978a, 1978b) have reported vapor levels of 11–1,100 $\mu g/m^3$ and from 0 (nondetected) to 44 $\mu g/m^3$ for naphthalene-containing particulate in a coke plant. Similar measurements in an aluminum reduction plant yielded somewhat lower levels of 0.72–310 $\mu g/m^3$ for vapor and 0.08–4 $\mu g/m^3$ for particulates. Higher levels would be anticipated in naphthalene-producing industries and naphthalene-using industries such as wood preserving, tanning, and ink and dye production. A NIOSH (1980) survey of worker exposures to polyaromatic hydrocarbons at a petroleum refinery in Tulsa, Oklahoma reported air concentrations of naphthalene as high as 10.2 $\mu g/m^3$ in an area sample and 19.3 $\mu g/m^3$ for a personal sample. For 2-methyl-naphthalene, 17.6 $\mu g/m^3$ was the maximum area concentration reported and 31.9 $\mu g/m^3$ was the highest value for a personal sample. A National Occupational Exposure Survey (NOES) conducted by NIOSH estimated that 112,702 and 4,358 workers are potentially exposed to naphthalene and 2-methyl-naphthalene, respectively (NIOSH 1991). The workers at greatest risk of exposure included mining machine operators, aircraft engine mechanics, and miscellaneous machine operators. The NOES data have become progressively dated, and as a consequence, less representative of current exposure situations. The number of workers exposed to naphthalene during its manufacture and subsequent use is estimated to be 250–500 in the UK and 1,500–2,000 in the European Union (EU). These estimates do not include operators handling creosote treated lumber or brush applicators or users of tar paints/membranes (EU 2002).

6.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans. Differences from adults in susceptibility to hazardous substances are discussed in Section 3.7, Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, put things in their mouths, sometimes eat inappropriate things (such as dirt or paint chips), and spend more time outdoors. Children also are closer to the ground, and they do not use the judgment of adults to avoid hazards (NRC 1993).

Children are likely to be exposed to naphthalene via the same routes that affect adults, such as inhalation of contaminated air, ingestion of contaminated groundwater used as a source of drinking water, ingestion of contaminated food, and dermal contact with contaminated soils or products treated with the compound. The EPA (1996c) calculated an estimated intake range of 0.0002–0.043 mg/kg/day of naphthalene for a 10-kg child, assuming an ingestion of 100 mg of soil per day. Assuming food ingestion of approximately 0.5–2.3 kg/day for children, an estimated daily intake of 204–940 ng/kg-day was calculated for a 10-kg child. An estimated average daily dose of 1,127 ng/kg-day was calculated, assuming an inhalation rate of 8.7 m^3/day for a 10-kg child.

Small children are more likely than adults to come into intimate contact with yard dirt, lawns, and dust from carpets. Dislodgeable pesticide residues in carpets or on uncovered floors may present a relatively important exposure route for infants and toddlers through dermal contact and oral ingestion. The tendency of young children to ingest soil, either intentionally through pica or unintentionally through hand-to-mouth activity, is well documented. These behavioral traits can result in ingestion of naphthalene present in soil and dust. Naphthalene has been detected in the house-dust in an 8-home pilot study (Chuang et al. 1995).

Dermal exposure to naphthalene may occur from handling or wearing clothing stored in naphthalene-containing moth repellents. No studies are available that describe the dermal absorption of naphthalene in

children. Experimental studies have shown that naphthalene can be dermally absorbed and systemically metabolized in rats (Turkall et al. 1994).

Inhalation exposure is a major source of exposure in both adults and children. Naphthalene has been detected in the indoor air of homes (Chuang et al. 1995, 1999; Kostianen 1995). Naphthalene has been detected in the smoke from charbroiling meat (Schauer et al. 1999a) and from the smoke from domestic fireplaces and wood burning stoves (McDonald et al. 2000; Schauer et al. 2001).

Naphthalene was among the chemicals detected at nine day care centers in Durham, Raleigh, and Chapel Hill, North Carolina (Wilson et al. 1999). Indoor and outdoor air was found to contain naphthalene at concentrations of 205 and 89.6 ng/m^{-3}, respectively. The concentrations were 0.011 ppm in soil, 0.008 ppm in dust, 0.94 ppb in liquid food, and 0.25 ppb in solid food samples. The differences in PAH concentrations between day care centers serving low-income clients and those serving middle-income clients were found to be small.

Naphthalene (mothballs) is commonly used as a moth repellant in clothes during storage and as a deodorizer in diaper pails. Acute hemolysis was reported in 21 children following a period of inhalation exposure of naphthalene. The source of naphthalene was woolen clothes and blankets that had been stored with mothballs over the summer (Valaes et al. 1963).

A potential source of exposure in infants is from the presence of naphthalene in breast milk or formula. Naphthalene was detected (concentrations not reported) in six of eight breast milk samples from women in four U.S. cities (Pellizzari et al. 1982).

Children may also be exposed to naphthalene from milk drinks that have been stored in LDPE bottles. Naphthalene concentrations of 0.02 µg/mL were found in milk drinks stored in LDPE bottles at the time of purchase, but increased to 0.1 µg/mL 30 days later and averaged 0.25 µg/mL at the expiration date of the milk drink (Lau et al. 1994). Residual naphthalene present in the LDPE packaging was hypothesized to be the source of naphthalene contamination. A later study by the same authors observed that the level of naphthalene in LDPE milk bottle material had been reduced to 0.1–0.4 µg/g due to new packaging material (Lau et al. 1995).

Accidental ingestion of household products containing naphthalene, such as mothballs or deodorant blocks, can occur in children. In 1990, 2,400 cases of accidental naphthalene ingestion were reported to

72 Poison Control Centers in the United States (Woolf et al. 1993). Nearly 90% of these cases occurred in children under 6 years of age.

6.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Members of the general population most likely to have high levels of exposure to naphthalene are users of naphthalene-containing moth repellents (including infants exposed to blankets or clothing stored in naphthalene-containing mothballs), smokers, and those in proximity to smokers. Workers in naphthalene-producing or naphthalene-using industries could be subject to heightened exposure, and individuals living or working near hazardous waste sites at which naphthalene has been detected could also be exposed to higher naphthalene concentrations if they came into contact with contaminated media.

6.8 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene are available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.8.1 Identification of Data Needs

Physical and Chemical Properties. The physical and chemical properties of naphthalene that are required to evaluate its behavior in the environment have been determined (EPA 1982e; HSDB 2004). Information that documented the physical and chemical properties of 1-methylnaphthalene and 2-methyl-

naphthalene are also available (HSDB 2004). However, measured Henry's law constants and log K_{oc} values for methylnaphthalenes would allow more accurate prediction of environmental fate processes.

Production, Import/Export, Use, Release, and Disposal. According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit substance release and off-site transfer information to the EPA. The TRI, which contains this information for 2002, became available in May of 2004. This database is updated yearly and should provide a list of industrial production facilities and emissions.

Naphthalene producers, production locations and volumes, uses, releases, and disposal practices are well documented (Lacson et al. 2000; SRI 2004; TRI02 2004). Disposal of naphthalene-containing wastes are regulated by EPA, and major spills or accidental releases must be reported to EPA. No data were located on production volume, releases, and disposal practices for 1-methylnaphthalene or 2-methylnaphthalene. This information would be helpful to predict the potential for human exposure to these chemicals.

Environmental Fate. Existing information indicates that most naphthalene is released to the atmosphere and undergoes rapid reaction with hydroxyl radicals (Atkinson et al. 1987; EPA 1982d; Howard 1989). Available data indicate that volatilization and biodegradation are important removal processes from water and soil (EPA 1982d; Howard 1989; Tabak et al. 1981; Wakeham et al. 1983). Additional studies on the rates of volatilization, degradation, and transport in groundwater would be helpful in assessing potential human exposure in the vicinity of industrial sources and chemical waste sites. Data describing the volatilization, biodegradation, and transport of 1-methylnaphthalene and 2-methylnaphthalene would be useful in predicting the potential for human exposure.

Bioavailability from Environmental Media. No studies were located on the bioavailability of naphthalene in various environmental media. Available toxicity data indicate that naphthalene present in contaminated air and ingested in drinking water or soil is probably absorbed. Confirmatory, quantitative data would be useful. Data on infants indicate that toxicologically significant amounts of naphthalene may be absorbed dermally from residues left on stored clothing, especially under circumstances where baby oil was used on the infants' skin (Schafer 1951). Quantitative studies of the dermal absorption of naphthalene from water and soil would be useful in determining potential exposure for populations living near hazardous waste sites.

No data have been located pertaining to the bioavailability of 1-methylnaphthalene or 2-methylnaphthalene in environmental media. Studies in laboratory animals to assess the absorption of this compound via the oral, inhalation, and dermal routes would be useful before bioavailability from each medium can be reasonably estimated.

Food Chain Bioaccumulation. Naphthalene is often readily degraded in the environment and is easily metabolized by a wide variety of organisms. Studies indicate that although naphthalene may bioconcentrate to a moderate degree for brief periods, it will not significantly bioaccumulate in organisms due to metabolism, and thus, is unlikely to biomagnify through the food chain (Howard 1989; Thomann 1989). Naphthalene has been found to be present in fish and shellfish (Bender and Huggett 1989; Hellou and Warren 1996; Miles and Roster 1999; Minyard and Roberts 199l; Neff and Burns 1996; Zitko et al. 1998). It has also been located in the flesh of fresh fruits and vegetables (Gomez et al. 1993; Kipopoulou et al. 1999; Seitz et al. 1999). Data were not located on 1-methylnaphthalene and 2-methylnaphthalene levels in foods. Additional data on naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene concentrations in foods and processed foods would be useful to assess the extent of human exposure via the food chain.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in the environment can be used in combination with the known body burden of naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

The concentrations of naphthalene in the air, water, and soil have been documented (Bradley et al. 1994; Chuang et al. 1999; EPA 1988g; Howard 1989; Miles and Delfino 1999; Richardson et al. 1994; Squillace et al. 1999; Wild et al. 1990; Yu et al. 1990; Zielinska et al 1998). In addition, indoor air levels have been measured (Chuang et al. 1991; Hung et al. 1992; Wilson et al. 1989). Additional information regarding exposure levels of 1-methylnaphthalene and 2-methylnaphthalene in environmental media would be useful for deriving exposure estimates for the general population.

Reliable monitoring data for the levels of naphthalene in contaminated media at hazardous waste sites are needed so that the information obtained on levels of naphthalene in the environment can be used in

combination with the known body burden of naphthalene to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Exposure Levels in Humans. A national survey of adipose tissue samples indicates that about 40% of the study subjects had measurable levels of naphthalene (EPA 1986g). Naphthalene was also detected in six of eight samples of human milk (Pellizzari et al. 1982). Data on the effect of cigarette filters on naphthalene uptake by the adipose tissues would be useful. Naphthalene has been detected in house dust (Chuang et al. 1995).

No data on exposure levels in humans were located for 1-methylnaphthalene or 2-methylnaphthalene. This information would be useful to determine whether any significant exposure to these chemicals occurs.

This information is necessary for assessing the need to conduct health studies on these populations.

Exposures of Children. No monitoring studies have been performed to investigate the exposure to, and the body burden of, naphthalene in children. No studies are available on the dermal absorption of naphthalene in infants and toddlers due to activities such as crawling, which will result in contact with the floor (carpet) and soil or from exposure to clothes stored with mothballs. Since naphthalene is likely to be adsorbed to these materials, more information would allow the estimation of a child's exposure to naphthalene to be more rigorously determined. Naphthalene has been detected in house dust (Chuang et al. 1995). The EPA has calculated estimated amounts of naphthalene inhaled and naphthalene ingested via the intake of food and soil for a 10-kg child (EPA 2002b). No studies on amounts of naphthalene present in foods eaten by children are available. Such studies may help to identify childhood-specific means of decreasing exposure to naphthalene.

Child health data needs relating to susceptibility are discussed in Section 3.12.2, Identification of Data Needs: Children's Susceptibility.

Exposure Registries. No exposure registries for naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene were located. These substances are not currently one of the compounds for which a sub-registry has been established in the National Exposure Registry. These substances will be considered in the future when chemical selection is made for sub-registries to be established. The information that is

amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

6.8.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2004) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 6.8.1. These studies are summarized in Table 6-2.

Table 6-2. Ongoing Studies on the Potential for Human Exposure to Naphthalene[a]

Investigator	Affiliation	Research description	Sponsor
Nylander French LA	University of North Carolina, Chapel Hill, North Carolina	Dermal exposure to benzene and naphthalene	National Institutes of Environmental Health Science
Aitken MD	University of North Carolina, Chapel Hill, North Carolina	Bacterial chemotaxis to naphthalene desorbing from non-aqueous phase liquid	National Science Foundation, Environmental Remediation Program
Atkinson R; Winer AM	University of California, Riverside, California	Photochemical and thermal reactions of combustion related particulate organic matter: A combined chemical and microbiological approach	ER-74 Office of Scientific and Technical Information
Boyd SA	Michigan State University, East Lansing, Michigan	Physicochemical and microbiological factors influencing the bioavailability of organic contaminants in subsoils	U.S. Department of Energy, Energy Research
Bryers JD	University of Connecticut Health Center, Farmington, Connecticut	Substrata surface chemistry, conformation of contaminant upon absorption, and availability for biodegradation	National Science Foundation, Environmental Remediation Program
Kilduff JE	Rensselaer Polytechnic Institute, Troy, New York	Collaborative research: Sorption reversibility of hydrophobic compounds in geosorbents investigated with model sorbents	National Science Foundation, Environmental Remediation Program
Thompson AF	Connecticut Agricultural Experimental Station, New Haven, Connecticut	Collaborative research: Sorption reversibility of hydrophobic compounds in geosorbents investigated with model sorbents	National Science Foundation, Environmental Remediation Program
Pulliam Holoman TR	University of Maryland, College Park, Maryland	Anaerobic degradation of polycyclic aromatic hydrocarbons in marine harbor sediments	National Science Foundation, Environmental Remediation Program
Sayler GS	University of Tennessee, Knoxville, Tennessee	On-line monitoring of aerobic bioremediation with bioluminescent reporter microbes	U.S. Department of Energy, Energy Research
Zylstra GJ	Rutgers University, New Brunswick, New Jersey	Molecular analysis of polycyclic aromatic hydrocarbon degradation by mycobacteria	National Science Foundation, Biomolecular Processes Cluster

Table 6-2. Ongoing Studies on the Potential for Human Exposure to Naphthalene[a]

Investigator	Affiliation	Research description	Sponsor
Pignatello JJ	Connecticut Agricultural Experimental Station, New Haven, Connecticut	Chain-transfer complexation of aromatic compounds with soil organic matter	U.S. Department of Agriculture
Inskeep WP	Montana State University, Bozeman, Montana	Fate and transport of chemicals in soils: linking chemical transformations	U.S. Department of Agriculture
Madsen EL	Cornell University, Ithaca, New York	Observing microbial diversity and horizontal gene transfer in a shallow aquifer	U.S. Department of Agriculture
Haggblom MM	Rutgers University, New Brunswick, New Jersey	Microbial degradation of PAHs in the rhizosphere of salt-marsh plants	U.S. Department of Agriculture
Pignatello JJ	Connecticut Agricultural Experimental Station, New Haven, Connecticut	Nonideal (specific) sorption of organic chemicals in soil organic matter	U.S. Department of Agriculture
Ogram AV; Hornsby AC	University of Florida, Gainesville, Florida	Pesticides and other toxic organics in soil and their potential for ground and surface water contamination	U.S. Department of Agriculture
Huang W	Drexel University, Philadelphia, Pennsylvania	Black carbon in soils and sediments and its interactions with organic pollutants	U.S. Department of Agriculture
Huwe JK; Hakk H; Shappell NW; Shlever WL; Larsen GL; Smith DJ	Agricultural Research Service, Fargo, South Dakota	Dioxins and other environmental contaminants in food	U.S. Department of Agriculture
Xing B	University of Massachusetts, Amrherst, Massachusetts	Effects of long-term tillage and cover crop systems on soil organic matter and pesticide sorption	U.S. Department of Agriculture
Chorover JD	Pennsylvania State University, University Park, Pennsylvania	Effects of mineral-organic interactions on chemical processes in soils	U.S. Department of Agriculture
Thompson ML; Sandor JA; Burras CL	Iowa State University, Ames, Iowa	Human impacts on soil; a pedogenic perspective	U.S. Department of Agriculture
Hyman MR	North Carolina State university, Raleigh, North Carolina	Physiology, biochemistry, and enzymology of microbial cometabolism	U.S. Department of Agriculture
Larson RA; Sims GK	University of Illinois, Urbana, Illinois	Phytoremediation of agrochemicals with aquatic and terrestrial plants	U.S. Department of Agriculture

Table 6-2. Ongoing Studies on the Potential for Human Exposure to Naphthalene[a]

Investigator	Affiliation	Research description	Sponsor
Simkins S	University of Massachusetts, Amherst, Massachusetts	Quantification of pesticide-derived organic carbon in microbial biomass and soil humic substances	U.S. Department of Agriculture
Pignatello JJ	Connecticut Agricultural Experimental Station, New Haven, Connecticut	Reducing the potential for environmental contamination by pesticides and other organic chemicals	U.S. Department of Agriculture
Scow KM	University of California, Davis, California	Reducing the potential for environmental contamination by pesticides and other organic chemicals	U.S. Department of Agriculture
Maier RM; Brusseau ML	University of Arizona, Tucson, Arizona	Reducing the potential for environmental contamination by pesticides and other organic chemicals	U.S. Department of Agriculture
Xing B	University of Massachusetts, Amherst, Massachusetts	Sorption of organic contaminants in soils; mechanisms and implications for desorption and bioavailability	U.S. Department of Agriculture
Rolston DE	University of California, Davis, California	Transport and transformation of trace gases in soil	U.S. Department of Agriculture

[a]FEDRIP 2004

7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring naphthalene, 1-methylnaphthalene, 2-methylnaphthalene,, its metabolites, and other biomarkers of exposure and effect to naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

Naphthalene is moderately volatile with a boiling point of 218 °C and low water solubility of 31.7 mg/L (20 °C). Its log octanol/water partition coefficient is 3.29, implying a moderate affinity for lipid tissues. It undergoes short-term bioaccumulation in tissues, but biochemical processes lead to its biodegradation and eventual elimination. Methylnaphthalenes have similar properties (see Table 4-2). All of these properties have implications for determination of naphthalene and methylnaphthalenes in biological materials.

Historically, diethyl ether has been a widely used solvent for the extraction of lipophilic organic analytes such as naphthalene from biological fluids (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are determined using solvent extraction, selective extraction and loss of compounds that have a low boiling point can cause errors. The commercial availability of highly purified solvents has largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Extraction is the first step in the overall cleanup process that places the analyte in a form and matrix suitable for introduction into the instrument used to quantitate it. Cleanup of biological samples may often be complex and involve a number of steps (Walters 1986). Directly coupled supercritical fluid extraction (SFE)-gas chromatography has been used for the

determination of polychlorinated biphenyls (Hawthorne 1988) and might also be applicable to determination of naphthalene and methylnaphthalenes in biological samples.

Naphthalene metabolites are less lipophilic than naphthalene itself. Metabolites are isolated from body fluids and tissue homogenates by extraction and separated by thin layer chromatography (TLC) and HPLC (Horning et al. 1980; Melancon et al. 1982; Stillwell et al. 1982). Final identification of metabolites, which include numerous oxygenated and sulfur-containing species, is accomplished by gas chromatography (GC) and mass spectrometry (MS).

New immunological methods are being developed for detecting selected naphthalene metabolites in urine or naphthalene protein adducts in the blood of lung lavage specimens (Cho et al. 1994b; Marco et al. 1993). Additional work in perfecting these techniques is necessary before they will be useful in research and clinical practice.

Analytical methods for the determination of naphthalene and for 1-methylnaphthalene and 2-methyl-naphthalene in biological samples are given in Table 7-1. A method for the determination of radiolabelled 2-methylnaphthalene in rat urine has been described by Melancon et al. (1982). TLC and HPLC were used to characterize 2-methylnaphthalene and its metabolites, including 2-naphthoylglycine, 2-naphthoic acid, and others.

7.2 ENVIRONMENTAL SAMPLES

Gas chromatography and HPLC are the analytical methods most commonly used for detection of naphthalene and methylnaphthalenes in environmental samples. Several variations of these methods using different collection, extraction, and/or cleanup procedures and different detection methods have been approved by EPA and NIOSH for analysis of naphthalene in ambient water, drinking water, waste water, soil, and air (EPA 1982a, 1982b, 1986a, 1986b, 1986c, 1986d, 1990a, 1990b, 1990c, 1990d, 1990e; NIOSH 1984a, 1984b). The American Public Health Association (APHA) has recommended standard methods for analysis of naphthalene in water and waste water, each of which has been accepted by EPA as equivalent to one of the EPA-approved methods (APHA 1992a, 1992b, 1992c, 1992d, 1992e, 1992f). Analytical methods for naphthalene and 2-methylnaphthalene are presented in Tables 7-2 and 7-3, respectively. Although no standard methods were located that provided information on detection

Table 7-1. Analytical Methods for Determining Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene in Biological Samples[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Extract; bulk lipid removal; Florisil® fractionation	HRGC/MS	9 ng/g	No data	Stanley 1986
Adipose tissue (human and bovine)	Extract with hexane; Florisil® cleanup	Capillary column GC/MS	10 ng/g	90 (human) 63 (bovine)	Liao et al. 1988
Human milk	Purge with helium; desorb thermally	Capillary column GC/MS	No data	No data	Pellizzari et al. 1982
Human urine (1-naphthol analysis	No data	TLC or GS/ unspecified spectroscopy	No data	No data	Bieniek 1994
Fish tissue	Purge and trap to carbon adsorption tube; extract with carbon disulfide	HRGC/FID	<10 µg/L	43–51	Murray and Lockhart 1988
Fish tissue	Saponification with potassium hydroxide; extraction with cyclopentane-dichloro-methane; adsorption enrichment with potassium silicate/silica gel; gel permeation chromatography enrichment	Capilliary column GC/PID	20 ng/g	76–202 (naphthalene) 77–82 (1-methyl-naphthalene) 75–131 (2-methyl-naphthalene)	Lebo et al. 1991
Rat urine	Extract with ammonium carbonate/ethyl acetate; evaporate under nitrogen stream; dissolve in pyridine	GC/MS	No data	No data	Horning et al. 1980
Mouse urine	Extract with ethyl acetate; evaporate under nitrogen stream; dissolve in pyridine	GC/MS	No data	No data	Stillwell et al. 1982
Burned tobacco	Extract with methanol/water and cyclohexane; enrich in dimethyl sulfoxide; fractional distillation and evaporation under dry nitrogen	GLC/MS	No data	85–95	Schmeltz et al. 1976

[a]Data are for naphthalene only unless otherwise specified.

FID = flame ionization detector; GC = gas chromatography; GLC = gas-liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; PID = photoionization detector; TLC = thin layer chromatography

Table 7-2. Analytical Methods for Determining Naphthalene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect in charcoal tube; elute with carbon disulfide	GC/FID	15 mg/m^3	No data	NIOSH 1977
Air	Collect in charcoal tube; elute with carbon disulfide	GC/FID	10 µg/sample	No data	NIOSH 1984a
Air	Collect in charcoal tube; elute with organic solvent	GC/FID	0.5 µg/sample	No data	NIOSH 1984b
Air	Collection filter or tube; extract with acetonitrile	HPLC/FD	0.080 µg/filter or 0.070 µg/tube	No data	Hansen et al. 1991
Indoor air	Medium flow rate samples; extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	250 pg/µL	No data	EPA 1990a
Indoor air	Medium flow rate samples; extract with methylene chloride	GC/MS	No data	No data	EPA 1990a
Water	Purge and trap	HRGC/PID	0.06 µg/L	102±6.3	Ho 1989
Water	Extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	1.8 µg/L	78±8.3	EPA 1982a
Water	Extract with methylene chloride at pH 11 and 2; concentrate	GC/MS	1.6 µg/L	75±35	EPA 1982b
Water	Adsorb on small bed volume Tenax® cartridges; thermally desorb	GC/MS	No data	No data	Pankow et al. 1988
Drinking water	Liquid-liquid extraction with methylene chloride; exchange to acetonitrile	HPLC/UV	3.3 µg/L	76–96	EPA 1990d
Drinking water	Liquid-solid extraction with methylene chloride; exchange to acetonitrile	HPLC/UV	2.2 µg/L	49.6–75.2	EPA 1990e
Drinking water	Purge and trap	Packed column GC/PID	0.01–0.05 µg/L	92	APHA1992e
Drinking water	Purge and trap	Capillary column GC/MS	0.02–0.2 µg/L	98–104	APHA 1992d
Drinking water	Purge and trap	Capillary column GC/PID	No data	102	APHA 1992f

Table 7-2. Analytical Methods for Determining Naphthalene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wastewater	Extract with methylene chloride	Isotope dilution, capillary column GC/MS	10 µg/L	75–149	EPA 1990c
Wastewater	Extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	1.8 µg/L	21.5–100	APHA 1992b
Water	Extract with methylene chloride	Capillary column GC/MS	10 µg/L[a]	No data	EPA 1986c
Wastes, non-water miscible	Extract with methylene chloride	Packed column GC/MS	160 mg/kg	No data	EPA 1986b
Soil	Extract with methylene chloride	Packed column GC/MS	1 mg/kg	No data	EPA 1986b
Soil, sediment	Extract with methylene chloride	Capillary column GC/MS	660 µg/kg	No data	EPA 1986c
Wastes, soil	Extract with methylene chloride	GC/FTIR	20 µg/L[a, b]	No data	EPA 1986d

[a]Identification limit in water. Detection limits for actual samples are several orders of magnitude higher, depending upon the sample matrix and extraction procedure employed.
[b]Based on a 2 µL injection of a 1 L sample that was extracted and concentrated to a volume of 1 mL.

FD = fluorescence detection; FID = flame ionization detector; FTIR = Fourier transform infrared spectrometry; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectroscopy; PID = photoionization detection; UV = ultraviolet spectrometry

Table 7-3. Analytical Methods for Determining 2-Methylnaphthalene in Environmental Samples[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery
Soil, sediment	Extract with methylene chloride	Capillary column GC/MS	660 µg/kg	No data
Water	Extract with methylene chloride	Capillary column GC/MS	10 µg/kg	No data

[a]EPA 1986c

GC = gas chromatography; MS = mass spectroscopy

limits or accuracy for 1-methylnaphthalene, this compound may be analyzed in environmental media by GC and HPLC methods (HSDB 1995).

Air samples for analysis may be collected on filters or charcoal tubes. Since naphthalene may exist in both the vapor phase and the particle phase in air (Harkov 1986), collection on a charcoal tube is the preferred method for sampling naphthalene from air for analysis (NIOSH 1977, 1984a, 1984b).

Naphthalene is usually extracted from the matrix with organic solvents (liquid-liquid or liquid-solid extraction) or by purge and trap with an inert gas. SFE techniques for extraction of organic compounds from environmental matrices are currently being studied by EPA. A protocol for SFE with carbon dioxide for many organic compounds, including naphthalene, from soils and sediments has been developed (EPA 1991f).

A technique for the detection of naphthalene in PAH-contaminated media has been developed (Heitzer et al. 1994). The technique measures bioluminescence in the genetically engineered microorganism *Pseudomonas fluorescens* HK44, which carries a transcriptional gene for naphthalene and salicylate metabolism. After the addition of the bacteria to sterile water, naphthalene was detected down to 1.55 µg/L, the lowest concentration studied. In an experiment using JP-4 jet fuel, naphthalene was detected down to 0.55 µg/L in the effluent of the biosensor (Heitzer et al. 1994).

Detectors used for identification and quantification of naphthalene and methylnaphthalenes include the flame ionization detector (FID), photoionization detector (PID), ultraviolet detection (UV), Fourier transform infrared detection (FTIR), and fluorescence detection (FD). Mass spectrometry is used for confirmation.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the health effects (and

techniques for developing methods to determine such health effects) of naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Sensitive and selective methods are available for the qualitative and/or quantitative measurement of naphthalene and many of its metabolites present in biological materials such as adipose tissue and urine (EPA 1986g; Horning et al. 1980; Liao et al. 1988). In contrast to the relative ease of measuring naphthalene once it has been isolated from its sample matrix, the development of improved techniques for sample preparation would be beneficial.

Metabolites of naphthalene in biological materials are not readily determined in routine practice because of the lack of standard methods for their quantification. Furthermore, there is a need for modern validated standard methods for analysis of naphthalene itself in biological materials. It would also be helpful to have a method that can be used to associate levels of naphthalene or its metabolites in biological media with levels of naphthalene exposure in the environment.

A method for the determination of 2-methylnaphthalene and its degradation products in rat urine has been reported (Melancon et al. 1982). It would be useful to determine if this method could also be applied to human urine and other biological samples.

Effect. There are currently no methods that can be used to correlate levels of naphthalene, 2-methyl-naphthalene, or their metabolites in biological tissues or fluid with the probable onset of adverse health effects. The development of such methods would be useful insofar as they estimate the doses required to produce cataracts and hemolytic effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining naphthalene in water, air, and waste samples with excellent selectivity and sensitivity have been developed and are undergoing constant improvement (EPA 1982a, 1982b, 1986a, 1986b, 1986c, 1986d, 1990a, 1990b, 1990c, 1990d, 1990e; NIOSH 1984a, 1984b). For each medium, the existing methods are adequate to measure background levels in the environment and levels at which health effects occur. Standard methods for 1-methylnaphthalene and 2-methylnaphthalene would be helpful in assessing data comparability.

It would be useful to have the means to rapidly and directly measure organic compounds such as naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in water and other environmental media without the necessity for tedious sample processing. The recently developed bioluminescent probe for naphthalene (Heitzer et al. 1994) may help satisfy this data need.

Degradation products of naphthalene in environmental media are difficult to determine. This difficulty is not so much an analytical problem as it is a problem of knowing the fundamental environmental chemistry of these compounds in water, soil, air, and biological systems.

There are some difficulties associated with sampling naphthalene from the atmosphere, where it is partially associated with particulate matter. High-volume sampling with glass fiber filters provides conditions conducive to artifact formation (Harkov 1986), thus introducing errors into the analysis of atmospheric naphthalene. This is an area in which further improvements would be useful.

7.3.2 Ongoing Studies

No ongoing studies involving analytical techniques of naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene were found in a search of the Federal Research in Progress database (FEDRIP 2003).

8. REGULATIONS AND ADVISORIES

The international, national, and state regulations and guidelines regarding naphthalene, 1-methylnaphthalene, and 2-methynaphthalene in air, water, and other media are summarized in Table 8-1.

As discussed in Chapter 2 and Appendix A, several MRLs for naphthalene (chronic-duration inhalation, acute-duration oral, and intermediate-duration oral) and chronic-duration oral MRLs for 1-methylnaphthalene and 2-methylnaphthalene have been derived.

An MRL of 0.0007 ppm ($3x10^{-3}$ mg/m^3) for chronic inhalation exposure to naphthalene is based on a LOAEL for nasal lesions in rats (Abdo et al. 2001; NTP 2000; $LOAEL_{[human\ equivalent\ concentration]}$= 0.2 ppm), and a total uncertainty factor of 300 (10 for the use of a LOAEL, 3 for extrapolation from animals to humans using dosimetric adjustment, and 10 for human variability). An MRL of 0.6 mg/kg/day for acute oral exposure to naphthalene is based on a minimal LOAEL of 50 mg/kg/day for clinical signs of toxicity in pregnant rats and a total uncertainty factor of 90 (3 for the use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 3 for human variability). The acute-duration oral MRL of 0.6 mg/kg/day is adopted as the intermediate-duration oral MRL for naphthalene.

For chronic-duration oral exposure to 1-methylnaphthalene, an MRL of 0.07 mg/kg/day was derived based on a LOAEL of 71.6 mg/kg/day for pulmonary alveolar proteinosis in female mice exposed to 1-methylnaphthalene in the diet for 81 weeks and an uncertainty factor of 1,000 (10 for using a LOAEL, 10 for extrapolating from animals to humans, and 10 for human variability).

For chronic-duration oral exposure to 2-methylnaphthalene, an MRL of 0.04 mg/kg/day was derived based on the lower 95% confidence limit on a benchmark dose associated with 5% extra risk ($BMDL_{05}$=4 mg/kg/day) for pulmonary alveolar proteinosis in male mice exposed to 2-methylnaphthalene in the diet for 81 weeks and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

The EPA calculated an oral exposure RfD of $2x10^{-2}$ mg/kg/day for naphthalene based on a NOAEL of 100 mg/kg/day for the absence of decreased mean terminal body weight in male rats exposed by gavage for 13 weeks (IRIS 2005; NTP 1980b). An inhalation RfC of $3x10^{-3}$ mg/m^3 for naphthalene was derived

based on a LOAEL of 10 ppm ($LOAEL_{[human\ equivalent\ concentration]}$=9.3 mg/m^3) for nasal lesions in mice exposed by inhalation for 2 years (IRIS 2005; NTP 1992a).

The EPA (2003r) calculated an oral exposure RfD of 0.004 mg/kg-day for 2-methylnaphthalene based on a value of 3.5 mg/kg-day for a 95% lower confidence limit on a dose associated with 5% extra risk ($BMDL_{05}$) for pulmonary alveolar proteinosis in mice exposed to 2-methylnaphthalene in the diet for 81 weeks.

The EPA is currently conducting a comprehensive review of the available environmental and toxicity data of naphthalene as part of its FIFRA re-registration process. The results of this review are expected in March 2008.

Table 8-1. Regulations and Guidelines Applicable to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Agency	Description	Information	Reference
INTERNATIONAL			
Guidelines:			
IARC	Carcinogenicity classification	Group 2B[a]	IARC 2002
WHO	Drinking water guideline	No data	
NATIONAL			
Regulations and Guidelines:			
a. Air:			
ACGIH	TLV (8-hour TWA)		ACGIH 2003
	Naphthalene[b]	10 ppm	
	STEL	15 ppm	
EPA	Hazardous air pollutant	Naphthalene	EPA 2003g 40 CFR 63, Table 1
	National emission standards for hazardous air pollutants		EPA 2003h 40 CFR 61.134
	Naphthalene processing, final coolers, and final-cooler cooling towers at coke by-product recovery plants	No (zero) emissions are allowed	
NIOSH	REL (10-hour TWA)		NIOSH 2003
	Naphthalene	10 ppm	
	STEL	15 ppm	
	IDLH	250 ppm	
OSHA	PEL (8-hour TWA) for general industry		OSHA 2003a 29 CFR 1910.1000,
	Naphthalene	10 ppm	Table Z-1
	PEL (8-hour TWA) for construction industry		OSHA 2003c 29 CFR 1926.55,
	Naphthalene	10 ppm	Appendix A
	PEL (8-hour TWA) for shipyard industry		OSHA 2003b 29 CFR 1915.1000
	Naphthalene	10 ppm	
USC	Hazardous air pollutant	Naphthalene	USC 2003 42 USC 7412
b. Water			
EPA	Drinking water health advisories		EPA 2002a
	1-day (10-kg child)	0.5 mg/L	
	10-day (10-kg child)	0.5 mg/L	
	DWEL[c]	0.7 mg/L	
	Life-time[d]	0.1 mg/L	
	Effluent guidelines and standards; toxic pollutants pursuant to Section 307(a)(1) of the Clean Water Act	Naphthalene	EPA 2003c 40 CFR 401.15
	Hazardous substance designated in accordance with Section 311 (b)(2)(A) of the Clean Water Act	Naphthalene	EPA 2003p 40 CFR 116.4

Table 8-1. Regulations and Guidelines Applicable to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
	Pollutants of initial focus in the Great Lakes Water Quality Initiative	Naphthalene	EPA 2003q 40 CFR 132, Table 6
	Reportable quantities of hazardous substances (naphthalene) designated pursuant to Section 311 of the Clean Water Act	100 pounds	EPA 2003j 40 CFR 117.3
c. Food	No data		
d. Other			
ACGIH	Carcinogenicity classification	A4[e]	
EPA	Carcinogenicity classification	Group C[f]	IRIS 2005
	RfD (oral)	$2.0x10^{-2}$ mg/kg/day	IRIS 2005
	RfC (inhalation)	$3.0x10^{-3}$ mg/m^3	IRIS 2005
	Community right-to-know; release reporting; effective date of reporting	01/01/87	EPA 2003m 40 CFR 372.65
	Criteria for municipal solid waste landfills; hazardous constituent	Naphthalene and 2-Methylnaphthalene	EPA 2003a 40 CFR 258, Appendix II
	Identification and listing of hazardous waste; hazardous waste number		EPA 2003d 40 CFR 261, Appendix VIII
	Naphthalene	U165	
	Land disposal restrictions; universal treatment standards for naphthalene		EPA 2003e 40 CFR 268.48
	Waste water standard	0.059 mg/L	
	Non-waste water standard	5.6 mg/L TCLP	
EPA	Landfills point source effluent limitations attainable by the application of the best practicable control technology currently available		EPA 2003f 40 CFR 445.11
	Maximum daily	0.059 mg/L	
	Maximum monthly average	0.022 mg/L	
	Reportable quantity of hazardous substance in accordance with Section 311 (b)(2) and 307(a) of the Clean Water Act, Section 112 of RCRA, and Section 112 of the Clean Air Act for naphthalene	100 pounds	EPA 2003b 40 CFR 302.4
	Standards for owners and operators of hazardous waste TSD facilities; groundwater monitoring	Suggested Method / PQL	EPA 2003k 40 CFR 264, Appendix IX
	Naphthalene	8100 / 200 µg/L 8270 / 10 µg/L	
	2-Methylnaphthalene	8270 / 10 µg/L	

Table 8-1. Regulations and Guidelines Applicable to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Agency	Description	Information	Reference
NATIONAL *(cont.)*			
	Standards for owners and operators of hazardous waste TSD facilities; health-based limits for exclusion of waste-derived residues; residue concentration limit	10 mg/kg	EPA 2003l 40 CFR 266, Appendix VII
	TSCA chemical information rules; health and safety data reporting for naphthalene		EPA 2003n 40 CFR 712.30
	Effective date	08/04/95	
	Reporting date[g]	10/03/95	
	TSCA health and safety data reporting for naphthalene[h]		EPA 2003o 40 CFR 716.120
	Effective date	08/04/95	
	Sunset date	10/03/95	
NTP	Carcinogenicity classification	Naphthalene is reasonably anticipated to be a human carcinogen (Group 2)	NTP 2005
STATE			
a. Air	No data		
b. Water			
Maine	Drinking water guideline	25 µg/L	HSDB 2004
Minnesota	Drinking water guideline	300 µg/L	HSDB 2004
New Jersey	Drinking water standard	300 µg/L	HSDB 2004
Washington	Drinking water guideline	14 µg/L	HSDB 2004
Wisconsin	Drinking water guideline	40 µg/L	HSDB 2004
Florida	Drinking water guideline	6.8 µg/L	HSDB 2004

Table 8-1. Regulations and Guidelines Applicable to Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene

Agency	Description	Information	Reference
STATE *(cont.)*			
c. Food	No data		
d. Other	No data		

[a]Group 2B: possibly carcinogenic to humans
[b]Skin notation: refers to the potential significant contribution to the overall exposure by the cutaneous route, including mucous membranes and the eyes, either by contact with vapors or, of probable greater significance, by direct skin contact with the substance.
[c]DWEL: a lifetime exposure concentration protection of adverse, non-cancer health effects, that assumes all of the exposure to a contaminant is from drinking water.
[d]Life-time: the concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for a lifetime of exposure. The lifetime HA is based on exposure of a 70-kg adult consuming 2 L water/day.
[e]A4: not classifiable as a human carcinogen
[f]Group C: a possible human carcinogen
[g]Reporting date: manufacturers and importers of naphthalene must submit a Preliminary Assessment Information Manufacturer's Report for each site at which they manufacture or import naphthalene by the reporting date.
[h]TSCATS health and safety data reporting: naphthalene is subject to all provisions of part 716. Manufacturers, importers, and processors of naphthalene are subject to the reporting requirements of subpart A.

ACGIH = American Conference of Governmental Industrial Hygienists; CFR = Code of Federal Regulations; DWEL = drinking water equivalent level; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; IARC = International Agency for Research on Cancer; IDLH = immediately dangerous to life or health; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PEL = permissible exposure limit; PQL = practical quantitation level; RCRA = Resource Conservation and Recovery Act; REL = recommended exposure limit; RfC = reference concentration; RfD = reference dose; STEL = short-term exposure limit; TCLP = toxicity characteristic leachate procedure; TLV = threshold limit values; TSCA = Toxic Substances Control Act; TSD = treatment, storage, and disposal; TWA = time-weighted average; USC = United States Code; WHO = World Health Organization

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10. GLOSSARY

Absorption—The taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD)—Usually defined as the lower confidence limit on the dose that produces a specified magnitude of changes in a specified adverse response. For example, a BMD_{10} would be the dose at the 95% lower confidence limit on a 10% response, and the benchmark response (BMR) would be 10%. The BMD is determined by modeling the dose response curve in the region of the dose response relationship where biologically observable data are feasible.

Benchmark Dose Model—A statistical dose-response model applied to either experimental toxicological or epidemiological data to calculate a BMD.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-controlled study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without outcome.

Case Report—Describes a single individual with a particular disease or exposure. These may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Describes the experience of a small number of individuals with the same disease or exposure. These may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome. At least one exposed group is compared to one unexposed group.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at one point in time.

Data Needs—Substance-specific informational needs that if met would reduce the uncertainties of human health assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the adverse effects.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurs. The terms, as used here, include malformations and variations, altered growth, and *in utero* death.

Environmental Protection Agency (EPA) Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Epidemiology—Refers to the investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one half of a quantity of a chemical from the body or environmental media.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 minutes without any escape-impairing symptoms or irreversible health effects.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

Immunological Effects—Functional changes in the immune response.

Incidence—The ratio of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—State of being diseased; morbidity rate is the incidence or prevalence of disease in a specific population.

Mortality—Death; mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations. A mutation is a change in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An OR of greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Organophosphate or Organophosphorus Compound—A phosphorus-containing organic compound and especially a pesticide that acts by inhibiting cholinesterase.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) allowable exposure level in workplace air averaged over an 8-hour shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests.

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic end points. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—Comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information: tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as air/blood partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which the pertinent observations are made on events occurring after the start of the study. A group is followed over time.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation reference concentration is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the no-observed-adverse-effect level (NOAEL, from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a chemical.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—The American Conference of Governmental Industrial Hygienists (ACGIH) maximum concentration to which workers can be exposed for up to 15 minutes continually. No more than four excursions are allowed per day, and there must be at least 60 minutes between exposure periods. The daily Threshold Limit Value-Time Weighted Average (TLV-TWA) may not be exceeded.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a Time Weighted Average (TWA), as a Short-Term Exposure Limit (STEL), or as a ceiling limit (CL).

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose$_{(50)}$ (TD$_{50}$)—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Toxicokinetic—The absorption, distribution, and elimination of toxic compounds in the living organism.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL) or Reference Dose (RfD) or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis, 3 being the approximate logarithmic average of 10 and 1.

Xenobiotic—Any chemical that is foreign to the biological system.

APPENDIX A. ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop F-32, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Naphthalene
CAS Number: 91-20-3
Date: June 2005
Profile Status: Final Post-Public Comment
Route: [x] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 6
Species: Rat

Minimal Risk Level: 0.0007 [ ] mg/kg/day [x] ppm

Reference(s): Abdo KM, Grumbein S, Chou BJ, et al. 2001. Toxicity and carcinogenicity study in F344 rats following 2 years of whole-body exposure to naphthalene vapors. Inhal Toxicol 13:931-950.

NTP. 1992a. Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in B6C3F1 mice (inhalation studies). Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. National Toxicology Program. NIH Publication No. 92-3141. Technical report series no. 410.

NTP. 2000. Toxicology and carcinogenesis studies of naphthalene (CAS No. 91-20-3) in F344/N rats (inhalation studies). National Toxicology Program. NTP TR 500, NIH Publ. No. 01-4434.

Experimental design: NTP 1992a: Groups of 75 B6C3F1mice of each sex were exposed by inhalation at concentrations of 0, 10, or 30 ppm. Exposure occurred 5 times/week, 6 hours/day for 104 weeks.

Abdo et al. 2001; NTP 2000: Groups of 49 male and 49 female F344/N rats were exposed to naphthalene at concentrations of 0, 10, 30, or 60 ppm for 6 hours/day, 5 days/week for 105 weeks.

Effects noted in study and corresponding doses: In mice, exposure to 10 or 30 ppm of naphthalene resulted in inflammation of the nose (males: 0/70, 67/69, 133/135; females: 1/69, 65/65, 135/135) and lungs (males: 0/70, 21/69, 56/135; females: 3/69, 13/65, 52/135), metaplasia of the olfactory epithelium (males: 0/70, 66/69, 134/135; females: 0/69, 65/65, 135/135), and hyperplasia of the nasal respiratory epithelium (males: 0/70, 66/69, 134/135; females: 0/69, 65/65, 135/135). Increased incidences of neoplastic lesions were restricted to the lung in females: alveolar/bronchiolar adenomas (5/69, 2/65, 28/135) and alveolar/bronchiolar carcinomas (0/69, 0/65, 1/135).

In rats, increased incidences of nonneoplastic and neoplastic lesions were restricted to the nose as shown in Table A-1.

Dose and end point used for MRL derivation: The lowest exposure level in both studies, 10 ppm, was a LOAEL in both sexes of both species for nonneoplastic lesions in nasal olfactory epithelium and respiratory epithelium. Applying EPA inhalation dosimetry (see below), a human equivalent LOAEL of 0.2 ppm, based on the rat LOAEL, was selected as the point of departure for the chronic inhalation MRL. Benchmark dose analyses were not conducted on the incidence data for nonneoplastic nasal lesions, because the data provided insufficient information on the shape of the dose-response relationship. The lowest exposure level in the principal study induced nasal lesions in essentially all of the rats.

[ ] NOAEL [x] LOAEL

Modifying Factors used in MRL derivation: N/A

Table A-1. Nonneoplastic and Neoplastic Lesions of the Nose in Male and Female F344/N Rats Exposed to Naphthalene 6 Hours/Day, 5 Days/Week for 105 Weeks

	Concentration (ppm)							
	0		10		30		60	
Lesion	M	F	M	F	M	F	M	F
Nonneoplastic lesions								
Olfactory epithelium								
Hyperplasia	0/49	0/49	48/49	48/49	45/48	48/49	46/48	43/49
Atrophy	3/49	0/49	49/49	49/49	48/48	49/49	47/48	47/49
Chronic inflammation	0/49	0/49	49/49	47/49	48/48	47/49	48/48	45/49
Hyaline degeneration	3/49	13/49	46/49	46/49	40/48	49/49	38/48	45/49
Respiratory epithelium								
Hyperplasia	3/49	0/49	21/49	18/49	29/48	22/49	29/48	23/49
Squamous metaplasia	0/49	0/49	15/49	21/49	23/48	17/49	18/48)	15/49
Hyaline degeneration	0/49	8/49	20/49	33/49	19/48	34/49	19/48	28/49
Goblet cell hyperplasia	0/49	0/49	25/49	16/49	29/48	29/49	26/48	20/49
Gland hyperplasia	1/49	0/49	49/49	48/49	48/48	48/49	48/48	42/49
Gland squamous metaplasia	0/49	0/49	3/49	2/49	14/48	20/49	26/48	20/49
Neoplastic lesions								
Respiratory epithelial adenoma	0/49	0/49	6/49	0/49	8/48	4/49	15/48	2/49
Olfactory epithelial neuroblastoma	0/49	0/49	0/49	2/49	4/48	3/49	3/48	12/49

F = female; M = male

Uncertainty Factors used in MRL derivation: Total Uncertainty Factor = 10x3x10=300

[x] 10 for use of a LOAEL
[x] 3 for extrapolation from animals to humans with dosimetric adjustment
[x] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose:
10 ppm x 6 hours/24 hours x 5 days/7 days=1.8 ppm (duration-adjusted LOAEL for nasal effects in rats or mice)
1.8 ppm x 128.18/24.45=9.4 mg/m^3

Following EPA (1994d) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*, equations for a category 1 gas producing nasal effects were used to derive human equivalent concentrations: HEC=Animal Concentration x $RGDR_{ET}$;

$RGDR_{ET}$= regional gas dose ratio in the extrathoracic (ET) region
= $(Dose_{ET})A/(Dose_{ET})H$= [minute volume/ETsurface area]$_A$÷[minute volume/ETsurface area]$_H$;

Reference minute volumes (L/min): 13.8 human, 0.137 rat, 0.0368 mouse;
Reference ET surface area (cm^2): 200 human, 15 rat, 3 mouse;

$RGDR_{ET}$(Rat to Human)=[0.137/15]÷[13.8/200]=0.132;
$LOAEL_{HEC}$=duration-adjusted LOAEL x 0.132=1.8 ppm x 0.132=0.2 ppm
$RGDR_{ET}$(Mouse to Human)=[0.0368/3]÷[13.8/200]=0.178;
$LOAEL_{HEC}$=duration-adjusted LOAEL x 0.132=1.8 ppm x 0.178=0.3 ppm

Using public health protection reasoning, the $LOAEL_{HEC}$ based on the rat data was selected as the point of departure for the chronic inhalation MRL.

Other additional studies or pertinent information which lend support to this MRL: Uncertainty in the MRL would likely be decreased with the development and application of hybrid computational fluid dynamics and physiologically based pharmacokinetic models that would estimate regional tissue doses of naphthalene metabolites in rats and humans. The models can incorporate species-specific information on nasal geometry, breathing patterns, and metabolism, as well as chemical-specific information on reactivity, partition coefficients, and diffusivity of the vapor in air and tissue. Such models have been developed for other gases that induce nasal lesions (see Frederick et al. 2001), but have not yet been developed for naphthalene.

Reactive naphthalene metabolites (1,2-naphthalene oxide, 1,2-naphthoquinone, 1,4-naphthoquinone, and 1,2-dihydroxy-3,4-epoxy-1,2,3,4-tetrahydronaphthalene) have been proposed to be involved in naphthalene's toxic modes of action (Buckpitt et al. 2002). CYP isozymes, which might be involved in naphthalene metabolism and bioactivation, have been demonstrated to exist in nasal respiratory epithelial and olfactory epithelial tissue from rodents and humans (Thornton-Manning and Dahl 1997). Studies designed to specifically characterize metabolism of naphthalene in nasal tissue, however, have not been conducted (e.g., which CYP isozymes catalyze naphthalene transformations in nasal tissue?, are there species differences in nasal tissue efficiencies and capabilities for metabolism and/or bioactivation of naphthalene?), with the exception of a single study that examined *in vitro* rates of metabolism of naphthalene to naphthalene oxides in postmitochondrial supernatants from mouse, rat, and hamster olfactory tissue (Buckpitt et al. 1992). In this study, metabolic rates (units of nmol/min/mg protein) showed the following order: mouse (87.1) > rat (43.5) > hamster (3.9). This order did not correspond with species differences in susceptibility to single intraperitoneal injections of naphthalene in a companion study (Plopper et al. 1992a). Rat nasal epithelial tissue (olfactory and respiratory epithelium) was more sensitive than tissue from mice and hamsters, which showed equivalent sensitivities.

Agency Contacts (Chemical Managers): Hisham El-Masri, Ph.D.; Moiz Mumtaz, Ph.D.; and G. Daniel Todd, Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Naphthalene
CAS Number: 91-20-3
Date: June 2005
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [x] Oral
Duration: [x] Acute [ ] Intermediate [ ] Chronic
Graph Key: 16
Species: Rat

Minimal Risk Level: 0.6 [x] mg/kg/day [ ] ppm

Reference: NTP. 1991a. Developmental toxicity of naphthalene (CAS No. 91-20-3) administered by gavage to Sprague-Dawley (CD) rats on gestational days 6 through 15. Research Triangle Park, NC: National Toxicology Program, National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. TER91006.

Experimental design: Groups of 25–26 pregnant female Sprague-Dawley rats received doses of 0, 50, 150, and 450 mg/kg/day by gavage on gestation days 6–15. There were two replicate groups of 12–13 animals.

Effects noted in study and corresponding doses: Rat dams in exposed groups showed one or more of several clinical signs of toxicity (slow respiration, lethargy, or prone body posture) on the first day of dosing (81, 96, and 96% of rats in the 50-, 150-, and 450-mg/kg/day groups). By the third day of dosing, these signs did not occur in any of the 50-mg/kg/day rats. A similar trend was noted in the 150-mg/kg/day group, but apparent tolerance did not develop until the sixth day of dosing. In the 450-mg/kg/day group, the incidence of rats exhibiting these signs of toxicity also declined during the exposure period, but did not fall below 15%. With the development of "tolerance", the slow respiration, lethargy, and prone body posture were replaced with rooting behavior, a common behavior of rodents following gavage administration of chemicals with strong odors or irritant properties. At the end of the exposure period (gestation day 15), incidence of rats showing rooting behavior was 0% for the control and 50-mg/kg/day groups, compared with 24 and 92% of dams in the 150- and 450-mg/kg/day groups, respectively. Weight gain during exposure (gestation days 6–15) was similar between the control and 50-mg/kg/day group, but was decreased by 31 and 53% in the 150- and 450-mg/kg/day groups, compared with controls. From these results, 50 mg/kg/day was judged to be a minimal less serious LOAEL for transient clinical signs of maternal toxicity in pregnant rat dams. At higher doses (150 and 450 mg/kg/day), these effects were more persistent and were accompanied by decreased weight gain.

No statistically significant exposure-related effects were observed on the average number of corpora lutea per dam, implantation sites per litter, live fetuses per litter, or average fetal body weight. The percent of fetuses malformed per litter (4, 4, 7, and 10% for control through 450 mg/kg/day) and the percent of litters with malformed fetuses (23, 27, 33, and 50%) both showed a statistically significant trend test, but pairwise comparisons between individual exposure groups and the control were not statistically significant. The investigators concluded that naphthalene was not fetotoxic or teratogenic in this assay.

Dose and end point used for MRL derivation: A minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity in pregnant rat dams.

[] NOAEL [x] minimal LOAEL

Modifying Factors used in MRL derivation: N/A

Uncertainty Factors used in MRL derivation: Total Uncertainty Factor= 3x10x3=90

[x] 3 for use of a minimal LOAEL
[x] 10 for extrapolation from animals to humans
[x] 3 for human variability

An uncertainty factor of 3 was selected for the use of a minimal LOAEL of 50 mg/kg/day. At this dose level, the only adverse effects observed in the pregnant rat dams were signs of maternal toxicity, which were only observed on the first 2 days of exposure.

An uncertainty factor of 10 was used for extrapolating from animals to humans.

An uncertainty factor of 3 was used for human variability because the critical effect is based on effects in a sensitive animal subpopulation. Pregnant rats appear to be more sensitive for the effects observed (clinical signs and decreased body weight gain) than nonpregnant rats. In 13-week gavage studies with nonpregnant rats (NTP 1980b), similar persistent clinical signs were not observed following administration of doses as high as 200 mg/kg/day, but were observed at 400 mg/kg/day. In nonpregnant rats exposed for 13 weeks, significant body weight decreases occurred at 200 mg/kg/day throughout exposure, but not at 100 mg/kg/day (NTP 1980b) or in nonpregnant mice exposed for 13 weeks to 133 mg/kg/day (Shopp et al. 1984) or 200 mg/kg/day (NTP 1980a). Mice in the NTP (1980a) study showed transient signs of toxicity (lethargy, rough hair coats, and decreased food consumption), but these only occurred between weeks 3 and 5 in the 200-mg/kg/day group.

Was a conversion used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

Other additional studies or pertinent information which lend support to this MRL: Neurologic symptoms have been reported in humans following ingestion of naphthalene at unknown, but presumably high dose levels. These include confusion (Ojwang et al. 1985) and listlessness and lethargy (Bregman 1954; Chusid and Fried 1955; Kurz 1987; Macgregor 1954; Zuelzer and Apt 1949), as well as decreased responses to painful stimuli and coma prior to death (Gupta et al. 1979; Kurz 1987). Persistent neurologic symptoms were not recorded in 13-week studies with rats or mice exposed to doses as high as 200 mg/kg/day (NTP 1980a, 1980b), but the highest exposure level tested in these studies, 400 mg/kg/day, produced lethargy in exposed rats (only rats were exposed to 400 mg/kg/day).

Hemolytic anemia has been identified in many human cases of acute accidental or intentional ingestion of naphthalene (e.g., Gidron and Leurer 1956; MacGregor 1954). Estimations of dose levels involved in these cases, however, are limited to a report (Gidron and Leurer 1956) of hemolytic anemia in a 16-year-old girl who swallowed 6 g of naphthalene (estimated dose=109 mg/kg, assuming body weight of 55 kg). Laboratory animals do not appear to be susceptible to the hemolytic activity of naphthalene. No pronounced changes in red-cell-related hematologic parameters were observed following 13-week oral exposures to doses up to 200 mg/kg/day in mice (NTP 1980a) and 400 mg/kg/day in rats (NTP 1980b), or in mice exposed by inhalation for 14 days to air concentrations as high as 30 ppm (NTP 1992a). Naphthalene-induced hemolytic anemia has been observed in dogs exposed to a single dose of 1,525 mg/kg or 263 mg/kg/day for 7 days (Zuelzer and Apt 1949), but more information on the dose-response relationship for hemolytic anemia in humans or animals acutely exposed to naphthalene is not available.

Another effect associated with acute or repeated oral exposure to naphthalene in animals is cataracts (Kojima 1992; Murano et al. 1993; Van Heyningen and Pirie 1976; Xu et al. 1992b). These effects, however, appear to occur at dose levels (in the range of 500–1,000 mg/kg/day) much higher than the lowest dose level (150 mg/kg/day) producing body weight gain decreases and clinical signs of toxicity in pregnant rats.

Agency Contacts (Chemical Managers): Hisham El-Masri, Ph.D.; Moiz Mumtaz, Ph.D.; and G. Daniel Todd, Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Naphthalene
CAS Number: 91-20-3
Date: June 2005
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [x] Intermediate [ ] Chronic
Graph Key: 16
Species: Rat

Minimal Risk Level: 0.6 [x] mg/kg/day [ ] ppm

Reference: NTP. 1991a. Developmental toxicity of naphthalene (CAS No. 91-20-3) administered by gavage to Sprague-Dawley (CD) rats on gestational days 6 through 15. Research Triangle Park, NC: National Toxicology Program, National Institute of Environmental Health Sciences, U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. TER91006.

Experimental design: See the worksheet for the acute-duration oral MRL.

Effects noted in study and corresponding doses: See the worksheet for the acute-duration oral MRL.

Dose and end point used for MRL derivation: A minimal LOAEL of 50 mg/kg/day for transient clinical signs of toxicity in pregnant rat dams.

[ ] NOAEL [x] minimal LOAEL

Modifying Factors used in MRL derivation: N/A

Uncertainty Factors used in MRL derivation: Total Uncertainty Factor =3x10x3=90

[x] 3 for use of a minimal LOAEL
[x] 10 for extrapolation from animals to humans
[x] 3 for human variability

See the worksheet for the acute-duration oral MRL for explanations of the uncertainty factors.

Was a conversion used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

Other additional studies or pertinent information which lend support to this MRL:

There are three intermediate-duration oral toxicity studies in laboratory animals that were considered for deriving the intermediate oral MRL for naphthalene. A 13-week comprehensive oral toxicity study in Fischer 344 rats found no adverse exposure related effects other than decreased body weight (NTP 1980b). This study identified 100 mg/kg/day as a NOAEL and 200 mg/kg/day as a LOAEL for decreased body weight in male and female rats. Another 13-week comprehensive oral toxicity study in B6C3F1 mice found no adverse effects in mice exposed to doses as high as 200 mg/kg/day (NTP 1980a). Another 90-day gavage study in mice focused on immune system variables and other toxicity variables (e.g., body

weight, organ weight, haematological parameters) and identified 133 mg/kg/day as a LOAEL and 53 mg/kg/day as a NOAEL for weight decreases in several organs (brain, liver, and spleen), but found no biologically significant exposure-related changes in other end points evaluated (Shopp et al. 1984). This study, however, did not include histopathological examination of tissues.

More detailed descriptions of the intermediate-duration oral toxicity studies follow. After the description of the studies, an analysis of their usefulness for MRL derivation is presented.

NTP. 1980b. Subchronic toxicity study: Naphthalene (C52904), Fischer 344 rats. Research Triangle Park, NC: U.S. Department of Health and Human Services, National Toxicology Program.

Naphthalene (>99% pure) in corn oil was administered by gavage to groups of 10 male and 10 female Fischer 344 rats at dose levels of 0, 25, 50, 100, 200, or 400 mg/kg/day, 5 days/week for 13 weeks (NTP 1980b). End points included weekly measurement of food consumption and body weight, twice daily observation for clinical signs of toxicity, measurement of hematological parameters for blood collected at termination (hemoglobin, hematocrit, total and differential white blood cell count, red blood cell count, mean cell volume, mean cell hemoglobin concentration), necropsy of all rats in the study, and complete histopathological examination of 27 organs and tissues (including the eyes, lungs, stomach, liver, reproductive organs, thymus, and kidneys) from all control and 400-mg/kg rats. Male kidneys and female thymuses from the 200-mg/kg group were also examined histopathologically (according to the histopathology tables; however, the report text states that the 100 mg/kg group was examined). Organ weight data were not reported.

At the highest dose level, two male rats died during the last week of treatment, and rats of both sexes displayed diarrhea, lethargy, hunched posture, and rough coats at intermittent intervals throughout the study. Food consumption was not affected by exposure. Mean terminal body weights were decreased by more than 10% relative to the controls in several groups (28 and 12% decrease in the 400- and 200-mg/kg males, respectively and 23% decrease in 400-mg/kg females). The terminal body weights at 13 weeks' exposure were 250.6, 306.7, 333.4, 351.2, 353.4, and 348.9 g for males and 156.7, 190.5, 197.2, 203.5, 197.8, and 203.4 g for females for the 400, 200, 100, 50, 25, and 0 dose groups, respectively. Differences between mean values of hematological parameters in exposed groups and those in control groups were <10% of control values, except for a 94% increase in numbers of mature neutrophils and a 25.1% decrease in numbers of lymphocytes in male 400 mg/kg rats and a 37.2% increase in mature neutrophils in 400 mg/kg females. Due to a lack of a consistent pattern of change in the hematologic parameters, the observed changes are not considered adverse. Histological examinations revealed low incidences of lesions in exposed male kidneys and exposed female thymuses; no lesions were observed in respective control kidneys or thymuses. Focal cortical lymphocytic infiltration or focal tubular regeneration were observed in kidneys in 2/10 male rats exposed to 200 mg/kg naphthalene, and diffuse renal tubular degeneration occurred in 1/10 male rats exposed to 400 mg/kg naphthalene. Lymphoid depletion of the thymus occurred in 2/10 females exposed to 400 mg/kg naphthalene, but not in any other females or in males. No other tissue lesions were detected. In this study, 100 mg/kg/day was a NOAEL, 200 mg/kg/day was a LOAEL, and 400 mg/kg/day was a serious LOAEL for decreased body weight in rats orally exposed to naphthalene for 13 weeks.

NTP. 1980a. Subchronic toxicity study: Naphthalene (C52904), B6C3F1 mice. Research Triangle Park, NC: U.S. Department of Health and Human Services, National Toxicology Program.

Ten male and 10 female B6C3F1 mice were administered gavage doses of naphthalene in corn oil at levels of 0, 12.5, 25, 50, 100, or 200 mg/kg, 5 days/week for 13 weeks (NTP 1980a). Seven mice (three males and two females of the 200 mg/kg group, one female of the 25 mg/kg group, and one control male) died during the second, third, and fourth weeks from gavage trauma or accident. Transient signs of

toxicity (lethargy, rough hair coats, and decreased food consumption) occurred between weeks 3 and 5 in the 200-mg/kg groups. Due to their transient nature, these effects are not considered to be adverse. All exposed male mice gained more weight during the study than did control males (weight gains expressed as a percentage of control weight gain were 154.3, 116.0, 125.9, 122.2, and 107.4 for the 12.5–200 mg/kg groups, respectively). In contrast, exposed female mice displayed decreased weight gain compared with controls (weight gains expressed as a percentage of control weight gain were 97.5, 81.5, 81.5, 77.8, and 76.5% for the 12.5–200 mg/kg groups, respectively). The average change in body weight between day 0 and the 13th week was 6.2 g/mouse for the 200-mg/kg female mice compared with 8.1 g/mouse for the control females. The investigators believed that a difference in weight gain of 1.9 g over a 13-week period "was not large enough to conclusively indicate a toxic effect." Respective mean terminal body weights (g) for control through the 200-mg/kg group were: 33.2, 37.7, 34.7, 34.7, 36.0, and 34.7 for males, and 26.7, 26.8, 25.4, 26.0, 26.1, and 25.6 for females. Mean terminal body weight values in exposed females were ≥95% of control values.

All mice were necropsied, and 27 organs (including the eyes, thymus, reproductive organs, and lungs) from the mice in the control and high-dose groups were examined histologically. No exposure-related lesions were observed in any organs. The highest incidence of lesions observed was for minimal to mild, focal or multifocal, subacute pneumonia in both controls (4/10 males and 2/10 females) and high-dose mice (4/10 males and 5/10 females). Organ weight data were not reported. Hematological analyses were performed on all groups. Exposed groups displayed mean values that were within 10% of the control means for the following parameters: hemoglobin, hematocrit, total white blood cells, and total red blood cells. An increase in lymphocytes (18% increase) and a decrease in segmented neutrophils (38.8% decrease) in high-dose males were not considered biologically significant by the authors. The highest dose in this study, 200 mg/kg/day, is judged to be a NOAEL for nonneoplastic lesions, hematologic changes, and adverse neurologic symptoms.

Shopp GM, White KL JR, Holsapple MP, et al. 1984. Naphthalene toxicity in CD-1 mice: General toxicology and immunotoxicology. Fundam Appl Toxicol 4:406-419.

Groups of male and female albino CD-1 mice (approximately 6 weeks old at the start) were administered gavage doses of 0, 5.3, 53, or 133 mg/kg naphthalene (99.3% pure) in corn oil for 90 consecutive days (Shopp et al. 1984). A naive control group and the 5.3 and 53 mg/kg dose groups each contained 76 male mice and 40 female mice. The vehicle control group contained 112 male mice and 76 female mice. The high-dose group contained 96 male mice and 60 female mice. Statistical analysis consisted of a one-way analysis of variance of means and Dunnett's t-test to compare control and treatment means using a significance level of $p<0.05$. Statistically significant chemical-related decreases in terminal body weights or survival were not observed in either sex. Respective mean terminal body weight values were (naïve, vehicle, 5.3, 53, and 133 mg/kg/day groups): 39.3, 37.3, 37.2, 36.2, and 36.8 g for male mice and 29.2, 29.0, 27.9, 27.0, and 27.1 g for female mice. No significant alterations in absolute or relative organ weights occurred in exposed male mice. Significant decreases in absolute weights of brain (9%), liver (18%), and spleen (28%) and relative weight of spleen (24%) occurred in high-dose females compared with controls. Histopathological examination of organs was not conducted, but the authors noted that cataracts were not formed in exposed mice (methods used to assess the presence of cataracts were not specified).

Examination of hematological parameters (including numbers of leucocytes, erythrocytes, and platelets and determination of hematocrit and hemoglobin) at termination revealed only slight, but statistically significant, increases in hemoglobin in high-dose females only; however, the hematological data were not shown in the available report. Chemical analysis of serum showed statistically significant decreased blood urea nitrogen in all exposed female groups. Compared with vehicle controls, the percent decreases in BUN were 16, 20, and 34% for the 5.3, 53, and 133 mg/kg/day groups, respectively. Increased serum

globulin (about 55%) and protein (about 40%) occurred in the two highest female dose groups compared with vehicle control values. Hepatic microsomal activities of aniline hydroxylase and aminopyrine N-demethylases were not statistically significantly changed in exposed versus control mice, but benzo[a]pyrene hydroxylase activities were statistically significantly decreased in exposed groups compared with control values (0.8, 0.62*, 0.55* and 0.41* nmol/min/mg protein for males in the control through high-dose group, and 1.40, 1.24, 1.13*, and 0.89* nmol/min/mg protein for females; statistically significant differences from control noted with *). The toxicological significance of the statistically significant changes in hematological parameters, hepatic enzyme activities, and serum chemical parameters is not clear, and these changes are not considered to be adverse.

No exposure-related responses were found in a battery of immunological assays (humoral immune response, lymphocyte responsiveness, delayed-type hypersensitivity response, popliteal lymph node response, and bone marrow function); immunotoxic responses were observed in positive controls given intraperitoneal injections of 50 mg/kg cyclophosphamide on days 87, 88, 89, and 90. The study identified a LOAEL of 133 mg/kg/day and a NOAEL of 53 mg/kg/day for statistically significant decreases in absolute weight of brain, liver, and spleen and relative weight of spleen in female mice, but not male mice. The biological significance of these changes, however, is uncertain because the effects were only observed in female mice, and histological changes in these organs were not observed in Fischer 344 rats (NTP 1980b) or B6C3F1 mice (NTP 1980a) exposed to naphthalene for 13 weeks.

Intermediate-Duration Oral MRL Derivation Considerations

The findings from the three intermediate-duration oral toxicity studies (one in rats and two in mice) do not collectively identify a clear, biologically significant, toxicity target other than body weight changes in rats. Consideration was given to basing the MRL on the NOAEL of 53 mg/kg/day and LOAEL of 133 mg/kg/day for decreases in absolute weight of brain, liver, and spleen, and in relative weight of spleen, in female mice (Shopp et al. 1984). However, the biological significance of these effects is uncertain because (1) small changes in organ weights are difficult to consistently measure in mice; (2) the effects were only observed in females; and (3) histological effects in the affected organs were not observed in the other 13-week oral studies with rats and mice. The biological significance of these effects in female, but not male, mice was less clearly biologically significant than the naphthalene-induced body weight changes observed in male and female rats.

In deriving a potential intermediate-duration MRL, the NOAEL of 100 mg/kg/day for decreased body weight in male and female rats should be adjusted to a continuous duration dose (100x5 days/7 days=71 mg/kg/day). The use of this adjusted dose and a total uncertainty factor of 100 (10 for extrapolating from rats to humans and 10 for human variability) arrives at a potential intermediate-duration oral MRL of 0.7 mg/kg/day, which is slightly larger than the acute-duration oral MRL for naphthalene, 0.6 mg/kg/day. Thus, the acute-duration oral MRL of 0.6 mg/kg/day is expected to be protective for intermediate-duration exposure scenarios and was adopted as the intermediate-duration oral MRL.

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APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 1-Methylnaphthalene
CAS Number: 90-12-0
Date: June 2005
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 46
Species: Mouse

Minimal Risk Level: 0.07 [x] mg/kg/day [ ] ppm

Reference: Murata Y, Denda A, Maruyama H, et al. 1993. Chronic toxicity and carcinogenicity studies of 1-methylnaphthalene in B6C3F1 mice. Fundam Appl Toxicol 21:44-51.

Experimental design: Groups of 50 B6C3F1 mice ingested the following doses (in mg/kg/day) over an 81-week period: 0 (M/F), 71.6 (M), 75.1 (F), 140.2 (M), and 143.7 (F). Tissues were examined histologically: brain, salivary glands, heart, thymus, lung, liver, pancreas, spleen, kidneys, testis, adrenals, trachea, stomach, small intestine, seminal vesicle, ovary, uterus, vagina, mammary gland, skeletal muscle, eye, Harderian glands, spinal cord, bone, and skin.

Effects noted in study and corresponding doses: Exposure-related lesions were restricted to the lung. Incidences for pulmonary alveolar proteinosis were (control through high-dose groups): 5/50, 23/50, and 17/49 for females and 4/49, 23/50, and 19/50 for males.

The only other exposure-related lesions found were lung tumors. Incidences for mice with adenomas were 4/50, 2/50, and 4/49 in females, and 2/49, 13/50, and 12/50 for males. Combined incidences for mice with lung adenomas or adenocarcinomas were: 5/50, 2/50, and 5/50 for females, and 2/49, 13/50, and 15/50 for males.

Dose and end point used for MRL derivation: Because the lowest exposure level was a LOAEL for increased incidence of alveolar proteinosis in male and female mice, benchmark dose analyses of the incidence data were conducted to determine a point of departure (POD) for the chronic-duration oral MRL. Available models in the EPA Benchmark Dose Software were fit to the incidence data for males and females, separately. None of the models provided adequate fit of the incidence data for females or for males, as assessed by chi-square goodness of fit statistics (p-values were <0.1). These results indicate that the data provide insufficient information to model the shape of the dose-response relationship. The lack of fit of the models to the data appears to be due to the apparent plateau of the response between the low- and high-dose levels. Thus, the LOAEL of 71.6 mg/kg/day for increased incidence of alveolar proteinosis in male mice was selected as the POD for the MRL.

[ ] NOAEL [x] LOAEL

Modifying Factors used in MRL derivation: N/A

Uncertainty Factors used in MRL derivation: Total Uncertainty Factor=10x10x10=1,000

[x] 10 for use of a LOAEL
[x] 10 for extrapolation from animals to humans
[x] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? If so, explain: Groups of 50 male and 50 female B6C3F1 mice were fed 0, 0.075, or 0.15% 1-methylnaphthalene (1-MN) in their diet for 81 weeks (567 days). Cumulative dose equivalents were provided by the investigators included: males: 0.075%=40,600 mg 1-MN/kg/body weight/567 days=71.6 mg/kg/day; 0.15%=79,500 mg 1-MN/kg/body weight/567 days=140.2 mg/kg/day; females: 0.075%=42,600 mg 1-MN/kg body weight/567 days=75.1 mg/kg/day; 0.15%=81,500 mg 1-MN/kg body weight/567 days=143.7 mg/kg/day.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

Other additional studies or pertinent information which lend support to this MRL: Increased incidence of pulmonary alveolar proteinosis has also been reported in B6C3F1 mice exposed to 2-methylnaphthalene in the diet for 81 weeks at dose levels of 50–54 and 108–114 mg/kg/day (Murata et al. 1997), and in mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene for 30–61 weeks (a mixture of 1- and 2-methylnaphthalene) (Emi and Konishi 1985; Murata et al. 1992).

Goodness-of-fit statistics [p-values for chi-square goodness of fit and the Akaike Information Criteria (AIC)] from the benchmark dose analyses of the incidence data for pulmonary alveolar proteinosis are summarized in the table below.

Table A-2. Goodness-of-fit Statistics From Benchmark Dose Analyses of Incidence Data for Male and Female Mice Exposed to 1-Methyl-naphthalene in the Diet for 81 Weeks (Murata et al. 1993).

	Male mouse data		Female mouse data	
Model	chi-square p-value	AIC	chi-square p-value	AIC
Log-logistic[b]	0.024	172.13	0.014	174.71
Gamma[a]	0.01	173.57	0.007	175.88
Multi-stage[c]	0.01	173.57	0.007	175.88
Quantal linear	0.01	173.57	0.007	175.88
Weibull[a]	0.01	173.57	0.007	175.88
Log-probit[b]	0.002	176.68	0.001	179.07
Probit	0.002	177.06	0.002	178.42
Logistic	0.001	177.45	0.002	178.71
Quantal quadratic	0.0002	181.03	0.0002	182.00

[a] = Restrict power >=1; [b] = Slope restricted to >1; [c] = Restrict betas >=0, Degree of polynomial = 1

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MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: 2-Methylnaphthalene
CAS Number: 91-57-6
Date: June 2005
Profile Status: Final Post-Public Comment
Route: [ ] Inhalation [x] Oral
Duration: [ ] Acute [ ] Intermediate [x] Chronic
Graph Key: 47
Species: Mouse

Minimal Risk Level: 0.04 [x] mg/kg/day [ ] ppm

Reference: Murata Y, Denda A, Maruyama H, et al. 1997. Chronic toxicity and carcinogenicity studies of 2-methylnaphthalene in B6C3F1 mice. Fundam Appl Toxicol 36(1):90-93.

Experimental design: Groups of 50 male and 50 female B6C3F1 mice were exposed to dietary levels of 0, 0.075, or 0.15% 2-MN for 81 weeks. Average intakes were reported as 0, 54.3, or 113.8 mg/kg/day for males and 0, 50.3, or 107.6 mg/kg/day for females.

Effects noted in study and corresponding doses: Survival and food consumption were not affected by exposure. Mean final body weights were decreased by 7.5 and 4.5% in high-dose males and females, respectively; these changes are not considered to be biologically significant. Histopathology only found exposure-related changes in the lung. Tissues examined were brain, heart, kidney, liver, lung, pancreas, salivary glands, spleen, testis, adrenals, bone, eye, Harderian glands, mammary gland, ovary, seminal vesicle, skeletal muscle, skin, small and large intestine, spinal cord, stomach, trachea, uterus, and vagina. No evidence of bronchiolar Clara cell necrosis or sloughing was found. Females showed statistically significantly decreased differential counts of stab and segmented form neutrophils and increased lymphocytes compared to controls, but biological significance of these changes is not clear due to a lack of reporting of the data (i.e., the report did not specify the response magnitudes or the dose levels at which they occurred).

Incidences for mice with pulmonary alveolar proteinosis were (control through high-dose groups): 5/50, 27/49, and 22/48 for females, and 4/49, 21/49, and 23/49 for males.

Incidences for mice with lung adenomas were: 4/50, 4/49, and 5/48 in females, and 2/49, 9/49, and 5/49 in males. Only the incidence in the male 54.3-mg/kg/day groups was significantly different from the control incidence. Combined incidences for lung adenomas or adenocarcinomas were: 5/50, 4/49, and 6/48 for females, and 2/49, 10/49, and 6/49 for males.

Dose and end point used for MRL derivation: Because the lowest exposure level was a LOAEL for increased incidence of alveolar proteinosis in male and female mice, benchmark dose (BMD) analyses of the incidence data were conducted to determine a point of departure (POD) for the chronic-duration oral MRL. Available models in the EPA Benchmark Dose Software were fit to the incidence data for males and females, separately. None of the models provided adequate fit of the incidence data for females, as assessed by chi-square goodness of fit statistics (p-values were <0.1). These results indicate that the female data provide insufficient information to model the shape of the dose-response relationship. The apparent plateau of the response between the low- and high-dose levels appears to contribute to the lack of fit of the models to the data. In contrast, the log-logistic and multi-stage models provided marginally adequate fits (p-values >0.1) to the male data, showing p-values of 0.23 and 0.11, respectively, for the chi-square goodness-of-fit statistic (Table A-3). The fitting algorithms for the gamma, quantal-linear, and

Weibull models provided identical model parameters and fit statistics as the multi-stage model. The Akaike Information Criteria (AIC) for the log-logistic model was lower than that for the multi-stage model indicating a better fit; thus the log-logistic model of the male data was selected to calculate the BMD POD for the MRL.

A benchmark response of 5% extra risk was selected over a default value of 10% extra risk in order to provide protection for children who may develop pulmonary alveolar proteinosis. This selection is supported by reports that children with pulmonary alveolar proteinosis (albeit of unknown etiology) experience more severe symptoms of respiratory dysfunction than do adults (EPA 2003r; Mazzone et al. 2001).

To derive the MRL of 0.04 mg/kg/day, the $BMDL_{05}$ of 4.3 mg/kg/day was divided by an uncertainty factor of 100 (10 for extrapolation from mice to humans and 10 for human variability).

An alternative NOAEL/LOAEL approach arrives at a similar value for the MRL. In the alternative approach, the LOAEL of 50.3 mg/kg/day for pulmonary alveolar proteinosis in female mice would be divided by an uncertainty factor of 1000 (10 for extrapolation from mice to humans, 10 for human variability, and 10 for extrapolation from a LOAEL to a NOAEL), arriving at a value of 0.05 mg/kg/day.

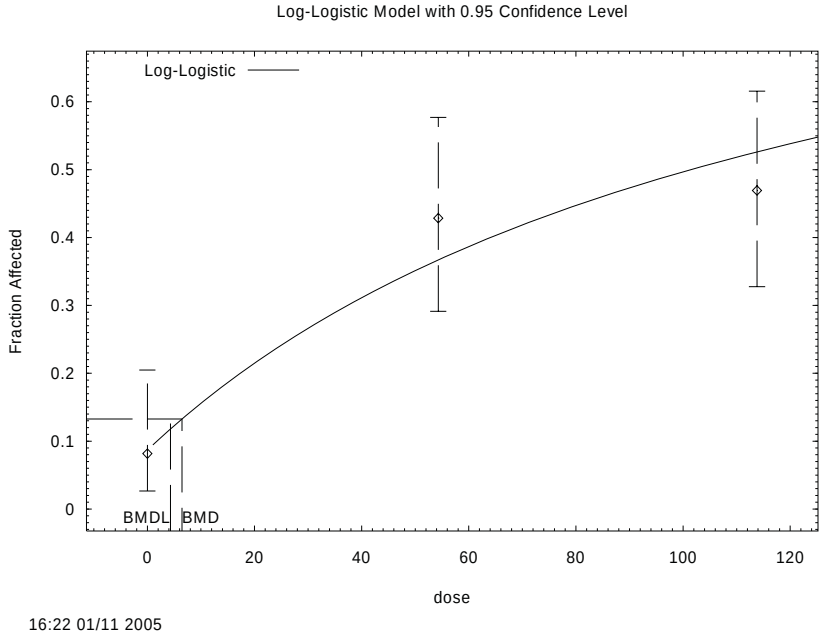
Table A-3. Benchmark Doses and Goodness-of-Fit Statistics from Modeling of Incidence Data for Pulmonary Alveolar Proteinosis in Male Mice Exposed to 2-Methylnaphthalene in the Diet for 81 Weeks (Murata et al. 1997)

	Benchmark doses (mg/kg/day)		Goodness-of-fit statistics	
Model	BMD (ED05)	BMDL (LED05)	chi-square p-value	AIC
Log-logistic[b]	6.47	4.30	0.23	167.81
Gamma[a]	8.76	6.4	0.11	168.93
Multi-stage[c]	8.76	6.4	0.11	168.93
Quantal linear	8.76	6.4	0.11	168.93
Weibull[a]	8.76	6.4	0.11	168.93
Log-probit[b]	20.92	15.95	0.03	170.99
Probit	17.23	13.8	0.01	172.4
Logistic	18.43	14.62	0.01	172.84
Quantal quadratic	32.73	26.51	0.001	175.87

[a] = Restrict power >=1; [b] = Slope restricted to >1; [c] = Restrict betas >=0, Degree of polynomial = 1

BMD(ED05) = predicted benchmark dose associated with 5% extra risk; BMDL (LED05) = 95% lower confidence limit on benchmark dose associated with 5% extra risk

Figure A-1. Observed and Predicted Incidence of Pulmonary Alveolar Proteinosis in Male Mice Exposed to 2-Methylnaphthalene in the Diet for 81 Weeks (Murata et al. 1997): Log-Logistic Model BMD=ED_{05}; BMDL=LED_{05}



Observed and predicted incidences of olfactory epithelial neuroblastomas in male rats exposed to naphthalene: Weibull model. BMD=EC_{10}; BMDL=LEC_{10}; dose unit= ppm.

[ ] NOAEL [ ] LOAEL [x] BMDL =

Modifying Factors used in MRL derivation: N/A

Uncertainty Factors used in MRL derivation: Total Uncertainty Factor=10x10=100

[ ] 10 for use of a LOAEL
[x] 10 for extrapolation from animals to humans
[x] 10 for human variability

Was a conversion used from ppm in food or water to a mg/body weight dose? No.

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: N/A

Other additional studies or pertinent information which lend support to this MRL: Increased incidence of pulmonary alveolar proteinosis has also been reported in B6C3F1 mice exposed to 1-methylnaphthalene in the diet for 81 weeks at dose levels as low as 71.6 mg/kg/day (Murata et al. 1993), and in mice dermally exposed to 30 or 119 mg/kg of methylnaphthalene for 30–61 weeks (a mixture of 1- and 2-methylnaphthalene) (Emi and Konishi 1985; Murata et al. 1992).

In a range-finding study, groups of B6C3F1 mice (10/sex/group) were fed diets containing 2-methylnaphthalene for 13 weeks delivering approximate average daily doses of 0, 31, 92, 276, 827, or 2,500 mg/kg/day (Murata et al. 1997). No histopathologic lesions were found in tissues and organs of male or female mice exposed to 827 or 2,500 mg/kg-day; tissues from mice in lower dose groups were not examined histologically. Decreased body weights, compared with control values, were seen at the three highest dose levels in both males and females, and were attributed to food refusal (Murata et al. 1997). The absence of pulmonary alveolar proteinosis in the prechronically exposed mice, which were exposed to much higher doses than those experienced by mice with this lesion in the chronic study, suggests that the development of pulmonary alveolar proteinosis from oral exposure to 2-methylnaphthalene requires chronic-duration exposure. The limited reporting of experimental details and results from this intermediate-duration study, however, precludes its use as the basis of an intermediate oral MRL for 2-methylnaphthalene.

The EPA (2003r) Toxicological Review of 2-Methylnaphthalene calculated an oral exposure RfD of 0.004 mg/kg-day for 2-methylnaphthalene based on a value of 3.5 mg/kg-day for a 95% lower confidence limit on a benchmark dose associated with 5% extra risk ($BMDL_{05}$) for pulmonary alveolar proteinosis in mice exposed to 2-methylnaphthalene in the diet for 81 weeks (Murata et al. 1992). The combined incidence data for this lesion in male and female mice in the control and low-dose groups were modeled with the quantal-linear model algorithm in the BMDS software (the high-dose data were excluded from the analysis, because when they were included adequate fit of models to the data were not obtained). A total uncertainty factor of 1,000 was used to derive the RfD: 10 for interspecies variability, 10 for interindividual variability, and 10 for database deficiencies.

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APPENDIX B. USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public, especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Relevance to Public Health

This chapter provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions:

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The chapter covers end points in the same order that they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, and dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this chapter.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal Risk Levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Chapter 3 Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, ATSDR has derived MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not

meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2, "Relevance to Public Health," contains basic information known about the substance. Other sections such as Chapter 3 Section 3.9, "Interactions with Other Substances," and Section 3.10, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive end point which, in its best judgment, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgment or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen end point are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables.

Chapter 3

Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, MRLs to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 3-1 and Figure 3-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See Sample LSE Table 3-1 (page B-6)

(1) Route of Exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Tables 3-1, 3-2, and 3-3, respectively). LSE figures are limited to the inhalation (LSE Figure 3-1) and oral (LSE Figure 3-2) routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures.

(2) Exposure Period. Three exposure periods—acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more)—are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect. The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the two "18r" data points in sample Figure 3-1).

(5) Species. The test species, whether animal or human, are identified in this column. Chapter 2, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 3.4, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration. The duration of the study and the weekly and daily exposure regimens are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to “Chemical x” via inhalation for 6 hours/day, 5 days/week, for 13 weeks. For a more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Nitschke et al. 1981).

(7) System. This column further defines the systemic effects. These systems include respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, one systemic effect (respiratory) was investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system,

which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific end point used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less Serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference. The complete reference citation is given in Chapter 9 of the profile.

(11) CEL. A CEL is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates that the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Sample Figure 3-1 (page B-7)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the acute and intermediate exposure periods are illustrated.

(14) Health Effect. These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL. In this example, the open circle designated 18r identifies a NOAEL critical end point in the rat upon which an intermediate inhalation exposure MRL is based. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL. Key number 38m is one of three studies for which CELs were derived. The diamond symbol refers to a CEL for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels. This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure. The Key explains the abbreviations and symbols used in the figure.

SAMPLE

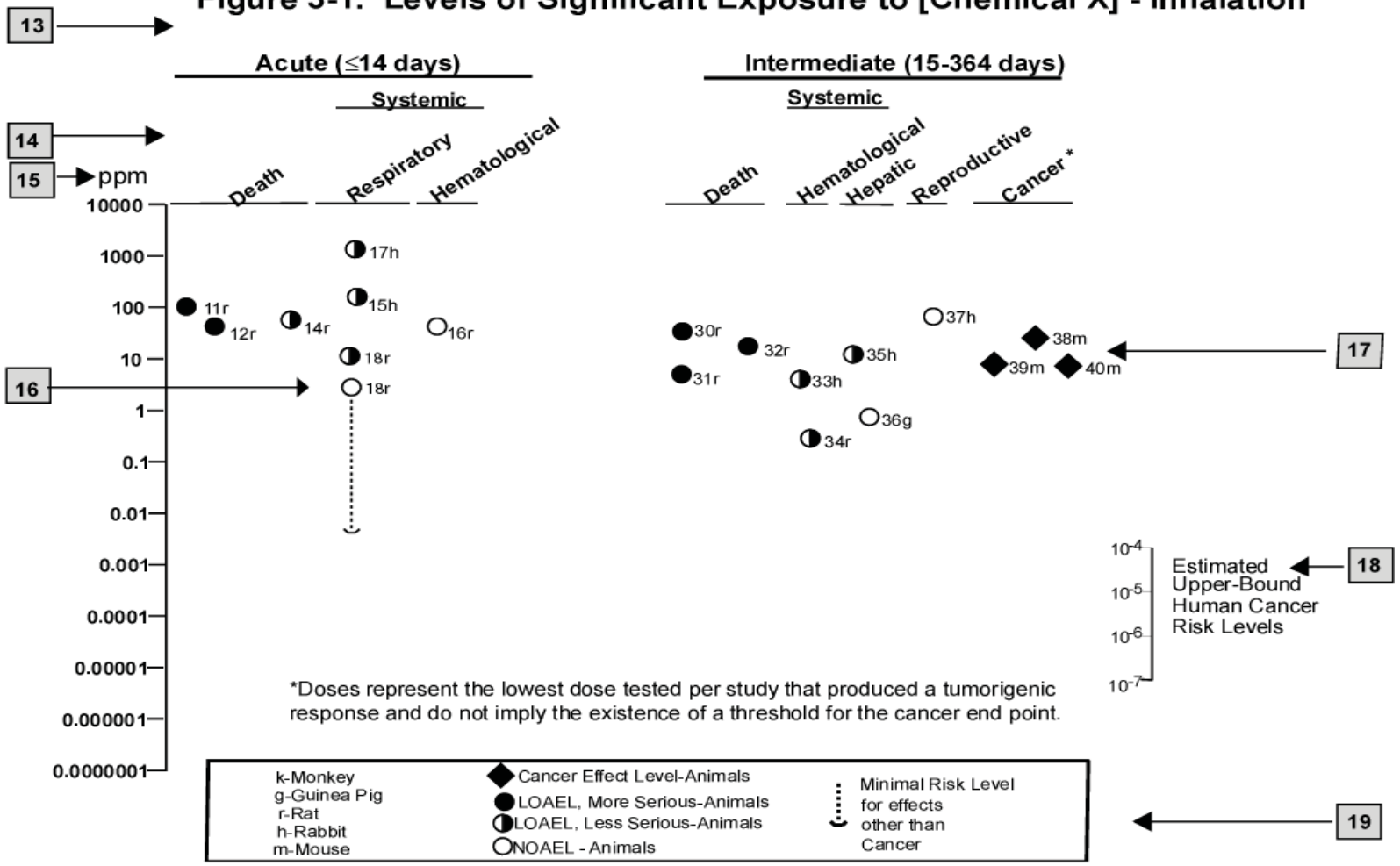
1 → **Table 3-1. Levels of Significant Exposure to [Chemical x] – Inhalation**

	Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	LOAEL (effect) Serious (ppm)	Reference
2 →	INTERMEDIATE EXPOSURE							
		5	6	7	8	9		10
3 →	Systemic	↓	↓	↓	↓	↓		↓
4 →	18	Rat	13 wk 5 d/wk 6 hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
	CHRONIC EXPOSURE							
	Cancer					11		
						↓		
	38	Rat	18 mo 5 d/wk 7 hr/d			20	(CEL, multiple organs)	Wong et al. 1982
	39	Rat	89–104 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
	40	Mouse	79–103 wk 5 d/wk 6 hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

12 → [a] The number corresponds to entries in Figure 3-1.
[b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of $5x10^{-3}$ ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

Figure 3-1. Levels of Significant Exposure to [Chemical X] - Inhalation



APPENDIX C. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AED	atomic emission detection
AFID	alkali flame ionization detector
AFOSH	Air Force Office of Safety and Health
ALT	alanine aminotransferase
AML	acute myeloid leukemia
AOAC	Association of Official Analytical Chemists
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
APHA	American Public Health Association
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BAT	best available technology
BCF	bioconcentration factor
BEI	Biological Exposure Index
BMD	benchmark dose
BMR	benchmark response
BSC	Board of Scientific Counselors
C	centigrade
CAA	Clean Air Act
CAG	Cancer Assessment Group of the U.S. Environmental Protection Agency
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CELDS	Computer-Environmental Legislative Data System
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
CL	ceiling limit value
CLP	Contract Laboratory Program
cm	centimeter
CML	chronic myeloid leukemia
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DOL	Department of Labor
DOT	Department of Transportation

DOT/UN/ NA/IMCO	Department of Transportation/United Nations/ North America/International Maritime Dangerous Goods Code
DWEL	drinking water exposure level
ECD	electron capture detection
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EEGL	Emergency Exposure Guidance Level
EPA	Environmental Protection Agency
F	Fahrenheit
F_1	first-filial generation
FAO	Food and Agricultural Organization of the United Nations
FDA	Food and Drug Administration
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FPD	flame photometric detection
fpm	feet per minute
FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GLC	gas liquid chromatography
GPC	gel permeation chromatography
HPLC	high-performance liquid chromatography
HRGC	high resolution gas chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
ILO	International Labor Organization
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
MA	*trans*,*trans*-muconic acid
MAL	maximum allowable level
mCi	millicurie
MCL	maximum contaminant level

MCLG	maximum contaminant level goal
MF	modifying factor
MFO	mixed function oxidase
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NATICH	National Air Toxics Information Clearinghouse
NATO	North Atlantic Treaty Organization
NCE	normochromatic erythrocytes
NCEH	National Center for Environmental Health
NCI	National Cancer Institute
ND	not detected
NFPA	National Fire Protection Association
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPD	nitrogen phosphorus detection
NPDES	National Pollutant Discharge Elimination System
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NSPS	New Source Performance Standards
NTIS	National Technical Information Service
NTP	National Toxicology Program
ODW	Office of Drinking Water, EPA
OERR	Office of Emergency and Remedial Response, EPA
OHM/TADS	Oil and Hazardous Materials/Technical Assistance Data System
OPP	Office of Pesticide Programs, EPA
OPPT	Office of Pollution Prevention and Toxics, EPA
OPPTS	Office of Prevention, Pesticides and Toxic Substances, EPA
OR	odds ratio
OSHA	Occupational Safety and Health Administration
OSW	Office of Solid Waste, EPA
OTS	Office of Toxic Substances
OW	Office of Water

OWRS	Office of Water Regulations and Standards, EPA
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PCE	polychromatic erythrocytes
PEL	permissible exposure limit
pg	picogram
PHS	Public Health Service
PID	photo ionization detector
pmol	picomole
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PSNS	pretreatment standards for new sources
RBC	red blood cell
REL	recommended exposure level/limit
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RQ	reportable quantity
RTECS	Registry of Toxic Effects of Chemical Substances
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SIC	standard industrial classification
SIM	selected ion monitoring
SMCL	secondary maximum contaminant level
SMR	standardized mortality ratio
SNARL	suggested no adverse response level
SPEGL	Short-Term Public Emergency Guidance Level
STEL	short term exposure limit
STORET	Storage and Retrieval
TD_{50}	toxic dose, 50% specific toxic effect
TLV	threshold limit value
TOC	total organic carbon
TPQ	threshold planning quantity
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
μm	micrometer
μg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

APPENDIX D. INDEX

Attachment

1I

12

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Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)

Office of Research and Development
Center for Public Health and Environmental Assessment

EPA/600/R-20/345F

Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)

U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC 20460

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PREFACE

This assessment titled *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid and Related Compound Potassium Perfluorobutane Sulfonate* is a toxicity assessment developed by the U.S. EPA's Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA).

The perfluorobutane sulfonic acid (PFBS) toxicity assessment is one of the key goals of the Agency's PFAS Action Plan (U.S. EPA, 2019) and provides qualitative and quantitative toxicity information that can be used along with exposure information and other important considerations to assess potential health risks to determine if, and when, it is appropriate to take action to address this chemical. This assessment is available for use across multiple U.S. EPA program and regional offices, other federal agencies, states, tribes, external stakeholders, and other entities as needed.

The PFBS human health toxicity values presented in this assessment were developed based on the best available science. The assessment provides high-quality evaluations and conclusions drawn from publicly available information on the toxicity of PFBS. This assessment is not a regulation; rather, it provides a critical part of the scientific foundation for risk assessment decision making. The PFBS assessment provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users, including risk assessors and risk managers, are advised to review the information, including potential uncertainties, provided in this document to ensure that the assessment is appropriate for the circumstances (e.g., exposure pathways, concentrations, presence of sensitive subpopulations) in question and the risk management decisions that would be supported by the risk assessment.

The PFBS toxicity assessment underwent a rigorous development and review process, as described below.

Overview of major steps in the PFBS assessment development and review process

- Draft assessment development
- Review by U.S. EPA program and regional offices (i.e., Intra-agency review)
- Review by other federal agencies (i.e., interagency review)
- External letter peer review
- Public comment period
- Second external letter peer review
- Intra-agency and interagency review

This assessment was provided for review to scientists in U.S. EPA's program and regional offices prior to external peer review and after external peer review. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection
Office of the Administrator/Office of Policy
Office of Chemical Safety and Pollution Prevention
Office of Land and Emergency Management
Office of Research and Development
Office of Water
Region 2, New York, NY
Region 3, Boston, MA
Region 4, Atlanta, GA
Region 5, Chicago, IL
Region 8, Denver, CO

This assessment was provided for review to other federal agencies prior to external peer review and after external peer review. Representatives from federal agencies and from the Environmental Council of the States (ECOS) were briefed during the assessment scoping and draft development process on March 9, 2018; May 2, 2018; and August 27, 2018. After public comment, interagency review was conducted by the Office of Management and Budget's PFAS Technical Working Group (TWG), an interagency group composed of career staff chief scientists or their equivalents from across the Executive Branch. Comments on this assessment were submitted by a subset of TWG representatives, namely:

Department of Defense
Department of Health and Human Services
  Agency for Toxic Substances and Disease Registry
  Food and Drug Administration
  National Institute of Environmental Health Sciences/National Toxicology Program
  National Institute of Occupational Safety and Health
Executive Office of the President
  Office of Management and Budget
National Aeronautics and Space Administration

This assessment was peer reviewed by independent, expert scientists external to U.S. EPA before and after the public comment period. The reports of the two external peer reviews and responses to comments on the U.S. EPA's draft Human Health Toxicity Values for PFBS, dated November 2018 and October 2020, are available at https://www.epa.gov/pfas/learn-about-human-health-toxicity-assessment-pfbs. Comments from external peer review were submitted by:

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This assessment was released for public comment from November 21, 2018 to January 22, 2019. The public comments are available on Regulations.gov in the Docket ID No. EPA-HQ-OW-2018-0614.

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

AEC	absolute eosinophil count
AFFF	aqueous film-forming foam
AIC	Akaike's information criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service registry number
CHO	Chinese hamster ovary (cell line)
CI	confidence interval
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
D3	deiodinase 3
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
ECP	eosinophilic cationic protein
GD	gestation day
GLP	Good Laboratory Practice
HAWC	Health Assessment Workspace Collaborative
HED	human equivalent dose
HPT	hypothalamic-pituitary-thyroid
i.v.	intravenous
ICR	Institute of Cancer Research
K^+PFBS	potassium perfluorobutane sulfonate
k_{elim}	serum elimination rate constant
LD	lactation day
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MW	molecular weight
NHANES	National Health and Nutrition Examination Survey
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OR	odds ratio
PECO	Population, Exposure, Comparator, and Outcome
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutane sulfonic acid
PFHxA	perfluorohexanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonic acid
PND	postnatal day
POD	point of departure
RfC	inhalation reference concentration
RfD	oral reference dose
ROS	reactive oxygen species
rT_3	reverse triiodothyronine
S-D	Sprague-Dawley
SD	standard deviation
T_2	3,5-diiodo-L-thyronine
T_3	triiodothyronine
T_4	thyroxine
TBG	thyroid-binding globulin
TSH	thyroid-stimulating hormone
TTR	transthyretin
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UFc	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
VLDL	very low-density lipoprotein

EXECUTIVE SUMMARY

SUMMARY OF OCCURRENCE AND HEALTH EFFECTS

The U.S. Environmental Protection Agency (U.S. EPA) is issuing subchronic and chronic oral toxicity values for perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service registry number [CASRN] 375-73-5) and its related salt, potassium perfluorobutane sulfonate (K^+PFBS) (CASRN 29420-49-3). The ionic state of per- and polyfluoroalkyl substances (PFAS) such as PFBS influence physicochemical properties such as water or lipid solubility and bioaccumulative potential, which in turn impact fate and transport in the environment and potential human health and ecological effects in exposed populations. K^+PFBS fully dissociates in aqueous solutions with pH levels ranging from 4–9; thus, the oral toxicity values derived in this document are also applicable to the deprotonated anionic form of PFBS (i.e., $PFBS^-$; CASRN 45187-15-3).

The toxicity assessment for PFBS includes toxicity values associated with potential noncancer health effects following oral exposure (in this case, oral reference doses [RfDs]). This assessment evaluates human health hazards. The toxicity assessment and the values contained within is not a risk assessment because it does not include an exposure assessment nor an overall risk characterization. Further, the toxicity assessment does not address the legal, political, social, economic, or technical considerations involved in risk management. The PFBS toxicity assessment can be used by U.S. EPA, states, tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, if, and when, it is necessary to take action to address potential risk associated with human exposures to PFBS.

PFBS and K^+PFBS are both four-carbon, fully fluorinated alkane members of a large and diverse class of linear and branched compounds known as "per- and polyfluoroalkyl substances," or PFAS. In the early 2000s, concerns grew over the environmental persistence, bioaccumulation potential, and long half-lives in humans of longer chain PFAS, in particular, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). As a result, shorter chain PFAS such as PFBS were developed and integrated into various consumer products and industrial applications, because PFBS has the desired properties and characteristics associated with this class of compounds but with faster elimination from the body than PFOA and PFOS. PFBS is associated with aqueous film-forming foam (foams (AFFFs) and used during chrome electroplating as a mist suppressant (See Section 1.2). It has also been found in food contact materials, dust, and source and finished drinking water. Accordingly, oral intake of water and food, inhalation, and dermal contact are plausible modes of PFBS exposure, with the oral route being the primary route of exposure. PFBS has been detected in humans, confirming exposure to this PFAS; however, the magnitude of human exposure likely depends on factors such as occupation (e.g., processing and/or manufacture of PFBS or PFBS-containing products and chrome electroplating) and living conditions (e.g., proximity to locations that make or use PFBS-containing products and nearby well-water use).

Human studies have examined possible associations between PFBS exposure and potential health outcomes such as alteration of menstruation, reproductive hormones or semen parameters, kidney function (uric acid production), lung function (induction of asthma), and lipid

profile. The ability to draw conclusions about associations is limited due to the small number of human studies per outcome. Of the examined health outcomes, only asthma and serum cholesterol levels in humans were found to exhibit a statistically significant positive association with PFBS exposure. No studies have been identified that evaluate the association between PFBS exposure and potential cancer outcomes. While the epidemiology studies were not influential to drawing evidence integration judgments or the derivation of toxicity values, the general findings identify potential areas of future research.

Animal studies of repeated-dose PFBS exposure have been exclusively via the oral route, used the potassium salt of PFBS (K^+PFBS) as the source exposure material, and have examined noncancer effects only. The available rat and mouse studies support identification of thyroid, developmental, and kidney endpoints as potential health effects following repeated exposures in utero and/or during adulthood. Animal studies have also evaluated other health outcomes, such as liver effects, reproductive parameters, lipid/lipoprotein homeostasis, and effects on the spleen and hematology; however, the available evidence does not support a clear association with PFBS exposure and these outcomes.

Noncancer Effects Observed Following Oral Exposure

Oral exposures to PFBS or its K^+ salt in adult and developing rats and mice have been shown to result in thyroid, developmental, and kidney effects. Thyroid effects in exposed adult rats and mice and in developing mice were primarily expressed through significant decreases in circulating levels of hormones such as thyroxine (T_4) and triiodothyronine (T_3). In early developmental life stages in mice (e.g., newborn), decreases in thyroid hormone were accompanied by other effects indicative of delayed maturation or reproductive development (e.g., vaginal patency and eyes opening). Kidney weight and/or histopathological alterations (e.g., renal tubular and ductal epithelial hyperplasia) were observed in rats following short-term and subchronic oral exposures. Many of the kidney effects, however, occurred at higher doses than did the thyroid and developmental effects. The limited number of human studies examining oral PFBS exposure does not inform the potential for effects in thyroid, developing offspring, or the renal system.

Oral Reference Doses for Noncancer Effects

Subchronic[1] and chronic[2] oral RfDs were derived for PFBS. The hazards of potential concern include thyroid, developmental, and kidney effects. From these identified targets of PFBS toxicity, perturbation of thyroid hormone levels (e.g., T_4) was used as the critical effect for deriving a subchronic and chronic RfD. Based on recommendations in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), chemical-specific toxicokinetic data (e.g., serum half-lives) were used to scale a toxicologically equivalent dose of orally administered PFBS from animals to humans. Following the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012),

[1]Subchronic exposure: Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the lifespan in humans (more than 30 days up to approximately 90 days in typically used laboratory animal species).

[2]Chronic exposure: Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the lifespan in humans (more than approximately 90 days to 2 years in typically used laboratory animal species). (https://ofmpub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&glossaryName=IRIS%20Glossary#formTop)

benchmark dose (BMD) modeling of thyroid effects in a developmental life stage following exposure to K^+PFBS in utero resulted in a $BMDL_{0.5SD}$ human equivalent dose (HED) of 0.095 milligrams per kilogram per day (mg/kg-day). This HED associated with thyroid effects served as the point of departure (POD) for deriving the subchronic and chronic RfDs.

The subchronic RfD for K^+PFBS was calculated by dividing the POD (HED) for decreased serum total T_4 observed in newborn (Postnatal Day [PND] 1) mice, in the study conducted by Feng et al. (2017), by a composite uncertainty factor (UF_C) of 100 to account for extrapolation from mice to humans (an interspecies uncertainty factor, or UF_A, of 3), for interindividual differences in human susceptibility (intraspecies uncertainty factor, or UF_H, of 10), and for deficiencies in the toxicity database (database uncertainty factor, or UF_D, of 3) (a value of 1 was applied for subchronic-to-chronic UF, or UF_S, and LOAEL-to-NOAEL uncertainty factor, or UF_L) (see Table 10), yielding a subchronic RfD of 0.00095 mg/kg-day rounded to 1×10^{-3} mg/kg-day. Because K^+PFBS is fully dissociated in water at the environmental pH range of 4–9 to the PFBS anion ($PFBS^-$) and the K^+ cation, data for K^+PFBS were used to derive a subchronic RfD for the free acid (PFBS) by adjusting for differences in molecular weight (MW) between K^+PFBS (338.19) and PFBS (300.10), yielding the value of 0.00085 mg/kg-day rounded to 9×10^{-4} mg/kg-day for the subchronic RfD for PFBS (free acid).

The chronic RfD for K^+PFBS associated with thyroid effects was calculated by dividing the POD (HED) for decreased serum total T_4 observed in newborn (PND 1) mice, in the study conducted by Feng et al. (2017), by a UF_C of 300 to account for extrapolation from mice to humans (UF_A of 3), for interindividual differences in human susceptibility (UF_H of 10), and deficiencies in the toxicity database (UF_D of 10) (a value of 1 was applied for UF_S and UF_L) (see Table 12), yielding a chronic RfD of 0.00032 mg/kg-day rounded to 3×10^{-4} mg/kg-day. Like the subchronic RfD for thyroid effect, based on the data for K^+PFBS, a chronic RfD for PFBS (free acid) of 0.00028 mg/kg-day rounded to 3×10^{-4} mg/kg-day was derived.

Confidence in the Oral RfDs

The overall confidence in the subchronic RfD for thyroid effects is medium. The gestational exposure study conducted by Feng et al. (2017) reported administration of K^+PFBS by gavage in pregnant Institute of Cancer Research (ICR) mice (10/dose) from Gestation Days (GDs) 1 to 20. This study was of good quality (i.e., high confidence) with adequate reporting and consideration of appropriate study design, methods, and conduct (click to see risk of bias analysis in HAWC[3]). Confidence in the oral toxicity database for derivation of the subchronic RfD is medium because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats, there are no PFBS studies available that have specifically evaluated health effect domains of emerging concern across the PFAS class such as immunotoxicity and mammary gland development (Dewitt et al., 2012; White et al., 2007). Further, neurodevelopmental effects are of particular concern when perturbations in thyroid hormone occur during a sensitive early life

[3]HAWC: A modular web-based interface to facilitate development of human health assessments of chemicals; see Appendix D for details.

stage, and the absence of a study evaluating neurodevelopmental effects following PFBS exposure is a source of uncertainty in the assessment.

The overall confidence in the chronic RfD for thyroid effects is low. Although the chronic RfD, like the subchronic RfD, was derived using data from the high-confidence principal study conducted by Feng et al. (2017), there is increased concern about the potential for identification of hazards following longer (i.e., chronic) duration PFBS exposures. In addition, because of the lack of studies that specifically evaluated health effect domains of emerging concern across the PFAS class, such as immunotoxicity, mammary gland development, or neurodevelopmental at any exposure duration—but particularly for chronic duration—confidence in the database specifically for a chronic RfD is low.

Effects Other Than Cancer Observed Following Inhalation Exposure

There are no studies available that examined toxicity in humans or experimental animals following inhalation exposure, thereby precluding the derivation of an inhalation reference concentration (RfC).

Evidence for Carcinogenicity

Under the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the Agency concluded that there is *"Inadequate Information to Assess Carcinogenic Potential"* for PFBS and K^+PFBS by either oral or inhalation routes of exposure. Therefore, the lack of data on the carcinogenicity of PFBS and the related compound K^+PFBS precludes the derivation of quantitative estimates for either oral (oral slope factor) or inhalation (inhalation unit risk) exposure.

1.0 BACKGROUND

1.1 PHYSICAL AND CHEMICAL PROPERTIES

Perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service registry number [CASRN] 375-73-5)[4] and its related salt, potassium perfluorobutane sulfonate (K^+PFBS) (CASRN 29420-49-3), are members of the group of per- and polyfluoroalkyl substances (PFAS), more specifically the short-chain perfluoroalkane sulfonates. For purposes of this assessment, "PFBS" will signify the ion, acid, or any salt of PFBS. Concerns about PFBS and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment (Sundström et al., 2012). The chemical formula of PFBS is $C_4HF_9O_3S$ and the chemical formula of K^+PFBS is $C_4F_9KO_3S$. Their respective chemical structures are presented in Figure 1. K^+PFBS differs from PFBS by being associated with a potassium ion. The reported water solubility of each species suggests that in aqueous environments the sulfonate would be the predominant form. The preferential use of K^+PFBS in laboratory studies is related to the optimal dissociation of the salt to the sulfonate (i.e., $PFBS^-$) at pH values ranging from 4 to 9 (see Table 1). Table 1 provides a list of the physicochemical properties for PFBS and K^+PFBS.

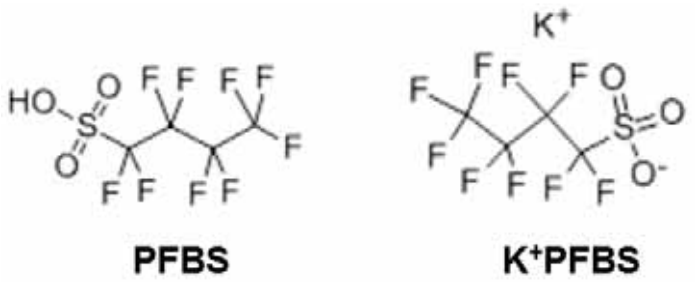


Figure 1. PFBS and K^+PFBS Chemical Structures

[4]The CASRN given is for linear PFBS; the source PFBS used in toxicity studies was assayed at ≥98% linear, suggesting some minor proportion of other chemicals, such as branched PFBS isomers, are present. Thus, observed health effects may apply to the total linear and branched isomers in a given exposure source.

Table 1. Physicochemical Properties of PFBS (CASRN 375-73-5) and Related Compound K^+PFBS (CASRN 29420-49-3)

Property (unit)	Value[a]	
	PFBS (free acid)[b]	K^+PFBS (potassium salt)[c]
Boiling point (°C)	152	447
Density (g/cm^3)	1.83 (predicted)	1.83 (predicted)
Vapor pressure (mm Hg)	0.104 (predicted)	1.12×10^{-8}
pH	ND	ND
Solubility in water (mol/L)	0.0017	0.08
Molecular weight (g/mol)	300.09	338.18
Dissociation constant	NA	Fully dissociated in water over the pH range of 4−9

[a]Values are experimentally determined unless otherwise indicated.
[b]U.S. EPA Chemistry Dashboard for CASRN 375-73-5.
[c]U.S. EPA Chemistry Dashboard for CASRN 29420-49-3.

K^+PFBS = potassium perfluorobutane sulfonate; NA = not applicable; ND = no data; PFBS = perfluorobutane sulfonic acid.

1.2 OCCURRENCE

PFBS-based compounds are surfactants used primarily in the manufacture of paints, cleaning agents, and water- and stain-repellent products and coatings. They serve as replacements for perfluorooctane sulfonic acid (PFOS) (3M, 2002b). Various sources report detection or occurrence in environmental media and consumer products, including drinking water, ambient water, dust, carpeting and carpet cleaners, floor wax, and food packaging. To assess potential health risks associated with these occurrences, an exposure assessment, which is beyond the scope of this document, would be necessary to determine the relative source contribution to human PFBS exposure from each reported occurrence and the relevance, if any, to human health.

Oral exposure via drinking water might be expected in areas where contamination has been reported. U.S. EPA Unregulated Contaminant Monitoring Rule data for public drinking water utilities in 2013−2015 showed levels of PFBS above the minimum reporting level (>0.09 micrograms per liter [µg/L]) in water systems serving Alabama, Colorado, Georgia, the Northern Mariana Islands, and Pennsylvania (U.S. EPA, 2017; Hu et al., 2016). These utilities used both ground and surface drinking water sources, with PFBS concentrations ranging from 0.09 to 0.37 µg/L. The estimated combined number of people served by these water systems is more than 340,000 (U.S. EPA, 2018).

Measurements from 37 surface water bodies in the northeastern United States (metropolitan New York area and Rhode Island) collected in 2014 showed an 85% site detection rate (Zhang et al., 2016). PFBS has also been identified in surface waters in Georgia, New

Jersey, North Carolina, and the Upper Mississippi River Basin (Post et al., 2013; Lasier et al., 2011; Nakayama et al., 2010; Nakayama et al., 2007). It has also been detected in wastewater treatment plant effluent, seawater, soil, and biosolids (Houtz et al., 2016; Zhao et al., 2012; Sepulvado et al., 2011).

PFBS contamination, which has been associated with the use of aqueous film-forming foams (AFFFs) (ESTCP, 2017; Anderson et al., 2016), was reported at Superfund sites and areas under assessment for Superfund designation. Contaminated sites include the former Wurtsmith Air Force Base, Ellsworth Air Force Base, and Dover Air Force Base (Aerostar SES LLC, 2017; Anonymous, 2017; ASTSWMO, 2015). At the Wurtsmith site, PFBS was detected at a concentration of 6.4 µg/L in groundwater contaminated by a PFAS plume originating from the fire training area (ASTSWMO, 2015). It is also present in some drinking water samples from nearby residential wells at low nanograms per liter concentrations, which were below the screening value cited by the Michigan Department of Community Health (MDCH, 2015). Other sources of PFAS and/or PFBS contamination include chrome plating operations, PFAS manufacture, and sites that use PFAS in product formulations such as textile and electronics facilities (Wang et al., 2013).

PFBS has also been detected in household dust and consumer products. There was a 92% detection frequency for PFBS among 39 household dust samples (10 from the United States) analyzed with levels ranging from 86 nanograms per gram (ng/g) for the 25th percentile to 782 ng/g for the 75th percentile (Kato et al., 2009). In a separate study, PFBS dust levels were measured in Boston area offices (n = 31), homes (n = 30), and vehicles (n = 13) with detection frequencies being relatively low—10, 3, and 0%, respectively—and ranging in the low parts per billion (Fraser et al., 2013). Consumer products could also be an exposure source. Limited quantitative testing showed the presence of PFBS in carpet and upholstery protectors (45.8 and 89.6 ng/g), carpet shampoo (25.7 and 911 ng/g), textiles (2 ng/g), and floor wax (143 ng/g) purchased in the United States (Liu et al., 2014).

PFBS is not authorized for use in food packaging. However, PFBS was detected in fast food packaging (7/20 samples) in one U.S. study (Schaider et al., 2017) although the magnitude of the detection was not reported.

The European Food Safety Authority reported the presence of PFBS in various food and drink items, including fruits, vegetables, cheese, and bottled water. For average adult consumers, the estimated exposure ranges for PFBS were 0.03−1.89 nanograms per kilogram per day (ng/kg-day) (minimum) to 0.10−3.72 ng/kg-day (maximum) (EFSA, 2012).

PFBS has been reported in serum of humans in the general population. In American Red Cross samples collected in 2015, 8.4% had a quantifiable serum PFBS concentration; the majority of samples were below the lower limit of quantitation (4.2 nanograms per milliliter [ng/mL]) (Olsen et al., 2017). The National Health and Nutrition Examination Survey (NHANES) included PFBS in consecutive biomonitoring cycles, including 2013–2014 where the 95th percentile reported for PFBS was at or below the level of detection (0.1 ng/mL). Considering the relatively rapid rate of elimination of PFBS (days to weeks), compared with longer chain PFAS (years), the lack of biomonitoring detects (e.g., NHANES 2013–2014 cycle) should not be interpreted as a lack of occurrence or exposure potential. Another study with a

lower limit of detection (0.013 ng/g) reported increasing levels of PFBS in serum from primiparous nursing women in Sweden from 1996 to 2010 (Glynn et al., 2012).

1.3 TOXICOKINETICS

1.3.1 Overview

Animal evidence has shown that PFBS, like other PFAS, is well absorbed following oral administration. PFBS distributes to all tissues of the body (Bogdanska et al., 2014), but a study evaluating the volume of distribution (V_d) concluded that distribution is predominantly extracellular (Olsen et al., 2009). Because of its resistance to metabolic degradation, PFBS is primarily eliminated unchanged in urine and feces.

Three sets of investigators have conducted toxicokinetic studies in rats and monkeys (Huang et al., 2019a; Chengelis et al., 2009; Olsen et al., 2009). Olsen et al. (2009) and Xu et al. (2020) have measured the half-life of PFBS in humans. Bogdanska et al. (2014) and Lau et al. (2020) have reported limited toxicokinetic information in mice. One study developed a physiologically based pharmacokinetic (PBPK) model that includes parameterization for PFBS (Fàbrega et al., 2015).

Results of all studies discussed in this section are summarized in Table 2.

Table 2. Summary of the Toxicokinetics of Serum PFBS (Mean ± SE)

Species/Sex	Study Design	Elimination Half-Life (hr)	AUC (µg-hr/mL)	Clearance	V_d (L/kg)	Reference
Mice						
Mice/male	Single oral dose (30 mg/kg)	3.7	1,515	0.019 (L/hr-kg)	0.129	Lau et al. (2020)
	Single oral dose (300 mg/kg)	6.0	7,178	0.039 (L/hr-kg)	0.291	Lau et al. (2020)
	Single oral dose (combined 30/300 mg/kg)	5.8		0.038 (L/hr-kg)	0.275	Lau et al. (2020)
Mice/female	Single oral dose (30 mg/kg)	4.4	520	0.056 (L/hr-kg)	0.145	Lau et al. (2020)
	Single oral dose (300 mg/kg)	4.6	4,587	0.064 (L/hr-kg)	0.308	Lau et al. (2020)
	Single oral dose (combined 30/300 mg/kg)	4.5		0.063 (L/hr-kg)	0.278	Lau et al. (2020)
Rats						
Rats/male	Single i.v. dose (10 mg/kg)	2.1	254	0.0394 (L/hr-kg)	0.118	Chengelis et al. (2009)
	Single i.v. dose (30 mg/kg)	4.51 ± 2.22[a]	294 ± 77	119 ± 34 (mL/hr)[b]	0.330 ± 0.032	Olsen et al. (2009)
	Single oral dose (30 mg/kg)	4.68 ± 0.43[a]	163 ± 10	NA	0.676 ± 0.055	Olsen et al. (2009)
	Single i.v. dose (4 mg/kg)	4.22 ± 0.28[c]	116 ± 7	0.0345 ± 0.002 (L/hr-kg)	0.188 ± 0.017[c]	Huang et al. (2019a)
	Single oral dose (4 mg/kg)	4.89 ± 1.67[c]	154 ± 15	0.0265 ± 0.003 (L/hr-kg)	0.174 ± 0.614[c]	Huang et al. (2019a)
	Single oral dose (20 mg/kg)	5.36 ± 1.24[c]	533 ± 45	0.0376 ± 0.003 (L/hr-kg)	0.167 ± 0.039[c]	Huang et al. (2019a)
	Single oral dose (100 mg/kg)	5.25 ± 1.19[c]	1,320 ± 100	0.0755 ± 0.006 (L/hr-kg)	0.335 ± 0.041[c]	Huang et al. (2019a)
Rats/female	Single i.v. dose (10 mg/kg)	0.64	32	0.311 (L/hr-kg)	0.288	Chengelis et al. (2009)
	Single i.v. dose (30 mg/kg)	3.96 ± 0.21[a]	65 ± 5	469 ± 40 (mL/hr)[d]	0.351 ± 0.034	Olsen et al. (2009)
	Single oral dose (30 mg/kg)	7.42 ± 0.79[a]	85 ± 12	NA	0.391 ± 0.105	Olsen et al. (2009)
	Single i.v. dose (4 mg/kg)	0.95 ± 0.10[c]	16 ± 1	0.252 ± 0.018 (L/hr-kg)	0.165 ± 0.015[c]	Huang et al. (2019a)
	Single oral dose (4 mg/kg)	1.50 ± 0.10[c]	29 ± 3	0.152 ± 0.020 (L/hr-kg)	0.328 ± 0.042[c]	Huang et al. (2019a)
	Single oral dose (20 mg/kg)	1.23 ± 0.12[c]	109 ± 23	0.183 ± 0.039 (L/hr-kg)	0.326 ± 0.073[c]	Huang et al. (2019a)
	Single oral dose (100 mg/kg)	1.11 ± 0.10[c]	387 ± 50	0.259 ± 0.033 (L/hr-kg)	0.415 ± 0.063[c]	Huang et al. (2019a)

Table 2. Summary of the Toxicokinetics of Serum PFBS (Mean ± SE)

Species/Sex	Study Design	Elimination Half-Life (hr)	AUC (µg-hr/mL)	Clearance	V_d (L/kg)	Reference
Monkeys[b]						
Cynomolgus macaque/male	Single i.v. dose (10 mg/kg)	15 (9.65)[e]	1,115 ± 859	0.016 (L/hr-kg)	0.209 ± 0.028	Chengelis et al. (2009)
	Single i.v. dose (10 mg/kg)	95.2 ± 27.1	24.3 ± 8.6	511 ± 141 (mL/hr)	0.254 ± 0.031	Olsen et al. (2009)
Cynomolgus macaque/female	Single i.v. dose (10 mg/kg)	8.1	489 ± 180	0.0229 ± 0.0099 (L/hr-kg)	0.248 ± 0.045	Chengelis et al. (2009)
	Single i.v. dose (10 mg/kg)	83.2 ± 41.9	35.4 ± 13.3	368 ± 120 (mL/hr)	0.255 ± 0.017	Olsen et al. (2009)
Humans						
Males and female	Occupational (n = 6)	619.2[f]	NA	NA	NA	Olsen et al. (2009)
Males	Occupational (n =5)	552[f]	NA	NA	NA	Olsen et al. (2009)
Female	Occupational (n = 1)	1,096.8	NA	NA	NA	Olsen et al. (2009)
Males and females	Occupational (n = 26)	1,056	NA	NA	NA	Xu et al. (2020)

[a]Olsen et al. (2009) reported $t_{1/2,\alpha}$ and $t_{1/2,\beta}$ in rats, presenting data for $t_{1/2,\beta}$.
[b]Body weights were reported to be 0.200−0.250 kg (with corresponding clearance of approximately 476 mL/hr-kg).
[c]Huang et al. (2019a) reported $t_{1/2,\alpha}$, $t_{1/2,\beta}$, and $t_{1/2}k_{10}$ in male rats (both oral and i.v.) and female rats (i.v. only); only $t_{1/2}k_{10}$ was reported in female rats (oral). Presenting data for $t_{1/2,\beta}$ for male rats (both oral and i.v.) and female rats (i.v.) and $t_{1/2}k_{10}$ for female rats (oral). The volume of distribution (V_d) was calculated as the sum of volume terms of the central compartment and that of the peripheral compartment except for orally exposed female rats. The volume of the peripheral compartment was not reported for orally exposed female rats, representing the volume of the central compartment only.
[d]The data were monitored 48 hours and 31 days postdosing for Chengelis et al. (2009) and Olsen et al. (2009), respectively.
[e]One male monkey had a serum concentration more than 10-fold higher than the others at 48 hours postdosing with an estimated PFBS half-life of 26 hours.
[f]Olsen et al. (2009) reported mean and geometric mean values for males only and all subjects, presenting data for geometric mean values.

AUC = area under the curve; i.v. = intravenous; NA = not available; PFBS = perfluorobutane sulfonic acid; SE = standard error; $t_{1/2}$ = half-life; V_d = volume of distribution.

1.3.2 Absorption

Olsen et al. (2009) conducted intravenous (i.v.) and oral uptake studies in rats (n = 3/sex) that were given a single dose (30 milligrams per kilogram [mg/kg]) of potassium PFBS (K^+PFBS). The serum area under the concentration curve (AUC) after i.v. administration was 294 ± 77 and 65 ± 5 (µg-hour/mL) in male and female rats, respectively, and 163 ± 10 and 85 ± 12 in males and females, respectively, after oral dosing. The large variance in AUC for male rats after i.v. dosing and greater AUC after oral dosing compared to i.v. dosing in females makes it difficult to interpret these results with certainty, but it seems that PFBS is 100% bioavailable in female rats, whereas the nominal bioavailability in male rats is only 55% based on AUC. Peak concentrations (C_{max}) occurred at 0.3−0.4 hours after oral dosing, showing that absorption was fairly rapid. Bioavailability based on C_{max} was 60% in male rats and 85% in female rats, suggesting a similar sex difference as estimated from the AUCs.

The above findings are generally confirmed by Huang et al. (2019a) who found that absorption of PFBS usually occurred within 24 hours, along with the time reaching the maximal plasma concentration (T_{max}) under 2.4 hours in male rats and under 1.4 hours in female rats, following a single dose of gavage administration in Hsd:Sprague-Dawley (S-D) rats (4, 20, 100 mg/kg of K^+PFBS). However, bioavailability calculated based on the AUC after i.v. and oral doses of 4 mg/kg reported by Huang et al. (2019a) was 75% in males and 60% in females. The C_{max} values of 45% and 27% in males and females, respectively, are qualitatively the opposite of the results from Olsen et al. (2009).

Given the range of estimated bioavailability from the results of Olsen et al. (2009) and Huang et al. (2019a), a difference in this parameter between male and female rats cannot be determined. Averaging the AUC-based values for both males and females from the two studies yields an overall average of 73%.

Notably, Huang et al. (2019a) also observed that the dose-adjusted AUC decreased with increasing doses for both males and females. However, this result could be attributed to saturation of renal resorption at higher doses, rather than a reduction in absorption.

Similar observations indicating rapid absorption of PFBS have been reported for CD-1 mice orally exposed to PFBS at 30 or 300 mg/kg, where T_{max} was estimated to occur between 1 and 2 hours after gavage (Lau et al., 2020).

1.3.3 Distribution

PFBS has been shown to distribute to tissues within 24 hours of exposure, with the liver and kidney being the organs with highest distribution. Lau et al. (2020) evaluated the pharmacokinetic properties of PFBS in CD-1 mice at 8 weeks of age. Male and female mice were given a single dose of 0, 30, or 300 mg/kg body weight PFBS via gavage. The liver and kidneys were harvested 24 hours postdosing. PFBS distributed to both organs readily in a dose-dependent manner but did not accumulate in either organ. Lau et al. (2020) reported similar combined V_d values of 0.275 or 0.278 liter per kilogram [L/kg] in male and female mice, respectively (Table 2).

Olsen et al. (2009) estimated volumes of distribution for K^+PFBS as 0.7 and 0.4 L/kg in male and female rats, respectively, and 0.25 L/kg in male and female cynomolgus macaques and

concluded that K^+PFBS is primarily distributed in the extracellular space. Consistent with the observations by Olsen et al. (2009), Huang et al. (2019a) found that the overall V_d for PFBS was generally comparable between male rats (0.167–0.335 L/kg) and female rats (0.165–0.415 L/kg). Chengelis et al. (2009) calculated a V_d of 0.248 L/kg in female cynomolgus macaques, consistent with females from Olsen et al. (2009). The male monkey V_d from Chengelis et al. (2009) was slightly lower (0.209 L/kg) than corresponding females and males from Olsen et al. (2009). These results indicate V_d is generally comparable between male and female monkeys. Huang et al. (2019a) also evaluated tissue concentrations in the liver, kidney, and brain of male and female rats and reported higher PFBS concentrations in the liver than the kidney, with the lowest concentrations occurring in the brain.

Bogdanska et al. (2014) characterized the tissue distribution of ^{35}S-labeled PFBS in male C57BL/6 mice. The animals (3/group) were exposed for either 1, 3, or 5 days to an average of 16 mg of PFBS/kg-day in the diet. Following 1, 3, and 5 days of exposure, the total estimated recovery of PFBS from all tissues evaluated was 10, 5, and 3.4% of the ingested dose, respectively. The declining recovery with time reflects the lack of accumulation in tissues after the first few days, with continued elimination in the urine. The study authors suggested that these low recovery rates most likely reflect rapid excretion of PFBS and/or potentially limited uptake of the compound; however, the results of Lau et al. (2020) and Olsen et al. (2009) suggest that limited tissue distribution is also a factor.

Bogdanska et al. (2014) found that blood levels of PFBS did not change when comparing values observed after 1 and 5 days of exposure. As with PFOS, PFBS was found to distribute to most of the 20 tissues examined at all exposure durations, but the levels of PFBS were significantly lower (fivefold to 40-fold lower) than those of PFOS in tissues after similar exposure to PFOS, especially in liver and lungs (Bogdanska et al., 2014). These differences might be attributed to chain-length-dependent active transport of perfluorinated chemicals (Weaver et al., 2010). Excluding stomach and fat tissue, PFBS tissue levels increased between 1 and 3 days of exposure, but there were no significant changes in tissue levels between 3 and 5 days of exposure in any tissue examined. As with PFOS, whole bone, liver, blood, skin, and muscle accounted for approximately 90% of the recovered PFBS at all time points. The highest tissue concentrations outside of blood, however, were found in the liver, GI tissues, kidney, and cartilage. The significant total PFBS mass found in muscle and skin was due to the large total volume of these tissues rather than the per unit concentration in them. The liver contained the highest tissue concentration of PFBS at all time points, while the brain contained the lowest.

Human studies were not available on lactational transfer of PFBS. Studies are sparse pertaining to the transplacental transfer of PFBS in humans; in a Spanish mother-child paired cohort, PFBS was not found in maternal blood samples or in corresponding cord blood during the first trimester of pregnancy (Manzano-Salgado et al., 2015). However, developmental studies in animals indicate the potential for effects in offspring following gestational exposure, suggesting direct (i.e., fetus) and/or indirect (maternal/pregnant dam) effects of PFBS on offspring (Feng et al., 2017; York, 2003a, 2002).

Volume of distribution is expected to be similar across mammalian species. For PFBS, the average value for male and female monkeys (0.23 L/kg) is in the range estimated for male

and female rats by Huang et al. (2019a) (0.17–0.42 L/kg), although estimates by Olsen et al. (2009) were slightly higher.

1.3.4 Metabolism

There is no evidence of biotransformation of PFBS. It is expected that PFBS, a short-chain (C4) of perfluoroalkyl acids (PFAAs), is metabolically inert because of the chemical stability that also exists in the longer chain PFAA chemicals, including perfluorohexane sulfonic acid (PFHxS) (C6), PFOS (C8), and perfluorooctanoic acid (PFOA) (C8).

1.3.5 Excretion

To facilitate comparison of differing studies for a given species, results for excretion are organized by species.

1.3.5.1 Mice

Lau et al. (2020) dosed male and female CD-1 mice with 0, 30, or 300 mg/kg body weight PFBS via a single gavage dose. Trunk blood was collected at 0.5, 1, 2, 4, 8, 16, 24, and 48 hours after dosing and urine at 24 hours after dosing. Within 24 hours of dosing, more than 95% of the PFBS measured in serum was excreted into urine. Although the rate of PFBS clearance was linear with administered doses, urine accounted for only 30–43% of the original gavage doses. The half-life of PFBS was estimated to be 4.5 hours in the female mice and 5.8 hours in the males. Sex difference in PFBS elimination was also noted in that the elimination rate of absorbed PFBS was about 28% faster in female mice than male mice. Similarly, AUC estimates for the serum, kidney, and liver compartments were higher in males than in females. The findings are generally comparable to previous studies on rats (Huang et al., 2019a; Olsen et al., 2009).

1.3.5.2 Rats

Chengelis et al. (2009) conducted a single-dose pharmacokinetic study in S-D rats, designed to compare the toxicokinetic behavior of PFBS with that of perfluorohexanoic acid (PFHxA), another PFAA. In this study, 12 male and 12 female rats were each administered a bolus dose of PFBS (10 mg/kg) via i.v. injection. Blood samples were collected from three animals per sex at 0.5, 1, 1.5, 2, 4, 8, and 24 hours after dosing. Additionally, to determine urinary excretion, three animals per sex were housed in metabolic cages following dose administration and their urine collected over the following time intervals: 0−6, 6−12, and 12−24 hours postdosing. Chengelis et al. (2009) fit the data to a noncompartmental model to calculate pharmacokinetic parameters. Female rats had an approximately threefold shorter mean elimination half-life of PFBS in serum (0.64 hour) than male rats (2.1 hour). This result could be in part due to the difference in clearance and V_d. The mean apparent clearance of PFBS from the serum was approximately eightfold higher for female rats (0.311 L/hour-kg) than for male rats (0.0394 L/hour-kg), and the mean apparent V_d for PFBS in the serum was approximately 2.4-fold higher for female rats (0.288 L/kg) than for male rats (0.118 L/kg). Approximately 70% of the administered dose of PFBS was recovered in the urine over 24 hours postdosing regardless of sex. Using the urine data, the mean half-life values for male rats and female rats were determined to be 3.1 and 2.4 hours, respectively; the finding of longer urinary half-lives in males is consistent with those observed for serum half-lives.

Olsen et al. (2009) evaluated the elimination of PFBS in S-D rats after i.v. and oral exposure to K^+PFBS. The terminal serum elimination half-lives following i.v. administration of 30 mg/kg K^+PFBS were 4.51 ± 2.22 hours for males and 3.96 ± 0.21 hours for females (mean ± standard deviation [SD]). Although there was no statistically significant difference between the terminal serum half-lives in male and female rats, there was a statistically significant difference in the urinary clearance rates ($p \leq 0.01$), with female rats (469 ± 40 mL/hour) having faster clearance rates than male rats (119 ± 34 mL/hour). Because clearance [CL] is calculated from the ratio of the volume of distribution [V_d] to the half-life [$t_{1/2}$], CL = 0.693 × V_d ÷ $t_{1/2}$, differences in V_d can lead to differences in CL, even when $t_{1/2}$ is similar between comparison groups. For rats receiving an oral dose, terminal serum K^+PFBS elimination half-lives were significantly different ($p \leq 0.05$) for males ($t_{1/2}$ = 4.68 ± 0.43 hour) versus females ($t_{1/2}$ = 7.42 ± 0.79 hour).

Huang et al. (2019a) also evaluated elimination of PFBS following a single i.v. or gavage dose in male or female Hsd:S-D rats (4, 20, 100 mg/kg of K^+PFBS). They reported elimination half-lives ($t_{1/2,\beta}$) following i.v. administration of PFBS in male and female rats of 4.22 and 0.95 hours, respectively. The data for male rats after both oral and i.v. dosing and female rats administered PFBS by i.v. fit a two-compartment model, whereas data in female rats dosed via gavage fit a one-compartment model. Thus, elimination half-lives were only reported for male rats following oral exposure and ranged from 4.89−5.36 hours. Overall plasma elimination half-lives ($t_{1/2}$ k_{10}) reported in female rats after oral administration were between 1.11−1.50 hours, approximately three to fourfold faster than in males that ranged from 4.89−5.36 hours. Similarly, clearance was three to sixfold higher in females than males given the same dose (26.5−75.5 mL/hour-kg in males, 152−259 mL/hour-kg in females).

The serum K^+PFBS elimination half-lives reported by Huang et al. (2019a) are consistent with the findings of Olsen et al. (2009) in male rats but not in female rats. In general, the elimination half-life of serum PFBS observed by Huang et al. (2019a) in female rats was two to fourfold shorter than seen by Olsen et al. (2009). Similarly, Chengelis et al. (2009) calculated half-lives using a one compartment model for each group, whereas Olsen et al. (2009) determined separate α and β phases via a two-compartment model. Thus, the half-life estimates of Olsen et al. (2009) following i.v. administration (4.51−3.96 hours) are higher than those estimated by Chengelis et al. (2009) based on urine data (0.64-2.1 hours).

1.3.5.3 Monkeys

Similar to their study in rats, Chengelis et al. (2009) investigated the toxicokinetic profile of PFBS through a series of experiments in the cynomolgus macaque (*Macaca fascicularis*). Monkeys (three males and three females) were each administered a bolus i.v. dose of 10 mg/kg PFBS. The controlled exposure to PFBS occurred 7 days after the same animals were each administered a bolus dose of PFHxA (10 mg/kg). Blood samples were collected at 0 hours (immediately prior to dosing) and at 1, 2, 4, 8, 24, and 48 hours after dose administration and were analyzed to determine PFBS concentration in serum. Only a single clearance half-life was estimated. The estimated half-life of PFBS in serum ranged from 5.8 to 26.0 hours in this experiment, and the median half-life was 9.55 hours for the six animals.

Olsen et al. (2009) also evaluated the elimination of PFBS (specifically, K^+PFBS) in cynomolgus macaques after i.v. dosing. A significant difference in design from the study of

Chengelis et al. (2009) is that Olsen et al. (2009) followed PFBS elimination for 31 days in monkeys (vs. 48 hours), allowing them to identify both an initial clearance half-life and a terminal-phase half-life. Olsen et al. (2009) did not observe statistically significant sex-related differences in half-life or clearance between male and female monkeys, unlike those observed in rats. In monkeys, the mean terminal serum elimination half-lives, after i.v. administration of 10 mg/kg K^+PFBS, were 95.2 ± 27.1 hours in males and 83.2 ± 41.9 hours in females.

The serum half-life data in Olsen et al. (2009) clearly show a slow elimination phase in monkeys that does not begin until 4−10 days after dosing. Chengelis et al. (2009) followed elimination for only 48 hours, hence could not have observed this terminal clearance phase. The initial elimination half-life ($t_{1/2,\beta}$) estimated by Olsen et al. (2009) in monkeys—13 hours for males, 11 hours for females—is essentially identical to the values estimated by Chengelis et al. (2009)—10 or 15 hours for males (without/with outlier) and 8 hours in females. Hence the two studies appear consistent in identifying an initial elimination half-life, but the difference in design precluded Chengelis and colleagues from identifying the longer (terminal) half-life of PFBS.

1.3.5.4 Humans

In addition to their experimental studies in rats and monkeys, Olsen et al. (2009) evaluated the elimination of human serum K^+PFBS in a group of workers with occupational exposure, with serum concentrations measured up to 180 days after cessation of further K^+PFBS work-related activity. Given that the workers had been occupationally exposed, distribution into the tissues is expected to have been complete before the observations began. The reported mean serum half-life was 23 days in males (n = 5) and 45.7 days in females (n = 1). Among the six subjects (five males, one female), the reported geometric mean serum elimination half-life for K^+PFBS was 25.8 days (95% confidence interval [CI]: 16.6−40.2 days). Because there was only one female subject, these data cannot be used to establish a significant sex difference in elimination. Urine appeared to be a major route of elimination in humans based on observed urine levels of PFBS in the study.

Xu et al. (2020) also measured PFBS elimination in a study population with previous occupational exposure, in this case airport employees who were exposed to firefighting foam that contained PFBS. Eleven male and six female employees provided repeated blood samples during a period of observation with minimal exposure, and the data were analyzed with a linear mixed-effects pharmacokinetic model. The average half-life was 44 days (95% CI: 37−55 days). Although Xu et al. (2020) evaluated age and sex as covariates of their statistical model, they did not report either as being a significant factor for PFBS elimination. The average half-life (44 days) is larger than that reported by Olsen et al. (2009) (25.8 days), but there is significant overlap: the range of Xu et al. (2020) is 21.6−87.2 days while the range of Olsen et al. (2009) is 13.1−45.7 days.

For the sake of comparison, the linear mixed model used by Xu et al. (2020) was also applied to the estimated serum PFBS elimination half-life for the population and each individual worker (five male, one female) who manufactured K^+PFBS, described in Olsen et al. (2009). In brief, a linear mixed effect model is an extension of simple linear models that can be used to estimate toxicokinetic parameters such as the serum elimination rate constant (k_{elim}) and half-life by assuming one-compartment first-order elimination kinetics. The details of the linear

mixed-effect model have been reported previously Li et al. (2018). Because of the limited sample size (only one female worker) and the lack of data on participant age for each worker in the study, age and sex were not included in the linear mixed model for reanalysis of the Olsen et al. (2009) data, whereas both were included in Xu et al. (2020). In general, the estimated half-life using the linear mixed effect model were similar to originally reported values in Olsen et al. (2009). For instance, as compared with the reported average of 25.8 days ranging from 13.1−45.7 days (Olsen et al., 2009), the estimated population elimination half-life for serum PFBS was 25.0 days with individual estimates of 14.6−42.9 days using the linear mixed effect model.

Although the estimated serum half-lives of PFBS in Olsen et al. (2009) overlapped with those of Xu et al. (2020) (mean = 43.8 days, range = 21.9−87.6 days), there is a statistically significant difference between these two studies as suggested by both parametric (one-way analysis of variance [ANOVA]) and nonparametric analyses (Kruskal-Wallis test). Overall, the estimated serum half-life of PFBS by Xu et al. (2020) is about twofold higher than Olsen et al. (2009).

Some of the difference between Xu et al. (2020) and Olsen et al. (2009) may be due to the difference in initial concentration, where the Olsen et al. (2009) subjects had initial concentrations ranging from 100−1,000 ng/mL PFBS, while the highest initial concentrations in Xu et al. (2020) was 1.3 ng/mL. It is possible that the higher serum levels in the Olsen et al. (2009) subjects resulted in saturation of renal resorption, hence more rapid excretion/shorter half-lives. However, to the extent that some ongoing low-level exposure occurred during the period of observation, such exposure would cause a greater bias towards over-estimation of the elimination half-life for the Xu et al. (2020) subjects than those of Olsen et al. (2009). The data of Olsen et al. (2009) might also have a greater signal:noise ratio than the data of Xu et al. (2020). Despite this uncertainty, the fact that the blood concentrations of the Xu et al. (2020) are more representative of environmental exposure, that their sample size was larger, and a significant statistical difference was observed, the two data sets will not be combined and the half-life estimated by Xu et al. (2020) is presumed to better predict human dosimetry at environmental levels.

The possibility that menstrual blood loss could contribute to overall clearance was evaluated, assuming that the concentration of PFBS in menstrual blood is the same as in the general circulation and that the V_d in humans is equal to the average value estimated for monkeys (0.23 L/kg). The results indicate that this avenue of loss is more than two orders of magnitude slower than that indicated by the measured PFBS half-life in humans. Thus, menstrual blood loss is unlikely to contribute significantly to overall PFBS elimination.

1.3.6 Physiologically Based Pharmacokinetic Models

Fàbrega et al. (2015) developed a physiologically based pharmacokinetic model to estimate the concentration of PFAS, including PFBS, in human tissues based on an existing model and experimental data on concentrations of PFAS in human tissues from individuals in Catalonia, Spain. Several uncertainties in the model limit the use for this assessment of PFBS.

There are three chemical-specific parameters that determine the rate of elimination: the free fraction in blood, the maximum rate of resorption in the kidney (T_m), and the saturation

constant for that resorption (K_t). No details beyond a rough description are provided on how these parameter values were identified. The data used for calibration are population samples in adults, who would essentially be at steady state, and only a single average level of exposure and corresponding blood concentration are reported, precluding the possibility of evaluating exposure or concentration dependence. In this situation it is not possible to uniquely identify the three parameters. This lack of identifiability is likely to be an underlying cause of the extreme variability in the individual parameter values (among the 11 PFAS evaluated) reported by Fàbrega et al. (2015).

In addition, the rate constant for elimination from the glomerular filtrate compartment to the urine "storage" compartment (i.e., the bladder) is the total glomerular filtration rate (GFR), which is approximately 10 L/hour in a 70 kg adult. But most of the glomerular flow is resorbed in the nephrons, and human urinary output is less than 2 L/day. Hence, the use of GFR for elimination is not realistic. Finally, note that while the model structure and the equations listed by Fàbrega et al. (2015) appear to be appropriate for most humans, excretion via lactation is not included.

Of considerable concern is the way in which partition coefficients (PCs) were identified. In particular, PCs were obtained by taking tissue concentration data from cadavers and comparing those to average blood concentrations from volunteer subjects, albeit from the same geographical area (county in Spain). The liver:blood PC for PFDA was thereby estimated to be 0.001 while the value for PFNA was 1.65. By contrast, Kim et al. (2019) obtained values of ~0.6–0.7 for PFDA in male and female rats, ~1.2 for PFNA in male rats, and ~0.5 for PFNA in female rats. Thus, there seems to be extreme inconsistency and hence uncertainty in these parameters as estimated by Fàbrega et al. (2015). Generally, human PCs should have values similar to those in rats.

The study authors do not compare model predictions for Tarragona County, Spain, with measured values for county residents (i.e., the data used for model calibration). Also, the study authors state that 20−30 years of simulated time are required to reach steady state. These steady-state estimates are inconsistent with the elimination data from Olsen et al. (2009), in which the half-life in males was 24 days, and in one female subject 46 days. These empirical half-lives are consistent with a time to steady state of less than a year, indicating that the predicted clearance from Fàbrega et al. (2015) may be an order of magnitude or more too low. At the same time, the simulated levels of five PFAS (average levels) were consistently lower than the averages in the validation data, four of these being lower by an order of magnitude or more.

Thus, predictions of the Fàbrega et al. (2015) model are considered highly uncertain, and data other than those used by the study authors will be needed to accurately estimate key pharmacokinetic (PK) parameters for PFBS and these other PFAS, a task that would require significant additional research.

1.3.7 Summary

Collectively, elimination half-lives appear to be similar for mice and rats, with potential sex-specific toxicokinetic differences being reported (i.e., females appearing to have a faster elimination rate). Humans have a longer serum elimination half-life (~weeks) than both rodents

(~hours) and monkeys (~days). Further, although V_d information is not available for humans, observations in male and female mice, rats, and monkeys exposed to comparable doses indicate comparability across species. Results of all studies discussed in this section are summarized in Table 2.

2.0 PROBLEM FORMULATION

2.1 CONCEPTUAL MODEL

A conceptual model was developed to summarize the availability of data to understand potential health hazards related to exposure to PFBS and/or K^+PFBS. The potential sources of these chemicals, the routes of exposure for biological receptors of concern (e.g., various human activities related to ingested drinking water, and food preparation and consumption), the potential organs and systems affected by exposure (e.g., effects such as developmental toxicity), and potential populations at risk due to exposure to PFBS and/or potassium salt are depicted in the conceptual diagram in Figure 2. Arrows indicate linkage between one or more boxes between levels of organization.

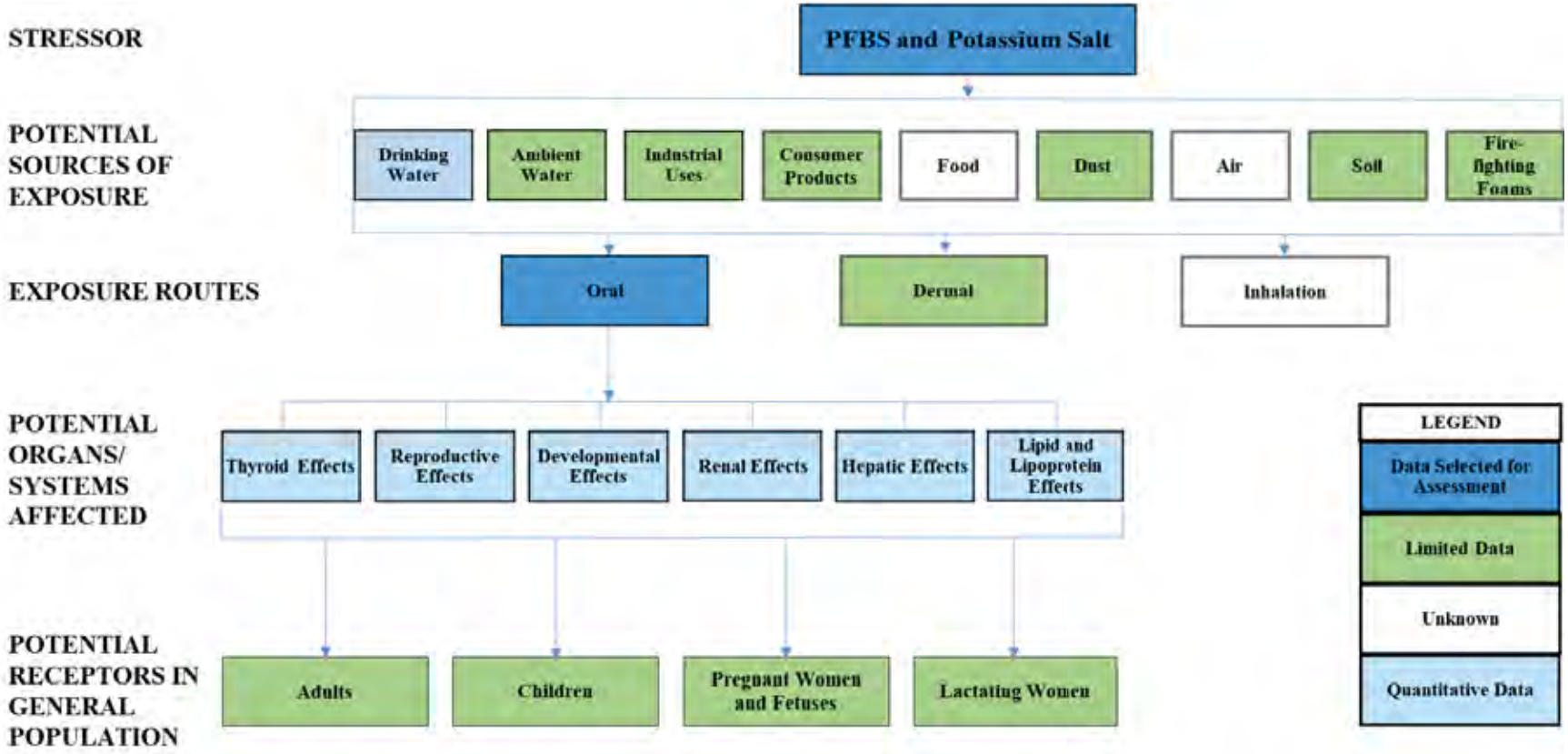


Figure 2. Conceptual Model for PFBS and/or Potassium Salt

2.2 OBJECTIVE

The overall objective of this assessment is to provide the health effects basis for the development of oral reference doses (RfDs) for PFBS (CASRN 375-73-5) and its related compound K^+PFBS (CASRN 29420-49-3), including the science-based decisions providing the basis for identifying potential human health effects and estimating PODs. Based on the needs of the U.S. EPA partner program offices, regions, states, and/or tribes as they pertain to diverse exposure scenarios and human populations, subchronic and chronic RfDs have been derived. The assessment includes studies and information previously provided in the 2014 PPRTV assessment (U.S. EPA, 2014f) and builds upon data from the literature published since that review.

2.3 METHODS

2.3.1 Literature Search

Four online scientific databases (PubMed, Web of Science, TOXLINE, and TSCATS via TOXLINE) were searched by the U.S. EPA's Health and Environmental Research Online (HERO) staff and stored in the HERO database.[5] The literature search focused on chemical name and synonyms with no limitations on publication type, evidence stream (i.e., human, animal, in vitro, and in silico), or health outcomes. Full details of the search strategy for each database are presented in Appendix A. The initial database searches were conducted on July 18, 2017 and updated on February 28, 2018; May 1, 2019; and May 15, 2020. Additional studies [e.g., Lau et al. (2020); Xu et al. (2020)] were identified during subsequent review periods and integrated into the assessment as appropriate. Studies were also identified from other sources relevant to PFBS, including studies submitted to the U.S. EPA by the manufacturer of PFBS (i.e., 3M) as part of the Toxic Substances Control Act (TSCA) premanufacture notices for other PFAS chemicals or as required under TSCA reporting requirements and studies referenced in prior evaluations of PFBS toxicity (MDH, 2020; ATSDR, 2015). In addition, on March 29, 2018, the National Toxicology Program (NTP) published study tables and individual animal data from a 28-day toxicity study of PFBS (http://doi.org/10.22427/NTP-DATA-002-01134-0003-0000-4), with a protocol outlining the NTP study methods available in HERO (https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/4309741) (NTP, 2011). The final *NTP Technical Report on the Toxicity Studies of Perfluoroalkyl Sulfonates Administered by Gavage to Sprague-Dawley Rats* was published in August, 2019 (NTP, 2019).

2.3.2 Screening Process

Two screeners independently conducted a title and abstract screening of the search results using DistillerSR[6] to identify study records that met the Population, Exposure, Comparator, and Outcome (PECO) eligibility criteria (see Appendix B for a more detailed summary):

[5]The U.S. EPA's HERO database provides access to the scientific literature behind U.S. EPA science assessments. The database includes more than 2,500,000 scientific references and data from the peer-reviewed literature used by the U.S. EPA to develop its regulations.

[6]DistillerSR is a web-based systematic review software used to screen studies available at https://www.evidencepartners.com/products/distillersr-systematic-review-software.

- Population: Human and nonhuman mammalian animal species (whole organism) of any life stage and in vitro models of genotoxicity.
- Exposure: Any qualitative or quantitative estimates of exposure of PFBS or K^+PFBS, via oral or inhalation routes of exposure. (Note: Nonoral and noninhalation studies are tracked as potential supplemental material and are presented in Section 4.8.2.)
- Comparator: A comparison or reference population exposed to lower levels or for shorter periods of time for humans. Exposure to vehicle-only or untreated control in animals.
- Outcome: Any examination of cancer or noncancer health outcomes.

In addition to the PECO criteria, the following additional exclusion criteria were applied, although these study types were tracked as supplemental material as described following the exclusion criteria:

- Records that do not contain original data such as other agency assessments, scientific literature reviews, editorials, and commentaries;
- Abstract only (e.g., conference abstracts); and
- Retracted studies.

Records that were not excluded based on title and abstract screening advanced to full-text review using the same PECO eligibility criteria. Studies that have not undergone peer review were included if the information could be made public and sufficient details of study methods and findings were included in the reports. Full-text copies of potentially relevant records identified from title and abstract screening were retrieved, stored in the HERO database, and independently assessed by the screeners using DistillerSR to confirm eligibility. At both title/abstract and full-text review levels, screening conflicts were resolved by discussion between the primary screeners in consultation with a third reviewer to resolve any remaining disagreements. During title/abstract or full-text level screening, studies that were not directly relevant to the PECO, but could provide supplemental information, were categorized (or "tagged") by the type of supplemental information they provided (e.g., review, commentary, or letter with no original data; conference abstract; toxicokinetics; mechanistic information aside from in vitro genotoxicity studies; other routes of exposure; exposure only). Conflict resolution was not required during the screening process to identify supplemental information (i.e., tagging by a single screener was sufficient to identify the study as potential supplemental information).

2.3.3 Study Evaluation

Study evaluation was conducted by one reviewer for epidemiological studies and by two independent reviewers for animal studies using the U.S. EPA's version of Health Assessment Workspace Collaborative (HAWC), a free and open source web-based software application designed to manage and facilitate the process of conducting literature assessments.[7] For pragmatic purposes, only one reviewer was considered necessary for epidemiological studies because it was apparent during literature screening that the animal evidence would be the most informative for deriving toxicity values. The available outcomes in the epidemiological studies were heterogeneous and unrelated to each other, and only a single study was available for each

[7]HAWC: A modular web-based interface to facilitate development of human health assessments of chemicals (https://hawcproject.org/).

outcome. This approach is consistent with recommendations from the National Academies of Science encouraging the U.S. EPA to explore ways to make systematic review more feasible, including a "rapid review in which components of the systematic review process are simplified or omitted (e.g., the need for two independent reviewers)" (NASEM, 2017). Study evaluation was not conducted for studies tagged during screening as supplemental information.

The general approach for evaluating epidemiology and animal toxicology was the same (see Figure 3), but the specifics of applying the approach differed. These evaluations were focused on the methodological approaches and completeness of reporting in the individual studies, rather than on the direction or magnitude of the study results. Evaluation of epidemiology studies was conducted for the following domains: exposure measures, outcome measures, participant selection, confounding, analysis, sensitivity, and selective reporting. For animal studies, the evaluation process focused on assessing aspects of the study design and conduct through three broad types of evaluations: reporting quality, risk of bias, and study sensitivity. A set of domains with accompanying core questions fall under each evaluation type and directed individual reviewers to evaluate specific study characteristics. For each domain evaluated for experimental animal studies (reporting quality, selection or performance bias, confounding/variable control, reporting or attrition bias, exposure methods sensitivity, and outcome measures and results display), basic considerations provided additional guidance on how a reviewer might evaluate and judge a study for that domain. Core and prompting questions used to guide the criteria and judgment for each domain are presented in Appendix C. Key concerns for the review of epidemiology and animal toxicology studies are potential sources of bias (factors that could systematically affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect).

For each study in each evaluation domain, reviewers reached a consensus rating regarding the utility of the study for hazard identification, with categories of *good*, *adequate*, *deficient*, *not reported*, or *critically deficient*. These ratings were then combined across domains to reach an overall classification of *high*, *medium*, or *low confidence* or *uninformative* (definitions of these classifications are available in Appendix C). The rationale for the classification, including a brief description of any identified strengths and/or limitations from the domains and their potential impact on the overall confidence determination, is documented and retrievable in HAWC. Uninformative studies were not used in evidence synthesis or dose-response analysis. Studies were evaluated for their suitability for each health outcome investigated and could receive different ratings for each outcome.

For epidemiological studies, exposure-specific criteria were developed prior to evaluation and are described in detail in Appendix C. In brief, standard analytical methods of measurement of PFBS in serum or whole-blood using quantitative techniques such as liquid chromatograph-triple quadrupole mass spectrometry and high-pressure liquid chromatography with tandem mass spectrometry were preferred. In addition, exposure must have been assessed in a relevant time window for development of the outcome.

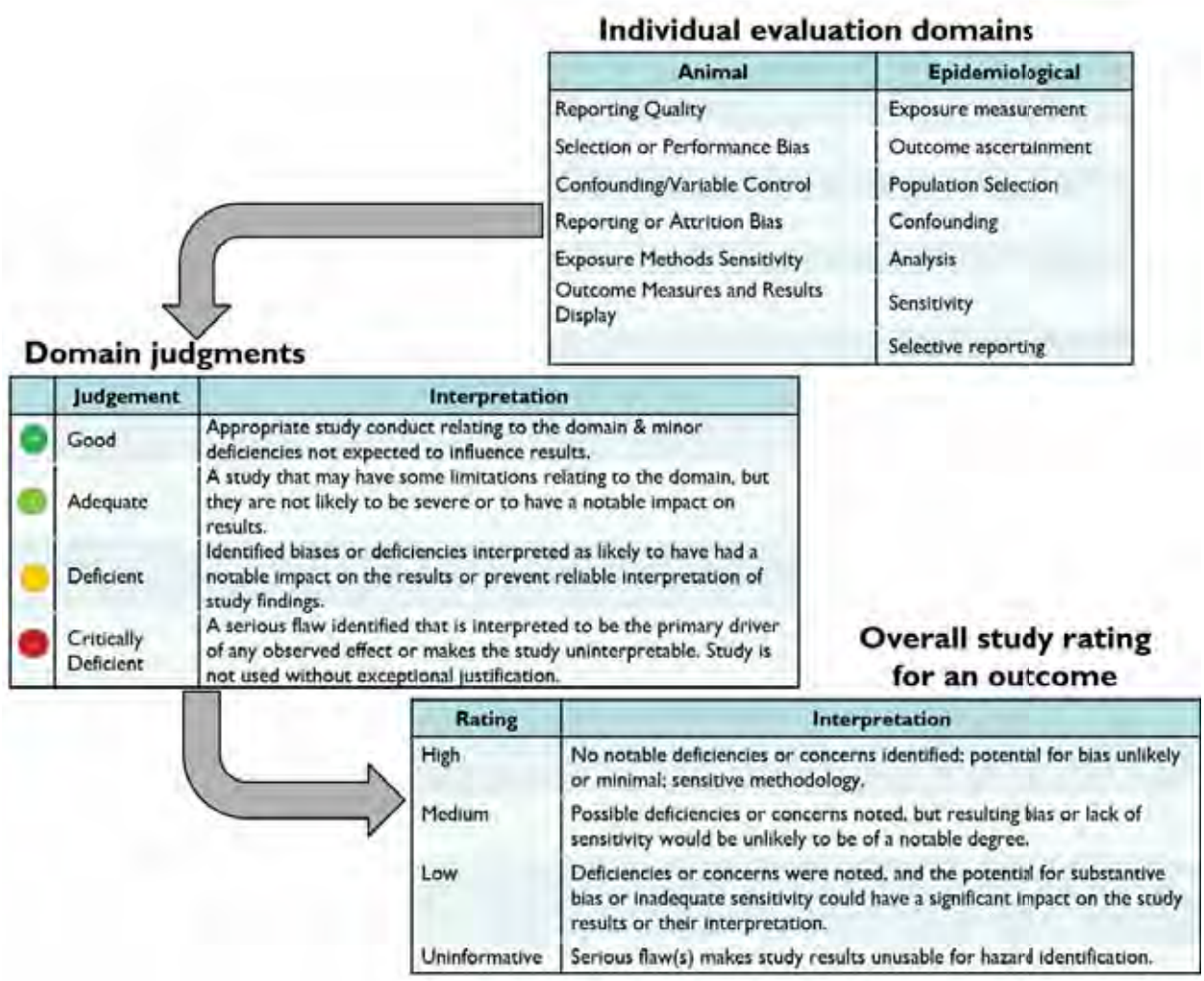


Figure 3. Approach for Evaluating Epidemiological and Animal Toxicology Studies

2.3.4 Data Extraction

Information on study design, methods, results, and data from animal toxicology studies were extracted into the HAWC and are available at https://hawcprd.epa.gov/assessment/100000037/. Visual graphics prepared from HAWC are embedded as hyperlinks and are fully interactive when viewed online by way of a "click to see more" capability. Clicking on content allows access to study evaluation ratings, methodological details, and underlying study data. The action of clicking on content contained in those visual graphics (e.g., data points, endpoint, and study design) will yield the underlying data supporting the visual content.[8] A HAWC user guide can be found in Appendix D. Study methods and findings from epidemiological studies were described in narratives, given the small size and heterogeneity of the evidence base. Data extraction was performed by one member of the evaluation team and checked by one to two other members. Any discrepancies in data extraction

[8]The following browsers are fully supported for accessing HAWC: Google Chrome (preferred), Mozilla Firefox, and Apple Safari. There are errors in functionality when viewed with Internet Explorer.

were resolved by discussion or consultation with a third member of the evaluation team. Digital rulers such as WebPlotDigitizer and Grab It! (https://automeris.io/WebPlotDigitizer/ and https://grab-it.soft112.com/, respectively) were used to extract numerical information from figures. Use of digital rulers was documented during extraction. Dose levels were extracted as reported in the study and converted to mg/kg-day (HED) for endpoints that were considered for use in the dose-response and derivation of toxicity values.

2.3.5 Evidence Synthesis

For the purposes of this assessment, after study evaluation, the informative evidence for each outcome was summarized from the available human studies and, separately, the available animal studies. This synthesis provides a short synopsis of the breadth of data available to inform each outcome and summarizes information on the general study design, doses tested, outcomes evaluated, and results for the endpoints of interest within each study. While the evidence synthesis describes inferences about the methodological rigor and sensitivity of the individual studies (i.e., study confidence) and discusses the pattern and magnitude of the experimental findings within studies, it does not include conclusions drawn across the sets of studies (see "Evidence Integration and Hazard Characterization," next).

2.3.6 Evidence Integration and Hazard Characterization

In this assessment, the evaluation of the available evidence from informative human and animal studies was described in an evidence integration narrative for each outcome, including overall evidence integration judgments as to whether the data provide evidence sufficient to support a hazard. These integrated judgments serve to characterize the extent of the available evidence for each outcome, including information on potential susceptible populations and life stages, as well as important uncertainties in interpreting the data.

The evidence integration for each health effect considered aspects of an association that might suggest causation first introduced by Austin Bradford Hill (Hill, 1965), including the consistency, exposure-response relationship, strength of association, biological plausibility, and coherence of the evidence. This involved weighing the PFBS-specific human and animal evidence relating to each of these considerations within or across studies, including both evidence that supports causation as well as evidence that indicates lack of support. For example, the evaluation of consistency examined the similarity of results across studies (e.g., direction and magnitude). When inconsistencies across studies were identified, the evaluation considered whether results were "conflicting" (i.e., unexplained positive and negative results in similarly exposed human populations or in similar animal models) or "differing" (i.e., mixed results explained by differences between human populations, animal models, exposure conditions, or study methods), based on analyses of potentially important explanatory factors such as confidence in the studies' results (the results of higher confidence studies were emphasized), exposure levels or duration, or differences in populations or species (including potential susceptible groups) across studies (U.S. EPA, 2005). While consistent evidence across studies increases support for a hazard, unexplained inconsistency or conflicting evidence decreases support for a hazard. The evaluations of these considerations were informed by U.S. EPA guidelines, including *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a) and *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996a).

The overall evidence integration judgments were developed using a structured framework based on evaluation of the considerations above (see Table 3). Using this framework, the human and animal evidence for each health effect was judged separately as *supports a hazard*, *equivocal*, or *supports no hazard*. Evidence integration judgments of *supports a hazard* span a range of supportive evidence bases that can be further differentiated by the quantity and quality of information available to rule out alternative explanations for the results. *Equivocal* evidence is limited in terms of the quantity, consistency, or confidence level of the available studies and serves to encourage additional research. *Supports no hazard* requires several high-confidence studies across potentially susceptible populations with consistent null results; this judgment was not reached in this assessment. Overall evidence integration judgments were made based on conclusions from both the animal and human data, considering the available information on the human relevance of findings in animals. Thus, for example, evidence in animals that *supports a hazard* alongside *equivocal* human evidence in the absence of information indicating that the responses in animals are unlikely to be relevant to humans would result in an overall judgment of *supports a hazard* for that outcome.

Table 3. Criteria for Overall Evidence Integration Judgments

	Animal	**Human**
Supports a hazard	The evidence for effects is consistent or largely consistent in at least one high- or medium-confidence experiment.[a] Although notable uncertainties across studies might remain, any inconsistent evidence or remaining uncertainties are insufficient to discount the cause for concern from the positive experiments. In the strongest scenarios, the set of experiments provide evidence supporting a causal association across independent laboratories or species. In other scenarios, including evidence for an effect in a single study, the experiment(s) demonstrate additional support for causality such as coherent effects across multiple related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; and/or consistent observations across exposure scenarios (e.g., route, timing, or duration), sexes, or animal strains.	One or more high- or medium-confidence independent studies reporting an association between the exposure and the health outcome. In general, the study results are largely consistent or any inconsistent results are insufficient to discount the cause for concern from the higher confidence study or studies, and there is reasonable confidence that alternative explanations, including chance, bias, and confounding, have been ruled out. In situations in which only a single study is available, the results of multiple studies are heterogeneous, or alternative explanations, including chance, bias and confounding, have not been ruled out, there is additional supporting evidence such as associations with biologically related endpoints in other human studies (coherence), large estimates of risk, or strong evidence of an exposure-response within or across studies.

Table 3. Criteria for Overall Evidence Integration Judgments

	Animal	Human
Equivocal	The evidence is generally inadequate to determine hazard. This includes a lack of relevant studies available or a set of low-confidence experiments. It also includes scenarios with a set of high- or medium-confidence experiments that are not reasonably consistent or not considered informative to the hazard question under evaluation. This category would also include a single high- or medium-confidence experiment with weak evidence of an effect (e.g., changes in one endpoint among several related endpoints, and without additional evidence supporting causality).	The evidence is considered inadequate to describe an association between exposure and the health outcome with confidence. This includes a lack of studies available in humans, only low-confidence studies, or considerable heterogeneity across medium- or high-confidence studies. This also includes scenarios in which there are serious residual uncertainties across studies (these uncertainties typically relate to exposure characterization or outcome ascertainment, including temporality) in a set of largely consistent medium- or high-confidence studies.
Supports no hazard	A set of high-confidence experiments examining the full spectrum of related endpoints within a type of toxicity, with multiple species, and testing a reasonable range of exposure levels and adequate sample size in both sexes, with none showing any indication of effects. The data are compelling in that the experiments have examined the range of scenarios across which health effects in animals could be observed, and an alternative explanation (e.g., inadequately controlled features of the studies' experimental designs) for the observed lack of effects is not available. The experiments were designed to specifically test for effects of interest, including suitable exposure timing and duration, post-exposure latency, and endpoint evaluation procedures, and to address potentially susceptible populations and life stages.	Several high-confidence studies, showing consistently null results (e.g., an OR of 1.0) ruling out alternative explanations including chance, bias, and confounding with reasonable confidence. Each of the studies should have used an optimal outcome and exposure assessment and adequate sample size (specifically for higher exposure groups and for sensitive populations). The set as a whole should include the full range of levels of exposures that human beings are known to encounter, an evaluation of an exposure response gradient, and at-risk populations and life stages and should be mutually consistent in not showing any indication of effect at any level of exposure.

[a]"Experiment" refers to measurements in a single population of exposed animals (e.g., a study that included separate evaluations of rats and of mice, or separate cohorts exposed at different life stages, would be considered as multiple experiments). Conversely, two papers or studies that report on the same cohort of exposed animals (e.g., examining different endpoints) would not be considered separate experiments.

OR = odds ratio.

The primary evidence and rationale supporting these decisions were summarized in a single evidence profile table to transparently convey the aspects of the evidence that were considered to increase or decrease the hazard support for each health effect. For the purposes of this assessment, only the integrated evidence that *supports a hazard* was considered for use in the dose-response analysis and derivation of toxicity values.

2.3.7 Derivation of Values

Development of the dose-response assessment for PFBS and/or the potassium salt has followed the general guidelines for risk assessment put forth by the National Research Council (NRC, 1983) and the U.S. EPA's *Framework for Human Health Risk Assessment to Inform*

Decision Making (U.S. EPA, 2014c). Other U.S. EPA guidelines and reviews considered in the development of this assessment include the following:

- *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006).
- *Exposure Factors Handbook* (U.S. EPA, 2011a).[9]
- *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
- *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (U.S. EPA, 2014d).
- *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012).
- *Child-Specific Exposure Scenarios Examples* (U.S. EPA, 2014a).

The U.S. EPA's *A Review of the Reference Dose and Reference Concentration Processes* describes a multistep approach to dose–response assessment, including analysis in the range of observation followed by extrapolation to lower levels (U.S. EPA, 2002). As described above, before deriving toxicity values, the U.S. EPA conducted a comprehensive evaluation of available human epidemiological and animal toxicity studies to identify potential health hazards and associated dose-response information through the literature search and screening, study evaluation, evidence synthesis, and evidence integration steps. This evaluation informed the selection of candidate key studies and critical effects for dose-response analysis, from which the U.S. EPA identified a critical effect and point of departure (POD) for subchronic and chronic reference value derivation and extrapolated a selected POD to a corresponding RfD (e.g., subchronic RfD). For dose-response analysis of PFBS and/or the potassium salt, the U.S. EPA used the BMD approach to identify a POD. The steps for deriving an RfD using the BMD approach are summarized below.

- **Step 1: Evaluate the data to identify and characterize endpoints related to exposure to PFBS chemicals.** This step involved determining the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data were collected, evaluated for study quality, and characterized for adverse outcomes, endpoints were selected that were judged to be relevant (i.e., for the purposes of this assessment, effects that were sufficient to *support a hazard*) and sensitive as a function of dose (typically defined by the no-observed-adverse-effect level [NOAEL] value). In this assessment, these decisions were directly informed by the evidence integration judgments arrived at for each assessed health outcome. Some of the most important considerations that influenced selection of endpoints for BMD modeling include data showing a dose-response relationship, percent change from controls, adversity of effect, and consistency across studies. For PFBS, thyroid, developmental, and kidney endpoints were considered for toxicity value derivations.

[9]Please note that specific updates to this handbook are available at https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=236252.

- **Step 2: Convert the adjusted daily doses to an HED.** The adjusted daily doses were converted to HEDs by considering U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
- **Step 3: Select the benchmark response (BMR) level.** The endpoints selected were modeled using the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012). The BMR is a predetermined change in the response rate of an adverse effect. It serves as the basis for obtaining the benchmark dose lower confidence limit (BMDL), which is the 95% lower bound of the BMD. BMRs were identified and applied consistent with quantal and continuous data and, when possible, informed by understanding of biological significance.
- **Step 4: BMD model the data.** This step involved fitting a statistical model to the dose-response data that describes the data set of the identified adverse effect. Typically, this involved selecting a family or families of models (e.g., polynomial continuous, Hill continuous, or exponential continuous) for further consideration based on the data and experimental design. In this step, a BMDL was derived by placing confidence limits (one- or two-sided) and a confidence level (typically 95%) on a BMD to obtain the dose that ensures with high confidence that the BMR is not exceeded.
- **Step 5: Determine a POD (HED).** If modeling was feasible, the estimated BMDL (HED)s were used as PODs (i.e., POD [HED]). If dose-response modeling was not feasible, NOAEL (HED)s or lowest-observed-adverse-effect level (LOAEL) (HED)s were identified.
- **Step 6: Provide rationale for selecting uncertainty factors.** Uncertainty factors were selected in accordance with U.S. EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the key study compared to a lifetime of the species studied, and the potential limitations of the toxicology database (U.S. EPA, 2014d, 2011b, 2002, 1994).
- **Step 7: Calculate the subchronic and chronic RfDs.** The RfDs were calculated by dividing a POD (HED) by the selected uncertainty factors.

$$\text{RfD} = \frac{\text{POD (HED)}}{\text{UF}_C}$$

where:

POD (HED) is calculated from the BMDL or NOAEL using a $BW^{3/4}$ allometric scaling approach consistent with U.S. EPA guidance (U.S. EPA, 2011b)

UF_C is established in accordance with U.S. EPA guidelines (U.S. EPA, 2014d, 2011b, 2002, 1994) considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the key study compared to a lifetime of the species studied, and the potential limitations of the toxicology database.

- **Step 8: Assignment of Confidence Levels.** In assessments in which an RfD or RfC is derived, characterization of the level of confidence in the principal study(ies), the database associated with that reference value, and the overall confidence in the reference value(s) are provided. Details on characterizing confidence are provided in Chapter 4

(specifically Section 4.3.9.2) of the U.S. EPA's *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). For example, the ranking of confidence in the database (low, medium, or high) reflects EPA's assessment of the degree to which the reference value (e.g., RfD) might potentially change (in either direction) with the acquisition of new data.

3.0 OVERVIEW OF EVIDENCE IDENTIFICATION FOR SYNTHESIS AND DOSE-RESPONSE ANALYSIS

3.1 LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 451 unique records, with 50 records identified from additional sources, such as TSCA submissions, posted NTP study tables, peer-review recommendations, and review of reference lists from other authoritative sources. Of the 501 studies identified, 377 were excluded during title and abstract screening, 124 were reviewed at the full-text level, and 42 were considered relevant to the PECO eligibility criteria (see Figure 4). This included 19 epidemiologic studies (described in 22 publications), 10 in vivo animal studies (described in 15 peer-reviewed and non-peer-reviewed publications), and 5 in vitro genotoxicity studies. The detailed search approach, including the query strings and PECO criteria, is provided in Appendix A and Appendix B, respectively.

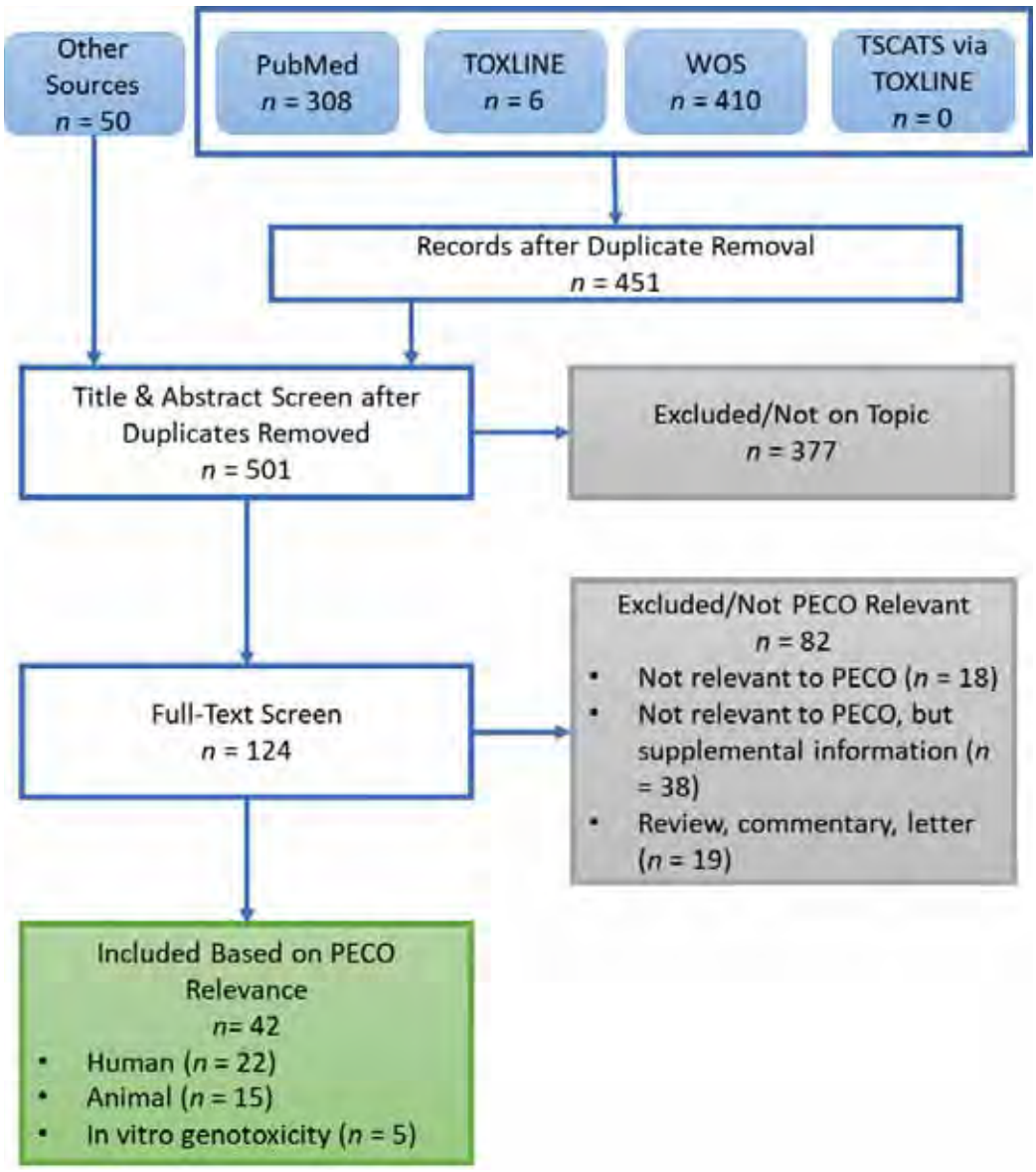


Figure 4. Literature Search and Screening Flow Diagram for PFBS (CASRN 375-73-5)

3.2 STUDY EVALUATION RESULTS

Based on the study evaluations, seven human epidemiology studies were considered uninformative and are not discussed any further in this assessment (see Table 4). All animal studies were considered informative and thus were identified as relevant during literature screening and included in the evidence synthesis and dose-response analysis. Overall, 12 epidemiologic studies (described in 15 publications) and 10 in vivo animal studies (described in 15 peer-reviewed and non-peer-reviewed publications) were included in the evidence synthesis and further evaluated for use in the development of toxicity values for PFBS. As shown in Figures 5 and 6, while the database of studies on PFBS is not large, several high- and medium-confidence oral exposure studies in animals were identified, as were several

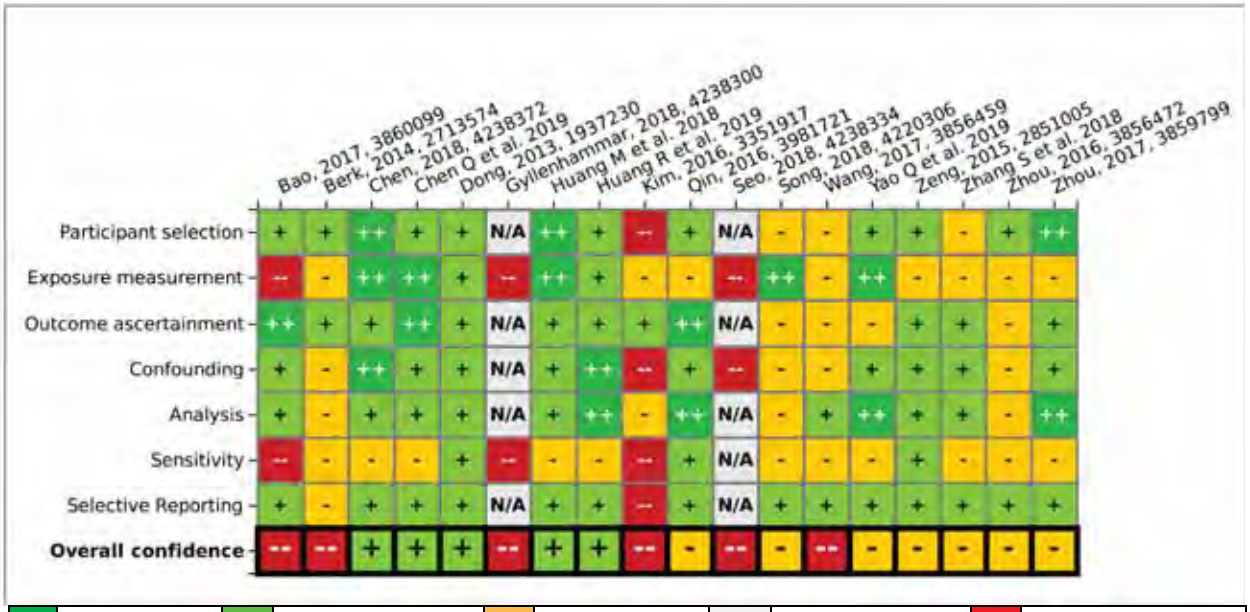
medium-confidence studies in humans. Multiple publications of the same study are not listed as independent studies in HAWC, they are reviewed together in one entry. In addition, Shiue (2016) was not evaluated because the outcome (i.e., sleep disturbances) was considered a nonspecific effect, and thus was not entered into HAWC. No studies were identified evaluating the toxicity of PFBS or K^+PFBS following inhalation exposure or on the carcinogenicity of PFBS or K^+PFBS in humans or animals.

Table 4. Epidemiological Studies Excluded Based on Study Evaluation

Reference	Outcome	Reason for Exclusion
Bao et al. (2017)	Blood pressure	Extremely poor sensitivity (96% of participants below the LOD for PFBS measurement) with no observed association.
Berk et al. (2014)	Depression	Serious concerns with temporality between exposure and outcome, confounding, and analysis.
Gyllenhammar et al. (2018)	Birth size, weight gain	Extremely poor sensitivity (median exposure = 0.01 ng/g, IQR LOD-0.04, 43% below the LOD for PFBS measurement) with no observed association.
Kim et al. (2016)	Congenital hypothyroidism	Excluded from full statistical analysis by study authors because of a high percentage below the LOD (72%) for PFBS measurement.
Seo et al. (2018)	Cholesterol, uric acid, diabetes, BMI, thyroid hormones	No consideration of potential confounding.
Shiue (2016)[a]	Sleep disturbances	Not evaluated because of nonspecific effect.
Wang et al. (2017)	Endometriosis-related infertility	Exposure measured concurrent with outcome for chronic outcome; serious concerns for exposure and outcome misclassification.

[a]Shiue (2016) was not evaluated because the outcome was sleep disturbances, which was considered a nonspecific effect, and thus was not entered in HAWC.

BMI = body mass index; HAWC = Health Assessment Workspace Collaborative; IQR = interquartile range; LOD = limit of detection; PFBS = perfluorobutane sulfonic acid.



++	Good (metric) or high (overall)	+	Adequate (metric) or medium (overall)	–	Deficient (metric) or low (overall)	N/A	Not assessed because of critical deficiency in other domain	- -	Critically deficient (metric) or uninformative (overall)

Figure 5. Evaluation Results for Epidemiological Studies Assessing Effects of PFBS (Click to see interactive data graphic for rating rationales)

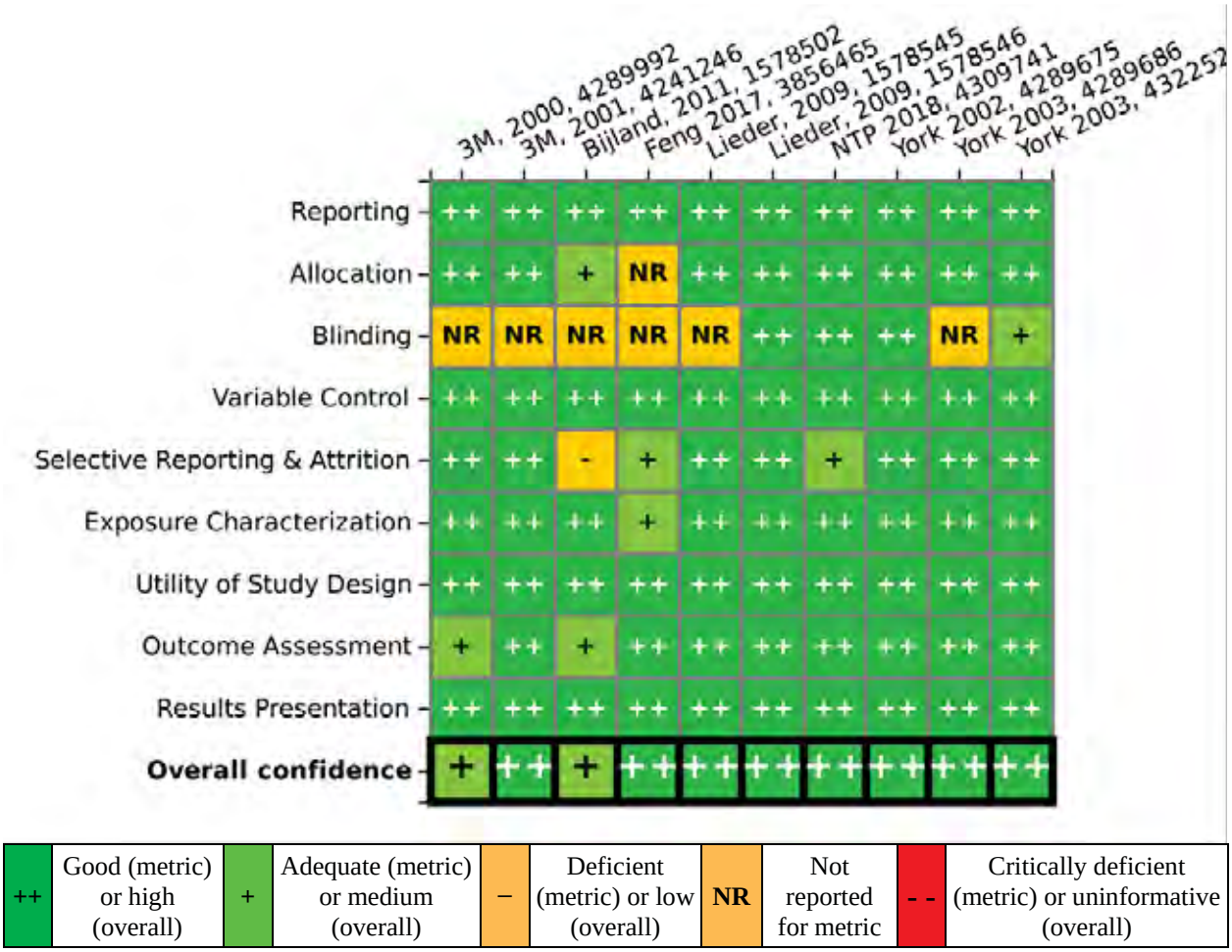


Figure 6. Evaluation Results for Animal Studies Assessing Effects of PFBS Exposure (Click to see interactive data graphic for rating rationales)

4.0 EVIDENCE SYNTHESIS: OVERVIEW OF INCLUDED STUDIES

The database of all repeated-dose oral toxicity studies for PFBS and the related compound K^+PFBS that are potentially relevant for deriving RfD values includes a short-term range-finding study in rats (3M, 2000d), two 28-day studies in rats (NTP, 2019; 3M, 2001), one subchronic study in rats (Lieder et al., 2009a; York, 2003b), one subchronic-duration lipoprotein metabolism study in mice (Bijland et al., 2011; 3M, 2010), three gestational exposure studies in mice and rats (Feng et al., 2017; York, 2003a, 2002), and a two-generation reproductive toxicity study in rats (Lieder et al., 2009b; York, 2003c, d, e). In addition, 19 epidemiological studies (described in 22 publications) were identified that report on the association between PFBS and human health effects. Specific study limitations identified during evaluation (see HAWC) are discussed only for studies interpreted as low confidence or if a limitation affected a specific inference for drawing conclusions.

Human and animal studies have evaluated potential effects on the thyroid, reproductive systems, development, kidneys, liver, and lipid and lipoprotein homeostasis following exposure to PFBS. The evidence base for these outcomes is presented in this section. For each potential health effect, the synthesis describes the database of human and animal studies, as well as an array of the animal results across studies. NOAELs and LOAELs presented in the figures and text are based on statistical significance and/or biological significance (e.g., directionality of effect [statistically significantly decreased cholesterol/triglycerides is of unclear toxicological relevance], abnormal or irregular dose-response relationship [nonmonotonicity], tissue-specific considerations for magnitude of effect [statistically nonsignificant increase of ≥10% in liver weight interpreted as biologically significant]). A summary of the available database is presented in Table 6 of Section 5. For information in this section, evidence to inform organ/system-specific effects of PFBS in animals following developmental exposure is discussed in the individual organ/system-specific sections (e.g., reproductive cycling endpoints after developmental exposure are discussed in the "Reproductive Effects" section). Other effects informing potential developmental effects (e.g., pup BW) are discussed in the "Offspring Growth and Early Development" section.

Evidence integration analyses and overall judgments on the hazard support for each outcome domain provided by the available human and animal studies are discussed in the "Evidence Integration and Hazard Characterization" section. Notably, in that section, the evidence informing organ/system-specific endpoints after developmental exposure was considered potentially informative to both the developmental effects outcome domain and the organ/system-specific outcome domain.

4.1 THYROID EFFECTS

4.1.1 Human Studies

One low-confidence study examined cross-sectional associations between PFBS exposure and thyroid hormones in women with premature ovarian insufficiency (Zhang et al., 2018) and reported no association with free T_3, free T_4, or thyroid-stimulating hormone. However, this study had poor sensitivity and methodological limitations that make interpreting these null results difficult; further, the results in this highly selected population may not be generalizable.

4.1.2 Animal Studies

Two high-confidence studies evaluated the effects of PFBS exposure on the thyroid, specifically thyroid hormone levels, thyroid histopathology, and thyroid weight (NTP, 2019; Feng et al., 2017) (see Figure 7). Dams exposed to K^+PFBS through gestation (GDs 1−20) exhibited a statistically significant decrease in total triiodothyronine (T_3), total thyroxine (T_4), and free T_4 (reduced 17, 21, and 12%, respectively, relative to control at 200 mg/kg-day and reduced 16, 20, and 11%, respectively, relative to control at 500 mg/kg-day) on GD 20 at doses of 200 and 500 mg/kg-day, but not at 50 mg/kg-day (Feng et al., 2017). Decreased total T_3 and total T_4 were also reported at PNDs 1, 30, and 60 in offspring gestationally exposed to K^+PFBS at the same doses (up to 37% reduction in T_3 and 52% reduction in T_4). Increased thyroid-stimulating hormone (TSH) was reported in dams and pubertal (PND 30) offspring (21 and 14% relative to control at 200 mg/kg-day, respectively) exposed gestationally to K^+PFBS. Statistically significant dose-dependent decreases in total T_3, total T_4, and free T_4 were also reported after exposure in male and female rats to K^+PFBS for 28 days at all doses tested (≥62.6 mg/kg-day) (NTP, 2019). The reported reductions in rat total T_3 were up to −57% (male) and −43% (female), in free T_4 up to −86% (male) and −77% (female), and in total T_4 up to −97% (male) and −71% (female). Dose-response graphics for T_4, T_3, and TSH, including effect size and variability, are included in Appendix E, Figures Figure E-1, Figure E-2, and Figure E-3, respectively. Thyroid gland weight, thyroid histopathology, and TSH levels were not changed after 28 days of PFBS exposure in male or female rats at doses up to 1,000 mg/kg-day (NTP, 2019).

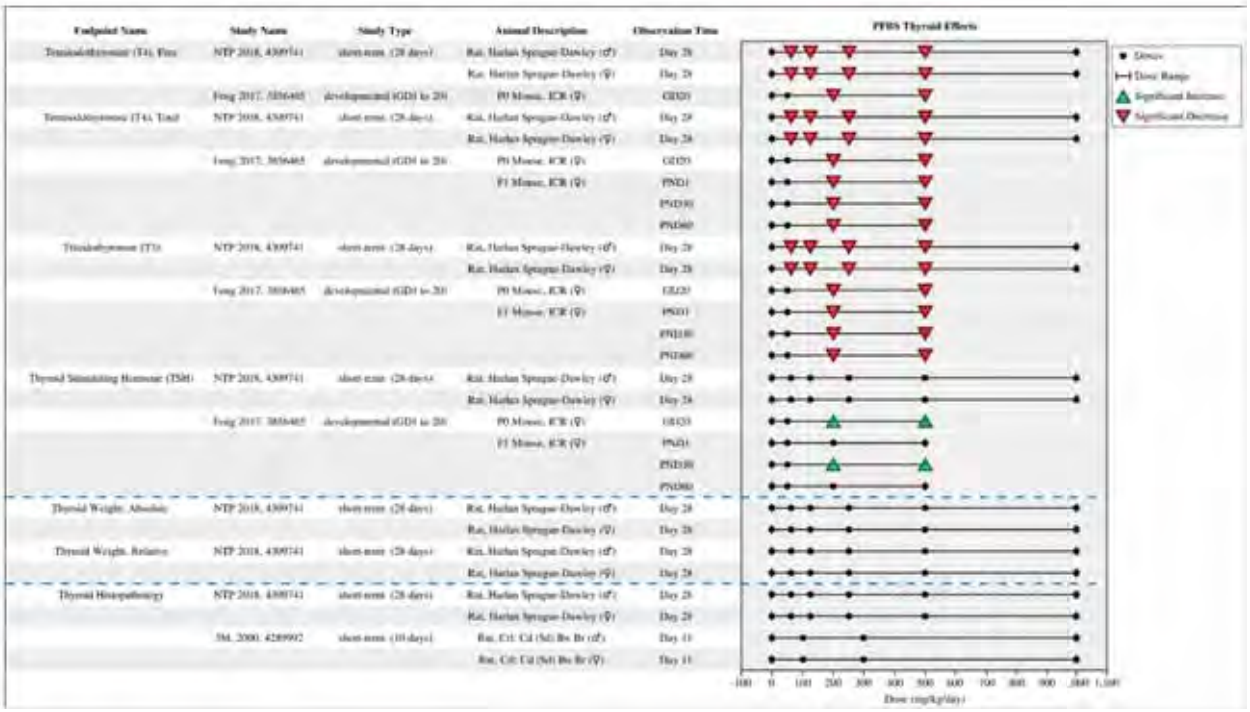


Figure 7. Thyroid Effects from K^+PFBS Exposure
(Click to see interactive data graphic and rationale for study evaluations for effects on the thyroid in HAWC)

4.2 REPRODUCTIVE EFFECTS

4.2.1 Human Studies

Five studies of populations in China and Taiwan examined different reproductive outcomes in women and men (Yao et al., 2019; Song et al., 2018; Zhang et al., 2018; Zhou et al., 2017a; Zhou et al., 2016).

Three low-confidence studies examined reproductive hormones in newborn boys and girls in China (Yao et al., 2019), adolescent boys and girls in Taiwan (Zhou et al., 2016), and adult women in China (Zhang et al., 2018). The study in newborns reported lower testosterone (β: −0.23; 95% CI: −0.46−0.01) and estradiol (β: −0.09; 95% CI: −0.2−0.01) in cord blood in male babies, but these differences were not statistically significant (Yao et al., 2019). The other two studies reported no clear associations between PFBS levels and reproductive hormones in women with premature ovarian insufficiency (Zhang et al., 2018) or in adolescents, either among the entire study population or stratified by sex (Zhou et al., 2016).

One low-confidence cross-sectional study (Song et al., 2018) examined the association between PFBS exposure and semen parameters. There was no indication of decreased semen quality in this study (correlation coefficients of −0.022 for semen concentration and 0.195 [$p < 0.05$] for progressive motility), although issues were noted regarding the ability of this study to detect an effect and important methodological details were missing.

Two studies examined other female reproductive effects: a cross-sectional study of menstrual cycle characteristics in a general population sample of women planning to become pregnant who were enrolled at preconception care clinics in China (Zhou et al., 2017a) and a case-control study in China of premature ovarian insufficiency (Zhang et al., 2018), defined by FSH level and oligo/amenorrhea. For any outcome related to menstruation, there is significant potential for reverse causation because menstruation is a potential mechanism by which PFAS are removed from the body (Wong et al., 2014; Zhang et al., 2013); therefore, both of these studies are considered low confidence. Zhou et al. (2017a) reported adjusted odds ratios (OR) of 1.30 (95% CI: 0.54−3.12) for menorrhagia and 1.48 (95% CI: 0.54−4.03) for hypomenorrhea in preconception women in China for each one unit increase in PFBS, but these results were not statistically significant. The study authors also reported inverse statistically nonsignificant associations for these two outcomes based on exposure quartiles (OR range: 0.61−0.84 for the highest quartiles relative to the referent) with no evidence of an exposure-response relationship, indicating that the associations are not robust. All of the analyses in this study examined continuous outcome measures. Zhang et al. (2018) reported no increase in odds of premature ovarian insufficiency with higher PFBS exposure (OR for tertile 2 vs. tertile 1: 0.84, 95% CI: 0.44–1.60; OR for tertile 3: 0.92, 95% CI: 0.48–1.76).

4.2.2 Animal Studies

Reproductive outcomes were evaluated in a high-confidence study of prenatal exposure to PFBS in mice (Feng et al., 2017), in two high-confidence gestational exposure studies in rats (York, 2003c, 2002), in high-confidence short-term and subchronic studies in rats [NTP (2019) and Lieder et al. (2009a), respectively], and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include fertility and

pregnancy outcomes, hormone levels, markers of reproductive development, and reproductive organ weights.

4.2.2.1 Female Fertility and Pregnancy Outcomes

Female fertility parameters were evaluated by both Feng et al. (2017) and Lieder et al. (2009b), who reported generally no effects in exposed parents, but some effects after gestational exposure in the F_1 offspring (click to see interactive graphic for female fertility effects in HAWC). Female fertility (e.g., fertility index and days in cohabitation) and delivery parameters (e.g., length of gestation, % deliveries, stillborn pups, and implantation sites) evaluated in Lieder et al. (2009b) were generally unaffected by K^+PFBS treatment for P_0- and F_1-generation dams at doses up to 1,000 mg/kg-day. The mean number of live born F_1 pups was statistically significantly decreased in the 30-mg/kg-day group, but this change was not dose dependent. The viability index in F_1 pups and the lactation index in F_1 and F_2 pups showed statistically significant changes at various doses but were not dose dependent (Lieder et al., 2009b). Similarly, no effects were observed in delivery and litter parameters (e.g., implantations, litter sizes, live fetuses, corpora lutea, and early resorptions) following prenatal exposure from GDs 6 to 20 (York, 2003c, 2002). Adult (PND 60) F_1 females gestationally exposed to PFBS at doses ≥200 mg/kg-day, however, exhibited fewer primordial, primary, secondary, early antral, antral, and preovulatory follicles, as well as fewer corpora lutea than control animals (Feng et al., 2017). Importantly, no effects on the health (e.g., weight gain) of the exposed dams were observed at any dose (Feng et al., 2017). Lieder et al. (2009b) evaluated ovarian follicles in F_1 females after they were mated and their pups had been weaned (i.e., Lactation Day [LD] 22) and observed no effects compared with controls at 1,000 mg/kg-day; however, no quantitative data were reported. Ovarian parameters were not evaluated in the study by York (2002).

4.2.2.2 Male Fertility

Two studies using S-D rats evaluated several potential responses in the male reproductive system (NTP, 2019; Lieder et al., 2009b). Male fertility parameters and reproductive effects (e.g., sperm parameters) were generally unaffected by K^+PFBS treatment in P_0- and F_1-generation males observed by Lieder et al. (2009b). At the highest dose, there were statistically significant increases in the percentage of abnormal sperm in F_1 animals and decreases in testicular sperm count in P_0-generation males. In addition, the study authors reported that the number of spermatids per gram testis was within the historical control of the testing facility. These effects were not statistically changed at lower doses. Alterations in parameters such as sperm count/number and morphology are considered indicative of adverse responses in the male reproductive system (Foster and Gray, 2013; Mangelsdorf et al., 2003; U.S. EPA, 1996a). A 28-day exposure study reported a decreased trend in testicular spermatid count per mg testis evaluated at the time of necropsy; however, no significant effects on other sperm measures were reported, including caudal epididymal sperm count and sperm motility (NTP, 2019). Note that a complete spermatogenesis cycle in male rats is typically 7 weeks in length, thus study designs of shorter duration could potentially miss effects of chemical exposure on some sperm parameters. Accordingly, the differences in responses observed in the two available studies might have been due to experimental design differences, because Lieder et al. (2009b) exposed P_0 animals for 70 days and F_1 animals during the entire period of gestation plus lactation, whereas NTP (2019) exposed animals for 28 days. Future studies should be conducted

to determine whether long-term and/or gestational exposure to PFBS significantly affects sperm measures in sexually mature and developing animals.

4.2.2.3 Reproductive Hormones (Female and Male)

Reproductive hormones were evaluated in mice (Feng et al., 2017) and, to a limited extent, in rats (NTP, 2019) (see Figure 8). Exposure to K^+PFBS for 28 days resulted in a significant trend for increased testosterone levels in females, but not in males (NTP, 2019). The increase in testosterone was not statistically significant when compared to control at any dose by pairwise analysis. Prenatal exposure to PFBS at and above 200 mg/kg-day resulted in statistically significant reduced serum estradiol levels and increased serum luteinizing hormone levels in pubertal offspring (i.e., PND 30) (Feng et al., 2017). The change in serum estradiol levels, but not luteinizing hormone, continued into adulthood in the K^+PFBS-exposed offspring (i.e., PND 60). Adult PFBS-exposed offspring also exhibited decreased serum progesterone levels at doses of 200 mg/kg-day and greater. PFBS exposure did not alter maternal estradiol-, progesterone-, or gonadotropin-releasing hormone. Reproductive hormone levels in males and females were not evaluated by Lieder et al. (2009b). The changes in follicle and corpora lutea development reported in the same study, however, may be associated with alterations in hormone production/levels because ovarian follicles and corpora lutea produce estrogen and progesterone, respectively (Foster and Gray, 2013; U.S. EPA, 1996b).

The hormonal effects observed in the NTP (2019) and Feng et al. (2017) studies might be associated with adverse reproductive effects reported in these studies. Androgens, luteinizing hormone, estradiol, and progesterone play an important role in normal development and in the functioning of the female reproductive system (Woldemeskel, 2017; Foster and Gray, 2013). Alterations in the levels and production of these reproductive hormones can disrupt endocrine signals at the hypothalamic-pituitary level and lead to delayed reproductive development and changes in functions (Rudmann and Foley, 2018; Woldemeskel, 2017; Foster and Gray, 2013).

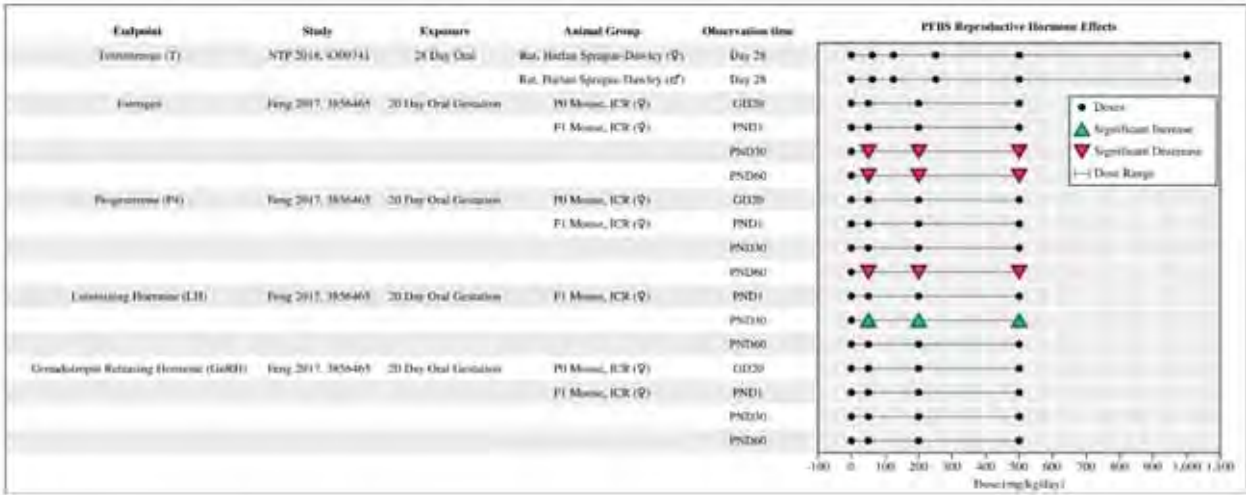


Figure 8. Reproductive Hormone Response to K^+PFBS Exposure
(Click to see interactive data graphic and rationale for study evaluations for reproductive hormone levels in HAWC)

4.2.2.4 Reproductive System Development, Including Markers of Sexual Differentiation and Maturation (Female and Male)

Several measures of female reproductive development were affected by gestational K^+PFBS exposure in mice (see Figure 9, Figure E-5, and Figure E-6). Feng et al. (2017) reported a delayed first estrous in female PFBS-exposed offspring (≥200 mg/kg-day) compared with control (see Figure E-5). Estrous cyclicity was also affected in K^+PFBS-exposed PNDs 40−60 offspring as exhibited by a prolongation of the diestrus stage compared with control. Estrous cycling was generally not statistically significantly altered in P_0- or F_1-generation females treated with K^+PFBS in the two-generation study by Lieder et al. (2009b). An increase in the number of rats with ≥6 consecutive days of diestrus was observed in the F_1 females exposed to 100 mg/kg-day; however, the increase was not present at higher doses (Lieder et al., 2009b). Estrous cyclicity was affected after adult exposure to K^+PFBS for 28 days as shown by a dose-dependent prolongation of diestrus at doses of 250 mg/kg-day and greater with marginal significance at the lowest dose tested (125 mg/kg-day) ($p = 0.063$) (NTP, 2019). Lieder et al. (2009b) reported a delay in the days to preputial separation in F_1 males of the 30- and 1,000-mg/kg-day groups;[10] however, the measure was no longer statistically significant when adjusted for BW. There was similarly no change in the days to vaginal patency in F_1 female rats (Lieder et al., 2009b). Unlike Lieder et al. (2009b), Feng et al. (2017) reported a delay in vaginal patency in F_1 females after gestational exposure of 200 mg/kg-day and greater (see Figure E-6).

[10]A marker of delayed reproductive development (Foster and Gray, 2013; U.S. EPA, 1996a).

Figure 9. Effects on Reproductive Development and Estrous Cycling Following PFBS Exposure (Click to see interactive data graphic)

4.2.2.5 Reproductive Organ Weights and Histopathology (Female and Male)

Studies have not consistently reported changes in reproductive organ weights (click to see interactive graphic for reproductive organ effects in HAWC). Reproductive organ weights, including testes, ovaries, and uterus, were unchanged in the two-generation reproductive study in P_0 and F_1 males and females (Lieder et al., 2009b) and following short-term and subchronic exposure to K^+PFBS (NTP, 2019; Lieder et al., 2009a; 3M, 2001, 2000d). F_1 females gestationally exposed to PFBS, however, exhibited decreased size and weight of the ovaries and uterus (Feng et al., 2017). In addition, the total uterine section diameter and endometrial and myometrial thickness were significantly reduced. There were no significant histopathological alterations in the male or female reproductive organs evaluated following exposure to K^+PFBS for 28 days (NTP, 2019) or in parental or offspring from the two-generation reproductive study (Lieder et al., 2009b).

4.3 OFFSPRING GROWTH AND EARLY DEVELOPMENT

4.3.1 Human Studies

No human studies were available to inform the potential for PFBS exposure to cause effects on the growth or early development of children.

4.3.2 Animal Studies

Evidence to inform organ/system-specific effects of PFBS in animals following developmental exposure are discussed in the individual hazard sections (e.g., reproductive cycling after developmental exposure is discussed in the "Reproductive Effects" section). This section is limited to discussion of other, specific developmental effects commonly evaluated in guideline developmental toxicity studies, including pup BW, developmental markers, and bone measures. Four high- or medium-confidence studies examined potential alterations in offspring growth and early development following PFBS exposure, including two gestational exposure studies in rats (York, 2003a, 2002) and one gestational exposure study in mice (Feng et al., 2017), as well as a two-generation study in rats (Lieder et al., 2009b; York, 2003c). (Click to see interactive graphic for developmental effects in HAWC.)

None of the studies identified significant effects in either rats or mice on measures of fetal morphology (i.e., malformations and variations). BW of female offspring of PFBS-exposed mice at doses greater than 200 mg/kg-day was statistically significantly lower than control at PND 1, and the pups remained underweight through weaning, pubertal, and adult periods, with decreases of approximately 25% observable in pups nearing weaning (Feng et al., 2017). At around PND 16, Feng et al. (2017) also reported an ~1.5-day developmental delay in eye opening in pups gestationally exposed to 200 mg/kg-day PFBS and greater. Importantly, no effects on the health of the exposed dams (e.g., weight gain) were observed at any dose (Feng et al., 2017). Dose-response graphics for eye opening, including effect size and variability, are included in Appendix E, Figure E-4. Fetal BWs (male and female) were also reduced (approximately 10%) compared with controls following gestational exposure from GDs 6 to 20 at the highest tested dose (1,000 mg/kg-day in York (2002) and 2,000 mg/kg-day in York (2003a)]). Parental BWs and organ weights, however, were also affected to a similar degree at those doses (Lieder et al., 2009b; York, 2003c, 2002), limiting the interpretation of the results. No statistically significant changes in F_1- and F_2-generation pup mean pup weight at birth and mean pup weight at weaning were reported by Lieder et al. (2009b) or York (2003c).

Several measures of thyroid hormone development and female reproductive development were affected by gestational PFBS exposure in mice and are described in more detail in the "Thyroid Effects" and "Reproductive Effects" sections, respectively.

4.4 RENAL EFFECTS

4.4.1 Human Studies

One low-confidence study (Qin et al. (2016), with additional details in Bao et al. (2014), selected 225 subjects ages 12−15 years old from a prior cohort study population in seven public schools in northern Taiwan (Tsai et al., 2010) and examined the association between PFBS exposure and uric acid concentrations. There was no association between ln(PFBS) concentration and uric acid concentrations in the total population (β: 0.0064 mg/dL increase in uric acid per 1 ln-µg/L increase in PFBS; 95% CI: −0.22−0.23). U.S. EPA identified that a nonsignificant positive association in boys was offset by a nonsignificant negative association in girls, and there is not enough information to determine whether there is a sex dependence. When PFBS exposure was analyzed for high uric acid (>6 mg/dL), the risk was somewhat elevated in boys (OR: 1.53; 95% CI: 0.92−2.54), but not in girls (OR: 0.99; 95% CI: 0.58−1.73). The potential for reverse causation (i.e., that renal function could influence the levels of PFBS in the blood) tempers any conclusions that might be drawn.

4.4.2 Animal Studies

Renal effects were evaluated in high-confidence short-term and subchronic-duration exposure studies in rats (NTP, 2019; Lieder et al., 2009a; 3M, 2001, 2000d) and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include kidney weights, histopathological changes, and serum biomarkers of effect (see Figure E-8 and Figure E-9). Dose-response graphics for histopathological effects, including effect size and variability, are included in Appendix E, Figure E-7.

Absolute and relative kidney weights of males and females were unchanged in S-D rats exposed daily for 90 days to K^+PFBS at doses up to 600 mg/kg-day compared with control rats (Lieder et al., 2009a). This lack of effect on kidney weight was also observed in parental and F_1 male and female rats of the same strain exposed to K^+PFBS at doses up to 1,000 mg/kg-day during a two-generation reproductive study (Lieder et al., 2009b). Although none of the findings reached statistical significance, an approximate 9% increase in absolute kidney weight was observed in female S-D rats exposed to 1,000 mg/kg-day K^+PFBS for 10 days (3M, 2000d); relative-to-body kidney weights were also increased approximately 6−9%. This organ-weight effect was not observed in corresponding males of the study. In a follow-up 28-day study by the same lab, a 9−11% increase in absolute and relative-to-body kidney weight was observed in female S-D rats exposed to 900 mg/kg-day K^+PFBS (3M, 2001), although these changes were not statistically significant. In this study, U.S. EPA also observed that smaller nonsignificant increases in kidney weight occurred in male rats. In another 28-day study, K^+PFBS exposure significantly increased absolute and relative right kidney weights in high-dose (500 mg/kg-day) male S-D rats (NTP, 2019). Only relative kidney weights were altered in female rats, but this effect was significant at all tested K^+PFBS doses (≥62.6 mg/kg-day). Click to see interactive graphic for kidney-weight effects in HAWC.

After 90 days of exposure, Lieder et al. (2009a) observed increased incidences of histopathological alterations of the kidneys of male and female rats of the high-dose group (600 mg/kg-day). Increased incidence of hyperplasia of the epithelium of renal papillary tubules and ducts was observed in rats of both sexes (see Figure E-7Figure E-8). A single incidence of papillary necrosis in both kidneys was observed in one male in the high-dose group. Further, focal papillary edema was observed in 3/10 rats of both sexes of the high-dose groups compared with no evidence of this effect in control rats. Similar histopathological alterations were observed in parental and F_1 male and female rats in the two-generation reproduction study (Lieder et al., 2009b). Compared with control rats, increased incidences of hyperplasia of the renal tubular and ductal papillary epithelium, and focal papillary edema were observed in parental male and female rats at PFBS doses ≥300 mg/kg-day. Hyperplastic foci in the same locations of the kidney were also observed in male and female F_1 rats exposed to ≥300 mg/kg-day PFBS across life stages from gestation to adulthood (Lieder et al., 2009b). Focal papillary edema was observed in male (≥1,000 mg/kg-day) and female (≥300 mg/kg-day) F_1 rats, although this specific alteration did not appear to be dose-dependent in females. Although kidney alterations such as hydronephrosis, mineralization, and tubular degeneration were observed in male or female S-D rats after just 10 days of oral K^+PFBS exposure, these effects were not significant compared to control and/or did not appear to be dose-dependent (3M, 2000d). The same histopathological lesions were noted in the 28-day rat study albeit with lack of statistical significance compared to control (3M, 2001). In another 28-day gavage study in S-D rats, chronic progressive nephropathy (CPN) was observed in all male and female PFBS treatment groups and control rats, with no evidence of dose dependence for this effect (NTP, 2019). Renal papillary necrosis was also observed in these rats but only at the highest exposure dose (1,000 mg/kg-day).

Serum levels of biomarkers indicative of kidney injury and/or function, including blood urea nitrogen (BUN) and creatinine, have been examined across multiple studies of varying exposure durations, and were found to be unchanged in male and female rats treated with K^+PFBS at doses up to 1,000 mg/kg-day (Lieder et al., 2009a; 3M, 2001, 2000d). After 28 days of gavage exposure in S-D rats, however, NTP (2019) observed significantly increased levels of BUN in males (≥250 mg/kg-day). This increased circulating BUN was not observed in female rats at doses up to 1,000 mg/kg-day. Click to see interactive graphic for other kidney effects in HAWC.

4.5 HEPATIC EFFECTS

4.5.1 Human Studies

No human studies were available to inform the potential for PFBS exposure to cause hepatic effects.

4.5.2 Animal Studies

Hepatic effects were evaluated in high-confidence short-term and subchronic studies in rats (NTP, 2019; Lieder et al., 2009a; 3M, 2001, 2000d) and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include liver weights, histopathological changes, and serum biomarkers of effect (see Figure E-10).

Ten days of daily gavage exposure to K^+PFBS significantly increased absolute, relative-to-body, and relative-to-brain weights of liver in adult male and female S-D rats exposed to 1,000 mg/kg-day (3M, 2000d). The absolute liver mass of male rats was increased by 36% compared with females (22%). A similar profile of liver-weight alteration in S-D rats was observed following 28 days of exposure wherein absolute and relative liver weights of high-dose (900 mg/kg-day) male rats had increased 25%−30% (3M, 2001). Female rats at the same treatment dose did not experience a similar magnitude increase in absolute or relative liver weights (4−6%). In another 28-day study in S-D rats, K^+PFBS exposure significantly increased absolute and relative liver weights in males (≥125 and ≥62.6 mg/kg-day, respectively) and females (≥250 and ≥125 mg/kg-day, respectively) (NTP, 2019). In contrast, the livers of male and female S-D rats exposed to K^+PFBS at doses up to 600 mg/kg-day for 90 days were not significantly changed compared with respective controls (Lieder et al., 2009a). In a two-generation reproduction study using the same strain of rat, however, increased absolute and relative liver weights were observed in male parental rats exposed to doses of K^+PFBS ≥300 mg/kg-day for approximately 70 days (Lieder et al., 2009b). In the F_1 adult males, only relative liver weight was significantly increased at the high dose (1,000 mg/kg-day), although terminal BW was significantly decreased in this group compared with control.

Histopathological examination of the livers of S-D rats across three separate gavage studies of increasing K^+PFBS exposure duration [10-day, 3M (2000d); 28-day, 3M (2001); 90-day, Lieder et al. (2009a)] did not reveal any significant dose-dependent alterations or lesions. For example, focal/multifocal hepatic inflammation was observed in 3/10 male and 4/10 female rats of the high-dose group (no incidence at the low or mid dose) compared to 6/10 male and female rats in the control groups (Lieder et al., 2009a). The Lieder et al. (2009b) two-generation reproduction gavage study did identify increased incidences of hepatocellular hypertrophy in parental and F_1 adult male rats at ≥300 mg/kg-day; however, this effect was absent in female rats at doses of K^+PFBS up to 1,000 mg/kg-day. NTP (2019) identified a significantly increased incidence of hepatocellular hypertrophy in male (≥125 mg/kg-day) and female (≥500 mg/kg-day) S-D rats after 28 days of K^+PFBS exposure. Further, significantly increased cytoplasmic alteration of hepatocytes was observed in these rats (male and female at ≥500 mg/kg-day). Hepatic necrosis was also observed but was not significant compared with control and only occurred at the high dose (1,000 mg/kg-day) in both sexes (NTP, 2019).

In general, serum biomarkers associated with altered liver function or injury, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were not significantly changed in male and female S-D rats across multiple gavage studies of varying exposure durations up to 90 days and at K^+PFBS doses up to 1,000 mg/kg-day (Lieder et al., 2009a; 3M, 2001, 2000d). NTP (2019), however, reported increased serum ALT and AST in male (500 mg/kg-day only) and female (≥250 mg/kg-day for ALT; ≥500 mg/kg-day for AST) rats exposed to K^+PFBS for 28 days. Click to see interactive graphic for liver effects in HAWC.

4.6 EFFECTS ON LIPIDS OR LIPOPROTEINS

4.6.1 Human Studies

One low-confidence study (Zeng et al., 2015) used the controls from the case-control study of asthma described below (Dong et al., 2013a) and examined the association between PFBS exposure and serum lipids. There was a statistically significant increase in total

cholesterol (β: 19.3 mg/dL increase per 1 µg/L increase in PFBS; 95% CI: 0.6−38.0) but when PFBS exposure was analyzed in quartiles, no exposure-response gradient was observed.

In addition, a medium-confidence birth cohort study in China examined associations with childhood adiposity (Chen et al., 2019). PFBS was measured in cord blood samples at birth and several measures of adiposity were collected at age 5 years. There was higher adiposity with higher exposure in girls, with significant exposure-response relationships across tertiles with waist circumference, fat mass, body fat percentage, and waist-to-height ratio. No association with adiposity was observed in boys. It is unlikely that the association in girls can be explained by confounding across the other PFAS measured in this study as the associations were strongest for PFBS, but it is possible that there is other unmeasured confounding.

4.6.2 Animal Studies

Beyond a single medium-confidence mouse study [Bijland et al. (2011); 3M (2010); summarized below], PFBS studies have not particularly focused on perturbations in lipids or lipoproteins as a potential health outcome, because studies have typically focused only on measures of serum cholesterol and triglyceride as part of a broader panel of clinical chemistry measures in high- or medium-confidence rat studies of 10, 28, and 90 days (see Figure E-11) [3M (2000d), 3M (2001), and Lieder et al. (2009a), respectively]. Circulating levels of cholesterol and triglycerides were unchanged in male and female S-D rats following daily gavage exposure to K^+PFBS for 10 days at doses up to 1,000 mg/kg-day (3M, 2000d). In a similarly designed study from the same laboratory, serum cholesterol and triglyceride levels were decreased in male rats but at the high dose only, and this effect was neither statistically significant compared with control nor observed in female rats of the same dose group (3M, 2001). Following exposure for up to 90 days, cholesterol and triglycerides were unchanged in male and female rats at doses up to 600 mg/kg-day (Lieder et al., 2009a). PFBS was included in a multi-PFAS study specifically designed to interrogate the mechanism of effect on lipid and lipoprotein metabolism in a transgenic mouse line (APOE*3-Leiden CETP) that is highly responsive to fat and cholesterol intake, consistent with human populations exposed to a western-type diet (containing 14% beef tallow, 1% corn oil, and 0.25% cholesterol) (Bijland et al., 2011; 3M, 2010). Adult male mice were fed a western-type, high-fat diet for 4 weeks prior to initiation of PFBS exposure and throughout the 4- to 6-week PFBS exposure period (at approximately 30 mg/kg-day). This study included several measures of lipid and lipoprotein synthesis, modification, and transport or clearance, such as circulating plasma levels, in vivo clearance of very low-density lipoprotein (VLDL)-like particles, fecal bile acid and sterol excretion, hepatic lipid levels, lipase activity, VLDL-triglyceride and VLDL-apoB production, and gene expression profiles. After 4 weeks of PFBS exposure, fasting plasma triglycerides, cholesteryl ester transfer protein, and glycerol were significantly decreased compared with mice on the control diet. Further, the half-life of VLDL-like particles and hepatic lipase activity, and hepatic cholesteryl ester and free cholesterol levels were decreased (Bijland et al., 2011; 3M, 2010). Hepatic uptake of VLDL-like particles (represents fatty acid/lipid transport into hepatic tissue) was modestly, but significantly, increased compared with control mice. This increased hepatic lipid uptake in the liver was accompanied by increased expression of genes associated with lipid binding, activation, and metabolism (e.g., β-oxidation).

4.7 OTHER EFFECTS

4.7.1 Human Studies

Two studies in China examined different immune outcomes in children (Chen et al., 2018; Dong et al., 2013a).

One medium-confidence study reported in five publications (Qin et al., 2017; Zhou et al., 2017b; Zhou et al., 2017a; Zhu et al., 2016; Dong et al., 2013b) examined the association between PFBS exposure and asthma, asthma symptoms, pulmonary function, and related immune markers (immunoglobulin E [IgE], absolute eosinophil count [AEC], eosinophilic cationic protein [ECP], T-helper cell-specific cytokines, and 16-kDa club cell secretory protein). The primary finding was a statistically significant (in the fourth quartile) positive association between incident asthma (i.e., diagnosis in the previous year) and PFBS exposure (OR for Q2: 1.3, 95% CI: 0.7−2.3; OR for Q3: 1.2, 95% CI: 0.7−2.2; OR for Q4: 1.9, 95% CI: 1.1−3.4). There were also increases in AEC and ECP with increased exposure (not statistically significant with the exception of AEC in children with asthma). There was no clear association with IgE or T-helper cell-specific cytokines. There was also no clear association with asthma severity or control of asthma symptoms (Dong et al., 2013a), or pulmonary function measured with spirometry among children with asthma (Qin et al., 2017). While reduced pulmonary function could be considered an outcome separate from asthma, the study authors noted no associations in pulmonary function (i.e., in nonasthmatics across the PFAS they studied), so for these purposes, it was considered an indicator of asthma severity.

One medium-confidence study (Chen et al., 2018) examined the association between PFBS exposure and atopic dermatitis and reported a statistically nonsignificant increase in atopic dermatitis with increased exposure (OR: 1.23; 95% CI: 0.74−2.04).

In addition, two studies examined cardiovascular effects (Huang et al., 2019b; Huang et al., 2018), but it is difficult to evaluate consistency across studies given the different outcomes in each.

One medium-confidence study (Huang et al., 2018) using data from NHANES cycles for 1999−2014 reported significantly higher odds of total cardiovascular disease with higher exposure (OR for above vs. below the LOD: 1.19; 95% CI: 1.06−1.32) and elevated, though not statistically significant, odds of individual types of cardiovascular disease (congestive heart failure, coronary heart disease, angina pectoris, heart attack, and stroke). There is potential in this study for confounding across the PFAS, because PFBS was highly correlated with some other PFAS with slightly stronger associations.

A medium-confidence cross-sectional study (Huang et al., 2019b) of hypertensive disorders of pregnancy reported higher odds for all such disorders in pregnancy (in the third tertile) (OR for Tertile 2 vs. Tertile 1: 0.89, 95% CI: 0.39−2.44; OR for Tertile 3: 2.26, 95% CI: 1.02−5.0; *p*-trend 0.03) and pre-eclampsia (OR for Tertile 2 vs. Tertile 1: 2.09, 95% CI: 0.51−8.53; OR for Tertile 3: 3.51, 95% CI: 0.94−13.2; *p*-trend 0.05), with both trends being statistically significant after mutual adjustment of PFAS.

4.7.2 Animal Studies

Other effects were evaluated following exposure to PFBS, including outcomes related to the spleen, hematological system, BW, neurotoxicity, and nonspecific clinical chemistry. These groups of outcomes were not synthesized because of inadequate available information, uncertain biological relevance, and/or inconsistencies across studies and sexes.

4.8 OTHER DATA

Other studies that used PFBS or K^+PFBS are described in this section. These studies are not adequate for determining RfD values and were considered supportive data. These data might include acute-duration exposures, genotoxicity, mechanistic, and other studies (see Table 5).

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Genotoxicity				
Mutagenicity test	*Salmonella typhimurium* (strains TA98 and TA100) and *Escherichia coli* (strain pKM101) in the presence or absence of S9. Concentrations of PFBS were between 0−5,000 μg/plate.	Test was negative for TA100 and pKM101 strains and equivocal for TA98 strain.	There is no in vitro evidence of PFBS mutagenicity.	NTP (2005)
Ames	*S. typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *E. coli* (strain WP2uvrA) were tested in the presence or absence of S9 and with or without a preincubation treatment. Concentrations of K^+PFBS were between 0−5,000 μg/plate.	The results of both mutation assays indicate that PFBS did not induce any significant increase in the number of revertant colonies for any of the tester strains in the presence or absence of induced rat liver S9.	There is no in vitro evidence of PFBS mutagenicity.	Pant (2001)
Genotoxicity test	Human hepatoma (HepG2) cells were treated with 0.4 μM to 2 mM PFBS. Intracellular ROS production was measured by use of 2′,7′-dichlorofluorescein diacetate and DNA damage was measured with the comet assay.	The amount of ROS and DNA strand breaks remained unaffected by PFBS treatment.	PFBS did not generate ROS or DNA damage in human liver cells.	Eriksen et al. (2010)
CHO chromosomal aberration	Cultures of CHO cells were treated with K^+PFBS at concentrations ranging from 0 to 5,000 μg/mL with or without exogenous metabolic activation. The in vitro exposure duration was 3 hr.	PFBS did not induce a statistically significant increase in the percentage of cells with aberrations at any of the concentrations tested, either with or without metabolic activation, in either assay when compared to the solvent controls.	Based on the negative results in the in vitro CA assay in CHO cells, PFBS is not considered to be a clastogenic agent.	Xu (2001)
Micronucleus assay	Male and female S-D rats (5/group) were exposed twice daily to K^+PFBS by gavage at doses of 31.3, 62.5, 125, or 250 mg/kg for 28 d.	PFBS did not induce a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes.	PFBS was negative for micronuclei in the blood of male and female rats, indicating a lack of genotoxic potential.	NTP (2012)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute duration and other routes of exposure				
Acute	10 rats/group, young adult male rat (strain not specified), administered PFBS by gavage, single dose, 50, 100, 300, 600, or 800 µL/kg and observed for 14 d postexposure.	Mortality: 0, 20, 60, 80, and 100% at 50, 100, 300, 600, and 800 µL/kg PFBS, respectively.	Acute oral PFBS rat LD_{50} in male rats is 236 µL/kg (corresponding to 430 mg/kg).	Bomhard and Löser (1996) Low confidence
Acute dermal	Adult (8 wk of age) male and female S-D rats (5/group) were exposed dermally (10% of body surface area) to 500, 1,000, or 2,000 mg/kg K^+PFBS for 24 hr and then observed for 15 d postexposure for signs of clinical toxicity, mortality, BW changes, or gross pathology (terminus of study).	No treatment-related observations were noted.	PFBS is not acutely toxic via the dermal route of exposure in rats.	3M (2000b)
Dermal irritation	Adult (14-wk of age) female NZW rabbits (3 rabbits total for study) were exposed dermally (6 cm^2 of skin) to 500 mg K^+PFBS for approximately 4 hr and then observed for 9 d postexposure for signs of clinical toxicity, mortality, or BW changes.	Draize scoring was performed on the patch site immediately following the exposure period and 24, 48, and 72 hr postexposure. No signs of dermal irritation were observed. No signs of clinical toxicity or mortality occurred. No treatment-related alterations in BW were noted.	PFBS did not induce erythema, edema, or other possible dermal findings during the scoring periods, indicating a lack of dermal irritant properties in rabbits.	3M (2000a)
Ocular sensitivity	Adult (16-wk of age) female NZW rabbits (3 rabbits total for study) were exposed to approximately 80 mg K^+PFBS via ocular installation in the left eye for 2 sec. Eyes were flushed with 0.9% saline after 24 hr and then observed and scored for up to 21 d postexposure. The rabbits were also followed for clinical signs of toxicity or mortality/moribundity.	Excessive lacrimation of the left eyes noted throughout study postexposure. Based on the laboratory scoring system, PFBS was "moderately" irritating at 24 and 72 hr postexposure.	PFBS is a moderate ocular irritant in rabbits.	3M (2000c)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Contact hypersensitivity	Adult male (10−12 wk old) and female (9 wk old) CRL:(HA)BR Hartley guinea pigs were injected intradermally with sterile water, Freund's adjuvant, or adjuvant containing 125 mg/mL K^+PFBS (induction phase). D 7 after induction, a petrolatum paste containing 0.5 g K^+PFBS was applied to the previous injection site of the guinea pigs for 48 hr (topical induction phase). D 22, a challenge dose of 0.5 g K^+PFBS (petrolatum paste) was applied to the shaved left cranial flank (right flanks were treated with petrolatum paste only) (challenge phase). This challenge procedure was repeated on D 29. Challenge sites were observed and scored following each challenge period (D 24−25 males and females and D 31−32 males only). Guinea pigs were also followed for signs of clinical toxicity, mortality/moribundity, or alterations in BW.	No mortalities, clinical signs of toxicity, or changes in BW associated with PFBS exposure were noted. Dermal scores were zero (no response) in females and did not exceed 1 in males (discreet or patchy edema), which was not considered significant compared with control guinea pigs exposed to Freund's adjuvant alone.	PFBS is not considered an allergen in the guinea pig maximization test.	3M (2002a)

BW = body weight; CA = chromosomal aberration; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; K^+PFBS = potassium perfluorobutane sulfonate; LD_{50} = median lethal dose; NZW = New Zealand White; PFBS = perfluorobutane sulfonic acid; ROS = reactive oxygen species; S-D = Sprague-Dawley.

4.8.1 Tests Evaluating Genotoxicity and Mutagenicity

Genotoxic, mutagenic, and clastogenic effects of PFBS have been tested in mammalian and prokaryotic cells in vitro (Eriksen et al., 2010; NTP, 2005; Pant, 2001; Xu, 2001), and in rats in vivo (NTP, 2019). PFBS was negative for mutagenicity in *Escherichia coli* strain pKM101 and *Salmonella typhimurium* strain TA100 (NTP, 2005). Mutagenicity test results were equivocal in *S. typhimurium* strain TA98. Pant (2001) tested PFBS at concentrations up to 5,000 μg/plate in *E. coli* strain WP2uvrA and *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence or absence of exogenous metabolic activation and found no evidence of mutagenic activity. In mammalian cells in vitro, PFBS did not generate reactive oxygen species (ROS) or oxidative deoxyribonucleic acid damage in HepG2 cells (Eriksen et al., 2010). PFBS also failed to induce chromosomal aberrations in Chinese hamster ovary cells, suggesting a lack of clastogenic activity (Xu, 2001). Adult male and female S-D rats exposed twice daily to oral PFBS at doses up to 250 mg/kg for 28 days did not experience any significant increases in micronucleated polychromatic erythrocytes, indicating a lack of genotoxic activity (see Table 5) (NTP, 2012).

4.8.2 Acute Duration and Other Routes of Exposure

Limited data are available to evaluate acute toxicity and effects from dermal exposure to PFBS (see Table 5). One low-confidence acute oral toxicity study on male rats administered PFBS by gavage reported a median lethal dose (LD_{50}) of 236 μL/kg (corresponding to 430 mg/kg) (Bomhard and Löser, 1996). One acute dermal toxicity study concluded that PFBS is not acutely toxic via the dermal route of exposure in rats, with no treatment-related observation at doses up to 2,000 mg/kg (3M, 2000b). PFBS was not reported to induce erythema, edema, or other possible dermal findings during the scoring periods, indicating a lack of dermal irritant properties in rabbits exposed to 500 mg K^+PFBS for approximately 4 hours (3M, 2000a). PFBS was found to be a moderate ocular irritant in rabbits exposed to 80 mg K^+PFBS via ocular installation (3M, 2000c). PFBS did not induce skin sensitization in the guinea pig maximization test with an intradermal injection of 125 mg/mL and topical induction of 0.5 g K^+PFBS (3M, 2002a).

5.0 EVIDENCE INTEGRATION AND HAZARD CHARACTERIZATION

The epidemiology database of studies of PFBS exposure and health effects consists of 19 epidemiologic studies (described in 22 publications), summarized in the previous section. The experimental animal database of all repeated-dose oral toxicity studies for PFBS and the related compound K^+PFBS includes a short-term range-finding study in rats (3M, 2000d), two 28-day studies in rats (NTP, 2019; 3M, 2001), one subchronic study in rats (Lieder et al., 2009a), one subchronic-duration lipoprotein metabolism study in mice (Bijland et al., 2011; 3M, 2010), three gestational exposure studies in mice and rats (Feng et al., 2017; York, 2003a, 2002), and a two-generation reproductive toxicity study in rats (Lieder et al., 2009b). Health outcomes evaluated across available studies included effects on the thyroid, reproductive organs and tissues, developing offspring, kidneys, liver, and lipids/lipoproteins following oral exposure to PFBS. Table 6 provides an overview of this database of potentially relevant studies and effects. This table includes only the high- and medium-confidence animal studies (a single, low-confidence animal study was not considered informative for drawing conclusions on potential health hazard[s]). The available epidemiology studies are also not included because their ability to inform conclusions about associations was limited because of the small number of studies (typically one) per outcome and poor sensitivity resulting from low exposure levels.

Following the summary of the available database in Table 6, narrative summaries describe the evidence integration judgments and the primary rationales supporting these decisions for each health effect. These narratives are supported by an evidence profile table that succinctly lays out the various factors that were judged to increase or decrease the support for a hazard. While the epidemiology studies were not influential in drawing evidence integration judgments (i.e., they were judged as equivocal for all outcomes) or the derivation of toxicity values (i.e., these studies are not discussed in the next section), the general findings are summarized below to provide context to the animal study findings and identify potential areas of future research.

Table 6. Summary of Noncancer Data for Oral Exposure to PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Exposure Duration[a]	Reference	Study Confidence	Number of Male/Female, Strain, Species, Study Type, Study Duration	Doses Tested (mg/kg-d)	Effects Observed at LOAEL	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Short term	3M (2000d)	Medium confidence	5/5, S-D rat, K^+PFBS administered by gavage, 10 d	0, 100, 300, 1,000	Increased absolute and relative liver weight.	300	1,000
Short term	3M (2001)	High confidence	10/10, S-D rat, K^+PFBS administered by gavage, 28 d	0, 100, 300, 900	Increased absolute and relative liver weight (male) and relative kidney weight (female).	300	900
Short term	NTP (2019)	High confidence	10/10, S-D rat, PFBS administered by gavage, twice/d, 28 d	0, 62.6, 125, 250, 500, 1,000[b]	Decreased T_3, free T_4, total T_4 in males and females. Increased relative liver weight in females and increased relative right kidney weight in males.	NDr	62.6
Subchronic	Lieder et al. (2009a); York (2003b)	High confidence	10/10, S-D rat, K^+PFBS administered by gavage, 7 d/wk, 90 d	0, 60, 200, 600	Increased incidence of renal hyperplasia in males and females.	200	600
Subchronic	Bijland et al. (2011); 3M (2010)	Medium confidence	6−8/0, Apoe*3-Leiden CETP mice, K^+PFBS in diet, 4−6 wk	0, 30	Alterations in lipid homeostasis (e.g., decreased hepatic lipase, triglycerides) is of uncertain biological significance.	NDr	NDr
Developmental	Feng et al. (2017)	High confidence	0/10, ICR mice, K^+PFBS administered by gavage, GDs 1−20	0, 50, 200, 500	Decreased T_3, free T_4, and total T_4 in dams and PND 1, 30, and 60 offspring. Increased TSH in maternal and offspring (PND 30 only). Delayed eyes opening, vaginal opening, and first estrous and decreased BW in pups.	50	200
Developmental	York (2003a)	High confidence	0/8, S-D rat, K^+PFBS administered by gavage, GDs 6−20	0, 100, 300, 1,000, 2,000	Decreased maternal feed consumption, BW gain, and gravid uterine weight. Decreased pup BW occurred at doses affecting maternal health, limiting the interpretation of the results; thus, developmental effect levels were not determined. (Limited endpoints evaluated—pilot study.)	P_0: 1,000 F_1: NDr	P_0: 2,000 F_1: NDr

Table 6. Summary of Noncancer Data for Oral Exposure to PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Exposure Duration[a]	Reference	Study Confidence	Number of Male/Female, Strain, Species, Study Type, Study Duration	Doses Tested (mg/kg-d)	Effects Observed at LOAEL	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Developmental	York (2002)	High confidence	0/25, S-D rat, K^+PFBS administered by gavage, GDs 6–20	0, 100, 300, 1,000	Decreased maternal feed consumption and BW gain. Decreased pup BW occurred at doses affecting maternal health, limiting the interpretation of the results; thus, developmental effect levels were not determined.	P_0: 300 F_1: NDr	P_0: 1,000 F_1: NDr
Reproductive	Lieder et al. (2009b); York (2003c); York (2003d); York (2003e)	High confidence	30/30, S-D rat, K^+PFBS administered by gavage, two-generation reproductive study	P_0 adults: 0, 30, 100, 300, 1,000 F_1 adults: 0, 30, 100, 300, 1,000	P_0 and F_1 adults: increased incidence of hyperplasia and focal papillary edema in the kidneys of males and females. F_2 pups: no dose-related effects at the highest dose tested (1,000 mg/kg-d).	P_0, F_1: 100 F_2: 1,000	P_0, F_1: 300 F_2: NDr

[a]Duration categories are defined as follows: Acute = exposure for ≤24 hours; short term = repeated exposure for 24 hours to ≤30 days; long term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (>30 days up to approximately 90 days in typically used laboratory animal species); chronic = repeated exposure for >10% lifespan for humans (>~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Rats were gavaged twice daily at administered doses of 0, 31.3, 62.6, 125, 250, and 500 mg/kg in NTP (2019).

BW = body weight; GD = gestation day; NDr = not determined; ICR = Institute of Cancer Research; K^+PFBS = potassium perfluorobutane sulfonate; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFBS = perfluorobutane sulfonic acid; PND = postnatal day; S-D = Sprague-Dawley; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid-stimulating hormone.

5.1 THYROID EFFECTS

PFBS-induced perturbation of the thyroid was consistently observed across two species, sexes, life stages, and exposure durations in two independent, high-confidence studies. These perturbations involved a coherent pattern of hormonal changes. Significant changes in tissue weight or histopathology were not observed.

Similar patterns of decreases in total T_3, total T_4, and free T_4 were observed in PFBS-exposed pregnant mice, nonpregnant adult female and adult male rats from a 28-day study, and gestationally exposed female mouse offspring (NTP, 2019; Feng et al., 2017). These decreases were statistically significant (~20% in dams and ~50% in offspring) and shown to persist at least 60 days after gestational exposure in offspring and exhibited dose dependence in both studies.

Development of numerous organ systems, including neuronal, reproductive, hepatic, and immune systems, is affected by altered thyroid homeostasis because adequate levels of thyroid hormones are necessary for normal growth and development in early life stages (Forhead and Fowden, 2014; Gilbert and Zoeller, 2010; Hulbert, 2000). Thus, the observed effects of PFBS exposure on thyroid hormone economy are biologically consistent with the reported delays and abnormalities in organ/system development discussed below. It is well established that the presence of sufficient thyroid hormones during the gestational and neonatal period is essential for brain development and maturation. Studies specifically evaluating the effect of PFBS on neurodevelopment were not identified, leaving uncertainty as to the potential for adverse developmental effects. Nonetheless, the coherence of these PFBS findings, in addition to the large number of xenobiotic exposure studies demonstrating associations between thyroid hormone economy and decrements in early life stage growth, development, and survival, provides support for thyroid hazard.

Taken together, the evidence in animals for thyroid effects *supports a hazard*. The single available study in humans did not report an association with thyroid hormones, but had severe limitations hindering its interpretation. This low-confidence cross-sectional study was conducted in a highly selected population (i.e., women with premature ovarian insufficiency), had poor sensitivity, and methodological limitations (Zhang et al., 2018). The limited evidence for thyroid effects in human studies is *equivocal*. Although there are some differences in hypothalamic-pituitary-thyroid (HPT) regulation across species (e.g., serum hormone-binding proteins, hormone turnover rates, and timing of in utero thyroid development), rodents are generally considered to be a good model for evaluating the potential for thyroid effects of chemicals in humans (Zoeller et al., 2007). For more details pertaining to HPT dynamics and the similarities and differences associated with thyroid hormone economy between rodents and humans, please refer to *A Literature Review of the Current State of the Science Regarding Species Differences in the Control of, and Response to, Thyroid Hormone Perturbations. Part 1: A Human Health Perspective* (Regulatory Science Associates, 2019). The pattern of decreased thyroid hormones in the absence of a coordinated reflex increase in TSH and commensurate alterations in thyroid tissue weight and/or histology, observed in PFBS studies [e.g., Feng et al. (2017)], is consistent with the human clinical condition referred to as "hypothyroxinemia," which is commonly associated with pregnancy in humans. Hypothyroxinemia has been defined as a low percentile value of FT_4 (ranging from the 2.5th percentile to the 10th percentile of FT_4), with a TSH level within the normal reference range (Hales et al., 2018; Alexander et al., 2017;

Lazarus et al., 2012; Negro et al., 2011). Overall, based on findings in animal models considered to be informative for evaluating the potential for thyroid effects in humans, the available evidence *supports a hazard,* and the thyroid is considered a potential target organ for PFBS toxicity in humans.

5.2 DEVELOPMENTAL EFFECTS

Overt effects on birth parameters and early development have generally not been observed in either rats or mice after PFBS exposure. Specifically, the available studies do not provide evidence of effects on endpoints relating to pregnancy loss, fetal survival, or fetal morphology (Feng et al., 2017; Lieder et al., 2009a; York, 2003a, c, 2002). While one mouse study indicated pronounced decreases in female offspring BW at several ages after gestational exposure (Feng et al., 2017), several other studies either did not observe decreases in offspring BW or only detected these changes when parental BWs were similarly affected (Feng et al., 2017; Lieder et al., 2009a; York, 2003a, c, 2002).

Delays in development have been reported following gestational PFBS exposure in mice, including delayed development of the female reproductive organs (i.e., ovaries, uterus, and vaginal patency), delayed and abnormal estrous cycling (i.e., first estrous and prolongation of diestrus), and delayed eye opening (Feng et al., 2017). Age at vaginal patency and ovarian follicle counts (i.e., in F_1 rat offspring after delivery of the F_2 generation) were unaffected at 1,000 mg/kg-day in a two-generation reproductive toxicity study (Lieder et al., 2009a). This observed lack of effects (i.e., on vaginal patency) is inconsistent with the findings in mice. However, Feng et al. (2017) also noted changes in reproductive hormones that might be relevant to the delays in female sexual development, including a decrease in serum estradiol and increased luteinizing hormone in pubertal offspring (i.e., PND 30 [Note: progesterone was decreased at a later age, PND 60, but not PND 30]). Because the changes reported in mice by Feng et al. (2017) were observed in parallel with effects on thyroid hormone levels (discussed above), it is plausible that these developmental delays and hormonal changes could represent sequalae of reduced thyroid function, although that was not directly tested.

For the most part, developmental effects have been reported in a single study and species (mouse); however, the findings are coherent with one another as well as with the consequences of decreased thyroid hormone levels. Because of the coherence across effects on the thyroid and several interrelated developmental effects in mice (i.e., delays and hormonal changes), the evidence in animals for developmental effects *supports a hazard*. There is no reason to expect that the specific developmental delays observed in mice would not be directly relevant to similar processes in humans. Thus, based on findings in animals that are presumed to be relevant to humans, the available evidence *supports a hazard* and the developing offspring is considered a potential target for PFBS toxicity in humans. Because no studies in humans were available that investigated these endpoints, this represents an area deserving of additional research.

5.3 REPRODUCTIVE EFFECTS

Reproductive outcomes, including male and female fertility, pregnancy outcomes, hormone levels, markers of reproductive development, and reproductive organ weights and histopathology, have been evaluated in a number of high-confidence studies in mice (Feng et al., 2017) and rats (NTP, 2019; Lieder et al., 2009a; Lieder et al., 2009b). In addition, five low-confidence human studies evaluated potential associations between PFBS exposure and

reproductive effects (Yao et al., 2019; Song et al., 2018; Zhang et al., 2018; Zhou et al., 2017a; Zhou et al., 2016).

PFBS exposure has resulted in no significant changes in male mating and fertility parameters, reproductive organ weights, or reproductive hormones. Although there were some slight, statistically significant effects on male reproductive endpoints in two rat studies [specifically, altered sperm parameters such as percentage of abnormal sperm or testicular sperm count (NTP, 2019; Lieder et al., 2009a) and delayed preputial separation at 1,000 mg/kg-day (Lieder et al., 2009a)], these findings were observed only at the highest doses and the levels of change were of questionable biological significance. No significant reproductive effects in men were noted across two human studies (Song et al., 2018; Zhou et al., 2016), although U.S. EPA noted a nonsignificant inverse association with testosterone and estradiol in male infants in one study (Yao et al., 2019).

In general, PFBS exposure in adults has also resulted in no significant alterations in female fertility or pregnancy outcomes in rats or mice (NTP, 2019; Feng et al., 2017; Lieder et al., 2009a; Lieder et al., 2009b) or in two human studies (Yao et al., 2019; Zhang et al., 2018; Zhou et al., 2017a; Zhou et al., 2016), and inconsistent changes in rodent reproductive organ weights were reported across studies regardless of duration and timing of exposure. However, changes in normal estrous cyclicity, specifically prolongation of the diestrus stage, have been reported in both nonpregnant adult rats exposed to PFBS (NTP, 2019) and adult mouse offspring exposed gestationally from GDs 1 to 20 (Feng et al., 2017). PFBS exposures in NTP (2019) began between 8 and 10 weeks of age; although the exposures might overlap with some aspects of reproductive development or changes in function during adolescence, these rats were sexually mature and thus the endpoints are considered in the context of reproductive, rather than developmental, effects. The mouse offspring in the study by Feng et al. (2017) also displayed delayed vaginal patency and histopathological markers of decreased fertility (i.e., decreased follicles and corpora lutea); however, the reproductive function of those offspring was not tested. While adult rat offspring (F_1) in a two-generation toxicity study also exhibited variable changes in estrous cyclicity (Lieder et al., 2009b), including prolonged diestrus at 100 mg/kg-day, this effect was not observed at higher doses, limiting interpretation, and no effects on vaginal patency were observed. Female reproductive hormones can inform the potential for effects on reproductive organ development, estrous cyclicity, and fertility. Changes in serum hormones included increased testosterone after exposure of female rats as adults (NTP, 2019), increased luteinizing hormone and decreased estradiol in pubertal mice after gestational exposure (Feng et al., 2017), and decreased estradiol and progesterone when these gestationally exposed mice were assessed as adults. Overall, the pattern and timing of hormonal changes after PFBS exposure is difficult to interpret and likely incomplete. However, the hormonal alterations after gestational PFBS exposure in mice are most relevant to conclusions about female reproductive health.

Taken together, the evidence indicates that the developing reproductive system, particularly in females, might be a target for PFBS toxicity. However, the potential for reproductive effects in adults was less clear, and significant impacts on mating or fertility parameters were not observed across the available studies. Therefore, the evidence in developing animals is considered most informative to conclusions relating to potential developmental effects (see above) and the evidence for reproductive effects (i.e., in adults) is *equivocal*. In the three studies of potential reproductive effects in humans, no clear associations

were observed, so the evidence in human studies is *equivocal*. Overall, based on *equivocal* human and animal evidence, the available evidence for reproductive effects is *equivocal*.

5.4 RENAL EFFECTS

Renal effects associated with oral exposure to PFBS have been observed in adult or developing rats across high- or medium-confidence gavage studies of various duration (NTP, 2019; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d).

Statistically significant increases in kidney weights have been observed in male and female rats after short-term exposure in one study (NTP, 2019), with strong dose-dependence for changes in relative weights in female rats at doses as low as 62.6 mg/kg-day. This study was likewise the only study to observe changes in serum markers of renal injury, specifically increased BUN in males at ≥250 mg/kg-day. However, while several other studies noted slight increases in weights, typically at higher PFBS doses (≥500 mg/kg-day), U.S. EPA found that these nonsignificant changes were not consistently observed across the set of available studies and no other studies reported changes in serum markers of renal injury (Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d).

Several kidney histopathology lesions (i.e., CPN, hydronephrosis, tubular degeneration, and tubular dilation) were unaffected by PFBS exposure in rats, although each of these endpoints was not assessed across several studies (NTP, 2019; Lieder et al., 2009a; 3M, 2000d). Mixed results were reported for mineralization and necrosis. Both of these endpoints were noted in females, but not males, after subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a), whereas mineralization was unaffected in male or female rats after short-term exposure (3M, 2000d), and necrosis was unaffected in male or female rats in short-term and two-generation (in both generations) studies (NTP, 2019; Lieder et al., 2009b). Multiple markers of inflammatory changes were consistently noted in the two longest exposure duration studies, which were the only studies to report on these endpoints. Specifically, increases in chronic pyelonephritis, tubular basophilia, and mononuclear cell infiltration were observed in female, but not male, rats following subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a). Similarly, increases in papillary edema and hyperplasia were observed in male and female rats after subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a), and in both generations of rats in the two-generation study at ≥300 mg/kg-day (Lieder et al., 2009b), with female rats being more sensitive than males.

Overall, the evidence in animals suggests an increased sensitivity of female rats (i.e., based on histopathology and organ-weight changes). Due primarily to the consistency and coherence in renal effects observed in the subchronic study by Lieder et al. (2009a) and the reproductive toxicity study by Lieder et al. (2009b) in male and female rats, the evidence in animals *supports a hazard*. There is insufficient evidence in the epidemiology studies of PFBS to inform the human relevance of these findings. Taken together, the renal histopathology evidence in rodents identifies a toxicologically significant spectrum of effects that is presumed to be relevant to similar changes known to occur in humans. Renal effects (i.e., uric acid) were evaluated in one low-confidence human study, and no clear association was observed; therefore, the evidence in human studies is *equivocal*. Overall, based on findings in animals that are presumed to be relevant to humans, the available evidence *supports a hazard* and indicates the kidney as a target organ of PFBS toxicity.

5.5 HEPATIC EFFECTS

Hepatic effects, including organ-weight changes and histopathology associated with oral exposures to PFBS, have been observed in high- or medium-confidence studies in adult or developing rats following short-term- and subchronic-duration exposures (NTP, 2019; Lieder et al., 2009a; 3M, 2001, 2000d) and in a two-generation reproductive study in rats (Lieder et al., 2009b). Increased absolute and/or relative liver weights were consistently observed in male and female rats after short-term and multigenerational exposure (NTP, 2019; Lieder et al., 2009b; 3M, 2001, 2000d). In some studies, the magnitude of the liver-weight changes and the doses at which effects occurred differed across sexes of rat, although the pattern across studies was unclear and did not consistently indicate one sex as more sensitive. Liver histopathology, including necrosis and inflammation, was not consistently observed across PFBS studies. One possible exception is increases in hepatocellular hypertrophy in male rats observed across two studies (NTP, 2019; Lieder et al., 2009b), although female rats were unaffected in the multigenerational study and this lesion was not observed at up to 600 mg/kg-day in the subchronic study by Lieder et al. (2009a). The only study to observe changes in serum markers of liver injury was NTP (2019), at ≥250 mg/kg-day in females and ≥500 mg/kg-day in males. The biological relevance or significance of the observed liver effects is not clear. In particular, the adversity of the variable changes in liver weight and observations of cellular hypertrophy is unclear. Further, the observed lesions either occurred in only one sex of rat, were not dose dependent compared with control, and/or occurred only at the highest PFBS dose tested. Thus, the evidence in animals is *equivocal*. Overall, based on *equivocal* animal evidence and a lack of human studies, the available evidence for hepatic effects is *equivocal*.

5.6 EFFECTS ON LIPIDS OR LIPOPROTEINS

Few studies have examined the effects of PFBS on circulating or hepatic lipid or lipoprotein homeostasis. It is recognized that increased circulating levels of lipids and lipoprotein products and/or increased hepatic lipid load are clinical observations of concern in humans. However, the lack of effect on lipid dynamics in most studies of rats exposed to high oral K^+PFBS doses for up to 90 days and the generally modest effects in transgenic mice, fed a high-fat, western-type diet renders this potential health outcome of unclear toxicological significance at this time. Thus, given the inconsistent, modest effects and the unclear biological relevance of these changes in isolation (i.e., lipids/lipoproteins were decreased, not increased) the evidence in animals is *equivocal*. Effects on serum lipids were evaluated in one low-confidence human study and childhood adiposity was evaluated in one medium-confidence study. Although an association was observed between increased PFBS exposure and increased total cholesterol and higher adiposity, this evidence in humans is *equivocal* due to lack of additional supportive evidence. Overall, based on *equivocal* evidence in both animal and human studies, the available evidence for effects on lipid or lipoprotein homeostasis is *equivocal*.

5.7 IMMUNE EFFECTS

Immune effects were observed in two human studies, including associations with asthma (Dong et al., 2013a) and atopic dermatitis (Chen et al., 2018). Exposure of human peripheral blood leukocytes or human promyelocytic THP-1 cells to PFBS, in culture, decreased cytokine (e.g., TNFα and IL-10) secretion following antigen challenge (Corsini et al., 2012). Because of the lack of additional evidence and some concerns about potential for residual confounding by other PFAS, the evidence in human studies is *equivocal*. Overall, based on *equivocal* evidence

in human studies and a lack of animal studies, the available evidence for immune effects is *equivocal*.

5.8 CARDIOVASCULAR EFFECTS

Cardiovascular effects were observed in two human studies, including associations with cardiovascular disease in adults (Huang et al., 2018) and hypertensive disorders in pregnancy (Huang et al., 2019b). The results are compelling, but as with the evidence for immune effects, there is a lack of additional supportive evidence and some concerns about potential for confounding; thus, the evidence in human studies is *equivocal.* Overall, based on *equivocal* evidence in human studies and a lack of animal studies, the available evidence for cardiovascular effects is *equivocal*.

5.9 EVIDENCE INTEGRATION AND HAZARD CHARACTERIZATION SUMMARY

Based on the evidence integration judgments regarding the potential for PFBS exposure to cause health effects (the narrative above is summarized in Table 7), the animal studies informing the potential effects of PFBS exposure on thyroid function, renal function, and development were concluded to *support a hazard*. Thus, for the purposes of this assessment, the animal data supporting these outcomes were considered for use in dose-response analysis, and other data were considered no further.

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Thyroid effects				
Human studies				*Supports a hazard* (animal evidence *supports a hazard*; human evidence is *equivocal*). The primary basis for this judgment is thyroid hormone decreases in mice and rats at ≥62.6 mg/kg-d.
• Low-confidence case-control study (Zhang et al., 2018)	• No factors noted.	• Single study of low confidence and poor sensitivity.	No association of PFBS with free T_3, free T_4, or thyroid stimulating hormone, but the study had poor sensitivity and other methodological limitations that hinder interpretability.	
Animal studies (all gavage)				
Mouse Studies: • High-confidence gestational (GDs 1−20) exposure study (Feng et al., 2017) Rat Studies: • High-confidence short-term (28-d) toxicity study (NTP, 2019)	• Consistent thyroid hormone decreases (i.e., for total T_3, total T_4, and free T_4) across two high-confidence studies of varied design. The findings were consistent across two species, sexes, life stages, and exposure durations. • Dose-response gradients were observed for those thyroid hormones. • Large magnitudes of effect (e.g., up to ~50% reductions in offspring serum hormones) were reported for those thyroid hormones.	• No factors noted.	Similar patterns of decreases in thyroid hormones (i.e., for total T_3, total T_4, and free T_4) were observed in PFBS-exposed pregnant mice and gestationally exposed female mouse offspring at ≥200 mg/kg-d (Feng et al., 2017) and in adult female and male rats at ≥62.6 mg/kg-d (NTP, 2019). Increased TSH was reported in mouse dams and in pubertal (PND 30) offspring following gestational exposure (Feng et al., 2017), but no changes were noted in rats exposed as adults (NTP, 2019). Thyroid weight and histopathology were not changed after short-term exposure in adult male or female rats (NTP, 2019).	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Developmental effects				
Human studies				*Supports a hazard* (animal evidence *supports a hazard*; human evidence is *equivocal*). The primary basis for this judgment is a set of persistent developmental delays and alterations in reproductive system maturation in female mice, generally at ≥200 mg/kg-d.
No studies available to evaluate.	--	--	--	
Animal studies (all gavage)				
Mouse Studies: • High-confidence gestational (GDs 1−20) exposure study (Feng et al., 2017) Rat Studies: • Two high-confidence gestational exposure (GDs 6−20) studies: a range-finding study and a follow-up study (York, 2003c, 2002) • High-confidence two-generation study (Lieder et al., 2009b)	• Biologically consistent spectrum of developmental effects in female offspring in a high-confidence mouse study at doses not causing maternal toxicity, including pronounced and persistent effects on BW, delays in developmental milestones and sexual maturation, concordant effects on reproductive organs, and altered serum hormones. • Concerning magnitude of effect (e.g., ~25% change in pup weight) and dose-dependence for several parameters. • Coherence of effects with thyroid hormone insufficiency (see above).	• Developmental effects were limited to changes in one study, sex, and species. • A high-confidence rat study reported some inconsistent evidence, including lack of a delay in vaginal patency and lack of clear effects on estrous cyclicity or ovarian morphology, although the latter endpoint was assessed in much older animals. These potential differences across species are not explainable based on toxicokinetics alone.	In the only mouse study (Feng et al., 2017), developmental effects and altered markers of female reproductive development or function were observed in female offspring after gestational PFBS exposure, including decreased BW, delayed eye opening, delayed vaginal opening, altered estrous cyclicity (including prolonged diestrus), altered reproductive hormones (e.g., decreased estradiol and progesterone), and effects on reproductive organs (e.g., weight and ovarian morphology). Most effects were observed at ≥200 mg/kg-d, with several changes noted at PND 60. Endpoints relating to fertility, pregnancy, survival, and fetal alterations were unchanged in both rats and mice across the four available studies, although this was not tested in mouse offspring (Feng et al., 2017). Developmental BW changes in rat offspring were either unchanged (Lieder et al., 2009b) or observed only at doses causing parental toxicity (York, 2003c, 2002).	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments				
Studies and Confidence	**Factors That Increase Support for Hazard**	**Factors That Decrease Support for Hazard**	**Summary of Findings**	**Overall Evidence Integration Judgment and Basis**
Continued:	Continued: Note: these effects were also coherent with effects on estrous cyclicity observed after short-term exposure in adult rats (NTP, 2019), but this was categorized as a reproductive effect (see below).	Continued:	Continued: In a rat two-generation study, while some statistically significant findings were noted for markers of female reproductive development or function, they were not dose-dependent or were of questionable biological relevance; thus, no clear changes in F_1 offspring were noted at doses up to 1,000 mg/kg-d regarding vaginal patency or estrous cycling at comparable ages to (Feng et al., 2017), or in ovarian morphology after the F_1 females gave birth to the F_2 pups.	Continued:
Reproductive effects				
Human studies				
Male reproductive effects				
• Low-confidence cohort study (Zhou et al., 2016) • Low-confidence cross-sectional study (Song et al., 2018) • Low-confidence cross-sectional study (Yao et al., 2019)	• No factors noted.	• Lack of clear association in studies of low confidence with poor sensitivity (i.e., due to low exposure levels, range).	No clear association between PFBS exposure and male reproductive hormones (Zhou et al., 2016) or semen parameters (Song et al., 2018) in adults. A study in newborns reported nonsignificant inverse associations between PFBS exposure and testosterone and estradiol (Yao et al., 2019).	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Female reproductive effects				*Equivocal* (*equivocal* human and animal evidence). Note: As the strongest evidence for female reproductive effects was in offspring that were gestationally exposed, these findings were considered most relevant to developmental, not reproductive, effects.
• Low-confidence cross-sectional study (Zhou et al., 2017a) • Low-confidence cohort study (Zhou et al., 2016) • Low-confidence cross-sectional study (Yao et al., 2019) • Low-confidence case-control study (Zhang et al., 2018)	• No factors noted.	• Lack of clear association in studies of low confidence with poor sensitivity (i.e., due to low exposure levels, range). • Potential for reverse causation for menstrual cycle characteristics and premature ovarian insufficiency.	No clear association between PFBS exposure and female reproductive hormones (Zhou et al., 2016) or menstrual cycle characteristics (Song et al., 2018).	
Animal studies *(all gavage)*				
Male reproductive effects				
Rat Studies: • High-confidence short-term (28-d) toxicity study (NTP, 2019) • High-confidence two-generation study (Lieder et al., 2009b) • High-confidence subchronic study (Lieder et al., 2009a)	• No factors noted.	• A few small, statistically significant changes were not dose-dependent or were of questionable biological relevance. • Lack of effects on male mating and fertility, hormones, or reproductive organs in rats.	Statistically significant effects on sperm health (NTP, 2019; Lieder et al., 2009a) and delayed preputial separation at 1,000 mg/kg-d (Lieder et al., 2009b) were not observed at lower doses, were within the normal range of historical controls for the laboratory, and/or were no longer significantly changed after correcting for other variables (e.g., BW). Other relevant parameters (e.g., organ weights, mating success, and so forth) were unchanged in the three studies.	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Female reproductive effects				
Mouse Studies: • High-confidence gestational (GDs 1−20) exposure study (Feng et al., 2017) Rat Studies: • High-confidence short-term (28-d) toxicity study (NTP, 2019) • High-confidence subchronic study (Lieder et al., 2009a) • High-confidence two-generation study (Lieder et al., 2009b)	• Effects on markers of female reproductive function (i.e., estrous cyclicity) were observed in high-confidence studies in rats and mice. • Changes in reproductive serum hormones were observed in female rats (i.e., increased testosterone) and mice (e.g., decreased estradiol and progesterone). Although the pattern of change is difficult to interpret and likely incomplete, there were no conflicting data.	• Lack of similar effects on reproductive function (i.e., estrous cyclicity) in a second high-confidence rat study. • Lack of effects on female fertility or pregnancy measures, although this was untested in prenatally exposed female mouse offspring. • Lack of organ-weight changes in three rat studies. Note: The lack of effects on ovarian follicles in rats did not decrease the support for hazard provided by findings in mice, as the age at endpoint assessment was not comparable.	See "Developmental effects" (above) for findings from Feng et al. (2017) and Lieder et al. (2009b). Altered estrous cyclicity (including prolonged diestrus) and increased serum testosterone were observed in female rats after short-term exposure, primarily at ≥250 mg/kg-d (NTP, 2019). Female reproductive organ weights were reduced in gestationally exposed mouse offspring (Feng et al., 2017), but were unchanged after short-term, subchronic, or two-generational exposure (NTP, 2019; Lieder et al., 2009a; Lieder et al., 2009b).	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Renal effects				
Human studies				*Supports a hazard.* (animal evidence *supports a hazard*; human evidence is *equivocal*). The primary basis for this judgment is kidney histopathology in rats, primarily females, at ≥300 mg/kg-d.
• Low-confidence cross-sectional study (Qin et al., 2016)	• No factors noted.	• Inconsistency across subpopulations in single study. • Single study of low confidence with concern for potential reverse causality.	Overall, there was no clear association for PFBS and uric acid. No association observed between PFBS and uric acid in the total population. Increase in uric acid with increased exposure in boys but decrease for girls (neither was statistically significant).	
Animal studies (all gavage)				
Rat Studies: • One high-confidence subchronic study (Lieder et al., 2009a) • Two high-confidence study (NTP, 2019; 3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study • One high-confidence two-generation study (Lieder et al., 2009b)	• Two high-confidence studies with the longest exposure durations reported consistent effects on kidney histopathology in male and female rats (females were more sensitive). • The histopathological effects related to inflammation were largely dose-dependent and of a concerning magnitude, although primarily at high doses (300 or 600 mg/kg-d).	• Inconsistency in kidney-weight changes across studies. • Findings are from a single laboratory and species. Note: The general lack of effects on other pathology endpoints in the shorter term studies was not considered to decrease support for hazard, as this was not interpreted as inconsistent.	Increases in kidney weight in male and female rats were observed in one short-term study at ≥ 62.6 mg/kg-d, but clear changes were not observed in the other short-term, subchronic, or two-generation rat studies. Kidney histopathology for some effects (i.e., CPN, hydronephrosis, tubular degeneration, and tubular dilation) was unchanged in single-study evaluations, and mixed results across studies were reported for mineralization and necrosis (NTP, 2019; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2000d). Multiple markers potentially related to inflammation and most notably papillary edema and hyperplasia were increased in the two longest duration studies (Lieder et al., 2009a; Lieder et al., 2009b), without contrary evidence.	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Continued:	Continued:	Continued:	Continued: Other markers of renal injury, including BUN and creatinine, were mostly unaffected across studies (NTP, 2019; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d), although the NTP study did observe effects on BUN in males at ≥250 mg/kg-d.	Continued:
Hepatic effects				
Human studies				*Equivocal* (*equivocal* human and animal evidence).
No studies available to evaluate	–	–	–	
Animal studies (all gavage)				
Rat Studies: • One high-confidence subchronic study (Lieder et al., 2009a) • Two high-confidence studies (NTP, 2019; 3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study • One high-confidence two-generation study (Lieder et al., 2009b)	• Consistent changes in liver weights in rats of both sexes across four studies. Although the pattern (e.g., by sex and dose) and magnitude of changes varied across studies, weights were consistently increased.	• Other than liver-weight changes, there were notable unexplained inconsistencies in the findings across studies. • One high-confidence study was entirely inconsistent.[a]	Absolute or relative liver weights were increased in all studies except the 90-d exposure component of the study by Lieder et al. (2009a), which tested doses up to 600 mg/kg-d. Note: 70 d of exposure in this study did elicit effects. Effects generally occurred at ≥300 mg/kg-d, although one study reported effects at lower doses (NTP, 2019; 3M, 2001), and two others (3M, 2001, 2000d) observed changes at ≥900 mg/kg-d. Serum markers of liver injury were unchanged in three studies (Lieder et al., 2009a; 3M, 2001, 2000d) and increased in one short-term study at ≥250 mg/kg-d (NTP, 2019).	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments				
Studies and Confidence	**Factors That Increase Support for Hazard**	**Factors That Decrease Support for Hazard**	**Summary of Findings**	**Overall Evidence Integration Judgment and Basis**
Continued:	Continued:	Continued:	Continued: Liver histopathology, specifically hepatocellular hypertrophy and cytoplasmic alterations in males and females (NTP, 2019) or hypertrophy in females only (Lieder et al., 2009a), were noted in two studies, but not in the others.	Continued:
Lipid or lipoprotein homeostasis				
Human studies				
• Low-confidence cross-sectional study (Zeng et al., 2015) • Medium-confidence study (Chen et al., 2019)	• Statistically significant association in medium-confidence study of adiposity. • Exposure response gradient observed across tertiles for adiposity.	• Single study per outcome. • Potential for residual confounding.	Increase in total cholesterol (statistically significant, β: 19.3 mg/dL increase per unit increase in PFBS) (Zeng et al., 2015). Higher adiposity in 5-year-old children associated with higher levels of PFBS in cord blood (Chen et al., 2019).	*Equivocal* (*equivocal* human and animal evidence).

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Animal studies				
Mouse Studies (diet): • Medium-confidence short-term (4−6 wk) study (Bijland et al., 2011); transgenic mice (human-like lipid metabolism) were fed a high-fat diet Rat Studies (all gavage): • One high-confidence subchronic study (Lieder et al., 2009a) • One high-confidence study (3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study	• Decreases in serum cholesterol and triglycerides were observed in male rats and mice.	• Inconsistent evidence in other rat studies and across sexes. • Small effect magnitudes and unclear direction (decreases) of changes are of questionable biological relevance and could not be informed by evaluating dose-dependency (i.e., only single-dose or high-dose effects were observed).	Serum lipids, specifically cholesterol and triglyceride levels, were slightly decreased (~20%) at 900 mg/kg-d in males, but not females, in one rat study (3M, 2001), but not in two other rat studies at up to 1,000 mg/kg-d. Serum and hepatic lipids and lipoproteins were also decreased in male mice exposed to ~30 mg/kg-d in diet.	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Immune effects				
Human studies				*Equivocal* (*equivocal* human and animal evidence).
Asthma				
• Medium-confidence case-control study (Zhou et al., 2016; Zhu et al., 2016; Dong et al., 2013b)	• Statistically significant association in a medium-confidence study. Note: Increases in eosinophil markers were not interpreted to increase support for hazard, because they were not statistically significant and other markers important to asthma etiology (e.g., IgE) were unchanged.	• Association was observed in a single study with concern regarding the potential for residual confounding (e.g., with other PFAS chemicals).	Statistically significant increase in odds of asthma diagnosis in the previous year (OR: 1.2−1.9) with increased PFBS exposure. Eosinophil markers (i.e., AEC and ECP) were increased with increased PFBS exposure in asthmatics and nonasthmatics; however, these increases did not reach statistical significance. IgE and T-helper cell-specific cytokines were unchanged (Zhu et al., 2016).	
Atopic dermatitis				
• Medium-confidence cohort study (Chen et al., 2018)	• No factors noted.	• Slight associations were not statistically significant in a single study with concern regarding the potential for residual confounding (e.g., with other PFAS chemicals).	Statistically nonsignificant increase in odds of atopic dermatitis (OR: 1.2) with increased PFBS exposure.	

Table 7. Summary of Hazard Characterization and Evidence Integration Judgments

Studies and Confidence	Factors That Increase Support for Hazard	Factors That Decrease Support for Hazard	Summary of Findings	Overall Evidence Integration Judgment and Basis
Animal studies				
No studies available to evaluate.	–	–	–	
Cardiovascular effects				
Human studies				
• Medium-confidence cross-section study (Huang et al., 2018) • Medium-confidence cross-sectional study (Huang et al., 2019b)	• Statistically significant associations in medium-confidence studies.	• Single study per outcome.	Higher odds of cardiovascular disease (total and individual types of disease) with PFBS exposure (Huang et al., 2018). Higher odds of hypertensive disorders in pregnancy with higher PFBS exposure (Huang et al., 2019b). There is potential for residual confounding that decreases confidence in the evidence.	*Equivocal* (*equivocal* human and animal evidence).
Animal studies				
No studies available to evaluate.	–	–	–	

[a]The lack of liver effects in the subchronic study was not interpreted to significantly reduce support for hazard because the maximum tolerated dose was 600 mg/kg-d, and other studies reported only liver effects at ≥900 mg/kg-d.

AEC = absolute eosinophil count; BUN = blood urea nitrogen; BW = body weight; CPN = chronic progressive nephropathy; ECP = eosinophilic cationic protein; GD = gestation day; IgE = immunoglobulin E; NTP = National Toxicology Program; OR = odds ratio; PFAS = per- and polyfluoroalkyl substances; PFBS = perfluorobutane sulfonic acid; PND = postnatal day; T_3 = triiodothyronine; T_4 = thyroxine; TSH = thyroid stimulating hormone.

6.0 DERIVATION OF VALUES

The hazard and dose-response database for PFBS and the potassium salt is primarily associated with the oral route of exposure. There are a limited number of dermal studies (see Table 5) and no known inhalation studies. There are no known studies evaluating potential cancer effects of PFBS. Therefore, only noncancer reference values are derived in this assessment for the oral route.

6.1 DERIVATION OF ORAL REFERENCE DOSES

The hazards of potential concern for oral PFBS exposure include thyroid, developmental, and kidney effects. Overall, the evidence *supports a hazard* for thyroid, developmental, and kidney effects based on the evidence from animal studies. The limited evidence for thyroid or renal effects in human studies is *equivocal*, and no studies evaluating developmental effects following PFBS exposure in humans were available. Thus, data in humans were not considered further, and the available animal studies that evaluated these effects are considered in the derivation of oral RfDs.

6.1.1 Derivation of the Subchronic Oral Reference Dose

6.1.1.1 Estimation of Points of Departure

Effects in the thyroid were considered when determining potential PODs for deriving a subchronic RfD. Similar patterns of decreases in total T_3, total T_4, and free T_4 were observed in PFBS-exposed pregnant mice, nonpregnant adult female rats, adult male rats, and gestationally exposed female mouse offspring (NTP, 2019; Feng et al., 2017). These decreases were significant (~20% in dams and ~50% in offspring), were shown to persist at least 60 days after gestational exposure in offspring, and they exhibited a clear dose dependence in both studies. Reflex increases in TSH in response to decreased T_4 or T_3 were not observed in male or female rats following 28 days of exposure (NTP, 2019). Such an increase in TSH was observed in pregnant mice (measured at GD 20) and their corresponding female offspring, at PND 30 only, with an irregular dose-response or time course (Feng et al., 2017). This pattern of decreased thyroid hormone without a concomitant increase in TSH is consistent with a human clinical condition referred to as "hypothyroxinemia" (Negro et al., 2011). Importantly, milder forms of thyroid perturbation are up to 10 times more prevalent in human populations than overt gestational hypothyroidism (Korevaar et al., 2016; Stagnaro-Green et al., 2011). Hypothyroxinemia has been associated with impairments in neurodevelopment and/or cognition later in life (Thompson et al., 2018; Min et al., 2016). Because the single available study in humans had severe limitations hindering the interpretation of the relationship between PFBS exposure and thyroid hormone alterations, at this time the available evidence in humans is not able to inform the potential for thyroid effects in humans. This hypothyroxinemia, rather than overt or subclinical hypothyroidism, is further supported by the lack of effect on thyroid weight or tissue architecture in rats after 28 days of PFBS exposure (NTP, 2019).

Developmental effects were considered in determining potential PODs for derivation of a subchronic RfD. Specifically, in Feng et al. (2017), developmental delays or abnormalities in growth (i.e., BW and eye opening), reproductive organs (i.e., ovaries, uterus, and vaginal opening), and reproductive cycling (i.e., first estrous and prolongation of diestrus) were observed

in mouse offspring. These effects were observed in mice from litters in which thyroid hormone deficiency occurred at PND 1 and then sustained through pubertal and adult periods (i.e., PNDs 30 and 60, respectively). These interrelated developmental effects in mice (i.e., delays and hormonal changes) are coherent with effects on the thyroid and presumed to be directly relevant to similar processes in humans; however, studies evaluating these outcomes in humans are not available.

Effects in the kidney were considered in determining potential PODs for deriving a subchronic RfD. Lieder et al. (2009a) reported mild to moderate hyperplasia in the kidneys of male and female rats following subchronic-duration exposure to PFBS, and Lieder et al. (2009b) found the same effects in the P_0- and F_1-generation animals in their reproductive toxicity study. Other studies evaluating effects in the kidney were of shorter duration and thus less suitable as a candidate principal study. Additional histopathological alterations accompanied the hyperplasia observed in the kidney, including papillary edema and inflammatory changes, specifically increases in chronic pyelonephritis, tubular basophilia, and mononuclear cell infiltration (Lieder et al., 2009a; Lieder et al., 2009b). Across the reported kidney histopathological effects following PFBS exposure, female rats were generally more sensitive than males.

Selected data sets from studies with multiple exposure levels for thyroid, developmental, and kidney effects were modeled using the U.S. EPA's Benchmark Dose Software (BMDS) Version 2.7. Consistent with the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change. Based on BMD guidance, in the absence of information regarding the level of change that is considered biologically significant, a BMR of 1 SD from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data is used to estimate the BMD and BMDL, and to facilitate a consistent basis of comparison across endpoints, studies, and assessments. For some types of effects (e.g., frank effects, developmental effects), biological considerations may warrant the use of a BMR of 0.5 SD or lower.

For effects in developing offspring, including thyroid hormone changes, a BMR of 0.5 SD change from the control mean is used for continuous data to account for effects occurring in a sensitive life stage. A 1 SD BMR is also presented as the basis for model comparison as directed in the U.S. EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012).

For thyroid hormone effects in offspring, a biological level of concern was considered in the identification of a BMR. Multiple lines of evidence regarding the degree of thyroid hormone disruption and developmental outcomes in offspring were evaluated. During developmental life stages, such as gestational/fetal and postnatal/early newborn, thyroid hormones are critical in many physiological processes associated with somatic growth and maturation and with life functions like thermogenesis, pulmonary gas exchange, and cardiac development (Sferruzzi-Perri et al., 2013; Hillman et al., 2012). Further, thyroid hormones are critically important in early neurodevelopment because they directly influence neurogenesis, synaptogenesis, and myelination (Rovet, 2014; Puig-Domingo and Vila, 2013; Stenzel and Huttner, 2013; Patel et al., 2011). Note that evidence from human epidemiological studies examining the association between thyroid hormone economy in pregnant mothers and neurodevelopment in their offspring is inconsistent. Several human epidemiologic studies have demonstrated key relationships between decreased

levels of thyroid hormones such as FT_4 in a pregnant woman and in utero and early postnatal life neurodevelopmental status. For example, children born euthyroid but who were exposed to thyroid hormone insufficiency in utero (e.g., ≤10th percentile free T_4), present with cognitive impairments (e.g., decreased intelligence quotient [IQ], increased risk of expressive language) and/or concomitant abnormalities in brain imaging (Levie et al., 2018; Korevaar et al., 2016; Henrichs et al., 2010; Lavado-Autric et al., 2003; Mirabella et al., 2000). Maternal hypothyroxinemia was also associated with adverse motor function and teacher-reported problems of behavior in offspring at 5 years of age (Andersen et al., 2018). Other human epidemiologic studies have not reported significant associations between thyroid hormone status during pregnancy and neurodevelopmental outcomes in offspring. For example, there was no statistically significant association between thyroid status and IQ decrements or neuropsychological parameters in children born to mothers screened and diagnosed with subclinical hypothyroidism (Hales et al., 2018; Lazarus et al., 2012) or mothers undergoing treatment for hypothyroxinemia during gestation (Casey et al., 2017). In these studies, the timing of maternal hypothyroxinemia during pregnancy may be a critical consideration for developmental health outcomes in offspring. Studies have observed a relationship between low free T_4 levels in women at 12 weeks gestation, but not 32 weeks gestation, and impaired psychomotor development in their offspring (Kooistra et al., 2006; Pop et al., 2003). In addition, differences in the type of maternal disruption of thyroid homeostasis may affect the interpretation of the human epidemiologic study results. Specifically, aside from overt primary hypothyroidism, there are two primary subcategories of hypothyroidism: (1) subclinical hypothyroidism; and (2) hypothyroxinemia. Subclinical hypothyroidism is characterized by *elevated TSH levels with normal serum T_4 and T_3 concentrations*. In contrast, hypothyroxinemia is characterized by *decreased T_4 with normal serum concentrations of TSH and T_3* (Alexander et al., 2017; Choksi et al., 2003). Maternal T_4 is the primary source of thyroid hormone for a developing human fetus in the first trimester (i.e., little if any maternal T_3 is transferred across the placenta primarily due to high levels of deiodinase 3 activity that catabolizes T_3 to a biologically inactive form). The first trimester is also a critical window for central nervous system development (e.g., neural tube, spinal cord, medulla, pons, thalamus/hypothalamus, etc.). It therefore stands to reason that the health implications may be different for early in utero development if associated with a condition where maternal T_4 (and T_3) concentrations are normal (subclinical hypothyroidism) versus one involving decreased levels of T_4 (hypothyroxinemia).

With regard to what level of decrease in thyroid hormone (e.g., T_4) is sufficient for anatomical and/or functional alterations, particularly in neurodevelopment in fetuses or newborns, several studies have identified a range of T_4 decrements associated with neurodevelopmental health outcomes across humans or experimental rodents. For example, neurodevelopmental and cognitive deficits have been observed in children who experienced a 25% decrease in maternal T_4 during the second trimester in utero (Haddow et al., 1999). In other studies, mild to moderate thyroid insufficiency in pregnant women was defined as having serum T_4 levels below the 10th percentile for the study population, which was associated with a 15−30% decrease relative to the corresponding median (Finken et al., 2013; Julvez et al., 2013; Román et al., 2013; Henrichs et al., 2010). In experimental animals, decreases in mean maternal T_4 levels of ~10−17% during pregnancy and lactation have been found to elicit neurodevelopmental toxicity in rat offspring (Gilbert et al., 2016; Gilbert, 2011). With regard to a general diagnostic criterion to delineate hypothyroxinemia from other types of clinical hypothyroidism, the Controlled Antenatal Thyroid Study (CATS), conducted in a large cohort of pregnant women in Europe, resulted in the identification of a condition referred to as “isolated hypothyroxinemia”

and is defined as the presence of free thyroxine (FT_4) below the 2.5th percentile with a thyrotropin (TSH) level within the reference range (Hales et al., 2018; Lazarus et al., 2012; Negro et al., 2011). However, there is no clear or consistent biological threshold for T_4 changes specifically associated with untoward developmental health outcomes, so a BMR of 0.5 SD was therefore identified as a default when performing BMD modeling on thyroid hormone alterations in offspring, consistent with U.S. EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). Further, while total T_4 (TT_4), free T_4 (FT_4), and TSH dose-response data are BMD modeled (see Table 9), important biological considerations are presented in Section 6.1.1.2 that delineate TT_4 as the key hormone metric for a developing fetus/neonate.

Significantly decreased thyroid hormone (e.g., T_4 and T_3) was observed in adult rats exposed twice daily to oral K^+PFBS (NTP, 2019) for 28-days, as well as the P_0 (maternal) mice of the Feng et al. (2017) study. No overt signs of traditional hypothyroidism such as increased TSH and increased thyroid tissue weight or histopathology were observed in either adult population. Adult rodents have a considerable reserve thyroid hormone capacity compared with the developing offspring, which depend on their supply from maternal T_4. While there is concern over decreases in thyroid hormone (i.e., hypothyroxinemia) in developmental life stages due to critical endocrine dependency of in utero and neonatal development, the levels at which there is concern for hypothyroxinemia in euthyroid adults is unclear. Therefore, for euthyroid adult rats and mice, a biologically significant level of change was not determined for the BMR because it is unclear what magnitude of hormone perturbation would be considered adverse. Therefore, for thyroid hormone effects in adult rodents, a default BMR of 1 SD from control mean was applied. Section 6.1.1.2 presents critical distinctions between perturbations in thyroid hormone economy in adults versus developing fetus/neonates, resulting in the use of different BMRs across life stages (e.g., 1 SD for adults, 0.5 SD for newborns).

For kidney hyperplasia data from the subchronic study by Lieder et al. (2009a) and the two-generation reproductive toxicity study by Lieder et al. (2009b), a BMR of 10% extra risk was used because it is the recommended approach for dichotomous data in the absence of information on the minimally significant level of change.

6.1.1.2 Approach for Animal-Human Extrapolation of Perfluorobutane Sulfonic Acid Dosimetry

As discussed in Section 1.3, toxicokinetic data exists for PFBS in relevant animal species (i.e., rats and mice) and humans, such that a data-informed adjustment approach for estimating the dosimetric adjustment factor (DAF) can be used. In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the U.S. EPA endorses a hierarchy of approaches to derive human equivalent oral exposures using data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches might include using chemical-specific information, without a complete physiologically based toxicokinetic model. In the absence of chemical-specific models or data to inform the derivation of human equivalent oral exposures, the U.S. EPA endorses $BW^{3/4}$ as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions.

The U.S. EPA concluded that data for PFBS are adequate to support derivation of data-informed dosimetric adjustment. Briefly, the ratio of the clearance (*CL*) in humans to

animals, CL_H:CL_A, can be used to convert an oral dose-rate in experimental animals (mg/kg-day) to a human equivalent dose rate. Assuming the exposure being evaluated is low enough to be in the linear (or first order) range of clearance, the average blood concentration (C_{AVG}) that results from a given dose is calculated as:

$$C_{AVG}\left(\text{mg}/\text{mL}\right) = f_{abs} \times \text{dose (mg/kg/hr)} \Big/ CL\ \text{(mL/kg/hr)}$$

where f_{abs} is the fraction absorbed and dose is the average dose rate expressed at an hourly rate. Assuming equal toxicity given equal C_{AVG} in humans as in mice or rats, and that f_{abs} is the same in humans as animals, the equitoxic dose, human equivalent dose (HED) (i.e., the human dose that should yield the same blood concentration (C_{AVG}) as the animal dose from which it is being extrapolated), is then calculated as follows:

$$HED = \frac{POD}{CL_A / CL_H} = POD \times \frac{CL_H}{CL_A}$$

Thus, the DAF could be calculated as simply CL_H:CL_A, the ratio of clearance in humans to clearance in the animal from which the POD is obtained. However, clearance values are not reported for humans in the available toxicokinetic studies for PFBS (Xu et al., 2020; Olsen et al., 2009). Because clearance is a measure of average elimination, to calculate clearance in the absence of the information, one also needs to evaluate a companion variable, the V_d. Neither Olsen et al. (2009) nor Xu et al. (2020) reported the V_d for humans. However, there is evidence suggesting that V_d for PFBS is relatively similar across species, including rodents (e.g., 0.12−0.29 L/kg across male and female rats following 10 mg/kg i.v. dose) and monkeys (e.g., 0.21−0.25 L/kg across male and female cynomolgus macaques following 10 mg/kg i.v. dose) (Chengelis et al., 2009; Olsen et al., 2009). Therefore, it is reasonable to assume V_d for humans is approximately equivalent to V_d for animals (i.e., $V_{d,H} = V_{d,A}$), in which case clearance and half-life are inversely related as follows:

$$CL\ \text{(mL/kg/hr)} = \ln(2) \times \frac{1}{t_{1/2}\text{(hr)}} \times V_d\ \left(\text{mL}/\text{kg}\right)$$

Because reliable measures of half-life in humans and animals are available for PFBS, the ratio of elimination half-life in animals from which the POD is obtained to that in humans, $t_{1/2,A}$:$t_{1/2,H}$, can be used to calculate the DAF, and the human equivalent dose (HED) can be calculated as follows:

$$HED = POD \times \frac{t_{1/2_A}}{t_{1/2_H}}$$

As described in Section 1.3, two studies evaluated the elimination of human serum K^+PFBS in human populations with previous occupational exposure (Xu et al., 2020; Olsen et al., 2009). Initial blood concentrations of PFBS in the population examined by Xu et al. (2020) are more representative of environmental exposure, and the population was larger, including 11 male and 6 female employees when compared to Olsen et al. (2009). While the estimated serum half-life of PFBS reported by Olsen et al. (2009) overlapped with that by Xu et al. (2020)

(mean: 43.8 days; range: 21.9−87.6 days), there is a statistically significant difference between these two studies. As such, the two data sets will not be combined and the half-life estimated by Xu et al. (2020) is presumed to better predict human dosimetry at environmental levels. The average half-life reported by Xu et al. (2020) (mean: 43.8 days or 1,050 hours) was assigned for $t_{½,H}$.

One study evaluated the elimination of serum PFBS in mice. Lau et al. (2020) reported serum terminal half-lives of 5.8 hours in male mice and 4.5 hours in female mice. Because the half-life estimates did not vary significantly between the doses (i.e., 30 and 300 mg/kg), these parameter estimates were combined. However, there was a statistically significant difference in the half-life estimates between sexes (female mice had a slightly shorter half-life [4.5 hours] compared to males [5.8 hours]), so sex-specific half-lives were assigned for $t_{1/2,A}$ for mice.

Two studies were used to calculate serum half-life estimates for dosimetric adjustment in rats (Huang et al., 2019a; Olsen et al., 2009). A numerical average of the terminal half-lives ($t_{1/2,\beta}$) measured in rats after oral and i.v. doses is identified in Olsen et al. (2009) as 4.6 hours in males and 5.7 hours in females. Olsen et al. (2009) reported sex-specific elimination differences in half-life values in rats. A numerical average of the $t_{1/2,\beta}$ measured in male rats after oral and i.v. doses in Huang et al. (2019a) is 4.9 hours. In male rats, half-life values reported in Olsen et al. (2009) and Huang et al. (2019a) are consistent, thus they were averaged for use in dosimetric adjustment, resulting in a geometric mean terminal serum half-life of 4.8 hours. The terminal half-life value reported by Huang et al. (2019a) in female rats after a 4-mg/kg i.v. dose of PFBS was 0.95 hours. Huang et al. (2019a) was not able to fit the data to a two-compartment model, thus they did not report a $t_{1/2,\beta}$ for rats following oral exposure. For this reason, the mean female $t_{1/2,\beta}$ value from Olsen et al. (2009) was used for dosimetric adjustment.

Table 8 presents the DAFs for converting rat and mice PODs to HEDs for PFBS.

Table 8. Mouse, Rat, and Human Half-Lives and Data-Informed DAFs

Species	Sex	Animal $t_{1/2}$ (hr)	Human $t_{1/2}$ (hr)	DAF ($t_{1/2,A}/t_{1/2,H}$)
Mouse	Male	5.8[a]	1,050[b]	0.0055
	Female	4.5[c]		0.0043
Rat	Male	4.8[d]		0.0046
	Female	5.7[e]		0.0054

[a]Terminal serum half-life of combined doses for male mice from Lau et al. (2020).
[b]Mean serum elimination half-life for humans (combined sexes) from Xu et al. (2020).
[c]Terminal serum half-life of combined doses for female mice from Lau et al. (2020).
[d]Geometric mean of terminal serum half-lives ($t_{1/2,\beta}$) measured after all oral and i.v. doses for male rats from Olsen et al. (2009) and Huang et al. (2019a).
[e]Mean of terminal serum half-lives ($t_{1/2,\beta}$) measured after oral and i.v. doses for female rats from Olsen et al. (2009).

DAF = dosimetric adjustment factor; i.v. = intravenous; $t_{1/2}$ = half-life.

Where modeling was feasible, the estimated BMDLs were identified as PODs (summarized in Table 9). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in HAWC and are discussed in Appendix F. Where dose-response modeling was not feasible, NOAELs or LOAELs were identified (summarized in Table 9).

Table 9. PODs Considered for Deriving the Subchronic RfD for K^+PFBS (CASRN 29420-49-3)

Endpoint/Reference	Species/Life Stage—Sex	POD (HED)[a] (mg/kg-d)	Comments[‡]
Thyroid effects			
Total T_4—Feng et al. (2017)	Mouse/P_0—female	$BMDL_{1SD}$ = 0.093	Adequate model fit
Free T_4—Feng et al. (2017)	Mouse/P_0—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
TSH—Feng et al. (2017)	Mouse/P_0—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
Total T_4 PND 1 (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate fit to the data, specifically variance
Total T_4 PND 1 (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.095 ($BMDL_{1SD}$ = 0.25)	Adequate model fit
Total T_4 PND 30—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
Total T_4 PND 60—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate fit to the data, specifically variance

Table 9. PODs Considered for Deriving the Subchronic RfD for K^+PFBS (CASRN 29420-49-3)

Endpoint/Reference	Species/Life Stage—Sex	POD (HED)[a] (mg/kg-d)	Comments[‡]
TSH PND 30—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
Total T_4—NTP (2019)	Rat—male	LOAEL = 0.29	No models provided adequate statistical or visual fit to mean responses
	Rat—female	$BMDL_{1SD}$ = 0.037	Adequate model fit
Free T_4—NTP (2019)	Rat—male	LOAEL = 0.34	No models provided adequate statistical or visual fit to mean responses
	Rat—female	$BMDL_{1SD}$ = 0.027	Adequate model fit
Developmental effects			
Eyes opening (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate fit to the data, specifically variance
Eyes opening (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.073 ($BMDL_{1SD}$ = 0.16)	Adequate model fit
Vaginal opening (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.15 ($BMDL_{1SD}$ = 0.35)	Adequate model fit
Vaginal opening (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.094 ($BMDL_{1SD}$ = 0.22)	Adequate model fit
First estrous (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
First estrous (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21	No models provided adequate statistical or visual fit to mean responses
Kidney effects			
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009a)	Rat—male	$BMDL_{10}$ = 0.49	Adequate model fit
	Rat—female	$BMDL_{10}$ = 0.30	Adequate model fit
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/P_0—male	$BMDL_{10}$ = 0.35	Adequate model fit
	Rat/P_0—female	$BMDL_{10}$ = 0.27	Adequate model fit

Table 9. PODs Considered for Deriving the Subchronic RfD for K^+PFBS (CASRN 29420-49-3)

Endpoint/Reference	Species/Life Stage—Sex	POD (HED)[a] (mg/kg-d)	Comments[‡]
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/F_1—male	$BMDL_{10}$ = 0.78	Adequate model fit
	Rat/F_1—female	$BMDL_{10}$ = 0.48	Adequate model fit

[a]Following U.S. EPA (2011b) and U.S. EPA (2014d) guidance, animal doses from candidate principal studies were converted to HEDs by applying a DAF, where HED = dose × DAF.
[b]Fetal endpoints from Feng et al. (2017) were modeled alternatively using dose-group sizes based either on total number of fetuses or dams. Given that Feng et al. (2017) seems not to have used the litter as the statistical unit of analysis, it is unclear whether the study-reported standard errors pertain to litters or fetuses. Alternatively, modeling fetal endpoints using litter *n* or fetal *n* provides two modeling results that bracket the "true" variance among all fetuses in a dose group (i.e., using the fetal *n* will underestimate the true variance while using the litter *n* will overestimate the true variance). Individual animal data were requested from study authors but were unable to be obtained.
[‡]BMD modeling methods and links to modeling inputs and results in HAWC are found in Appendix F. HAWC visualization: Candidate PODs for subchronic and chronic RfD.

$BMDL_{0.5SD}$ = benchmark dose lower confidence limit for 0.5 SD change from the control; $BMDL_{10}$ = 10% benchmark dose lower confidence limit; $BMDL_{1SD}$ = benchmark dose lower confidence limit for 1 SD change from the control; DAF = dosimetric adjustment factor; HAWC = Health Assessment Workspace Collaborative; HED = human equivalent dose; K^+PFBS = potassium perfluorobutane sulfonate; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PND = postnatal day; POD = point of departure; RfD = oral reference dose; SD = standard deviation; T_4 = thyroxine; TSH = thyroid stimulating hormone.

6.1.1.3 Considerations in Selecting the Critical Effect for Deriving Oral Reference Doses

The evidence for the thyroid, developmental, and kidney effect domains *support a hazard* via the oral exposure route (see Table 7). However, there are qualitative and quantitative differences in the strength of evidence between these effect domains (see Table 9). PFBS-induced perturbation of the thyroid was consistently observed across two species, sexes, life stages, and exposure durations in two independent, high-confidence studies. These perturbations involved a coherent pattern of hormonal changes with similar sensitivity in the POD ranges across life stages (e.g., maternal and PND 1/newborn $BMDL_{05}$s of 0.093 and 0.095 mg/kg-day, respectively). Developmental effects (e.g., delayed eyes opening, vaginal opening, or first estrous) were observed in mouse litters in which decrements in thyroid hormone occurred and with similar sensitivity in the ranges of POD estimates (i.e., 0.073−0.21 mg/kg-day) (Feng et al., 2017). However, these developmental effects have been reported in a single study and species (mouse). Kidney effects in adult animals (Lieder et al., 2009a; Lieder et al., 2009b) were observed in adult or developing rats across high- or medium-confidence gavage studies of various duration; however, were less sensitive at 0.27 mg/kg-day and above.

In deriving a subchronic RfD, both the Feng et al. (2017) and NTP (2019) studies were considered as potential principal studies because of the observed sensitivity of thyroid hormone decrements. However, the biological significance of hypothyroxinemia (i.e., decreased T_4) in

adult euthyroid animals, absent additional signs of overt thyroid toxicity (e.g., reflex increase in TSH and/or alterations in tissue weight or histology), is unclear; therefore, the thyroid effects from the NTP (2019) rat study were not selected as a critical effect. The gestational exposure study in mice was selected as the principal study for deriving the subchronic RfD based on thyroid effects. The gestational exposure study conducted by Feng et al. (2017) reported administration of K^+PFBS by gavage in ICR mice (10/dose) from GDs 1 to 20. This study was of good quality (i.e., high confidence) with adequate reporting and consideration for appropriate study design, methods, and conduct (click to see risk of bias analysis in HAWC). Feng et al. (2017) reported statistically significantly decreased total T_3, total T_4, and free T_4, as well as increased TSH in dams and offspring (increased TSH PND 30 only) gestationally exposed to PFBS.

The critical effect from the Feng et al. (2017) study was decreased serum total thyroxine (T_4) in newborn (PND 1) mice. T_4 and T_3 are essential for normal growth of developing offspring across animal species [for review see Forhead and Fowden (2014)]. And, previous studies have shown that exposure to other PFAS during pregnancy results in lower T_4 and T_3 levels in pregnant women and fetuses or neonates (Yang et al., 2016; Wang et al., 2014). The selection of total T_4 as the critical effect is based on a number of key considerations (see below) that account for cross-species correlations in thyroid physiology and hormone dynamics particularly within the context of a developmental life stage.

A key consideration for selecting total T_4 is that this represents the aggregate of potential thyroid endocrine signaling (i.e., free T_4 + protein bound T_4) at any given time. In humans, FT_4 represents approximately 0.03% of circulating hormone, indicating that as much as 99.97% of all T_4 is protein bound (e.g., albumin; TBG). Although T_3 is the active hormone form in respondent somatic tissues, the formation of T_3 is contingent upon the deiodination of free T_4. A critical consideration in pregnant females is that T_4, not T_3, is the thyroid hormone that crosses the placenta of humans and rodents. Although free T_4 might be considered a suitable measure of thyroid hormone status in nondevelopmental (e.g., adult) life stages, there are some important factors associated with maintenance of the microenvironment for developing offspring in utero that supports using total T_4 as the critical effect. A tightly regulated transfer of maternal thyroid hormone to a fetus is paramount to proper development of multiple tissues and organ systems (e.g., nervous system), especially during the early trimesters. The placenta has transporters and deiodinases that collectively act as a gatekeeper to maintain an optimal T_4 microenvironment in the fetal compartment (Fisher, 1997; Koopdonk-Kool et al., 1996). For example, deiodinase 3 (D3) is highly expressed in human uterus, placenta, and amniotic membrane, where it serves a critical role of regulating thyroid hormone transfer to the fetus through the deiodination of T_4 to transcriptionally inactive reverse triiodothyronine (rT_3) or T_3 to inactive 3,5-diiodo-L-thyronine (T_2). Similarly, Wasco et al. (2003) showed that D3 is highly expressed in the rodent uterus and is highly induced during pregnancy. Further, the *Dio3* gene that encodes D3 has been shown to be imprinted in the mouse (Hernandez et al., 2002), suggesting a pivotal role for this specific deiodinase in the mouse as well. Indeed, the human and rodent placenta have been shown to be similarly permeable to T_4 and T_3 (Fisher, 1997; Calvo et al., 1992). Due to placental barrier functionality, free T_4 levels in a pregnant dam might not be entirely representative of actual T_4 status in a developing fetus. Further, the American Thyroid Association published a guidelines document in 2017 in which they stated: “Current uncertainty around FT_4 estimates in pregnancy has led some to question the wisdom of relying on any FT_4 immunoassays during pregnancy. In

contrast, measurement of TT_4 and the calculated FT_4 index do show the expected inverse relationship with serum TSH. This finding suggests that TT_4 measurements may be superior to immunoassay measurement of FT_4 measurements in pregnant women" (Alexander et al., 2017). Thus, decreased total T_4 in offspring (and dams during pregnancy/at delivery) is expected to be more representative of PFBS-mediated thyroid effects and potentially associative developmental effects.

There are some differences in HPT development and functional maturation and regulation during early life stages (e.g., timing of in utero and early postnatal thyroid development) between humans and rodents [for a comprehensive overview see Regulatory Science Associates (2019)]. Human thyroid development occurs in three phases in utero which entails initial development of the gland between Embryonic Day 10 to Gestational Week 11 (Phase I), maturation of the fetal thyroid system from Gestational Weeks 11−35 (Phase II), and further refinement of hypothalamic-pituitary-thyroid axis functionality during the latter portion of gestation up to approximately 4 weeks into the postnatal period (Phase III) (Klein et al., 1982; Fisher and Klein, 1981). Importantly, in utero development of the rodent thyroid gland occurs in the same phases and order as humans, the difference being that rodents are essentially born during Phase II, with Phase III occurring almost exclusively postnatally; whereas in humans, Phase III is well underway in utero and completes postnatally. Accordingly, rodent neurodevelopment in the early postnatal phase is analogous to the third trimester of human development in utero (Gilbert et al., 2012). Further, fetal development of rodents in utero is entirely dependent on maternal thyroid hormone until approximately GD 17−18, whereas in humans, fetal development transitions from complete reliance on maternal thyroid hormone during the first trimester (i.e., thyroid development Phase I) to a mix of fetal thyroid hormone synthesis and maternal transplacental hormone transfer beginning in the second trimester (i.e., thyroid development Phase II) through the in utero portion of Phase III (Fisher and Klein, 1981).

Within the context of early developmental life stages, there are several commonalities in HPT dynamics between humans and rodents such as similar profiles of (1) thyroid hormone binding proteins, (2) hormone functional reserve, and (3) placental deiodinase. For example, two carrier proteins—thyroid binding globulin (TBG) and transthyretin (TTR)—are primarily responsible for storage and transit of T_4 in mammals (Rabah et al., 2019). TBG is the primary carrier of T_4 in humans across all life stages (Savu et al., 1991). Importantly, in fetal and infant rats, TBG is also the primary carrier of T_4 (Savu et al., 1989). As rats transition to adulthood, TTR takes over as the primary carrier of T_4. In addition, as a relatively highly abundant carrier protein, albumin also plays a role in thyroid hormone binding and transit in humans and rodents; however, the relative affinity for binding is lower than either TBG or TTR.

Life-stage-specific differences in thyroid hormone reserve capacity between adults and neonates have been noted. On average, intrathyroidal thyroglobulin stores in adults are on the order of months, whereas in neonates the functional reserve is approximated at less than 1 day (Gilbert and Zoeller, 2010; Savin et al., 2003; van den Hove et al., 1999). This suggests that the adult thyroid has compensatory abilities not present in early life stages, making fetal/neonatal populations particularly sensitive to perturbations in thyroid hormone economy (e.g., hypothyroxinemia). And although the timing of thyroid development can vary between species (Forhead and Fowden, 2014), the dynamic reserve capacity of T_4 between humans and

rodents near birth and in early postpartum might not be significantly different. For example, human neonates have a serum half-life of T_4 of approximately 3 days (Vulsma et al., 1989), and thyroid tissue stores of T_4 are estimated to be less than 1 day (van den Hove et al., 1999). Because the developing rodent thyroid does not begin producing its own hormone until late in gestation (≥GD 17), newborn rodent T_4 levels are primarily a reflection of transplacentally translocated maternal hormone; and adult rats have been shown to have a serum T_4 half-life of 0.5–1 day (Choksi et al., 2003). For this reason, significant differences in functional thyroid reserve capacity between human and rodent neonates are not anticipated.

Accounting for the information presented above, the subchronic RfD, based on the $BMDL_{0.5SD}$ (HED) of 0.095 mg/kg-day for decreased serum total T_4 in newborn (PND 1) mice, is derived as follows:

Subchronic RfD for K^+PFBS = $BMDL_{0.5SD}$ (HED) ÷ UF_C
= 0.095 mg/kg-day ÷ 100
= 0.00095mg/kg-day
= **1×10^{-3} mg/kg-day**

Table 10 summarizes the uncertainty factors for the subchronic RfD for K^+PFBS based on effects in the thyroid.

Table 10. Uncertainty Factors for the Subchronic RfD for Thyroid Effects for K^+PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between mice and humans following oral K^+PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b). However, some residual uncertainty remains in the relative cross-species sensitivity in toxicodynamics (e.g., thyroid signaling). Thus, in the absence of chemical-specific data to quantify these uncertainties, U.S. EPA's guidance recommends use of a UF_A of 3.
UF_D	3	A UF_D of 3 is applied due to database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2019; Bijland et al., 2011; 3M, 2010; Lieder et al., 2009a; 3M, 2001, 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002). However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, because other health effect domains such as immunotoxicity and mammary gland development are effects of increasing concern across several members of the larger PFAS family (Grandjean, 2018; Liew et al., 2018; White et al., 2007), the lack of studies evaluating these outcomes following PFBS exposure is a limitation in the database.
UF_H	10	A UF_H of 10 is applied to account for interindividual variability in the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (life style) factors that can influence the response to dose. In the absence of chemical-specific data to quantify this variability in the toxicokinetics and toxicodynamics of K^+PFBS/PFBS in humans, U.S. EPA recommends using a UF_H of 10.

Table 10. Uncertainty Factors for the Subchronic RfD for Thyroid Effects for K^+PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR was selected based on evidence that it represented a minimal biologically significant response level in susceptible populations such as developing offspring.
UF_S	1	A UF_S of 1 is applied because the POD comes from a developmental study in mice. The developmental period is recognized as a susceptible life stage in which exposure during certain time windows (e.g., gestational) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991a).
UF_C	100	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

BMDL = benchmark dose lower confidence limit; BMR = benchmark response; DAF = dosimetric adjustment factor; HED = human equivalent dose; K^+PFBS = potassium perfluorobutane sulfonate; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFAS = per- and polyfluoroalkyl substances; PFBS = perfluorobutane sulfonic acid; POD = point of departure; RfD = oral reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

The data for K^+PFBS can be used to derive a subchronic RfD for the free acid (PFBS), as K^+PFBS is fully dissociated in water at the environmental pH range of 4–9 (NICNAS, 2005). To calculate the subchronic RfD for the free acid, the subchronic RfD for the potassium salt is adjusted to compensate for differences in MW between K^+PFBS (338.19) and PFBS (300.10). The subchronic RfD for PFBS (free acid) is calculated as follows:

Subchronic RfD for PFBS (free acid) = RfD for K^+PFBS salt × (MW free acid ÷ MW salt)
= 0.00095 mg/kg-day × (300.10 ÷ 338.19)
= 0.00095 mg/kg-day × (0.89)
= 0.00085 mg/kg-day
= **9×10^{-4} mg/kg-day**

Confidence in the subchronic RfD for PFBS and K^+PFBS for thyroid effects is medium, as explained in Table 11.

Table 11. Confidence Descriptors for the Subchronic RfD for PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Confidence Categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.

Table 11. Confidence Descriptors for the Subchronic RfD for PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Confidence Categories	Designation	Discussion
Confidence in database	M	Confidence in the oral toxicity database for derivation of the candidate subchronic RfD for thyroid effects is medium because although there are multiple developmental toxicity studies in mice and rats, no studies are available that have specifically evaluated neurodevelopmental, immunological, or mammary gland effects. In addition, available toxicokinetic studies are limited (e.g., one mouse toxicokinetic study) and toxicokinetic data do not exist for PFBS at all life stages, including neonates, infants, and children. Additionally, studies are not available to estimate the relative cross-species sensitivity in toxicodynamics (e.g., thyroid signaling).
Confidence in candidate subchronic RfD	M	The overall confidence in the candidate subchronic RfD for thyroid effects is medium.

H = high; HAWC = Health Assessment Workspace Collaborative; K^+PFBS = potassium perfluorobutane sulfonate; M = medium; PFBS = perfluorobutane sulfonic acid; RfD = oral reference dose.

The subchronic RfD is derived to be protective of all types of effects across studies and species following oral subchronic exposure and is intended to protect sensitive subpopulations and life stages.

6.1.2 Derivation of the Chronic Oral Reference Dose

There are no chronic studies available for PFBS and K^+PFBS. Therefore, based on the same database and similar considerations as the subchronic RfD, the noncancer chronic RfD is derived, based on the same $BMDL_{0.5SD}$ (HED) of 0.095 mg/kg-day for decreased serum total T_4 in newborn (PND 1) mice (Feng et al., 2017), as follows:

Chronic RfD for K^+PFBS $= BMDL_{0.5SD}\text{ (HED)} \div UF_C$
$= 0.095\text{ mg/kg-day} \div 300$
$= 0.00032\text{ mg/kg-day}$
$= \mathbf{3 \times 10^{-4}\text{ mg/kg-day}}$

Table 12 summarizes the uncertainty factors for the chronic RfD for K^+PFBS based on effects in the thyroid.

Table 12. Uncertainty Factors for the Chronic RfD for Thyroid for K^+PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between mice and humans following oral K^+PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the U.S. EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b). However, some residual uncertainty remains in the relative cross-species sensitivity in toxicodynamics (e.g., thyroid signaling). Thus, in the absence of chemical-specific data to quantify these uncertainties, U.S. EPA's guidance recommends using a UF_A of 3.
UF_D	10	A UF_D of 10 is applied to account for database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2019; Bijland et al., 2011; Lieder et al., 2009a; 3M, 2001, 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002). However, because thyroid hormone is known to be critical during developmental life stages, particularly for neurodevelopment, the database is limited by the lack of developmental neurotoxicity studies. Further, because of the lack of chronic studies, there is additional uncertainty regarding how longer-term exposures might affect hazard identification and dose-response assessment for PFBS via the oral route (e.g., potentially more sensitive effects). Lastly, because immunotoxicity and mammary gland development are effects of increasing concern across several members of the larger PFAS family (Grandjean, 2018; Liew et al., 2018; White et al., 2007), the lack of studies evaluating these outcomes following PFBS exposure is a limitation in the database.
UF_H	10	A UF_H of 10 is applied to account for interindividual variability in the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (lifestyle) factors that can influence the response to dose. In the absence of chemical-specific data to quantify this variability in the toxicokinetics and toxicodynamics of K^+PFBS/PFBS in humans, U.S. EPA recommends using a UF_H of 10.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR was selected based on evidence that it represented a minimal biologically significant response level in susceptible populations such as developing offspring.
UF_S	1	A UF_S of 1 is applied because the POD comes from a developmental study of mice. The developmental period is recognized as a susceptible life stage in which exposure during certain time windows (e.g., gestational) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991b). The additional concern over potential hazards following longer term (chronic) exposures is accounted for under the UF_D above.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

BMDL = benchmark dose lower confidence limit; BMR = benchmark response; DAF = dosimetric adjustment factor; HED = human equivalent dose; K^+PFBS = potassium perfluorobutane sulfonate; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFAS = per- and polyfluoroalkyl substances; PFBS = perfluorobutane sulfonic acid; POD = point of departure; RfD = oral reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

The data for K^+PFBS can be used to derive a chronic RfD for the free acid (PFBS), because K^+PFBS is fully dissociated in water at the environmental pH range of 4−9 (NICNAS, 2005). To calculate the chronic RfD for the free acid, the chronic RfD for the potassium salt is

adjusted to compensate for differences in MW between K^+PFBS (338.19) and PFBS (300.10). The chronic RfD for PFBS (free acid) for thyroid effects is the same as the value for the K^+PFBS salt. The chronic RfD for PFBS (free acid) is calculated as follows:

Chronic RfD	=	RfD for K^+PFBS salt × (MW free acid ÷ MW salt)
for PFBS (free acid)	=	0.00032 mg/kg-day × (300.10 ÷ 338.19)
	=	0.00032 mg/kg-day × (0.89)
	=	0.00028 mg/kg-day
	=	**3×10^{-4} mg/kg-day**

Confidence in the chronic RfD for PFBS and K^+PFBS for thyroid effects is low, as explained in Table 13 below.

Table 13. Confidence Descriptors for Chronic RfD for PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Confidence Categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.
Confidence in database	L	Confidence in the oral toxicity database for deriving the chronic RfD is low because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, one acceptable two-generation reproductive toxicity study in rats, and multiple developmental toxicity studies in mice and rats, the database lacks any chronic-duration exposure studies or studies that have evaluated neurodevelopmental, immunological, or mammary gland effects. In addition, available toxicokinetic studies are limited (e.g., one mouse toxicokinetic study) and toxicokinetic data do not exist for PFBS at all life stages, including neonates, infants, and children. Additionally, studies are not available to estimate the relative cross-species sensitivity in toxicodynamics (e.g., thyroid signaling).
Confidence in candidate chronic RfD	L	The overall confidence in the candidate chronic RfD for thyroid effects is low.

H = high; HAWC = Health Assessment Workspace Collaborative; K^+PFBS = potassium perfluorobutane sulfonate; L = low; PFBS = perfluorobutane sulfonic acid; RfD = oral reference dose.

The chronic RfD is derived to be protective of all types of effects across studies and species following oral chronic exposure and is intended to protect the population as a whole, including potentially susceptible populations and life stages (U.S. EPA, 2002). This value should be applied in general population risk assessments. Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific life stages of concern. For example, fluctuations in exposure levels that result in elevated exposures during development could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD.

6.2 DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No published studies investigating the effects of subchronic- or chronic-duration inhalation toxicity of PFBS and the related compound K^+PFBS in humans or animals have been identified.

6.3 CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR AND DERIVATION OF CANCER RISK VALUES

No studies evaluating the carcinogenicity of PFBS or K^+PFBS in humans or animals have been identified. In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the U.S. EPA concluded that there is *"Inadequate Information to Assess Carcinogenic Potential"* for PFBS and K^+PFBS by any route of exposure. Therefore, the lack of data on the carcinogenicity of PFBS and the related compound K^+PFBS precludes the derivation of quantitative estimates for either oral (oral slope factor) or inhalation (inhalation unit risk) exposure.

6.4 SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Early life stages as well as pregnant women are potentially susceptible to PFBS exposure. PFBS has been detected in blood serum of nursing mothers, which might indicate a potential for lactational exposure (Glynn et al., 2012); however, information on the kinetics of lactational transfer are lacking and represents a key data gap for future research.

The available information suggests sex-specific variation in the toxicokinetics of PFBS in rodents. Studies in mice and rats generally report clearance and elimination half-lives to be faster for females than for males (see the "Toxicokinetics" section). For example, Lau et al. (2020) reports statistically significant differences in half-life between the sexes with female mice exhibiting a shorter half-life than males. Similar sex-specific variation in elimination has been reported in rats. Olsen et al. (2009) reported a statistically significant difference in the urinary clearance rates ($p \leq 0.01$), with female rats (469 ± 40 mL/hour) having faster clearance rates than male rats (119 ± 34 mL/hour). Huang et al. (2019a) also reported higher clearance in female rats than in male rats given the same dose (26.0−75.5 mL/hour-kg in males, 152−259 mL/hour-kg in females). Chengelis et al. (2009) reported that the mean apparent clearance of PFBS from the serum was approximately eightfold higher for female rats (0.311 L/hour-kg) than for male rats (0.0394 L/hour-kg). Statistically significant sex-related differences in half-life or clearance were not observed between male and female monkeys (Olsen et al., 2009). Differences in the toxicokinetics in rodents could result in sex-specific differences in toxicity studies.

In vivo toxicity studies report that PFBS exposure can alter thyroid hormone levels in parental and F_1 generation animals (see the "Thyroid Effects" section). Thyroid hormones play a critical role in coordinating complex developmental processes for various organs/systems (e.g., reproductive and nervous system), and disruption of thyroid hormone production/levels in a pregnant woman or neonate can have persistent adverse health effects for the developing offspring (Ghassabian and Trasande, 2018; Foster and Gray, 2013; Julvez et al., 2013; Román et al., 2013).

Animal studies also provide evidence that gestationally exposed females might be a susceptible subpopulation because of potential effects on female reproduction, including evidence of altered ovarian follicle development and delayed vaginal opening (see the "Reproductive Effects" section). Furthermore, gestationally exposed females also had significantly reduced BWs and delayed eye opening. These findings suggest that developmental landmarks indicative of adverse responses can be affected after PFBS exposure (see the "Offspring Growth and Early Development" section).

APPENDIX A. LITERATURE SEARCH STRATEGY

This appendix presents the full details of the literature search strategy used to identify primary, peer-reviewed literature pertaining to perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service registry number [CASRN] 375-73-5) and/or the potassium salt (K^+PFBS) (CASRN 29420-49-3) and the deprotonated anionic form of PFBS (i.e., $PFBS^-$; CASRN 45187-15-3). Initial database searches were conducted on July 18, 2017 using four online scientific databases (PubMed, Web of Science [WOS], TOXLINE, and TSCATS via TOXLINE) and updated on February 28, 2018; May 1, 2019; and May 15, 2020. The literature search focused on chemical name and synonyms (see Table A-1) with no limitations on publication type, evidence stream (i.e., human, animal, in vitro, and in silico) or health outcomes. Beyond database searches, references were also identified from studies submitted under the Toxic Substances Control Act (TSCA) and from review of other government documents (e.g., Agency for Toxic Substances and Disease Registry [ATSDR]) and combined with the results of the database search. Search results are retained in the U.S. EPA's Health and Environmental Research Online (HERO) database.

Table A-1. Synonyms and MeSH Terms

ChemID	375-73-5 1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid 1-Perfluorobutanesulfonic acid Nonafluoro-1-butanesulfonic acid Nonafluorobutanesulfonic acid Perfluorobutanesulfonic acid PFBS 1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid
PubMed (new only)	Perfluorobutane sulfonic acid Perfluorobutanesulfonate Perfluorobutane sulfonate
EPA Spreadsheet	1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid 1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro- 1-Butanesulfonic acid, nonafluoro- 1-Perfluorobutanesulfonic acid Nonafluoro-1-butanesulfonic acid Nonafluorobutanesulfonic acid PFBS Perfluoro-1-butanesulfonate Perfluorobutane sulfonate Perfluorobutanesulfonate Perfluorobutanesulfonic acid Perfluorobutylsulfonate 45187-15-3

MeSH = medical subject headings; PFBS = perfluorobutane sulfonic acid.

A.1. LITERATURE SEARCH STRINGS

PubMed

375-73-5[rn] OR 45187-15-3[rn] "nonafluorobutane-1-sulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw] OR "Perfluorobutanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonate"[tw] OR "Perfluorobutane sulfonate"[tw] OR "1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-"[tw] OR "1-Butanesulfonic acid, nonafluoro-"[tw] OR "Perfluoro-1-butanesulfonate"[tw] OR "Perfluorobutylsulfonate"[tw] OR "Eftop FBSA"[tw] OR (PFBS[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw]))

WOS

TS="1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR TS="1-Perfluorobutanesulfonic acid" OR TS="Nonafluoro-1-butanesulfonic acid" OR TS="Nonafluorobutanesulfonic acid" OR TS="Perfluorobutanesulfonic acid" OR TS="1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR TS="Perfluorobutane sulfonic acid" OR TS="Perfluorobutanesulfonate" OR TS="Perfluorobutane sulfonate" OR TS="1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-" OR TS="1-Butanesulfonic acid, nonafluoro-" OR TS="Perfluoro-1-butanesulfonate" OR TS="Perfluorobutylsulfonate" OR TS="Eftop FBSA" OR (TS=PFBS AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA))

TOXLINE

((375-73-5 [rn] OR 45187-15-3 [rn] OR "1 1 2 2 3 3 4 4 4-nonafluoro-1-butanesulfonic acid" OR "1-perfluorobutanesulfonic acid" OR "nonafluoro-1-butanesulfonic acid" OR "nonafluorobutanesulfonic acid" OR "perfluorobutanesulfonic acid" OR "1 1 2 2 3 3 4 4 4-nonafluorobutane-1-sulphonic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonate" OR "perfluorobutane sulfonate" OR "1-butanesulfonic acid 1 1 2 2 3 3 4 4 4-nonafluoro-" OR "1-butanesulfonic acid nonafluoro-" OR "perfluoro-1-butanesulfonate" OR "perfluorobutylsulfonate" OR "eftop fbsa" OR (pfbs AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR pfas OR pfos OR pfoa)))) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]

TSCATS

375-73-5[rn] AND tscats[org]; 45187-15-3[rn] AND tscats[org]

APPENDIX B. DETAILED PECO CRITERIA

Table B-1. Population, Exposure, Comparator, and Outcome Criteria

PECO Element	Evidence
Population	**Human:** Any population (occupational; general population including children, pregnant women, and other sensitive populations). The following study designs will be considered most informative: controlled exposure, cohort, case-control, or cross-sectional. Note: Case reports and case series are not the primary focus of this assessment and will be tracked as supplemental material during the study screening process. **Animal:** Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, in utero, lactation, peripubertal, and adult stages). **In vitro models of genotoxicity:** The studies will be considered PECO relevant. All other in vitro studies will be tagged as "non-PECO relevant, but supplemental material." **Nonmammalian model systems/in vitro/in silico NOT related to genotoxicity:** Nonmammalian model systems (e.g., fish, amphibians, birds, and *Caenorhabditis elegans*); studies of human or animal cells, tissues, or biochemical reactions (e.g., ligand binding assays) with in vitro exposure regimens; bioinformatics pathways of disease analysis; and/or high throughput screening data. These studies will be classified as non-PECO relevant, but have supplemental information.
Exposure	**Human:** Studies providing qualitative or quantitative estimates of exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational-setting measures (e.g., water levels or air concentrations), residential location, job title or other relevant occupational information. Human "mixture" studies are considered PECO relevant as long as they have the PFAS of interest. **Animal:** Studies providing qualitative and quantitative estimates of exposure based on administered dose or concentration. Oral and inhalation studies are considered PECO relevant. Nonoral and noninhalation studies are tagged as supplemental. Experimental mixture studies are included as PECO relevant only if they include a PFBS-only arm. Otherwise, mixture studies are tagged as supplemental. All studies must include exposure to PFBS, CASRN 375-73-5. Studies of precursor PFAS that identify any of the targeted PFAS as metabolites will also be included.
Comparator	**Human:** A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. For D-R purposes, exposure-response quantitative results must be presented in sufficient detail such as regression coefficients presented with statistical measure of variation such as RR, HR, OR, or SMR or observed cases vs. expected cases (common in occupational studies); slope or linear regression coefficient (i.e., per unit increase in a continuous outcome); difference in the means; or report means with results of *t*-test, mean comparison by regression, or other mean-comparing hypothesis test. **Animal:** Quantitative exposure versus lower or no exposure with concurrent vehicle control group.
Outcome	Cancer and noncancer health outcomes. In general, endpoints related to clinical diagnostic criteria, disease outcomes, histopathological examination, genotoxicity, or other apical/phenotypic outcomes will be prioritized for evidence synthesis. Based on preliminary screening work and other assessments, the systematic review is anticipated to focus on liver (including serum lipids), developmental, reproductive, neurological, developmental neurotoxicity, thyroid disease/disruption, immunological, cardiovascular, and musculoskeletal outcomes.

D-R = dose-response; HR = hazard ratio; OR = odds ratio; PECO = Population, Exposure, Comparator, and Outcome; PFAS = per- and polyfluoroalkyl substances; PFBS = perfluorobutane sulfonic acid; RR = risk ratio; SMR = standardized mortality ratio

APPENDIX C. STUDY EVALUATION METHODS

For each outcome in a study, in each domain, reviewers reached a consensus judgment of *good, adequate, deficient, not reported,* or *critically deficient*. Questions used to guide the development of criteria for each domain in epidemiology studies are presented in Table C-1 and experimental animal toxicology studies in Table C-3. These categories were applied to each evaluation domain for each study as follows:

- *Good* represents a judgment that the study was conducted appropriately in relation to the evaluation domain and any deficiencies, if present, are minor and would not be expected to influence the study results.
- *Adequate* indicates a judgment that there are methodological limitations relating to the evaluation domain, but that those limitations are not likely to be severe or to have a notable impact on the results.
- *Deficient* denotes identified biases or deficiencies that are interpreted as likely to have had a notable impact on the results or that prevent interpretation of the study findings.
- *Not reported* indicates that the information necessary to evaluate the domain was not available in the study. Generally, this term carries the same functional interpretation as *deficient* for the purposes of the study confidence classification. Depending on the number and severity of other limitations identified in the study, it may or may not be worth reaching out to the study authors for this information.
- *Critically deficient* reflects a judgment that the study conduct introduced a serious flaw that makes the observed effect(s) uninterpretable. Studies with a determination of critically deficient in an evaluation domain will almost always cause the study to be considered overall *uninformative*.

Once the evaluation domains were rated, the identified strengths and limitations were considered to reach a study confidence rating of *high, medium, low,* or *uninformative* for a specific health outcome. This was based on the reviewer judgments across the evaluation domains and included consideration of the likely impact the noted deficiencies in bias and sensitivity, or inadequate reporting, have on the results. The ratings, which reflect a consensus judgment between reviewers, are defined as follows:

- *High*: A well-conducted study with no notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology. *High* confidence studies generally reflect judgments of *good* across all or most evaluation domains.
- *Medium*: A satisfactory (acceptable) study in which deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree. Generally, *medium* confidence studies will include *adequate* or *good* judgments across most domains, with the impact of any identified limitation not being judged as severe.
- *Low*: A substandard study in which deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. Typically, *low* confidence studies would have a *deficient* evaluation for one or more domains, although some *medium* confidence studies could have a *deficient* rating in domain(s) considered to have less influence on the magnitude or

direction of effect estimates. Generally, *low* confidence results are given less weight than *high* or *medium* confidence results during evidence synthesis and integration and are generally not used as the primary sources of information for hazard identification or derivation of toxicity values unless they are the only studies available. Studies rated as *low* confidence only because of sensitivity concerns about bias towards the null require additional consideration during evidence synthesis. Observing an effect in these studies could increase confidence, assuming the study was otherwise well-conducted.

- *Uninformative*: An unacceptable study in which serious flaw(s) make the study results unusable for informing hazard identification. Studies with *critically deficient* judgments in any evaluation domain will almost always be classified as *uninformative* (see explanation above). Studies with multiple *deficient* judgments across domains might also be considered *uninformative*. *Uninformative* studies will not be considered further in the synthesis and integration of evidence for hazard identification or dose-response but might be used to highlight possible research gaps.

Table C-1. Questions Used to Guide the Development of Criteria for Each Domain in Epidemiology Studies

Core Question	Prompting Questions	Follow-Up Questions
Exposure measurement Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?	For all: • Does the exposure measure capture the variability in exposure among the participants, considering intensity, frequency, and duration of exposure? • Does the exposure measure reflect a relevant time window? If not, can the relationship between measures in this time and the relevant time window be estimated reliably? • Was the exposure measurement likely to be affected by a knowledge of the outcome? • Was the exposure measurement likely to be affected by the presence of the outcome (i.e., reverse causality)? For case-control studies of occupational exposures: • Is exposure based on a comprehensive job history describing tasks, setting, time period, and use of specific materials? For biomarkers of exposure, general population: • Is a standard assay used? What are the intra- and interassay coefficients of variation? Is the assay likely to be affected by contamination? Are values less than the limit of detection dealt with adequately? What exposure time period is reflected by the biomarker? If the half-life is short, what is the correlation between serial measurements of exposure?	Is the degree of exposure misclassification likely to vary by exposure level? If the correlation between exposure measurements is moderate, is there an adequate statistical approach to ameliorate variability in measurements? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

Table C-1. Questions Used to Guide the Development of Criteria for Each Domain in Epidemiology Studies

Core Question	Prompting Questions	Follow-Up Questions
Outcome ascertainment Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?	For all: • Is outcome ascertainment likely to be affected by knowledge of, or presence of, exposure (e.g., consider access to health care, if based on self-reported history of diagnosis)? For case-control studies: • Is the comparison group without the outcome (e.g., controls in a case-control study) based on objective criteria with little or no likelihood of inclusion of people with the disease? For mortality measures: • How well does cause of death data reflect occurrence of the disease in an individual? How well do mortality data reflect incidence of the disease? For diagnosis of disease measures: • Is diagnosis based on standard clinical criteria? If based on self-report of diagnosis, what is the validity of this measure? For laboratory-based measures (e.g., hormone levels): • Is a standard assay used? Does the assay have an acceptable level of interassay variability? Is the sensitivity of the assay appropriate for the outcome measure in this study population?	Is there a concern that any outcome misclassification is nondifferential, differential, or both? What is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Participant selection Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?	For longitudinal cohort: • Did participants volunteer for the cohort based on knowledge of exposure and/or preclinical disease symptoms? Was entry into the cohort or continuation in the cohort related to exposure and outcome? For occupational cohort: • Did entry into the cohort begin with the start of the exposure? • Was follow-up or outcome assessment incomplete, and if so, was follow-up related to both exposure and outcome status? • Could exposure produce symptoms that would result in a change in work assignment/work status ("healthy worker survivor effect")? For case-control study: • Were controls representative of population and time periods from which cases were drawn? • Are hospital controls selected from a group whose reason for admission is independent of exposure? • Could recruitment strategies, eligibility criteria, or participation rates result in differential participation relating to both disease and exposure? For population-based survey: • Was recruitment based on advertisement to people with knowledge of exposure, outcome, and hypothesis?	Were differences in participant enrollment and follow-up evaluated to assess bias? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)? Were appropriate analyses performed to address changing exposures over time in relation to symptoms? Is there a comparison of participants and nonparticipants to address whether differential selection is likely?

Table C-1. Questions Used to Guide the Development of Criteria for Each Domain in Epidemiology Studies

Core Question	Prompting Questions	Follow-Up Questions
Confounding Is confounding of the effect of the exposure likely?	Is confounding adequately addressed by considerations in… a. …participant selection (matching or restriction)? b. …accurate information on potential confounders and statistical adjustment procedures? c. …lack of association between confounder and outcome or confounder and exposure in the study? d. …information from other sources? Is the assessment of confounders based on a thoughtful review of published literature, potential relationships (e.g., as can be gained through directed acyclic graphing), minimizing potential overcontrol (e.g., inclusion of a variable on the pathway between exposure and outcome)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Analysis Do the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?	• Are missing outcome, exposure, and covariate data recognized and, if necessary, accounted for in the analysis? • Does the analysis appropriately consider variable distributions and modeling assumptions? • Does the analysis appropriately consider subgroups of interest (e.g., based on variability in exposure level, duration, or susceptibility)? • Is an appropriate analysis used for the study design? • Is effect modification considered, based on considerations developed a priori? • Does the study include additional analyses addressing potential biases or limitations (i.e., sensitivity analyses)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Sensitivity Is there a concern that sensitivity of the study is not adequate to detect an effect?	• Is the exposure range adequate? • Was the appropriate population included? • Was the length of follow-up adequate? Is the time/age of outcome ascertainment optimal given the interval of exposure and the health outcome? • Are there other aspects related to risk of bias or otherwise that raise concerns about sensitivity?	
Selective reporting Is there reason to be concerned about selective reporting?	• Are the results needed for the IRIS analysis presented (based on a priori specification)? If not, can these results be obtained? • Are only statistically significant results presented?	

IRIS = Integrated Risk Information System.

C.1. EXPOSURE MEASUREMENT EVALUATION CRITERIA

The criteria used to evaluate exposure measurement for PFBS (Table C-2) are adapted from the criteria developed by the National Toxicology Program (NTP) Office of Health Assessment and Translation for their assessment of the association between perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and immune effects (NTP, 2016, 2015) and were established prior to beginning study evaluation. Standard analytical methods for evaluating individual per- and polyfluoroalkyl substances (PFAS) in serum or whole-blood using quantitative techniques such as liquid chromatography-triple quadrupole mass spectrometry are preferred (CDC, 2018; U.S. EPA, 2014b, e; ATSDR, 2009; CDC, 2009). The estimated serum half-life of PFBS is approximately 1 month (Lau, 2015; Olsen et al., 2009), so unlike for some other PFAS with longer half-lives, current exposure might not be indicative of past exposures. Little data is available on repeated measures of PFBS in humans over time, so the reliability of a single measure is unclear. The timing of the exposure measurement is considered in relation to the etiologic window for each outcome being reviewed.

Table C-2. Criteria for Evaluation of Exposure Measurement in Epidemiology Studies

Exposure Measurement Rating	Criteria
Good	All of the following: • Evidence that exposure was consistently assessed using well-established methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma). • Exposure was assessed in a relevant time window for development of the outcome (i.e., temporality is established, and sufficient latency occurred prior to disease onset). • There is evidence that a sufficient proportion of the exposure data measurements are above the limit of quantification for the assay so that different exposure groups can be distinguished based on the analyses conducted. • The laboratory analysis included standard quality control measures with demonstrated precision and accuracy. • There is sufficient specificity/sensitivity and range or variation in exposure measurements that would minimize potential for exposure measurement error and misclassification by allowing exposure classifications to be differentiated (i.e., can reliably categorize participants into groups such as high vs. low exposure).

Table C-2. Criteria for Evaluation of Exposure Measurement in Epidemiology Studies

Exposure Measurement Rating	Criteria
Adequate	• Evidence that exposure was consistently assessed using well-established methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma), but there were some minor concerns about quality control measures or other potential for nondifferential misclassification. OR • Exposure was assessed using indirect measures (e.g., drinking water concentrations and residential location/history, questionnaire, or occupational exposure assessment by a certified industrial hygienist) that have been validated or empirically shown to be consistent with methods that directly measure exposure (i.e., intermethods validation: one method vs. another). Note: This could be *good* if the validation was sufficient. All studies for PFBS used direct measures. And all of the following: • Exposure was assessed in a relevant time window for development of the outcome. • There is evidence that a sufficient proportion of the exposure data measurements are above the limit of quantification for the assay. • There is sufficient specificity/sensitivity and range or variation in exposure measurements that would minimize potential for exposure measurement error and misclassification by allowing exposure classifications to be differentiated (i.e., can reliably categorize participants into groups such as high vs. low exposure), but there might be more uncertainty than in *good*.
Deficient	Any of the following: • Some concern, but no direct evidence, that the exposure was assessed using poorly validated methods. • There is insufficient information provided about the exposure assessment, including precision, accuracy, and level of quantification, but no evidence for concern about the method used. • Exposure was assessed in a relevant time window for development of the outcome. There could be concerns about reverse causation between exposure and outcome, but there is no direct evidence that it is present. • There is some concern over insufficient specificity/sensitivity and range or variation in exposure measurements that may result in considerable exposure measurement error and misclassification when exposure classifications are compared (i.e., data do not lend themselves to reliably categorize participants into groups such as high vs. low exposure, and/or there is considerable uncertainty in exposure values that do not allow for confidence in the examination of small per unit changes in continuous exposures).

Table C-2. Criteria for Evaluation of Exposure Measurement in Epidemiology Studies

Exposure Measurement Rating	Criteria
Critically deficient	Any of the following: • Exposure was assessed in a time window that is unknown or not relevant for development of the outcome. This could be due to clear evidence of reverse causation between exposure and outcome, or other concerns such as the lack of temporal ordering of exposure and disease onset, insufficient latency, or having exposure measurements that are not reliable measures of exposure during the etiologic window. • Direct evidence that bias was likely because the exposure was assessed using methods with poor validity. • Evidence of differential exposure misclassification (e.g., differential recall of self-reported exposure). • There is evidence that an insufficient proportion of the exposure data measurements are above the limit of quantification for the assay.

PFAS = per- and polyfluoroalkyl substances; PFBS = perfluorobutane sulfonic acid.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type	Domain– Core Question	Prompting Questions	Basic Considerations
Reporting Quality	**Reporting quality–** Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest? *Notes:* *Reviewers should reach out to study authors to obtain missing information when studies are considered key for hazard evaluation and/or dose-response.* *This domain is limited to reporting. Other aspects of the exposure methods, experimental design, and endpoint evaluation methods are evaluated using the domains related to risk of bias and study sensitivity.*	Does the study report the following? • **Critical information** necessary to perform study evaluation: o Species, test article name, levels and duration of exposure, route (e.g., oral, inhalation), qualitative or quantitative results for at least one endpoint of interest. • **Important information** for evaluating the study methods: o Test animal: strain, sex, source, and general husbandry procedures. o Exposure methods: source, purity, method of administration. o Experimental design: frequency of exposure, animal age, and life stage during exposure and at endpoint/outcome evaluation. o Endpoint evaluation methods: assays or procedures used to measure the endpoints/outcomes of interest.	These considerations typically do not need to be refined by assessment teams, although in some instances the **important information** may be refined depending on the endpoints/outcomes of interest or the chemical under investigation. A judgment and rationale for this domain should be given for the study. Typically, these will not change regardless of the endpoints/outcomes investigated by the study. **In the rationale, reviewers should indicate whether the study adhered to GLP, OECD, or other testing guidelines.** • *Good*: All critical and **important information** is reported or inferable for the endpoints/outcomes of interest. • *Adequate*: All **critical information** is reported but some **important information** is missing. However, the missing information is not expected to significantly impact the study evaluation. • *Deficient*: All **critical information** is reported but **important information** is missing that is expected to significantly reduce the ability to evaluate the study. • *Critically deficient*: Study report is missing any pieces of **critical information**. Studies that are *critically deficient* for reporting are *uninformative* for the overall rating and considered no further for evidence synthesis and integration.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Risk of Bias	**Selection and performance bias**	**Allocation–** Were animals assigned to experimental groups using a method that minimizes selection bias?	For each study: • Did each animal or litter have an equal chance of being assigned to any experimental group (i.e., random allocation)? • Is the allocation method described? • Aside from randomization, were any steps taken to balance variables across experimental groups during allocation?	These considerations typically do not need to be refined by assessment teams. A judgment and rationale for this domain should be given for each cohort or experiment in the study. • *Good*: Experimental groups were randomized, and any specific randomization procedure was described or inferable (e.g., computer-generated scheme). (Note that normalization is not the same as randomization [see response for *adequate*].) • *Adequate*: Study authors report that groups were randomized but do not describe the specific procedure used (e.g., "animals were randomized"). Alternatively, the study authors used a nonrandom method to control for important modifying factors across experimental groups (e.g., body-weight normalization). • *Not reported* (interpreted as *deficient*): No indication of randomization of groups or other methods (e.g., normalization) to control for important modifying factors across experimental groups. • *Critically deficient*: Bias in the animal allocations was reported or inferable.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Risk of Bias	**Selection and performance bias**	**Observational bias/blinding–** Did the study implement measures to reduce observational bias?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the study report blinding or other methods/procedures for reducing observational bias? • If not, did the study use a design or approach for which such procedures can be inferred? • What is the expected impact of failure to implement (or report implementation) of these methods/procedures on results?	These considerations typically do not need to be refined by the assessment teams. (Note that it can be useful for teams to identify highly subjective measures of endpoints/outcomes where observational bias may strongly influence results prior to performing evaluations.) A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *Good*: Measures to reduce observational bias were described (e.g., blinding to conceal treatment groups during endpoint evaluation; consensus-based evaluations of histopathology lesions).[a] • *Adequate*: Methods for reducing observational bias (e.g., blinding) can be inferred or were reported but described incompletely. • *Not reported*: Measures to reduce observational bias were not described. o Interpreted as *adequate*—The potential concern for bias was mitigated based on use of automated/computer-driven systems; standard laboratory kits; relatively simple, objective measures (e.g., body or tissue weight); or screening-level evaluations of histopathology. o Interpreted as *deficient*—The potential impact on the results is major (e.g., outcome measures are highly subjective). • *Critically deficient*: Strong evidence for observational bias that could have impacted results.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Risk of Bias	**Confounding/ variable control**	**Confounding–** Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?	For each study: • Are there differences across the treatment groups (e.g., co-exposures, vehicle, diet, palatability, husbandry, health status, and so forth) that could bias the results? • If differences are identified, to what extent are they expected to impact the results?	These considerations may need to be refined by assessment teams, as the specific variables of concern can vary by experiment or chemical. A judgment and rationale for this domain should be given for each cohort or experiment in the study, noting when the potential for confounding is restricted to specific endpoints/outcomes. • *Good*: Outside of the exposure of interest, variables that are likely to confound or modify results appear to be controlled for and consistent across experimental groups. • *Adequate*: Some concern that variables that were likely to confound or modify results were uncontrolled or inconsistent across groups but are expected to have a minimal impact on the results. • *Deficient*: Notable concern that potentially confounding variables were uncontrolled or inconsistent across groups and are expected to substantially impact the results. • *Critically deficient*: Confounding variables were presumed to be uncontrolled or inconsistent across groups and are expected to be a primary driver of the results.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Risk of Bias	**Reporting and attrition bias**	**Selective reporting and attrition–** Did the study report results for all prespecified outcomes and tested animals? *Note:* *This domain does **not** consider the appropriateness of the analysis/results presentation. This aspect of study quality is evaluated in another domain.*	For each study: *Selective reporting bias:* • Are all results presented for endpoints/outcomes described in the methods (see note)? *Attrition bias:* • Are all animals accounted for in the results? • If there are discrepancies, do study authors provide an explanation (e.g., death or unscheduled sacrifice during the study)? • If unexplained results, omissions, and/or attrition are identified, what is the expected impact on the interpretation of the results?	These considerations typically do not need to be refined by assessment teams. A judgment and rationale for this domain should be given for each cohort or experiment in the study. • *Good*: Quantitative or qualitative results were reported for all prespecified outcomes (explicitly stated or inferred), exposure groups, and evaluation time points. Data not reported in the primary article is available from supplemental material. If results, omissions, or animal attrition are identified, the study authors provide an explanation, and these factors are not expected to impact the interpretation of the results. • *Adequate*: Quantitative or qualitative results are reported for most prespecified outcomes (explicitly stated or inferred), exposure groups, and evaluation time points. Omissions and/or attrition are not explained but are not expected to significantly impact the interpretation of the results. • *Deficient*: Quantitative or qualitative results are missing for many prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation time points and/or high animal attrition; omissions and/or attrition are not explained and may significantly impact the interpretation of the results. • *Critically deficient*: Extensive results omission and/or animal attrition is identified and prevents comparisons of results across treatment groups.

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Sensitivity	**Exposure methods sensitivity**	**Chemical administration and characterization–** Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods? *Note:* *Consideration of the appropriateness of the route of exposure is not evaluated at the individual study level. Relevance and utility of the routes of exposure are considered in the PECO criteria for study inclusion and during evidence synthesis.*	For each study: • Does the study report the source, purity, and/or composition (e.g., identity and percent distribution of different isomers) of the chemical? If not, can the purity and/or composition be obtained from the supplier (e.g., as reported on the website)? • Was independent analytical verification of the test article purity and composition performed? • Did the study authors take steps to ensure the reported exposure levels were accurate? o For inhalation studies: Were target concentrations confirmed using reliable analytical measurements in chamber air? o For oral studies: If necessary, based on consideration of chemical-specific knowledge (e.g., instability in solution; volatility) and/or exposure design (e.g., the frequency and duration of exposure), were chemical concentrations in the dosing solutions or diet analytically confirmed? • Are there concerns about the methods used to administer the chemical (e.g., inhalation chamber type, gavage volume, etc.)?	It is essential that these criteria are considered and potentially refined by assessment teams, as the specific variables of concern can vary by chemical. A judgment and rationale for this domain should be given for each cohort or experiment in the study. • *Good*: Chemical administration and characterization is complete (i.e., source, purity, and analytical verification of the test article are provided). There are no concerns about the composition, stability, or purity of the administered chemical or the specific methods of administration. For inhalation studies, chemical concentrations in the exposure chambers are verified using reliable analytical methods. • *Adequate*: Some uncertainties in the chemical administration and characterization are identified but these are expected to have minimal impact on interpretation of the results (e.g., source and vendor-reported purity are presented, but not independently verified; purity of the test article is suboptimal but not concerning). For inhalation studies, actual exposure concentrations are missing or verified with less reliable methods. • *Deficient*: Uncertainties in the exposure characterization are identified and expected to substantially impact the results (e.g., source of the test article is not reported, levels of impurities are substantial or concerning, deficient administration methods such as use of static inhalation chambers or a gavage volume considered too large for the species and/or life stage at exposure). • *Critically deficient*: Uncertainties in the exposure characterization are identified, and there is reasonable certainty that the results are largely attributable to factors other than exposure to the chemical of interest (e.g., identified impurities are expected to be a primary driver of the results).

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Sensitivity	**Exposure methods sensitivity**	**Exposure timing, frequency and duration–** Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the exposure period include the critical window of sensitivity? • Was the duration and frequency of exposure sensitive for detecting the endpoint of interest?	Considerations for this domain are highly variable depending on the endpoint(s)/outcome(s) of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *Good*: The duration and frequency of the exposure was sensitive, and the exposure included the critical window of sensitivity (if known). • *Adequate*: The duration and frequency of the exposure was sensitive, and the exposure covered most of the critical window of sensitivity (if known). • *Deficient*: The duration and/or frequency of the exposure is not sensitive and did not include the majority of the critical window of sensitivity (if known). These limitations are expected to bias the results towards the null. • *Critically Deficient*: The exposure design was not sensitive and is expected to strongly bias the results towards the null. The rationale should indicate the specific concern(s).

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Sensitivity	**Outcome measures and results display**	**Endpoint sensitivity and specificity–** Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest? *Note:* *Sample size alone is not a reason to conclude an individual study is critically deficient.*	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Are there concerns regarding the specificity and validity of the protocols? • Are there serious concerns regarding the sample size (see note)? • Are there concerns regarding the timing of the endpoint assessment?	Considerations for this domain are highly variable depending on the endpoint(s)/outcome(s) of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. Examples of potential concerns include: • Selection of protocols that are insensitive or nonspecific for the endpoint of interest. • Use of unreliable methods to assess the outcome. • Assessment of endpoints at inappropriate or insensitive ages, or without addressing known endpoint variation (e.g., due to circadian rhythms, estrous cyclicity, etc.). • Decreased specificity or sensitivity of the response due to the timing of endpoint evaluation, as compared to exposure (e.g., short-acting depressant or irritant effects of chemicals, insensitivity due to prolonged period of nonexposure prior to testing).

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type		Domain– Core Question	Prompting Questions	Basic Considerations
Sensitivity	**Outcome measures and results display**	**Results presentation–** Are the results presented in a way that makes the data usable and transparent?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the level of detail allow for an informed interpretation of the results? • Are the data analyzed, compared, or presented in a way that is inappropriate or misleading?	Considerations for this domain are highly variable depending on the outcomes of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. Examples of potential concerns include: • Nonpreferred presentation such as developmental toxicity data averaged across pups in a treatment group when litter responses are more appropriate. • Failure to present quantitative results. • Pooled data when responses are known or expected to differ substantially (e.g., across sexes or ages). • Failure to report on or address overt toxicity when exposure levels are known or expected to be highly toxic. • Lack of full presentation of the data (e.g., presentation of mean without variance data; concurrent control data are not presented).

Table C-3. Questions Used to Guide the Development of Criteria for Each Domain in Experimental Animal Toxicology Studies

Evaluation Type	Domain– Core Question	Prompting Questions	Basic Considerations
Overall Confidence	**Overall Confidence–** Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest? *Note:* *Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias towards the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? • If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects?	The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias, and sensitivity on the results. A confidence rating and rationale should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *High confidence*: No notable concerns are identified (e.g., most or all domains rated *good*). • *Medium confidence*: Some concerns are identified, but expected to have minimal impact on the interpretation of the results (e.g., most domains rated *adequate* or *good*; may include studies with *deficient* ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis. • *Low confidence*: Identified concerns are expected to significantly impact the study results or their interpretation (e.g., generally, *deficient* ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note). • *Uninformative*: Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, *critically deficient* rating in any domain; many *deficient* ratings). *Uninformative* studies are considered no further in the synthesis and integration of evidence.

[a]For nontargeted or screening-level histopathology outcomes often used in guideline studies, blinding during the initial evaluation of tissues is generally not recommended because masked evaluation can make "the task of separating treatment-related changes from normal variation more difficult" and "there is concern that masked review during the initial evaluation may result in missing subtle lesions." Generally, blinded evaluations are recommended for targeted secondary review of specific tissues or in instances when there is a predefined set of outcomes that is known or predicted to occur (Crissman et al., 2004).

GLP = Good Laboratory Practice; OECD = Organisation for Economic Cooperation and Development.

APPENDIX D. HAWC USER GUIDE AND FREQUENTLY ASKED QUESTIONS

D.1 WHAT IS HAWC AND WHAT IS ITS PURPOSE?

The Health Assessment Workspace Collaborative (HAWC) is an interactive, expert-driven, content management system for human health assessments that is intended to promote transparency, trackability, data usability, and understanding of the data and decisions supporting an environmental and human health assessment. Specifically, HAWC is an interface that allows the data and decisions supporting an assessment to be managed in modules (e.g., study evaluation, summary study data, etc.) that can be publicly accessed online (see Section D.2 below and Figure D-1). Following the literature search and screening that are conducted using HERO and DistillerSR, HAWC manages each study included in an assessment and makes the extracted information available via a web link that takes a user to a web page displaying study-specific details and data (e.g., study evaluation, experimental design, dosing regime, endpoints evaluated, dose-response data, etc., described in further detail below in Sections D.3 to D.6). Finally, all data managed in HAWC is fully downloadable using the blue "Download datasets" link (highlighted in the red box below) also located in the gray navigation bar located on the assessment home page (discussed in Section D.7). Note that a user may quickly navigate HAWC by clicking on the file path (highlighted in the orange, dashed box below) given in the gray row below the HAWC icon and menu bar (see Figure D-1). HAWC aims to facilitate team collaboration by scientists who develop these assessments and enhance transparency of the process by providing online access (no user account required) to the data and expert decisions used to evaluate potential human health hazard and risk of chemical exposures.

Figure D-1. HAWC Homepage for the Public PFBS Assessment

D.2 HOW DO I ACCESS HAWC?

HAWC is an open-source, online application that may be accessed using the following link—https://hawcprd.epa.gov/assessment/public/—and then selecting an available assessment. The following browsers are fully supported for accessing HAWC: Google Chrome (preferred), Mozilla Firefox, and Apple Safari. There are errors in functionality when viewed with Internet Explorer. No user account is required for access to public HAWC assessments. The assessments located in HAWC are meant to accompany a textual expert synthesis of the data managed in HAWC. Each written assessment document contains embedded URL links to the evidence in HAWC (e.g., study evaluation, summary study data, visualizations, etc.) supporting

the assessment text. The links embedded in an assessment document can be accessed by a mouse click (or hover while pressing CTRL + right click).

D.3 WHAT CAN I FIND IN HAWC?

HAWC contains a comprehensive landscape of study details and data supporting an assessment. Note that links are provided in the assessment text to guide the reader, but a user may also navigate to the HAWC homepage for an assessment on their own. Once a user lands on an assessment homepage, all studies included in an assessment can be viewed by clicking the blue "Study list" link (highlighted in the red box below) in the gray navigation pane (see Figure D-2). By clicking the study name listed in blue (under "Short citation") a user can view the full study details, study evaluation, and experimental details and data. For example, in Figure D-2, a user may click on "3M, 2000, 4289992" (highlighted in the orange, dashed box below). This will take the user to the 3M (2000d) study details page that includes a link to the study in HERO along with study details, study evaluation, and available experimental (animal) and study population (epidemiologic) groups.

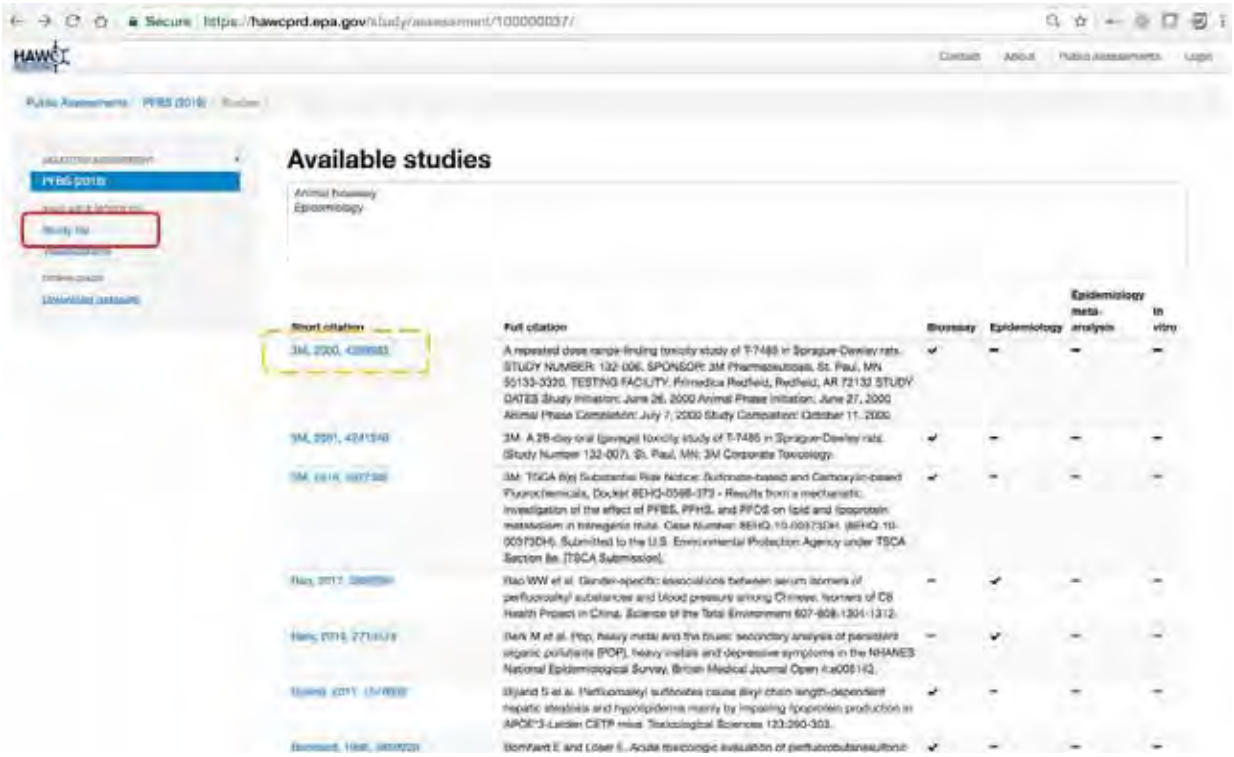


Figure D-2. Representative Study List

D.4 HOW DO I ACCESS STUDY EVALUATION(S)?

Study evaluation is performed to ensure that the studies used in the assessment are conducted in such a manner that the results are credible for each outcome and the ratings are outcome specific. The study evaluation criteria and decisions are fully documented in HAWC and displayed for each study on the study details page. Study evaluation is depicted as a pie chart with each domain and rating making up a piece of the pie that is colored according to the

rating. A user may hover over each piece of the pie, which causes rating metric text to populate to the right of the pie graph (see Figure D-3). For full domain and rating details the user may click the blue "View details" button (highlighted in the red box below). [Note that this example is given for the 3M (2000d)].

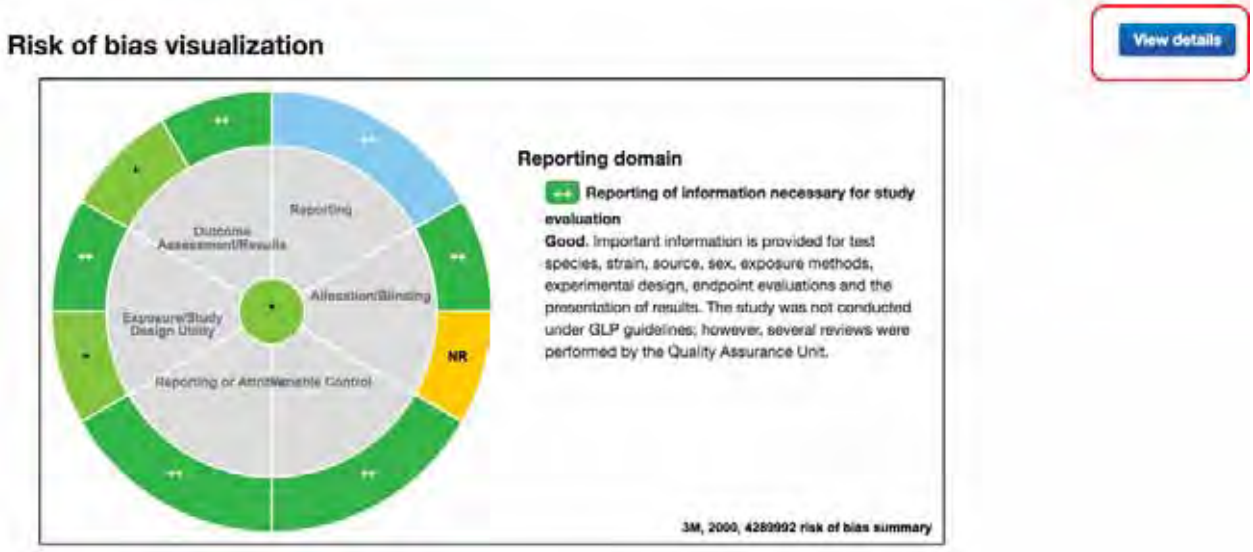


Figure D-3. Representative Study Evaluation Pie Chart with the Reporting Domain Selected and Text Populating to the Right of Pie Chart

D.5 HOW DO I ACCESS STUDY-SPECIFIC INFORMATION ON EXPERIMENTAL AND STUDY POPULATION DETAILS AND EXTRACTED ENDPOINT DATA?

Specific information on experimental design, dosing (if animal bioassay), outcomes and exposure (if epidemiology), and extracted endpoint data can be accessed from the study details page by clicking on [for the 3M (2000d) study] "available animal bioassay experiments" at the bottom of the study details page. A user may click on the experiment name (highlighted in blue, 10-day oral) to view dosing/exposure details and available groups. Clicking on available animal groups (e.g., male Sprague-Dawley or female Sprague-Dawley) will take the reader to a new page with experimental group information (e.g., species/strain/sex, dosing regimen information, and available/additional endpoints information for animal studies; and outcome and exposure information for epidemiologic studies). If a study reports data, then the data are extracted and managed as "available endpoints." If the study authors include endpoints in the methods and results but do not report data, the endpoint is listed under "additional endpoints" without dose-response data. All endpoints are also clickable and contain an endpoint description, methods, and (if data are reported) a clickable data plot (e.g., alanine aminotransferase [ALT]). The description of endpoints, methods, and data are often copied directly from the study report and, therefore, can contain study author judgments and may not necessarily include U.S. EPA judgments on the endpoint data that would be included in the assessment.

D.6 WHAT ARE VISUALIZATIONS AND HOW DO I ACCESS THEM?

The data managed in HAWC is displayed using visualizations that are intended to support textual descriptions within an assessment. All visualizations can be accessed using the blue "Visualizations" link (highlighted in the red box below) also found in the gray navigation pane (see Figure D-4A). *Note that the available visualizations are at the discretion of the chemical manager and are meant to accompany the assessment text*. Visualizations are fully interactive. Hovering and clicking on records in the rows and columns and data points on a plot will cause a pop-up window to appear (see Figure D-5B). This pop-up window is also interactive and clicking on blue text within this pop-up will open a new web page with descriptive data.

Figure D-4A. Visualization Example for PFBS
(Note that the records listed under each column [study, experiment endpoint, units, study design, observation time, dose] and data within the plot are interactive.)

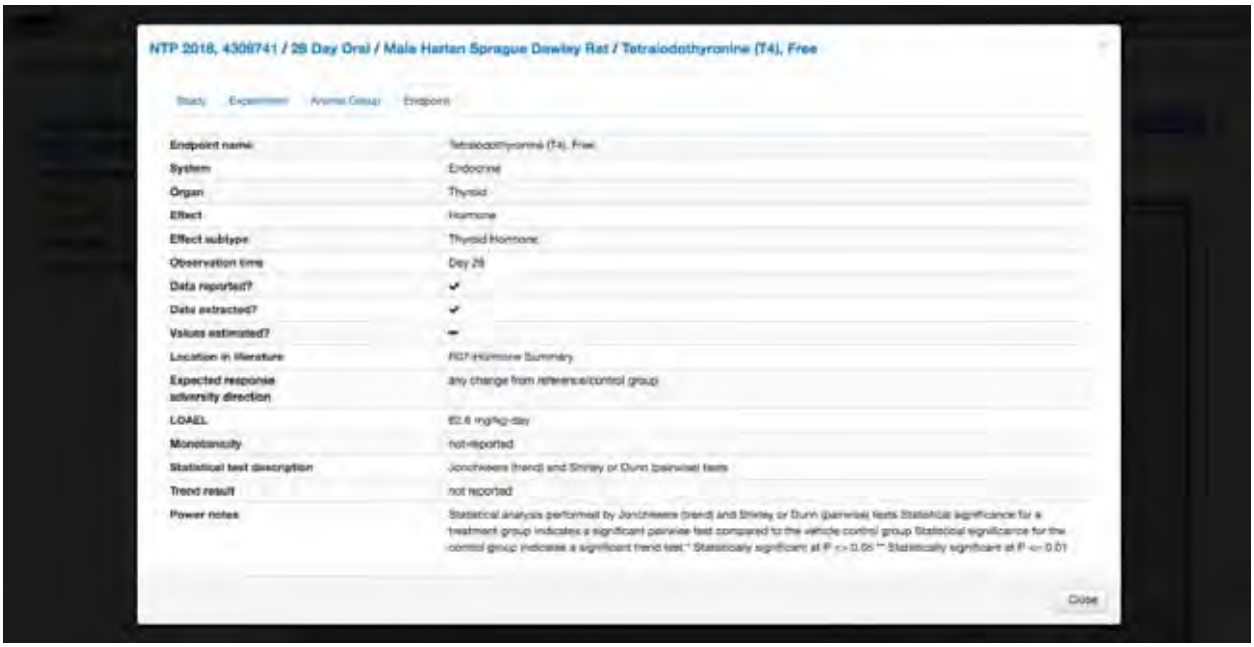


Figure D-4B. Example Pop-Up Window after Clicking on Interactive Visualization Links (In Figure D-4A, the red circle for study NTP (2019); male at a dose of 500 mg/kg-day was clicked leading to the pop-up shown above. Clicking on the blue text will open a new window with descriptive data.)

D.7 HOW DO I DOWNLOAD DATA SETS?

A user may download any available data set by first clicking on the blue "Download datasets" link (highlighted in the red box below) in the gray navigation pane on the assessment homepage. This takes the user to a new page where the desired data set may be selected for download as an Excel file (see representative image in Figure D-6).

Figure D-5. Representative Data Download Page

D.8 HOW DO I ACCESS THE BENCHMARK DOSE MODELING OUTPUTS?

Benchmark dose (BMD) modeling is performed on an endpoint-by-endpoint basis at the discretion of the chemical manager. Those endpoints for which BMD modeling has been completed are referenced in the assessment text and are available for viewing. To access BMD modeling outputs the user can click on links included in the assessment text. Alternatively, the user may navigate to the BMD modeling outputs by clicking on a study [e.g., Feng et al. (2017)] of interest from the study list, an available animal bioassay experiment (in this example, the 20-day oral gestation study), an available animal group (P_0 female ICR mice), and an endpoint of interest (tetraiodothyronine [T_4], free). Next navigate to the blue "Actions" button, click, and scroll to "View session" (highlighted in the red box below) under BMD Modeling (see Figure D-7A). The BMD setup, results, and model recommendation and selection (highlighted in the orange, dashed box in Figure D-8B) are available for viewing. Selecting the BMD setup tab will display the modeled dose-response data, the selected models and options, and all benchmark modeling responses (BMRs). The results tab will display the BMD modeling output summary for all models. A user may hover over a selected model row to visualize the model fit to the data. In addition, a user may obtain the Benchmark Dose Software (BMDS) output text by clicking the "View" button under the "Output" column for each model that was run. The "Model recommendation and selection" tab displays all models, warnings when appropriate, and the recommendation for which models are valid, questionable, or failed to fit.

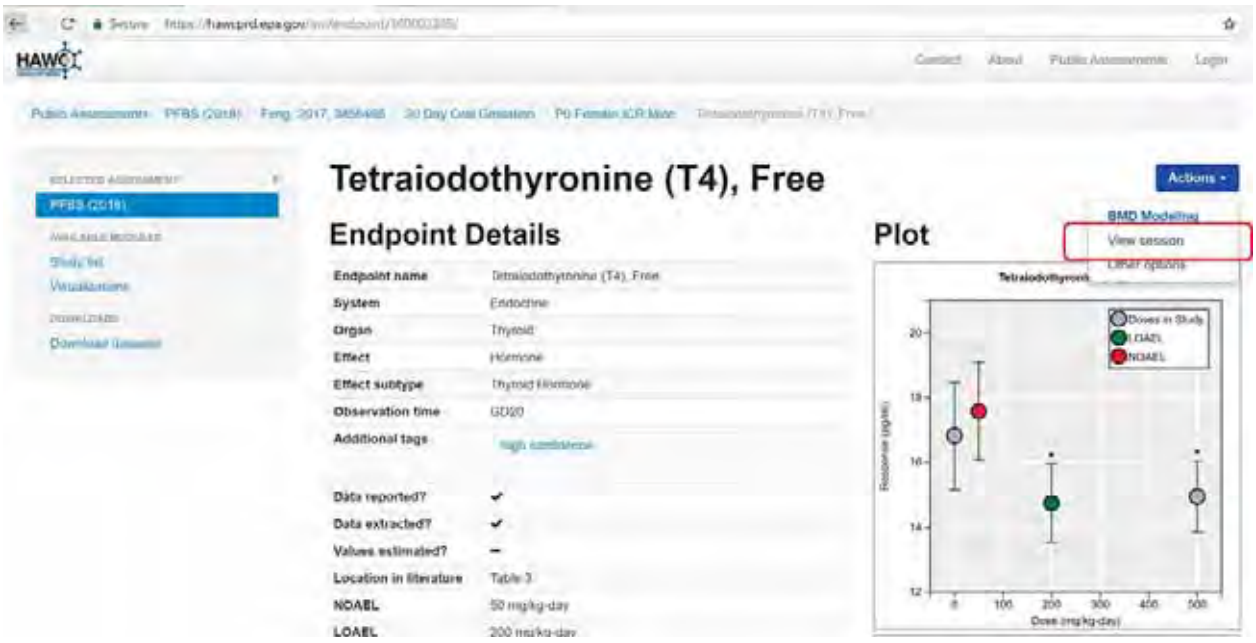


Figure D-6A. Example BMD Modeling Navigation

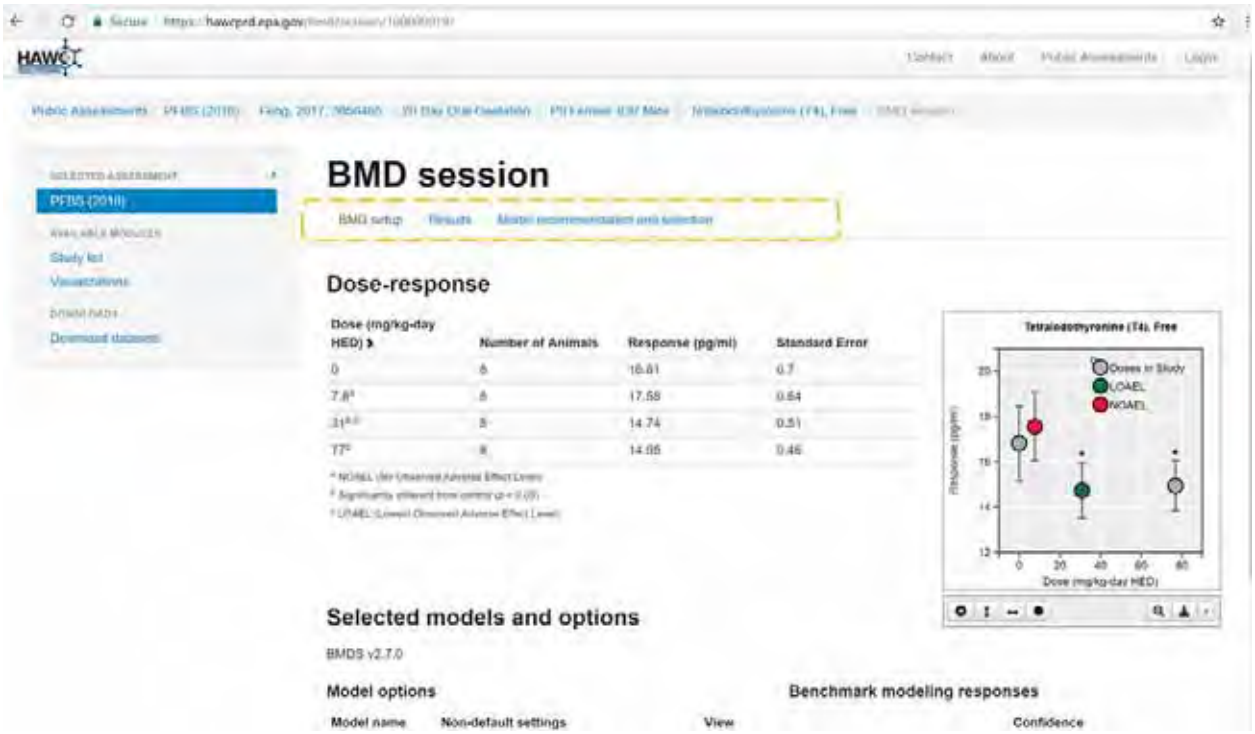


Figure D-6B. Example BMD Session

APPENDIX E. ADDITIONAL DATA FIGURES

Figure E-1. Serum Free and Total Thyroxine (T_4) Response in Animals Following K^+PFBS Exposure
(Click to see interactive data graphic)

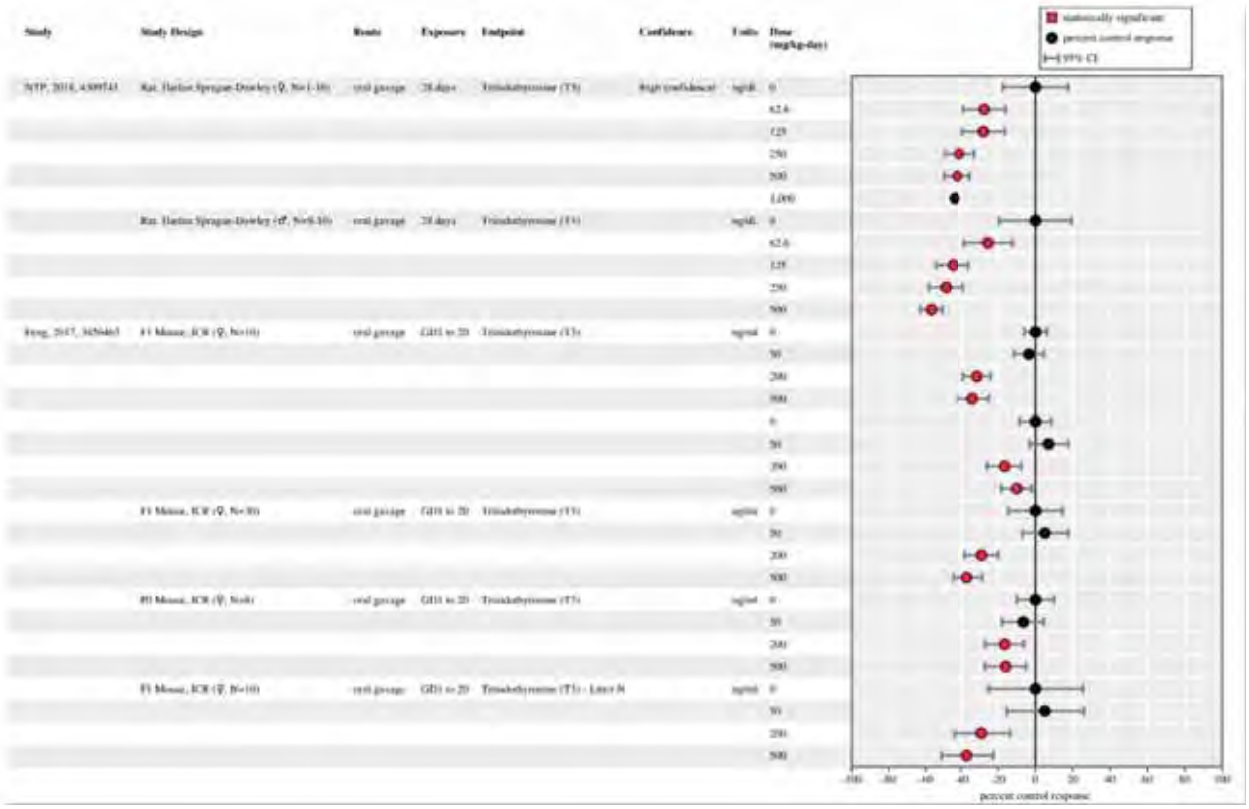


Figure E-2. Serum Total Triiodothyronine (T_3) Response in Animals Following K^+PFBS Exposure
(Click to see interactive data graphic)

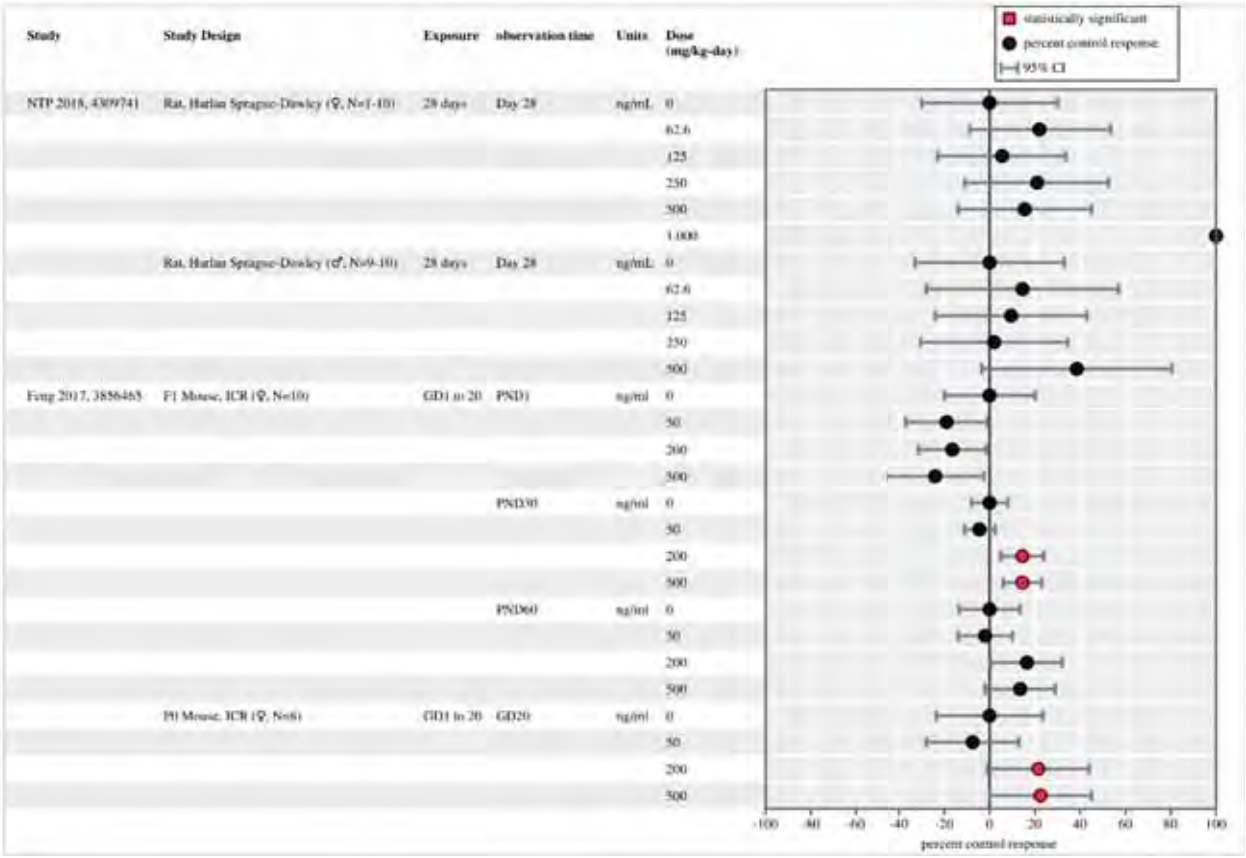


Figure E-3. Serum Thyroid-Stimulating Hormone (TSH) Response in Animals Following K^+PFBS Exposure
(Click to see interactive data graphic)

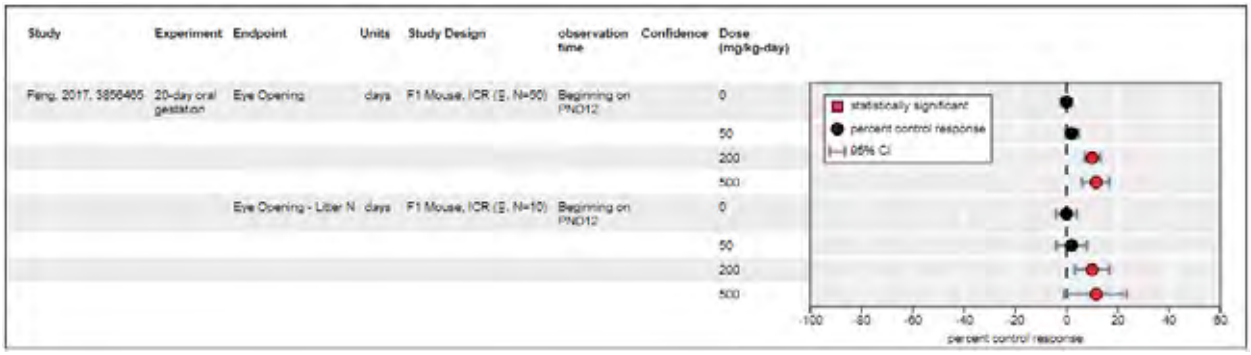


Figure E-4. Developmental Effects (Eye Opening) Following K^+PFBS Exposure in Rats
(Click to see interactive data graphic)

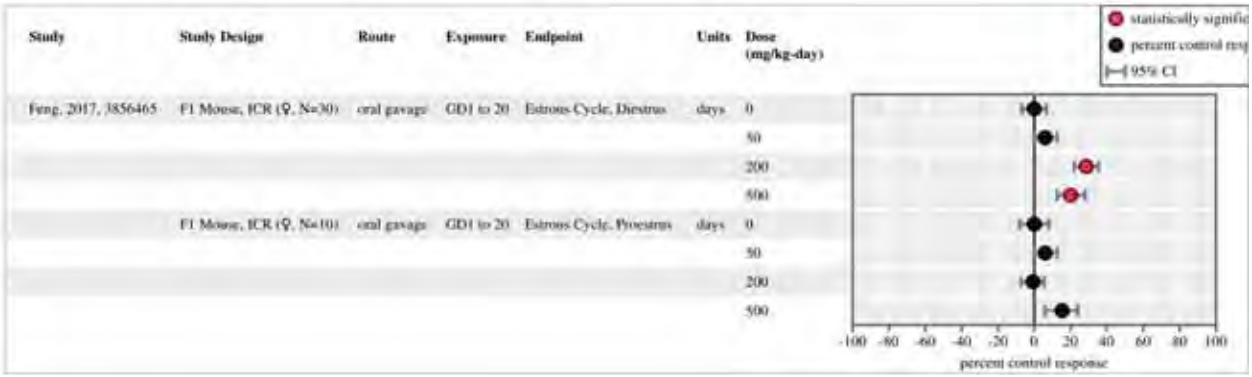


Figure E-5. Developmental Effects (First Estrus) Following K^+PFBS Exposure in Rats (Click to see interactive data graphic)

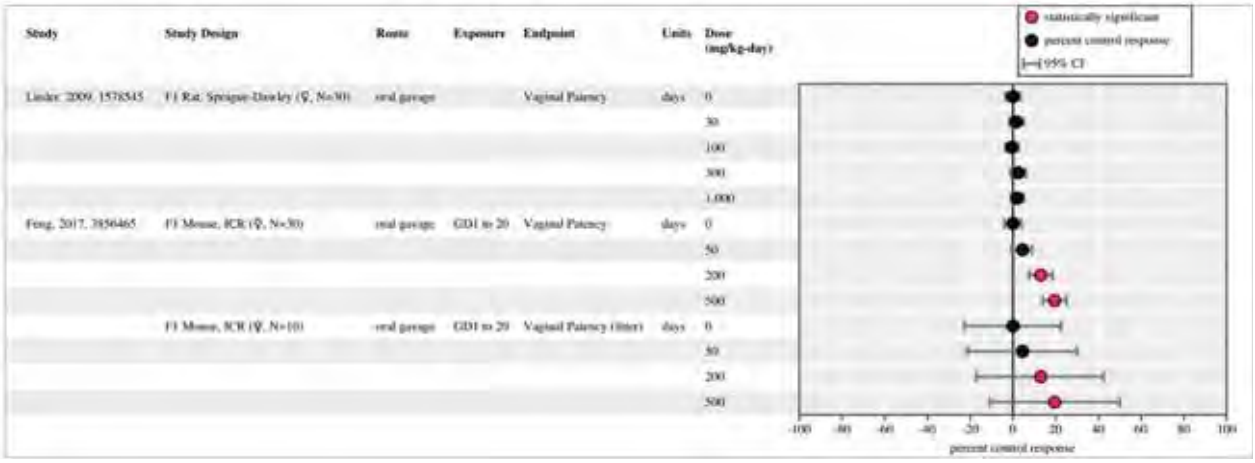


Figure E-6. Developmental Effects (Vaginal Patency) Following K^+PFBS Exposure in Rats (Click to see interactive data graphic)

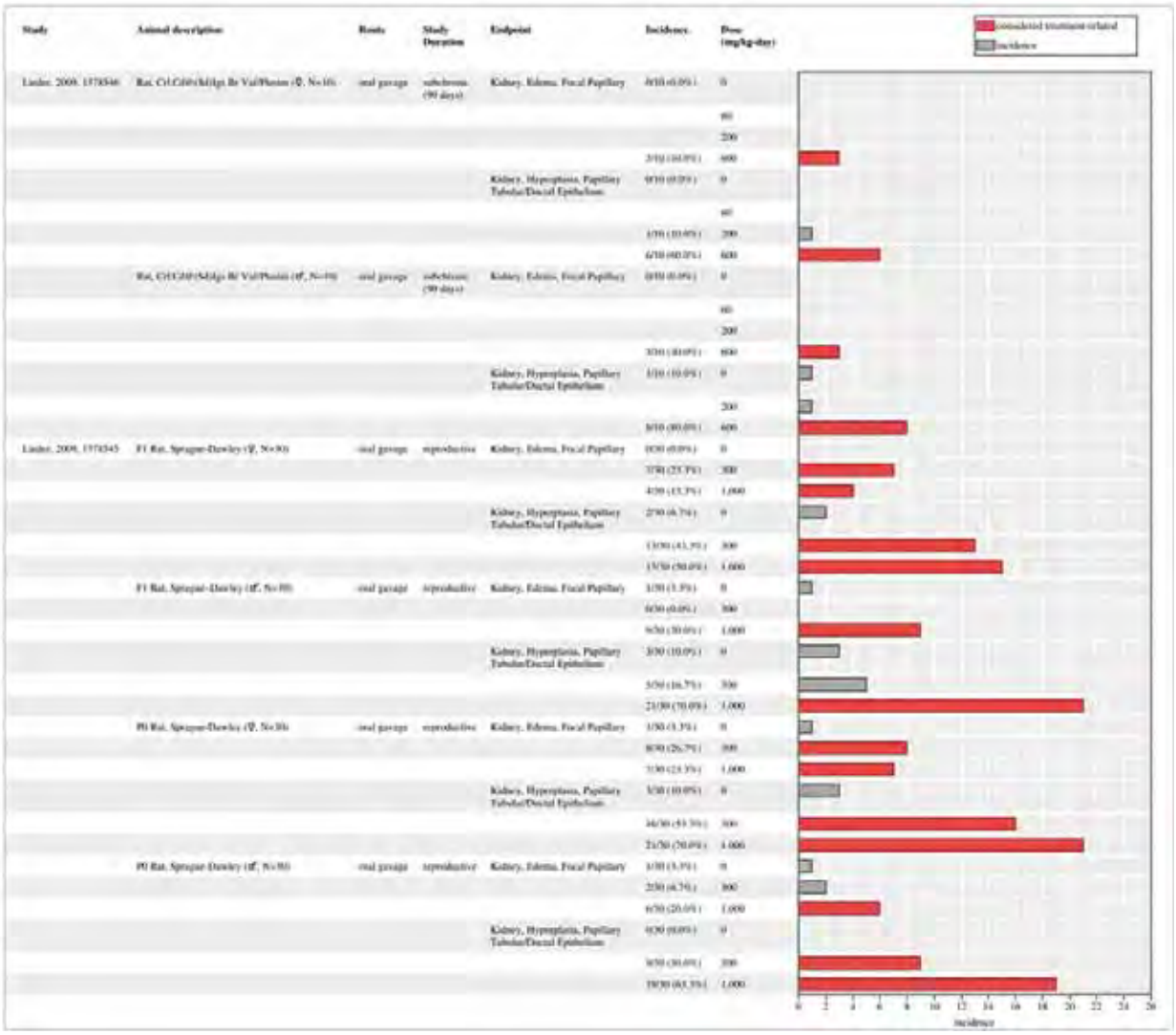


Figure E-7. Kidney Histopathological Effects Following K^+PFBS Exposure in Rats (Click to see interactive data graphic)

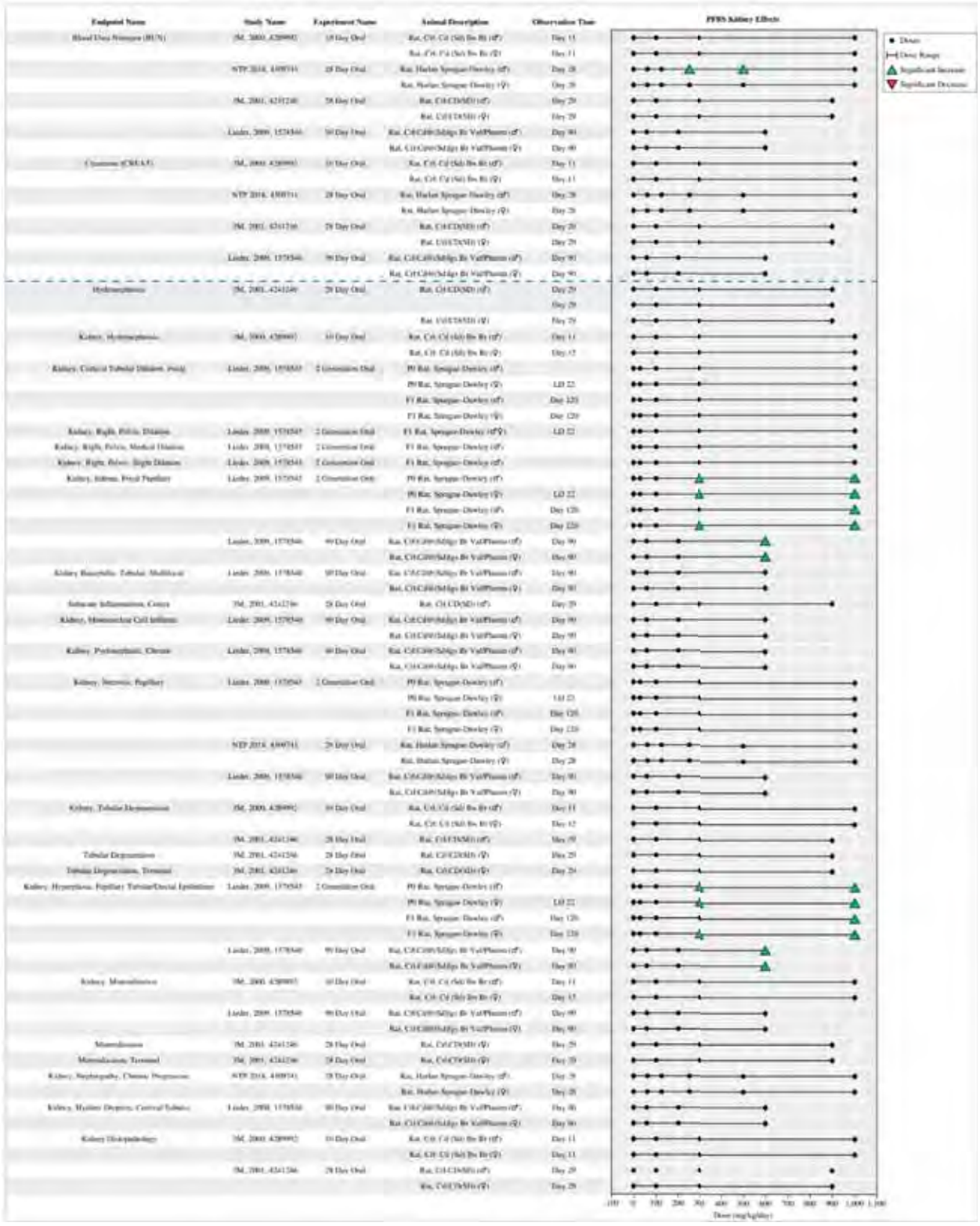


Figure E-8. Renal Effects Following K^+PFBS Exposure in Rats
(Click to see interactive data graphic)

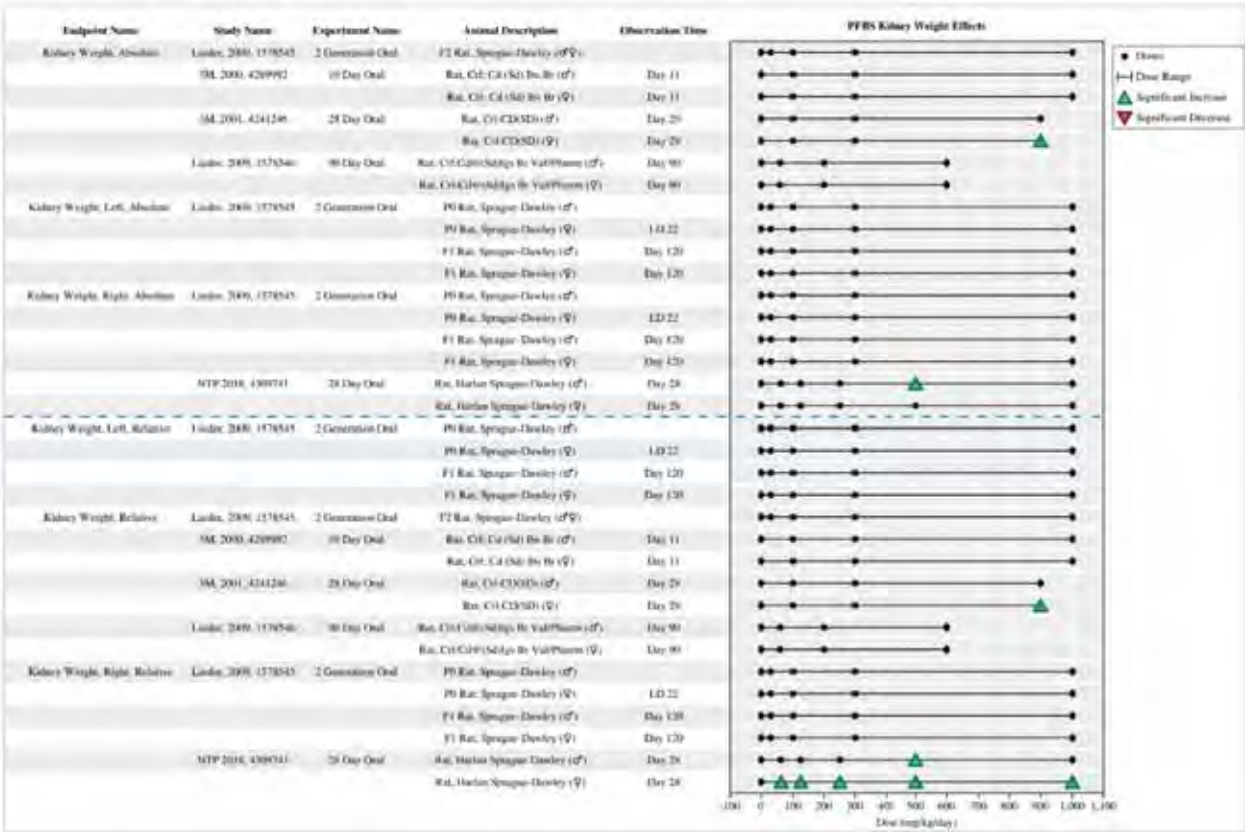


Figure E-9. Kidney-Weight Effects Following K^+PFBS Exposure in Rats
(Click to see interactive data graphic)

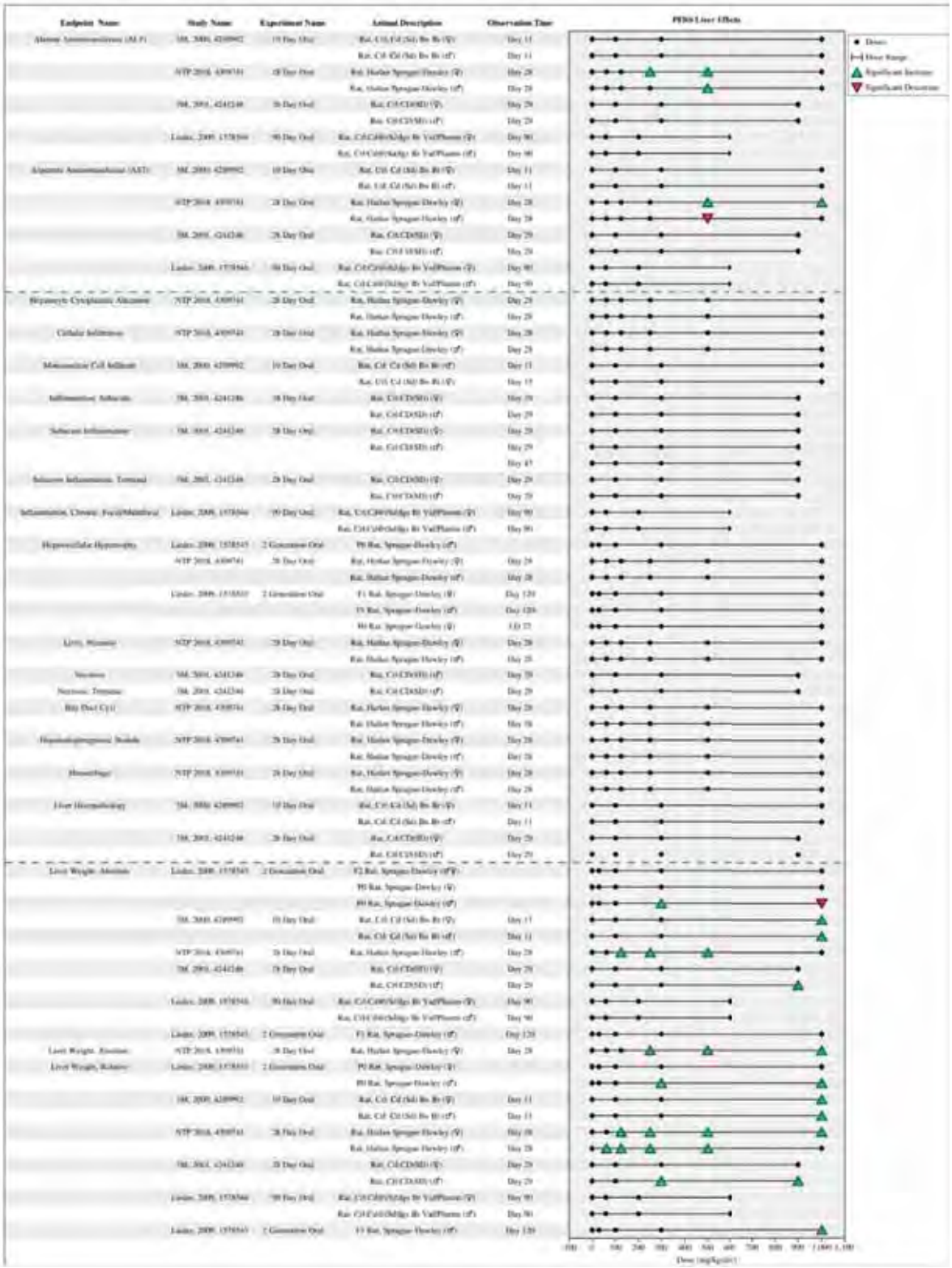


Figure E-10. Liver Effects Following K^+PFBS Exposure in Rats
(Click to see interactive data graphic)

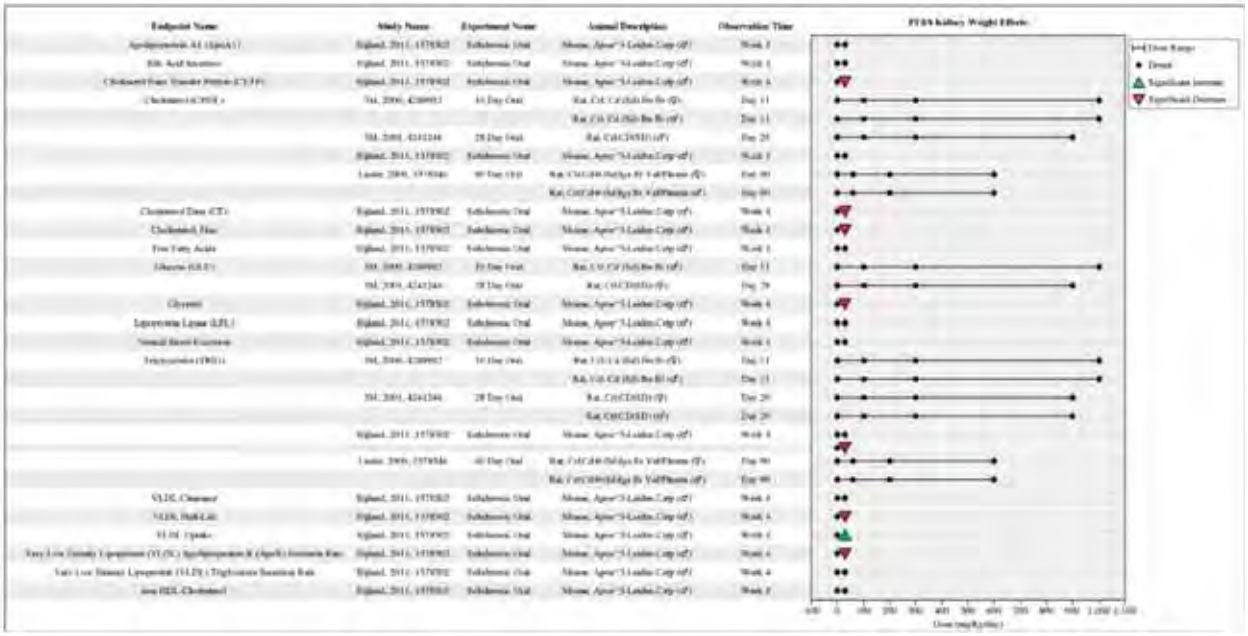


Figure E-11. Effects on Lipids and Lipoproteins Following K^+PFBS Exposure in Rats and Mice
(Click to see interactive data graphic)

APPENDIX F. BENCHMARK DOSE MODELING RESULTS

F.1. MODELING OF NONCANCER ENDPOINTS

As discussed in the body of the report under "Derivation of Oral Reference Doses," the endpoints selected for benchmark dose (BMD) modeling were incidence of renal papillary epithelial tubular/ductal hyperplasia in rats from Lieder et al. (2009a) and Lieder et al. (2009b); thyroid hormones in pregnant mice and offspring at Postnatal Days (PNDs) 1, 30, and 60 from Feng et al. (2017) and adult rats from NTP (2019); and developmental effects (i.e., eye opening, first estrus, vaginal opening) from Feng et al. (2017). The animal doses in the study, converted to human equivalent doses (HEDs), were used in the BMD modeling; the data are available for download in Health Assessment Workspace Collaborative (HAWC). BMD modeling was conducted by experts in quantitative Benchmark Dose Software (BMDS) analysis and interpretation. Links to the data and modeling output are included in Table F-1. The selected point of departure (POD) (HED) listed in Table F-1 represents the best-fitting model for each endpoint; if the data were determined not to be amenable to BMD modeling, the no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) is listed. Figure F-1 illustrates the doses examined and NOAEL, LOAEL, BMD, and benchmark dose lower confidence limit (BMDL) values for the potential critical effects.

Table F-1. Candidate PODs for the Derivation of the Subchronic and Chronic RfDs for PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Endpoint/Reference	Species/Life Stage—Sex	Selected POD (HED)[a] (mg/kg-d)
Kidney effects		
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009a)	Rat—male	$BMDL_{10}$ = 0.489
	Rat—female	$BMDL_{10}$ = 0.300
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/P_0—male	$BMDL_{10}$ = 0.351
	Rat/P_0—female	$BMDL_{10}$ = 0.265
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/F_1—male	$BMDL_{10}$ = 0.776
	Rat/F_1—female	$BMDL_{10}$ = 0.478
Thyroid effects		
Total T_4—NTP (2019)	Rat—male	LOAEL = 0.34
	Rat—female	$BMDL_{1SD}$ = 0.037
Free T_4—NTP (2019)	Rat—male	LOAEL = 0.34
	Rat—female	$BMDL_{1SD}$ = 0.027
Total T_4—Feng et al. (2017)	Mouse/P_0—female	$BMDL_{1SD}$ = 0.093
Free T_4—Feng et al. (2017)	Mouse/P_0—female	NOAEL = 0.21
TSH—Feng et al. (2017)	Mouse/P_0—female	NOAEL = 0.21
Total T_4 PND 1 (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
Total T_4 PND 1 (litter *n*)[b]—Feng et al. (2017)	**Mouse/F_1—female**	**$BMDL_{0.5SD}$ = 0.095 ($BMDL_{1SD}$ = 0.25)**

Table F-1. Candidate PODs for the Derivation of the Subchronic and Chronic RfDs for PFBS (CASRN 375-73-5) and the Related Compound K^+PFBS (CASRN 29420-49-3)

Endpoint/Reference	Species/Life Stage—Sex	Selected POD (HED)[a] (mg/kg-d)
Total T_4 PND 30—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
Total T_4 PND 60—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
TSH PND 30—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
Developmental effects		
Eyes opening (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
Eyes opening (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.073 ($BMDL_{1SD}$ = 0.16)
Vaginal opening (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.15 ($BMDL_{1SD}$ = 0.35)
Vaginal opening (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	$BMDL_{0.5SD}$ = 0.094 ($BMDL_{1SD}$ = 0.22)
First estrous (fetal *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21
First estrous (litter *n*)[b]—Feng et al. (2017)	Mouse/F_1—female	NOAEL = 0.21

[a]Following U.S. EPA (2011b) guidance, animal doses from candidate principal studies were converted to HEDs through the application of a DAF, where HED = dose × DAF. See Table 8 in the assessment for full details. Links are to the HAWC BMDS session containing full modeling results for that endpoint.
[b]Fetal endpoints from Feng et al. (2017) were modeled alternatively using dose group sizes based either on total number of fetuses or dams. Given that it appears that Feng et al. (2017) did not use the litter as the statistical unit of analysis, it is unclear if the study-reported standard errors pertain to litters or fetuses. Alternatively, modeling fetal endpoints using litter *n* or fetal *n* provides two modeling results that bracket the "true" variance among all fetuses in a dose group (i.e., using the fetal *n* will underestimate the true variance while using the litter *n* will overestimate the true variance). Individual animal data were requested from study authors but were unable to be obtained.

BMDL = benchmark dose lower confidence limit; BMDS = benchmark dose software; DAF = dosimetric adjustment factor; HAWC = Health Assessment Workspace Collaborative; HED = human equivalent dose; K^+PFBS = potassium perfluorobutane sulfonate; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; PFBS = perfluorobutane sulfonic acid; PND = postnatal day; POD = point of departure; RfD = oral reference dose; SD = standard deviation; T_4 = total thyroxine; TSH = thyroid-stimulating hormone.

study name	experiment name	animal description	endpoint name	observation time
Lieder, 2009, 1578546	90 Day Oral	Rat, Crl:CD®(Sd)Igs Br Va0/Plusrm (♂)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	90.0 days
		Rat, Crl:CD®(Sd)Igs Br Va0/Plusrm (♀)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	90.0 days
Lieder, 2009, 1578545	2 Generation Oral	P0 Rat, Sprague-Dawley (♂)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	None not reported
		P0 Rat, Sprague-Dawley (♀)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	None not reported
		F1 Rat, Sprague-Dawley (♂)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	120.0 days
		F1 Rat, Sprague-Dawley (♀)	Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	120.0 days
NTP, 2018, 4309741	28 Day Oral	Rat, Harlan Sprague-Dawley (♂)	Tetraiodothyronine (T4), Free	None not reported
		Rat, Harlan Sprague-Dawley (♀)	Tetraiodothyronine (T4), Free	None not reported
		Rat, Harlan Sprague-Dawley (♂)	Tetraiodothyronine (T4), Total	None not reported
		Rat, Harlan Sprague-Dawley (♀)	Tetraiodothyronine (T4), Total	None not reported
Feng 2017, 3856465	20 Day Oral Gestation	P0 Mouse, ICR (♀)	Tetraiodothyronine (T4), Free	20.0 gestational day (GD)
			Tetraiodothyronine (T4), Total	20.0 gestational day (GD)
		F1 Mouse, ICR (♀)	Tetraiodothyronine (T4), Total	1.0 post-natal day (PND)
				30.0 post-natal day (PND)
				60.0 post-natal day (PND)
			Tetraiodothyronine (T4), Total - Litter N	1.0 post-natal day (PND)
		P0 Mouse, ICR (♀)	Thyroid Stimulating Hormone (TSH)	20.0 gestational day (GD)
		F1 Mouse, ICR (♀)	Thyroid Stimulating Hormone (TSH)	1.0 post-natal day (PND)
				30.0 post-natal day (PND)
				60.0 post-natal day (PND)
			Eye Opening - Fetal N	42.0 post-natal day (PND)
			Eye Opening - Litter N	42.0 post-natal day (PND)
			First Estrous - Fetal N	24.0 post-natal day (PND)
			First Estrous - Litter N	24.0 post-natal day (PND)
			Vaginal Opening - Fetal N	24.0 post-natal day (PND)
			Vaginal Patency - Litter N	24.0 post-natal day (PND)

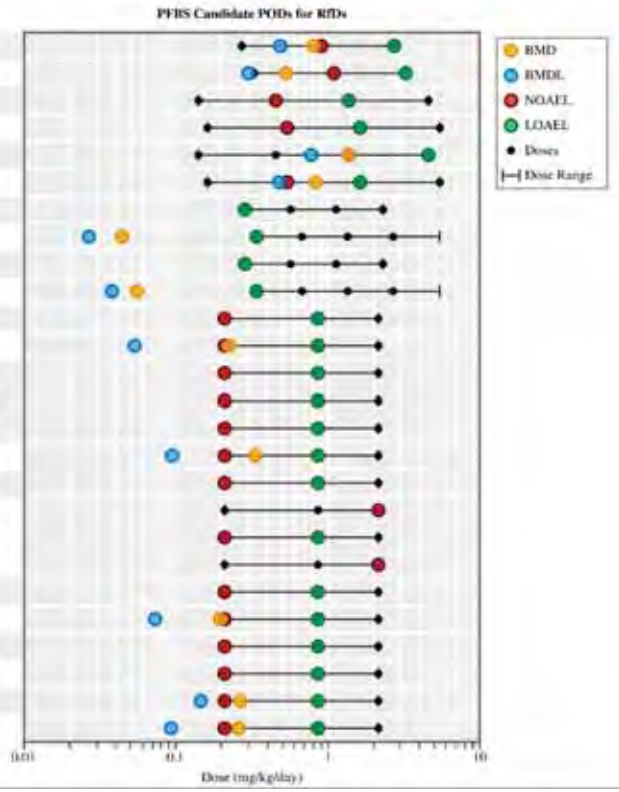


Figure F-1. Candidate PODs for the Derivation of the Subchronic and Chronic RfDs for PFBS
(Click to see interactive data graphic)

F.2. MODELING PROCEDURE FOR CONTINUOUS NONCANCER DATA

BMD modeling of continuous data was conducted on the HAWC website using the U.S. Environmental Protection Agency's (U.S. EPA's) BMDS (Version 2.7). All continuous models available within the software were fit using a benchmark response (BMR) of 1 standard deviation (SD). For continuous data of effects in developing offspring, including thyroid hormone changes, a BMR of 0.5 SD change from the control mean is used to account for effects occurring in a sensitive life stage. A 1 SD BMR is also presented as the basis for model comparison as directed in the U.S. EPA *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). An adequate fit is judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.1$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.1$), the data set is considered unsuitable for BMD modeling. In cases in which a model with # parameters = # dose-groups was fit to the data set, all parameters were estimated, and no *p*-value was calculated, that model was not considered for estimating a POD unless no other model provided adequate fit. Among all models providing adequate fit, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD from which to derive the oral reference dose/inhalation reference concentration (RfD/RfC).

F.2.1 Modeling Predictions for Serum Total T_4 in PND 1 Female Offspring (litter *n*)

The modeling results for total T_4 in PND 1 female offspring (litter *n*) exposed Gestation Days (GDs) 1−20 are shown in Table F-2. The Exponential 4 model (see Figure F-2) was selected given appropriate fit to the data and that the BMDL values differed by greater than threefold. The output for the U.S. EPA's BMDS model run is also provided below.

Table F-2. Modeling Results for Total T_4 in PND 1 Female Offspring (Litter *n*) Exposed GDs 1−20[a]

Model	Global *p*-Value	AIC	$BMD_{0.5SD}$ (HED) (mg/kg-d)	$BMDL_{0.5SD}$ (HED) (mg/kg-d)	BMD_{1SD} (HED) (mg/kg-d)	$BMDL_{1SD}$ (HED) (mg/kg-d)	Residual of Interest
Linear	0.5652	−4.74898	0.7778	0.5120	1.5557	1.0241	0.348
Polynomial	0.5652	−4.74898	0.7778	0.5120	1.5557	1.0241	0.348
Power	0.5652	−4.74898	0.7778	0.5120	1.5557	1.0241	0.348
Hill	−999	−1.89	0.368	0.0704	0.8677	0.2294	-6.01×10^{-7}
Exponential-M2	0.77	−5.3672	0.5546	0.3017	1.2555	0.6694	−0.5752
Exponential-M3	0.77	−5.3672	0.5546	0.3017	1.2555	0.6694	−0.5752
Exponential-M4[b]	**0.8583**	**−3.8581**	**0.3346**	**0.0951**	**0.8708**	**0.2498**	**−0.08305**
Exponential-M5	−999	−1.89	0.3807	0.0958	0.8669	0.2517	-4.356×10^{-7}

[a]Feng et al. (2017).
[b]Selected model. Exponential 4 model was selected given appropriate fit to the data and that the BMDL values differed by greater than threefold. The Hill and Exponential 5 models were not selected because they did not return a *p*-value.

AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., 0.5 SD = exposure concentration associated with 0.5 SD change from the control mean); BMR = benchmark response; GD = gestation day; HED = human equivalent dose; PND = postnatal day; SD = standard deviation; T_4 = thyroxine.

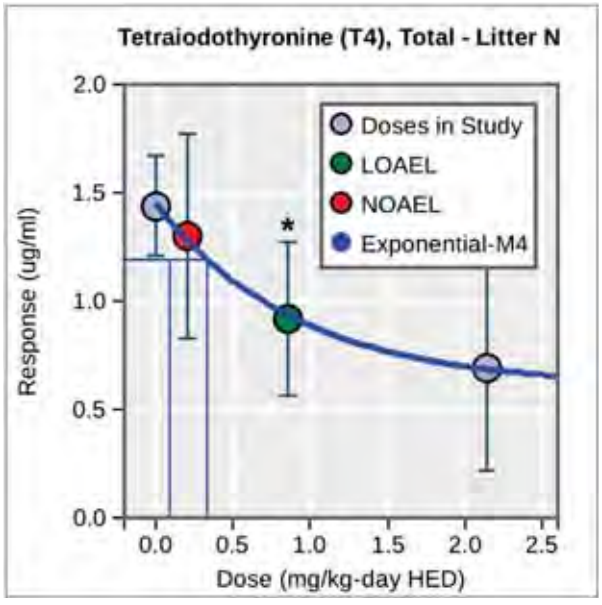


Figure F-2. Exponential (Model 4) for Total T_4 in PND 1 Female Offspring (Litter *n*) Exposed GDs 1−20 (Feng et al., 2017)

```
================================================================
          Exponential Model.  (Version: 1.11;  Date: 03/14/2017)
          Input Data File: C:\Windows\TEMP\bmds-dfile-k4vsthrz.(d)
          Gnuplot Plotting File:
          Mon Aug 17 15:16:06 2020
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.

   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008

   MLE solution provided: Exact


                  Initial Parameter Values
```

Variable	Model 4	
lnalpha	-1.29725	
rho	0	Specified
a	1.512	
b	1.50054	
c	0.434618	
d	1	Specified

Parameter Estimates

Variable	Model 4	Std. Err.
lnalpha	-1.29645	0.0611565
a	1.45283	0.148029
b	1.10398	1.13864
c	0.417162	0.225239

NC = No Convergence

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	1.44	0.329
0.21	10	1.3	0.657
0.86	10	0.92	0.493
2.14	10	0.69	0.657

Estimated Values of Interest

Dose	Est Mean	Est Std	Scaled Residual
0	1.453	0.523	-0.07759
0.21	1.278	0.523	0.1354
0.86	0.9337	0.523	-0.08305
2.14	0.6858	0.523	0.02529

```
  Other models for which likelihoods are calculated:

    Model A1:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma^2

    Model A2:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma(i)^2

    Model A3:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

    Model  R:        Yij = Mu + e(i)
                Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                    Model      Log(likelihood)      DF         AIC
                   -------    -----------------    ----   ------------
                       A1        5.944999            5     -1.889998
                       A2        8.698072            8     -1.396144
                       A3        5.944999            5     -1.889998
                        R       0.3138778            2      3.372244
                        4        5.929054            4     -3.858109


   Additive constant for all log-likelihoods =     -36.76.   This constant added to
the
   above values gives the log-likelihood including the term that does not
   depend on the model parameters.


                               Explanation of Tests

   Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
   Test 2:  Are Variances Homogeneous? (A2 vs. A1)
   Test 3:  Are variances adequately modeled? (A2 vs. A3)

   Test 6a: Does Model 4 fit the data? (A3 vs 4)


                             Tests of Interest
```

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	16.77	6	0.01017
Test 2	5.506	3	0.1383
Test 3	5.506	3	0.1383
Test 6a	0.03189	1	0.8583

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is greater than .1. A homogeneous variance model appears to be appropriate here.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD = 0.87078

BMDL = 0.249811

BMDU = 21400

==========

Exponential Model. (Version: 1.11; Date: 03/14/2017)
Input Data File: C:\Windows\TEMP\bmds-dfile-17lffb4f.(d)
Gnuplot Plotting File:

Mon Aug 17 15:16:07 2020

```
================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function by Model:
     Model 2:     Y[dose] = a * exp{sign * b * dose}
     Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
     Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
     Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

   Note: Y[dose] is the median response for exposure = dose;
         sign = +1 for increasing trend in data;
         sign = -1 for decreasing trend.

     Model 2 is nested within Models 3 and 4.
     Model 3 is nested within Model 5.
     Model 4 is nested within Model 5.


  Dependent variable = Response
  Independent variable = Dose
  Data are assumed to be distributed: normally
  Variance Model: exp(lnalpha +rho *ln(Y[dose]))
  rho is set to 0.
  A constant variance model is fit.

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 500
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

  MLE solution provided: Exact


                Initial Parameter Values

                Variable          Model 4
                --------          --------
                  lnalpha          -1.29725
                      rho                 0 Specified
                        a             1.512
```

```
                        b           1.50054
                        c          0.434618
                        d                 1 Specified



                  Parameter Estimates

                Variable          Model 4          Std.  Err.
                --------          -------          ---------
                 lnalpha            -1.29645           0.0611565
                       a             1.45283            0.148029
                       b             1.10398             1.13864
                       c            0.417162            0.225239

  NC = No Convergence


         Table of Stats From Input Data

  Dose      N         Obs Mean     Obs Std Dev
  -----    ---       ----------   -------------
      0     10         1.44        0.329
   0.21     10          1.3        0.657
   0.86     10         0.92        0.493
   2.14     10         0.69        0.657


              Estimated Values of Interest

   Dose      Est Mean      Est Std     Scaled Residual
 ------    ----------    ---------    ----------------
      0         1.453        0.523         -0.07759
   0.21         1.278        0.523           0.1354
   0.86        0.9337        0.523         -0.08305
   2.14        0.6858        0.523          0.02529



Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
    Model A2:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = Sigma(i)^2

    Model A3:        Yij = Mu(i) + e(ij)
                Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

    Model  R:        Yij = Mu + e(i)
                Var{e(ij)} = Sigma^2


                              Likelihoods of Interest

                   Model      Log(likelihood)      DF         AIC
                  -------    -----------------    ----   ------------
                      A1        5.944999           5     -1.889998
                      A2        8.698072           8     -1.396144
                      A3        5.944999           5     -1.889998
                       R       0.3138778           2      3.372244
                       4        5.929054           4     -3.858109


  Additive constant for all log-likelihoods =     -36.76.   This constant added to
the
  above values gives the log-likelihood including the term that does not
  depend on the model parameters.


                               Explanation of Tests

  Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)
  Test 2:  Are Variances Homogeneous? (A2 vs. A1)
  Test 3:  Are variances adequately modeled? (A2 vs. A3)

  Test 6a: Does Model 4 fit the data? (A3 vs 4)


                            Tests of Interest

    Test          -2*log(Likelihood Ratio)       D.  F.          p-value
  --------        ------------------------      ------     --------------
    Test 1                        16.77           6             0.01017
    Test 2                        5.506           3              0.1383
```

```
 Test 3                        5.506           3             0.1383
Test 6a                      0.03189           1             0.8583


 The p-value for Test 1 is less than .05.   There appears to be a
 difference between response and/or variances among the dose
 levels, it seems appropriate to model the data.

 The p-value for Test 2 is greater than .1.   A homogeneous
 variance model appears to be appropriate here.

 The p-value for Test 3 is greater than .1.   The modeled
 variance appears to be appropriate here.

 The p-value for Test 6a is greater than .1.   Model 4 seems
 to adequately describe the data.


Benchmark Dose Computations:

  Specified Effect = 0.500000

         Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

               BMD =      0.33455

              BMDL =    0.0950923

              BMDU =      1.22544
```

F.3 MODELING PROCEDURE FOR DICHOTOMOUS NONCANCER DATA

BMD modeling of dichotomous noncancer data (see Figure F-1) was conducted on the HAWC website using the U.S. EPA's BMDS Version 2.7. For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, and Weibull dichotomous models available within the software were fit using a BMR of 10% extra risk. The Multistage model is run for all polynomial degrees up to $n - 2$, where n is the number of dose groups including control. Adequacy of model fit was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control) closest to the predefined BMR (absolute value < 2.0), and visual inspection of the model fit. In the cases where no best model was found to fit to the data, use of a reduced data set without the high-dose group was further attempted for modeling and the result was presented along with that of the full data set. In cases in which a

model with # parameters = # dose-groups was fit to the data set, all parameters were estimated, and no *p*-value was calculated, that model was not considered for estimating a POD *unless* no other model provided adequate fit. Among all models providing adequate fit, the BMDL from the model with the lowest AIC was selected as a potential POD when BMDL values were sufficiently close (within threefold) (see Table F-1). Otherwise, the lowest BMDL was selected as a potential POD.

APPENDIX G. QUALITY ASSURANCE

U.S. EPA has an agency-wide quality assurance (QA) policy, and that policy is outlined in the *EPA Quality Manual for Environmental Programs* (see CIO 2105-P-01-0) and follows the specifications outlined in U.S. EPA Order CIO 2105.0. The goal of the QA policy is to assure that environmental data used to support Agency decisions are of adequate quality and usability for their intended purpose.

As required by CIO 2105.0, ORD maintains a Quality Management Program, which is documented in an internal Quality Management Plan (QMP). The latest version was developed in 2013 using the *Guidance for Developing Quality Systems for Environmental Programs (QA/G-1).* An NCEA-specific QMP was also developed in 2013 as an appendix to the ORD QMP. Quality assurance for products developed within CPHEA is managed under the ORD QMP and applicable appendices.

This assessment has been designated as High Profile and is classified as QA Category A. Category A designations require reporting of all critical QA activities, including audits.

Another requirement of the Agency quality system includes the use of project-specific planning documents referred to as Quality Assurance Project Plans (QAPPs) that describe how specific data collection efforts will be planned, implemented, and assessed. Specific management of quality assurance in this assessment is documented in an Umbrella Quality Assurance Project Plan, which was developed using the U.S. EPA Guidance for Quality Assurance Project Plans (QA/G-5). The latest approved version of the QAPP is dated September 2019. During assessment development, additional QAPPs may be applied for quality assurance management. They include:

Title	Document Number	Date
Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents	L-CPAD-0032718-QP	October 2015 (last updated 2020)
Umbrella Quality Assurance Project Plan for NCEA PFAS Toxicity Assessments	B-IO-0031652-QP-1-2	July 2018 (last updated September 2019)
Quality Assurance Project Plan (QAPP) for Enhancements to Benchmark Dose Software (BMDS)	B-003742-QP-1-0	July 2019

During assessment development, this project underwent quality audit:

Date	Type of Audit	Major Findings	Actions Taken
September 18, 2020	Technical System Audit	None	None

During assessment development, the assessment was subjected to external reviews by individual letters from expert peer reviewers and by other federal agency partners including the Executive Offices of the President. Peer-review reports during these review steps are available at https://www.epa.gov/pfas/learn-about-human-health-toxicity-assessment-pfbs. In addition, the assessment underwent public comment from November 21, 2018 to January 22, 2019. The public comments are available in the Docket ID No. EPA-HQ-OW-2018-0614. Prior to release, the final draft assessment was submitted to management and QA clearance. During this step the CPHEA QA director and QA managers review the project QA documentation and ensure U.S. EPA QA requirements have been met.

APPENDIX H. REFERENCES

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Attachment

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13

United States Environmental Protection Agency

EPA/690/R-08/008F
Final
8-25-2008

Provisional Peer Reviewed Toxicity Values for

Cobalt
(CASRN 7440-48-4)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR COBALT (CASRN 7440-48-4)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).

2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.

3. Other (peer-reviewed) toxicity values, including:

   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a five-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically

updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

The Integrated Risk Information System (IRIS) does not report a Reference Dose (RfD) for cobalt (U.S. EPA, 2007). The Health Effects Assessment Summary Tables (HEAST) (U.S. EPA, 1997a) and Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) likewise do not contain an RfD for cobalt. The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991, 1994a) report a Health Effect Assessment (HEA) for cobalt (U.S. EPA, 1987). The 1987 HEA derived a chronic RfD of 0.005 mg cobalt/kg-day based on a no-observed-adverse-effect level (NOAEL) of 5 mg cobalt/kg-day for testicular effects in a subchronic rat study (Nation et al., 1983). The Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for cobalt and its compounds reports an oral Minimal Risk Level (MRL) for intermediate exposure of $1x10^{-2}$ mg/kg-day (ATSDR, 2004), based on a lowest-observed-adverse-effect level (LOAEL) of approximately 1 mg cobalt/kg-day for polycythemia in humans (Davis and Fields, 1958). ATSDR (2004) did not derive an oral MRL for chronic exposure. This MRL for intermediate exposure was based on the polycythemic effect of cobalt exposure (1 mg cobalt/kg-day, Davis and Fields, 1958) by application of an UF of 10 for a LOAEL and an UF of 10 for human variability. The World Health Organization (WHO, 2005) has not published an Environmental Health Criteria (EHC) document on cobalt. An International Agency for Research on Cancer (IARC) Monograph on cobalt and its compounds (IARC, 2006) and the National Toxicology Program (NTP) Status Reports (NTP, 2005) were searched for relevant information.

IRIS (U.S. EPA, 2007) does not report a Reference Concentration (RfC) for cobalt. The HEAST (U.S. EPA, 1997a) likewise does not list an RfC for cobalt. The cobalt HEA (U.S. EPA, 1987) derived a subchronic inhalation RfC of $9x10^{-5}$ mg/m^3 based on a LOAEL of 0.1 mg/m^3 for

respiratory effects in a 3-month study in swine (Kerfoot et al., 1975). A chronic inhalation RfC of $9x10^{-6}$ mg/m^3 was derived from the same study. The ATSDR Toxicological Profile for cobalt and its compounds reports an inhalation MRL for chronic exposure of $1x10^{-4}$ mg/m^3 (ATSDR, 2004), based on a NOAEL of 0.0053 mg cobalt/m^3 for decreased pulmonary function in humans (Nemery et al., 1992). The American Conference of Governmental Industrial Hygienists (ACGIH, 2004) has set a Threshold Limit Value-Time-Weighted Average (TLV-TWA) of 0.02 mg/m^3 for cobalt and inorganic cobalt compounds, expressed as cobalt, based on respiratory and cardiovascular effects. The National Institute for Occupational Safety and Health (NIOSH, 2005) Recommended Exposure Limit (REL) TWA for cobalt is 0.05 mg/m^3, based on effects in the respiratory system. The Occupational Safety and Health Administration (OSHA, 2005) Permissible Exposure Limit (PEL) is 0.1 mg/m^3.

IRIS (U.S. EPA, 2007) does not report a cancer classification, slope factor or unit risk for cobalt. The HEAST (U.S. EPA, 1997a) and Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) likewise do not report carcinogenicity assessments for cobalt. The CARA lists (U.S. EPA, 1991, 1994a) do not report a cancer classification or an estimate of the carcinogenic potency of stable cobalt compounds due to a lack of pertinent data. An IARC Monograph on cobalt and its compounds (IARC, 2006) classified cobalt sulfate and other soluble cobalt (II) salts as "possibly carcinogenic to humans." ACGIH (2004) has classified cobalt in category A3 – confirmed animal carcinogen with unknown relevance to humans.

Literature searches for studies relevant to the derivation of provisional toxicity values for cobalt were conducted initially through 2000 in TOXLINE (supplemented with BIOSIS and NTIS updates), MEDLINE, TSCATS, RTECS, CCRIS, DART, EMIC/EMICBACK, HSDB, GENETOX and CANCERLIT and subsequently from 2000 to August 2005 in MEDLINE, TOXLINE (NTIS subfile), TOXCENTER, TSCATS, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS and Current Contents. An updated literature search was performed in MEDLINE from 2005 to June 2008.

REVIEW OF PERTINENT DATA

Human Studies

Overview

Indicators of adverse health effects in humans following oral exposure to cobalt include increased erythrocyte number and hemoglobin (Taylor et al., 1977; Duckham and Lee, 1976; Davis and Fields, 1958), cardiomyopathy (Morin et al., 1971; Alexander, 1969, 1972) and decreased iodine uptake by the thyroid (Roche and Layrisse, 1956). Cardiomyopathy is an endpoint of concern for cobalt in humans; however, it is highly likely that alcohol consumed in "beer-cobalt cardiomyopathy," as well as other factors, such as smoking, played a role in the effects that were observed. Cobalt is a sensitizer in humans by any route of exposure. Sensitized individuals may react to inhalation of cobalt by developing asthma; ingestion or dermal contact with cobalt may result in development of dermatitis. Several studies have suggested that

cross-sensitization may occur between cobalt and nickel (Shirakawa et al., 1990; Lammintausta et al., 1985; Bencko et al., 1983; Rystedt and Fisher, 1983).

Respiratory effects, including respiratory irritation, wheezing, asthma, pneumonia and fibrosis, have been widely reported in humans exposed to cobalt by inhalation (for review, see Barceloux, 1999; Lison, 1996). Epidemiology studies show decreased pulmonary function in workers exposed to inhaled cobalt (Nemery et al., 1992; Gennart and Lauwerys, 1990). Results of studies investigating cancer incidence in workers exposed to inhaled cobalt are suggestive of a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983).

Oral Exposure

In humans, cobalt stimulates production of red blood cells through increased production of the hormone erythropoietin and has been explored for use in the treatment of anemia (Smith and Fisher, 1973; Duckham and Lee, 1976). Increases in red blood cell counts and blood hemoglobin have been reported in non-anemic volunteers (Davis and Fields, 1958) and in anephric anemic patients (Taylor et al., 1977; Duckham and Lee, 1976).

Reversible polycythemia (increase in blood cell number) was reported (see Table 1) in six healthy adult males following treatment with 150 mg cobalt chloride per day for 22 days (Davis and Fields, 1958). Five subjects received 150 mg cobalt chloride/day for the entire exposure period and a sixth subject initially received 120 mg cobalt chloride/day, which was later increased (time not specified) to 150 mg/day. Cobalt chloride was administered as a 2% solution diluted in either water or milk. Assuming an average body weight of 70 kg, 150 mg cobalt chloride/day corresponds to approximately 1 mg cobalt/kg-day. Outcomes assessed in this study were red blood cell count, hemoglobin percentage, leukocyte count, reticulocyte percentage and thrombocyte count. Polycythemia was observed in all six patients within 7 to 22 days of treatment as demonstrated by increases in red blood cell counts ranging from 0.5 to 1.19 million (approximately 16-20% increase above pre-treatment levels) and increases in hemoglobin levels ranging from 6 to 11% above pretreatment values. In five of the six subjects, reticulocyte levels were elevated, reaching at least twice the pre-experiment values. Thrombocyte and total leukocyte counts were not significantly different from pretreatment values. Erythrocyte counts returned to pre-treatment levels within 9 to 15 days after cobalt administration was discontinued. The fact that leucocyte counts remained relatively constant throughout the experiment supports the concept that this is a true polycythemia. As such, based on the results of this study, 1 mg cobalt/kg-day was identified as a LOAEL for cobalt-induced polycythemia in humans.

Table 1. Hematopoietic, Thyroid and Developmental Effects of Cobalt (Co) via Oral Route

Target Organ	Species	Effect	Dosage (mg Co/kg-day)
Hematopoietic Effects	Human Human Rat	Reversible Effect (Polycythemia) ↑ Hemoglobin and ↑ RBC Hematopoietic effect	1.0 0.16 – 0.32* 0.5 – 32.0
Thyroid	Human Mice	↓ Iodine uptake Histopathological changes in thyroid	1.0 48.0
Fetus	Rat	Developmental toxicity	5.2 – 21.0
Heart	Rat	↓ Myocardial function	8.0

*Therapeutic doses for anemic patients

Duckham and Lee (1976) treated 12 anephric patients on dialysis with 25 to 50 mg cobalt chloride daily for approximately 12 weeks. Assuming an average body weight of 70 kg, doses of 25 and 50 mg cobalt chloride/day are equivalent to 0.16 and 0.32 mg cobalt/kg-day, respectively. During the exposure period, patients also received daily treatment with 100 mg ferrous sulfate and 50 mg ascorbic acid. Within approximately 2 months of initiation of treatment with cobalt, an increase in hemoglobin of 26-70% was observed in patients treated with 0.32 mg cobalt/kg-day. Serum cobalt levels appeared to reach steady state within 2 months of exposure (approximately 40-100 μg cobalt/100 mL). In a subgroup of three patients, continuation of treatment with 0.16 mg cobalt/kg-day for approximately 3 months maintained elevated hemoglobin levels. Hemoglobin levels decreased rapidly when cobalt therapy was discontinued. The authors did not report whether therapy with ferrous sulfate and ascorbic acid was discontinued at the same time. Results of this study are difficult to interpret because patients were anephric and on dialysis, which may have altered cobalt pharmacokinetics and dose-effect relationships. Furthermore, since it is well established that treatment with ferrous sulfate alone increases hemoglobin concentration (Hillman, 2001), concomitant therapy with iron is a confounding factor. Since this study did not evaluate the response of patients treated with ferrous sulfate alone, it is not possible to determine the relative contributions of iron and cobalt to the observed increases in hemoglobin. Thus, adverse effect levels cannot be confidently determined for cobalt. In a separate study, a group of eight anephric patients with refractory anemia were treated with 25 to 50 mg cobalt chloride daily for 12 to 36 weeks (Taylor et al., 1977). Increased hemoglobin concentration and decreased requirement for blood transfusions were observed (Taylor et al., 1977). Data on hemoglobin concentrations (or other indicators of polycythemia) were not reported.

Pregnant women given 75 to 100 mg cobalt chloride/day with no other treatment for 90 days to 6 months did not experience pregnancy-induced reductions in hematocrit and hemoglobin levels, compared to untreated controls (Holly, 1955). However, daily treatment with 1 g ferrous sulfate alone or combined daily treatment with 60 to 90 mg cobalt chloride and 0.8 to 1.2 g ferrous sulfate prevented pregnancy-related decreases in hematocrit and hemoglobin levels. The response to combined cobalt chloride and iron therapy was more pronounced than the

response to iron therapy alone. In patients treated with iron only, decreases in hemoglobin and hematocrit were prevented in approximately 80% of patients, compared to 100% of patients treated with combined cobalt chloride and iron.

Cardiomyopathy has been observed in association with consumption of large quantities of beer containing cobalt chloride (introduced into the beer to stabilize the foam) (Alexander, 1969, 1972; Morin et al., 1971). Exposure estimates in reported cases range from 0.04 to 0.14 mg cobalt/kg-day (corresponding to approximately 8-30 pints of beer daily) over a period of years (Alexander, 1969, 1972; Morin et al., 1971). The cardiomyopathy in the beer drinkers, referred to in the literature as "beer-cobalt cardiomyopathy," was fatal to 43% of the subjects within several years, with approximately 18% of these deaths occurring within the first several days following diagnosis. Beer-cobalt cardiomyopathy appeared to be similar to alcoholic cardiomyopathy and beriberi; however, the onset of the beer-cobalt cardiomyopathy was much more abrupt. The practice of adding cobalt to beer to stabilize the foam has been discontinued. It should be noted, however, that the cardiomyopathy may also have been due to the fact that the beer drinkers had protein-poor diets and may have had prior or concurrent cardiac and hepatic damage from alcohol abuse. Due to the potential adverse effects of poor nutrition and/or chronic ethanol exposure on cardiovascular health, it is difficult to delineate the contribution of oral cobalt exposure to the observed cardiomyopathy. As such, no adverse effects levels can be determined for cobalt-induced cardiotoxicity.

The thyroid also appears to be a target organ for cobalt (see Table 1). Treatment of 12 euthyroid (normal thyroid) patients with 150 mg cobalt chloride/day (equivalent to 1 mg cobalt/kg-day, assuming a body weight of 70 kg) for 2 weeks resulted in a greatly reduced uptake of 48-hour radioactive iodine by the thyroid when measured after 1 week of exposure to cobalt, with uptake nearly abolished completely by the second week of exposure to cobalt (Roche and Layrisse, 1956). It should be noted that when cobalt treatment was discontinued, iodine uptake returned to pre-treatment reported values. No other clinical details were provided for the human subjects. Therefore, based on the results of this study, a LOAEL of 1 mg cobalt/kg-day was identified for decreased radioactive iodine uptake in human thyroid following oral cobalt exposure. In another small clinical study (Paley et al., 1958), decreased radioactive iodine uptake was reported in two of four (3 males, 1 female) euthyroid patients orally administered 37.5 mg cobalt/day as cobalt chloride (equivalent to 0.54 mg cobalt/kg-day, assuming a body weight of 70 kg) for 10 to 14 days. One of the two subjects with reported decreased iodine uptake had received i.v. cobalt in addition to oral cobalt intake, and had been previously diagnosed with hyperthyroidism (although was clinically euthyroid at the time of study). The i.v. dosing may have raised the internal cobalt concentration to a level greater than the reported 0.54 mg dosage based upon oral dosing of 37.5 mg/day in other subjects that did not receive i.v. cobalt. Of the remaining three subjects, 24-hour iodine uptake was not significantly decreased following oral cobalt exposure compared to corresponding pre-treatment values (based on pairwise t-test). The oral cobalt dose of 0.54 mg cobalt/kg-day represents a NOAEL for thyroid effects in humans. It should be noted that the Roche and Layrisse (1956) and Paley et al. (1958) studies lack details pertinent to other clinical conditions (e.g. including effects on thyroid stimulating hormone [TSH]) of these patients; thus the mechanism for the effect of cobalt on thyroidal iodine uptake cannot be ascertained. However, cobalt appears to increase thiocyanate-

induced release of radioiodine from the thyroid, suggesting a possible effect on binding of iodine (e.g., iodination of thyroglobulin) in the thyroid gland.

Cobalt has been found to be a sensitizer in humans. Individuals are sensitized following dermal or inhalation exposure, but flares of dermatitis may be triggered following cobalt ingestion. In a small clinical study, several patients with eczema of the hands were challenged orally with 1 mg cobalt sulfate (0.005 mg cobalt/kg-day, assuming a body weight of 70 kg) in tablet form once per week for 3 weeks; this translates to an estimated average daily dose of 0.0007 mg cobalt/kg-day (1 day a week/7 days a week × 0.005 mg cobalt/kg-day). 28/47 patients had a flare of dermatitis following the oral challenge (Veien et al., 1987). All 47 patients had positive dermal patch tests to cobalt (13 to cobalt alone and 34 to nickel and cobalt) and 7 of the 13 patients who had patch-tested positive to cobalt alone reacted to the oral challenge. These results suggest that cobalt allergy can be induced from oral ingestion exposures to cobalt. Although the exposure levels associated with sensitization to cobalt following inhalation or dermal exposure have not been established, interrelationships have been found to exist between cobalt and nickel sensitization (Bencko et al., 1983; Rystedt and Fisher, 1983; Veien et al., 1987). In guinea pigs, nickel and cobalt sensitization appear to be interrelated and mutually enhancing (Lammintausta et al., 1985). Therefore, it is possible that in people sensitized by nickel, exposure to cobalt may result in an allergic reaction.

Inhalation Exposure

Numerous studies have investigated health effects in workers occupationally exposed to cobalt-bearing dust (Linna et al., 2003; Swennen et al., 1993; Auchincloss et al., 1992; Cugell, 1992; Nemery et al., 1992; Prescott et al., 1992; Gennart and Lauwerys, 1990; Meyer-Bisch et al., 1989; Raffn et al., 1988; Shirakawa et al., 1988, 1989; Sprince et al., 1988; Kusaka et al., 1986a,b; Demedts et al., 1984; Davison et al., 1983). However, many of these studies are of limited utility for risk assessment due to inadequate characterization of exposure and/or effects. Four studies were considered to be potentially suitable for RfC derivation. Two of these focused exclusively on respiratory effects (Nemery et al., 1992; Gennart and Lauwerys, 1990); one studied only thyroid effects (Prescott et al., 1992) and one considered multiple endpoints (Swennen et al., 1993). The populations studied included diamond-cobalt saw manufacturers, diamond polishers, plate painters and cobalt production workers. All four studies were cross-sectional design.

Several studies have examined the effects of hard metal, a mixture containing approximately 20% cobalt with the remainder being primarily tungsten carbide. Exposure of humans to hard metal has been shown to result in an increase in cancer mortality (Moulin et al., 1998; Lasfargues et al., 1994) as well as a number of other diseases, including asthma and pulmonary fibrosis (for reviews, see Barceloux, 1999; Lison, 1996). There is substantial evidence from animal studies that tungsten, although it acts as an inert dust by itself, can potentiate the effects of cobalt on the respiratory tract (Lasfargues et al., 1995; Lison et al., 1995, 1996; Swennen et al., 1993). For this reason, studies of hard metal were not given further consideration.

Gennart and Lauwerys (1990) studied ventilatory function in workers at a plant producing diamond-cobalt circular saws. The form of cobalt used in diamond polishing is primarily metallic cobalt powder; specific cobalt species contained in this powder were not identified. The exposed population consisted of 48 workers (34 males and 14 females) who agreed to participate in the study (an additional 27 workers declined). Exposure duration for these workers ranged from 0.1 to 32 years, with an average of approximately 6 years. The work involved weighing and mixing cobalt powder and microdiamond particles (and possibly small amounts of other undisclosed substances), cold pressing, heating and hot pressing. After sintering, the pieces were welded onto steel disks. These operations were performed in two rooms called the mixing room and the oven room, where all the examined workers spent most of their time. Controls consisted of 23 workers (11 males and 12 females) from other factories in the same area who were not exposed to known pneumotoxic chemicals. Personal air samples were collected at different workplaces during half a workshift. Subjects filled out a questionnaire regarding occupational and medical histories, smoking habits and pulmonary symptoms; gave a urine sample for cobalt determination; and participated in lung function tests. Cobalt concentrations varied from 9.4 to 2875 $\mu g/m^3$ in the mixing room (geometric mean=135.5 $\mu g/m^3$) and from 6.2 to 51.2 $\mu g/m^3$ in the oven room (geometric mean=15.2 $\mu g/m^3$). The prevalence of respiratory symptoms, such as cough, sputum and dyspnea, were significantly increased in the exposed workers compared to the control group (numeric data not reported). Mean predicted values of FEV_1 (forced expiratory volume in 1 second adjusted for body size) and FVC (forced vital capacity) were significantly lower, and the prevalence of abnormal values was higher in the cobalt exposed workers (both smokers and non-smokers) compared to the control group. In controls, FEV_1 and FVC were 95.4 and 101.6 percent of predicted values, respectively. Mean percent predicted FEV_1 and FVC in exposed non-smokers were 87.1 and 92.3, respectively, and in exposed smokers were 83.9 and 93.4, respectively. Among non-smokers, all measures of pulmonary function were lower in workers exposed for 5 years or more than in those exposed to cobalt for a shorter period of time.

Nemery et al. (1992) conducted a cross-sectional study of cobalt exposure and respiratory effects in diamond polishers who were primarily exposed to metallic cobalt-containing dust; species of cobalt in the dust samples were not identified. The study group was composed of 194 polishers working in 10 different workshops. In two of these workshops (#1, 2), the workers used cast iron polishing disks almost exclusively, and in the others, they primarily used cobalt-containing disks. The number of subjects from each workshop varied from 6 to 28 and the participation rate varied from 56 to 100%. The low participation in some workshops reflects the fact that only workers who used cobalt disks were initially asked to be in the study; low participation is not due to a high refusal rate (only eight refusals were documented). More than a year after the polishing workshops were studied, an additional three workshops with workers engaged in sawing diamonds, cleaving diamonds or drawing jewelry were studied as an unexposed control group (n=59 workers). Subjects were asked to fill out a questionnaire regarding employment history, working conditions, medical history, respiratory symptoms and smoking habits; to give a urine sample for cobalt determination; and to undergo a clinical examination and lung function tests. Both area air samples and personal air samples were collected (always on a Thursday). Sampling for area air determinations started 2 hours after work began and continued until 1 hour before the end of the work day. Personal air samples were collected from the breathing zone of a few workers per workshop for four successive

1-hour periods. Air samples were analyzed for cobalt and iron. In addition, personal air samplers were used to sample the air 1 cm above the polishing disks. These samples were analyzed for the entire spectrum of mineral and metallic compounds. Air samples were not obtained at one of the polishing workshops (#4); however, this workshop was reported to be almost identical to an adjoining workshop (#3) for which samples were obtained. Urinary cobalt levels were similar between workers in these two workshops, so exposure was considered to be similar as well.

Results of area and personal air sampling were strongly correlated (R=0.92), with area air sampling reporting lower concentrations than personal air samples in all workshops except one (#9) (Nemery et al., 1992). In this workshop, personal air samples appeared to be artificially low in comparison to area air samples and urinary cobalt levels of the workers. When this workshop was excluded, a strong correlation (R=0.85-0.88) between urinary cobalt and cobalt in the air was observed. Based on urinary cobalt levels, the predicted concentration of cobalt expected in personal air samples from workshop #9 was approximately 45 $\mu g/m^3$ (the mean value actually reported was 6 $\mu g/m^3$). The polishing workshops were divided into two groups: those with low exposure to cobalt (#1-5, n=102) and those with high exposure to cobalt (#6-10, n=91). Mean cobalt exposure concentrations were 0.4, 1.6 and 10.2 $\mu g/m^3$ by area air sampling and 0.4, 5.3 and 15.1 $\mu g/m^3$ by personal air sampling in the control, low-exposure and high-exposure groups, respectively. The inclusion of the apparently biased personal air samples from workshop #9 means that the reported mean cobalt exposure in the high-exposure group obtained by personal air sampling (15.1 $\mu g/m^3$) may be lower than the true value. Air concentrations of iron were highest in the two polishing workshops that used iron disks and the sawing workshop (highest value=62 $\mu g/m^3$), and were not correlated with cobalt levels. Analysis of samples taken near the disks showed the presence of cobalt, with occasional traces of copper, zinc, titanium, manganese, chromium, silicates and silicon dioxide. No tungsten was detected. Some workers may have previously been exposed to asbestos since pastes containing asbestos had been used in the past to glue the diamonds onto holders. However, since the asbestos was in its non-friable form, exposure was insufficient to produce functional impairment. Smoking habits were similar in workers from the high-exposure, low-exposure and control groups. Duration of exposure was not discussed.

Workers in the high-exposure group were more likely than those in the other groups to complain about respiratory symptoms; the prevalences of eye, nose and throat irritation and cough, and the fraction of these symptoms related to work, were significantly increased in the high-exposure group (Nemery et al., 1992). Workers in the high-exposure group also had significantly lower lung function compared to controls and low-exposure group workers, as assessed by FVC, FEV_1, MMEF (forced expiratory flow between 25 and 75% of the FVC) and mean PEF (peak expiratory flow rate), although the prevalence of abnormal values did not differ significantly between exposure categories. In controls, FVC, FEV_1 and MMEF were approximately 110, 107 and 94 percent of predicted values, respectively, compared to approximately 105, 104 and 87 percent of predicted values, respectively, in the high-exposure group workers. Results in the low-exposure group did not differ from controls. The effect on spirometric parameters in the high exposure group was present in both men and women. Women seemed to be affected more than men; however, the interaction between exposure and sex was not significant (two-way analysis of variance). Smoking was found to exert a strong effect on

lung function; however, lung function level remained negatively correlated with exposure to cobalt, independent of smoking.

A cobalt dose-effect relationship is evident from the Nemery et al. (1992) study, based on a multivariate regression analysis of urinary cobalt and lung function measurements. Increasing urinary cobalt concentration (approximate range <1-70 μg cobalt/g creatinine) was significantly (p<0.05) associated with co-variate-adjusted decreasing forced expiratory volume (FEV1%) and forced vital capacity (FVC%). Significant co-variates retained in the regression analysis included gender and smoking. The model predicted 3% and 4% decreases in FEV1% and FVC%, respectively, in association with a 10-fold increase in urinary cobalt concentration. The approximate mean urinary cobalt levels of the control and high exposure groups were 2 and 20 μg cobalt/g creatinine, respectively. The magnitude of the cobalt effect was similar to the predicted effect of smoking, approximately 3-4% decrease in FEV1% and FVC%. Cobalt concentration determined from personal air sampling may be more representative of airborne cobalt exposure than area sampling. As such, 5.3 $\mu g/m^3$ and 15.1 $\mu g/m^3$ represent a NOAEL and LOAEL, respectively, for decreased pulmonary function and increased symptoms of airway irritation.

Swennen et al. (1993) conducted a cross-sectional study of workers exposed to metallic cobalt and various inorganic cobalt salts and oxides (specific species not identified) at a cobalt plant producing these materials from cobalt metal cathodes and scrap metal. The study group included 82 male workers from the cobalt plant who had no history of lung disease prior to employment and who had never been exposed to other pneumotoxic chemicals. Methods for selection or exclusion of subjects in constructing the cohort and participation were not reported. The control group comprised 82 age-matched workers from the mechanical workshop of a nearby plant owned by the same company. Workers filled out a questionnaire regarding occupational history, respiratory complaints and smoking habits; received a routine clinical examination; participated in lung function tests; had a chest radiograph taken; and gave blood and urine samples (before and after working on Monday and Friday of one week) for determination of cobalt content as well as hematological and serum chemistry analyses. Exposure was monitored by personal air samplers worn by each cobalt worker for 6 hours on both Monday and Friday.

Workers in the cobalt plant were exposed to cobalt concentrations ranging from 1 to 7772 $\mu g/m^3$ (Swennen et al., 1993). The geometric mean exposure concentration was 125 $\mu g/m^3$. Exposure duration ranged from 0.3 to 39.4 years, with an average exposure of 8.0 years. A significantly higher number of exposed workers reported dyspnea than did controls. The increase occurred primarily among smokers although no significant interaction was found between smoking and exposure to cobalt. Based on a logistic regression model, the probability of dyspnea during exercise was significantly associated with increasing cobalt concentration in the air or urine. The parameters of the model were not reported. The clinical examinations detected significantly increased prevalence of skin disorders (eczema, erythema) (51 vs. 25%) and wheezing (16 vs. 6%) in the exposed group compared to controls. Lung function tests did not differ between the two groups; however, a few significant trends were noted: the FEV_1/VC (forced expiratory volume in one second/vital capacity) ratio decreased with increasing concentration of cobalt in the air and urine, and the RV (residual volume) and TLC (total lung

capacity) increased with increasing duration of exposure. No lung abnormalities were found by chest radiographs in either group. Blood analyses did not show polycythemia, and in fact, there were slight, but significant, decreases in red blood cell count, hemoglobin and hematocrit in the exposed workers. White blood cell counts were significantly increased. Serum levels of the thyroid hormone T3 (triiodothyronine) were slightly (7%), but significantly, decreased in the exposed group, while T4 (thyroxine) and TSH (thyrotropin) were not affected. Serum markers for cardiomyopathy (i.e., myocardial creatine kinase) were unchanged.

Prescott et al. (1992) conducted a cross-sectional study to investigate the effects of cobalt exposure on thyroid volume in female plate painters. The test group included 61 female plate painters exposed to cobalt blue dyes in two porcelain factories. The control group consisted of 48 unexposed women working at the same factories. The dyes used in the two factories differed; factory I (36 workers) used cobalt aluminate, which is insoluble, and factory II (25 workers) used cobalt-zinc silicate, which was reported to be "semi-soluble." Workers were exposed to cobalt during the painting procedure when the plates were spray-painted (under a fume hood) two or three times with the water-based cobalt blue underglaze and when the excess color was removed with a brush after drying. Cobalt concentrations were reported to be approximately 0.05 mg/m^3 in the workplaces (no further details on air levels were reported). The average duration of exposure was 14.6 years in group I workers and 16.2 years in group II workers. Subjects filled out a questionnaire regarding health, use of medicines, day of menstrual cycle, employment information and smoking habits and agreed to give blood and urine samples for determination of thyroid hormone levels (e.g. thyroxine (T4), triiodothyronine (T3), and thyroid stimulating hormone) and cobalt concentration, respectively, and to undergo ultrasonography to determine volume of the thyroid gland.

Urinary cobalt levels were similar in group I exposed workers and controls (Prescott et al., 1992). Group II workers exposed to semi-soluble cobalt-zinc silicate had urinary cobalt levels that were approximately 10-fold higher than controls. Group I workers did not differ from controls for any of the thyroid parameters measured; however, Group II workers had a significant 22% increase in serum T4 (thyroxine) levels. Mean thyroid volume was lower in this group as well, although the difference from controls (16.1 mL in group II vs. 19.2 mL in controls and 18.7 mL in group I) was not statistically significant. The occurrence of respiratory effects in these workers was not reported.

Results of three studies investigating cancer incidence in workers exposed to cobalt by the inhalation route (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983) are suggestive of a possible association between exposure to cobalt and respiratory tumors. Morgan (1983) investigated the health and causes of death of 49 men occupationally exposed to cobalt salts and oxides (specific species not identified) in a manufacturing plant in South Wales. During the study period, 33 men died (five with lung cancer and three with cancer at other sites). The expected number of deaths was 3.0 for lung cancer and 4.1 for cancers at other sites, based on national statistics, resulting in mortality ratios of 1.7 and 0.73, respectively (statistical analysis of data not reported).

Mur et al. (1987) analyzed the mortality of a cohort of 1143 workers in a plant that refined and processed cobalt and sodium. The plant workers may have been involved in multiple processing applications utilizing different forms of cobalt including cobalt chloride, oxides and

other salts (specific species not identified). An increase in deaths [Standard Mortality Ratio (SMR) = 4.66; 95% confidence interval (CI) = 1.46-10.64] resulting from lung cancer was observed in workers based on four cases observed in the exposed group and one case expected based on French national statistics. In a study within the cohort that controlled for age and smoking habits, 44% (four workers) in the exposed group and 17% (three workers) in the control group died of lung cancer. The authors indicated that the differences were not statistically significant and that the workers were exposed to arsenic and nickel in addition to cobalt. The exposure levels of cobalt were not reported.

Tuchsen et al. (1996) analyzed the cancer incidence of a cohort of 874 women who worked in one of two factories (382 from one factory, 492 from a second factory) applying a cobalt-based (cobalt-aluminate spinel) plate underglaze. From unexposed areas of factory I, 520 referents were selected. Both groups were compared to statistics for all Danish women in the same calendar year. During the 5-year follow-up period, the overall cancer incidence was only slightly elevated in exposed workers, while the incidence of lung cancers was significantly increased [Standard Incidence Ratio (SIR) = 2.35; 95% CI = 1.01-4.6]. The incidence of lung cancers in the referents (not exposed to cobalt) was greater than that of all Danish women, but the difference was not statistically significant. Exposure characterization prior to 1980 was not described, while exposures after 1980 were variable and reported as a mean concentration for a given year. Exposures were generally in the range of 0-1 mg cobalt/m^3 except for 2 years, during which they were greater.

Animal Studies

Overview

Studies in animals show that oral exposure to cobalt produces effects similar to those observed in humans, including increases in red blood cells and hemoglobin (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955; Stanley et al., 1947), thyroid effects (Shrivastava et al., 1996) and cardiac effects (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970). Other findings in animals not reported in humans include neurobehavioral changes (Singh and Junnarkar, 1991; Bourg et al., 1985; Krasovskii and Fridlyand, 1971) and testicular toxicity (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Developmental toxicity studies in rats and mice provide evidence that high oral doses of cobalt may produce developmental effects in animals, in some cases in the absence of overt maternal toxicity (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985).

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic inhalation exposure to cobalt resulted in cytotoxicity and reparative proliferation in all regions of the respiratory tract in rats and mice (NTP, 1991; Bucher et al., 1990). Available chronic animal studies have demonstrated the carcinogenic potential of inhaled cobalt in male and female rats and mice, with alveolar and bronchiolar tumors being the most prevalent (Bucher et al., 1999; NTP, 1998).

Oral Exposure

Studies in rats show that subchronic oral exposure to cobalt chloride increases red blood cell counts and hemoglobin levels with NOAELs ranging from 0.05 to 0.62 mg cobalt/kg-day (Krasovskii and Fridlyand, 1971; Stanley et al., 1947) and LOAELs ranging from 0.5 to 32 mg cobalt/kg-day (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955; Stanley et al., 1947). In general, effects in animal studies were observed at higher exposure levels than those reported in humans.

Effects of cobalt on red blood cells and hemoglobin were investigated in Sprague-Dawley rats treated with 2.5, 10, and 40 mg cobalt chloride hexahydrate/kg-day (equivalent to 0.62, 2.5, and 9.9 mg cobalt/kg-day, respectively) for 8 weeks (Stanley et al., 1947). After 8 weeks of exposure, increases in hemoglobin and red blood cell number were observed in the 2.5 and 9.9 mg cobalt/kg-day treatment groups. Statistical significance was not reported.

Hemoglobin and hematocrit were significantly increased in male Sprague-Dawley rats exposed to 500 ppm cobalt chloride in drinking water, equivalent to approximately 32 mg cobalt/kg-day (assuming a water intake of 0.139 L/kg-day for male Sprague-Dawley rats; U.S. EPA, 1988), for 3 months (Domingo et al., 1984). Compared to controls, hematocrit and hemoglobin were both increased by approximately 30% at the end of the 3-month exposure period, with increases observed within the first 2 weeks of exposure (numeric data not presented). Following the 3-month exposure period, histopathological examination showed no treatment-related morphological or ultrastructural changes to any organ. Increased tissue weights were observed for spleen, heart and lungs, and testicular weight was decreased compared to controls. Based on the results of this study, 32 mg cobalt/kg-day was identified as a subchronic LOAEL for increased hematocrit and hemoglobin and decreased testicular weight in rats.

In rats exposed to 40 mg cobalt chloride/kg-day (equivalent to 18 mg cobalt/kg-day) for 4 months, hemoglobin and red blood cell count were increased by 37 and 21%, respectively, compared to controls (Holly, 1955). Similar effects were observed following concomitant administration of 40 mg cobalt chloride/kg-day and 200 mg ferrous sulfate, with increases of 30% for hemoglobin and 32% for red blood cell count, compared to controls. Statistical significance was not reported.

Oral exposure of rats to 10 mg cobalt/kg-day (as cobalt chloride) for 5 months resulted in increases in hemoglobin, hematocrit and red blood cell count compared to untreated controls, with effects reaching a plateau after approximately 60 days of exposure (Murdock, 1959). Statistical significance was not reported. No changes were observed for mean corpuscular hemoglobin concentration and mean cell volume compared to untreated controls, indicating that stimulation of erythropoiesis by cobalt did not result in the production of abnormal red blood cells.

The effects of exposure to 0.05, 0.5, and 2.5 mg cobalt/kg-day (as cobalt chloride) for 7 months were examined in rats (Krasovskii and Fridlyand, 1971). Treatment with 0.5 and 2.5 mg cobalt/kg-day, but not 0.05 mg cobalt/kg-day, for 7 months increased red blood cells and hemoglobin. Stimulation of hematopoiesis was more pronounced in the 2.5 mg cobalt/kg-day

group than in the 0.5 mg cobalt/kg-day group, with polycythemia in the 0.5 mg cobalt/kg-day group described as mild and transient. Results of this study are difficult to evaluate since numeric data and statistical analyses were not reported.

Studies in animals have noted cardiac effects following cobalt (cobalt sulfate) exposure (Haga et al., 1996; Pehrsson et al., 1991; Mohiuddin et al., 1970), although at higher exposure levels than observed in human studies. The effect of cobalt on myocardial function was examined in rats exposed to 8.4 mg cobalt/kg-day for 16 or 24 weeks (Haga et al. 1996). After 24 weeks of exposure, decreased left ventricular systolic and diastolic function was observed. An increase in the ventricular weight to body weight ratio indicates that left ventricular hypertrophy is a contributory factor in cobalt-induced myocardial dysfunction although a mechanism was not identified. Significant effects on cardiac function were not observed following 16 weeks of exposure. In guinea pigs, exposure to 20 mg cobalt/kg-day as cobalt sulfate in the diet for 5 weeks resulted in decreased absolute and relative heart weights and a greater incidence of abnormal electrocardiograms compared to animals fed on diets not supplemented with cobalt (Mohiuddin et al., 1970). Cardiac arrhythmias, including bradycardia, and repolarization abnormalities, were observed in 65% of cobalt-treated animals compared to 5% of control animals. Cellular alterations, observed at the light and electron microscopic levels, in cardiac tissues included pericardial thickening and inflammation, myocardial degeneration and vacuolization, endocardial thickening and myofibrillar damage. In contrast, no effects on cardiac function were observed in male rats (12/group) exposed to protein-restricted diets containing 8.4 mg cobalt/kg-day for 8 weeks (Pehrsson et al., 1991). Treated rats showed a significant decrease in body weight but no differences in left ventricular function relative to animals treated with protein-restricted diets without added cobalt. Although the results from the Pehrsson et al. (1991) and Haga et al. (1996) rat studies conflict, it appears that oral cobalt-induced myocardial injury/dysfunction may have a significant time-dependence. Oral cobalt (as cobalt sulfate) at the same dose level (8.4 mg cobalt/kg-day) did not appear to alter cardiac structure or function following exposure for up to 16 weeks (Pehrsson et al., 1991; Haga et al., 1996). However, ventricular hypertrophy with a concomitant decrease in left ventricular systolic and diastolic function was observed in rats after 24 weeks of oral cobalt (Haga et al., 1996). Thus, based on the results of this study, 8.4 mg cobalt/kg-day represents a subchronic LOAEL for myocardial toxicity in rats; Based on the results of the Mohiuddin et al. (1970) study, a LOAEL of 20 mg cobalt/kg-day was identified for myocardial toxicity in guinea pigs.

Histopathological changes in the thyroid gland have been observed following exposure of female mice to 400 ppm cobalt chloride (~48 mg cobalt/kg-day, assuming an average water intake of 0.265 L/kg-day for female mice; U.S. EPA, 1988) in drinking water for 15 to 45 days (Shrivastava et al., 1996). The severity of effect increased with exposure duration. After 15 days of exposure, a reduction in thyroid epithelial cell height with degenerated nuclei and reduced amount of colloid with peripheral resorption vacuoles was observed, with more pronounced effects after 30 days of exposure. More significant degenerative changes were observed after 45 days of exposure, including necrotic epithelial cells, reduced connective tissue between follicles, lymphocytic infiltrate and larger amounts of colloid within the lumen. Based upon significant thyroid toxicity observed in this study, a LOAEL of 48 mg cobalt/kg-day was identified in mice.

Developmental effects of orally administered cobalt have been studied in rats, rabbits and mice (Szakmary et al., 2001; Pedigo and Vernon, 1993; Paternain et al., 1988; Seidenberg et al., 1986; Domingo et al., 1985; Elbetieha et al., 2008). Szakmary et al. (2001) evaluated the developmental effects of oral cobalt sulfate exposure in rats, mice and rabbits. Exposure of pregnant rats to 5.2-21.0 mg cobalt/kg-day (oral gavage) decreased perinatal growth and survival, retarded skeletal development and produced skeletal and urogenital malformations, with a LOAEL of 5.2 mg cobalt/kg-day. Maternal toxicity (increased relative liver, adrenal, spleen weights; increased BUN, serum creatinine) was only observed at the highest dose (21.0 mg cobalt/kg-day). Thus, embryotoxicity in rats was observed at exposure levels below the LOAEL for maternal toxicity. In pregnant mice exposed to 10.5 mg cobalt/kg-day, retarded skeletal development and malformations of the eye, kidney and skeleton were observed in the absence of maternal toxicity. In pregnant rabbits exposed to 4.2 mg cobalt/kg-day, 20% mortality was observed in dams. Fetal resorptions were observed in 30% of surviving dams. Results of the studies in rats and mice provide evidence that adverse developmental effects can occur in the absence of maternal toxicity, and that rabbits are more sensitive to oral cobalt.

Domingo et al. (1985) treated pregnant female rats (15 animals/group) with 5.4 to 21.8 mg cobalt/kg-day as cobalt chloride from gestation day 14 through lactation day 21. Offspring were examined for mortality, body weight, body and tail length and general signs of toxicity after 1, 4 and 21 days of nursing. In contrast to the study by Szakmary et al. (2001), results of the Domingo et al. (1985) study reported maternal toxicity at all doses that produced adverse developmental effects (specific maternal effects observed were not reported). Fetal effects at 5.4 mg cobalt/kg-day included stunted growth of the pups of both sexes, decreased body length and tail length in male offspring and decreased spleen and liver weight in female offspring. Effects at the 10.9 mg cobalt/kg-day dose included decreased body weight in female pups, while at 21.8 mg cobalt/kg-day, decreased number of living young and decreased survival were seen. Blood parameters (liver enzymes, bilirubin, total protein, uric acid, urea, creatinine, hemoglobin and hematocrit) in pups did not show any treatment-related changes. No signs of toxicity were observed in surviving pups in any of the cobalt exposure groups.

No significant effects on fetal growth or survival were found in rats exposed to 6.2 to 24.8 mg cobalt/kg-day as cobalt chloride (oral gavage) during gestation days 6-15 (Paternain et al., 1988). The incidence of stunted fetuses was higher in the animals treated with 12.4 or 24.8 mg cobalt/kg-day (0.3 stunted fetuses per litter in the 12.4 mg cobalt/kg-day group; 1.0 stunted fetuses per litter in the 24.8 mg cobalt/kg-day group) compared to the control group (0 stunted fetuses per litter); however, the differences were not statistically significant. No treatment-related effects were observed for the number of corpora lutea, total implants, resorptions, the number of dead and live fetuses or fetal size parameters. No gross external abnormalities, skeletal malformations or other signs of fetal toxicity were observed. Maternal effects, including reduced body weight gain and food consumption and altered hematological parameters (increased hematocrit, hemoglobin and reticulocytes), were reported at all exposure levels. No fetal effects were reported in mice exposed to 81.7 mg cobalt/kg-day (oral gavage) during gestation days 8-12 (Seidenberg et al., 1986), but a significant ($p<0.05$) decrease in maternal weight was found. Additional details were not reported.

Pedigo and Vernon (1993) exposed male B6C3F1 mice to 400 ppm cobalt chloride (~45 mg cobalt/kg-day, assuming a water intake of 0.247 L/kg-day for male B6C3F1 mice; U.S. EPA, 1988) in the drinking water for 10 weeks, after which the males were mated with control females to examine for dominant lethal effects. Relative to the control group, the cobalt treatment group had a lower percentage of pregnant females (control, 29/32; cobalt, 18/31), lower number of implantations per female (control, 8.3; cobalt, 6.5) and higher preimplantation losses (control, 0.43; cobalt, 2.4). At the end of the 10-week treatment period, sperm concentration was decreased to 15.3% and motility decreased to 18.3% of controls. Several measures of sperm velocity were also depressed relative to controls. All sperm parameters, except sperm concentration, returned to control levels 8 weeks after the cobalt exposure was terminated. The increase in preimplantation losses in the dominant lethal assay appears related to adverse effects on spermatogenesis rather than to effects on preimplantation development of embryos.

Several studies reported testicular degeneration and atrophy in rats exposed to 11.7 to 46.9 mg cobalt/kg-day as cobalt chloride for 2-3 months in the diet or in the drinking water (Anderson et al., 1992, 1993; Pedigo et al., 1988; Corrier et al., 1985; Mollenhauer et al., 1985; Domingo et al., 1984; Nation et al., 1983). Pedigo et al. (1988) exposed male CD-1 mice to 100, 200 or 400 ppm of cobalt chloride (~11.7, 23.4 or 46.9 mg cobalt/kg-day, respectively, assuming an average water intake of 0.258 L/kg-day for male mice; U.S. EPA, 1988) in the drinking water for 13 weeks. High-dose animals showed a significantly decreased testicular weight beginning at week 9 of treatment and a decreased epididymal sperm concentration by week 11 of treatment. All dose groups showed significantly decreased testicular weight and epididymal sperm concentration and increased serum testosterone levels by week 12 of exposure, with the magnitude increasing with dose. Effects on serum testosterone levels may be secondary to effects on spermatogenesis and related to inhibition of local inhibitory feed-back mechanisms. Based on the results of this study, 11.7 mg cobalt/kg-day was identified as a subchronic LOAEL for decreased testicular weight and epididymal sperm concentration in male rats.

Anderson et al. (1992, 1993) exposed groups of male CD-1 mice to 400 ppm of cobalt chloride (~46.9 mg cobalt/kg-day, assuming an average water intake of 0.258 L/kg-day for male mice; U.S. EPA, 1988) in the drinking water for up to 13 weeks. A decrease in testicular weight and a progressive degeneration of the seminiferous tubules were seen beginning at 9 weeks of exposure. Initial changes were vacuolization of Sertoli cells and abnormal spermatid nuclei, followed by sloughing of cells, shrinkage of tubules and thickened endothelium. No recovery was reported after a 20-week non-exposure recovery period. Co-administration of 800 ppm of zinc chloride provided a partial protection against the effects of cobalt. Based on the results of this study, 46.9 mg cobalt/kg-day was identified as a subchronic LOAEL for decreased testicular weight and degeneration of seminiferous tubules in male mice. Similar histology (degeneration of the testes, particularly the seminiferous tubules) was noted in Sprague-Dawley rats exposed to 20 mg cobalt/kg-day in the diet for up to 98 days (Corrier et al., 1985; Mollenhauer et al., 1985). Decreased testicular weight was seen in Sprague-Dawley rats exposed to 500 ppm cobalt chloride (~32 mg cobalt/kg-day, assuming a water intake of 0.139 L/kg-day for male Sprague-Dawley rats; U.S. EPA, 1988) for 3 months (Domingo et al., 1984).

Elbetieha et al. (2008) examined the potential effects of cobalt on male fertility in forty adult (60 day-old) male Swiss mice exposed to cobalt chloride hexahydrate via drinking water at concentrations of 200, 400, or 800 ppm for 12 weeks. Based on daily water intake reported in the study, daily average doses of cobalt chloride were estimated at 26, 47, or 93 mg/kg-day (equivalent to 6.5, 11.7, or 23 mg cobalt/kg-day); control animals received untreated tap water. Mice were observed daily for signs of clinical toxicity during the exposure period. At the end of the 12-week cobalt exposure period, male mice were separated into individual cages containing two virgin Swiss female mice and given *ad libitum* access to food and untreated tap water. Mice were cohabitated for 10 days during which it was estimated that the females completed two estrus cycles. Male control and cobalt-treated mice were necropsied after day 10 of cohabitation and testes, seminal vesicles, epididymides and preputial glands were harvested, weighed, and prepared for analysis. The left testis and epididymis from each male mouse was processed for determination of sperm count, while the right testis was processed for histopathology. Ten days later, female mice were necropsied and examined for number of pregnancies, number of implantation sites, number of viable fetuses, total number of resorptions, and incidence rate of resorptions.

Ingestion of cobalt chloride was associated with 1/10 and 2/10 deaths in the mid- and high-dose treatment groups, respectively, during week 10 of exposure. Average body weight gain was significantly reduced in all cobalt treatment groups ($p < 0.01$). No other signs of clinical toxicity were observed in surviving male mice. Relative to the control group, the number of pregnant females mated with male mice from the mid- and high-dose groups was significantly ($p < 0.05$) reduced (control, 19/20; mid-dose, 12/18; high-dose, 7/16). The number of implantation sites was significantly ($p < 0.01$) reduced in females mated with low- and mid-dose males (control, 7.89; low-dose, 5.67; mid-dose, 5.42), and the number of viable fetuses was significantly ($p < 0.05$) reduced in females mated with males from all cobalt treatment groups (control, 7.74; low-dose, 5.0; mid-dose, 4.67; high-dose, 5.83). In addition, the total number of resorptions (control, 3/150; low-dose, 9/81; mid-dose, 9/65; high-dose, 10/45) and the number of animals with resorptions (control, 3/19; low-dose, 10/15; mid-dose, 10/16; high-dose, 5/7) were significantly ($p < 0.05$) increased in females mated with males from all three cobalt-treatment groups. Analysis of male reproductive organs revealed a significant ($p < 0.005$) decrease in absolute epididymal weight in mice of the high-dose treatment group. Testes weights were significantly ($p < 0.01$) reduced in males at all doses of cobalt, and a significant ($p < 0.005$) increase in the absolute weight of seminal vesicles of the mid- and high-dose males only. Compared to controls, testicular sperm counts and daily sperm production were decreased in the mid- and high-dose males, but not in the low-dose animals. Epididymal sperm counts were decreased in male mice from all three cobalt treatment groups. Histopathological examination of testis tissue from males of the mid- and high-dose revealed a number of abnormalities including necrosis of the seminiferous tubules and interstitium, congested blood vessels, hypertrophy of the interstitial Leydig cells, and degeneration of the spermatogonial cells; incidence rate of these observations was not reported. These testicular histopathologies were not observed in the testes of control and low-dose treated males. Based on the results of this study, a LOAEL of 6.5 mg/kg-day was identified for decreased testicular weight, epididymal sperm counts, and associated reproductive abnormalities in pregnant females. A NOAEL was not identified.

Nation et al. (1983) exposed groups (n=6) of male Sprague-Dawley rats (weighing 200-210 g) to diets containing 0, 5 or 20 mg cobalt/kg-day as cobalt chloride for a total of 69 days. Following 14 days of exposure, animals were trained for scheduled (operant) or conditioned suppression neurobehavioral tests. Other than two seizures in the same high-dose animal, no overt signs of neurotoxicity were reported at any exposure level. A trend toward a decreased response rate in the schedule training behavior was observed in both the exposed groups but only attained statistical significance in the high-dose animals near the end of the operant testing period (sessions 28-35, on exposure days 44-51). A trend toward decreased conditioned suppression behavior did not attain statistical significance in either group. Animals exposed to 20 mg cobalt/kg-day, but not 5 mg cobalt/kg-day, showed a significantly decreased weight of the testes following 69 days of exposure. Based on the results of this study, a NOAEL of 5 mg cobalt/kg-day and a LOAEL of 20 mg cobalt/kg-day was identified for decreased testicular weight and changes in operant behavior in male Sprague-Dawley rats.

Several other studies have examined the effects of cobalt on neurobehavioral parameters (Singh and Junnarkar, 1991; Krasovskii and Fridlyand, 1971; Bourg et al., 1985). In groups of male Sprague-Dawley rats (n=8) exposed to 20 mg cobalt/kg-day as cobalt chloride for 57 days in the drinking water, cobalt enhanced behavioral reactivity to stress (the animals were less likely to descend from a safe platform to an electrified grid) (Bourg et al., 1985). Singh and Junnarkar (1991) reported a moderate reduction in spontaneous activity and mild hypothermia in rats exposed orally to cobalt chloride (approximately 8 mg cobalt/kg-day) or cobalt sulfate (approximately 35 mg cobalt/kg-day). Krasovskii and Fridlyand (1971) exposed groups of rats (number and sex not specified) to 0.05, 0.5 or 2.5 mg cobalt/kg-day as cobalt chloride for up to 7 months. Neurobehavioral tests showed that treatment with cobalt resulted in a significant (p<0.05) increase in the latent reflex period at 0.5 mg cobalt/kg and above, and a pronounced neurotropic effect (disturbed conditioned reflexes) at 2.5 mg cobalt/kg.

Inhalation Exposure

In a subchronic inhalation study, groups of 10 F344/N rats and 10 B6C3F1 mice of each sex were exposed to cobalt sulfate hexahydrate aerosol (MMAD=0.83-1.10 μm; σ_g not reported) at concentrations of 0, 0.3, 1, 3, 10 or 30 mg/m^3 (equivalent to 0, 0.067, 0.22, 0.67, 2.2 or 6.7 mg cobalt/m^3) 6 hours/day, 5 days/week for 13 weeks (Bucher et al., 1990; NTP, 1991). Although this report indicates that exposure was to cobalt sulfate heptahydrate aerosol, detailed analysis of the cobalt aerosol in the 2-year continuation study (Bucher et al., 1999; NTP, 1998) reports that the aerosol was actually composed of cobalt sulfate hexahydrate; thus, exposure to the hexahydrate form is assumed for the 13-week study. Animals were monitored for body weight and observed for clinical signs during the exposure period. Urine samples for urinalysis and cobalt determination were collected from rats prior to sacrifice. Following termination of exposure, all animals were sacrificed and necropsied. Blood samples were collected and analyzed for hematological parameters (rats and mice) and serum chemistry and thyroid function parameters (rats only). The major organs were weighed. Animals from the control and high-dose groups received comprehensive histopathological examinations, while those from the lower dose groups received more limited examinations focused on the respiratory tissues.

All rats survived until scheduled necropsy (NTP, 1991; Bucher et al., 1990). Gross evidence of toxicity was noted only in rats exposed to 6.7 mg cobalt/m^3, and they displayed clinical signs of toxicity (ruffled fur, hunched posture) and reduced body weights. Polycythemia, indicated by significant increases in red blood cell count, hemoglobin and hematocrit, was noted in males exposed to $\geq$0.67 mg cobalt/m^3 and females exposed to $\geq$2.2 mg cobalt/m^3. In addition, platelets were significantly reduced in rats of both sexes at $\geq$2.2 mg cobalt/m^3 and reticulocytes were increased in females at 6.7 mg cobalt/m^3. Leukocyte counts and differentials were unaffected. Serum cholesterol was significantly reduced in males at $\geq$2.2 mg cobalt/m^3 and females at 6.7 mg cobalt/m^3. No other serum chemistry parameters were affected, including creatine kinase isozymes indicative of damage to cardiac muscle cells. Among the thyroid hormones, T3 (triiodothyronine) was significantly reduced in females at 2.2 mg cobalt/m^3 (83% of control) and males at 6.7 mg cobalt/m^3 (62% of control) and TSH (thyrotropin) was significantly reduced in males at 6.7 mg cobalt/m^3 (30% of control), but T4 (thyroxine) was not affected in either sex at any dose and the researchers concluded that thyroid function was not consistently affected in this study. Urinalysis revealed a dose-related increase in the number of epithelial cells and granular casts in the urine of many exposed male rats (3-7 per group exposed to $\geq$0.67 mg cobalt/m^3) but not in the urine of control male rats. The researchers interpreted this finding as indicating minimal nephropathy in exposed male rats although histopathological lesions were not detected in the kidney. No effects on sperm counts, sperm motility or the incidence of abnormal sperm were noted. Average estrus cycle of females exposed to 6.7 mg cobalt/m^3 was slightly longer than controls, but the difference was not significant. Absolute and relative lung weights were significantly increased in both male and female rats at $\geq$0.22 mg cobalt/m^3. Other organ weights were not affected by treatment. Compound-related lesions were found only in the respiratory tissues of exposed rats. Degenerative, inflammatory and regenerative lesions were found throughout the respiratory tract (see Table 2). Incidence and severity of lesions were similar in males and females. The most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels.

Among mice, 2/10 males exposed to 6.7 mg cobalt/m^3 died during the study (NTP, 1991; Bucher et al., 1990). The only clinical signs of toxicity observed were rapid breathing and skin discoloration in one of the mice that died. Body weights were reduced throughout the study in both males and females exposed to 6.7 mg cobalt/m^3. No dose-related hematological effects were found. Absolute and relative lung weights were significantly increased in male and female mice exposed to $\geq$2.2 mg cobalt/m^3. Respiratory lesions were similar to those observed in rats. As with rats, the most sensitive tissue was the larynx, with squamous metaplasia present at all exposure levels. Reproductive system effects were more prominent in mice than rats. Males had significantly decreased testicular weight (48% compared to control), decreased epididymal weight (81% compared to control), testicular atrophy consisting of loss of germinal epithelium in the seminiferous tubules and foci of mineralization and an increased percentage of abnormal sperm at 6.7 mg cobalt/m^3 (295% compared to control). Significant reductions in sperm motility of 90, 87 and 54% were observed in the 0.67, 2.2 and 6.7 mg cobalt/m^3 exposure groups, respectively (lower doses were not tested). Females had a significantly increased length of the estrus cycle at 6.7 mg cobalt/m^3 (119% longer compared to control).

Table 2. Rats with Selected Lesions in the 13-Week Cobalt Sulfate Inhalation Study[a]

Site	Lesion	Exposure Group (mg Cobalt (Co) per m^3)					
		Control	0.067 mg Co/m^3	0.22 mg Co/m^3	0.67 mg Co/m^3	2.2 mg Co/m^3	6.7 mg Co/m^3
Larynx	Inflammation	M: 0 F: 1	M: 2 F: 2	M: 8 [c] F: 7 [c]	M: 9 [c] F: 10 [c]	M: 9 [c] F: 10 [c]	M: 9 [c] F: 10 [c]
	Squamous metaplasia	M: 0 F: 1	M: 9 [c] F: 7 [c]	M: 10 [c] F: 10 [c]	M: 10 [c] F: 10 [c]	M: 10 [c] F: 10 [c]	M: 10 [c] F: 10 [c]
Lung	Inflammation	M: 0 F: 0	M: 0 F: 0	M: 6 [b] F: 2	M: 10 [c] F: 9 [c]	M: 10 [c] F: 10 [c]	M: 10 [c] F: 10 [c]
	Fibrosis	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 1	M: 1 F: 4 [b]	M: 10 [c] F: 5 [b]
	Bronchiolar epithelium regeneration	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 7 [c] F: 5 [b]

M: number of males with lesions out of 50 animals.
F: number of females with lesions out of 50 animals.
[a]NTP, 1991; Bucher et al., 1990
[b]$p<0.05$ vs controls by Fisher exact test
[c]$p<0.01$ vs controls by fisher exact test

Other studies in animals have also reported respiratory lesions and altered respiratory function following inhalation exposure to cobalt. Kyono et al. (1992) observed mild pulmonary lesions in rats exposed to 2.12 mg/m^3 of cobalt aerosols (generated from an aqueous suspension of ultrafine metallic cobalt particles) 5 hours/day for 4 days. Lesions were characterized by focal hypertrophy of the epithelium, abnormal macrophages, vacuolization of type I epithelial cells and proliferation of type II epithelial cells, which are indicative of an initial inflammatory response. Kerfoot et al. (1975) exposed groups of five miniature swine to 0, 0.1 or 1.0 mg/m^3 of pure cobalt metal powder for 6 hours/day, 5 days/week for 3 months. Wheezing was observed in animals from both cobalt groups after 4 weeks of exposure (numeric data not reported). Tidal volume was decreased to 73% and 64% of controls in the low and high dose groups, respectively, and total respiratory compliance was decreased relative to controls (low dose, 66% of control; high dose, 56% of control). Statistical significance was not reported. Examination of lung tissue by electron microscopy revealed septa thickened by collagen, elastic tissue and fibroblasts in both exposure groups, with more pronounced effects in the high dose group. Johansson et al. (1987) exposed rabbits (8/group) to 0.4 or 2 mg cobalt/m^3 as cobalt chloride, 6 hours/day, 5 days/week for 14-16 weeks. Nodular accumulation of alveolar type II cells (8/8 rabbits in both cobalt groups), abnormal accumulation of enlarged, vaculolated alveolar macrophages (5/8 in the low dose group and 8/8 in the high dose group) and interstitial inflammation (4/8 rabbits in the low dose group and 8/8 rabbits in the high dose group) were observed, with more pronounced effects in the high dose group.

The carcinogenicity of inhaled cobalt was investigated in groups of 50 F344/N rats and 50 B6C3F1 mice of each sex exposed to cobalt sulfate hexahydrate aerosol (MMAD=1.4-1.6 μm; σ_g=2.1-2.2) at concentrations of 0, 0.3, 1 or 3 mg/m^3 (equivalent to 0,

0.067, 0.22 or 0.67 mg cobalt/m^3) 6 hours/day, 5 days/week for 105 weeks (Bucher et al., 1999; NTP, 1998). Animals were monitored for body weight and observed for clinical signs during the exposure period. Following termination of exposure, all animals were sacrificed and necropsied. At necropsy, all organs and tissues were examined for gross lesions, trimmed and examined histologically.

In F344 rats, there were no changes in survival or mean body weights in males or females of any exposure group (Bucher et al., 1999; NTP, 1998). Irregular breathing was noticed more frequently in female rats exposed to 0.67 mg cobalt/m^3 than in controls or other treatment groups; no changes in clinical signs were noted in any of the treated male rats. Incidence of selected neoplasms and nonneoplastic lesions of the lung in rats is summarized in Table 3. Both male and female rats in all exposure groups showed a high incidence (94% or greater) of squamous metaplasia of the alveolar epithelium, fibrosis of the pulmonary interstitium and granulomatous inflammation, with all lesions increasing in severity with increasing exposure level. Significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in high-dose male rats, while significant increases in alveolar/bronchiolar adenomas or carcinomas were seen in the mid- and high-dose female rats. The combined incidence of alveolar/bronchiolar neoplasms (adenoma and carcinoma) in male rats and female rats was significantly greater than that in control animals, and a significant linear trend occurred in both sexes. Rats of both sexes showed treatment-related increases in hyperplasia of the lateral nasal wall, atrophy of the olfactory epithelium and squamous metaplasia of the larynx. A significant increase in the incidence of pheochromocytoma in 0.67 mg cobalt/m^3 dosed females was also noted (2/48, 1/49, 4/50 and 10/50 in control, 0.067, 0.22 and 0.67 mg cobalt/m^3 groups, respectively). A marginally increased incidence of pheochromocytoma in males exposed to 0.22 mg cobalt/m^3, but not in those exposed to 0.67 mg cobalt/m^3, was considered by the study authors not to be related to treatment.

In B6C3F1 mice, no changes in survival were observed in any exposure group (Bucher et al., 1999; NTP, 1998). Male mice exposed to 0.67 mg cobalt/m^3 showed a decreased mean body weight relative to controls from week 96 through the end of the study (105 weeks). Mean body weights of exposed female mice were generally greater than those of controls throughout the study. Irregular breathing was noted slightly more frequently in female mice exposed to 0.22 mg cobalt/m^3 than in controls or other exposed groups. Incidence of selected neoplasms and nonneoplastic lesions of the lung in mice is summarized in Table 4. A dose-related increase in the occurrence of cytoplasmic vacuolization of the bronchus was seen in both sexes of mice, with incidences at all exposure levels being significantly different from controls. As in rats, both sexes of mice showed a significant linear trend toward increased alveolar/bronchiolar tumors, with the 0.67 mg cobalt/m^3 male and the 0.22- and 0.67 mg cobalt/m^3 female groups attaining statistical significance. Mice of both sexes showed significantly increased incidences of squamous metaplasia of the larynx ($p<0.05$) at all exposure levels examined. In male mice, but not in females, the incidence of hemangiosarcoma was significantly elevated in animals exposed to 0.22 mg cobalt/m^3, but not in other exposure groups (2/50, 4/50, 8/50 and 7/50 in the control, 0.067, 0.22 and 0.67 mg cobalt/m^3 groups, respectively).

Table 3. Incidence of Selected Neoplasms and Nonneoplastic Lesions in the Respiratory Tract of Rats in the 2-Year Inhalation Study of Cobalt Sulfate[a]

Site	Lesion Type	Exposure Group (mg Cobalt (Co) per m^3)			
		Control	0.067 mg Co/m^3	0.22 mg Co/m^3	0.67 mg Co/m^3
Lung	Alveolar epithelium hyperplasia	M: 9 F: 15	M: 20 [b] F: 7	M: 20 [b] F: 20	M: 23 [c] F: 33 [c]
	Alveolar epithelium metaplasia	M: 0 F: 2	M: 50 [c] F: 47 [c]	M: 48 [c] F: 50 [c]	M: 49 [c] F: 49 [c]
	Inflammation granulomatous	M: 2 F: 9	M: 50 [c] F: 47 [c]	M: 48 [c] F: 50 [c]	M: 50 [c] F: 49 [c]
	Alveolar/bronchiolar adenoma	M: 1 F: 0	M: 4 F: 1	M: 1 F: 10 [c]	M: 6 F: 9 [c]
	Alveolar/bronchiolar carcinoma	M: 0 F: 0	M: 0 F: 2	M: 3 F: 6 [b]	M: 1 F: 6 [b]
	A/B adenoma or carcinoma	M: 1 F: 0	M: 4 F: 3	M: 4 F: 15 [c]	M: 7 [b] F: 15 [c]
	Squamous cell carcinoma	M: 0 F: 0	M: 0 F: 0	M: 0 F: 1	M: 0 F: 1
Nose	Lateral wall hyperplasia	M: 2 F: 1	M: 14 [c] F: 8 [b]	M: 21 [c] F: 26 [c]	M: 21 [c] F: 38 [c]
	Olfactory epithelium atrophy	M: 8 F: 5	M: 24 [c] F: 29 [c]	M: 42 [c] F: 46 [c]	M: 48 [c] F: 47 [c]
Larynx	Squamous metaplasia	M: 0 F: 1	M: 10 [c] F: 22 [c]	M: 37 [c] F: 39 [c]	M: 50 [c] F: 48 [c]

M: Incidence of lesions in male rats out of 50 animals.
F: Incidence of lesions in female rats out of 50 animals.
[a] Bucher et al., 1999; NTP, 1998
[b] $p<0.05$ compared to control by logistic regression test
[c] $p<0.01$ compared to control by logistic regression test

Table 4. Incidence of Selected Neoplasms and Nonneoplastic Lesions in the Respiratory Tract of Mice in the 2-Year Inhalation Study of Cobalt Sulfate[a]

Site	Lesion Type	Exposure Group (mg cobalt (Co) per cubic meter)			
		Control	0.067 mg Co/m^3	0.22 mg Co/m^3	0.67 mg Co/m^3
Lung	Bronchus cytoplasmic vacuolization	M: 0 F: 0	M: 18 [c] F: 6 [b]	M: 34 [c] F: 31 [c]	M: 38 [c] F: 43 [c]
	Alveolar/bronchiolar adenoma	M: 9 F: 3	M: 12 F: 6	M: 13 F: 9	M: 18 [b] F: 10 [b]
	Alveolar/bronchiolar carcinoma	M: 4 F: 1	M: 12 F: 1	M: 13 F: 4	M: 18 [b] F: 9 [c]
	A/B adenoma or carcinoma	M: 11 F: 4	M: 14 F: 7	M: 19 F: 13 [c]	M: 28 [c] F: 18 [c]
Nose	Olfactory epithelium atrophy	M: 0 F: 0	M: 0 F: 2	M: 28 [c] F: 12 [c]	M: 48 [c] F: 46 [c]
	Hyperplasia	M: 0 F: 0	M: 0 F: 0	M: 0 F: 0	M: 10 [c] F: 30 [c]
Larynx	Squamous metaplasia	M: 0 F: 0	M: 37 [c] F: 45 [c]	M: 48 [c] F: 40 [c]	M: 44 [c] F: 50 [c]

M: Incidence of lesions in male mice out of 50 animals.
F: Incidence of lesions in female mice out of 50 animals.
[a] Bucher et al., 1999; NTP, 1998
[b] $p<0.05$ compared to control by logistic regression test
[c] $p<0.01$ compared to control by logistic regression test

Wehner et al. (1977, 1979) exposed 2-month-old male Syrian golden hamsters to inhaled cobalt oxide at 0 or 10 mg/m^3 (51 animals/group), 7 hours/day, 5 days/week for approximately 15 months. The incidence of tumors in treated hamsters was not statistically different from controls. There was "limited" histopathologic and ultrastructural examination in the study. No developmental toxicity studies were located following inhalation exposure to cobalt.

Other Studies

Parenteral Administration

Heath (1956) injected groups of 10 male and 20 female rats with a single intramuscular 28 mg dose of powdered cobalt in the thigh. Injection-site sarcomas appeared in 18 (60%) of the treated rats within 5-12 months. Similar results were observed in Wistar rats by Gilman (1962) and Gilman and Ruckerbauer (1962), with single intramuscular doses of 20 mg of cobalt oxide and cobalt sulfide. Cobalt oxide and cobalt sulfide given intramuscularly at doses twice those used in rats did not induce sarcomas in mice (Gilman and Ruckerbauer, 1962). Shabaan et al. (1977) observed a high incidence of fibrosarcomas in rats given subcutaneous injections of cobalt chloride at 40 mg/kg-day for 10 days. Tumors developed in 8-12 months. Stoner et al. (1976) tested cobalt acetate in the strain A mouse pulmonary tumor test. Groups of 20 mice/sex

received three times per week intraperitoneal injections for a total of 19 cumulative doses of 0, 95, 237 or 475 mg/kg. Survival was high over the 30-week observation period, and the incidence of lung tumors in treated mice was not statistically different from controls.

Genotoxicity Studies

The genetic toxicity of cobalt was reviewed by Beyersman and Hartwig (1992) and more recently by De Boeck et al. (2003b), Hartwig and Schwerdtle (2002) and Lison et al. (2001). Cobalt compounds have generally tested negative in bacterial mutagenicity assays, with occasional positive results occurring with the addition of an exogenous metabolic system. In contrast, cobalt compounds have generally tested positive in yeast and plant cells. In mammalian cell systems, cobalt has been shown to induce DNA strand breaks, sister-chromatid exchanges and morphological cell transformation.

Results of *in vitro* studies using human peripheral blood mononucleated cells show that cobalt metal and cobalt chloride induced DNA strand breaks at non-cytotoxic concentrations (De Boeck et al., 1998, 2003a). Evidence demonstrating mutagenic activity of cobalt *in vivo* in humans is lacking. No significant change in DNA strand breaks were observed in lymphocytes from nonsmoking workers who had been occupationally exposed to cobalt or hard metal dust although a positive association was observed between DNA strand breaks and smoking (De Boeck et al., 2000).

Experimental data in animals provide evidence of genotoxicity following *in vivo* exposure to cobalt. Single oral exposure of male Swiss mice to 0, 4.96, 9.92 or 19.8 mg cobalt/kg-day, as cobalt chloride, resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells, with significant linear trends toward increasing aberrations with increased exposure (Palit et al., 1991a,b,c,d). Thirty hours following single intraperitoneal injection of cobalt chloride at doses of 6.19, 12.4, or 22.3 mg cobalt/kg in BALB/c mice, an increase in micronucleus formation was seen in the mid- and high-dose mice but not in low-dose mice (Suzuki et al., 1993). Single injection of 12.4 mg cobalt/kg resulted in significantly increased micronucleus formation at 24 hours post-injection but not at 12, 48, 72 or 96 hours. Pedigo and Vernon (1993) reported that treatment with 400 ppm cobalt chloride (~45 mg cobalt/kg-day, assuming a water intake of 0.247 L/kg-day for male B6C3F1 mice; U.S. EPA, 1988) in the drinking water of male B6C3F1 mice for 10 weeks resulted in an increase in dominant lethal effects as indicated by changes in the number of pregnant females, percentage of live embryos and number of pre-implantation losses per female.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR COBALT

Indicators of human health effects following oral exposure to cobalt (Co) include increased erythrocyte production and hemoglobin levels, decreased iodine uptake by the thyroid gland, elicitation of dermatitis in sensitized individuals and cardiomyopathy. Observations in humans for effects on the heart, blood and the thyroid gland are supported by results of studies in animals. Other effects, including neurobehavioral, developmental and testicular toxicity were

observed in animals and at relatively high doses; these endpoints were not considered further for the development of the subchronic or chronic provional RfD (p-RfD).

Cardiomyopathy was considered as an endpoint of concern for cobalt exposure in humans; however, it is probable that alcohol consumed in "beer-cobalt cardiomyopathy," as well as other associated factors such as nutritional deficiency, played a role in the cardiotoxic effects observed. Therefore, a dose-response relationship could not be determined for cobalt exposure from these studies. Studies in animals have noted cardiac effects following cobalt exposure at higher exposure levels than observed in human studies of "beer-cobalt cardiomyopathy." On this basis, cardiomyopathy was not selected as the critical endpoint for p-RfD derivation.

Allergic response in cobalt-sensitized workers was considered as a potential critical endpoint for the derivation of an oral p-RfD. However, the available data provide no information on the dose-response relationship of cobalt sensitization, nor is a no observable adverse effect level (NOAEL) for the elicitation of an allergic response in humans defined. Interrelationships also exist between cobalt and nickel (Ni) sensitization so that people sensitized by (Ni) may have an allergic reaction following cobalt exposure. Allergic response was, therefore, not chosen as the critical effect for p-RfD derivation.

Cobalt has been shown to induce polycythemia which is characterized by an increase in erythrocyte number and hemoglobin levels through stimulation of erythropoietin, a hormone produced primarily in the kidney. The hematological effects of cobalt treatment have been reported in healthy, non-anemic adults (Davis and Fields, 1958) and in anephric anemic dialysis patients (Taylor et al., 1977; Duckham and Lee, 1976). However, the effects observed in healthy adults were reversible and erythrocyte counts returned to pre-treatment levels within 9 to 15 days after cobalt administration was discontinued. In anephric dialysis patients, treatment with cobalt resulted in an increase in hemoglobin from levels clinically described as "anemic" to levels at or near "normal." Thus, the effect of cobalt administration in these patients was clinically beneficial. Furthermore, the results of this study are difficult to interpret due to confounding factors, including the anephric status of patients and the concomitant administration of iron. Hematologic effects of cobalt were also found in several studies in rats (Domingo et al., 1984; Krasovskii and Fridlyand, 1971; Murdock, 1959; Holly, 1955, Stanley et al., 1947), supporting the plausibility for the effects observed in humans. However, the effects in animals were generally observed at higher doses than that used in the Davis and Fields (1958) human study. It is not known whether cobalt exposure in humans at higher dose levels would increase erythrocytes sufficiently above normal physiological levels to significantly increase the risk of cardiovascular effects. Therefore, polycythemia was not chosen as the critical effect for p-RfD derivation.

Effects of cobalt on thyroidal iodine uptake were identified as an endpoint of concern in humans, based on a preliminary report by Roche and Layrisse (1956). This report showed that oral exposure to cobalt (1 mg cobalt/kg-day) for 2 weeks markedly inhibited radioactive iodine uptake in the human thyroid. In a smaller human clinical study, reduced iodine uptake was reported in 2 of 4 euthyroid patients exposed to 0.54 mg cobalt/kg-day by the oral route for up to 14 days (Paley et al., 1958). A confounding factor in this study is that one of the two subjects reported to have reduced iodine uptake had received intravenous (i.v.) cobalt in addition to oral cobalt intake. The i.v. loading dose regimen may have raised the internal concentration of cobalt

to a level greater than the estimated 0.54 mg cobalt/kg-day based on oral intake alone, rendering the Paley et al. (1958) study inappropriate for consideration. Importantly, long-term cobalt exposure (up to 7 months) at 2-4 mg/kg-day in anemic children has been reported to cause goiter (Gross et al., 1954; Kriss et al., 1955; Little and Sunico, 1958). Therefore, while reduced iodine uptake is reported in humans following short-term exposures at low doses (Roche and Layrisse, 1956; Paley et al., 1958), potentially more severe thyroid lesions may occur as a function of increased duration or dose. Based on observations from rodent models of cobalt exposure, the severity of thyroid toxicity appears to be related to duration of exposure. Indeed, necrosis and inflammation of the thyroid has been reported in mice exposed to approximately 48 mg cobalt/kg-day with an increase in severity over a period of 15-45 days (Shrivastava et al., 1996).

Subchronic provisional RfD

Although cobalt exposure induces decreased radioactive iodine uptake in the thyroid (Roche and Layrisse, 1956), and polycythemia (Davis and Fields, 1958) in humans at similar daily exposure levels (1 mg/kg-day and 0.97 mg/kg-day, respectively), thyroid toxicity is chosen as the critical effect for derivation of provisional oral reference values. Cobalt-induced polycythemia and decreased iodine uptake by the thyroid were reversible following relatively short-term exposure in humans, however supporting studies indicate the potential for more severe thyroid effects (e.g., Kriss et al., 1955). The point of departure (POD) of 1 mg cobalt/kg-day for decreased iodine uptake in human thyroid is the LOAEL; dividing this POD by a composite uncertainty (UF) of 300 yields a **subchronic p-RfD of 3E-3 mg/kg-day** as follows:

Subchronic p-RfD = LOAEL ÷ UF
= 1 mg/kg-d ÷ 300
= **0.003 or 3E-3 mg/kg-day**

The composite UF of 300 is composed of three uncertainty factors: An UF of 10 for LOAEL to NOAEL extrapolation was applied because the POD is based on a LOAEL. An UF of 10 was applied due to the lack of data regarding inter-individual human variability or information on sensitive subpopulations. Specifically, because the critical study (Roche and Layrisse, 1956) for oral cobalt was based on healthy (euthyroid) adults, an UF of 10 was applied to protect sensitive human populations. The available database includes several short-term human studies, multiple developmental studies in animals and animal studies investigating hematological, cardiac, neurological, neurobehavioral, and thyroid endpoints. The lack of a multi-generation reproductive toxicity study is of particular concern because the database includes several animal studies indicating effects on sperm function and testicular degeneration which raises concerns that cobalt exposure may affect reproductive capability. Therefore, an UF of 3 was applied to account for lack of a multi-generation toxicity study.

Chronic provisional RfD

Using the same LOAEL of 1 mg/kg-day for decreased iodine uptake in humans, and an additional UF of 10 for extrapolating from subchronic to chronic duration (composite UF of 3000), a **chronic p-RfD of 3E-4 mg/kg-day** is derived as follows:

p-RfD = LOAEL ÷ UF
= 1 mg/kg-d ÷ 3000
= **0.0003 or 3E-4 mg/kg-day**

An UF of 10 for extrapolation from subchronic to chronic duration was applied because the critical effect was chosen from a principal study of a relatively short duration (2 weeks) of oral exposure in humans. The temporal relationship between cobalt-induced decreased radioactive iodine uptake and more severe thyroid toxicity should be considered carefully. One postulated temporal relationship is that chronic exposure may have no greater effect than that resulting from short-term exposure, because if the precursor event of inhibition of iodine uptake does not occur, then there may be no change in thyroid function in the short- or long-term. Prolonged cobalt exposure could have less of an effect because of the compensatory response of the pituitary-thyroid axis to iodine deficiency, via increasing iodine uptake. However, although plausible, there are no data to suggest that this postulated temporal relationship exist for cobalt-induced thyroid toxicity. Indeed, a limited number of clinical observations primarily in children exposed to oral cobalt at doses of 2-4 mg/kg-day for up to 7 months suggest the potential for more severe thyroid toxicity (e.g., Kriss et al., 1955). In addition, cobalt may not be readily eliminated from the body; for example, the biological half-life of cobalt chloride in rats is 25 hours (Rosenberg, 1993). Therefore, an UF of 10 for extrapolation from subchronic to chronic duration was applied.

Confidence in the principal study is low-to-medium. Roche and Layrisse (1956) examined twelve subjects over a two-week exposure period. Since only a single dose level was evaluated, a NOAEL for decreased iodine uptake was not identified. Other human and animal studies support the plausibility of cobalt producing thyroid toxicity (Paley et al., 1958; Prescott et al., 1992; Shirivistava et al., 1996). Confidence in the database is low-to-medium. Although some studies (Gross et al., 1954; Kriss et al., 1955; Little and Sunico, 1957) of longer duration reported increased severity of thyroid effects (e.g., goiter) in children exposed to cobalt at higher doses (2-4 mg cobalt/kg-day), critical details of these studies are unavailable for assessment. Therefore, a temporal relationship between prolonged oral cobalt exposure and increased severity of thyroid effects in humans (or experimental animals) is not clear, based upon available data. As such, a low confidence in the provisional subchronic and chronic RfDs results.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR COBALT

The human and animal database indicates that respiratory effects are sensitive endpoints of inhaled cobalt. Symptoms of respiratory tract irritation and altered pulmonary function have been widely reported in workers exposed to cobalt-containing airborne media. Of the four human epidemiology studies discussed above, the study by Nemery et al. (1992) provides the strongest basis for derivation of a provisional RfC (p-RfC). Workers in this study were exposed to lower air concentrations of metallic cobalt dust than in the studies by Gennart and Lauwerys (1990), Prescott et al. (1992) and Swennen et al. (1993). The values obtained from personal air samples from the Nemery et al. (1992) study, indicate a NOAEL of 5.3 $\mu g/m^3$ and a LOAEL of 15.1 $\mu g/m^3$. Furthermore, the Nemery et al. (1992) study demonstrated a dose-effect relationship

on lung function which correlated with urinary cobalt-levels, after adjusting for effects of smoking and gender.

Animal data support the conclusion that the respiratory tract is the critical target for inhaled cobalt (NTP, 1991; Bucher et al., 1990; Wehner et al., 1977). Subchronic and chronic inhalation exposure to cobalt resulted in inflammation, fibrosis, and bronchiolar regeneration in all regions of the respiratory tract in both rats and mice (NTP, 1991, 1998; Bucher et al., 1990, 1999) at doses higher than those identified in the Nemery et al. (1992) study. The NTP (1991) study further demonstrated that cobalt can produce testicular effects in male mice following inhalation exposure, but the effects were produced only at high dose levels. Oral studies have also identified the testes as a target for cobalt toxicity. Multi-generation reproduction studies following inhalation or oral exposure to cobalt are not available. Although developmental toxicity studies following inhalation exposure to cobalt are not available, oral studies provide evidence that high oral doses of cobalt may produce developmental effects in animals (Szakmary et al., 2001; Paternain et al., 1988; Domingo et al., 1985).

Decreased pulmonary function and respiratory tract irritation were identified as the co-critical effects for derivation of the subchronic and chronic p-RfCs. Assuming the personal air samples to be more representative of worker exposure than the area air samples, the study by Nemery et al. (1992) identified a NOAEL of 5.3 $\mu g/m^3$ and a LOAEL of 15.1 $\mu g/m^3$ for metallic cobalt for effects on pulmonary function (e.g. forced expiratory volume (FEV), forced vital capacity (FVC) and forced expiratory flow [referred to as MMEF]) and an increased prevalence of symptoms of respiratory tract irritation (e.g. nose/throat irritation, cough, phlegm, dyspnea). Although the LOAEL may be biased low due to inclusion of data from workshop #9, this does not affect the p-RfC derivation. A NOAEL/LOAEL approach is taken for the derivation of inhalation RfC values because the critical effect data are not amenable to benchmark dose modeling. For example, workers in the low cobalt exposure group experienced a slight but non-statistically significant increase in ventilatory function compared to controls, whereas a significant decrease in ventilatory function was observed in the high cobalt exposure group compared to both the control and the low cobalt exposure groups. The NOAEL for occupational exposure was adjusted to continuous exposure as follows:

$$5.3\ \mu g/m^3\ (10\ m^3/day\ /\ 20\ m^3/day)\ (5\ days\ /\ 7\ days) = 1.9\ \mu g/m^3$$

Using the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$ as the POD, the subchronic p-RfC and chronic p-RfC for cobalt was derived as shown below.

Subchronic p-RfC

Dividing the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$ by a composite UF of 100 yields a **subchronic p-RfC of 2E-5 mg/m^3** for metallic cobalt as follows:

$$\begin{aligned} \text{Subchronic p-RfC} &= NOAEL_{ADJ} \div UF \\ &= 1.9\ \mu g/m^3 \div 100 \\ &= \mathbf{0.00002\ or\ 2E\text{-}5\ mg/m^3} \end{aligned}$$

The composite UF of 100 is composed of two uncertainty factors: 10 for database insufficiencies and 10 for inter-individual variability. Nemery et al. (1992) did not report exposure duration for any worker in this study; an assumption is made that worker exposure was at least of subchronic duration. A factor of 10 was applied to account for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. A factor of 10 was applied to account for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This subchronic p-RfC may not be protective for people with hypersensitivity to cobalt.

Chronic p-RfC

Dividing the $NOAEL_{ADJ}$ of 1.9 $\mu g/m^3$ by a composite UF of 300 yields a **chronic p-RfC of 6E-6 mg/m^3** for metallic cobalt as follows:

$$\begin{aligned} \text{Chronic p-RfC} &= NOAEL_{ADJ} \div UF \\ &= 1.9\ \mu g/m^3 \div 300 \\ &= \mathbf{0.000006\ or\ 6E\text{-}6\ mg/m^3} \end{aligned}$$

The composite UF of 300 is composed of three uncertainty factors: 3 to account for extrapolating from an assumed subchronic exposure duration to a chronic exposure duration, 10 for database insufficiencies and 10 for human inter-individual variability. A factor of 3 is applied to account for extrapolating from an assumed subchronic to chronic exposure duration. Since Nemery et al. (1992) did not report duration for any worker in this study, it is possible that exposure duration may have been subchronic or longer for some workers. A factor of 10 is applied to account for database insufficiencies due to the lack of inhalation developmental toxicity studies and a multi-generation reproduction study. A factor of 10 is applied to account for human variability, including sensitive subgroups. Individuals with underlying respiratory diseases (asthma, chronic obstructive pulmonary disease) may be more sensitive to the respiratory effects of inhaled cobalt. This chronic p-RfC may not be protective for people with hypersensitivity to cobalt.

Confidence in the key study (Nemery et al., 1992) is low because this cross-sectional study:

- looked at only respiratory endpoints;
- included a control group that was studied more than 1 year after the exposed population;
- included a study group exposed to iron and diamond dust in addition to cobalt (and possibly to asbestos in the past);
- did not report duration of exposure; and
- encountered a number of procedural difficulties during its course (e.g., construction of control group).

Confidence in the database is medium. The choice of the critical endpoint is well supported by other studies in humans and animals. Subchronic exposure studies in rats and mice (NTP, 1991) found histopathological changes in the upper respiratory tract. Other studies in animals support these findings. Reproductive and developmental effects have not been adequately studied. Furthermore, oral studies reported large doses were required to produce reproductive or

developmental effects. It would be difficult to get a large enough internal dose via inhalation to produce these effects. For these reasons, there is medium-to-low confidence in the subchronic and chronic p-RfCs.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR COBALT

Weight-of-Evidence Descriptor

Under the 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a), cobalt sulfate (soluble) is described as "likely to be carcinogenic to humans by the inhalation route," based on both the limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals as shown by a statistically significant increased incidence of alveolar/bronchiolar tumors in both sexes of rats and mice, pheochromocytomas in female rats, and hemangiosarcomas in male mice (Bucher et al., 1999). While available studies in humans have suggested a possible association between exposure to cobalt and respiratory tumors in cobalt workers (Tuchsen et al., 1996; Mur et al., 1987; Morgan et al., 1983), limitations within these studies, including small numbers of subjects, inadequate exposure assessment and potential exposure to other chemicals make them inadequate for assessing the carcinogenic potential of cobalt. Studies for evaluation of the oral carcinogenic potential for cobalt were not located.

Mode-of-Action Discussion

The U.S. EPA (2005a) Guidelines for Carcinogen Risk Assessment defines mode of action as "a sequence of key events and processes, starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation." Examples of possible modes of carcinogenic action, in general, include mutagenic, mitogenic, anti-apoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immunologic suppression.

While the mode of action of cobalt-induced carcinogenicity has not been determined, data suggests a number of potential biological events that might be involved including non-mutagenic genotoxicity (e.g. clastogenicity). A recent review by Lison et al. (2001) of *in vitro* and *in vivo* experiments in animal models indicates that two different mechanisms of genotoxicity may contribute to the carcinogenic potential of cobalt compounds: DNA strand breakage and inhibition of DNA repair. DNA strand breaks have been reported at non-cytotoxic concentrations in human peripheral blood monocytes (De Boeck et al., 1998, 2003a). Furthermore, oral exposure of mice to cobalt chloride resulted in significantly increased percentages of both chromosomal breaks and chromosomal aberrations in bone marrow cells (Palit et al., 1991a,b,c,d). Mechanistic studies suggest cobalt-induced oxidative stress may be involved. Exposure to cobalt compounds increases indices of oxidative stress, including diminished levels of reduced glutathione, increased levels of oxidized glutathione, increased levels of oxygen radicals and increased free-radical-induced DNA damage (Kawanishi et al., 1994; Lewis et al., 1991; Kadiiska et al., 1989; Zhang et al., 1998; Moorehouse et al., 1985). To compound the potential DNA strand breaking effects of cobalt, it appears that cobalt may also inhibit the repair of such genetic damage. A review by Hartwig and Schwerdtle (2002)

concluded that cobalt may specifically target zinc finger structures in DNA repair proteins, interfering with base and nucleotide excision repair. Collectively, while data indicate that cobalt induces DNA damage and repair inhibition, there is weak evidence to suggest direct or indirect mutagenicity in bacterial or mammalian systems.

Potential for a Mutagenic Mode of Action

Key events

The precise mechanism of cobalt-induced carcinogenicity has not been fully determined. There is evidence that cobalt is capable of eliciting genotoxic effects. While evaluations for mutagenic effects in bacteria have generally yielded negative results, results in several mammalian cell systems have suggested that cobalt is genotoxic in mammalian cells. Limited data from *in vivo* animal studies show that cobalt induces genotoxic effects, including chromosomal breaks, chromosomal aberrations and micronucleus formation. The most likely mechanisms for the genotoxic effects of cobalt are DNA strand breakage and the inhibition of DNA repair.

Strength, consistency, specificity of association

Although the carcinogenic potential of inhaled cobalt has been demonstrated in rats and mice by increased incidence of alveolar/bronchiolar tumors (Bucher et al., 1999; NTP, 1998), direct evidence demonstrating that cobalt can induce mutagenic changes in cells of the respiratory tract is lacking. *In vivo* exposure to hard metal dust containing 6.3% cobalt, 84% tungsten and 5.4% carbon induced DNA strand breaks in rat type II epithelial lung cells (De Boeck et al., 2003c). Chromosome/genome mutations were observed within 12 hours of exposure to a single intratracheal instillation of 16.6 mg hard metal dust/kg body weight. Since the mutagenic potential of cobalt alone was not evaluated in this study, a causal relationship between type II epithelial cell mutations and cobalt exposure could not be established. Potential mutagenic changes in respiratory tract cells could also be mediated through activated oxygen species released by inflammatory cells (e.g., macrophages, polymorpohnuclear neutrophils), rather than directly by cobalt (Lison et al., 2001).

Dose-response concordance

A dose-response concordance has not been established between the development of bronchoalveolar tumors and mutagenesis following inhalation exposure to cobalt. Dose-response information on mutagenicity is available for acute oral and parenteral exposure to cobalt in mice (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). No carcinogenicity data are available for the oral or parenteral routes upon which to base a dose-response concordance. Furthermore, no data are available on the mutagenic potential of cobalt in respiratory tract cells following *in vitro* or *in vivo* exposure.

Temporal relationships

In vivo studies in animals show that acute oral and parenteral exposure to cobalt produces genotoxicity to bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Due to the lack of data on the mutagenic potential of cobalt in respiratory tract cells, the temporal relationship between potential mutagenic mechanisms and the development of bronchoalveolar tumors cannot be assessed. Development of lung tumors in animals exposed to cobalt occurred following chronic exposure (NTP, 1998).

Biological plausibility and coherence

In vivo mutagenicity studies in mice show that oral and intraperitoneal exposure to single doses of cobalt chloride induced mutagenic changes in bone marrow cells (Suzuki et al., 1993; Palit et al., 1991a,b,c,d). Although it has been hypothesized that the bronchoalveolar tumors are the result of genotoxicity (De Boeck et al., 2003b; Hartwig and Schwerdtle, 2002; Lison et al., 2001), no direct evidence is available linking cobalt-induced mutagenesis to the development of cancer. Carcinogenicity through an indirect mutagenic mode of action may be mediated by activated inflammatory cells (macrophages, polymorpohnuclear neutrophils) (Lison et al., 2001).

Other Potential Mode(s) of Action: Cytotoxicity and Cellular Regeneration

Subchronic and chronic inhalation studies (Bucher et al., 1990, 1999; NTP, 1991, 1998) in rodents provide some evidence that cobalt causes cell injury with subsequent reparative cell proliferation, which may be involved in the development of bronchoalveolar tumors. Following inhalation exposure to cobalt sulfate hexahydrate aerosol at concentrations of 0.3 to 30 mg/m^3 (equivalent to 0.067 to 6.7 mg cobalt/m^3) for 3 months, rats and mice developed several lesions indicative of cell damage and proliferation throughout the entire respiratory tract, including nasal epithelial degeneration and metaplasia, laryngeal inflammation and metaplasia, bronchiolar epithelial regeneration and ectasia, alveolar hyperplasia and lung fibrosis (NTP, 1991; Bucher et al., 1990). Squamous hyperplasia of the larynx was the most sensitive effect (LOAEL=0.067 mg cobalt/m^3). The results of the 2-year carcinogenesis study (Bucher et al., 1999; NTP, 1998) in rats and mice revealed a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas in the 0.67 mg cobalt/m^3 group, but not in the 0.067 and 0.22 mg cobalt/m^3 groups for male rats and mice. In female rats and mice, a statistically significant increase in combined alveolar/bronchiolar adenomas and carcinomas was observed in the 0.22 and 0.67 mg cobalt/m^3 groups, but not in the 0.067 mg cobalt/m^3 group. In this same study, granulomatous inflammation of the lung was observed at all exposure levels (0.067, 0.22 and 0.67 mg cobalt/m^3) in rats. Other markers of cell damage and proliferation, including hyperplasia, metaplasia and fibrosis, were observed in the 0.22 and 0.67 mg cobalt/m^3 exposure groups. Compared to rats, mice appeared to be less sensitive to cobalt-induced cytotoxic changes. Results of this study show that bronchoalveolar tumors develop at exposure levels that also produce cell damage and reparative proliferation, although cell damage and repair are also observed at lower exposure levels than tumorigenesis. These observations suggest the possibility that cell injury in the respiratory tract may have preceded the development of cancers although direct evidence for this assertion is lacking.

Although limited evidence of carcinogenicity in humans is available, results of several epidemioloic studies suggest a possible association between exposure to cobalt and respiratory tumors (Tuchsen et al., 1996; Mur et al., 1987; Morgan, 1983). Subchronic exposure studies in cobalt workers show an association between cobalt exposure and diminished pulmonary function (Nemery et al., 1992; Gennart and Lauwerys, 1990). Taken together, results of studies in rodents and humans suggest that inhaled cobalt may produce a cytotoxic response in the respiratory tract that may contribute to decreases in pulmonary function and the development of bronchoalveolar tumors.

Sustained cell proliferation, in response to cytotoxicity, can be a significant risk factor for cancer (Correa, 1996). Sustained cytotoxicity and regenerative cell proliferation may result in the perpetuation of mutations (spontaneous or directly or indirectly induced by the chemical), resulting in uncontrolled growth. It is also possible that continuous proliferation may increase the probability that damaged DNA will not be repaired. No data on cobalt are available to directly evaluate the relationship between cell damage and reparative proliferation and the development of bronchoalveolar tumors.

Conclusions Regarding Cancer Mode of Action

Limited evidence supports genotoxicity and cytotoxicity followed by cellular regeneration as potential modes of action for cobalt tumorigenicity. *In vitro* and *in vivo* studies provide evidence that cobalt is capable of eliciting genotoxic effects in mammalian cells; however, two key uncertainties remain:

(1) No direct evidence linking cobalt-induced mutagenesis to the development of cancer is available and (2) the mutagenic potential of cobalt in respiratory cells has not been evaluated.

Results of the 3-month and 2-year inhalation studies in rats and mice (Bucher et al., 1990, 1999; NTP, 1991, 1998) are also consistent with the hypothesis that cobalt acts through a mode of action involving cytotoxicity and cellular regeneration, based on the observations that these effects occur following subchronic exposure and bronchoalveolar tumors develop at exposure levels that produce cytotoxicity and reparative proliferation. These observations suggest the possibility that cell injury in the respiratory tract may have preceded the development of cancers although direct evidence for this assertion is lacking. No mode of action data are available to explain the statistically significant increases in the incidences of pheochromocytomas and hemangiosarcomas that were observed in female rats and male mice, respectively.

Because a mutagenic mode of action is plausible, but cannot be clearly established for carcinogenicity of inhaled cobalt, it is recommended that an age-dependent adjustment factor not be applied to the unit risk to account for possible age-dependence of carcinogenic potency as described in U.S. EPA (2005b).

Quantitative Estimates of Carcinogenic Risk

Oral Exposure

Human or animal studies examining the carcinogenicity of cobalt following oral exposure were not located. Therefore, derivation of an oral slope factor is precluded.

Inhalation Exposure

As available human inhalation studies were not sufficiently detailed, particularly with regards to analysis of exposure, the NTP (1998; Bucher et al., 1999) 2-year carcinogenicity study in rats and mice was chosen as the principal study for the derivation of an inhalation unit risk, based on the dose-response relationship for statistically significant increased incidences of alveolar/bronchiolar (A/B) neoplasms (adenoma and carcinoma). Although statistically significant increases in the incidences of pheochromocytomas and hemangiosarcomas were observed in female rats and male mice, respectively, these tumors were not considered for the derivation of the inhalation unit risk because a higher and more consistent response across species was observed for alveolar/bronchiolar tumors. The exposure concentrations in this study were adjusted to continuous exposure as follows:

$$Conc_{[ADJ]} = Conc \times \frac{5\ days/\ week}{7\ days/\ week} \times \frac{6\ hours/\ day}{24\ hours/\ day}$$

This adjustment resulted in duration-adjusted concentrations of 0, 0.012, 0.040 and 0.120 mg cobalt/m^3, respectively, for exposure to cobalt sulfate hexahydrate at 0.0, 0.3, 1.0 and 3.0 mg/m^3 exposure levels. Using the RDDR computer program, as specified in the RfC guidelines (U.S. EPA, 1994b), human equivalent concentrations (HECs, in mg cobalt/m^3) were calculated at each exposure level for each species and sex using body weight default values (U.S. EPA, 1994b), assuming exposure to particulates (MMAD=1.5 μm, σ_g=2.2) with effects occurring in the thoracic region of the respiratory tract. Table 5 shows the resulting HECs.

Table 5. Human Equivalent Concentrations (mg Cobalt/m^3) Corresponding to Exposure Concentrations in the NTP (1998; Bucher et al., 1999) Chronic Cancer Bioassay

Study	Male Rat	Female Rat	Male Mouse	Female Mouse
RDDR Multiplier	0.83	0.79	1.48	1.44
Control	0	0	0	0
Low	0.010	0.0095	0.018	0.017
Medium	0.033	0.032	0.059	0.058
High	0.10	0.095	0.18	0.17

All models for quantal data in the U.S. EPA Benchmark Dose (BMD) software (version 1.3.2) were fit to incidence for tumors (combined A/B adenomas and carcinomas), in rats and mice; males and females were modeled separately. All data sets modeled showed a statistical trend for increased tumor incidence with increasing exposure concentration. In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of 10% increase in extra risk was used as the basis for the BMD, with the BMDL represented by the 95% lower confidence limit on the BMD. Models were run using the default restrictions on parameters built into the BMD software. Table 6 shows the exposure concentration and incidence data that were modeled.

Table 6. Neoplasm Incidence Observed in the NTP (1998; Bucher et al., 1999) Chronic Cancer Bioassay

Animal/Strain/Site	Incidence of Neoplasms			
	Human Equivalent Concentration of Cobalt (mg/m^3)			
F-344 Rats (male)	**0**	**0.010**	**0.033**	**0.10**
Lung: A/B adenoma or carcinoma	1/50	4/50	4/48	7/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
F-344 Rats (female)	**0**	**0.0095**	**0.032**	**0.095**
Lung: A/B adenoma or carcinoma	0/50	3/49	15/50	15/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
B6C3F1 Mice (male)	**0**	**0.018**	**0.059**	**0.18**
Lung: A/B adenoma or carcinoma	11/50	14/50	19/50	28/50
	Human Equivalent Concentration of Cobalt (mg/m^3)			
B6C3F1 Mice (female)	**0**	**0.017**	**0.058**	**0.17**
Lung: A/B adenoma or carcinoma	4/50	7/50	13/50	18/50

Table 7 summarizes the BMD modeling results. BMDLs shown were derived from acceptable model fits (p>0.5). As is shown in Table 7, BMDLs were similar across study groups (range: 0.011-0.035 mg/m^3). Lung tumors in female rats were chosen as the endpoint for use as a point of departure for derivation of the inhalation unit risk. The BMDL for this endpoint was the lowest for all study groups (i.e., male and female rats and mice) and was based on a model that showed a good fit to the data (p=0.84), as reflected in the proximity of the BMDL to the BMD, after dropping the high exposure group. Dropping the high exposure group is recommended according to U.S. EPA (2000) procedure when no models achieve adequate fit using all exposure levels. Although this left only two exposure levels (in addition to the control), these exposure levels are in the low-dose portion of the curve within the region of the dose-response relationship in which response is increasing with exposure level (i.e., the region of interest for deriving the point of departure) and bracket the derived BMD. Appendix A presents the results from all model runs used to support this toxicity assessment.

Table 7. Summary of BMD Modeling Results for Cobalt Cancer Data

Tumor	Species	Sex	BMD (mg/m^3)	BMDL (mg/m^3)
Lung: A/B adenoma or carcinoma	rat	male	0.085	0.035
Lung: A/B adenoma or carcinoma	rat	female	0.014[a]	0.011[a]
Lung: A/B adenoma or carcinoma	mouse	male	0.026	0.015
Lung: A/B adenoma or carcinoma	mouse	female	0.038	0.023

[a] Based on control, low and middle exposure levels; high exposure level was dropped due to failure of models to achieve adequate fit using all exposure levels.

In the absence of mode of action data to inform the low dose extrapolation for cobalt, an inhalation cancer unit risk was calculated by linear extrapolation of the BMDL to zero exposure level (U.S. EPA, 2005a). The provisional **inhalation unit risk of 9 $(mg/m^3)^{-1}$** for cobalt sulfate (soluble) was calculated as follows:

$$\begin{aligned} \text{Provisional Unit Risk} &= \text{BMR / BMDL} \\ &= 0.1 / 0.011 \\ &= \mathbf{9\ (mg/m^3)^{-1}} \end{aligned}$$

Table 8 shows continuous life-time exposure concentrations that correspond with specified risk levels (i.e., $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$).

Table 8. Continuous Life-time Exposure Concentrations Corresponding to Specified Cancer Risk

Exposure Concentration at $1x10^{-4}$ Risk	$1.1x10^{-5}$ mg/m^3
Exposure Concentration at $1x10^{-5}$ risk	$1.1x10^{-6}$ mg/m^3
Exposure Concentration at $1x10^{-6}$ Risk	$1.1x10^{-7}$ mg/m^3

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APPENDIX A: SUMMARY OF BMD MODELING OF TUMOR INCIDENCE DATA IN MALE AND FEMALE RATS AND MICE (NTP, 1998; BUCHER ET AL., 1999)

Male rat – A/B adenoma or carcinoma:

All models show acceptable fit ($p > 0.1$)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.035 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.502	111.12	0.087	0.043
logistic	0.446	111.52	0.099	0.066
log logistic (slope ≥1)	0.510	111.07	0.085	**0.035**
2 degree polynomial (pos betas)	0.502	111.12	0.087	0.043
1 degree polynomial (pos betas)	0.502	111.12	0.087	0.043
probit	0.453	111.47	0.098	0.063
log probit (slope ≥1)	0.357	112.11	0.104	0.064
quantal linear	0.502	111.12	0.087	0.043
quantal quadratic	0.373	111.99	0.010	0.069
weibull (power ≥1)	0.502	111.12	0.087	1.043

Output from BMD v1.3.2 is shown below:

```
================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\PROJECTS\COBALT\BMDS\RAMALULOG.(D)
        Gnuplot Plotting File:  C:\PROJECTS\COBALT\BMDS\RAMALULOG.plt
                                          Fri Sep 09 11:46:38 2005
================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

  Dependent variable = INRM
  Independent variable = ECRM
  Slope parameter is restricted as slope ≥ 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to 1e-008
```

Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.02
intercept = 0.683504
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point or have been specified by the user
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.63
intercept	-0.63	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.0398603	0.0231667
intercept	0.272287	0.592931
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value
Full model	-52.8567			
Fitted model	-53.5353	1.35715	2	0.5073
Reduced model	-55.5862	5.45902	3	0.1411

AIC: 111.071

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0399	1.993	1	50	-0.7178
0.0100	0.0523	2.615	4	50	0.8797
0.0330	0.0797	3.827	4	48	0.09207
0.1000	0.1513	7.565	7	50	-0.2228

Chi-square = 1.35 DF = 2 p-value = 0.5099

Benchmark Dose Computation

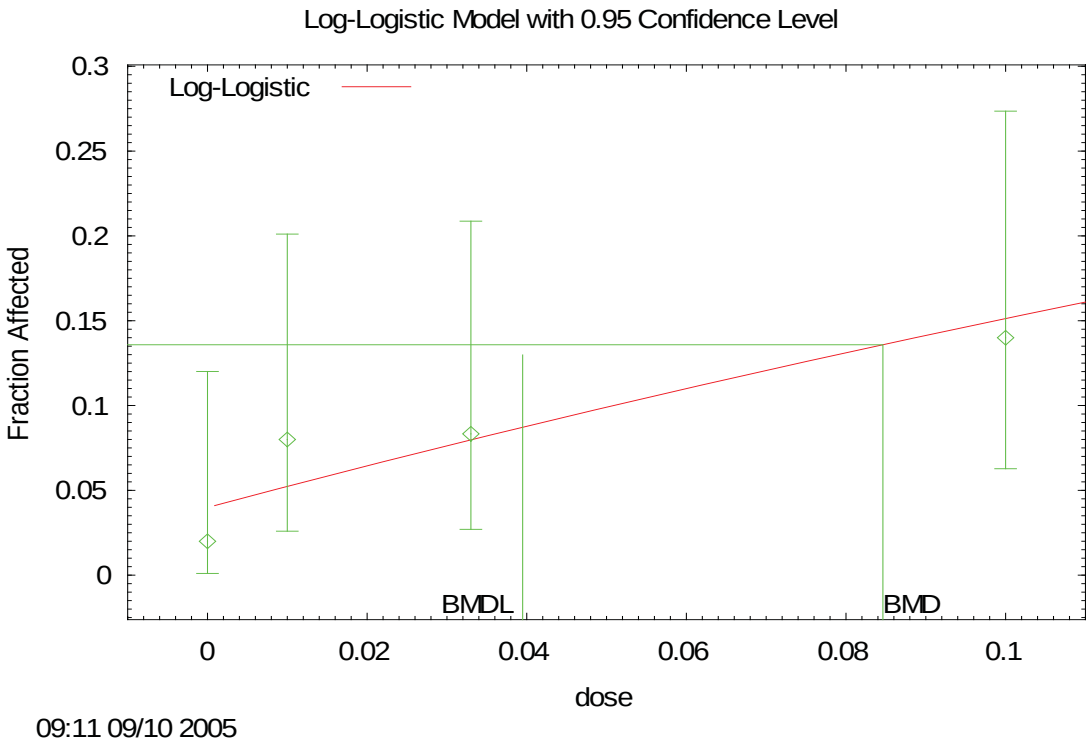
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0846262

BMDL = 0.0394914


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.3
0.25
0.2
0.15
0.1
0.05
0
BMDL
BMD
0
0.02
0.04
0.06
0.08
0.1
dose
09:11 09/10 2005

Female rat – A/B adenoma or carcinoma:

Most models showed poor fit ($p < 0.05$) with highest exposure level included (no increase in incidence at the highest exposure level.
The log-logistic model showed the best fit (*p*=0.11, lowest AIC)

Model	*p*	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.025	155.21	0.018	0.043
logistic	0.000	167.02	0.045	0.036
log logistic (slope ≥1)	0.090	152.86	0.015	**0.011**
2 degree polynomial (pos betas)	0.025	155.21	0.018	0.014
1 degree polynomial (pos betas)	0.025	155.21	0.018	0.014
probit	0.000	166.25	0.042	0.033
log probit (slope ≥1)	0.000	166.51	0.032	0.023
quantal linear	0.025	155.21	0.018	0.014
quantal quadratic	0.000	170.16	0.052	0.040
weibull (power ≥1)	0.025	155.21	0.018	0.014

Omitting the data from the highest exposure level improved fit of all models ($p > 0.1$)
Log-probit model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL=0.011 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	1.000	87.66	0.013	0.0077
logistic	0.242	89.69	0.020	0.0164
log logistic (slope ≥1)	1.000	87.66	0.013	0.0071
2 degree polynomial (pos betas)	0.710	87.66	0.014	0.0077
1 degree polynomial (pos betas)	1.000	86.40	0.011	0.0073
probit	0.289	89.31	0.019	0.0152
log probit (slope ≥1)	0.843	85.98	0.014	**0.0110**
quantal linear	0.710	86.40	0.011	0.0073
quantal quadratic	0.535	86.70	0.017	0.0139
weibull (power ≥1)	1.000	87.66	0.014	0.0077

Output from BMD v1.3.2 (all data included) is shown below:

```
==========================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.(D)
        Gnuplot Plotting File:
C:\\PROJECTS\COBALT\BMDS\RAFELU\RAFELULOGLOG.plt
```

Fri Sep 09 16:34:38 2005

==========

BMDS MODEL RUN

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The form of the probability function is

P[response] = background + (1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = INRF
Independent variable = ECRF
Slope parameter is restricted as slope $\geq$ 1

Total number of observations = 4
Total number of records with missing values = 0
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = 1.93572
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates
~~~~~~~~~~

Variable	Estimate	Std. Err.
background	0	NA
intercept	1.98253	0.20995
slope	1	NA

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-72.3723			
Fitted model	-75.4299	6.1152	3	0.1061
Reduced model	-89.3929	34.0413	3	<.0001

AIC: 152.86

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0000	0.000	0	50	0
0.0095	0.0645	3.162	3	49	-0.09415
0.0320	0.1885	9.427	15	50	2.015
0.0950	0.4082	20.411	15	50	-1.557

Chi-square = 6.49 DF = 3 p-value = 0.0900

Benchmark Dose Computation

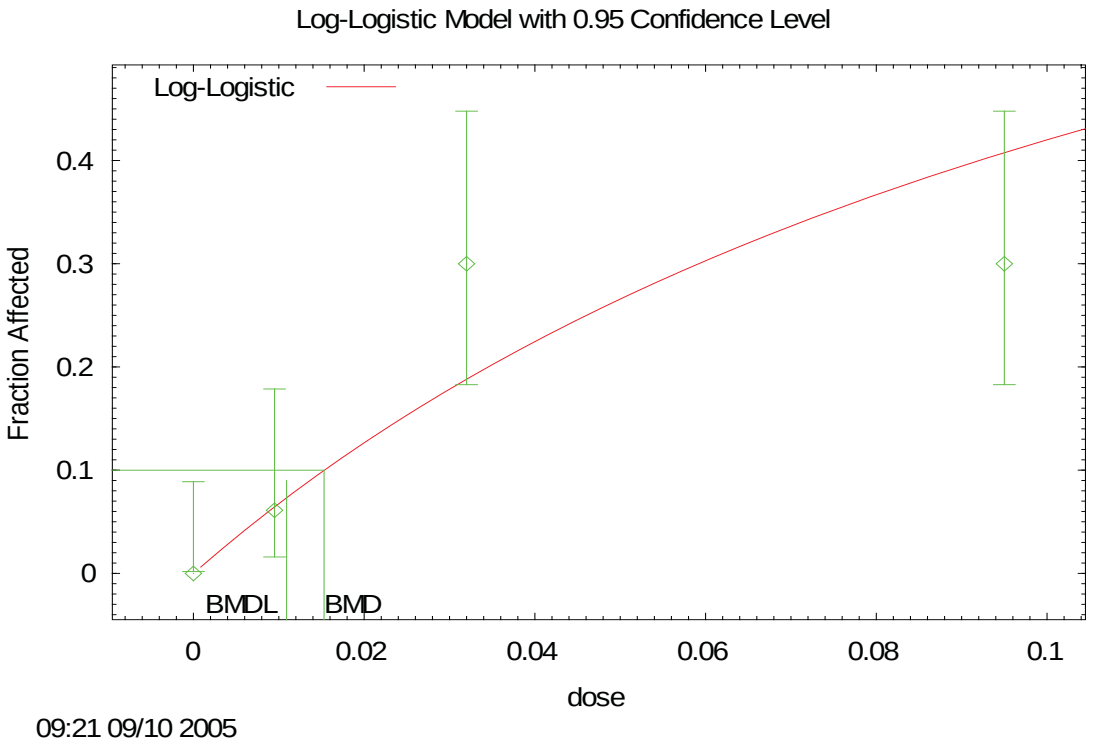
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0153022

BMDL = 0.0109172



Output from BMD v1.3.2 (highest exposure level excluded) is shown below:

```
 ================================================================
          Probit Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:53 $
          Input Data File:
C:\PROJECTS\COBALT\BMDS\RAFELUSE\RAFELUSEPROLOG.(D)
          Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\RAFELUSE\RAFELUSEPROLOG.plt
                                                Fri Sep 09 16:41:28 2005
 ================================================================

 BMDS MODEL RUN
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   The form of the probability function is:

   P[response] = Background
           + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = INRF
```

Independent variable = ECRF
Slope parameter is restricted as slope ≥ 1

Total number of observations = 4
Total number of records with missing values = 1
Maximum number of iterations = 250
Relative Function Convergence has been set to 1e-008
Parameter Convergence has been set to 1e-008

User has chosen the log transformed model

Default Initial (and Specified) Parameter Values
background = 0
intercept = 3.0285
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0	NA
intercept	2.97347	0.157916
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-41.8291			
Fitted model	-41.9887	0.319256	2	0.8525
Reduced model	-54.9105	26.1628	2	<.0001

AIC: 85.9774

Goodness of Fit

Dose	Est._Prob.	Expected	Scaled Observed	Size	Residual
0.0000	0.0000	0.000	0	50	0
0.0095	0.0462	2.263	3	49	0.5015
0.0320	0.3197	15.985	15	50	-0.2987

Chi-square = 0.34 DF = 2 p-value = 0.8434

Benchmark Dose Computation

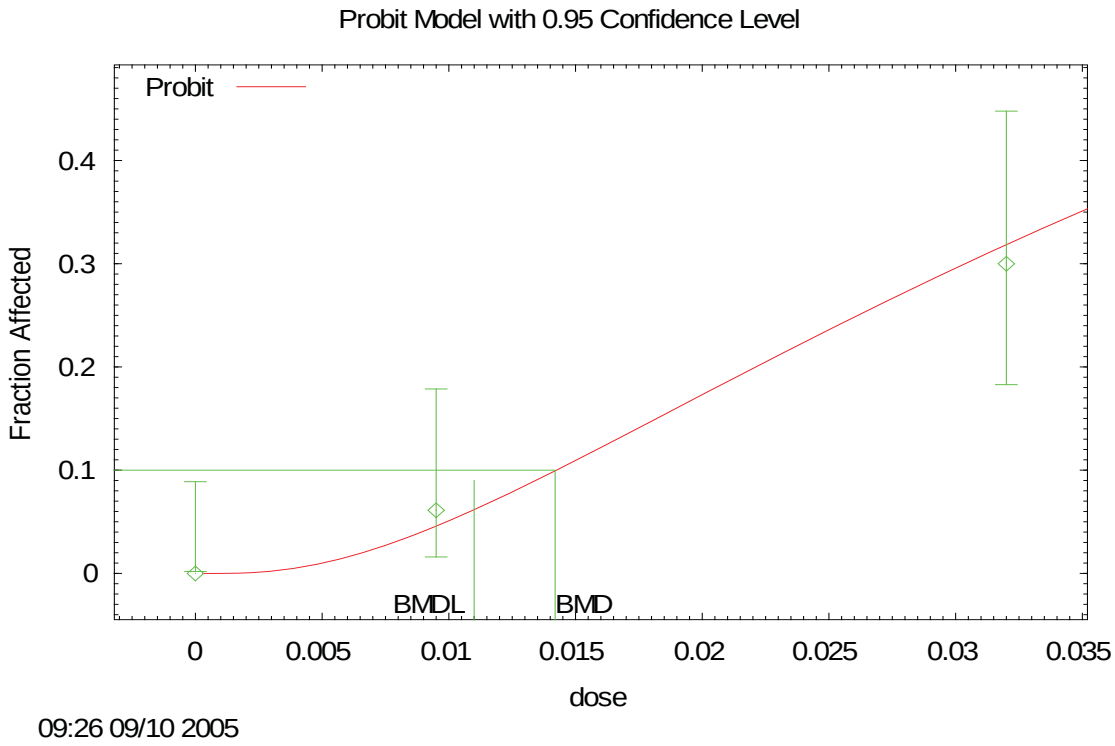
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0141927

BMDL = 0.0109984


Probit Model with 0.95 Confidence Level
Probit
Fraction Affected
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
0.005
0.01
0.015
0.02
0.025
0.03
0.035
dose
09:26 09/10 2005

Male mouse – A/B adenoma or carcinoma:

All models show acceptable fit (p > 0.1)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.015 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.944	251.10	0.033	0.0215
logistic	0.759	251.54	0.048	0.0359
log logistic (slope ≥1)	0.999	250.99	0.026	**0.0150**
2 degree polynomial (pos betas)	0.944	251.10	0.033	0.0215
1 degree polynomial (pos betas)	0.944	251.10	0.033	0.0215
probit	0.775	251.50	0.046	0.0349
log probit (slope ≥1)	0.594	252.03	0.059	0.0397
quantal linear	0.944	251.10	0.033	0.0215
quantal quadratic	0.412	252.76	0.080	0.0633
weibull (power ≥1)	0.944	251.10	0.033	0.0215

Output from BMD v1.3.2 is shown below:

```
================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\ROJECTS\COBALT\BMDS\MOMALU\MOMALULOGLOG.(D)
        Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOMALU\MOMALULOGLOG.plt
                                        Fri Sep 09 16:57:38 2005
================================================================

BMDS MODEL RUN
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  The form of the probability function is

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = INMM
  Independent variable = ECMM
  Slope parameter is restricted as slope ≥ 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to 1e-008
  Parameter Convergence has been set to 1e-008
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.22
intercept = 1.47367
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.62
intercept	-0.62	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.22179	0.0478621
intercept	1.45848	0.375385
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value
Full model	-123.493			
Fitted model	-123.494	0.00271986	2	0.9986
Reduced model	-130.684	14.3818	3	0.002429

AIC: 250.988

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.2218	11.090	11	50	-0.03047
0.0180	0.2777	13.884	14	50	0.03649
0.0590	0.3793	18.963	19	50	0.0109
0.1800	0.5613	28.065	28	50	-0.0185

Chi-square = 0.00 DF = 2 p-value = 0.9986

Benchmark Dose Computation

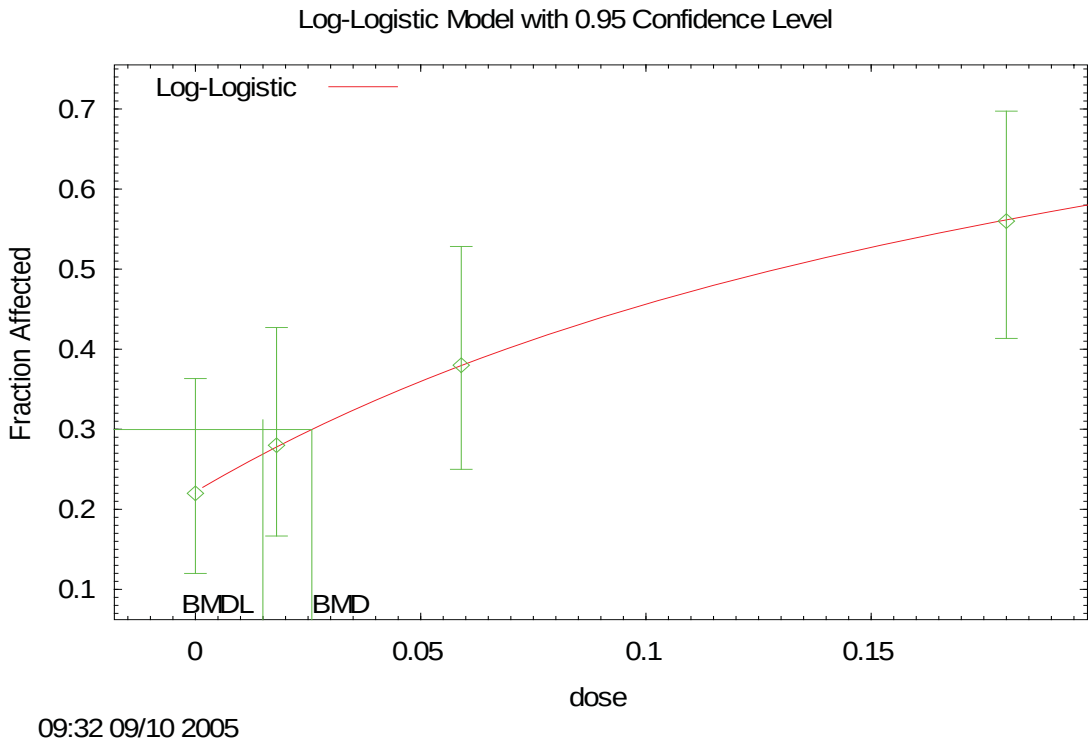
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0258434

BMDL = 0.0149697


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.7
0.6
0.5
0.4
0.3
0.2
0.1
BMDL
BMD
0
0.05
0.1
0.15
dose
09:32 09/10 2005

Male mouse – A/B adenoma or carcinoma:

All models show acceptable fit (p > 0.1)
Log-logistic model yielded best fit (highest p-value and lowest AIC)
Best estimate of BMDL = 0.023 mg/m^3

Model	p	AIC	BMD mg/m^3	BMDL mg/m^3
gamma (power ≥1)	0.571	196.12	0.0455	0.0296
logistic	0.273	197.61	0.0735	0.0562
log logistic (slope ≥1)	0.700	195.72	0.0384	**0.0231**
2 degree polynomial (pos betas)	0.571	196.12	0.0455	0.0296
1 degree polynomial (pos betas)	0.571	196.12	0.0455	0.0296
probit	0.300	197.42	0.0697	0.0528
log probit (slope ≥1)	0.167	198.57	0.0768	0.0524
quantal linear	0.571	196.12	0.0455	0.0296
quantal quadratic	0.117	199.26	0.0959	0.0739
weibull (power ≥1)	0.571	196.12	0.0455	0.0296

Output from BMD v1.3.2 is shown below:

```
================================================================
        Logistic Model $Revision: 2.1 $ $Date: 2000/02/26 03:38:20 $
        Input Data File: C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.(D)
        Gnuplot Plotting File:
C:\PROJECTS\COBALT\BMDS\MOFELU\MOFELULOGLOG.plt
                                        Fri Sep 09 17:05:08 2005
================================================================

BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is

  P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


  Dependent variable = INMF
  Independent variable = ECMF
  Slope parameter is restricted as slope ≥ 1

  Total number of observations = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to 1e-008
  Parameter Convergence has been set to 1e-008
```

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.08
intercept = 1.17812
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.6
intercept	-0.6	1

Parameter Estimates

Variable	Estimate	Std. Err.
background	0.0920048	0.035283
intercept	1.06119	0.354864
slope	1	NA

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	p-value
Full model	-95.5104			
Fitted model	-95.8619	0.702985	2	0.7036
Reduced model	-102.791	14.5619	3	0.002232

AIC: 195.724

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Scaled Size	Residual
0.0000	0.0920	4.600	4	50	-0.2937
0.0170	0.1345	6.726	7	50	0.1135
0.0580	0.2223	11.117	13	50	0.6403
0.1700	0.3911	19.556	18	50	-0.451

Chi-square = 0.71 DF = 2 p-value = 0.7003

Benchmark Dose Computation

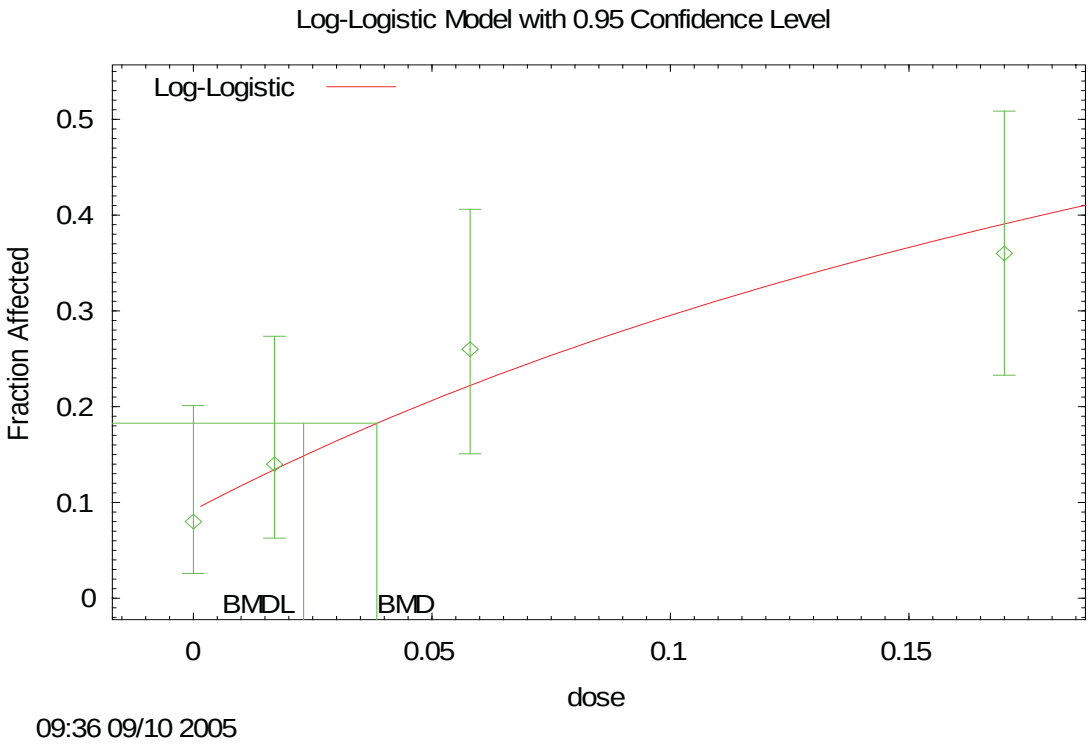
Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0384492

BMDL = 0.0231


Log-Logistic Model with 0.95 Confidence Level
Log-Logistic
Fraction Affected
0.5
0.4
0.3
0.2
0.1
0
BMDL
BMD
0
0.05
0.1
0.15
dose
09:36 09/10 2005

Attachment

1I

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Nickel, soluble salts; CASRN Various

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Nickel, soluble salts

File First On-Line 09/30/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	12/01/1991
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	not evaluated; message	08/01/1994

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Nickel, soluble salts
CASRN — Various
Last Revised — 12/01/1991

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Decreased body and organ weights **Rat Chronic Oral Study** **Ambrose et al., 1976**	NOAEL: 100 ppm diet (5 mg/kg/day) LOAEL: 1000 ppm diet (50 mg/kg/day)	300	1	2E-2 mg/kg/day

*Conversion Factors -- 1 ppm = 0.05 mg/kg/day (assumed rat consumption)

I.A.2. Principal and Supporting Studies (Oral RfD)

Ambrose, A.M., D.S. Larson, J.R. Borzelleca and G.R. Hennigar, Jr. 1976. Long-term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181-187.

Ambrose et al. (1976) reported the results of a 2-year feeding study using rats given 0, 100, 1000 or 2500 ppm nickel (estimated as 0, 5, 50 and 125 mg Ni/kg bw) in the diet. Body weights in the high-dose male and female rats were significantly decreased compared with controls. Body weight was also reduced at 1000 ppm. This reduction was significant for females at week 6 and from weeks 26 through 104, whereas males showed body weight reduction only at 52 weeks. Groups of female rats on the 1000 or 2500 ppm nickel diets (50 and 125 mg Ni/kg bw) had significantly higher heart-to-body weight ratios and lower liver-to-body weight ratios than controls. No significant effects were reported at 100 ppm (5 mg Ni/kg bw). The dose of 1000 ppm (50 mg Ni/kg bw) represents a LOAEL for this study, while the dose of 100 ppm (5 mg Ni/kg bw) is a NOAEL. In this study, 2-year survival was poor, particularly in control rats of both sexes (death: 44/50), raising some concern about the interpretation of the results of this study. A subchronic study conducted by American Biogenics Corp. (ABC, 1986) also found 5 mg/kg/day to be a NOAEL, which supports the Ambrose et al. (1976) chronic NOAEL of 5 mg/kg/day.

Dietary exposure of dogs to 2500 ppm Ni (about 63 mg/kg/day) resulted in depressed body weight gain; no effects were seen at either 100 ppm (about 2.5 mg/kg/day) or 1000 ppm Ni (about 25 mg/kg/day) in the diet (Ambrose et al., 1976). This study demonstrates that rats are the more sensitive of the two species.

ABC (1986) conducted the 90-day study with nickel chloride in water (0, 5, 35 and 100 mg/kg/day) administered by gavage to both male and female CD rats (30 animals/sex/group). The data generated in this study included clinical pathology, ophthalmological evaluations, serum biochemistry, body and organ weight changes and histopathological evaluations of selected organs (heart, kidney, liver).

The body weight and food consumption values were consistently lower than those of controls for the 35 and 100 mg/kg/day dosed males. Female rats in both high-dose groups had lower body weights than controls, but food consumption was unaffected by the test article. Clinical signs of toxicity, such as lethargy, ataxia, irregular breathing, cool body temperature, salivation and discolored extremities, were seen primarily in the 100 mg/kg/day group; these signs were less severe in the 35 mg/kg/day group. The 5 mg/kg/day group did not show any significant clinical signs of toxicity. There was 100% mortality in the high-dose group; 6/30 males and 8/30 females died in the mid-dose group (35 mg/kg/day). Histopathologic evaluation indicated that deaths of 3/6 males and 5/8 females in the mid-dose group were due to gavage errors. At sacrifice, kidney, liver and spleen weights for 35 mg/kg/day treated males and right kidney weights for 35 mg/kg/day treated females were significantly lower than controls. Based on the results obtained in this study, the 5 mg/kg/day nickel dose was a NOAEL, whereas 35 mg/kg/day was a LOAEL for decreased body and organ weights.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 10 is used for interspecies extrapolation and 10 to protect sensitive populations. An additional uncertainty factor of 3 is used to account for inadequacies in the reproductive studies (RTI, 1987; Ambrose et al., 1976; Smith et al., 1990) (see Additional Comments section). During the gestation and postnatal development of F1b litters in the RTI (1987) study, temperatures were about 10 degrees F higher than normal at certain times, which makes evaluation of this part of the reproductive study impossible. In the Ambrose et al. (1976) study, statistical design limitations included small sample size and use of pups rather than litters as the unit for comparison. There were also problems with the statistical analysis of the Smith et al. (1990) study.

The Ni dietary study by Ambrose et al. (1976) identifying a NOAEL of 100 ppm (5 mg/kg/day) is supported by the subchronic gavage study in water (ABC, 1986), which indicated the same NOAEL (5 mg/kg/day).

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

In addition to the effects on organ weights described in the critical study, two other sensitive endpoints exist: neonatal mortality and dermatotoxicity. While no reproductive effects have been associated with nickel exposure to humans, several studies in laboratory animals have demonstrated fetotoxicity. These studies are described below.

Following the reproductive studies is a discussion of nickel-induced dermatotoxicity in hypersensitive humans. While nickel has long been recognized as a contact irritant, many studies have also demonstrated dermal effects in sensitive humans resulting from ingested nickel. The weight-of- evidence from these studies indicates that ingested nickel may invoke an eruption or worsening of eczema; however, a dose-response relationship is difficult to establish. A few representative studies and review articles are cited below.

While the systemic toxicity data (as manifested in organ weight changes) was used as the critical study for the RfD determination, the reproductive/fetotoxicity and the dermatotoxicity were both considered as possible endpoints upon which to base the quantitative risk assessment of nickel. The data for effects on the latter two endpoints do not demonstrate consistent dose-response relationships, and in both cases the available studies are sufficiently flawed so as to prevent their selection as the basis for the oral RfD. It is noted, however, that the RfD based on the Ambrose et al. (1976) study is considered to be protective of all endpoints with the possible exception of hypersensitive individuals as described below.

In addition to the 2-year feeding study used as the basis for the RfD, Ambrose et al. (1976) also reported reproductive toxicity of nickel. The study had some statistical design limitations including small sample size and use of pups rather than litters as the unit for comparison. Furthermore, the results were equivocal and did not clearly define a NOAEL or LOAEL. Because nickel was administered in a laboratory chow diet rather than drinking water, quantifying analogous nickel exposure via drinking water was problematic.

In a 2-generation study (RTI, 1987) nickel chloride was administered in drinking water to male and female CD rats (30/sex/dose) at dose levels of 0, 50, 250 and 500 ppm (0, 7.3, 30.8 and 51.6 mg/kg/day, estimated) for 90 days before breeding (10 rats/sex/group comprised a satellite subchronic nonbreeder group). At the 500 ppm dose level there was a significant decrease in the Po maternal body weight, along with absolute and relative liver weights. Thus, 250 ppm (30.8 mg/kg/day) was a NOAEL for Po breeders. Histopathology was performed for liver, kidney, lungs, heart, pituitary, adrenals and reproductive organs to make this assessment. This NOAEL is

higher than the NOAEL derived from the chronic Ambrose et al. (1976) and subchronic gavage (ABC, 1986) assays.

In the RTI (1987) F1a generation (postnatal days 1-4) at the 500 ppm dose level the number of live pups/litter was significantly decreased, pup mortality was significantly increased, and average pup body weight was significantly decreased in comparison with controls. Similar effects were seen with F1b litters of Po dams exposed to 500 ppm nickel. In the 50 and 250 ppm dose groups increased pup mortality and decreased live litter size was observed in the F1b litters. However, these effects seen with F1b litters are questionable because the room temperature tended to be 10 degrees F higher than normal at certain times (gestation-postnatal days) along with much lower levels of humidity. As evidenced in the literature, temperatures that are 10 degrees F above normal during fetal development cause adverse effects (Edwards, 1986). Therefore, the above results seen at 50 and 250 ppm cannot be considered to be genuine adverse effects.

F1b males and females of the RTI (1987) study were randomly mated on postnatal day 70 and their offspring (F2a and F2b) were evaluated through postnatal day 21. This phase included teratological evaluations of F2b fetuses. Evaluation of the data indicated that the 500 ppm dose caused significant body weight depression of both mothers and pups, and increased neonatal mortality during the postnatal development period. The intermediate dose, 250 ppm nickel, produced transient depression of maternal weight gain and water intake during gestation of the F2b litters. The 50 ppm nickel exposure caused a significant increase in short ribs (11%). However, since this effect was not seen in both the higher dose groups, the reported incidence of short ribs in the 50 ppm group is not considered to be biologically significant.

Schroeder and Mitchener (1971) conducted a 3-generation study in which 5 mating pairs of rats were provided drinking water containing 5 mg Ni/L (estimated as 0.43 mg/kg bw). Results of this study indicated significant increases in neonatal mortality and in the number of runts born to exposed rats compared with controls. The major weakness of this study, however, is that the end result is based on a total of five matings. The matings were not randomized and the males were not rotated. The Schroeder and Mitchener (1971) study was conducted in an environmentally controlled facility where rats had access to food and water containing minimal levels of essential trace metals. Because of the interactions of nickel with other trace metals, the restricted exposure to trace metals (chromium was estimated as inadequate) may have contributed to the toxicity of nickel.

Smith et al. (1990) also studied the reproductive and fetotoxic effects of nickel. Four groups of 34 female Long-Evans rats were given drinking water containing nickel chloride in the following concentrations of nickel: 0, 10, 50 or 250 ppm (0, 1.3, 6.8 or 31.6 mg/kg/day) for 11 weeks prior to mating and during two successive gestation periods (G1, G2) and lactation periods (L1, L2).

Maternal body weight gain was reduced during G1 in mid- and high-dose females. The reproductive performance of the exposed rats was not affected. Pup birth weight was unaltered by treatment, and weight gain was reduced only in male pups exposed to 50 ppm nickel during L1. The most significant toxicological finding was the increased incidence of perinatal mortality. The proportion of dead pups per litter was elevated at the high dose in L1 and at 10 and 250 ppm in L2. While the perinatal mortality reported in this study is consistent with other reproductive studies on nickel, it is hard to define a NOAEL and LOAEL because of the absence of a clear dose-response trend at the lower doses.

Many studies have been published regarding nickel sensitivity in humans. Of the general population, approximately 8-10% of women and 1-2% of men demonstrate a sensitivity to nickel as determined by a patch test (North American Contact Dermatitis Group, 1973; Prystowsky et al., 1979). Initial sensitization to nickel is believed to result from dermal contact, but recurring flares of eczema, particularly of the hands, may be triggered by ingestion.

The human studies described below are difficult to interpret for several reasons: very small numbers of subjects (mostly women already determined to be sensitive to nickel by a patch test) were used in the studies; many investigators reported a placebo effect; many studies were not conducted in a double-blind manner, thereby introducing investigator bias; and it was often not specified whether subjects had been fasted overnight or whether there were other dietary restrictions. It is important to note that the way in which nickel is consumed may greatly affect its bioavailability. Sunderman et al. (1989) demonstrated that 27+/-17% of the nickel in drinking water was absorbed by healthy humans whereas only 0.7+/-0.4% of the same dose of nickel ingested in food was absorbed (a 40-fold difference). One final point to bear in mind in interpreting these studies is that the subjects were generally given a bolus dose of nickel. The absorption and biokinetics following such an exposure may be quite different from an exposure which is given incrementally throughout the day.

Following an overnight fast, groups of 5 nickel-sensitive women were given 100 mL of water along with one oral dose of nickel sulfate containing 0.6, 1.25 or 2.5 mg nickel (Cronin et al., 1980). The clinical response was observed for the next 24 hours. Worsening of hand eczema was reported in 2/5 female subjects that received 0.6 mg, 3/5 at 1.25 mg and 5/5 at 2.5 mg. Erythema was observed in 1/5 (0.6 mg), 4/5 (1.25 mg) and 4/5 (2.5 mg) women. While there appears to be a good dose-response relationship, this study did not report controls. The response observed at the lowest dose may well be within background levels.

Numerous other studies have been conducted to attempt to establish the relationships between nickel exposure and dermal irritation. Kaaber et al. (1978, 1979) reported worsening of eczema following an oral challenge with 2.5 mg nickel. In the 1978 study, 17/28 subjects experienced aggravation of dermatitis following nickel ingestion. Nine of the 17 that experienced adverse

effects from the nickel found that their condition improved when they adopted a low nickel diet. In the 1979 study 9/14 subjects responded negatively to nickel treatment.

Studies conducted by Gawrodger et al. (1986), Burrows et al. (1981) and Jordan and King (1979) offer different results. Jordan and King's double blind, placebo controlled investigation suggested that 0.5 mg supplement to a normal diet was safe with the possible exception of extremely sensitive individuals. Gawrodger et al. (1986) reported that 5/10 women responded to both the 0.4 and 2.5 mg doses of nickel, but 10/26 also reacted to a placebo. They determined the LOAEL of their experiment to be 5.6 mg of nickel, a dose at which 100% of the women responded. Burrows et al. (1981) administered 0.5 mg nickel twice a day on two consecutive days to 22 patients, each of whom served as her own control. There was no significant difference between the number of individuals responding to a placebo as compared to nickel. However, the placebo response was high (12/22). The authors concluded that there is probably no connection between nickel in an ordinary diet and exacerbation of dermatitis but that a higher level may aggravate dermatitis in some individuals.

Nielsen (1989) describes a study in which 12 nickel-sensitive women were challenged for a 4-day period with a diet providing 490 ug Ni/day. No changes were observed before the start of the nickel challenge to day 0 (start of challenge). On day 4, the eczema of 6 patients was considered to be worse according to both the patients' impressions and a dermatologist's evaluation. The delayed reaction in this study may be attributed to the fact that the dose of nickel was ingested in the diet throughout the day as opposed to studies which employed a bolus dose. This difference may greatly affect the pharmacokinetics of ingested nickel.

While the previous studies on humans with a hypersensitivity to nickel were considered in developing the RfD, none of them were adequate to serve as the basis for the quantitative risk assessment. The RfD is believed to be set at a level which would not cause individuals to become sensitized to nickel; however, those who have already developed a hypersensitivity (e.g., from a dermal exposure) may not be fully protected.

One final point to bear in mind in establishing an RfD for nickel is that nickel has been shown to be an essential trace element for several animal species. Rats deprived of nickel exhibit retarded growth and low hemoglobin levels (Schnegg and Kirchgessner, 1977). A requirement for nickel has not been conclusively demonstrated in humans, but nickel is considered to be a normal constituent of the diet. Typical daily intake of nickel ranges from 100-300 ug/day.

I.A.5. Confidence in the Oral RfD

Study — Low
Database — Medium
RfD — Medium

The chronic study (Ambrose et al., 1976) was properly designed and provided adequate toxicological endpoints; however, high mortality occurred in the controls (44/50). Therefore, a low confidence is recommended for the study. The database provided adequate supporting subchronic studies, one by gavage and the other in drinking water (Po animals of the RTI subchronic study, 1986). A medium confidence level in the database is recommended since there are inadequacies in the remaining reproduction data.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 1986, 1991

The information contained in the Quantification of Toxicologic Effects for Nickel was reviewed by the Science Advisory Board in August 1990.

Other EPA Documentation — None

Agency Work Group Review — 04/16/1987, 05/20/1987, 07/16/1987, 05/17/1990, 08/14/1991

Verification Date — 07/16/1987

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Nickel, soluble salts
CASRN — Various

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Nickel, soluble salts
CASRN — Various

The U.S. EPA has not evaluated soluble salts of nickel, as a class of compounds, for potential human carcinogenicity. However, nickel refinery dust and specific nickel compounds - nickel carbonyl and nickel subsulfide - have been evaluated. Summaries of these evaluations are on IRIS.

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — Nickel, soluble salts
CASRN — Various

VI.A. Oral RfD References

Ambrose, A.M., P.S. Larson, J.R. Borzelleca and G.R. Hennigar, Jr. 1976. Long-term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181-187.

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VI.B. Inhalation RfD References

None

VI.C. Carcinogenicity Assessment References

None

VII. Revision History

Substance Name — Nickel, soluble salts
CASRN — Various

Date	Section	Description
12/01/1991	I.A.4.	Text significantly revised; additional studies added
08/01/1994	II.	Message added
12/10/1998	I., II.	This chemical is being reassessed under the IRIS Program.

VIII. Synonyms

Substance Name — Nickel, soluble salts
CASRN — Various
Last Revised — 09/30/1987

- 7440-02-0
- C.I. 77775
- NICHEL
- Nickel
- Nickel, soluble salts

Attachment

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EPA/635/R-05/002

TOXICOLOGICAL REVIEW

OF

ZINC AND COMPOUNDS

(CAS No. 7440-66-6)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

July 2005

U.S. Environmental Protection Agency
Washington D.C.

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS —TOXICOLOGICAL REVIEW OF ZINC AND COMPOUNDS (CAS No. 7440-66-6)

LIST OF TABLES

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to zinc and compounds. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of zinc and compounds.

In Section 6, *Major Conclusions in the Characterization of Hazard and Dose Response*, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response by addressing knowledge gaps, uncertainties, quality of data, and scientific controversies. This discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 202-566-1676.

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REVIEWERS

This document and the accompanying IRIS Summary have been peer reviewed by EPA scientists and independent scientists external to EPA. Comments from all peer reviewers were evaluated carefully and considered by the Agency during the finalization of this assessment. During the finalization process, the IRIS Program Director achieved common understanding of the assessment among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Economics, and Innovation; Office of Children's Health Protection; Office of Environmental Information, and EPA's regional offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

This document presents background information and justification for the Integrated Risk Information System (IRIS) Summary of the hazard and dose-response assessment of zinc and compounds. IRIS Summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action. The RfD is an estimate of an oral exposure for [a given duration], to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a statistical lower confidence limit on the benchmark dose (BMDL), a no-observed-adverse effect-level (NOAEL), a lowest-observed-adverse-effect level (LOAEL), or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used. The RfD is expressed in units of mg/kg-day. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways to better facilitate their use: (1) generally, the *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day of oral exposure; (2) the *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $µg/m^3$ continuous airborne exposure; and (3) the 95% lower bound and central estimate on the estimated concentration of the chemical substance in drinking water or air that presents cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for zinc and compounds has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment

include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. 1995a), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998), and *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through October, 2004.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Some of the chemical and physical properties of zinc and zinc-containing compounds are presented in Table 2-1.

Table 2-1. Chemical and physical properties of zinc and selected zinc compounds

	Zinc	Zinc oxide	Zinc chloride	Zinc sulfate	Zinc sulfide
CAS Registry Number	7440-66-6	1314-13-2	7646-85-7	7733-02-0	1314-98-3
Molecular formula	Zn	ZnO	$ZnCl_2$	$ZnSO_4$	ZnS
Molecular weight	65.38	81.38	136.29	161.44	97.44
Melting point, ^oC	419.5	100 (decomposes)	283	600 (decomposes)	~1700
Boiling point, ^oC	908	No data	732	No data	No data
Water solubility, g/L (25^oC)	Insoluble	$\sim 2x10^{-3}$	$4.3x10^3$	$1.7x10^3$	$\sim 7x10^{-3}$
Density (g/cm^3)	7.14	5.607	2.907	3.54	~4.1

Source: ATSDR, 1995; Barceloux, 1999.

Zinc is ubiquitous in the environment and occurs in the earth's crust at an average concentration of about 70 mg/kg (Thomas, 1991). Zinc metal is not found freely in nature; rather it occurs in the +2 oxidation state primarily as various minerals such as sphalerite (zinc sulfide), smithsonite (zinc carbonate), and zincite (zinc oxide). Fifty-five zinc containing minerals are known to exist. The most important commercial minerals, their molecular composition and zinc percentages are listed in Table 2-2.

Table 2-2. Zinc commercial minerals, molecular composition, and percentage of zinc

Name	Composition	% Zinc
Sphalerite	ZnS	67.0
Hemimorphite	$Zn_4Si_2O_7(OH)_2H_2O$	54.2
Smithsonite	$ZnCO_3$	52.0
Hydrozincite	$Zn_5(OH)_6(CO_3)_2$	56.0
Zincite	ZnO	80.3
Willemite	Zn_2SiO_4	58.5
Franklinite	$(Zn,Fe,Mn)(Fe,Mn)_2O_4$	15-20

Source: Goodwin, 1998.

The primary anthropogenic sources of zinc in the environment are from metal smelters and mining activities (ATSDR, 1995). The production and use of zinc in brass, bronze, die castings metal, alloys, rubbers, and paints may also lead to its release to the environment through various waste streams.

Elemental zinc is a lustrous, blue-white to grey metal that is virtually insoluble in water. It has a melting point of 419.5°C and boiling point of 908°C (ATSDR, 1995). Pure zinc is usually produced by an electrolytic process in which zinc oxide is leached from the roasted or calcined ore with sulfuric acid to form zinc sulfate solution which is electrolyzed in cells to deposit zinc on cathodes (Lewis, 1993). The primary application of zinc in metallurgy is its use as a corrosion protector for iron and other metals.

Zinc salts have numerous applications and are used in wood preservation, catalysts, corrosion control in drinking water systems, photographic paper, vulcanization acceleration for rubber, ceramics, textiles, fertilizers, pigments, batteries, and as nutritional supplements or medicines (ATSDR, 1995). Zinc chloride is a primary ingredient in smoke bombs used for crowd dispersal, in fire-fighting exercises (by both military and civilian communities), and by the military for screening purposes. Zinc chloride, zinc sulfate, zinc oxide, and zinc sulfide have dental, medical, and household applications. Zinc chloride and zinc sulfate are also used in herbicides (ATSDR, 1995). Zinc compounds are usually colorless which is advantageous since

they do not color paints, plastics, rubber, or cosmetics to which they might be added. However, zinc oxide and zinc sulfide exhibit luminescence when excited by UV-Vis radiation.

Zinc ions are strongly adsorbed to soils at pH 5 or greater and are expected to have low mobility in most soils (Christensen et al., 1996; Gao et al., 1997). Zinc is taken up by plants and vegetables and the normal zinc content is in the range of 15 to 100 mg/kg (Thomas, 1991).

In natural waters, zinc can be found in several chemical forms, such as hydrated ions, metal-inorganic complexes, or metal-organic complexes (U.S. EPA, 1979). Hydrated zinc cations may be hydrolyzed to form zinc hydroxide or zinc oxide (U.S. EPA, 1979). In anaerobic environments, Zinc sulfide may be formed (U.S. EPA, 1979). Zinc accumulates in aquatic organisms, and bioconcentration factor values for freshwater fish and marine fish were reported as 1000 and 2000, respectively (U.S. EPA, 1979).

As discussed in Section 4.1, zinc is an essential element in humans. In adults, the greatest dietary sources of zinc are meats, dairy products, grains, and mixed dishes (Pennington et al., 1989), while fruits, nuts, fats, sweeteners, and beverages contribute comparatively small amounts of zinc to the diet.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

3.1.1. Gastrointestinal Absorption

Numerous studies have assessed zinc absorption in healthy humans under a variety of dietary conditions. The North American adult diet contains about 8-15 mg Zn/day based on data from the 1988-1994 National Health and Nutrition Examination Survey (IOM, 2001). Zinc uptake from a normal diet ranges from 26-33% (Sandstrom and Abrahamson, 1989; Knudsen et al., 1995; Hunt et al., 1998) when taken with food, but is higher (i.e., 68-81%) when subjects have fasted (Istfan et al., 1983; Sandstrom and Abrahamson, 1989). Within a 5-25 mg dose range, zinc absorption, expressed as a percent of the total dose administered, decreases as the dose increases; for example, in human volunteers, 61% of a 24.5 mg dose of zinc (as zinc chloride) was absorbed, compared to 81% of a 4.5 mg dose (Istfan et al., 1983).

Within the digestive tract, zinc is primarily absorbed in the small intestine. Ligation studies in rats have suggested that absorption is mainly in the duodenum (Methfessel and Spencer, 1973; Davies, 1980), with approximately 60% of the absorption occurring in the duodenum, 30% in the ileum, 8% in the jejunum, and 3% through the colon and cecum (Davies, 1980). However, more recent studies in humans (Lee et al., 1989) have suggested a greater rate of transport across the jejunum than across any other intestinal segment. As discussed in a review by Lönnerdal (2000), it is possible that while there is a greater rate of absorption in the jejunum, the fact that oral zinc first passes through the duodenum allows for a greater absolute absorption in that segment, despite a greater transport rate in the jejunum. However, the quantitative importance of the different intestinal segments is not yet clearly defined. Gastrointestinal absorption of zinc is biphasic, with an initial rapid phase followed by a saturable slow phase (Davies, 1980; Gunshin et al., 1991). It is notable that these studies generally used water-soluble forms of zinc; as zinc appears to be absorbed as zinc ion, less soluble forms would be expected to show a lower level of gastrointestinal absorption.

Zinc appears to be absorbed by both passive diffusion and a saturable carrier-mediated process (Tacnet et al., 1990). The carrier-mediated mechanism appears to be most important at low zinc levels, and involves a saturable cysteine-rich intestinal protein (CRIP) (Hempe and Cousins, 1991, 1992). CRIP binds zinc during transmucosal transport and may function as an intracellular zinc carrier. There is also some evidence that CRIP binds zinc in competition with

metallothionein (Hempe and Cousins, 1991). The binding capacity of CRIP for zinc is limited, and CRIP becomes saturated at high intestinal concentrations of zinc (Hempe and Cousins, 1991). Metallothionein may be involved in zinc homeostasis at higher zinc concentrations (Richards and Cousins, 1975; Hempe and Cousins, 1992). Metallothionein production is increased in response to an increase in zinc levels as well as by other heavy metals (Richards and Cousins, 1975; Cousins, 1985). The exact role of metallothionein in zinc absorption is not known, but it is thought to regulate zinc availability by sequestering it in the intestinal mucosal cells, thereby preventing absorption and providing an exit route for excess zinc as these cells are shed and excreted in the feces (Foulkes and McMullen, 1987). It has been proposed that as zinc enters the cells of the intestinal mucosa it is initially associated with CRIP, with only a small fraction binding to metallothionein, but as zinc concentrations rise, the binding to CRIP becomes saturated, the proportion of zinc binding to CRIP decreases, and more zinc is bound to metallothionein (Hempe and Cousins, 1992).

Evans (1976) proposed that zinc bound to ligands is transported into epithelial cells where the metal is transferred to the binding site on the plasma membrane. Metal-free albumin then interacts with the plasma membrane and removes zinc from the receptor site. The quantity of metal-free albumin available probably determines the amount of zinc removed from the epithelial cell, and thus regulates the quantity of zinc that enters the body. Several dietary factors can influence zinc absorption, including other trace elements (e.g., copper, iron, lead, calcium, cadmium, cobalt; see Section 4.6.2), amino acids, simple and complex carbohydrates, and protein. High levels of phytate or phosphate in the diet can decrease the amount of zinc absorbed (Pecoud et al., 1975; Larsson et al., 1996; Oberleas, 1996). Oberleas (1996) suggested that the phytate in the food provided to test subjects complexes with endogenous zinc ions secreted from the pancreas, thus preventing its reabsorption and increasing fecal zinc elimination. In general, low molecular weight substances, such as amino acids, increase the absorption of zinc (Wapnir and Stiel, 1986). Imidazole, tryptophan, proline, and cysteine increased zinc absorption from various regions of the gastrointestinal tract. Wapnir and Stiel (1986) suggested that the increase was due to the presence of both mediated and non-mediated transport mechanisms for amino acids. Absorption is inhibited by certain proteins (e.g., bovine serum albumin and dephytinized soyabean protein isolate), is unaffected by others (e.g., bovine whey) (Davidsson et al., 1996), and enhanced by others (e.g., casein) (Hunt et al., 1991; Davidsson et al., 1996).

Physiological factors also appear to influence zinc absorption. The primary factor influencing zinc absorption appears to be the body's ability to alter zinc excretion and absorption efficiency in order to maintain zinc homeostasis (Johnson et al., 1993). Zinc absorption is enhanced in humans with low zinc levels; 93% of a 1.19 mg dose of zinc was absorbed in subjects maintained on a low zinc diet (1.4 mg/day) as compared to 81% absorption of the same test dose in subjects on an adequate zinc diet (15 mg/day) (Istfan et al., 1983). A study in mice (He et al., 1991) suggests that zinc absorption decreases with age. Fractional absorption was significantly lower in young adult mice (70 days of age) and in adult mice (100 days of age) compared to weanling mice (1 day of age); fractional absorption in adolescent mice (20 days of age) was similar to that found in weanlings.

3.1.2. Respiratory Tract Absorption

Hamdi (1969) found elevated levels of zinc in the urine and blood of workers exposed to zinc oxide fumes, relative to non-exposed workers. Although this study did not estimate zinc absorption efficiency, it does provide evidence that zinc is absorbed following inhalation exposure. Similarly, Drinker and Drinker (1928) found elevated levels of zinc in the gall bladder, kidney, and pancreas of cats, rabbits, and rats exposed to airborne zinc oxide.

Studies by Sturgis et al. (1927) and Gordon et al. (1992) examined lung retention following inhalation exposure to zinc oxide. Retention is reflective of deposition of zinc oxide in the lung rather than systemic absorption (Hirano et al., 1989). Species differences in retention have been observed; guinea pigs, rats, and rabbits retained 20, 12, and 5%, respectively, following nose-only exposure to 11.3, 4.3, or 6.0 mg/m^3 of zinc oxide, respectively, for 3 hours (guinea pigs and rats) or 6 hours (rabbits) (Gordon et al., 1992).

3.2. DISTRIBUTION

Zinc is an essential human nutrient, a cofactor for over 300 enzymes, and is found in all tissues. In humans, the highest concentrations of zinc have been found in bone, muscle, prostate, liver, and kidneys (Schroeder et al., 1967; Wastney et al., 1986). Similar distributions have been found in animals (Ansari et al., 1975, 1976; Llobet et al., 1988). Less than 10% of the body's total zinc is readily exchanged with plasma (Miller et al., 1994) and most of this is from the slow exchange of zinc located in bone and muscle. In blood, zinc is found in plasma, erythrocytes, leukocytes, and platelets. Approximately 98% of serum zinc is bound to proteins; 85% is bound to albumin, 12% to α_2-macroglobulin, and the remainder to amino acids (Giroux et al., 1976). In

erythrocytes, zinc is predominantly found as a component of carbonic anhydrase (87%) and Cu, Zn-superoxide dismutase (5.4%) (Ohno et al., 1985).

Ansari et al. (1975) examined the heart, liver, kidneys, muscle, tibia, and small intestine for changes in tissue zinc concentration following the addition of 600 ppm supplemental zinc to the diet of male rats for up to 42 days. While small increases in tissue zinc levels relative to controls were reported, only occasionally were the differences statistically significant, and no pattern with increasing tissue zinc with time was noted. In a later study, Ansari et al. (1976) exposed male rats to up to 8400 ppm supplemental zinc as zinc oxide in the diet for 21 days then examined the liver, kidney, heart, tibia, and muscle for tissue zinc concentrations. Exposure to 1200 ppm had no significant effect on tissue zinc levels relative to controls; the amount of stable zinc in liver, kidney, and bone was increased at 2400 ppm and higher, but reached a plateau (2400-7200 ppm; approximately 200-625 mg/kg-day). Exposure at the highest level (8400 ppm) caused additional increases in liver, kidney, and bone, as well as an increase in zinc level in the heart. No changes in zinc concentration were seen in the skeletal muscle. Similar results for the accumulation of zinc in organs have been found in mice (He et al., 1991), rabbits (Bentley and Grubb, 1991), and wood mice *(Apodemus sylvaticus L.*) (Cooke et al., 1990).

In a series of animal experiments carried out by Drinker and Drinker (1928), the fate of inhaled zinc oxide from the lungs of animals (cats, rabbits and rats) was assessed. Increased zinc levels were found in the lungs, pancreas, liver, kidney, and gall bladder.

3.3. METABOLISM

Zinc is a metallic element that is found in the body as a divalent cation. Accordingly, it does not undergo metabolism. It interacts electrostatically with anions (i.e., carbonate, hydroxide, oxalate, phytate) and negatively charged moieties on macromolecules such as proteins. It can also form soluble chelation complexes with amino acids and multidentate organic acids such as ethylenediaminetetraacetic acid.

3.4. ELIMINATION AND EXCRETION

Following oral exposure, zinc is primarily excreted via the gastrointestinal tract and eliminated in the feces; approximately 70-80% of an ingested dose is excreted in the feces (Davies and Nightingale, 1975). Oberleas (1996) found that the pancreas secretes into the

duodenum two to four times the amount of zinc that is typically consumed in an average day; most of this secreted zinc is reabsorbed. Zinc is also excreted in the urine. In humans, approximately 14% of the eliminated zinc was excreted in urine; when zinc intake was increased, urinary excretion accounted for 25% of the eliminated zinc (Wastney et al., 1986). Other minor routes of elimination are sweat (Prasad et al., 1963), saliva secretion (Greger and Sickles, 1979), and incorporation into hair (Rivlin, 1983).

The rate at which zinc is excreted is dependant on both current zinc intake and past zinc intake, probably via an effect on body stores (Johnson et al., 1988). Age also affects the rate at which zinc is excreted. He et al. (1991) reported higher fecal excretion of zinc in adult mice following an intraperitoneal dose of ^{65}Zn, as compared to weanling, adolescent, or young adult mice.

3.5. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

Physiologically based toxicokinetic models have been developed to assess environmental exposure levels for other metals such as cadmium and lead. However, no toxicokinetic models have been developed for zinc in either human or animal species.

4. HAZARD IDENTIFICATION

4.1. ESSENTIALITY OF ZINC

While the focus of this document, and the values derived in Chapter 5, is on the effects of excess zinc exposure, rather than the effects of insufficient zinc intake, a discussion of the importance of zinc as a dietary nutrient is relevant when considering the effects of zinc exposure. The essentiality of zinc was established over 100 years ago. Zinc is essential for the function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu, Zn-superoxide dismutase, carboxypeptidase, δ-aminolevulinic acid dehydratase (ALAD), carbonic anhydrase, deoxyribonucleic acid (DNA) polymerases (DNA polymerase alpha, DNA polymerase III), and reverse transcriptase (Vallee and Falchuk, 1993; Sandstead, 1994). A list of key enzymes containing zinc or affected by zinc status are provided in Table 4-1. Zinc has three functions in these metalloenzymes: participation in catalytic functions, maintenance of structural stability, and regulatory functions (Vallee and Falchuk, 1993; Walsh et al., 1994). Zinc is also involved in DNA and ribonucleic acid (RNA) synthesis and cell proliferation. The zinc coordinates with cysteine and histidine residues of certain peptides and produces a tertiary structure which has an affinity for unique segments of DNA in promoter gene regions (Prasad, 1993). The configurations include the zinc finger, the most common zinc motif, and the zinc thiolate cluster (Walsh et al., 1994). Other physiological roles of zinc include enhancement of the affinity of growth hormone for its binding receptors, modulation of synaptic transmissions by interacting with specific sites on ionotrophic neurotransmitter receptor proteins, and induction of metallothionein (Walsh et al., 1994).

A wide range of clinical symptoms have been associated with zinc deficiency in humans (Abernathy et al., 1993; Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc absorption or in patients receiving total parenteral nutrition lacking in adequate zinc, include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Symptoms associated with moderate zinc deficiency include growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing. Neurosensory changes (hypogeusia, decreased dark adaptation), impaired neuropsychological functions (dysosmia, irritability, and reduced cognitive function), oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune function (alterations in T-cell subpopulations,

decreased natural killer cell activity) have been observed in individuals with mild or marginal zinc deficiency.

As reviewed by Mahomed et al. (1989), severe zinc deficiency in animals has been associated with reduced fertility, fetal nervous system malformations, and growth retardation in late pregnancy. In humans, labor abnormalities, congenital malformations, and preterm labor have been reported in otherwise healthy women with low maternal serum zinc concentrations. Numerous studies have examined pregnancy outcomes following zinc supplementation. For example, Simmer et al. (1991) found significant intrauterine growth retardation and fewer inductions of labor (generally associated with poor fetal growth), and non-statistically significant decreases in birth weight and placental weights in zinc-deficient women compared to women receiving a supplement containing 100 mg zinc citrate (22.5 mg zinc). The women receiving the supplement had been selected because they were determined to be at risk of delivering small-for-gestational age babies. However, Mahomed et al. (1989) did not find any statistically significant differences in gestation duration, details of labor and delivery, fetal development, or neonatal health among 246 randomly selected pregnant women receiving 20 mg Zn/day as zinc sulfate (66 mg zinc sulfate) tablets beginning before the 20th week of pregnancy as compared to 248 women receiving placebo tablets. While the zinc supplement and placebo group had marginal zinc intake (approximately 10 mg/day) prior to supplementation, the zinc supplementation did not appear to influence pregnancy outcome. The author commented that the women recruited in this study were from mid-socioeconomic groups. Endogenous stores of zinc could possibly have met the need for fetal development.

Table 4-1. Key enzymes containing zinc or affected by zinc status

Enzyme name (symbol)	Alternative titles (symbol)	Reaction catalyzed	Cofactor(s)	Enzyme commission number (EC)[a]
Cu, Zn-superoxide dismutase	Superoxide dismutase, cytosolic; Superoxide dismutase 1	$2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$	Copper and zinc	1.15.1.1
Erythrocyte Cu, Zn-superoxide dismutase	Superoxide dismutase, cytosolic; Superoxide dismutase 1	$2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$	Copper and zinc	1.15.1.1
Extracellular Cu, Zn-superoxide dismutase	Superoxide dismutase, extracellular	$2\ O_2^{\cdot-} + 2\ H^+ <=> O_2 + H_2O_2$	Copper and zinc	1.15.1.1
Cytochrome c oxidase	Ferrocytochrome c oxidase	4 ferrocytochrome c + $O_2 <=> 2H_2O$ + 4 ferricytochrome c	Copper	1.9.3.1
Ceruloplasmin	Ferroxidase	$4\ Fe^{2+} + 4\ H^+ + O_2 <=> 4\ Fe^{3+} + 2\ H_2O$	Copper	1.16.3.1
Metallothionein	Metallothionein 1A	Cysteine residues complex with zinc, cadmium, and copper to form mercaptide linkages	N/A[b]	N/A

[a] EC numbers specify enzyme catalyzed reactions, not specific enzymes.
[b] Not applicable

Sources: McKusick, 1998; Bairoch and Apweiler, 1999.

The zinc content of a typical mixed diet of North American adults is approximately 10-15 mg/day (IOM, 2001). The U.S. Food and Drug Administration's (FDA) Total Diet Study (Pennington and Schoen, 1996) found zinc intakes of 7.25, 9.74, 15.42, 9.38, and 15.92 mg/day in children (2 years of age), girls (14-16 years), boys (14-16 years), women (25-30 years), and men (25-30 years), respectively. The 2000 recommended dietary allowances (RDAs) for zinc (IOM, 2001) are presented in Table 4-2.

4.2. STUDIES IN HUMANS

Human studies have investigated the effects of dietary zinc supplementation. High doses can cause clinical symptoms of gastrointestinal distress, while low doses primarily affect the status of other essential nutrients such as copper and iron.

4.2.1. Oral Exposure

In a double-blind crossover trial, Samman and Roberts (1987, 1988) gave zinc sulfate tablets (150 mg supplemental Zn/day in three divided doses at mealtimes) to healthy adult volunteers (21 men and 26 women) for 6 weeks; identical capsules containing lactose were given to the same group of volunteers for 6 weeks as the placebo. Using the reported average body weights, the zinc doses averaged 2 mg Zn/kg-day for the men and 2.5 mg Zn/kg-day for the women. Adverse symptoms, including abdominal cramps, vomiting, and nausea, occurred in 84% of the women and 18% of the men. Five females withdrew from the trial because of gastric irritation. A dose-related increase in clinical symptoms was observed when doses were expressed on a mg/kg-day basis. Ingestion of zinc tablets alone (contrary to instructions) or with small meals increased the incidence of adverse effects. Zinc administration for 6 weeks had no effect on plasma levels of copper, total cholesterol, or high-density lipoprotein (HDL)-cholesterol in males or females, but significantly decreased the plasma level of low-density lipoprotein (LDL)-cholesterol in females only. An apparent inverse linear relationship between plasma zinc levels and LDL-cholesterol levels was found in the females. Hematocrit values were unaffected by zinc ingestion in males and females. Specific measures of copper status (ferroxidase activity of serum ceruloplasmin, antioxidant activity of erythrocyte Cu, Zn-superoxide dismutase [ESOD] activity) were apparently unaffected in males. However, females, who received higher mg/kg-day doses of zinc than males, exhibited a significant reduction in the activity of two copper metalloenzymes: serum ceruloplasmin and ESOD. Other indicators of copper status were not affected.

Table 4-2. Recommended dietary allowances (RDA) by life stage group and gender

Life stage group	RDA (mg/day)	
	Male	Female
0 through 6 months	2[a]	2[a]
7 through 12 months	3	3
1 through 3 years	3	3
4 through 8 years	5	5
9 through 13 years	8	8
14 through 18 years	11	9
19 through 50 years	11	8
>51 years	11	8
Pregnancy		
≤18 years		12
19 through 50 years		11
Lactation		
<18 years		13
19 through 50 years		12

[a]Acceptable daily intake. No RDA value was reported.

Source: IOM, 2001.

Fischer et al. (1984) instructed groups of 13 healthy adult male volunteers (ages not specified) to take capsules containing 0 (cornstarch) or 25 mg supplemental zinc (as zinc gluconate) twice daily for 6 weeks; using a reference body weight of 70 kg for an adult male, average daily dose was 0.71 mg supplemental Zn/kg-day. Nonfasting blood samples were taken at the beginning and at biweekly intervals and tested for measures of copper status. Plasma copper levels and levels of ceruloplasmin's ferroxidase activity did not change during the course of the study. However, ESOD activity decreased after 4 weeks in the supplement group and was significantly lower than controls by 6 weeks. An inverse correlation between plasma zinc levels and ESOD activity was also observed at 6 weeks.

A 10-week study of zinc supplementation in 18 healthy women, aged 25-40 years, given zinc gluconate supplements twice daily (50 mg supplemental Zn/day, or 0.83 mg supplemental Zn/kg-day) resulted in a decrease of ESOD activity (Yadrick et al., 1989). ESOD activity declined over the 10-week supplementation period and, at 10 weeks, was significantly different ($p<0.05$) from values during the pretreatment period. By 10 weeks, ESOD activity had declined to 53% of pretreatment levels. This change in enzyme activity is considered a better indicator of altered copper status than a measure of metal concentration in tissue or plasma. This has been documented by studies in rats which were fed copper-deficient or high-zinc diets, in which treatment-related changes in copper metalloenzyme activity are greater and precede changes in plasma or tissue levels of copper (L'Abbe and Fischer, 1984a, b). Ceruloplasmin activity was not altered. Serum zinc was significantly increased. There was also a significant decline in serum ferritin and hematocrit values at 10 weeks. Such a decrease could pose a significant risk to the iron status of women.

Recently, Davis et al. (2000) and Milne et al. (2001) have reported the results of exposure of a group of postmenopausal women (aged 50-76, mean of 64.9 ± 6.7 years) to varying concentrations of zinc and copper in the diet. Average height was 159.6 ± 7.6 cm, and mean body weight was 65.1 ± 9.5 kg. Subjects were kept in a metabolic ward for a 200-day period, and fed a controlled basal diet that contained 0.6 mg copper and 3 mg zinc. For the first 10 days, all subjects consumed an equilibration diet, which consisted of the basal diet supplemented with 1.4 mg copper (2 mg total) and 6 mg zinc (9 mg total). Following an initial 10-day equilibration, one group (n=12) was exposed to the basal diet supplemented with 0.4 mg Cu/day (1 mg Cu/day total) and the other group (n=13) was fed the basal diet supplemented with 2.4 mg Cu/day (3.0 mg Cu/day total). The remaining 190 days were divided into two 90-day study periods for both groups: the copper-supplemented basal diet (1 mg Cu/day, total) with no zinc supplement was fed for the first 90-day period and the copper-supplemented (1 mg Cu/day, total) basal diet supplemented with 50 mg Zn/day was fed for the second 90-day period. The two 90-day periods were separated by an additional equilibration period, identical to the one performed at the beginning of the study.

During each of the equilibration periods, and twice monthly during the exposure periods, blood was drawn from the subjects after an overnight fast, and evaluated for changes in cells and cell elements (erythrocytes, platelets, mononuclear cells [MNC], neutrophils), plasma and blood levels of copper and zinc, and a variety of blood proteins and factors (alkaline phosphatase activity, superoxide dismutase activities [ESOD and extracellular Cu, Zn-superoxide dismutase

(EC-SOD)], 5'-nucleotidase activity, triiodothyronine, thyroxine, and thyroid-stimulating hormone levels, and amyloid precursor protein [APP] levels). Copper and zinc levels were determined for urine, feces, and diet. Alcohol tolerance tests were performed at the end of the first equilibration period and at the end of the low- and high-zinc exposures. Data were analyzed by a two-way (dietary zinc and copper) repeated-measures analysis of variance, and Tukey's contrasts were used to test for differences among means.

Plasma zinc concentrations were significantly lower, relative to the equilibration levels, and platelet zinc concentrations tended to be lower, though not significantly, in subjects fed 3 mg Zn/day than in those fed 53 mg Zn/day; plasma zinc was not lowered from equilibration levels when subjects were fed 3 mg Zn/day, but was elevated in those fed 53 mg Zn/day. Zinc supplementation increased Zn levels in the feces and urine, but did not appear to affect plasma Cu levels. Neither erythrocyte zinc levels nor erythrocyte membrane zinc concentrations were significantly altered by changes in dietary zinc.

High-zinc subjects showed significant increases in bone-specific alkaline phosphatase activity, relative to the equilibration period, but not in plasma alkaline phosphatase or erythrocyte membrane alkaline phosphatase. Zinc supplementation significantly increased mononuclear white cell 5'-nucleotidase activity and decreased plasma 5'-nucleotidase activity; the difference in 5'-nucleotidase activity was apparent when subjects were fed the high-copper diet, but not when they were fed the low-copper diet.

EC-SOD activity, but not ESOD activity, was significantly increased by zinc supplementation; this was more apparent in the low-copper group. ESOD activity was significantly decreased relative to equilibration levels in low-copper subjects and significantly increased in high-copper subjects; in both cases, zinc supplementation caused a statistically insignificant decrease in ESOD activity.

Erythrocyte glutathione peroxidase activity was increased by low dietary zinc and decreased by high dietary zinc; however, the decrease did not result in a return to initial equilibration activity. Plasma free thyroxine concentrations, but not total thyroxine concentrations, were significantly increased in the zinc-supplemented groups; no other effects on thyroid-related endpoints were noted.

During the low-zinc period, there was an increase in total cholesterol; this increase was reversed with high-zinc treatment, resulting in lower total cholesterol. LDL-cholesterol changes were similar to the total cholesterol changes, while HDL-cholesterol, very low density lipoprotein-cholesterol, and triglycerides were not affected. Zinc supplementation significantly decreased platelet APP expression in subjects fed the low-copper diet; however, technical problems prevented many of these samples from being properly analyzed, so the sample size for APP expression was very small. Most indicators of iron status were not affected by the changes in dietary zinc or copper during the 90-day period; the exception was a small drop in hemoglobin (Hb) levels, which the investigators attributed to the effects of accumulated blood loss due to blood draws conducted during the study.

Hale et al. (1988) carried out an epidemiological study of the effect of zinc supplements on the development of cardiovascular disease in elderly subjects who were participants in an ongoing longitudinal geriatric health screening program. Noninstitutionalized, ambulatory subjects between the ages of 65 and 91 (average 78) years were evaluated using questionnaire, electrocardiogram, hematological, and drug-use data. A group of subjects (38 women and 31 men) that had ingested zinc supplements (20 to 150 mg supplemental Zn/day) for at least one year was compared to a control group (1195 women and 637 men) from the same screening program. Approximately 85% of the study group reported taking <50 mg supplemental Zn/day; for the 15% that reported an average intake of 60-150 mg supplemental Zn/day, the average duration was 8 years. The overall duration of zinc usage by the study group was: ≤2 years, 30%; >2≤10 years, 55%; and >10 years, 15%. Based on the results of the questionnaire and hematological parameters, the incidence of anemia was reported to have decreased with an increase in zinc dose. There were no differences between zinc and control groups with respect to electrocardiographic results or the incidence of adverse cardiovascular events (heart attack, heart failure, hypertension, or angina). The zinc group had a lower mean serum creatinine, lower total serum protein, lower serum uric acid, and a higher mean corpuscular Hb. Red blood cell counts were significantly lower in the women, but not in the men, of the zinc group.

Three groups of healthy white men were administered 0 (n=9), 50 (n=13), or 75 (n=9) mg/day supplemental zinc as zinc gluconate for 12 weeks (Black et al., 1988). The subjects were given instructions to avoid foods high in calcium, fiber, and phytic acid, dietary constituents that are known to decrease zinc absorption. Subjects were also told to restrict their intake of zinc-rich foods in order to minimize the variation in daily dietary zinc. Three-day dietary records were collected on a biweekly basis. These records indicated that the dietary zinc intakes of the

three treatment groups were 12.5, 14.0, and 9.5 mg Zn/day for the groups receiving the 0, 50, and 75 mg/day supplements, respectively. Based on the average body weights for each treatment group, total zinc intakes were 0.16, 0.85, and 1.10 mg Zn/kg-day for the 0, 50, and 75 mg/day groups, respectively. Biweekly blood samples were collected from all subjects and analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, zinc, and copper. Urinary zinc and copper values were also determined. There was a general decline in the mean serum HDL-cholesterol for the 75-mg supplement group between weeks 6 and 12. HDL values for this group were significantly lower than those for the placebo group at weeks 6 and 12 (p <0.05). There was also a decline in the HDL values for the 50-mg group between weeks 8 through 12; however, this decline was not significantly different from that for the controls until the 12th week of treatment. When the mean HDL-cholesterol level of these subjects was compared to population percentile norms (Simko et al., 1984), there was a decline from the 92nd to the 77th percentile in 6 weeks, followed by a relative stabilization of HDL values for the remaining 6-week test period. Over the 12-week period, the HDL values for the 50-mg supplemental zinc group declined from the 90th to the 77th population percentile norms. Serum zinc, copper, total cholesterol, LDL-cholesterol, and triglycerides did not appear to be affected by treatment.

In another study, 12 healthy men (23 to 35 years) with normal serum cholesterol levels received a zinc sulfate capsule twice a day with meals (160 mg supplemental Zn/day or ~2 mg supplemental Zn/kg-day, assuming a 70 kg reference body weight) for 5 weeks and 8 subjects received placebo capsules (Hooper et al., 1980). Fasting lipid levels were measured weekly for 7 weeks and at week 16 in the zinc group, and biweekly for 6 weeks in the control group. There were no statistically significant differences in total serum cholesterol, triglyceride, and LDL-cholesterol between the zinc and control groups. After 5 weeks of zinc ingestion, serum HDL-cholesterol had been reduced by 17%; although no further zinc was administered, the serum HDL-cholesterol level continued to decline and was reduced by 26% at week 7, relative to the values for the placebo group. The rise in plasma zinc concentration did not correlate with the fall in HDL-cholesterol. Serum HDL-cholesterol returned to near baseline levels 11 weeks after the end of zinc supplementation.

Bogden et al. (1988) exposed groups of healthy elderly (age 60-89) to 0, 15, or 100 mg supplemental Zn/day for 3 months. At the end of the study, blood was drawn, and evaluated for changes in zinc levels in plasma, erythrocytes, MNCs, polymorphonuclear cells, and platelets. Serum samples were also evaluated for cholesterol, HDL cholesterol, alkaline phosphatase, and albumin. No statistically significant changes in any of the evaluated serum parameters were

reported, with the exception of an decrease in the ratio of plasma zinc to plasma copper in the high-dose group.

Chandra (1984) gave 11 healthy men 300 mg of supplemental zinc as zinc sulfate in two divided doses daily for 6 weeks (~4 mg supplemental Zn/kg-day using a 70 kg reference body weight). Fasting blood samples were taken prior to exposure, after 2, 4, and 6 weeks of exposure, and at 2 and 10 weeks following cessation of exposure. Effects of zinc ingestion included a 19% reduction in HDL levels at 4 weeks, and a 30% decrease in HDL levels and a 15% increase in LDL levels at 6 weeks, relative to pre-exposure values. Total serum cholesterol and triglycerides were unchanged. Zinc ingestion also adversely affected several indices of polymorphonuclear leukocyte function: chemotactic migration was reduced by 53% and the amount of phagocytosis of bacteria was reduced by 49%, although the bactericidal capacity was unchanged. In addition, the lymphocyte stimulation response to phytohemagglutinin was reduced by approximately 60-70%.

Freeland-Graves et al. (1982) exposed groups of eight healthy women to 0, 15, 50, or 100 mg supplemental zinc as zinc acetate daily for 60 days (approximately 0, 0.25, 0.83, or 1.7 mg supplemental Zn/kg-day, assuming a reference female body weight of 60 kg) and evaluated effects on serum zinc and cholesterol levels. Zinc exposure resulted in significant, dose-related increases in serum zinc. In the highest exposure group only, plasma HDL-cholesterol was significantly reduced at 4 weeks of exposure, but not at any other timepoint examined. A direct correlation between dietary zinc and whole-blood copper was observed in treated subjects. The study authors noted that in the 50 and 100 mg groups, some bloating, nausea, and abdominal cramps were noted unless the supplement was taken with a large glass of water at mealtime.

Prasad et al. (1978) fed a patient with sickle cell anemia supplements of 150 to 200 mg Zn/day for 2 years. The supplement resulted in copper deficiency; serum copper and plasma ceruloplasmin levels were decreased. When copper was administered, the plasma ceruloplasmin levels became normal. In a follow-up study of 13 patients on zinc therapy (similar treatment levels assumed), 7 patients had ceruloplasmin levels at the lower limit of normal after 24 weeks of dosing.

In a recent study by Prasad et al. (2004), the antioxidant effect of zinc was studied in humans. Twenty healthy subjects (9 males and 11 females, ages 19 - 50 years) were randomly assigned into two groups. Ten subjects received oral placebo, and 10 received oral zinc (45 mg

zinc as zinc gluconate) daily for 8 weeks. Blood was drawn from the subjects both before and after the treatment period, and the following parameters were examined: plasma zinc concentration, lipid peroxidation, DNA oxidation, tumor necrosis factor alpha (TNF-α) and interleukin-1β mRNA levels, and nuclear factor kappa-B (NF-κB) DNA binding. A statistically significant increase in plasma zinc concentrations was observed in the zinc-supplemented group. Plasma markers of lipid peroxidation (i.e., 4-hydroxynonenol and malondialdehyde) and DNA oxidation (i.e., 8-hydroxy-2'-deoxyguanosine) were significantly decreased in the zinc-supplemented group ($p<0.05$). Ex vivo studies were performed to determine the effects of zinc supplementation on the ability of MNCs to modulate relative mRNA levels of pro-inflammatory cytokines (i.e., TNF-α and interleukin-1β) in response to lipopolysaccharide (LPS) stimulation. LPS-treated MNCs from the zinc-supplemented group had a statistically significant decrease in the levels of TNF-α and interleukin-1β mRNA versus placebo controls. The activation and DNA binding of NF-κB following ex vivo treatment of MNCs with TNF-α was used as a model for induction of oxidative stress. A 50% decrease in the DNA binding of NF-κB was shown with MNCs from the zinc-supplemented group compared to placebo controls ($p<0.05$).

4.2.2. Inhalation Exposure

Most of the available information on the toxicity of inhaled zinc focuses on metal fume fever, a collection of symptoms observed in individuals exposed to freshly formed zinc oxide fumes or zinc chloride from smoke bombs. The earliest symptom of metal fume fever (also referred to as zinc fume fever, zinc chills, brass founder's ague, metal shakes, or Spelter's shakes) is a metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-like symptoms, chills, fever, profuse sweating, headache, and weakness follow (Drinker et al., 1927a, b; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur within several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An increase in tolerance develops with repeated exposure; however, this tolerance is lost after a brief period without exposure, and symptoms are most commonly reported at the beginning of the work week and after holidays. There are many reports of metal fume fever in the literature; however, most describe individual cases and exposure levels are not known. It is beyond the scope of this document to describe all of these reports. Below is a discussion of some of the studies which provide useful information on critical exposure levels or describe the clinical sequelae.

Drinker et al. (1927a) described the case of a worker exposed to zinc oxide on two successive days. On the first day, the worker was exposed for 5 hours to an average

concentration of 52 mg Zn/m^3. The worker reported feeling an oncoming fever four hours after exposure began, and elevated temperature, chill, and fatigue were reported several hours after exposure termination. No adverse symptoms were reported after the second day of exposure, even though zinc oxide levels were higher on the second day (330 mg Zn/m^3). To further examine this apparent tolerance, Drinker et al. (1927a) experimentally exposed another man with previous zinc oxide exposure to 430 mg/m^3 for 8 minutes on day 1 and to 610 mg/m^3 for 8 minutes on day 2. On day 1, the subject's temperature gradually increased and peaked 13 hours after exposure (101.2°F versus 98.5°F prior to exposure). The subject reported chills and feeling feverish, weak, and somewhat debilitated 10-15 hours after exposure. As with the occupational exposure, these symptoms were not observed after the second exposure.

Brown (1988) described the case of a shipyard worker who sprayed zinc onto steel surfaces. The worker complained of aches and pains, dyspnea, dry cough, lethargy, a metallic taste, and fever. Chest radiographs taken at the time of admission into a hospital revealed multiple nodules measuring 3-4 mm in size. The symptoms had resolved after 3 days, and the chest radiograph was normal after 4 days.

There is evidence to suggest that exposure to zinc oxide fumes may impair lung function. Malo et al. (1990, 1993) present case reports of two workers exhibiting symptoms of metal fume fever with evidence of functional lung involvement. In the first case (Malo et al., 1990), a worker exposed to zinc oxide fumes reported chills with muscle aches and dyspnea; a chest radiograph revealed diffuse interstitial shadows. After a 10-day period of non-exposure, the chest radiograph was normal. A lung function test was performed after the worker was away from work for 30 days; forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), and the FEV_1/FVC ratio were normal. The worker was then exposed to his usual work environment for 1 hour on two consecutive days. Significant decreases in FEV_1 (16-20%) and FVC (10-11%) were observed on both days, 4-6 hours after exposure; buccal temperature was also increased and the worker experienced malaise and general muscle ache. In the second case (Malo et al., 1993), lung function tests were performed 3 months after the worker left work and after the worker returned to work for 1 day. A decrease in FEV_1 (24%) was observed after the worker returned to work (lung function was normal prior to returning to work). Total zinc concentrations in the work environment were 0.26-0.29 mg/m^3.

In a series of experiments by Drinker et al. (1927b), a group of five men and three women received face-only exposure to various concentrations of zinc oxide for 6-40 minutes.

Two of the men were exposed to several different concentrations; the remaining subjects were exposed to only one concentration. Body temperature was used as an indicator of metal fume fever. The magnitude of the increase in body temperature appeared to be concentration-related. Based on the results of this study and epidemiology data, the study authors concluded that workers exposed to less than 15 mg Zn/m^3 in the air were not likely to develop metal fume fever.

The results of more recent studies suggest that metal fume fever will occur at lower concentrations. In a study by Fine et al. (1997), a group of 13 healthy, non-smoking subjects without any previous exposure to zinc oxide fumes were exposed to 0, 2.5, or 5 mg/m^3 furnace-generated zinc oxide for 2 hours. The subjects were exposed to all three concentrations; each exposure was separated by a 48-hour non-exposure period. Significant increases in oral temperature were observed 6-12 hours after exposure to 2.5 or 5 mg/m^3 zinc oxide fume. A statistically significant increase in the number of symptoms reported was also observed after exposure to 5 mg/m^3. The symptoms occurred 6-9 hours after exposure, and all symptoms were resolved by the next day after exposure. The commonly reported symptoms were fatigue, muscle ache, and cough. Levels of plasma interleukin-6 were significantly increased after exposure to 2.5 or 5 mg/m^3; peak levels were observed 6 hours after exposure.

Gordon et al. (1992) exposed four adults to 5 mg/m^3 zinc oxide fumes or furnace gases for 2 hours. All subjects reported symptoms 4-8 hours after zinc oxide exposure; the symptoms included chills, muscle/joint pain, chest tightness, dry throat, and headache. No significant alterations in lung function were observed following zinc oxide exposure.

Martin et al. (1999) described a cohort of 20 Chinese workers who were exposed to zinc oxide over a single 8-hour workday. Subjects were given an examination by a physician, a spirometric evaluation, and chest radiographs before beginning work, immediately after the shift, and 24 hours after the start of exposure. Exposure concentrations, measured twice per individual during the 8-hour shift, ranged from 0-36.3 mg/m^3. However, as no significant association between airborne zinc measurements and serum zinc levels was present, the reliability of these measurements in reflecting actual zinc exposure is uncertain. No subject showed signs of metal fume fever. Chest radiographs likewise did not reveal any changes over the period examined. Similarly, no changes in respiratory parameters, assessed by spirometry, were reported as a result of exposure.

Zerahn et al. (1999) described the effects of an accidental exposure of 13 soldiers (11 men and 2 women) to an unknown level of zinc chloride smoke during a combat exercise. Blood samples were obtained on day 2, as well as after 1, 2, 4, and 8 weeks. Blood samples from 10/13 subjects were available on day 0, and at week 29. Spirometric analyses of lung function parameters were performed on day 1 postexposure, as well as 1, 2, 4, 8, and 29 weeks after the exposure. Radiographs were taken from day 1 after exposure and during followup. Significant decreases in lung diffusion capacity were observed from 1 week postexposure through the end of the study, with the lowest value occurring at week 4. A significant decrease in total lung capacity was seen at week 4 only, and a decrease in vital capacity at week 2 only. Plasma levels of fibrinogen were also elevated from weeks 1-8 postexposure.

Pettilä et al. (2000) described three cases of patients who inhaled an unknown level of zinc chloride smoke for 1-5 minutes and developed acute respiratory distress syndrome. Two of the three died as a result of exposure; autopsy revealed edema, pulmonary sepsis, emphysematic changes, and necrosis in both cases. The third patient developed respiratory distress on day 2 postexposure, and received supportive therapy. Four months after smoke inhalation, pulmonary function tests were 41-44% of the expected values, and revealed severe restrictive pulmonary dysfunction.

4.3. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

4.3.1. Oral Exposure

As with the human studies, oral animal studies have identified several critical targets of zinc toxicity. The sensitive targets of toxicity include alterations in copper status (Straube et al., 1980; L'Abbe and Fischer, 1984a, b; Bentley and Grubb, 1991), hematology (Straube et al., 1980; Maita et al., 1981; Bentley and Grubb, 1991; Zaporowska and Wasilewski, 1992), and damage to the kidneys (Straube et al., 1980; Maita et al., 1981; Llobet et al., 1988), pancreas (Aughey et al., 1977; Maita et al., 1981), and gastrointestinal tract (Maita et al., 1981).

Maita et al. (1981) exposed groups of 12 male and 12 female Wistar rats and ICR mice to 0, 300, 3000, or 30,000 ppm zinc sulfate (hydration state not reported) in the diet for 13 weeks. The study authors estimated zinc sulfate intakes of male rats to be 23.2, 234, and 2514 mg/kg-day (5.3, 53, and 572 mg supplemental Zn/kg-day). In the case of females, the authors estimated the doses as 24.5, 243, and 2486 mg $ZnSO_4$/kg-day (5.6, 55, and 565 mg supplemental Zn/kg-

day). For male mice the estimated doses were 42.7, 458, and 4927 mg $ZnSO_4$/kg-day (9.7, 104, and 1119 mg supplemental Zn/kg-day) and 46.4, 479, and 4878 mg $ZnSO_4$/kg-day (10.5, 109, and 1109 mg supplemental Zn/kg-day) for female mice. Zinc intakes from the control diet were not estimated.

In rats, no adverse clinical signs or increases in mortality were observed (Maita et al., 1981). Body weight gain was decreased in the high-dose male rats, as was food and water intake. Several statistically significant alterations in hematology and serum clinical chemistry parameters were observed in the high-dose rats; these included decreases in hematocrit and Hb levels in males, decreases in leukocyte levels in males and females, decreases in serum total protein, cholesterol, and calcium levels in males, and decreases in serum calcium levels in females. Significant decreases in absolute and relative liver and spleen weights were observed in the high-dose male rats; decreases in absolute weight were also observed in a number of other organs in the high-dose males which were probably related to the decreased body weight. No other consistent alterations in organ weights were observed. Histopathological lesions were limited to the pancreas of high-dose rats; however, significant increases in the incidence of degeneration and necrosis of acinar cells, decreased number of acinar cells, clarification of centroacinar cells and "ductule-like" metaplasia of acinar cells, and interstitial fibrosis were observed. Incidences of these lesions were not reported.

In mice, an increase in mortality was observed in the high-dose group (5/24 mice died); impairment of the urinary tract and regressive changes (decreased number of acinar cells) in the pancreas were observed in the animals dying early (Maita et al., 1981). Decreases in body weight gain were also observed in both sexes of high-dose mice. In the low- and mid-dose male mice, there were significant increases in Hb and erythrocyte levels. Significant decreases in hematocrit, Hb, and erythrocyte levels were observed in the high-dose male and female mice; a significant decrease in hematocrit level was also observed in the mid-dose male mice. Total leukocyte levels were also decreased in the high-dose male mice. Several statistically significant alterations in serum clinical chemistry parameters were observed in the high-dose mice, including slight-to-moderate decreases in total protein, glucose, and cholesterol and moderate-to-marked increases in alkaline phosphatase and urea nitrogen. Decreases in total protein and increases in alkaline phosphatase and urea nitrogen were also observed in the mid-dose male mice, although the study authors stated that the values were within acceptable historical limits. Histological alterations were observed in the pancreas, gastrointestinal tract, and kidneys of high-dose mice; incidences were not reported. Pancreatic alterations included an increased

number of acinar cells, many displaying necrosis, swollen nuclei, and/or ductule-like metaplasia. Slight-to-moderate ulcerative lesions in the boundary of the forestomach, inflammation of the mucous membranes of the "upper intestine" with proliferation of epithelial cells, and edema at the lamina propria were observed.

In a study by L'Abbe and Fischer (1984a), groups of 10 weanling male Wistar rats were fed a basal diet supplemented with 15, 30, 60, 120, or 240 ppm zinc as anhydrous zinc sulfate for 6 weeks; the 30 ppm group served as the control group. Using a reference body weight of 0.217 kg and food intake of 0.020 kg/day (U.S. EPA, 1988), daily doses of 1.4, 2.8, 5.5, 11, and 22 mg supplemental Zn/kg-day were estimated. Although a linear relationship between zinc intake and serum ceruloplasmin levels was not established, the number of animals with abnormal ceruloplasmin levels increased with increasing doses. Abnormal ceruloplasmin levels were observed in 0, 0, 11, 30, and 100% of the animals in the 15, 30, 60, 120, and 240 ppm groups, respectively. The study authors estimated that the ED_{50} for low ceruloplasmin levels was approximately 125 ppm. Dose-related decreases in liver Cu, Zn-superoxide dismutase and heart cytochrome c oxidase activities were observed at dietary zinc levels greater than 30 ppm, reaching statistical significance in the 120 and 240 ppm groups. Heart Cu, Zn-superoxide dismutase and liver cytochrome c oxidase activities were not affected.

In a second study, L'Abbe and Fischer (1984b) fed groups of 10 weanling male Wistar rats diets containing normal (30 mg Zn/kg diet) or supplemented (240 mg Zn/kg diet) zinc (as zinc sulfate) and normal (6 mg Cu/kg diet) or deficient (0.6 mg Cu/kg diet) copper for up to 6 weeks. Groups of rats were sacrificed at 2, 4, and 6 weeks. Blood, heart, and liver samples were collected for analysis. No significant differences in body weight or food consumption were noted among treated groups. Similarly, no differences were seen in Hb levels. Serum and heart copper levels were significantly decreased in rats fed either zinc-supplemented or copper-deficient diets. In both the high zinc and copper-deficient groups, activity levels of serum ceruloplasmin, liver and heart Cu, Zn-superoxide dismutase, and liver and heart cytochrome c oxidase were significantly reduced relative to control animals by 2 weeks of exposure, and remained reduced throughout the study.

Zaporowska and Wasilewski (1992) exposed groups of 13 male and 16 female Wistar rats to 0 or 0.12 mg Zn/mL as zinc chloride in the drinking water for 4 weeks. The study authors estimated the daily drinking water dose to be 11.66 mg Zn/kg-day in males and 12.75 mg Zn/kg-day for females. Although significant decreases in food and water intake were observed, body

weight gain was not significantly different from controls. Significant alterations were observed in several hematological endpoints including decreases in erythrocyte and Hb levels, increases in total and differential (neutrophils and lymphocytes) leukocyte levels, and increases in the percentage of reticulocytes and polychromatophilic erythrocytes.

Bentley and Grubb (1991) fed groups of seven-eight male New Zealand white rabbits diets containing 0, 1000, or 5000 μg supplemental zinc/g as zinc carbonate (0, 34, 170 mg supplemental Zn/kg-day using an estimated time-weighted-average body weight of 2.5 kg and an allometric equation for food intake [U.S. EPA, 1988]) for 8 (1000 μg/g group) or 22 weeks (5000 μg/g group); the basal diet contained 105.5 μg Zn/g. No adverse alterations in body weight gain were observed. A significant decrease in Hb levels were observed in the 5000 μg/g group. Significant decreases in serum copper and increases in serum and tissue (liver, kidney, brain, testis, pancreas, thymus, skin, bone, and hair) zinc levels were also observed in the 5000 μg/g group. No effects were reported at other dose levels.

de Oliveira et al. (2001) exposed groups of 9 or 12 male and female Swiss mice to 0 or 1% hydrated zinc acetate (0 or 793 mg Zn/kg-day), assuming reference body weight and drinking water consumption values from U.S. EPA (1988), beginning in the first month of life and lasting for 60 days. Animals were evaluated using a shock avoidance behavioral test at the end of their 60-day exposure period. The animals were placed in a two-compartment chamber where one compartment was dark and the other lighted. When placed in the lighted compartment, the mice (who prefer the dark) moved into the dark compartment where they received an electric shock upon contact with the dark room floor. On the next day when the animals were placed in the lighted compartment, the time before they moved into the dark compartment increased significantly from the time on the first day, signifying that they had learned from the adverse day zero experience. There was no significant difference in the time before dark room entry between the control and zinc-exposed animals on test day 1. Entry into the dark chamber did not result in shock treatment on test day 1.

The control and zinc-exposed animals continued to be tested on days 7, 14, 21, and 28. No shock was given on any of these test days. The initial period in the lit room before entering the dark room decreased over time for both the control and the zinc-exposed groups. However, the decrease over time was greater in the zinc-exposed group signifying a more rapid extinction of the learned avoidance response. The time spent in the lighted chamber before entry into the dark room was significantly lower (about half of that for the controls) for the zinc-exposed

animals on day 28. Accordingly, postnatal zinc exposure appeared to have a negative effect on the retention of a learned behavioral response.

Llobet et al. (1988) examined the effects of subchronic oral administration of zinc in Sprague-Dawley rats. Forty female rats were exposed to 0, 160, 320, and 640 mg/kg-day zinc acetate dihydrate in the drinking water (0, 48, 95, and 191 mg Zn/kg-day) for 12 weeks. Sugar was added to all drinking water of all groups to reduce unpalatability. Food and water were provided *ad libitum*. Food and water consumption, volume of urine, and weight of excreted feces were measured daily and body weights were measured weekly. After 12 weeks of treatment, blood samples were collected and analyzed for hematocrit, Hb, glucose, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, urea, and creatinine concentrations. The brain, heart, lungs, spleen, liver, and kidneys were weighed, analyzed for zinc concentration, and (all but the brain) examined histologically. Zinc concentrations were also determined for bone, abdominal muscle, and blood. Clinical signs noted were apathy and two deaths in the 640 mg/kg-day group. Statistically significant decreases in water intake and urine output were observed in the 640 mg/kg-day group; a decrease in urine output was also observed in the 320 mg/kg-day group for 3 of the 6 two-week measurement periods. No alterations in body weight gain or organ weights were observed. Increases in blood urea and creatinine levels in the 640 mg/kg-day group were the only significant alterations in hematological or serum clinical chemistry parameters. Zinc concentrations were significantly increased in the liver, kidneys, heart, bone, and blood of rats in the 320 and 640 mg/kg-day groups. The study authors noted that the "most severe histological alterations were observed in kidneys," but it is unclear, from the limited reporting of the histological results, if lesions were observed in other tissues. The described renal lesions included flattened epithelial cells in the Bowman's capsule, desquamation of the proximal convoluted tubules, and pyknotic nuclei in the 640 mg/kg-day group.

Straube et al. (1980) examined the effects of excess dietary zinc in ferrets. Adult ferrets (six males, nine females), weighing 500-700 g, were divided into four groups and fed a basal diet of canned dog food (that contained 27 ppm zinc and 3.3 ppm copper) plus 0 (five animals), 500 ppm (three animals), 1500 ppm (four animals), or 3000 ppm (three animals) supplemental zinc as zinc oxide. Doses of 0, 142, 425, and 850 mg supplemental Zn/kg-day, respectively, are estimated using the midpoint of the range of initial body weights and the amount of food given to each animal (170 g per day, assumed to be consumed completely each day). Animals in the 1500 and 3000 ppm groups showed signs of severe toxicity and were sacrificed or died within

the first 3 weeks. Animals in the 500 ppm group were sacrificed on days 48, 138, and 191, and the controls were sacrificed on days 27, 48, 138, 147, and 197. The following parameters were used to assess toxicity: hematology (Hb, packed cell volume, erythrocyte, leukocyte, and reticulocyte levels), serum clinical chemistry (urea nitrogen, bilirubin, ceruloplasmin oxidase activity, and blood glucose), and histopathology (kidney, liver, pancreas, lung, heart, stomach, intestine, spleen, bone marrow, and brain). Severe decreases in food intake (80%) and body weight loss (12-50%) were observed in the 1500 and 3000 ppm groups. Additional effects observed in the 1500- and 3000-ppm groups included: macrocytic hypochromic anemia, increased reticulocyte count, diffuse nephrosis, and the presence of protein, glucose, blood, and bilirubin in the urine. The 500 ppm group showed no clinical signs of toxicity. Increases in tissue zinc levels, decreases in copper levels, and decreased ceruloplasmin oxidase activity were observed at all three dietary concentrations.

Aughey et al. (1977) investigated the effects of supplemental zinc on endocrine glands in groups of 75 male and 75 female C3H mice by administering 0 or 0.5 g/L zinc (as zinc sulfate) in the drinking water for up to 14 months. The authors reported that the body weight in the control group ranged from 21 to 30 g, and the mean weight of the zinc-fed mice was approximately 1 g higher. Using the midpoint of the body weight range (0.022 to 0.031 kg), a water intake of 0.0069 L/day was calculated (U.S. EPA, 1988), resulting in average daily drinking water doses of 0 or 135 mg Zn/kg-day. At 1 month intervals, five mice in each of the treated and control groups were killed. After 6 months of exposure to zinc, there were no significant changes in plasma insulin or glucose levels as compared to controls. Histological alterations were observed in the pancreas, pituitary gland, and adrenal gland of zinc-exposed mice. The histological changes in the mice were first observed after 3 months of exposure to zinc. In the zinc-supplemented mice, the pancreatic islets were enlarged and had a vacuolated appearance. The β-cells of the pancreatic islets were larger with enlarged mitochondria and prominent Golgi apparatus. The severity of the pancreatic lesions appeared to increase with increasing exposure durations. Pituitary alterations consisted of changes in the adrenocorticotrophic hormone-producing cells that indicated increased synthesis and secretion, including increased number and size of granules and more prominent rough endoplasmic reticulum and Golgi apparatus. Hypertrophy of the adrenal zona fasciculata and increased adrenal cortical lipid and cholesterol deposition were also observed. No tumors were reported in the pancreas, pituitary gland, or adrenal gland of zinc-exposed mice; data on other organs were not reported.

In a 1-year study, an unspecified number of newborn Chester Beatty stock mice (sex not reported) were administered 0, 1000, or 5000 ppm zinc (approximately 0, 170, or 850 mg/kg/day) as zinc sulfate in drinking water (Walters and Roe, 1965). A separate group of mice received zinc oleate in the diet at an initial dose of 5000 ppm supplemental zinc; this dose was reduced to 2500 ppm after 3 months and to 1250 ppm after an additional 3 months because of mortality due to anemia. An epidemic of the ectromelia virus caused the deaths of several mice during the first 8 weeks; consequently, additional control and test-diet groups were established. There was no difference in body weight gain between control and treated groups, except for the dietary zinc group which became anemic. Survival was not reported in treated compared with control groups. An apparent increase in the incidence of hepatomas was observed in treated mice surviving for 45 weeks or longer relative to controls (original and replacement mice were pooled). The hepatoma incidences in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 3/24 (12.5%), 3/28 (10.7%), 3/22 (13.6%), and 7/23 (30.4%), respectively. Incidences of malignant lymphoma in the control, low-dose drinking water, high- dose drinking water, and test-diet groups were 3/24 (12.5%), 4/28 (14.3%), 2/22 (9%), and 2/23 (8.7%), respectively. Incidences of lung adenoma in the control, low-dose drinking water, high-dose drinking water, and test-diet groups were 10/24 (41.7%), 9/28 (32.1%), 5/22 (22.7%), and 9/23 (39.1%), respectively. None of these were significantly elevated in a statistical analysis of these data performed by the EPA.

Halme (1961) exposed tumor-resistant and tumor-susceptible strains of mice to zinc in drinking water. In a 3-year, 5-generation study, zinc chloride was added to the water of tumor-resistant mice (strain not specified); the groups received 0, 10, 20, 50, 100, or 200 mg Zn/L. The spontaneous tumor frequency for this strain of mice was 0.0004%. The tumor frequencies in the generations were reported as: F0=0.8%, F1=3.5%, F1 and F2=7.6%, and F3 and F4=25.7%. Most of the tumors occurred in the 10- and 20-mg Zinc dose groups. No statistical analyses and no individual or group tumor incidence data were reported. In the tumor-susceptible mice, strains C3H and A/Sn received 10-29 mg Zn/L in their drinking water for 2 years; 33/76 C3H strain mice developed tumors (31 in females) and 24/74 A/Sn strain mice developed tumors (20 in females). Most of the tumors were reported to be adenocarcinomas, but the tissues in which they occurred were not reported. The numbers of specific tumor types were not reported. The overall tumor frequencies (43.4% for C3H and 32.4% for A/Sn, both sexes combined) were higher than the spontaneous frequency (15% for each strain), although no statistical analyses were reported.

4.3.2. Inhalation Exposure

As with most of the human inhalation studies, inhalation studies in animals have focused exclusively on the toxicity of zinc from acute exposures. No relevant subchronic or chronic animal inhalation studies of zinc compounds were located.

In a multispecies study, Gordon et al. (1992) exposed an unspecified number of male Hartley guinea pigs, Fischer 344 rats, and New Zealand rabbits to freshly generated zinc oxide particles. The guinea pigs and rats received nose-only exposure to 0, 2.5, or 5.0 mg/m^3 zinc oxide for 3 hours; the rabbits received nose-only exposure to 0 or 5.0 mg/m^3 zinc oxide for 2 hours. Animals were sacrificed 0, 4, or 24 hours following cessation of exposure. The lungs were lavaged, and the lavage fluid and recovered cells were examined for evidence of inflammation. Significant increases in lavage fluid parameters (lactate dehydrogenase, β-glucuronidase, and protein content) were observed 24 hours after the guinea pigs and rats were exposed to 2.5 or 5.0 mg/m^3. No significant alterations in lavage parameters were observed in the rabbits. The ability of alveolar macrophages to phagocytize particles was assessed in guinea pigs and rabbits. In the guinea pigs exposed to 5.0 mg/m^3, there was a significant reduction in phagocytic capacity (percentage of viable macrophages engulfing four or more particles), but no effect on phagocytic index (percentage of macrophages engulfing particles). Phagocytic ability was not adversely affected in the rabbits. The authors suggested that the reason rabbits were less affected was a lower retention of the inhaled zinc particles (4.7% in rabbits, compared to 11.5% in rats and 19.8% in guinea pigs), resulting in a lower dose per unit tissue mass.

Lam et al. (1988) exposed groups of seven-eight male Hartley guinea pigs to 2.7 or 7 mg/m^3 (average concentrations) freshly formed ultrafine zinc oxide aerosols (count median diameter of 0.05 μm; geometric standard deviation of 2.0) for 3 hours/day for 5 days. Two groups of eight guinea pigs were exposed to furnace gases for 3 hours on one of two days; the two groups were combined and served as the control group. No significant alterations in tidal volume, functional residual capacity, residual volume, respiratory frequency, airway resistance, or compliance were observed. Gradual decreases in total lung capacity (significant after day 4), vital capacity (significant after day 2), and single-breath diffusing capacity for carbon monoxide (significant after day 4), relative to controls, were observed in the 7 mg/m^3 group, but not in the 2.7 mg/m^3 group. Significant increases in relative and absolute lung weights were also observed in the 7 mg/m^3 group.

Lam et al. (1988) also assessed the effect of a single high peak of zinc oxide on lung function. In the first of the two experiments, eight male Hartley guinea pigs were exposed to 4.0 mg/m^3 zinc oxide for 3 hours on day 1; on day 2, the animals were exposed to 34 mg/m^3 for the first hour and to 4.0 mg/m^3 for the remaining 2 hours. Significant decreases in total lung capacity and vital capacity were observed on days 2, 3, 4, and 5; apparent alveolar volume was decreased on day 3. Relative lung weights were decreased on days 2-5. In general, the decrements in lung function parameters and lung weight changes peaked at day 3. Increase in respiratory resistance and decrease in respiratory compliance were observed on days 1 and 2. Increases in absolute and relative lung weights were observed on days 2-5.

In the second experiment, eight male Hartley guinea pigs were exposed to 6 mg/m^3 (average concentration) 3 hours/day for 5 days; the animals were exposed to 25 mg/m^3 during the first hour of exposure on day 1. Several lung function parameters were significantly altered, including decreases in vital capacity and total lung capacity on days 1-5, decreases in functional residual capacity and residual volume on days 2-5, a decrease in apparent alveolar volume on day 3, and increases in single-breath diffusing capacity for carbon monoxide on days 1-5. A gradual, but statistically significant increase in respiratory resistance and decrease in respiratory compliance was observed on days 1-5. Increases in absolute and relative lung weights were observed on days 2-5.

Amdur et al. (1982) exposed groups of 23 male Hartley guinea pigs to 0.91 mg/m^3 freshly-generated zinc oxide for 1 hour. A significant decrease in respiratory compliance was observed immediately after exposure and 1 hour postexposure. No alterations in respiratory frequency, tidal volume, or minute volume were observed. Similar results were observed in another study by this group in which seven guinea pigs were exposed to 0.90 mg/m^3 zinc oxide for 1 hour. This study showed that compliance continued to decrease between the first and second postexposure hours.

4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.4.1. Oral Exposure

4.4.1.1. *Reproductive and Developmental Studies in Humans*

No human studies were identified which examined the potential of zinc to induce reproductive or developmental effects. Studies which examined the influence of zinc supplementation in pregnant women with marginal zinc intakes are discussed in Section 4.1.

4.4.1.2. *Reproductive Studies in Animals*

The reproductive and developmental toxicity of zinc has been investigated in several animal studies. Studies in rats provide evidence that high oral doses of zinc (>25 mg/kg-day) adversely affect spermatogenesis (Saxena et al., 1989; Evenson et al., 1993) and result in impaired fertility (decreased number of implantation sites and increased number of resorptions) in exposed females (Sutton and Nelson, 1937; Schlicker and Cox, 1968; Kumar, 1976; Pal and Pal, 1987).

In two separate experiments, Saxena et al. (1989) exposed an unspecified number of adult male Sprague-Dawley rats to 0 or 500 ppm of supplemental zinc (zinc form not specified) in the diet for 3 or 6 weeks. Using averages of the weekly body weight and food intake data provided, the supplemental zinc intake is calculated to have been 20 mg/kg-day for the 3-week experiment and 28 mg/kg-day for the 6-week experiment. In general, there were no adverse effects on food intake or body weight gain in the rats fed the high zinc diet for 3 or 6 weeks. The study authors noted an increase in swelling of the cervical and pectoral girdle lymph nodes and lameness of the forelimbs in the zinc-exposed animals, and that the degree of swelling increased with exposure duration; however, no data were provided to assess the statistical significance of this effect. General loss of hair and roughness of fur with subcutaneous hematomas were also noted in the rats exposed for 6 weeks. With the exception of a statistically significant increase in caput epididymis weight in the rats exposed for 3 weeks, there were no significant alterations in relative weights of reproductive tissues (testes, caput epididymis, cauda epididymis, seminal vesicles, prostate). Zinc intake significantly affected enzyme activities in tissues of the male reproductive system. Significant decreases in lactic dehydrogenase were observed in the testes, caput epididymis, cauda epididymis (6 weeks only), seminal vesicles, and prostate (6 weeks only) after 3 or 6 weeks of exposure. Increases in arylsulfatase activity were observed in the seminal vesicles after 3 or 6 weeks of exposure and in the cauda and caput epididymis after 6 weeks of exposure. Leucyl aminopeptidase activity was significantly increased in the testes, caput epididymis (3 weeks only), cauda epididymis, seminal vesicles (3 weeks only), and prostate gland after 3 or 6 weeks of exposure. Histological examination of the gonads of rats consuming increased levels of zinc for 3 weeks revealed meiotic arrest at the primary spermatocyte stage, degenerating secondary spermatocytes, fluid accumulation within the

seminiferous tubules, and reduced epithelial cell height in the epididymis. After 6 weeks of exposure, histological examination of the testes revealed additional evidence of arrested spermatogenesis. The germinal epithelium contained only spermatogonia, one layer of primary spermatocytes, and a few pyknotic secondary spermatocytes; no mature spermatozoa were present in the cauda epididymis. Necrotic nuclei were observed among Sertoli cells, Leydig cells, and in the epithelia of prostatic follicles and seminal vesicles. Fertility tests were not carried out in this study.

Evenson et al. (1993) fed groups of 10 male Sprague-Dawley rats a diet containing deficient, adequate or excessive amounts of zinc (4, 12, or 500 mg total Zn/kg food) for 8 weeks; using the average of the initial and terminal body weight data provided in this paper and an allometric equation for food intake (U.S. EPA, 1988), the average dosages of zinc are estimated to be 0.4, 1, or 49 mg total Zn/kg-day. Body weight gain was directly related to the zinc dose, but there was no effect on the relative testicular weight. Flow cytometric data revealed that excess zinc caused abnormalities in the chromosome structure of sperm. The authors suggested that excess zinc, represented by the highest dose group, destabilizes disulfide bonds and complexes with protamine (a basic protein in the sperm) molecules, leading to a destabilization of sperm chromatin quaternary structure and greater susceptibility to DNA denaturation. No fertility tests were carried out in this study.

Sutton and Nelson (1937) maintained groups of young female (n=3) and male (n=2) rats on basal diets supplemented with 0, 0.10, 0.50, or 1.0% zinc as zinc carbonate for 10-39 weeks. Using reference values for body weight (0.124 kg) and food intake (14 g) (U.S. EPA, 1988), supplemental zinc intake is estimated as 0, 113, 565, or 1130 mg/kg-day. Hematological alterations consisting of a 20% decrease in Hb level in the 0.50% group, a 42-57% decrease in Hb level in the 1.0% group, and 15-28% decrease in erythrocyte level in the 1.0% group were observed. No hematological alterations were observed in the 0.10% group. Growth, reproduction, and development were reported to be normal for the 0.10% group over several generations. Adverse reproductive effects were observed in the 0.50% group; there were several stillbirths in the first pregnancy, after which there were no live young born. Rats in this group ceased to become pregnant after 5 months, although their body weights appeared normal. Reproduction and development were reported to have returned to normal in this group after excess zinc was withheld from the diet. No data were presented in support of this statement, so the timeframe of recovery is not known. Most of the animals on the 1.0% zinc diet failed to grow normally and some died within 4 weeks; no reproduction occurred in this dose group.

Since both males and females were treated with zinc, but no histopathological examination of the gonads was performed, it is not possible to determine the immediate cause of reproductive failure at higher dose levels.

Pal and Pal (1987) added 4000 ppm of zinc as zinc sulfate to the diet of 12 Charles-Foster female rats for 18 days beginning immediately after coitus. Using the reference values for food intake and body weight (U.S. EPA, 1988), supplemental zinc intake is estimated at 450 mg/kg-day. The incidence of conception in the treated group was significantly reduced compared to controls (5/12 vs. 12/12). In those animals that did conceive, the number of implantation sites per pregnant female was not significantly altered. Zinc treatment had no effect on the number of resorption sites and there were no stillbirths or malformations among the offspring of treated rats. In a separate experiment in which female rats were fed 4000 ppm supplemental zinc for 3 weeks prior to mating, the incidence of conception and fetal outcome were not adversely affected by treatment.

In a series of four studies conducted by Schlicker and Cox (1968), groups of 10-20 female Sprague-Dawley rats were fed a control diet or a diet containing supplemental zinc oxide prior to mating and/or during gestation. The exposure protocols for the four studies were as follows: (1) 10 rats fed 0 or 0.4% dietary zinc on gestational days 0 through 15 or 16, (2) 20 rats fed 0 or 0.4% supplemental zinc on gestational days 0 through 18 or 20, (3) 20 rats fed 0 or 0.4% supplemental zinc for 21 days prior to mating through delivery, and (4) 10 rats fed 0 or 0.2% supplemental zinc for 21 days prior to mating through gestational day 15. Using initial body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), excess zinc intake by dams is estimated as 0, 200, or 400 mg/kg-day for the 0, 0.2, and 0.4% dietary concentrations, respectively. Dams were sacrificed on the final day of exposure, and the fetuses removed for examination. A 4-29% fetal resorption rate was observed in the dams exposed to 0.4% zinc beginning on gestational day 0 (studies 1 and 2). In rats exposed to 0.4% zinc prior to mating and during gestation, there was a 100% resorption of the fetuses. Significant decreases in body weight were observed in the fetuses of rats exposed to 0.4% zinc on gestational days 0-15 16, 18, or 20, but not in the 0.2% group exposed prior to mating and during gestational days 0-15. No external malformations were observed in the 0.4% group exposed during gestation or in the 0.2% group exposed prior to and during gestation.

In a single-generation study of reproductive performance, Khan et al. (2001) exposed groups (n=5-7) of male and female Sprague-Dawley rats to 0, 3.6, 7.2, 14.4, or 28.8 mg Zn/kg-

day, as zinc chloride, by gavage. Animals were exposed 7 days per week for 77 days prior to cohabitation and throughout the 21-day cohabitation period; females were also exposed during each of the 21-day gestation and lactation periods. Evaluated reproductive parameters included fertility, viability index, weaning index, litter size, and pup body weight. No significant changes were seen in body weights of the exposed rats prior to birth, but postpartum dam body weights for the mid- and high-dose groups were significantly decreased, relative to controls. The fertility indices in all dose groups were significantly lower than in the control group, though no dose-related trends were noted. At the highest two dose levels, the number of live pups per litter, but not total pups per litter, was significantly decreased, as was live pup weight at postnatal day 21, though not at days 4, 7, or 14. No other changes in reproductive parameters were noted, and no effects on serum clinical chemistry endpoints were reported.

Kumar (1976) compared the effect of different levels of dietary zinc on pregnancy in an unspecified strain of rats. Beginning on day 1 of pregnancy, 12 control rats were fed a basal diet containing 30 ppm of zinc (3.39 mg/kg-day), and 13 rats were fed the basal diet plus 150 ppm supplemental zinc (as zinc sulfate, ~20 mg/kg-day total zinc). The dams were sacrificed on gestational day 18. No alterations in the number of implantation sites were found, but a statistically significant increase in the number of resorptions (9.5%) was observed in the zinc-supplemented group.

Kinnamon (1963) fed groups of five Sprague-Dawley female rats a diet containing 0 or 0.5% supplementary zinc as zinc carbonate for 5 weeks prior to mating with untreated males and for the first 2 weeks of gestation. At the end of the 7-week period, the rats were injected with radiolabelled zinc chloride, then housed in metabolism cages for 4 days prior to sacrifice. Using the body weight data provided and an allometric equation for food intake (U.S. EPA, 1988), supplemental zinc doses of 0 or 500 mg/kg-day were calculated. No significant differences in number of fetuses per litter, wet weight of the litter, or average weight per fetus were observed.

4.4.1.3. *Developmental Studies in Animals*

Several studies have examined the developmental toxicity of zinc. Studies by Schlicker and Cox (1968) and Ketcheson et al. (1969) have found decreases in body weights in the offspring of rats exposed to high doses of zinc in the diet. Additionally, alopecia and achromotrichia have been observed in the offspring of mice and mink exposed to high doses of zinc during gestation and lactation (Bleavins et al., 1983; Mulhern et al., 1986).

Ketcheson et al. (1969) fed groups of 10 pregnant female Sprague-Dawley rats a basal diet containing 9 ppm of zinc or 0.2% or 0.5% supplemental zinc as zinc oxide, throughout gestation and lactation day 14. Using an estimated body weight of 0.300 kg and reported food intake data, estimated maternal supplemental zinc doses are 120 and 280 mg/kg-day during gestation in the 0.2 and 0.5% groups, respectively, and 150 and 400 mg/kg-day during lactation. No significant alterations in maternal body weight or food intake were observed in the zinc-supplemented groups relative to controls. No significant alterations in duration of gestation or the number of viable pups per litter were observed. Significant alterations in newborn and 14-day-old pup body weights were observed; the alterations consisted of an increase in the 0.2% group and a decrease in the 0.5% group. The increase in pup body weight at the 0.2% dietary level suggests that the basal diet did not provide a sufficient amount of zinc to support pregnancy and lactation. No external malformations were reported.

Uriu-Hare et al. (1989) fed groups of eight-nine Sprague-Dawley rats diet containing low, adequate (control group), or high amounts of zinc (4.5, 24.5, or 500 ppm total zinc) during gestational days 1-20. Using estimates of body weight (0.285 kg) and food intake (17 g/day) data presented in graphs, the total dietary intake of zinc is estimated to have been 0.27, 1.45, or 30 mg/kg-day. No adverse effects on maternal body weight gain, hematocrit levels, or the incidences of resorptions, malformations, fetal body weight, or fetal length were observed in the high zinc group, as compared to the adequate zinc group. Adverse effects, including decreases in maternal body weight and increases in resorptions, malformations, and fetal growth were observed in the low-zinc group only.

Mulhern et al. (1986) fed an unspecified number of female weanling C57BL/6J mice a diet containing 50 (normal) or 2000 (high) ppm of zinc as zinc carbonate and, at age 6 weeks, mated them with unexposed males. Each dam and her offspring were assigned to one of 10 groups receiving 50 or 2000 ppm total zinc during gestation, lactation, and postweaning until age 8 weeks. Decreases in hematocrit and body weight were observed in the F_1 mice exposed to 2000 ppm zinc during gestation, lactation, and postweaning. The study authors noted that decreases in body weight gain were observed in other groups; however, the magnitude and statistical significance were not reported. Alopecia was observed in all groups of F_1 mice exposed to 2000 ppm during lactation, regardless of gestational exposure. The mice began to lose hair between 2 and 4 weeks of age, and exhibited severe alopecia at 5 weeks. Exposure to 2000 ppm during lactation and/or post weaning resulted in achromotrichia, which the authors suggest may result from the effects of zinc-induced copper deficiency.

Bleavins et al. (1983) fed groups of adult mink (11 females and 3 males) a basal diet containing 20.2 ppm of zinc or the basal diet supplemented with 500 ppm of zinc as zinc sulfate heptahydrate. After 2 months the animals were mated during an 18-day period; since no clinical signs of zinc toxicity or copper deficiency were noted for the 500-ppm group, 3 days before the end of the mating period, the high dose of zinc was increased to 1000 ppm. Using the reference body weight and an allometric equation for food intake (U.S. EPA, 1988), the intake of zinc is calculated to have been 56 mg/kg-day. Fewer dams (8/11) on the high-zinc diet produced offspring than those on the control diet (11/11); however, gestational length, litter size, birth weights and kit mortality to weaning were not affected. Zinc had no effect on body, liver, spleen or kidney weights, or on hematological parameters (leukocyte, erythrocyte, Hb, hematocrit) in adults. Clinical signs associated with copper deficiency (alopecia, anemia, achromotrichia) were also not observed in adults. However, 3- to 4-week-old kits exhibited achromotrichia around the eyes, ears, jaws, and genitals, with a concomitant loss of hair and dermatosis in these areas. Subsequently, achromotrichia and alopecia spread over much of the body. At 8 weeks, treated kits had lower hematocrit and lower lymphocyte counts, but higher numbers of band neutrophils. At 8 weeks, treated kits exhibited signs of immunosuppression (significantly lowered thymidine incorporation by lymphocytes after stimulation by concanavalin A). Treated male kits had lower body weights than controls at 12 weeks. After weaning, the kits were placed on the basal diet, and within several weeks they recovered.

4.4.2. Inhalation Exposure

No studies examining the reproductive/developmental toxicity of zinc in humans or animals were identified.

4.5. OTHER STUDIES

4.5.1. Acute Toxicity Data

4.5.1.1. *Oral Exposure*

Brewer et al. (2000) reported on the use of zinc supplementation for the treatment of Wilson's disease. Wilson's disease results in an accumulation of copper within the body, eventually leading to hepatic changes and, in some patients, neurologic effects as well. The study authors discussed the results of 26 pregnancies in 19 women with Wilson's disease who received oral zinc acetate (from 25-150 mg Zn/day) prior to and during pregnancy. Urinary

copper, a reliable indicator of body copper status, was able to be maintained within normal levels with zinc supplementation, and hepatic and neurological signs in the affected women returned to normal while treatment continued. Of 26 pregnancies, there were four miscarriages, and two fetal abnormalities; one major (microcephaly) and one minor (surgically correctable heart defect). This study did not include any control subjects; thus these adverse effects cannot be fully correlated to either Wilson's disease or to zinc supplements.

4.5.1.2. *Inhalation Exposure*

Fine et al. (2000) exposed a group of 11 control subjects and a group of 10 sheet metal workers to 5 mg/m^3 of zinc oxide fume for 2 hours on each of 3 consecutive days. Naive subjects showed a number of slight to moderate symptoms following the first exposure, including chills, flushing, fatigue, muscle and stomach aches, dyspnea, and nausea. Following the second and third exposures, the incidence of symptoms among naive subjects were significantly lower than following the first exposure. Similarly, the increase in temperature was greatest among naive subjects after the first exposure, and decreased after the second and third exposures; after the third exposure, the temperature increase was significantly lower than after the first exposure. The temperature changes and incidence of symptoms for sheet metal workers were not significantly different from exposure to control air. Both the response of naive subjects to multiple exposures and the response of sheet metal workers to zinc oxide exposure were cited as evidence of the development of tolerance to zinc fume fever.

4.5.1.3. *Other Methods of Exposure*

In a short-term in vivo assay, Stoner et al. (1976) injected strain A/Strong mice (20/sex/dose) intraperitoneally with zinc acetate 3 times/week for a total of 24 injections (total doses were 72, 180, or 360 mg/kg). Controls (20/sex/group) consisted of an untreated group, a vehicle control group administered 24 injections of saline, and a positive control group administered a single injection of urethane (20 mg/mouse). Mice were sacrificed 30 weeks after the first injection; survival was comparable for all groups. There was no increase in number of lung tumors per mouse in treated animals relative to the pooled controls. While four thymomas were observed in zinc acetate-treated groups and none in controls, the occurrence of these tumors was not statistically significantly elevated.

Guthrie (1956) injected 0.15-0.20 mL of 10% zinc sulfate into the testis of 19 four-month-old rats and 0.15 mL of 5% zinc chloride into the testis of 29 three-month-old rats

(strain not specified). No testicular tumors were observed in either group at sacrifice 15 months after injection. No controls were described.

4.5.2. Genotoxicity

The results of short-term genotoxicity assays for zinc are equivocal. Zinc acetate and/or zinc-2,4-pentanedione have been analyzed in four short-term mutagenicity assays (Thompson et al., 1989). In the Salmonella assay (with or without hepatic homogenates), zinc acetate was not mutagenic over a dose range of 50-7200 μg/plate, but zinc 2,4-pentanedione was mutagenic to strains TA1538 and TA98 at 400 μg/plate. The addition of hepatic homogenates diminished this response in a dose-dependent manner. In the mouse lymphoma assay, zinc acetate gave a dose-dependent positive response with or without metabolic activation; the mutation frequency doubled at 10 μg/mL. In the Chinese hamster ovary cell in vitro cytogenetic assay, zinc acetate gave a dose-dependent positive response with or without metabolic activation, but the presence of hepatic homogenates decreased the clastogenic effect. Neither zinc acetate nor zinc-2,4-pentanedione were positive in the unscheduled DNA synthesis assay in rat hepatocytes over a dose range of 10-1000 μg/mL.

Zinc chloride has been reported to be positive in the Salmonella assay (Kalinina et al., 1977), negative in the mouse lymphoma assay (Amacher and Paillet, 1980), and a weak clastogen in stimulated human lymphocyte cultures (Deknudt and Deminatti, 1978). Zinc sulfate was not mutagenic in the Salmonella/microsome assay (Gocke et al., 1981), and zinc acetate did not induce chromosomal aberrations in unstimulated human lymphocyte cultures (Gasiorek and Bauchinger, 1981). Crebelli et al. (1985) found zinc oxide (99% purity) (1000-5000 μg/plate) not to be mutagenic for reverse mutation in *Salmonella typhimurium*.

Responses in mutagenicity assays are thought to depend on the form (e.g., inorganic or organic salt) of the zinc tested. For example, inorganic salts tend to dissociate and the zinc becomes bound with culture media constituents. Salts that dissociate less readily (i.e., zinc-2,4-pentanedione) tend to be transported into the cell and are postulated to cause a positive response (Thompson et al., 1989).

Zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer protection (Woo et al., 1988). Zinc deficiency enhanced methylbenzylnitrosamine (MBN)-induced carcinoma of the esophagus in male rats (Fong et al., 1978), but retarded the

development of oral cancer induced by 4-nitroquinoline-N-oxide (4-NQO) in 4-week-old female rats (Wallenius et al., 1979). In a study that examined both zinc deficiency and supplementation, Mathur et al. (1979) found that animals with a deficient diet (5.9 mg/kg) and animals with a diet supplemented with excessively high levels of zinc (200-260 mg/kg) had fully developed carcinomas of the palatial mucosa. While the rats were on the specific diets, the palatial mucosa was painted with 4-NQO, 3 times/week for 20 weeks. In the zinc-deficient group, 2/25 rats developed cancer of the palatial mucosa; 2/25 rats in the excessive zinc group also developed this form of cancer. Animals supplemented with moderate levels of zinc in the diet (50 mg/kg) developed only moderate dysplasia. Thus, zinc's modifying effect on carcinogenesis may be dose-dependent.

4.6. INTERACTIONS

Numerous studies have examined the interactions of zinc and other metals; however, the vast majority of these have examined the effect of co-exposure to zinc on the toxicity of the other metal. The few studies that have been conducted on the effect of other metals on the toxicity of zinc are not adequate to support dose-response assessments for the interactions, or even qualitative assessments of the type or direction of the interaction (e.g., antagonism, synergism), particularly under subchronic or chronic exposure conditions. Interactions between zinc and other metals are highly plausible given that the ligand binding reactions of zinc are similar to those of a variety of other essential or toxic divalent cations (Andersen, 1984). These include a relatively high reactivity with thiolate anions (ionized functional groups from cysteine) and formation of relatively stable chelation complexes with multidentate carboxylic acid ligands (similar to calcium and lead). Thus, competition for reactions with sulfhydryls proteins and ligand exchange reactions are potential mechanisms of interaction that may exert effects at the level of zinc transport, binding, catalysis, or stabilization of zinc-dependent enzymes. The displacement of zinc from ALAD by lead is a good example of such an interaction, and is the basis for one aspect of the toxicity of lead (the inhibition of ALAD and heme synthesis) and the ability of zinc to attenuate this effect of lead (Finelli et al., 1975; Simons, 1995).

Binding to and induction of the synthesis of metallothionein appears to play an important role in the physiologic regulation of zinc levels and, possibly, zinc's reactivity as a potential binder of hydroxyl radicals (Li et al., 1980; Udom and Brady, 1980; Goering and Fowler, 1987; Kelly et al. 1996; Liu et al., 1996). A variety of divalent cations including, cadmium, cobalt,

copper, lead, and zinc bind to metallothionein (Stillman, 1995). Expression of metallothionein resulting from cadmium exposure may result in increased liver content of zinc and decreased plasma zinc concentrations; this could potentially give rise to interactions that have toxicologic consequences. For example, displacement of zinc from weakly bound extracellular proteins by cadmium is thought to be involved in the mechanism by which cadmium (and possibly other divalent metals) induces the synthesis of metallothionein (Palmiter, 1994). When cells are deprived of zinc they become very sensitive to zinc and relatively insensitive to cadmium. The increased sensitivity could be due either to increased transport of zinc or to a change in the relative amounts or affinities of metallothionein. The decreased sensitivity of cadmium is predicted if zinc is the only effective inducer, because during zinc starvation the low affinity pool of extracellular zinc would be depleted first; thus addition of small amounts of cadmium would fill this pool without liberating any zinc. Addition of more cadmium would displace zinc from higher affinity pools; thus further depletion would lead to cell death. Induction of metallothionein by zinc has been shown to alter the physiologic disposition of copper and the toxicity of cadmium (Waalkes and Pérez-Ollé, 2000). Recent characterization of divalent metal ion transporters in epithelia, including that of mammalian small intestine, suggest that zinc may share absorptive mechanisms with a variety of divalent cations, including cadmium, copper, iron, and lead (Gunshin et al., 1997; Fleming et al., 1999). This provides at least one mechanism by which co-exposure with other divalent metals could affect zinc absorption, and possibly transport of absorbed zinc in other tissues.

For the most part, however, definitive evidence for any of the above mechanisms giving rise to antagonism or synergism of the toxicity of zinc has not been reported. Information on interactions relevant to the toxicity of zinc and compounds is presented below.

4.6.1. Interactions with Essential Trace Elements

4.6.1.1. *Copper and Zinc*

As discussed above, the most sensitive effects of high supplementary levels of zinc in humans are alterations in the levels of copper-containing enzymes (e.g., Cu, Zn-superoxide dismutase and serum ceruloplasmin) and plasma LDL cholesterol levels. Although studies by Samman and Roberts (1987, 1988), Fischer et al. (1984) and Yadrick et al. (1989) failed to find decreases in plasma copper levels, these studies did find alterations in serum ceruloplasmin and ESOD activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels. Animal studies reported by L'Abbe and Fischer (1984a, b) have demonstrated the reduction of Cu, Zn-superoxide dismutase

activity in the liver and heart as the most sensitive indicator of copper status in rats fed high levels of zinc in their diet. These observations were correlated with similar Cu, Zn-superoxide dismutase activities in the liver and heart of animals fed a copper deficient diet. It is believed that the copper deficiency results from a zinc-induced decrease in copper absorption, although the exact mechanisms are not understood. Excess dietary zinc results in induction of intestinal metallothionein synthesis; because metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells may be sequestered by metallothionein and not absorbed systemically (Walsh et al., 1994).

The above considerations suggest that increased intakes of copper may decrease toxic effects of zinc that are related to copper deficiency; however, this possibility has not been rigorously explored experimentally. Smith and Larson (1946) reported that co-exposure to copper resulted in a partial attenuation of the microcytic and hypochromic anemia resulting from exposure to high levels of dietary zinc. This would be consistent with copper replenishment after zinc-induced copper depletion. Several studies have demonstrated that increased levels of copper can decrease the absorption of zinc. Oestreicher and Cousins (1985) reported that dietary levels of zinc and copper did not affect absorption of zinc or copper in an isolated, perfused rat small intestine model. However, low levels of copper in the perfusion medium resulted in an increased absorption of zinc, while medium and high copper levels resulted in decreased zinc absorption. Kinnamon (1963) reported a significant decrease in uptake of a single gavage dose of radiolabeled zinc in rats fed a diet high in copper for 5 weeks prior to exposure. Gachot and Poujeol (1992) reported exposure of primary rabbit proximal tubule cells to both 15 and 50 μM copper resulted in noncompetitive inhibition of zinc absorption into the cells. Zinc and copper are substrates for a divalent metal transport protein that has been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of zinc and copper has not been determined. However, Klevay (1973) reported that rats fed a diet with a 40:1 ratio of zinc:copper gained less weight than those fed a normal 5:1 ratio, indicating the importance of the relative levels of both zinc and copper in the diet.

4.6.1.2. *Calcium and Zinc*

Hwang et al. (1999) reported that administration of calcium acetate to hemodialysis patients did not result in changes in hair or serum zinc relative to baseline levels, though both levels were lower than normal controls. A review by Lönnerdal (2000) provides evidence that calcium levels do not directly influence the absorption of zinc. It appears, however, that calcium

aggravates zinc deficiency when it is added to diets based on plant products that might be expected to be high in phytate (reviewed in O'Dell, 1969). Heth and Hoekstra (1965) reported a decreased absorption of zinc when calcium was co-administered in the diet, and that increased dietary calcium resulted in an increased rate of zinc loss (shortened clearance half-time).

4.6.1.3. *Iron and Zinc*

O'Brien et al. (2000) reported that percentage zinc absorption was significantly lower in pregnant women who received iron-containing prenatal supplements (60 mg/day) relative to women who had not received iron-containing supplements. Plasma zinc concentrations were also significantly lower after iron supplementation, but not if the supplement also contained 15 mg of zinc. Bouglé et al. (1999) reported a significant correlation between zinc absorption and iron content in the diet, with increased dietary iron resulting in diminished absorption of zinc. However, Lönnerdal (2000) has suggested that at lower iron intake levels, iron has no effect on the absorption of zinc. Zinc and iron are substrates for a divalent metal transport protein that has been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of zinc has not been determined.

4.6.2. Interactions with Other Heavy Metals

4.6.2.1. *Cadmium and Zinc*

Numerous studies have demonstrated that zinc can decrease the carcinogenicity and toxicity of cadmium (Gunn et al., 1963; Waalkes et al., 1989; Coogan et al., 1992; Brzoska et al., 2001), possibly through decreased cadmium absorption or alterations in metallothionein levels (for review, see Krishnan and Brodeur, 1991). Less is known about the effects of cadmium on the pharmacokinetics and toxicity of zinc.

Toxic levels of cadmium may inhibit zinc absorption (Lönnerdal, 2000). Studies conducted in isolated cells or membranes from kidney proximal tubule or small intestine indicate that zinc and cadmium may share common transport and/or binding mechanisms in transporting epithelia (Tacnet et al., 1990, 1991; Prasad and Nath, 1993; Prasad et al., 1996; Endo et al., 1997). For example, Gachot and Poujeol (1992) assessed the effect of cadmium on the uptake of zinc by isolated rabbit proximal tubule cells. At low concentrations (15 μM), cadmium acts as a competitive inhibitor of carrier-mediated zinc uptake, while at higher concentrations (50 μM) it also exhibits noncompetitive inhibition of an unsaturable pathway. Similar results were reported by King et al. (2000) who found that injection of cadmium chloride in mice reduced the uptake of ^{65}Zn by 56% in testes and 47% in brain. Exposure of rats whose diets contained normal

(12 mg/kg) or elevated (60 mg/kg) levels of zinc to 5 mg Cd/L in the drinking water did not alter the amount of zinc or copper in the plasma or liver (Bebe and Panemangalore, 1996). Levels of copper in the kidneys were decreased in animals that were exposed to high-dosages of zinc and cadmium, but not in animals that received normal zinc diets and cadmium; cadmium had no effect on kidney zinc levels. Brzoska et al. (2001) reported that treatment of rats with cadmium resulted in decreased levels of zinc in the tibia; zinc supplementation restored the levels to normal.

4.6.2.2. *Lead and Zinc*

A sizable database on the effects of zinc on lead toxicity exists. However, a detailed discussion of the effects of exposure to zinc on the toxicity of lead is beyond the scope of this document. The effects of zinc on the toxicity of lead are discussed in a review by Krishnan and Brodeur (1991).

Administration of zinc in the diet, but not through injection, has been shown to decrease the toxicity of dietary lead (Cerklewski and Forbes, 1976; El-Gazzar et al., 1978), possibly due to zinc decreasing the intestinal absorption of lead (Cerklewski and Forbes, 1976; Cerklewski, 1979). It is not known if lead will affect the absorption of zinc. However, exposure of rats whose diets contained normal (12 mg/kg) or elevated (60 mg/kg) levels of zinc to drinking water containing 20 mg Pb/L did not alter the amount of zinc or copper in the plasma, kidney, or liver (Bebe and Panemangalore, 1996). This would suggest, though it is hardly conclusive, that lead exposure does not alter zinc absorption. Both zinc and lead have been shown to bind to the N-methyl-D-aspartate receptor site in rats, but lead does not appear to bind to the zinc allosteric site (Lasley and Gilbert, 1999). As noted previously, zinc and lead are substrates for a divalent metal transport protein that has been shown to participate in the absorption of iron (Gunshin et al., 1997). The relative importance of this protein in the absorptive transport of lead or zinc has not been determined.

4.6.2.3. *Cobalt and Zinc*

Anderson et al. (1993) reported that exposure to 400 ppm cobalt chloride in the drinking water of mice for 13 weeks resulted in seminiferous tubule damage and degeneration (vacuole formation, sloughing of cells, giant cell formation) in the testes. Co-exposure to 800 ppm zinc chloride resulted in 90% of the animals exhibiting complete or partial protection against the testicular toxicity of cobalt. No studies examining the potential effects of cobalt compounds on the toxicity of zinc were identified.

4.7. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION – ORAL AND INHALATION

4.7.1. Oral Exposure

The essentiality of zinc was established over 100 years ago. Zinc is essential for the function of more than 300 enzymes, including alkaline phosphatase, alcohol dehydrogenase, Cu, Zn-superoxide dismutase, carboxypeptidase, ALAD, carbonic anhydrase, RNA polymerase, and reverse transcriptase (Vallee and Falchuk, 1993; Sandstead, 1994). A wide range of clinical symptoms have been associated with zinc deficiency in humans (Abernathy et al., 1993; Prasad, 1993; Sandstead, 1994; Walsh et al., 1994). The clinical manifestations of severe zinc deficiency, seen in individuals with an inborn error of zinc absorption or in patients receiving total parenteral nutrition without adequate zinc, include bullous pustular dermatitis, diarrhea, alopecia, mental disturbances, and impaired cell-mediated immunity resulting in intercurrent infections. Symptoms associated with moderate zinc deficiency include growth retardation, male hypogonadism, skin changes, poor appetite, mental lethargy, abnormal dark adaptation, and delayed wound healing. Neurosensory changes, impaired neuropsychological functions, oligospermia, decreased serum testosterone, hyperammonemia, and impaired immune function (alterations in T-cell subpopulations, decreased natural killer cell activity) have been observed in individuals with mild or marginal zinc deficiency. Severe zinc deficiency in animals has been associated with reduced fertility, fetal neurological malformations, and growth retardation in late pregnancy (Mahomed et al., 1989).

Increased zinc consumption, as supplemental zinc, has been associated with changes in health effects in humans, including decreased copper metalloenzyme activity (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989; Davis et al., 2000; Milne et al., 2001), hematological effects such as anemia, neutropenia (Hale et al., 1988), decreases in cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982; Chandra, 1984; Black et al., 1988; Davis et al., 2000; Milne et al., 2001), immunotoxicity (Chandra, 1984), and gastrointestinal effects (Freeland-Graves et al., 1982; Samman and Roberts, 1987, 1988).

Although the decreased copper metalloenzyme activities and cholesterol levels are not necessarily adverse in themselves, they are likely to be indicators of more severe effects occurring at greater dose levels. Several human studies provide evidence that excess zinc intake may induce copper deficiency. Severe copper deficiency has been observed in individuals

ingesting very high doses of zinc for over one year (Patterson et al., 1985; Hoffman et al., 1988). At lower zinc doses, more subtle signs of impaired copper status, such as alterations in copper metalloenzyme activities, are evident. Copper deficiency is thought to result from a zinc-induced decrease in copper absorption. Excess dietary zinc results in induction of intestinal metallothionein synthesis; because metallothionein has a greater binding capacity for copper than for zinc, copper absorbed into the intestinal mucosal cells is sequestered by metallothionein and not absorbed systemically (Walsh et al., 1994). Zinc and copper may also be substrates for a divalent metal transport protein (i.e., CRIP) induced by copper in the small intestine (Gunshin et al., 1997). Although studies by Davis et al. (2000), Milne et al. (2001), Samman and Roberts (1987, 1988), Fischer et al. (1984), and Yadrick et al. (1989) failed to find decreases in plasma copper levels after zinc supplementation, these studies did find alterations in indicators of body copper status, including decreases in serum ceruloplasmin, EC-SOD, and ESOD activities. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels.

While the exact function of HDL is not known, it is thought to function in the transfer of cholesterol from extrahepatic tissue to the liver. Bile acids are synthesized from cholesterol in the liver and carry cholesterol breakdown products to the intestines with the bile, thus providing an excretory pathway for cholesterol. The results of epidemiology studies suggest an association between high concentrations of HDL with a reduced risk of coronary heart disease. As compared to all lipids and lipoproteins measured, HDL may have the largest impact on risk of coronary heart disease in individuals over 50 years old (Simko et al., 1984). Normal levels of HDL-cholesterol are 45.5 mg/dL in men and 55.5 mg/dL in women. HDL-cholesterol levels below 35 mg/dL have been associated with an increased risk of coronary heart disease (Simko et al., 1984). Collectively, the human data suggest that short-term ($\leq$12 weeks) increases in zinc intake result in decreases in HDL-cholesterol levels. In the Hooper et al. (1980) and Chandra (1984) studies, in which subjects received daily doses of 2 or 4 mg supplemental Zn/kg-day for up to 6 weeks, the HDL-cholesterol levels dropped below 35 mg/dL. Although zinc-induced decreases in HDL-cholesterol have been observed, a relationship between increased zinc intake and an increased risk of coronary heart disease has not been established. Additionally, not all human studies have confirmed effects on HDL-cholesterol levels following zinc supplementation (Davis et al., 2000; Milne et al., 2001).

Following high-level oral exposure, zinc appears to exert adverse health effects primarily through interaction with copper. Specifically, high levels of zinc can result in a saturation of the

carrier-mediated pathway of zinc absorption and a shift to metallothionein-mediated absorption (Hempe and Cousins, 1992). It is believed that the copper deficiency results from a zinc-induced decrease in copper absorption. Zinc-induced copper deficiency is consistent with numerous reports of effects of zinc on various biomarkers of copper nutritional status following exposures to elevated levels of zinc in humans and animals, as well as by reports indicating that copper supplementation can result in an attenuation of zinc-induced toxicity.

While co-exposure to zinc has been demonstrated to alter the toxicity of a number of other metals, few studies have been conducted on the effects of co-exposure to metals (other than copper) on zinc toxicity. The available studies suggest the plausibility that co-exposure to other divalent metals may decrease absorption of zinc, but offer only limited insight as to potential effects of these metals on zinc toxicity. The few studies that have been conducted on the effect of other metals on the toxicity of zinc are not adequate to support dose response assessments for the interactions, or even qualitative assessments of the type or direction of the interactions (e.g., antagonism, synergism), particularly under subchronic or chronic exposure conditions.

4.7.2. Inhalation Exposure

Most of the available information on the toxicity of inhaled zinc has focused on metal fume fever, a collection of symptoms observed in individuals exposed to freshly formed zinc oxide fumes or zinc chloride from smoke bombs. The earliest symptom of metal fume fever (also referred to as zinc fume fever, zinc chills, brass founder's ague, metal shakes, or Spelter's shakes) is a metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-like symptoms, chills, fever, profuse sweating, headache, and weakness follows (Drinker et al., 1927a; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur within several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An increase in tolerance develops with repeated exposure; however this tolerance is lost after a brief non-exposure period, and symptoms are most commonly reported on Mondays and after holidays. There are many reports of metal fume fever in the literature; however, most describe individual cases and exposure levels are not known.

In animals, exposure to zinc oxide results in similar effects as those reported in humans. Gordon et al. (1992) examined the effects of zinc oxide in rabbits, rats, and guinea pigs, and reported changes in lavage parameters which appeared to correlate with pulmonary retention of the zinc particles. In a series of studies in guinea pigs, Lam et al. (1988) reported that ultrafine zinc oxide particles resulted in significant respiratory effects, including decreased lung function

and increased lung weight. However, subchronic or chronic studies of the toxicity of zinc following inhalation exposure in animals are not available. Similarly, no studies examining the effects of inhaled zinc on reproductive or developmental endpoints were located.

The mechanisms behind metal fume fever are not known, but are thought to involve several different factors. Exposure to zinc oxide particles has been shown to elicit the release of a number of proinflammatory cytokines, leading to a persistent pulmonary inflammation which could result in some of the reported symptoms of metal fume fever, including decreased lung function and bronchoconstriction. An allergic response to zinc particles, leading to an asthma-like response, has also been proposed as a possible mechanism. However, additional mechanistic information will be required in order to adequately determine the mechanisms involved in the toxicity of inhaled zinc.

4.8. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *inadequate information to assess carcinogenic potential* of zinc, because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available, and results of genotoxic tests of zinc have been equivocal.

Adequate studies examining the carcinogenicity of zinc in orally-exposed humans are not available. Prasad et al. (1978) reported on sickle cell anemia patients who were treated with zinc for 2 years; however, carcinogenic endpoints were not evaluated. Aughey et al. (1977) did not find pancreatic, pituitary, or adrenal tumors in C3H mice exposed to zinc sulfate in the drinking water for up to 14 months; however, histopathology of other organs was not reported. Additional data on the carcinogenicity of zinc following oral exposure are not available. While a number of studies of the effects of short-term exposure to zinc in the workplace are available, the vast majority of these focus on the more acute effects of zinc, particularly metal fume fever and its resulting sequelae. No studies adequately examining the carcinogenic effects of zinc in humans or animals were located in the available literature.

Either zinc deficiency or excessively high levels of zinc may enhance susceptibility to carcinogenesis, whereas supplementation with low to moderate levels of zinc may offer protection (Mathur, 1979; Woo et al., 1988). For example, zinc deficiency enhanced carcinomas

of the esophagus induced by MBN (Fong et al., 1978) but retarded the development of oral cancer induced by 4-NQO (Wallenius et al., 1979). Thus, zinc's modifying effect on carcinogenesis may depend on the dose of zinc as well as the carcinogen being affected. The mutagenicity of zinc, particularly in *S. typhimurium*, appears to depend greatly on the chemical form.

4.9. SUSCEPTIBLE POPULATIONS

4.9.1. Possible Childhood Susceptibility and Susceptible Diabetics

Data in humans are not available that examine whether children are more susceptible to the toxicity of zinc than adults. However, the RDA for children, expressed in terms of mg/kg-day, is greater than that for adults. Animal studies have, however, suggested that neonates and/or developing animals may be more susceptible to the toxic effects of excess zinc. Bleavins et al. (1983) reported that in minks exposed to 56 mg Zn/kg-day throughout gestation and weaning, no changes were seen in exposed adults, but 3-4 week-old kits exhibited achromotrichia, thought to be associated with copper deficiency. Signs of copper deficiency progressed as zinc exposure continued.

ESOD, formerly known as erythrocuprein, contains two atoms of zinc and copper each as cofactors and acts as a scavenger of singlet oxygen species. As reported by Arai et al. (1987), this enzyme is known to be glycosylated, and glycosylation is significantly increased in diabetics. Furthermore, this glycosylation significantly decreases ESOD activity compared to the activity of non-glycosylated form of ESOD. Thus diabetics may be sensitive to high dietary levels of zinc. Several other studies have examined the effects of zinc exposure in young animals, but have not provided data on adult animals similarly exposed for comparison. Additional data will be required to adequately assess the susceptibility of children to zinc exposure, relative to adults.

4.9.2. Possible Gender Differences

Several studies in humans have suggested that females may be more sensitive to the adverse effects of excess zinc than males. For example, Samman and Roberts (1987, 1988) reported that women experienced adverse symptoms more frequently (84% in women vs. 18% in men) as well as being more susceptible to zinc-induced changes in LDL cholesterol levels, serum ceruloplasmin, and ESOD. However, women in this study received a higher average dose

(2.5 mg/kg-day) than did the corresponding men (2.0 mg/kg-day). In contrast, Hale et al. (1988) reported that in elderly subjects, zinc-exposed women did not experience the same reduction in the incidence of anemia as was seen in zinc-exposed men. The studies of Yadrick et al. (1989) and Fischer et al. (1984) reported similar effect levels on ESOD levels, expressed as mg total Zn/kg-day, in men and women. Further data examining the potential difference in response between men and women were not located.

In animal studies, it appears that if any differences between sexes were noted, the male is the more susceptible gender. For example, Maita et al. (1981) reported changes in body weight, altered clinical chemistry, and decreased liver and spleen weights in male rats, but not in female rats, exposed to 572 mg Zn/kg-day. Studies of reproductive function have demonstrated alterations in spermatogenesis at zinc exposure levels below those inducing alterations in female reproductive parameters (Sutton and Nelson, 1937; Pal and Pal, 1987; Saxena et al., 1989; Evenson et al., 1993). Other studies (Aughey et al., 1977; Zaporowska and Wasilewski, 1992) have not reported significant differences between male and female animals exposed to zinc. Additional studies will be required to determine whether sex-specific differences in adverse responses to zinc exist.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation of developing organisms of all animal species. Thus, insufficient as well as excessive oral intake can cause toxicity and disease and a quantitative risk assessment must take essentiality into account. The principal studies examine dietary supplements of zinc and the interaction of zinc with other essential trace metals, specifically copper, to establish a safe daily intake level of zinc for the general population, including pregnant women and children, without compromising normal health and development.

5.1.1. Choice of Principal Study and Critical Effect

Available studies of oral zinc toxicity have identified a number of zinc-induced physiological changes in humans, including decreased copper metalloenzyme activities (Fischer et al., 1984; Samman and Roberts, 1987, 1988; Yadrick et al., 1989; Davis et al., 2000; Milne et al., 2001), hematological effects (Hale et al., 1988), decreases in HDL-cholesterol levels (Hooper et al., 1980; Freeland-Graves et al., 1982; Chandra, 1984; Black et al., 1988), immunotoxic effects (Chandra, 1984), and gastrointestinal effects (Samman and Roberts, 1987, 1988). The available data indicate that the most sensitive effects of zinc are alterations in copper status. It is thought that the copper deficiency results from a zinc-induced decrease in copper absorption. As discussed in Fischer et al. (1984), copper metalloenzyme activities are a more sensitive indicator of copper status than plasma copper levels. For example, although studies by Samman and Roberts (1987, 1988), Fischer et al. (1984), Yadrick et al. (1989), Davis et al. (2000), and Milne et al. (2001) failed to find significant decreases in plasma copper levels, these studies did find alterations in other indicators of copper status, including activities of serum ceruloplasmin, ESOD, and/or EC-SOD. Some 60% or more of total erythrocyte copper is associated with ESOD. The identity of this protein, originally called erythrocuprein, from human tissues has been reported by McCord and Fridovich (1969). This protein contains two atoms, each, of zinc and copper.

Erythrocuprein functions as a superoxide dismutase having the ability to catalyze the dismutation of monovalent superoxide anion radicals into hydrogen peroxide and oxygen.

These proteins are also present in phagocytic cells and known to act as scavengers of singlet oxygen, thus preventing oxidative tissue damage. It follows that while the decreased copper metalloenzyme activities seen in several of the human studies are not necessarily adverse in themselves, they signal a decrease in the body's defenses against free radical oxidation. The consequences of the decrease in the enzyme activity would vary depending on the status of other components of the free radical defense system, such as the dietary adequacy of vitamins C, E, A, and selenium. Additional support for the selection of the critical endpoint comes from the rat study of L'Abbe and Fischer (1984a), which noted that changes in indicators of copper status (e.g., serum ceruloplasmin and cytochrome c oxidase activity and liver and heart Cu, Zn-superoxide dismutase activity) in rats exposed to supplemental zinc in the diet for 6 weeks were dose-related.

Of the available studies in humans, the studies of Davis et al. (2000), Milne et al. (2001), Fischer et al. (1984), and Yadrick et al. (1989) have identified effects on indicators of copper status at similar daily exposure levels.

In the study reported by Davis et al. (2000) and Milne et al. (2001), a population of postmenopausal women consumed a total of 53 mg Zn/day (3 mg/day in the controlled diet plus 50 mg/day as supplements), resulting in a total average daily dose of 0.81 mg/kg-day (using a mean body weight of 65.1 kg provided in the manuscripts). Bone-specific alkaline phosphatase activity was increased following zinc exposure, and ESOD activity and plasma free thyroxine were significantly decreased following exposure to zinc for 90 days.

Fischer et al. (1984) examined a group of adult male volunteers exposed to 50 mg supplemental Zn/day; adding in an average daily dietary consumption of 15.92 mg Zn/day (from the U.S. FDA Total Diet Study from 1982-1986 [Pennington et al., 1989]), the total exposure level from Fischer et al. (1984) was 65.92 mg Zn/day, or 0.94 mg/kg-day assuming a reference male body weight of 70 kg. ESOD activity was decreased by 4 weeks of exposure, with an inverse correlation between plasma zinc and ESOD activity apparent at 6 weeks.

The study of Yadrick et al. (1989) exposed a group of healthy adult women to 50 mg supplemental Zn/day; adding in an average daily dietary consumption of 9.38 mg/day (from the FDA Total Diet Study from 1982-1986 [Pennington et al., 1989]), the total exposure level from the Yadrick et al. (1989) study was 59.38 mg Zn/day, or 0.99 mg/kg-day assuming a reference

female body weight of 60 kg. ESOD activity declined steadily over the treatment period, and was statistically lower than pretreatment values at the end of the 10-week exposure.

In establishing an RfD for zinc, the data on essentiality were combined with the data on toxicity to define a level that would meet physiological requirements without causing toxic responses when consumed daily for a lifetime. The exposure values that were considered in determining the RfD suggest that there is only one order of magnitude between the minimum amount of zinc that will maintain physiological function (5.5 mg/day, King, 1986) and the amount associated with appearance of potentially adverse effects (60 mg/day, Cantilli et al., 1994).

As the four studies identified physiological changes on similar, sensitive endpoints (indicators of body copper status) at similar dose levels (0.81-0.99 mg Zn/kg-day) in a variety of human subject groups (adult males, adult females, postmenopausal females), the studies of Davis et al. (2000), Milne et al. (2001), Yadrick et al. (1989), and Fischer et al. (1984) were selected as co-principal studies.[1]

5.1.2. Methods of Analysis

A NOAEL/LOAEL approach was applied to derive the RfD. A benchmark dose approach was considered, but was not utilized for this assessment. All of the co-principal studies examined only one dose level, apart from controls, and therefore did not provide sufficient information to describe the dose-response function. Therefore, the studies are not suitable for benchmark analysis.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UF)

In selecting the point of departure for the RfD, the effect levels from the principal studies were evaluated. As described in Section 5.1.1 above, the studies identified effect levels of 0.81

[1] The studies by Davis et al. (2000) and Milne et al. (2001) were approved by the Institutional Review boards of the University of North Dakota and the US Department of Agriculture and followed Guidelines of the Department of Health and Human Services and the Helsinki Declaration regarding the use of human subjects. The study by Yadrick et al. (1989) was approved by the Institutional Review Board of Oklahoma State University and informed consent was obtained from each participant. Finally, the study by Fischer et al. (1984) was approved by the Human Studies Committee of the Health Protection Branch, Health and Welfare Canada, and a consent form was signed by all participants.

mg Zn/kg-day (Davis et al., 2000; Milne et al., 2001), 0.94 mg Zn/kg-day (Fischer et al., 1984), and 0.99 mg Zn/kg-day (Yadrick et al., 1989) for changes in indicators of body copper status. Since the four studies have similar methodologies and outcomes with regard to effects, they were averaged together to obtain the point of departure (0.81+0.94+0.99=2.74/3=0.91 mg/kg-day).

The RfD of 0.3 mg/kg-day was derived by dividing the point of departure of 0.91 mg Zn/kg-day by a total uncertainty factor of 3 as follows:

RfD = NOAEL ÷ UF
= 0.91 mg/kg-day ÷ 3
= 0.3 mg/kg-day.

When considered within the context of the RDA and reference daily intake (RDI) values shown in Table 5-1, the RfD allows for some flexibility in the dietary intake (i.e., the RfD is 1.2 to 2.3 times the RDA). For essential elements such as zinc, the RDA provided the lower bound for determination of the RfD.

An interspecies uncertainty factor (UF_A) was not necessary for extrapolation from an animal study to the human population. The principal studies were conducted in human volunteers.

A threefold intraspecies uncertainty factor (UF_H) was applied to account for variability in susceptibility in human populations. The critical effect for zinc is decreased copper uptake, leading to a decrease in the activity of Cu, Zn-SOD enzymes that function as part of the body's system to protect against free radicals and oxidative stress. This system is complex, involving the SOD, catalase, glutathione, glutathione peroxidase, glutathione reductase, and the antioxidant vitamins (A, C, and E) providing several layers of protection. However, there is variability within the human population. Individuals with genetic catalase deficiency and glucose-6-phosphate dehydrogenase deficiencies have reduced capacities to metabolically cope with oxidative stress. Poor nutrition can also compromise the ability to respond to free radicals and oxidative stress. It is, accordingly, prudent to allow a threefold factor for human variability since the individuals used in the critical studies were apparently healthy adults. The use of a 10-fold uncertainty factor for intrahuman variability would result in an RfD below the RDA.

In the case of zinc and other nutritionally required elements, it is important that the RfD not be set at a value that would suggest that people should consume diets with insufficient zinc. Recommended dietary levels, expressed as intake both in mg Zn/day and in mg Zn/kg-day (calculated by adjusting with reference body weights of 13 kg for young children, 61 kg for women [pregnant, lactating, or general adult], or 70 kg for men), are presented in Table 5-1. Use of a threefold factor results in an RfD value that exceeds the dietary values by factors from 1.2 to 2.3. A smaller margin between the RfD and RDA cannot be recommended. RDA values are established for healthy individuals, and thus there are instances when additional dietary zinc is recommended such as during the recovery from surgery and other circumstances where active tissue repair is necessary.

Table 5-1. Estimated nutritional requirements of zinc at various life stages, expressed as mg/day and mg/kg-day

Life stage	Recommended intake (mg Zn/day)	Reference body weight (kg)	Recommended intake (mg Zn/kg-day)
1-3 years	3 (RDA[a])	13	0.23
Adulthood (>18 years) Male Female	 11 (RDA) 8 (RDA)	 76 61	 0.15 0.13
Pregnant women	11 (RDA)	61	0.18
Lactating women	12 (RDA)	61	0.2
U.S. FDA RDI[b] Values Male Female	 15 mg (RDI) 15 mg (RDI)	 70 60	 0.21 0.25

[a]RDA values and reference body weights are from IOM (2001).
[b]RDI values are established by the U.S. FDA and are used in the labeling of nutritional supplements.

An uncertainty factor to account for extrapolation from a subchronic study to estimate chronic exposure conditions (UF_S) was not necessary. Zinc is an essential element and therefore chronic exposures of zinc are required for proper nutrition. Exposure at the level of the RfD is expected to be without adverse effects when zinc is consumed on a daily basis over the life-span of the individual, neither inducing nutritional deficiency nor resulting in toxic effects in healthy non-pregnant adult humans consuming an average American diet.

There is extensive experience with humans receiving chronic dietary exposures from the diet plus nutritional supplements that do not exceed the 15 mg/day RDI which demonstrates that these levels are not adverse. For example, Hale et al. (1988) studied hematological parameters in elderly subjects who were supplemented with zinc for an average duration of 8 years. In general, no significant alterations were found between the zinc-supplemented group and controls. On the other hand, Prasad et al. (1978) studied a patient given 150-200 mg Zn/day for 2 years. The patient developed copper deficiency which was reversed with copper supplementation. Additionally, pharmacokinetic data on zinc absorption, distribution, and elimination suggest that steady-state levels will be reached within the time periods evaluated by the principal studies. Therefore, an uncertainty factor for extrapolation from a study of less than chronic duration to a lifetime exposure scenario was not determined to be necessary.

An uncertainty factor for extrapolation from a LOAEL to a NOAEL (UF_L) was determined to not be necessary. The RfD was based on a minimal effect level for a sensitive biological indicator, i.e., decreased ESOD activity, which is reflection of zinc-associated alterations in copper homeostasis that could lead to oxidative tissue damage. As discussed in the section on intrahuman variability, there is redundancy in the physiological free radical defense system that argues against describing the decreased activity of Cu, Zn-SOD as definitively adverse. Protection for variability in the status of this defense system is accommodated by the threefold factor allowed for intrahuman variability.

The deficit in copper absorption in the presence of excess zinc can also not be categorized as requiring the application of a LOAEL to NOAEL UF. As discussed in Fischer et al. (1984), copper metalloenzyme activities are more sensitive indicators of copper status than plasma copper levels. They are an early biomarker for a subclinical copper deficiency. Most importantly, the application of a threefold UF for a LOAEL to NOAEL adjustment, when combined with a threefold factor for intraspecies variability, would lower the RfD to below the RDA.

A database uncertainty factor (UF_D) to account for uncertainties due to lack of information in the database was not necessary. The database contains a considerable number of well-conducted human studies in a diverse group of human subjects. There are numerous reproductive and developmental toxicity studies performed in different species. Animal studies demonstrate that effects on reproductive and/or developmental endpoints are not the most sensitive endpoints for zinc toxicity.

The additional use of a daily vitamin supplement containing 15 mg zinc, such as is found in a standard multivitamin tablet, in conjunction with a diet adequate in zinc would result in a total adult daily exposure on the order of 0.4 mg Zn/kg-day[2], which is above the RfD. However, daily multivitamins also contain copper (2 mg/day), which could be expected to counteract the effects of excess zinc intake resulting from daily multivitamin use. Therefore, the use of a daily multivitamins, or similar balanced supplements, is not contraindicated by exposure at the level of the RfD.

5.1.4. Previous IRIS Assessment

In the previous assessment for zinc, the oral RfD was based on a single clinical study (Yadrick et al., 1989) which investigated the effects of oral zinc supplements (50 mg/day) on copper and iron balance. The total exposure level in this study (as discussed in Section 5.1.3) was 0.99 mg Zn/kg-day. The RfD of 0.3 mg/kg-day was derived by dividing this dose (0.99 mg/kg-day) by a total uncertainty factor of 3 based on a minimal LOAEL from a moderate-duration study of the most sensitive humans and consideration of a substance that is an essential dietary nutrient.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

Available data on humans exposed to zinc compounds by inhalation are limited to reports of acute exposures to zinc oxide or zinc chloride. Similarly, available studies in animals have been of acute duration, and, therefore, are not suitable for use in derivation of an RfC. A route-to-route extrapolation from the oral data was considered, but was not attempted as available data from acute inhalation studies suggest that significant portal of entry effects will occur. Lacking suitable data, derivation of an inhalation RfC for zinc compounds is precluded.

[2] A typical over-the-counter zinc supplement, such as a daily multivitamin, contains 15 mg Zn. For a healthy adult male, this would add an additional 0.21 mg Zn/kg-day, or 0.25 mg Zn/kg-day for a healthy adult female; thus, with a zinc-sufficient diet, the total zinc intake for a male consuming one multivitamin daily would be 0.36 mg/kg-day. Values for normal and lactating females consuming the same multivitamin would be 0.38 mg/kg-day and 0.45 mg/kg-day, respectively. Each of these values falls within the order of magnitude range about the RfD and can be considered to be without risk.

5.3. CANCER ASSESSMENT

5.3.1. Oral Slope Factor

Data are inadequate for the derivation of an oral slope factor for zinc. No human studies examining the oral carcinogenicity of zinc or zinc compounds were located. A 1-year study in mice (Walters and Roe, 1965) did not find increases of malignant lymphoma, lung adenoma, or hepatoma. The study did not report on the incidence of any other types of tumors, nor did it perform adequate histologic analysis of other tissues. Similarly, Aughey et al. (1977) did not observe increases in tumors of the pancreas, pituitary gland, or adrenal gland in mice exposed to zinc for 14 months; however, observations from other organs were not reported. A study by Halme (1961) reported potential increases in zinc-induced tumors in a multi-generation study in rats, but was not sufficiently descriptive to allow for a complete evaluation of the study. No other animal studies of the oral carcinogenicity of zinc were identified. Therefore, lack of data precludes the derivation of an oral slope factor.

5.3.2. Inhalation Unit Risk

Data are inadequate for the derivation of an inhalation unit risk for zinc. No suitable human or animal studies were identified which examined the carcinogenicity of zinc following chronic inhalation exposure.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Zinc is an essential element, necessary for the function of more than 300 enzymes. A wide range of clinical symptoms have been associated with zinc deficiency in humans (Prasad, 1993; Sandstead, 1994; Walsh et al., 1994), though generally only with chronically severe or moderately severe deficiency. Oral exposure to high levels of zinc in humans can result in several systemic effects, the most sensitive of which are related to diminished copper status. As discussed in Fischer et al. (1984), copper metalloenzyme activity is a more sensitive indicator of copper status than plasma copper levels. These sensitive indicators of copper status, which may not be adverse in themselves, can be considered as precursor events to more severe copper-deficiency-induced changes.

The majority of the inhalation data on zinc focuses on short-term inhalation of zinc oxide or zinc chloride, resulting in metal fume fever. The earliest symptoms of metal fume fever are a metallic taste in the mouth accompanied by dryness and irritation of the throat. Flu-like symptoms, chills, fever, profuse sweating, headache, and weakness follow (Drinker et al., 1927a, b; Sturgis et al., 1927; Rohrs, 1957; Malo et al., 1990). The symptoms usually occur within several hours after exposure to zinc oxide fumes and persist for 24 to 48 hours. An increase in tolerance develops with repeated exposure; however, this tolerance is lost after a brief non-exposure period. Studies of the health effects of subchronic or chronic exposure to inhaled zinc compounds were not located in the available literature.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) there is *inadequate information to assess carcinogenic potential* of zinc in humans, because studies of humans occupationally exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the carcinogenicity of zinc are not available, and tests of the genotoxic effects of zinc have been equivocal.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

The most sensitive effects of oral exposure to excess zinc in humans involve the copper status of the body. Zinc exposure can result in a decreased absorption of copper, leading to low systemic copper levels and subsequent health effects, including decreased copper metalloenzyme activity, hematological effects, decreases in cholesterol levels, immunotoxicity, and gastrointestinal effects. While changes such as decreased copper metalloenzyme levels may not be adverse in themselves, they have been demonstrated to be precursor events for more severe effects. The study of Yadrick et al. (1989) established a minimal LOAEL of 0.99 mg Zn/kg-day for decreased levels of ESOD, an indicator of body copper status, in women exposed for 10 weeks, while the study of Fischer et al. (1984) established a minimal LOAEL of 0.94 mg Zn/kg-day for the same endpoint in men exposed for 6 weeks, and the study of Davis et al. (2000) and Milne et al. (2001) identified a minimal LOAEL of 0.81 mg Zn/kg-day for changes in ESOD and plasma free thyroxine. These four studies in human volunteers were considered to be co-principal studies, and the minimal LOAEL (0.91 mg Zn/kg-day, average of these LOAELs) was selected as the point of departure. An uncertainty factor of 3 (discussed in Section 5.1.3, above), representing the uncertainties associated with human variability and the need for an adequate dietary level of zinc, was then applied to the minimal LOAEL of 0.91 mg Zn/kg-day to give the RfD of 0.3 mg Zn/kg-day.

6.2.2. Noncancer/Inhalation

Data on the effects of inhaled zinc are primarily limited to short-term studies examining metal fume fever in occupationally-exposed humans. Studies in animals are not sufficient for the derivation of an RfC, owing mainly to insufficient duration or other study limitations. Lacking suitable data, derivation of an inhalation RfC is precluded.

6.2.3. Cancer/Oral and Inhalation

Data in both humans and animals are inadequate to evaluate potential associations between zinc exposure and cancer. Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is *inadequate information to assess the carcinogen potential* of zinc, because studies of humans occupationally-exposed to zinc are inadequate or inconclusive, adequate animal bioassays of the possible carcinogenicity of zinc are not available, and tests of the genotoxic effects of zinc have been equivocal.

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APPENDIX A. EXTERNAL PEER REVIEW—SUMMARY OF COMMENTS AND DISPOSITION

The support document and IRIS summary for zinc have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. Public comments were read and considered. The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. All three external peer reviewers recommended that this document and the accompanying assessments were acceptable with minor revisions. A summary of significant comments made by the external reviewers and EPA's response to these comments follows.

(1) General Questions for Peer Reviewers

General Question For the RfD, has the most appropriate critical effect been chosen? For the cancer assessment, are the tumors observed biologically significant? Relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

Comment All three reviewers agreed that the document is concise and clearly written, and the choice of critical study and critical effects are appropriate. Some of the concerns reviewers presented include: balancing adverse effects with both deficiencies and level of concern for effects of deficiency or the effects below the RfD, specifically for children; adverse effects resulting from other metal interactions, such as iron and or copper; uncertainty associated with a higher RfD than the currently derived RfD, concerns for different forms of zinc exposure, enhancement of NOAEL/LOAEL information from animal studies; clear presentation of zinc status as essential element in IRIS Summary; additional studies recommended by two reviewers.

Response to Comment Section 5.1.3 of the Toxicological Review was added to provide an enhanced discussion of the RfD, relevance to the RDA, and effects below RfD in sensitive populations, such as children. Although limited data are available, information on the potential adverse effects in children were included in Section 4.9 of the Toxicological Review. Table 3 presents diet, age, gender and body-weight-specific zinc requirements followed by a discussion of the effects that may occur below the RfD and the uncertainties associated with the RfD.

An enhanced discussion of the chosen UF of 3 has been added in Section 5.1.3 of the Toxicological Review and clearly describes the rational for the chosen UF; however, the suggested uncertainty factor of 1.5 was not implemented. This decision was based on the threshold effect of decreased ESOD activity and the uncertainty as to whether decreased ESOD activity may predispose a cell to an accumulation of oxidative damage due to decreased

quenching of free radicals. Although the recent antioxidant effects of zinc supplementation have been reported (Prasad et al., 2004), the study did not determine whether the decreased levels of serum markers of oxidative stress (e.g., 8-hydroxy-2'-deoxyguanosine) were due to a decreased level of oxidative DNA damage or a decrease in the removal of this lesion within nucleated cells.

Metal-metal interactions are discussed in Section 4.6 of the Toxicological Review and information on zinc speciation and their relevance to environmental exposure are included Section 3.1 of the Toxicological Review.

Because available animal studies present information on supplementary levels of dietary zinc and no additional dosages, it is not possible to clearly discuss proper NOAEL/LOAELs from these studies; therefore, this was not discussed in Chapter 4 of the Toxicological Review.

Section 5.1 of the Toxicological Review and Section I.A.2 of the IRIS Summary have been revised to include information, as suggested by one reviewer, on the relevance of the RfD and environmental levels of zinc and the significance of zinc as an essential element to help risk assessors and managers make meaningful risk assessment decisions, as follows:

> The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation of developing organisms of all animal species. Thus, insufficient as well as excessive oral intake can cause toxicity and disease and a quantitative risk assessment must take essentiality into account. The principal studies examine dietary supplements of zinc and the interaction of zinc with other essential trace metals, specifically copper, to establish a safe daily intake level of zinc for the general population, including pregnant women children, without compromising normal health and development.

Suggested new studies have been added in the Toxicological Review and the relevant literature has been reviewed and updated through October 2004.

(2) RfD Derivation

General Question The RfD for zinc is based on human clinical studies to establish daily nutritional requirements. The human studies examined dietary supplements of zinc and the interaction of zinc with other metals, such as copper, to establish a safe daily intake of zinc for children, adults, and pregnant women. Do you consider this RfD to be protective of adverse effects in children and pregnant women? Do you agree with the method of analysis used to evaluate dose-response data for zinc?

A. Comment Is the RfD protective of adverse effects in children and pregnant women?

Reviewers did not consider the RfD to be protective for adverse effects in children because it was below the RDA and they suggested expanding the discussions in the Toxicological Review.

Response to Comment The paragraph regarding the protective effect of the RfD in children is not accurate and has been removed. An enhanced discussion of the RfD relative to the RDA has been included. For transparency, the dose conversion and body weight have been added in Table 5-1, Section 5.1.3 of the Toxicological Review. As suggested by one reviewer, effects of multivitamins were also included, and a paragraph addressing the relevance of the RfD for children and pregnant woman has been added.

B. Comment Are appropriate uncertainty factors applied to the point of departure?

The reviewers, while suggesting the UF of 1.5 instead of 3, recommended expanding the discussions on uncertainties in the Toxicological Review.

Response to Comment The discussion of the rationale for an uncertainty factor of 3 has been enhanced. Since the RfD was based on a toxicity threshold dose-response, standard uncertainty factors have been used to develop the RfD.

(3) RfC Derivation

General Question Data for derivation of RfC are considered inadequate. Do you agree?

Comment All reviewers agreed. One reviewer suggested that an overview statement be provided in the IRIS Summary regarding the inadequacy of the data.

Response to Comment The summary sheet was modified per reviewer's recommendations, and the following statement was included in Section I.B of the IRIS Summary:

> Available data are not suitable for the derivation of an RfC for zinc. A number of case reports of metal fume fever have been reported in humans, however exposure levels are not known. The data in animals is limited to a few studies of acute duration, no subchronic or chronic inhalation studies of zinc are available at this time.

(4) Cancer Weight-of-Evidence (WOE) Classification

General Question The WOE classification for zinc has been discussed in Chapter 4 of the Toxicological Review. Have appropriate criteria been applied from the EPA draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999)?

Comment All reviewers agreed that the application of the guidelines and presentation of data in support of the WOE was appropriate.

Comment Two reviewers had specific editorial comments and one reviewer provided annotated changes in each chapter of the Toxicological Review and IRIS Summary.

Response to Comment All editorial and annotated changes were incorporated.

RECOMMENDATIONS

Comment Two reviewers recommended acceptance with major revisions as suggested for Chapters 5 and 6 of the Toxicological Review and for the IRIS Summary while the third reviewer recommended acceptance with minor revision.

Response to Comment All major editorial changes, addition of new studies, and revisions to the text in both the Toxicological Review and the IRIS Summary were incorporated. Corrections were made to reflect adverse effects at or below the RfD to protect children.

The uncertainty section was completely revised and expanded statements were provided in support of the UF of 3. This value was considered the most protective for preventing zinc deficiency and toxicity. When considered within the context of the RDA and RDI values shown in Table 5-1, Section 5.1.3 of the Toxicological Review, the RfD is 50% greater than the nearest RDA values (for young children and pregnant or lactating women), and 20% greater than the RDI values. For essential elements such as zinc, the RDA provides the lower bound for determination of the RfD. Based on these reasons, the UF of 3 was considered to be protective against adverse effects that may occur from deficiency or excess, and the recommendation by two reviewers to reduce the UF to 1 or 1.5 due to a concern for deficiency were considered to be adequately addressed by the UF of 3.

Attachment

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cadmium out of its milk, and Miller concluded that most major animal products, including meat and milk, seemed quite well protected against cadmium accumulation.

Interactions of cadmium with several other trace elements (Hill et al. 1963,[172] Gunn and Gould 1967,[160] Mason and Young 1967)[189] somewhat confuse the matter of establishing criteria.

Recommendation

From the available data on the occurrence of cadmium in natural waters, its toxicity, and its accumulation in body tissues, an upper limit of 50 μg/l allows an adequate margin of safety for livestock and is recommended.

Chromium

In a five-year survey of lake and river waters of the United States (Kopp and Kroner 1970),[182] the highest level found in over 1,500 samples was about 0.1 mg/l, the average being about 0.001 mg/l. In another similar survey (Durum et al. 1971)[141] of 700 samples, none contained over 0.05 mg/l of chromium VI and only 11 contained more than 0.005 mg/l. A number of industrial processes however use the element, which then may be discharged as waste into surface waters, possibly at rather high levels.

Even in its most soluble forms, the element is not readily absorbed by animals, being largely excreted in the feces; and it does not appear to concentrate in any particular mammalian tissue or to increase in these tissues with age (Mertz 1967,[194] Underwood 1971[254]).

Hexavalent chromium is generally considered more toxic than the trivalent form (Mertz 1967).[194] However, in their review of this element, McKee and Wolf (1963)[193] suggested that it has a rather low order of toxicity. Further, Gross and Heller (1946)[158] found that for rats the maximum nontoxic level, based on growth, for chromium VI in the drinking water was 500 mg/l. They also found that this concentration of the element in the water did not affect feed utilization by rabbits. Romoser et al. (1961)[226] found that 100 ppm of chromium VI in chick diets had no effect on the performance of the birds over a 21-day period.

In a series of experiments, Schroeder et al. (1963a,[238] 1963b,[239] 1964,[234] 1965[235]) administered water containing 5 mg/l of chromium III to rats and mice on low-chromium diets over a life span. At this level, the element was not toxic, but instead it had some beneficial effects. Tissue levels did not increase significantly with age.

As a result of their review of chromium toxicity, McKee and Wolf (1963)[193] suggested that up to 5 mg/l of chromium III or VI in livestock drinking water should not be harmful. While this may be reasonable, it may be unnecessarily high when the usual concentrations of the element in natural waters is considered.

Recommendation

An upper allowable limit of 1.0 mg/l for livestock drinking waters is recommended. This provides a suitable margin of safety.

Cobalt

In a recent survey of surface waters in the United States (Durum et al. 1971)[141] 63 per cent of over 720 samples were found to contain less than 0.001 mg/l of cobalt. One sample contained 4.5 mg/l, one contained 0.11 mg/l, and three contained 0.05–0.10 mg/l.

Underwood (1971)[254] reviewed the role of cobalt in animal nutrition. This element is part of the vitamin B_{12} molecule, and as such it is an essential nutrient. Ruminants synthesized their own vitamin B_{12} if they were given oral cobalt. For cattle and sheep a diet containing about 0.1 ppm of the element seemed nutritionally adequate. A wide margin of safety existed between the required and toxic levels for sheep and cattle, which were levels of 100 times those usually found in adequate diets being well tolerated.

Nonruminants required preformed vitamin B_{12}. When administered to these animals in amounts well beyond those present in foods and feeds, cobalt induced polycythemia (Underwood 1971).[254] This was also true in calves prior to rumen development; about 1.1 mg of the element per kg of body weight administered daily caused depression of appetite and loss of weight.

Cobalt toxicity was also summarized by McKee and Wolf (1963).[193]

Recommendation

In view of the data available on the occurrence and toxicity of cobalt, an upper limit for cobalt in livestock waters of 1.0 mg/l offers a satisfactory margin of safety, and should be met by most natural waters.

Copper

The examination of over 1,500 river and lake waters in the United States (Kopp and Kroner 1970)[182] yielded, at the highest, 0.28 mg/l of copper and an average value of 0.015 mg/l. These rather low values were probably due in part to the relative insolubility of the copper ion in alkaline medium and to its ready adsorbability on colloids (McKee and Wolf 1963).[193] Where higher values than those reported above are found, pollution from industrial sources or mines can be suspected.

Copper is an essential trace element. The requirement for chicks and turkey poults from zero to eight weeks of age is 4 ppm in the diet (NRC 1971b).[206] For beef cattle on rations low in molybdenum and sulfur, 4 ppm in the diet is adequate; but when these elements are high, the copper requirement is doubled or tripled (NRC 1970).[204] A dietary level of 5 ppm in the forage is suggested for pregnant and

lactating ewes and their lambs (NRC 1968b[203]). A level of 6 ppm in the diet is considered adequate for swine (NRC 1968a).[202]

Swine are apparently very tolerant of high levels of copper, and 250 ppm or more in the diet have been used to improve liveweight gains and feed efficiency (Nutrition Reviews 1966a[210]; NRC 1968a).[202] On the other hand, sheep were very susceptible to copper poisoning (Underwood 1971),[254] and for these animals a diet containing 25 ppm was considered toxic. About 9 mg per animal per day was considered the safe tolerance level (NRC 1968b).[203]

Several reviews of copper requirements and toxicity have been presented (McKee and Wolf 1963,[133] Nutrition Reviews 1966a,[210] Underwood 1971).[254] There is very little experimental data on the effects of copper in the water supply on animals, and its toxicity must be judged largely from the results of trials where copper was fed. The element does not appear to accumulate at excessive levels in muscle tissues, and it is very readily eliminated once its administration is stopped. While most livestock tolerate rather high levels, sheep do not (NRC 1968b).[203]

Recommendation

It is recommended that the upper limit for copper in livestock waters be 0.5 mg/l. Very few natural waters should fail to meet this.

Fluorine

The role of fluorine as a nutrient and as a toxin has been thoroughly reviewed by Underwood (1971).[254] (Unless otherwise indicated, the following discussion, exclusive of the recommendation, is based upon this review.) While there is no doubt that dietary fluoride in appropriate amounts improved the caries resistance of teeth, the element has not yet been found essential to animals. If it is a dietary essential, its requirement must be very low. Its ubiquity probably insures a continuously adequate intake by animals.

Chronic fluoride poisoning of livestock has, on the other hand, been observed in several areas of the world, resulting in some cases from the consumption of waters of high fluoride content. These waters come from wells in rock from which the element has been leached, and they often contain 10–15 mg/l. Surface waters, on the other hand, usually contain considerably less than 1 mg/l.

Concentrations of 30–50 ppm of fluoride in the total ration of dairy cows is considered the upper safe limit, higher values being suggested for other animals (NRC 1971a).[205] Maximum levels of the element in waters that are tolerated by livestock are difficult to define from available experimental work. The species, volume, and continuity of water consumption, other dietary fluoride, and age of the animals, all have an effect. It appears, however, that as little as 2 mg/l may cause tooth mottling under some circumstances. At least a several-fold increase in its concentration seems, however, required to produce other injurious effects.

Fluoride from waters apparently does not accumulate in soft tissues to a significant degree. It is transferred to a very small extent into the milk and to a somewhat greater degree into eggs.

McKee and Wolf (1963)[133] have also reviewed the matter of livestock poisoning by fluoride, concluding that 1.0 mg/l of the element in their drinking water did not harm these animals. Other more recent reports presented data suggesting that even considerably higher concentrations of fluoride in the water may, with the exception of tooth mottling, caused no animal health problems (Harris et al. 1963,[100] Shupe et al. 1964,[246] Nutrition Reviews 1966b,[211] Saville 1967,[231] Schroeder et al. 1968a[237]).

Recommendation

An upper limit for fluorides in livestock drinking waters of 2.0 mg/l is recommended. Although this level may result in some tooth mottling it should not be excessive from the standpoint of animal health or the deposition of the element in meat, milk, or eggs.

Iron

It is well known that iron (Fe) is essential to animal life. Further, it has a low order of toxicity. Deobald and Elvehjem (1935)[128] found that iron salts added at a level of 9,000 mg Fe/kg of diet caused a phosphorus deficiency in chicks. This could be overcome by adding phosphate to the diet. Campbell (1961)[124] found that soluble iron salt administered to baby pigs by stomach tube at a level of 600 mg Fe/kg of body weight caused death within six hours. O'Donovan et al. (1963)[212] found very high levels of iron in the diet (4,000 and 5,000 mg/kg) to cause phosphorus deficiency and to be toxic to weanling pigs. Lower levels (3,000 mg/kg) apparently were not toxic. The intake of water by livestock may be inhibited by high levels of this element (Taylor 1935).[250] However, this should not be a common or a serious problem. While iron occurs in natural waters as ferrous salts which are very soluble, on contact with air it is oxidized and it precipitates as ferric oxide, rendering it essentially harmless to animal health.

It is not considered necessary to set an upper limit of acceptability for iron in water. It should be noted, however, that even a few parts per million of iron can cause clogging of lines to stock watering equipment or an undesirable staining and deposit on the equipment itself.

Lead

Lake and river waters of the United States usually contain less than 0.05 mg/l of lead (Pb), although concentrations in excess of this have been reported (Durum et al. 1971,[141] Kopp and Kroner 1970).[182] Some natural waters in areas where galena is found have had as much as 0.8 mg/l of the

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lactating ewes and their lambs (NRC 1968b[205]). A level of 6 ppm in the diet is considered adequate for swine (NRC 1968a).[202]

Swine are apparently very tolerant of high levels of copper, and 250 ppm or more in the diet have been used to improve liveweight gains and feed efficiency (Nutrition Reviews 1966a[210]; NRC 1968a).[202] On the other hand, sheep were very susceptible to copper poisoning (Underwood 1971),[254] and for these animals a diet containing 25 ppm was considered toxic. About 9 mg per animal per day was considered the safe tolerance level (NRC 1968b).[205]

Several reviews of copper requirements and toxicity have been presented (McKee and Wolf 1963,[196] Nutrition Reviews 1966a,[210] Underwood 1971).[254] There is very little experimental data on the effects of copper in the water supply on animals, and its toxicity must be judged largely from the results of trials where copper was fed. The element does not appear to accumulate at excessive levels in muscle tissues, and it is very readily eliminated once its administration is stopped. While most livestock tolerate rather high levels, sheep do not (NRC 1968b).[205]

Recommendation

It is recommended that the upper limit for copper in livestock waters be 0.5 mg/l. Very few natural waters should fail to meet this.

Fluorine

The role of fluorine as a nutrient and as a toxin has been thoroughly reviewed by Underwood (1971).[254] (Unless otherwise indicated, the following discussion, exclusive of the recommendation, is based upon this review.) While there is no doubt that dietary fluoride in appropriate amounts improved the caries resistance of teeth, the element has not yet been found essential to animals. If it is a dietary essential, its requirement must be very low. Its ubiquity probably insures a continuously adequate intake by animals.

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United States Environmental Protection Agency

2018 Edition of the Drinking Water Standards and Health Advisories Tables

The 2012 Drinking Water Standards and Health Advisories (DWSHA) Tables were amended March 2018 to fix typographical errors and add health advisories published after 2012.

2018 Edition of the Drinking Water Standards and Health Advisories

EPA 822-F-18-001

Office of Water
U.S. Environmental Protection Agency
Washington, DC

March 2018

The Health Advisory (HA) Program, sponsored by the EPA's Office of Water (OW), publishes concentrations of drinking water contaminants at Drinking Water Specific Risk Level Concentration for cancer (10^{-4} Cancer Risk) and concentrations of drinking water contaminants at which noncancer adverse health effects are not anticipated to occur over specific exposure durations - One-day, Ten-day, and Lifetime - in the *Drinking Water Standards and Health Advisories* (DWSHA) tables. The One-day and Ten-day HAs are for a 10 kg child and the Lifetime HA is for a 70 kg adult. The daily drinking water consumption for the 10 kg child and 70 kg adult are assumed to be 1 L/day and 2 L/day, respectively. The Lifetime HA for the drinking water contaminant is calculated from its associated Drinking Water Equivalent Level (DWEL), obtained from its RfD, and incorporates a drinking water Relative Source Contribution (RSC) factor of contaminant-specific data or a default of 20% of total exposure from all sources. Maximum Contaminant Levels (MCLs) and Maximum Contaminant Level Goals (MCLGs) for some regulated drinking water contaminants are also published.

HAs serve as the informal technical guidance for unregulated drinking water contaminants to assist Federal, State and local officials, and managers of public or community water systems in protecting public health as needed. They are not to be construed as legally enforceable Federal standards. EPA's OW has provided MCLs, MCLGs, RfDs, One-Day HAs, Ten-day HAs, DWELs, Lifetime HAs, Drinking Water Specific Risk Level Concentration for cancer (10^{-4} Cancer Risk), and Cancer Descriptors in the DWSHA tables. HAs are intended to protect against noncancer effects. The 10^{-4} Cancer Risk level provides information concerning cancer effects. The MCL values for specific drinking water contaminants must be used for regulated contaminants in public drinking water systems.

The DWSHA tables are revised periodically by the OW so that the benchmark values are consistent with the most current Agency assessments. Reference dose (RfD) values are updated to reflect the values in the Integrated Risk Information System (IRIS) and the Office of Pesticide Programs (OPP) Reregistration Eligibility Decisions (REDs) documents. The associated DWEL is recalculated accordingly. The 2018 DWSHA tables **do not** reflect assessments from IRIS or OPP published from 2012 to 2018. The DWSHA tables are currently undergoing a modernization effort to move the relevant HA information into a web-based format. This posting of the 2018 DWSHA tables is an intermediate step to address typographical errors and include health advisories published since the 2012 tables were published.

A Lifetime noncancer benchmark is made available to risk assessment managers for comparison to the cancer risk level drinking water concentration (10^{-4} Cancer Risk) and to determine whether the noncancer Lifetime HA or the cancer risk level drinking water concentration provides a more meaningful scenario-specific risk reduction. In this regard, the Office of Water defines the Lifetime HA as the concentration in drinking water that is not expected to cause any adverse noncarcinogenic effects for a lifetime of exposure, whereas the 10^{-4} Cancer Risk is the concentration of the chemical contaminant in drinking water that is associated with a specific probability of cancer. The Office of Water also advises consideration of the more conservative cancer risk levels (10^{-5}, 10^{-6}), found in the IRIS or OPP RED source documents, if it is considered more appropriate for exposure-specific risk assessment.

Many of the values on the DWSHA tables have been revised since the original HAs were published. Revised RfDs, 10^{-4} Cancer Risk values, and cancer designations or descriptors obtained from Integrated Risk Information System (IRIS) are presented in **BOLD** type. Revised RfDs, 10^{-4} Cancer Risk values, and cancer designations or descriptors obtained from Office of Pesticide Program's Registration Eligibility Decision (OPP RED) are presented in ***BOLD ITALICS*** type.

The summaries of IRIS Toxicological Reviews from which the RfDs and cancer benchmarks, as well as the associated narratives and references can be accessed at: http://www.epa.gov/IRIS. Those from OPP REDs can be accessed at: http://www.epa.gov/pesticides/reregistration/status.htm.

In some cases, there is an HA value for a contaminant but there is no reference to an HA document. Such HA values can be found in the Drinking Water Criteria Document for the contaminant.

With a few exceptions, the RfDs, Health Advisories, and Cancer Risk values have been rounded to one significant figure following the convention adopted by IRIS.

For unregulated chemicals with current IRIS or OPP REDs RfDs, the Lifetime Health Advisories are calculated from the associated DWELs, using the RSC values published in the HA documents for the contaminants.

The DWSHA tables may be reached from the Water Science home page at: http://www.epa.gov/waterscience/. The DWSHA tables are accessed under the Drinking Water icon.

Copies of the Tables may be ordered free of charge from

SAFE DRINKING WATER HOTLINE
1-800-426-4791
Monday thru Friday, 9:00 AM to 5:30 PM EST

DEFINITIONS

The following definitions for terms used in the DWSHA tables are not all-encompassing, and should not be construed to be "official" definitions. They are intended to assist the user in understanding terms used in the DWSHA tables.

Action Level: The concentration of a contaminant which, if exceeded, triggers treatment or other requirements which a water system must follow. For example, it is the level of lead or copper which, if exceeded in over 10% of the homes tested, triggers treatment for corrosion control.

Cancer Classification: A descriptive weight-of-evidence judgment as to the likelihood that an agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Under the 2005 EPA *Guidelines for Carcinogen Risk Assessment*, Cancer Descriptors replace the earlier alpha numeric Cancer Group designations (US EPA 1986 guidelines). The Cancer Descriptors in the 2005 EPA *Guidelines for Carcinogen Risk Assessment* are as follows:

- "carcinogenic to humans" (**H**)
- "likely to be carcinogenic to humans" (**L**)
- "likely to be carcinogenic above a specified dose but not likely to be carcinogenic below that dose because a key event in tumor formation does not occur below that dose" (**L/N**)
- "suggestive evidence of carcinogenic potential" (**S**)
- "inadequate information to assess carcinogenic potential" (**I**)
- "not likely to be carcinogenic to humans" (**N**)

The letter abbreviations provided parenthetically above are now used in the DWSHA tables in place of the prior alpha numeric identifiers for chemicals that have been evaluated under the new guidelines (the 2005 guidelines or the 1996 and 1999 draft guidelines) or whose records in the DWSHA tables have been revised.

Cancer Group: A qualitative weight-of-evidence judgment as to the likelihood that a chemical may be a carcinogen for humans. Each chemical was placed into one of the following five categories (US EPA 1986 guidelines). The Cancer Group designations are given in the Tables for chemicals that have not yet been evaluated under the new guidelines or whose records in the DWSHA tables have been revised.

Group Category

A	Human carcinogen
B	Probable human carcinogen: **B1** indicates limited human evidence **B2** indicates sufficient evidence in animals and inadequate or no evidence in humans
C	Possible human carcinogen
D	Not classifiable as to human carcinogenicity
E	Evidence of noncarcinogenicity for humans

10^{-4} Cancer Risk: The concentration of a chemical in drinking water corresponding to an excess estimated lifetime cancer risk of 1 in 10,000.

Drinking Water Advisory: A nonregulatory concentration of a contaminant in water that is likely to be without adverse effects on health and aesthetics for the period it is derived.

DWEL: Drinking Water Equivalent Level. A DWEL is a drinking water lifetime exposure level, assuming **100%** exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur.

HA: Health Advisory. An estimate of acceptable drinking water levels for a chemical substance based on health effects information; an HA is not a legally enforceable Federal standard, but serves as technical guidance to assist Federal, State, and local officials.

> **One-Day HA:** The concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for up to one day of exposure. The One-Day HA is intended to protect a 10-kg child consuming 1 liter of water per day.
>
> **Ten-Day HA:** The concentration of a chemical in drinking water that is not expected to cause any adverse noncarcinogenic effects for up to ten days of exposure. The Ten-Day HA is also intended to protect a 10-kg child consuming 1 liter of water per day.
>
> **Lifetime HA:** The concentration of a chemical in drinking water that is not expected to cause any adverse **noncarcinogenic effects** for a lifetime of exposure, incorporating a drinking water RSC factor of contaminant-specific data or a default of 20% of total exposure from all sources. The Lifetime HA is based on exposure of a 70-kg adult consuming 2 liters of water per day. For Lifetime HAs developed for drinking water contaminants before the Lifetime HA policy change to develop Lifetime HAs for all drinking water contaminants regardless of carcinogenicity status in this DWSHA update, the Lifetime HA for Group C carcinogens, as indicated by the 1986 Cancer Guidelines, includes an uncertainty adjustment factor of 10 for possible carcinogenicity.

MCLG: Maximum Contaminant Level Goal. A non-enforceable health benchmark goal which is set at a level at which no known or anticipated adverse effect on the health of persons is expected to occur and which allows an adequate margin of safety.

MCL: Maximum Contaminant Level. The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to the MCLG as feasible using the best available analytical and treatment technologies and taking cost into consideration. MCLs are enforceable standards.

Oral cancer slope factor: The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day.

RfD: Reference Dose. An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

Risk Specific Level Concentration: The concentration of the chemical contaminant in drinking water or air providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

SDWR: Secondary Drinking Water Regulations. Non-enforceable Federal guidelines regarding cosmetic effects (such as tooth or skin discoloration) or aesthetic effects (such as taste, odor, or color) of drinking water.

TT: Treatment Technique. A required process intended to reduce the level of a contaminant in drinking water.

Unit Risk: The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed.

ABBREVIATIONS

D	Draft
DWEL	Drinking Water Equivalent Level
DWSHA	Drinking Water Standards and Health Advisories
F	Final
HA	Health Advisory
I	Interim
IRIS	Integrated Risk Information System
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
NA	Not Applicable
NOAEL	No-Observed-Adverse-Effect Level
OPP	Office of Pesticide Programs
OW	Office of Water
P	Proposed
Pv	Provisional
RED	Registration Eligibility Decision
Reg	Regulation
RfD	Reference Dose
TT	Treatment Technique

Drinking Water Standards and Health Advisories

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor[1]
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
ORGANICS												
Acenaphthene	83-32-9	-	-	-	-	-	-	0.06	2	-	-	-
Acifluorfen (sodium)	62476-59-9		-	-	F '88	2	2	0.01	0.4	-	0.1	***L/N***
Acrylamide	79-06-1	F	zero	TT[2]	F '87	1.5	0.3	**0.002**	0.07	-	-	**L**
Acrylonitrile	107-13-1		-	-	-	-	-	-	-	-	0.006	B1
Alachlor	15972-60-8	F	zero	0.002	F '88	0.1	0.1	0.01	0.4	-	***0.04***	***B2***
Aldicarb[3]	116-06-3	F[4]	0.001	0.003	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldicarb sulfone[3]	1646-88-4	F[4]	0.001	0.002	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldicarb sulfoxide[3]	1646-87-3	F[4]	0.001	0.004	F '95	0.01	0.01	0.001	0.035	0.007	-	D
Aldrin	309-00-2	-	-	-	F '92	0.0003	0.0003	0.00003	0.001	-	0.0002	B2
Ametryn	834-12-8	-	-	-	F '88	9	9	0.009	0.3	0.06	-	D
Ammonium sulfamate	7773-06-0	-	-	-	F '88	20	20	0.2	8	2	-	D
Anthracene (PAH)[5]	120-12-7	-	-	-	-	-	-	0.3	10	-	-	D
Atrazine	1912-24-9	F	0.003	0.003	F '88	-	-	***0.02***	0.7	-	-	***N***
Baygon	114-26-1	-	-	-	F '88	0.04	0.04	0.004	0.1	0.003	-	C
Bentazon	25057-89-0	-	-	-	F '99	0.3	0.3	0.03	1	0.2	-	E
Benz[a]anthracene (PAH)	56-55-3	-	-	-	-	-	-	-	-	-	-	B2
Benzene	71-43-2	F	zero	0.005	F '87	0.2	0.2	**0.004**	0.1	**0.003**	**1 to 10**	**H**
Benzo[a]pyrene (PAH)	50-32-8	F	zero	0.0002	-	-	-	-	-	-	0.0005	B2
Benzo[b]fluoranthene (PAH)	205-99-2	-	-	-	-	-	-	-	-	-	-	B2
Benzo[g,h,i]perylene (PAH)	191-24-2	-	-	-	-	-	-	-	-	-	-	D
Benzo[k]fluoranthene (PAH)	207-08-9	-	-	-	-	-	-	-	-	-	-	B2
Bis(2-chloro-1-methylethyl) ether	108-60-1	-	-	-	F '89	4	4	**0.04**	1	0.3	-	-
Bromacil	314-40-9	-	-	-	F '88	5	5	***0.1***	3.5	0.07	-	C
Bromobenzene	108-86-1	-	-	-	D '86	4	4	**0.008**	0.3	**0.06**	-	**I**

[1] Chemicals evaluated under the 2005 Cancer Guidelines or the 1996 or 1999 drafts are demoted by an abbreviation for their weight-of-the-evidence descriptor (see page iii). If the agency has not completed a new assessment for the chemical, the 1986 Guidelines Group designation (see page iii) is given in the Cancer Descriptor column.

[2] When Acrylamide is used in drinking water systems, the combination (or product) of dose and monomer level shall not exceed that equivalent to a polyacrylamide polymer containing 0.05% monomer dosed at 1 mg/L.

[3] The MCL value for any combination of two or more of these three chemicals should not exceed 0.007 mg/L because of a similar mode of action.

[4] Administrative stay of the effective date.

[5] PAH = Polycyclic aromatic hydrocarbon.

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Bromochloromethane	74-97-5	-	-	-	F '89	50	1	0.01	0.5	0.09	-	D
Bromodichloromethane (THM)	75-27-4	F	zero	0.08[1]	-	1	0.6	0.003	0.1	-	0.1	L
Bromoform (THM)	75-25-2	F	zero	0.08[1]	-	5	0.2	0.03	1	-	0.8	L
Bromomethane	74-83-9	-	-	-	D '89	0.1	0.1	0.001	0.05	0.01	-	D
Butyl benzyl phthalate	85-68-7	-	-	-	-	-	-	0.2	7	-	-	C
Butylate	2008-41-5	-	-	-	F '89	2	2	0.05	2	0.4	-	D
Carbaryl	63-25-2	-	-	-	F '88	1	1	***0.01***	0.4	-	4	***L***
Carbofuran	1563-66-2	F	0.04	0.04	F '87	-	-	***0.00006***	-	-	-	***N***
Carbon tetrachloride	56-23-5	F	zero	0.005	F '87	4	0.2	**0.004**	0.1	**0.03**	**0.05**	**L**
Carboxin	5234-68-4	-	-	-	F '88	1	1	0.1	3.5	0.7	-	D
Chloramben	133-90-4	-	-	-	F '88	3	3	0.015	0.5	0.1	-	D
Chlordane	12798-03-6	F	zero	0.002	F '87	0.06	0.06	**0.0005**	0.02	**0.004**	**0.01**	B2
Chloroform (THM)	67-66-3	F	0.07	0.08[1]	-	4	4	**0.01**	0.35	0.07	-	**L/N**
Chloromethane	74-87-3	-	-	-	F '89	9	0.4	-	-	-	-	**I**
Chlorophenol (2-)	95-57-8	-	-	-	D '94	0.5	0.5	0.005	0.2	0.04	-	D
Chlorothalonil	1897-45-6	-	-	-	F '88	0.2	0.2	0.015	0.5	-	0.15	B2
Chlorotoluene o-	95-49-8	-	-	-	F '89	2	2	0.02	0.7	0.1	-	D
Chlorotoluene p-	106-43-4	-	-	-	F '89	2	2	0.02	0.7	0.1	-	D
Chlorpyrifos	2921-88-2	-	-	-	F '92	0.03	0.03	***0.0003***	0.01	0.002	-	***D***
Chrysene (PAH)	218-01-9	-	-	-	-	-	-	-	-	-	-	B2
Cyanazine	21725-46-2	-	-	-	D '96	0.1	0.1	0.002	0.07	0.001	-	

[1] 1998 Final Rule for Disinfectants and Disinfection By-products: The total for trihalomethanes (THM) is 0.08 mg/L.

Drinking Water Standards and Health Advisories

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Cyanogen chloride[1]	506-77-4	-	-	-	-	0.05	0.05	0.05	2	-	-	D
2,4-D (2,4-dichlorophenoxyacetic acid)	94-75-7	F	0.07	0.07	F '87	1	0.3	***0.005***	0.2	-	-	***D***
DCPA (Dacthal)	1861-32-1	-	-	-	F '08	2	2	***0.01***	0.35	0.07	-	***C***
Dalapon (sodium salt)	75-99-0	F	0.2	0.2	F '89	3	3	0.03	0.9	0.2	-	D
Di(2-ethylhexyl)adipate	103-23-1	F	0.4	0.4	-	20	20	0.6	20	0.4	3	C
Di(2-ethylhexyl)phthalate	117-81-7	F	zero	0.006	-	-	-	0.02	0.7	-	0.3	B2
Diazinon	333-41-5	-	-	-	F '88	0.02	0.02	***0.0002***	0.007	0.001	-	***E***
Dibromochloromethane (THM)	124-48-1	F	0.06	0.08[2]	-	0.6	0.6	0.02	0.7	0.06	0.08	S
Dibromochloropropane (DBCP)	96-12-8	F	zero	0.0002	F '87	0.2	0.05	-	-	-	0.003	B2
Dibutyl phthalate	84-74-2	-	-	-	-	-	-	**0.1**	4	-	-	D
Dicamba	1918-00-9	-	-	-	F '88	-	-	***0.5***	18	4	-	*N*
Dichloroacetic acid	76-43-6	F	zero	0.06[3]	-	**3**	**3**	**0.004**	0.1	**0.03**	**0.07**	**L**
Dichlorobenzene o-	95-50-1	F	0.6	0.6	F '87	9	9	0.09	3	0.6	-	D
Dichlorobenzene — [4]	541-73-1	-	-	-	F '87	9	9	0.09	3	0.6	-	D
Dichlorobenzene p-	106-46-7	F	0.075	0.075	F '87	11	11	0.1	4	0.075	-	C
Dichlorodifluoromethane	75-71-8	-	-	-	F '89	40	40	0.2	5	1	-	D
Dichloroethane (1,2-)	107-06-2	F	zero	0.005	F '87	0.7	0.7	-	-	-	0.04	B2
Dichloroethylene (1,1-)	75-35-4	F	0.007	0.007	F '87	2	1	**0.05**	2	**0.4**	**0.006**	**S**
Dichloroethylene (cis-1,2-)	156-59-2	F	0.07	0.07	F '90	4	**3**	**0.002**	0.07	**0.01**	-	**I**
Dichloroethylene (trans-1,2-)	156-60-5	F	0.1	0.1	F '87	20	**2**	**0.02**	0.7	0.1	-	**I**
Dichloromethane	75-09-2	F	zero	0.005	D '93	10	2	0.06	2	**0.2**	0.5	**L**
Dichlorophenol (2,4-)	120-83-2	-	-	-	D '94	0.03	0.03	0.003	0.1	0.02	-	E
Dichloropropane (1,2-)	78-87-5	F	zero	0.005	F '87	-	0.09	-	-	-	0.06	B2
Dichloropropene (1,3-)	542-75-6	-	-	-	F '88	0.03	0.03	**0.03**	1	-	**0.04**	**L**
Dieldrin	60-57-1	-	-	-	F '88	0.0005	0.0005	0.00005	0.002	-	0.0002	B2
Diethyl phthalate	84-66-2	-	-	-	-	-	-	0.8	30	-	-	D

[1] Under review.
[2] 1998 Final Rule for Disinfectants and Disinfection By-products: The total for trihalomethanes is 0.08 mg/L.
[3] 1998 Final Rule for Disinfectants and Disinfection By-products: The total for five haloacetic acids is 0.06 mg/L.
[4] The values for m-dichlorobenzene are based on data for o-dichlorobenzene.

Drinking Water Standards and Health Advisories

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Diisopropylmethylphosphonate	1445-75-6	-	-	-	F '89	8	8	0.08	3	0.6	-	D
Dimethrin	70-38-2	-	-	-	F '88	10	10	0.3	10	2	-	D
Dimethyl methylphosphonate	756-79-6	-	-	-	F '92	2	2	0.2	7	0.1	0.7	C
Dimethyl phthalate	131-11-3	-	-	-	-	-	-	-	-	-	-	D
Dinitrobenzene (1,3-)	99-65-0	-	-	-	F '91	0.04	0.04	0.0001	0.005	0.001	-	D
Dinitrotoluene (2,4-)	121-14-2	-	-	-	F '08	1	1	0.002	0.1	-	0.005	L
Dinitrotoluene (2,6-)	606-20-2	-	-	-	F '08	0.4	0.04	0.001	0.04	-	0.005	L
Dinitrotoluene (2,6 & 2,4)[1]		-	-	-	F '92	-	-	-	-	-	0.005	B2
Dinoseb	88-85-7	F	0.007	0.007	F '88	0.3	0.3	0.001	0.035	0.007	-	D
Dioxane p-	123-91-1	-	-	-	F '87	4	0.4	**0.03**	1	0.2	**0.035**	**L**
Diphenamid	957-51-7	-	-	-	F '88	0.3	0.3	0.03	1	0.2	-	D
Diquat	85-00-7	F	0.02	0.02	-	-	-	***0.005***	0.02	-	-	***E***
Disulfoton	298-04-4	-	-	-	F '88	0.01	0.01	***0.0001***	0.0035	0.0007	-	***E***
Dithiane (1,4-)	505-29-3	-	-	-	F '92	0.4	0.4	0.01	0.4	0.08	-	D
Diuron	330-54-1	-	-	-	F '88	1	1	***0.003***	0.1	-	***0.2***	***L***
Endothall	145-73-3	F	0.1	0.1	F '88	0.8	0.8	***0.007***	0.25	0.05	-	***N***
Endrin	72-20-8	F	0.002	0.002	F '87	0.02	0.005	**0.0003**	0.01	0.002	-	I
Epichlorohydrin	106-89-8	F	zero	TT[2]	F '87	0.1	0.1	0.002	0.07	-	**0.3**	B2
Ethylbenzene	100-41-4	F	0.7	0.7	F '87	30	3	0.1	3	0.7	-	D
Ethylene dibromide (EDB)[3]	106-93-4	F	zero	0.00005	F '87	0.008	0.008	**0.009**	0.3	-	**0.002**	**L**
Ethylene glycol	107-21-1	-	-	-	F '87	20	6	**2**	70	14	-	D
Ethylene Thiourea (ETU)	96-45-7	-	-	-	F '88	0.3	0.3	***0.0002***	0.007	-	***0.06***	B2
Fenamiphos	22224-92-6	-	-	-	F '88	0.009	0.009	***0.0001***	0.0035	0.0007	-	***E***

[1] Technical grade.
[2] When epichlorohydrin is used in drinking water systems, the combination (or product) of dose and monomer level shall not exceed that equivalent to an epichlorohydrin-based polymer containing 0.01% monomer dosed at 20 mg/L.
[3] 1,2-dibromoethane.

Drinking Water Standards and Health Advisories

Chemicals	CAS Number	Standards			Status HA Standards	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Fluometuron	2164-17-2	-	-	-	F '88	2	2	0.01	0.5	0.09		D
Fluorene (PAH)	86-73-7	-	-	-	-	-	-	0.04	1	-	-	D
Fonofos	944-22-9	-	-	-	F '88	0.02	0.02	0.002	0.07	0.01	-	D
Formaldehyde	50-00-0	-	-	-	D '93	10	5	**0.2**	7	1	-	B1[1]
Glyphosate	1071-83-6	F	0.7	0.7	F '88	20	20	***2***	70	-	-	D
Heptachlor	76-44-8	F	zero	0.0004	F '87	0.01	0.01	0.0005	0.02	-	0.0008	B2
Heptachlor epoxide	1024-57-3	F	zero	0.0002	F '87	0.01	-	0.00001	0.0004	-	0.0004	B2
Hexachlorobenzene	118-74-1	F	zero	0.001	F '87	0.05	0.05	0.0008	0.03	-	0.002	B2
Hexachlorobutadiene[2]	87-68-3	-	-	-	-	0.3	0.3	0.0003	0.01	-	0.09	L
Hexachlorocyclopentadiene	77-47-4	F	0.05	0.05	-	-	-	**0.006**	0.2	-	-	**N**
Hexachloroethane	67-72-1	-	-	-	F '91	5	5	0.001	0.04	0.001	**0.3**	C
Hexane (n-)	110-54-3	-	-	-	F '87	10	4	-	-	-	-	**I**
Hexazinone	51235-04-2	-	-	-	F '96	3	2	***0.05***	2	0.4	-	D
HMX[3]	2691-41-0	-	-	-	F '88	5	5	0.05	2	0.4	-	D
Indeno[1,2,3,-c,d]pyrene (PAH)	193-39-5	-	-	-	-	-	-	-	-	-	-	B2
Isophorone	78-59-1	-	-	-	F '92	15	15	0.2	7	0.1	4	C
Isopropyl methylphosphonate	1832-54-8	-	-	-	F '92	30	30	0.1	3.5	0.7	-	D
Isopropylbenzene (cumene)	98-82-8	-	-	-	D '87	11	11	0.1	4	-	-	D
Lindane[4]	58-89-9	F	0.0002	0.0002	F '87	1	1	***0.005***	0.2	-	-	***S***
Malathion	121-75-5	-	-	-	F '92	0.2	0.2	***0.07***	2	0.5	-	***S***
Maleic hydrazide	123-33-1	-	-	-	F '88	10	10	0.5	20	4	-	D
MCPA[5]	94-74-6	-	-	-	F '88	0.1	0.1	***0.004***	0.14	0.03	-	***N***
Methomyl	16752-77-5	-	-	-	F '88	0.3	0.3	0.025	0.9	0.2	-	E
Methoxychlor	72-43-5	F	0.04	0.04	F '87	0.05	0.05	**0.005**	0.2	0.04	-	D
Methyl ethyl ketone	78-93-3	-	-	-	F '87	75	7.5	**0.6**	20	4	-	D
Methyl parathion	298-00-0	-	-	-	F '88	0.3	0.3	***0.0002***	0.007	0.001	-	***N***

[1] Carcinogenicity based on inhalation exposure.
[2] Regulatory Determination Health Effects Support Document for Hexachlorobutadiene (http://www.epa.gov/safewater/ccl/pdfs/reg_determine1/support_cc1_hexachlorobutadiene_healtheffects.pdf).
[3] HMX = octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine.
[4] Lindane = γ – hexachlorocyclohexane.
[5] MCPA = 4 (chloro-2-methoxyphenoxy) acetic acid.

Drinking Water Standards and Health Advisories

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Metolachlor	51218-45-2	-	-	-	F '88	2	2	***0.1***	3.5	0.7	-	***C***
Metribuzin	21087-64-9	-	-	-	F '88	5	5	***0.01***	0.35	0.07	-	***D***
Monochloroacetic acid	79-11-8	F	0.07	0.06[1]	-	0.2	0.2	0.01	0.35	0.07	-	I
Monochlorobenzene	108-90-7	F	0.1	0.1	F '87	4	4	**0.02**	0.7	0.1	-	D
Naphthalene	91-20-3	-	-	-	F '90	0.5	0.5	**0.02**	0.7	0.1	-	**I**
Nitrocellulose[2]	9004-70-0	-	-	-	F '88	-	-	-	-	-	-	-
Nitroguanidine	556-88-7	-	-	-	F '90	10	10	0.1	3.5	0.7	-	D
Nitrophenol p-	100-02-7	-	-	-	F '92	0.8	0.8	0.008	0.3	0.06	-	D
N-nitrosodimethylamine		-	-	-	-	-	-	-	-	-	**0.00007**	**B2**
Oxamyl (Vydate)	23135-22-0	F	0.2	0.2	F '05	0.01	0.01	0.001	0.035		-	N
Paraquat	1910-42-5	-	-	-	F '88	0.1	0.1	***0.0045***	0.2	0.03	-	***E***
Pentachlorophenol	87-86-5	F	zero	0.001	F '87	1	0.3	**0.005**	0.2	0.04	**0.009**	**L**
PFOA	335-67-1	-	-	-	F '16	-	-	2×10^{-5}	3.7×10^{-4}	7×10^{-5}	5×10^{-2}	S
PFOS	1763-23-1	-	-	-	F '16	-	-	2×10^{-5}	3.7×10^{-4}	7×10^{-5}	-	S
Phenanthrene (PAH)	85-01-8	-	-	-	-	-	-	-	-	-	-	D
Phenol	108-95-2	-	-	-	D '92	6	6	**0.3**	11	2	-	D
Picloram	1918-02-1	F	0.5	0.5	F '88	20	20	***0.02***	0.7	-	-	D
Polychlorinated biphenyls (PCBs)	1336-36-3	F	zero	0.0005	D '93	-	-	-	-	-	0.01	B2
Prometon	1610-18-0	-	-	-	F '88	0.2	0.2	***0.05***	2	0.4	-	***N***
Pronamide	23950-58-5	-	-	-	F '88	0.8	0.8	***0.08***	3	-	***0.1***	***B2***
Propachlor	1918-16-7	-	-	-	F '88	0.5	0.5	***0.05***	2	-	0.1	***L***
Propazine	139-40-2	-	-	-	F '88	-	-	***0.02***	0.7	0.01	-	***N***
Propham	122-42-9	-	-	-	F '88	5	5	0.02	0.6	0.1	-	D
Pyrene (PAH)	129-00-0	-	-	-	-	-	-	0.03	-	-	-	D
RDX[3]	121-82-4	-	-	-	F '88	0.1	0.1	0.003	0.1	0.002	0.03	C
Simazine	122-34-9	F	0.004	0.004	F '88	-	-	***0.02***	0.7	-	-	***N***
Styrene	100-42-5	F	0.1	0.1	F '87	20	2	0.2	7	0.1	-	C
2,4,5-T (Trichlorophenoxy-acetic acid)	93-76-5	-	-	-	F '88	0.8	0.8	0.01	0.35	0.07	-	D

[1] 1998 Final Rule for Disinfectants and Disinfection By-products: the total for five haloacetic acids is 0.06 mg/L.
[2] The Health Advisory Document for nitrocellulose does not include HA values and describes this compound as relatively nontoxic.
[3] RDX = hexahydro -1,3,5-trinitro-1,3,5-triazine.

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
2,3,7,8-TCDD (Dioxin)	1746-01-6	F	zero	3E-08	F '87	1E-06	1E-07	1E-09	4E-08	-	2E-08	B2
Tebuthiuron	34014-18-1	-	-	-	F '88	3	3	0.07	2	0.5	-	D
Terbacil	5902-51-2	-	-	-	F '88	0.3	0.3	0.01	0.4	0.09	-	E
Terbufos	13071-79-9	-	-	-	F '88	0.005	0.005	***0.00005***	0.002	0.0004	-	D
Tetrachloroethane (1,1,1,2-)	630-20-6	-	-	-	F '89	2	2	0.03	1	0.07	0.1	C
Tetrachloroethane (1,1,2,2-)	79-34-5	-	-	-	F '08	3	3	0.01	0.4	-	0.04	L
Tetrachloroethylene[1]	127-18-4	F	zero	0.005	F '87	2	2	0.01	0.5	0.01	-	-
Tetrachloroterephthalic acid	236-79-0	-	-	-	F '08	100	100	-	-	-	-	I
Trichlorofluoromethane	75-69-4	-	-	-	F '89	7	7	0.3	10	2	-	D
Toluene	108-88-3	F	1	1	D '93	20	2	**0.08**	3	-	-	**I**
Toxaphene	8001-35-2	F	zero	0.003	F '96	0.004	0.004	0.0004	0.01	-	0.003	B2
2,4,5-TP (Silvex)	93-72-1	F	0.05	0.05	F '88	0.2	0.2	0.008	0.3	0.05	-	D
Trichloroacetic acid	76-03-9	F	0.02	0.06[2]	-	3	3	0.03	1	0.02	-	S
Trichlorobenzene (1,2,4-)	120-82-1	F	0.07	0.07	F '89	0.1	0.1	**0.01**	0.35	0.07	-	D
Trichlorobenzene (1,3,5-)	108-70-3	-	-	-	F '89	0.6	0.6	0.006	0.2	0.04	-	D
Trichloroethane (1,1,1-)	71-55-6	F	0.2	0.2	F '87	100	40	**2**	70	-	-	**I**
Trichloroethane (1,1,2-)	79-00-5	F	0.003	0.005	F '89	0.6	0.4	0.004	0.1	0.003	0.06	C
Trichloroethylene [1]	79-01-6	F	zero	0.005	F '87	-	-	0.007	0.2	-	0.3	B2
Trichlorophenol (2,4,6-)	88-06-2	-	-	-	D '94	0.03	0.03	0.0003	0.01	-	0.3	B2
Trichloropropane (1,2,3-)	96-18-4	-	-	-	F '89	0.6	0.6	**0.004**	0.1	-	-	**L**
Trifluralin	1582-09-8	-	-	-	F '90	0.08	0.08	***0.02***	0.7	0.01	0.4	***C***
Trimethylbenzene (1,2,4-)	95-63-6	-	-	-	D '87	-	-	-	-	-	-	D
Trimethylbenzene (1,3,5-)	108-67-8	-	-	-	D '87	10	-	-	-	-	-	D
Trinitroglycerol	55-63-0	-	-	-	F '87	0.005	0.005	-	-	0.005	0.2	-
Trinitrotoluene (2,4,6-)	118-96-7	-	-	-	F '89	0.02	0.02	0.0005	0.02	0.002	0.1	C
Vinyl chloride	75-01-4	F	zero	0.002	F '87	3	3	**0.003**	0.1	-	**0.002**	**H**
Xylenes	1330-20-7	F	10	10	D '93	40	40	**0.2**	7	-	-	**I**

[1] Under review.
[2] 1998 Final Rule for Disinfectants and Disinfection By-products: The total for five haloacetic acids is 0.06 mg/L.

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life-time (mg/L)	mg/L at 10^{-4} Cancer Risk	
INORGANICS												
Ammonia	7664-41-7	-	-	-	D '92	-	-	-	-	30	-	D
Antimony	7440-36-0	F	0.006	0.006	F '92	0.01	0.01	0.0004	0.01	0.006	-	D
Arsenic	7440-38-2	F	zero	0.01	-	-	-	**0.0003**	0.01	-	**0.002**	A
Asbestos (fibers/l >10Fm length)	1332-21-4	F	7 MFL[1]	7 MFL	-	-	-	-	-	-	700-MFL	A[2]
Barium	7440-39-3	F	2	2	D '93	0.7	0.7	**0.2**	7	-	-	**N**
Beryllium	7440-41-7	F	0.004	0.004	F '92	30	30	**0.002**	0.07	-	-	-
Boron	7440-42-8	-	-	-	F '08	3	3	**0.2**	7	6	-	**I**
Bromate	7789-38-0	F	zero	0.01	D '98	0.2	-	**0.004**	0.14	-	0.005	B2
Cadmium	7440-43-9	F	0.005	0.005	F '87	0.04	0.04	0.0005	0.02	0.005	-	D
Chloramine[3]	10599-90-3	F	4[4]	4[4]	D '95	-	-	0.1	3.5	3.0	-	-
Chlorine	7782-50-5	F	4[4]	4[4]	D '95	3	3	0.1	5	4	-	D
Chlorine dioxide	10049-04-4	F	0.8[4]	0.8[4]	D '98	0.8	0.8	0.03	1	0.8	-	D
Chlorite	7758-19-2	F	0.8	1	D '98	0.8	0.8	0.03	1	0.8	-	D
Chromium (total)	7440-47-3	F	0.1	0.1	F '87	1	1	**0.003**[5]	0.1	-	-	D
Copper (at tap)	7440-50-8	F	1.3	TT[6]	D '98	-	-	-	-	-	-	D
Cyanide	143-33-9	F	0.2	0.2	F '87	0.2	0.2	**0.0006**[7]	-	-	-	**I**
Fluoride	7681-49-4	F	4	4	-	-[8]	-	**0.06**[9]	-	-	-	-
Lead (at tap)	7439-92-1	F	zero	TT[6]	-	-	-	-	-	-	-	B2
Manganese	7439-96-5	-	-	-	F"04	1	1	0.14[10]	1.6	0.3	-	D
Mercury (inorganic)	7487-94-7	F	0.002	0.002	F '87	0.002	0.002	**0.0003**	0.01	0.002	-	D
Molybdenum	7439-98-7	-	-	-	D '93	0.08	0.08	0.005	0.2	0.04	-	D
Nickel	7440-02-0	F	-	-	F '95	1	1	0.02	0.7	0.1	-	-

[1] MFL = million fibers per liter.
[2] Carcinogenicity based on inhalation exposure.
[3] Monochloramine; measured as free chlorine.
[4] 1998 Final Rule for Disinfectants and Disinfection By-products: MRDLG=Maximum Residual Disinfection Level Goal; and MRDL=Maximum Residual Disinfection Level.
[5] IRIS value for chromium VI.
[6] Copper action level 1.3 mg/L; lead action level 0.015 mg/L.
[7] This RfD is for hydrogen cyanide.
[8] In case of overfeed of the fluoridation chemical see CDC Guidelines in Engineering and Administrative Recommendations on Water Fluoridation www.cdc.gov/mmwr/preview/mmwrhtml/00039178.htm. Elevated F levels ≥ 10mg/L require action by the water system operator.
[9] Based on dental fluorosis in children, a cosmetic effect. MCLG based on skeletal fluorosis.
[10] Dietary manganese. The lifetime health advisory includes a 3 fold modifying factor to account for increased bioavailability from drinking water.

Chemicals	CASRN Number	Standards			Status HA Document	Health Advisories						Cancer Descriptor
						10-kg Child						
		Status Reg.	MCLG (mg/L)	MCL (mg/L)		One-day (mg/L)	Ten-day (mg/L)	RfD (mg/kg/day)	DWEL (mg/L)	Life- time (mg/L)	mg/L at 10^{-4} Cancer Risk	
Nitrate (as N)	14797-55-8	F	10	10	D '93	10[1]	10[1]	1.6	-	-	-	-
Nitrite (as N)	14797-65-0	F	1	1	D '93	1[1]	1[1]	0.16	-	-	-	-
Nitrate + Nitrite (both as N)		F	10	10	D '93	-	-	-	-	-	-	-
Perchlorate[2]	14797-73-0	-	-	-	I '08	-	-	0.007	0.025	0.015	-	L/N
Selenium	7782-49-2	F	0.05	0.05	-	-	-	0.005	0.2	0.05	-	D
Silver	7440-22-4	-	-	-	F '92	0.2	0.2	0.005[3]	0.2	0.1[3]	-	D
Strontium	7440-24-6	-	-	-	D '93	25	25	**0.6**	20	4	-	D
Thallium	7440-28-0	F	0.0005	0.002	F '92	0.007	0.007	-	-	-	-	**I**
White phosphorous	7723-14-0	-	-	-	F '90	-	-	0.00002	0.0005	0.0001		D
Zinc	7440-66-6	-	-	-	D '93	6	6	0.3	10	2	-	**I**
RADIONUCLIDES												
Beta particle and photon activity (formerly man-made radionuclides)		F	zero	4 mrem/yr	-	-	-	-	-		4 mrem/yr	A
Gross alpha particle activity		F	zero	15 pCi/L	-	-	-	-	-	-	15 pCi/L	A
Combined Radium 226 & 228	7440-14-4	F	zero	5 pCi/L	-	-	-	-	-	-	-	A
Radon	10043-92-2	P	zero	300 pCi/L AMCL[4] 4000 pCi/L	-	-	-	-	-	-	150 pCi/L	A
Uranium	7440-61-1	F	zero	0.03	-	-	-	0.0006[5]	0.02	-	-	A

[1] These values are calculated for a 4-kg infant and are protective for all age groups.
[2] Subchronic value for pregnant women.
[3] Based on a cosmetic effect.
[4] AMCL = Alternative Maximum Contaminant Level.
[5] Soluble uranium salts. Radionuclide Rule.

Secondary Drinking Water Regulations

Chemicals	CAS Number	Status	SDWR
Aluminum	7429-90-5	F	0.05 to 0.2 mg/L
Chloride	7647-14-5	F	250 mg/L
Color	NA	F	15 color units
Copper	7440-50-8	F	1.0 mg/L
Corrosivity	NA	F	non-corrosive
Fluoride	7681-49-4	F	2.0 mg/L
Foaming agents	NA	F	0.5 mg/L
Iron	7439-89-6	F	0.3 mg/L
Manganese	7439-96-5	F	0.05 mg/L
Odor	NA	F	3 threshold odor numbers
pH	NA	F	6.5 – 8.5
Silver	7440-22-4	F	0.1 mg/L
Sulfate	7757-82-6	F	250 mg/L
Total dissolved solids (TDS)	NA	F	500 mg/L
Zinc	7440-66-6	F	5 mg/L

Microbiology

	Status Reg.	Status HA Document	MCLG	MCL	Treatment Technique
Cryptosporidium	F	F 01	zero	TT	Systems that filter must remove 99% of *Cryptosporidium*
Cylindrospermosin	-	F 15	-	-	-
Cyanobacterial Microcystin Toxins	-	F 15	-	-	-
Giardia lamblia	F	F 98	zero	TT	99.9% killed/inactivated
Legionella	F[1]	F 01	zero	TT	No limit; EPA believes that if *Giardia* and viruses are inactivated, *Legionella* will also be controlled
Heterotrophic Plate Count (HPC)	F[1]	-	NA	TT	No more than 500 bacterial colonies per milliliter.
Mycobacteria	-	F 99	-	-	-
Total Coliforms	F	-	zero	5%	No more than 5.0% samples total coliform-positive in a month. Every sample that has total coliforms must be analyzed for fecal coliforms; no fecal coliforms are allowed.
Turbidity	F	-	NA	TT	At no time can turbidity go above 5 NTU (nephelometric turbidity units)
Viruses	F[1]	-	zero	TT	99.99% killed/inactivated

[1] Regulated under the surface water treatment rule.

Drinking Water Advisory Table

Chemicals	Status	Health-based Value	Taste Threshold	Odor Threshold
Ammonia	**D '92**	**Not Available**	**30 mg/L**	
Methyl tertiary butyl ether (MtBE)	**F '98**	**Not Available**	**40 μg/L**	**20 μg/L**
Sodium	**F '03**	**20 mg/L (for individuals on a 500 mg/day restricted sodium diet).**	**30-60 mg/L**	
Sulfate	**F '03**	**500 mg/L**	**250 mg/L**	

Taste Threshold: Concentration at which the majority of consumers do not notice an adverse taste in drinking water; it is recognized that some sensitive individuals may detect a chemical at levels below this threshold.

Odor Threshold: Concentration at which the majority of consumers do not notice an adverse odor in drinking water; it is recognized that some sensitive individuals may detect a chemical at levels below this threshold.

Attachment

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Comprehensive reviews of literature dealing with trace element effects on plants are provided by McKee and Wolf (1963),[436] Bolland and Butler (1966),[278] and Chapman (1966).[386] Hodgson (1963)[417] presented a review dealing with reactions of trace elements in soils.

In developing a workable program to determine acceptable limits for trace elements in irrigation waters, three considerations should be recognized:

- Many factors affect the uptake of and tolerance to trace elements. The most important of these are the natural variability in tolerances of plants and of animals that consume plants, in reactions within the soil, and in nutrient interactions, particularly in the plant.
- A system of tolerance limits should provide sufficient flexibility to cope with the more serious factors listed above.
- At the same time, restrictions must be defined as precisely as possible using presently available, but limited, research information.

Both the concentration of the element in the soil solution, assuming that steady state may be approached, and the total amount of the element added in relation to quantities that have been shown to produce toxicities were used in arriving at recommended maximum concentrations. A water application rate of 3 acre feet/acre/year was used to calculate the yearly rate of trace elements added in irrigation water.

The suggested maximum trace element concentrations for irrigation waters are shown in Table V-13.

The suggested maximum concentrations for continuous use on all soils are set for those sandy soils that have low capacities to react with the element in question. They are generally set at levels less than the concentrations that produce toxicities when the most sensitive plants are grown in nutrient solutions or sand cultures. This level is set, recognizing that concentration increases in the soil as water is evapotranspired, and that the effective concentration in the soil solution, at near steady state, is higher than in the irrigation water. The criteria for short-term use are suggested for soils that have high capacitites to remove from solution the element or elements being considered.

The work of Hodgson (1963)[417] showed that the general tolerance of the soil-plant system to manganese, cobalt, zinc, copper, and boron increased as the pH increased, primarily because of the positive correlation between the capacity of the soil to inactivate these ions and the pH. This same relationship exists with aluminum and probably exists with other elements such as nickel (Pratt et al. 1964)[449] and boron (Sims and Bingham 1968).[495] However, the ability of the soil to inactivate molybdenum decreases with increase in pH, such that the amount of this element that could be added without producing excesses was higher in acid soils.

TABLE V-13—Recommended Maximum Concentrations of Trace Elements in Irrigation Waters[a]

Element	For waters used continuously on all soil	For use up to 20 years on fine textured soils of pH 6.0 to 8.5
	mg/l	mg/l
Aluminum	5.0	20.0
Arsenic	0.10	2.0
Beryllium	0.10	0.50
Boron	0.75	2.0
Cadmium	0.010	0.050
Chromium	0.10	1.0
Cobalt	0.050	5.0
Copper	0.20	5.0
Fluoride	1.0	15.0
Iron	5.0	20.0
Lead	5.0	10.0
Lithium	2.5[b]	2.5[b]
Manganese	0.20	10.0
Molybdenum	0.010	0.050[d]
Nickel	0.20	2.0
Selenium	0.020	0.020
Tin[c]		
Titanium[c]		
Tungsten[c]		
Vanadium	0.10	1.0
Zinc	2.0	10.0

[a] These levels will normally not adversely affect plants or soils.
[b] Recommended maximum concentration for irrigating citrus is 0.075 mg/l.
[c] See text for a discussion of these elements.
[d] For only acid fine textured soils or acid soils with relatively high iron oxide contents.

In addition to pH control (i.e., liming acid soils), another important management factor that has a large effect on the capacity of soils to adsorb some trace elements without development of plant toxicities is the available phosphorus level. Large applications of phosphate are known to induce deficiencies of such elements as copper and zinc and greatly reduce aluminum toxicity (Chapman 1966).[386]

The concentrations given in Table V-13 are for ionic and soluble forms of the elements. If insoluble forms are present as particulate matter, these should be removed by filtration before the water is analyzed.

Aluminum

The toxicity of this ion is considered to be one of the main causes of nonproductivity in acid soils (Coleman and Thomas 1967,[392] Reeve and Sumner 1970,[493] Hoyt and Nyborg 1971a[419]).

At pH values from about 5.5 to 8.0, soils have great capacities to precipitate soluble aluminum and to eliminate its toxicity. Most irrigated soils are naturally alkaline, and many are highly buffered with calcium carbonate. In these situations aluminum toxicity is effectively prevented.

With only a few exceptions, as soils become more acid (pH <5.5), exchangeable and soluble aluminum develop by dissolution of oxides and hydroxides or by decomposition of clay minerals. Thus, without the introduction of aluminum, a toxicity of this element usually develops as soils are acidified, and limestone must be added to keep the soil productive.

Attachment

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Selenium

Selenium is toxic at low concentrations in nutrient solutions, and only small amounts added to soils increase the selenium content of forages to a level toxic to livestock. Broyer et al. (1966)[384] found that selenium at 0.025 mg/l in nutrient solutions decreased the yields of alfalfa.

The best evidence for use in setting water quality criteria for this element is application rates in relation to toxicity in forages. Amounts of selenium in forages required to prevent selenium deficiencies in cattle (Allaway et al. 1967)[386] ranged between 0.03 and 0.10 mg/kg (depending on other factors), whereas concentrations above 3 or 4 mg/kg were considered toxic (Underwood 1966).[471] A number of investigators (Hamilton and Beath 1963,[410] Grant 1965,[407] Allaway et al. 1966)[467] have shown that small applications of selenium to soils at a rate of a few kilograms per hectare produced plant concentrations of selenium that were toxic to animals. Gissel-Nielson and Bisbjerg (1970)[406] found that applications of approximately 0.2 kg/hectare of selenium produced from 1.0 to 10.5 mg/kg in tissues of forage and vegetable crops.

Recommendation

With the low levels of selenium required to produce toxic levels in forages, the recommended maximum concentration in irrigation waters is 0.02 mg/l for continuous use on all soils. At a rate of 3 acre feet of water per acre per year this concentration represents 3.2 pounds per acre in 20 years. The same recommended maximum concentration should be used on neutral and alkaline fine textured soils until greater information is obtained on soil reactions. The relative mobility of this element in soils in comparison to other trace elements and slow removal in harvested crops provide a sufficient safety margin.

Tin, Tungsten, and Titanium

Tin, tungsten, and titantium are effectively excluded by plants. The first two can undoubtedly be introduced to plants under conditions that can produce specific toxicities. However, not enough is known at this time about any of the three to prescribe tolerance limits. (This is true with other trace elements such as silver.) Titantium is very insoluble, at present it is not of great concern.

Vanadium

Gericke and Rennenkampff (1939)[405] found that vanadium at 0.1, 1.0, and 2.0 mg/l added to nutrient solutions as calcium vanadate slightly increased the growth of barley, whereas at 10 mg/l vanadium was toxic to both tops and roots and that vanadium chloride at 1.0 mg/l of vanadium was toxic. Warington (1954,[476] 1956[477]) found that flax, soybeans, and peas showed toxicity to vanadium in the concentration range of 0.5 to 2.5 mg/l. Chiu (1953)[383] found that 560 pounds per acre of vanadium added as ammonium metavanadate to rice paddy soils produced toxicity to rice.

Recommendations

Considering the toxicity of vanadium in nutrient solutions and in soils and the lack of information on the reaction of this element with soils, a maximum concentration of 0.10 mg/l for continued use on all soils is recommended. For a 20-year period on neutral and alkaline fine textured the recommended maximum concentration is 1.0 mg/l.

Zinc

Toxicities of zinc in nutrient solutions have been demonstrated for a number of plants. Hewitt (1948)[413] found that zinc at 16 to 32 mg/l produced iron deficiencies in sugar beets. Hunter and Vergnano (1953)[421] found toxicity to oats at 25 mg/l. Millikan (1947)[433] found that 2.5 mg/l produced iron deficiency in oats. Earley (1943)[399] found that the Peking variety of soybeans was killed at 0.4 mg/l, whereas the Manchu variety was killed at 1.6 mg/l.

The toxicity of zinc in soils is related to soil pH, and liming acid soil has a large effect in reducing toxicity (Barnette 1936,[471] Gall and Barnette 1940,[404] Peech 1941,[440] Staker and Cummings 1941,[468] Staker 1942,[467] Lee and Page 1967[426]). Amounts of added zinc that produce toxicity are highest in clay and peat soils and smallest in sands.

On acid sandy soils the amounts required for toxicity would suggest a recommended maximum concentration of zinc of 1 mg/l for continuous use. This concentration at a water application rate of 3 acre feet/acre/year would add 813 pounds per acre in 100 years. However, if acid sandy soils are limed to pH values of six or above, the tolerance level is increased by at least a factor of two (Gall and Barnette 1940).[404]

Recommendations

Assuming adequate use of liming materials to keep pH values high (six or above), the recommended maximum concentration for continuous use on all soils is 2.0 mg/l. For a 20-year period on neutral and alkaline soils the recommended maximum is 10 mg/l. On fine textured calcareous soils and on organic soils, the concentrations can exceed this limit by a factor of two or three with low probability of toxicities in a 20-year period.

PESTICIDES (IN WATER FOR IRRIGATION)

Pesticies are used widely in water for irrigation on commercial crops in the United States (Sheets 1967).[502] Figures on production, acreage treated, and use patterns indicate insecticides and herbicides comprise the major agricultural pesticides. There are over 320 insecticides and 127 herbicides registered for agricultural use (Fowler 1972).[498]

Attachment

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Silver; CASRN 7440-22-4

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the IRIS assessment development process. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website.

STATUS OF DATA FOR Silver

File First On-Line 01/31/1987

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	12/01/1991
Inhalation RfC (I.B.)	not evaluated	
Carcinogenicity Assessment (II.)	yes	06/01/1989

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Silver
CASRN — 7440-22-4
Last Revised — 12/01/1991

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of

information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
Argyria **2- to 9-Year Human i.v. Study** **Gaul and Staud, 1935**	NOEL: None LOAEL: 1 g (total dose); converted to an oral dose of 0.014 mg/kg/day	3	1	5E-3 mg/kg/day

* Conversion Factors: Based on conversion from the total i.v. dose to a total oral dose of 25 g (i.v. dose of 1 g divided by 0.04, assumed oral retention factor; see Furchner et al., 1968 in Additional Comments section) and dividing by 70 kg (adult body weight) and 25,500 days (a lifetime, or 70 years).

I.A.2. Principal and Supporting Studies (Oral RfD)

Gaul, L.E. and A.H. Staud. 1935. Clinical spectroscopy. Seventy cases of generalized argyrosis following organic and colloidal silver medication. J. Am. Med. Assoc. 104: 1387-1390.

The critical effect in humans ingesting silver is argyria, a medically benign but permanent bluish-gray discoloration of the skin. Argyria results from the deposition of silver in the dermis and also from silver-induced production of melanin. Although silver has been shown to be uniformly deposited in exposed and unexposed areas, the increased pigmentation becomes more pronounced in areas exposed to sunlight due to photoactivated reduction of the metal. Although the deposition of silver is permanent, it is not associated with any adverse health effects. No pathologic changes or inflammatory reactions have been shown to result from silver deposition. Silver compounds have been employed for medical uses for centuries. In the nineteenth and early twentieth centuries, silver arsphenamine was used in the treatment of syphillis; more recently it has been used as an astringent in topical preparations. While argyria occurred more commonly before the development of antibiotics, it is now a rare occurrence. Greene and Su (1987) have published a review of argyria.

Gaul and Staud (1935) reported 70 cases of generalized argyria following organic and colloidal silver medication, including 13 cases of generalized argyria following intravenous silver arsphenamine injection therapy and a biospectrometric analysis of 10 cases of generalized argyria classified according to the quantity of silver present. In the i.v. study, data were presented for 10 males (23-64 years old) and for two females (23 and 49 years old) who were administered 31-100 i.v. injections of silver arsphenamine (total dose was 4-20 g) over a 2- to 9.75-year period. Argyria developed after a total dose of 4, 7 or 8 g in some patients, while in others, argyria did not develop until after a total dose of 10, 15 or 20 g. In the biospectrometric analysis of skin biopsies from 10 cases of generalized argyria, the authors confirmed that the degree of the discoloration is directly dependent on the amount of silver present. The authors concluded that argyria may become clinically apparent after a total accumulated i.v. dose of approximately 8 g of silver arsphenamine. The book entitled "Argyria. The Pharmacology of Silver" reached the same conclusion, that a total accumulative i.v. dose of 8 gm silver arsphenamine is the limit beyond which argyria may develop (Hill and Pillsbury, 1939). However, since body accumulates silver throughout life, it is theoretically possible for amounts less than this (for example, 4 g silver arsphenamine) to result in argyria. Therefore, based on cases presented in this study, the lowest i.v. dose resulting in argyria in one patient, 1 g metallic silver (4 g silver arsphenamine x 0.23, the fraction of silver in silver arsphenamine) is considered to be a minimal effect level for this study.

Blumberg and Carey (1934) reported argyria in an emaciated chronically ill (more than 15 years) 33-year-old female (32.7 kg) who had ingested capsules containing silver nitrate over a period of 1 year. The patient reported ingesting 16 mg silver nitrate three times a day (about 30 mg silver/day) for alternate periods of 2 weeks. Spectrographic analysis of blood samples revealed a blood silver level of 0.5 mg/L 1 week after ingestion of silver nitrate capsules ceased, and there was only a small decrease in this level after 3 months. The authors noted that this marked argyremia was striking because even in cases of documented argyria, blood silver levels are not generally elevated to this extent. Normal levels for argyremic patients were reported to range from not detected to 0.005 mg Ag/l blood. Heavy traces of silver in the skin, moderate amounts in the urine and feces, and trace amounts in the saliva were reported in samples tested 3 months after ingestion of the capsules stopped; however, despite the marked argyremia and detection of silver in the skin, the argyria at 3 months was quite mild. No obvious dark pigmentation was seen other than gingival lines which are considered to be characteristic of the first signs of argyria. The authors suggested that this may have been because the woman was not exposed to strong light during the period of silver treatment. This study is not suitable to serve as the basis for a quantitative risk assessment for silver because it is a clinical report on only one patient of compromised health. Furthermore, the actual amount of silver ingested is based on the patient's recollection and cannot be accurately determined.

In a case reported by East et al. (1980), argyria was diagnosed in a 47-year- old woman (58.6 kg) who had taken excessively large oral doses of anti-smoking lozenges containing silver acetate over a period of 2.5 years. No information was provided as to the actual amount of silver ingested. Symptoms of argyria appeared after the first 6 months of exposure. Based on whole body neutron activation analysis, the total body burden of silver in this female was estimated to be 6.4 (plus or minus 2) g. Both the total body burden and concentration of silver in the skin were estimated to be 8000 times higher than normal. In a separate 30-week experiment, the same subject retained 18% of a single dose of orally-administered silver, a retention level much higher than that reported by other investigators. East et al. (1980) cited other studies on this particular anti-smoking formulation (on the market since 1973) which demonstrated that "within the limits of experimental error, no silver is retained after oral administration." However, this may not hold true for excessive intakes like that ingested by this individual. As with the study by Blumberg and Carey (1934), this study is not suitable to serve as the basis for a quantitative risk assessment. It is a clinical report on only one patient and the actual amount of silver ingested can only be estimated.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF — An uncertainty factor of 3 is applied to account for minimal effects in a subpopulation which has exhibited an increased propensity for the development of argyria. The critical effect observed is a cosmetic effect, with no associated adverse health effects. Also, the critical study reports on only 1 individual who developed argyria following an i.v. dose of 1 g silver (4 g silver arsphenamine). Other individuals did not respond until levels five times higher were administered. No uncertainty factor for less than chronic to chronic duration is needed because the dose has been apportioned over a lifetime of 70 years.

MF — None

I.A.4. Additional Studies/Comments (Oral RfD)

In the study by East et al. (1980) (see section 1.A.2.), one human was found to retain 18% of a single oral dose. However, the authors acknowledge that this high level of retention is not consistent with data published in other laboratories. For ethical reasons, the experiment could be not repeated to determine the validity of the results.

Humans are exposed to small amounts of silver from dietary sources. The oral intake of silver from a typical diet has been estimated to range from 27-88 ug/day (Hamilton and Minski, 1972/1973; Kehoe et al., 1940). Tipton et al. (1966) estimated a lesser intake of 10-20 ug/day in two subjects during a 30- day observation period. Over a lifetime, a small but measurable amount of silver is accumulated by individuals having no excessive exposure. Gaul and Staud

(1935) estimated that a person aged 50 years would have an average retention of 0.23-0.48 g silver (equivalent to 1-2 g silver arsphenamine). Petering et al. (1991) estimated a much lower body burden of 9 mg over a 50- year period based on estimated intake, absorption, and excretion values; however, it is not clear how the final estimate was calculated. Furchner et al. (1968) studied the absorption and retention of ingested silver (as silver nitrate, amount not specified) in mice, rats, monkeys and dogs. In all four species, very little silver was absorbed from the GI tract. Cumulative excretion ranged from 90 to 99% on the second day after ingestion, with <1% of the dose being retained in <1 week in monkeys, rats and mice. Dogs had a slightly greater retention. The authors used the data from the dog to estimate how much silver ingested by a 70 kg human would be retained. An "equilibrium factor" of 4.4% was determined by integrating from zero to infinity a retention equation which assumes a triphasic elimination pattern for silver with the initial elimination of 90% coming from the dog data. The first elimination half-time of 0.5 days was used "arbitrarily"; subsequent half-times of 3.5 days and 41 days were taken from a metabolic study by Polachek et al. (1960). Furchner et al. (1968) considered their calculated equilibrium factor of 4.4% to be a conservative estimate for the amount of silver which would be retained by a 70 kg human. This figure was rounded to 4% and was used in the dose conversion (i.v. dose converted to oral intake) for the calculation of the RfD.

In addition to silver arsphenamine, any silver compound (silver nitrate, silver acetate, argyrol, Neosilvol and Collargol, etc.), at high dose, can cause argyria. Another important factor predisposing to the development of argyria is the exposure of the skin to light.

Argyria, the critical effect upon which the RfD for silver is based, occurs at levels of exposure much lower than those levels associated with other effects of silver. Argyrosis, resulting from the deposition of silver in the eye, has also been documented, but generally involves the use of eye drops or make-up containing silver (Greene and Su, 1987). Silver has been found to be deposited in the cornea and the anterior capsule of the lens. The same deposition pattern was seen in the eyes of male Wistar rats following administration of a 0.66% silver nitrate solution to the eyes for 45 days (Rungby, 1986). No toxicological effects were reported.

Toxic effects of silver have been reported primarily for the cardiovascular and hepatic systems. Olcott (1950) administered 0.1% silver nitrate in drinking water to rats for 218 days. This exposure (about 89 mg/kg/day) resulted in a statistically significant increase in the incidence of ventricular hypertrophy. Upon autopsy, advanced pigmentation was observed in body organs, but the ventricular hypertrophy was not attributed to silver deposition.

Hepatic necrosis and ultrastructural changes of the liver have been induced by silver administration to vitamin E and/or selenium deficient rats (Wagner et al., 1975; Diplock et al., 1967; Bunyan et al., 1968). Investigators have hypothesized that this toxicity is related to a silver-induced selenium deficiency that inhibits the synthesis of the seleno-enzyme glutathione

peroxidase. In animals supplemented with selenium and/or vitamin E, exposures of silver as high as 140 mg/kg/day (100 mg Ag/L drinking water) were well- tolerated (Bunyan et al., 1968).

I.A.5. Confidence in the Oral RfD

Study — Medium
Database — Low
RfD — Low

The critical human study rates a medium confidence. It is an old study (1935) which offers fairly specific information regarding the total dose of silver injected over a stated period of time. One shortcoming of the study is that only patients developing argyria are described; no information is presented on patients who received multiple injections of silver arsphenamine without developing argyria. Therefore, it is difficult to establish a NOAEL. Also, the individuals in the study were being treated for syphilis and may have been of compromised health.

Confidence in the database is considered to be low because the studies used to support the RfD were not controlled studies. For clinical case studies of argyria (such as Blumberg and Carey, 1934; East et al., 1980), it is especially difficult to determine the amount of silver that was ingested.

Confidence in the RfD can be considered low-to-medium because, while the critical effect has been demonstrated in humans following oral administration of silver, the quantitative risk estimate is based on a study utilizing intravenous administration and thus necessitates a dose conversion with inherent uncertainties.

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — This assessment is not presented in any existing U.S. EPA document.

Other EPA Documentation — None

Agency Work Group Review — 10/09/1985, 02/05/1986, 04/18/1990, 02/20/1991, 07/18/1991

Verification Date — 07/18/1991

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the RfD for silver conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new

studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

Substance Name — Silver
CASRN — 7440-22-4

Not available at this time.

II. Carcinogenicity Assessment for Lifetime Exposure

Substance Name — Silver
CASRN — 7440-22-4
Last Revised — 06/01/1989

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per ug/L drinking water or risk per ug/cu.m air breathed. The third form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000 or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

Classification — D; not classified as to human carcinogenicity

Basis — In animals, local sarcomas have been induced after implantation of foils and discs of silver. However, the interpretation of these findings has been questioned due to the phenomenon of solid-state carcinogenesis in which even insoluble solids such as plastic have been shown to result in local fibrosarcomas.

II.A.2. Human Carcinogenicity Data

No evidence of cancer in humans has been reported despite frequent therapeutic use of the compound over the years.

II.A.3. Animal Carcinogenicity Data

Inadequate. Local sarcomas have been induced after subcutaneous (s.c.) implantation of foils and discs of silver and other noble metals. Furst (1979, 1981), however, cited studies showing that even insoluble solids such as smooth ivory and plastic result in local fibrosarcomas and that tin when crumbled will not. He concluded that i.p. and s.c. implants are invalid as indicators of carcinogenicity because a phenomenon called solid-state carcinogenesis may complicate the interpretation of the cause of these tumors. It is difficult to interpret these implantation site tumors in laboratory animals in terms of exposure to humans via ingestion. Within these constraints there are two studies given below in which silver per se appeared to induce no carcinogenic response.

Schmahl and Steinhoff (1960) reported, in a study of silver and of gold, that colloidal silver injected both i.v. and s.c. into rats resulted in tumors in 8 of 26 rats which survived longer than 14 months. In 6 of the 8, the tumor was at the site of the s.c. injection. In about 700 untreated rats the rate of spontaneous tumor formation of any site was 1 to 3%. No vehicle control was reported.

Furst and Schlauder (1977) evaluated silver and gold for carcinogenicity in a study designed to avoid solid-state carcinogenesis. Metal powder was suspended in trioctanoin and injected monthly, i.m., into 50 male and female Fischer 344 rats per group. The dose was 5 mg each for 5 treatments and 10 mg each for 5 more treatments for a total dose of 75 mg silver. The treatment regimen included a vehicle control (a reportedly inert material), and cadmium as a positive control. Injection site sarcomas were found only in vehicle control (1/50), gold (1/50) and

cadmium (30/50); no tumors (0/50) appeared at the site of injection in the silver-treated animals. A complete necropsy was performed on all animals. The authors mentioned the existence of spontaneous tumors in Fischer 344 rats, but reported only injection site tumors. They concluded that finely divided silver powder injected i.m. does not induce cancer.

II.A.4. Supporting Data for Carcinogenicity

Further support for the lack of silver's ability to induce or promote cancer stems from the finding that, despite long standing and frequent therapeutic usage in humans, there are no reports of cancer associated with silver. In a recent Proceedings of a Workshop/Conference on the Role of Metals in Carcinogenesis (1981) containing 24 articles on animal bioassays, epidemiology, biochemistry, mutagenicity, and enhancement and inhibition of carcinogenesis, silver was not included as a metal of carcinogenic concern.

No evidence of the mutagenicity of silver was shown in two available studies. Demerec et al. (1951) studied silver nitrate for the possible induction of back-mutations from streptomycin dependence to nondependence in Eschericha coli. Silver nitrate was considered nonmutagenic in this assay. Nishioka (1975) screened silver chloride with other chemicals for mutagenic effects using a method called the rec-assay. Silver chloride was considered nonmutagenic in this assay.

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

Not available.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Not available.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Document — U.S. EPA, 1988

The 1988 Drinking Water Criteria Document for Silver has received Agency Review.

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Work Group Review — 09/22/1988

Verification Date — 09/22/1988

Screening-Level Literature Review Findings — A screening-level review conducted by an EPA contractor of the more recent toxicology literature pertinent to the cancer assessment for silver conducted in August 2003 did not identify any critical new studies. IRIS users who know of important new studies may provide that information to the IRIS Hotline at hotline.iris@epa.gov or 202-566-1676.

II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

III. [reserved]
IV. [reserved]
V. [reserved]

VI. Bibliography

Substance Name — Silver
CASRN — 7440-22-4

VI.A. Oral RfD References

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VI.B. Inhalation RfC References

None

VI.C. Carcinogenicity Assessment References

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VII. Revision History

Substance Name — Silver
CASRN — 7440-22-4

Date	Section	Description
06/01/1989	II.	Carcinogen summary on-line
08/01/1991	I.A.	Withdrawn; new oral RfD verified (in preparation)
12/01/1991	I.A.	Oral RfD summary replaced; RfD changed
10/28/2003	I.A.6, II.D.2	Screening-Level Literature Review Findings message has been added.

VIII. Synonyms

Substance Name — Silver
CASRN — 7440-22-4
Last Revised — 06/01/1989

- 7440-22-4
- ARGENTUM CREDE
- COLLARGOL
- Silver

Attachment

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Table A – Calculated Health-Based Standards and Corresponding LLOQs/LCMRLs for the Constituents Proposed for Updates

CASRN	Constituent	Calculated Health-Based Standard (mg/L)	Cancer / Noncancer Health-Based Basis	LLOQ / LCMRL (mg/L)	Method
Section 620.410(a) - Inorganics					
7429-90-5	Aluminum	1.9	Noncancer	0.020	EPA 200.8
7440-48-4	Cobalt	0.0012	Noncancer	0.0001	EPA 200.8
7439-93-2	**Lithium**	0.0077	Noncancer	0.04	EPA 200.7
7439-98-7	Molybdenum	0.019	Noncancer	0.0001	EPA 200.8
7440-02-0	Nickel	0.077	Noncancer	0.002	EPA 200.8
14797-73-0	Perchlorate	0.0081	Noncancer	0.002	EPA 314.0
7440-22-4	Silver	0.058	Noncancer	0.0002	EPA 200.8
7440-62-2	Vanadium	0.00027	Noncancer	0.00005	EPA 1640
7440-66-6	Zinc	1.2	Noncancer	0.010	EPA 200.8
Section 620.410(b) – Organics					
83-32-9	Acenaphthene	0.23	Noncancer	0.0001	EPA 8270C SIM
67-64-1	Acetone	3.5	Noncancer	0.005	EPA 8260B
120-12-7	Anthracene	1.2	Noncancer	0.0001	EPA 8270C SIM
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)	0.000012	Cancer	0.00001	EPA 8081A
56-55-3	Benzo(a)anthracene	0.00025	Cancer	0.0001	EPA 8270C SIM
205-99-2	Benzo(b)fluoranthene	0.00025	Cancer	0.0001	EPA 8270C SIM
207-08-9	Benzo(k)fluoranthene	0.0025	Cancer	0.0001	EPA 8270C SIM
65-85-0	Benzoic acid	15	Noncancer	0.1	EPA 8270C
78-93-3	2-Butanone (methyl ethyl ketone)	2.3	Noncancer	0.005	EPA 8260B
75-15-0	Carbon disulfide	0.38	Noncancer	0.001	EPA 8260B
218-01-9	Chrysene	0.025	Cancer	0.0001	EPA 8270C SIM
53-70-3	**Dibenzo(a,h)anthracene**	0.000025	Cancer	0.0001	EPA 8270C SIM
1918-00-9	Dicamba	0.12	Noncancer	0.00075	EPA 8151A
75-71-8	Dichlorodifluoromethane	0.77	Noncancer	0.001	EPA 8260B
75-34-3	1,1-Dichloroethane	0.77	Noncancer	0.001	EPA 8260B

CASRN	Constituent	Calculated Health-Based Standard (mg/L)	Cancer / Noncancer Health-Based Basis	LLOQ / LCMRL (mg/L)	Method
84-66-2	Diethyl phthalate	3.1	Noncancer	0.001	EPA 8270C
84-74-2	Di-*n*-butyl phthalate	0.38	Noncancer	0.001	EPA 8270C
99-65-0	**1,3-Dinitrobenzene**	0.00039	Noncancer	0.001	EPA 8270C
121-14-2	**2,4-Dinitrotoluene**	0.00025	Cancer	0.001	EPA 8270C
606-20-2	**2,6-Dinitrotoluene**	0.000052	Cancer	0.001	EPA 8270C
123-91-1	1,4-Dioxane (*p*-dioxane)	0.00078	Cancer	0.0001	EPA 8270C SIM
206-44-0	Fluoranthene	0.15	Noncancer	0.0001	EPA 8270C SIM
86-73-7	Fluorene	0.15	Noncancer	0.0001	EPA 8270C SIM
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	0.000012	Noncancer	0.000002	EPA 537.1
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	0.77	Noncancer	0.001	EPA 8330A
193-39-5	Indeno(1,2,3-c,d)pyrene	0.00025	Cancer	0.0001	EPA 8270C SIM
98-82-8	Isopropylbenzene (cumene)	0.38	Noncancer	0.001	EPA 8260B
93-65-2	**MCPP (Mecoprop)**	0.0039	Noncancer	0.1	EPA 8151A
1634-04-4	MTBE (methyl tertiary-butyl ether)	0.038	Noncancer	0.003	EPA 8260B
90-12-0	1-Methylnaphthalene	0.27	Noncancer	0.0001	EPA 8270C SIM
91-57-6	2-Methylnaphthalene	0.015	Noncancer	0.0001	EPA 8270C SIM
95-48-7	2-Methylphenol (*o*-cresol)	0.19	Noncancer	0.001	EPA 8270C SIM
91-20-3	Naphthalene	0.077	Noncancer	0.0001	EPA 8270C SIM
98-95-3	Nitrobenzene	0.0077	Noncancer	0.001	EPA 8330A
375-73-5	PFBS (perfluorobutanesulfonic acid)	0.0012	Noncancer	0.000002	EPA 537.1
355-46-4	PFHxS (perfluorohexanesulfonic acid)	0.000077	Noncancer	0.000002	EPA 537.1
375-95-1	PFNA (perfluorononanoic acid)	0.000012	Noncancer	0.000002	EPA 537.1
335-67-1	**PFOA (perfluorooctanoic acid)**	0.00000054	Cancer	0.000002	EPA 537.1
1763-23-1	PFOS (perfluorooctanesulfonic acid)	0.0000077	Noncancer	0.000002	EPA 537.1
129-00-0	Pyrene	0.12	Noncancer	0.0001	EPA 8270C SIM

CASRN	Constituent	Calculated Health-Based Standard (mg/L)	Cancer / Noncancer Health-Based Basis	LLOQ / LCMRL (mg/L)	Method
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	0.062	Noncancer	0.001	EPA 8330A
118-96-7	TNT (2,4,6-trinitrotoluene)	0.0077	Noncancer	0.001	EPA 8330A
75-69-4	Trichlorofluoromethane	1.2	Noncancer	0.001	EPA 8260B
99-35-4	1,3,5-Trinitrobenzene	0.46	Noncancer	0.01	EPA 8270C

Constituents highlighted in bold have LLOQs/LCMRLs greater than the calculated health-based standard.
LCMRL = Lowest Concentration Minimum Reporting Level
LLOQ = Lower Limit of Quantitation

Attachment

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Class II Groundwater Quality Standard (GQS) Updates						Treatment Factor (TF) Determination Parameters	
CASRN	**Constituent**	**Current Class II GQS mg/L**	**Current Class II GQS Basis / TF Applied to Class I GQS**	**Proposed Updated Class II GQS mg/L**	**Updated Class II GQS Basis / TF Applied to Class I GQS**	**Organic Carbon Partition Coefficient (K_{oc}) L/kg**	**Dimensionless Henry's Law Constant at 20 ^{0}C (H') unitless**
Part 620.420(a) - Inorganics							
Part 620.420(a)(1)							
7681-49-4	Fluoride (sodium fluoride)	4.0	Class I GQS	2.0	Livestock	----	----
7439-93-2	Lithium	----	----	2.5	Irrigation	----	----
7439-98-7	Molybdenum	----	----	0.05	Irrigation	----	----
14797-73-0	Perchlorate	0.0049	Class I GQS	0.0081	Class I GQS	----	----
Part 620.420(a)(2)							
7429-90-5	Aluminum	----	----	5	Livestock	----	----
7440-50-8	Copper	0.65	Lead/Copper Rule	0.5	Livestock	----	----
7440-14-4	Radium (combined 226+228) (pCi/L)	----	----	5	Class I GQS	----	----
7782-49-2	Selenium	0.05	Class I GQS	0.02	Irrigation	----	----
7440-22-4	Silver	----	----	0.058	Class I GQS	----	----
Part 620.420(b) - Organics							
83-32-9	Acenaphthene	2.1	TF = 5	1.2	TF = 5	5.03E+03	4.82E-03
67-64-1	Acetone	6.3	No TF Applied	3.5	No TF Applied	2.36E+00	1.17E-03
120-12-7	Anthracene	10.5	TF = 5	6	TF = 5	1.64E+04	1.37E-03
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)'	0.00055	TF = 5	0.00006	TF = 5	2.81E+03	2.74E-04
56-55-3	Benzo(a)anthracene	0.00065	TF = 5	0.0013	TF = 5	1.77E+05	2.54E-04
205-99-2	Benzo(b)fluoranthene	0.0009	TF = 5	0.0013	TF = 5	5.99E+05	1.47E-05
207-08-9	Benzo(k)fluoranthene	0.006	TF = 5	0.013	TF = 5	5.87E+05	1.19E-05
65-85-0	Benzoic acid	28.0	No TF Applied	15	No TF Applied	6.00E-01	1.00E-06
78-93-3	2-Butanone (methyl ethyl ketone)	4.2	No TF Applied	2.3	No TF Applied	4.51E+00	1.86E-03
75-15-0	Carbon disulfide	3.5	TF = 5	1.9	TF = 5	2.17E+01	4.95E-01
218-01-9	Chrysene	0.06	TF = 5	0.13	TF = 5	1.81E+05	1.07E-04
53-70-3	Dibenzo(a,h)anthracene	0.0015	TF = 5	0.0005	TF = 5	1.91E+06	2.52E-06
1918-00-9	Dicamba'	0.21	No TF Applied	0.12	No TF Applied	2.90E+01	8.91E-08

Class II Groundwater Quality Standard (GQS) Updates						Treatment Factor (TF) Determination Parameters	
CASRN	Constituent	Current Class II GQS mg/L	Current Class II GQS Basis / TF Applied to Class I GQS	Proposed Updated Class II GQS mg/L	Updated Class II GQS Basis / TF Applied to Class I GQS	Organic Carbon Partition Coefficient (K_{oc}) L/kg	Dimensionless Henry's Law Constant at 20 ^{0}C (H') unitless
75-71-8	Dichlorodifluoromethane	7.0	TF = 5	3.9	TF = 5	4.39E+01	1.27E+01
75-34-3	1,1-Dichloroethane	7.0	TF = 5	3.9	TF = 5	3.18E+01	1.89E-01
84-66-2	Diethyl phthalate	5.6	No TF Applied	3.1	No TF Applied	1.05E+02	1.45E-05
84-74-2	Di-*n*-butyl phthalate	3.5	TF = 5	1.9	TF = 5	1.16E+03	3.30E-05
99-65-0	1,3-Dinitrobenzene	0.0007	No TF Applied	0.001	No TF Applied	3.52E+02	1.15E-06
121-14-2	2,4-Dinitrotoluene	0.0001	No TF Applied	0.005	TF = 5	5.76E+02	1.19E-06
606-20-0	2,6-Dinitrotoluene	0.00031	No TF Applied	0.005	TF = 5	5.87E+02	1.71E-05
123-91-1	1,4-Dioxane (*p*-dioxane)	0.0077	No TF Applied	0.00078	No TF Applied	2.63E+00	1.53E-04
206-44-0	Fluoranthene	1.4	TF = 5	0.75	TF = 5	5.55E+04	2.08E-04
86-73-7	Fluorene	1.4	TF = 5	0.75	TF = 5	9.16E+03	2.46E-03
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)'	----	----	0.000012	No TF Applied	3.04E+02	8.38E-03
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)'	1.4	No TF Applied	3.9	TF = 5	5.32E+02	3.54E-08
193-39-5	Indeno(1,2,3-c,d)pyrene	0.0022	TF = 5	0.0013	TF = 5	1.95E+06	6.55E-06
98-82-8	Isopropylbenzene (cumene)	3.5	TF = 5	1.9	TF = 5	6.98E+02	3.34E-01
93-65-2	MCPP (Mecoprop)'	0.007	No TF Applied	0.1	No TF Applied	4.85E+01	7.44E-07
1634-04-4	MTBE (methyl tertiary-butyl ether)	0.07	No TF Applied	0.038	No TF Applied	1.16E+01	1.99E-02
90-12-0	1-Methylnaphthalene	----	----	0.27	No TF Applied	1.29E-02	2.10E-02
91-57-6	2-Methylnaphthalene	0.14	TF = 5	0.015	No TF Applied	1.35E-02	2.12E-02
95-48-7	2-Methylphenol (*o*-cresol)	0.35	No TF Applied	0.19	No TF Applied	3.07E+02	3.40E-05
91-20-3	Naphthalene	0.22	TF = 5	0.39	TF = 5	1.54E+03	1.27E-02
98-95-3	Nitrobenzene	0.014	No TF Applied	0.0077	No TF Applied	2.26E+02	6.81E-04
375-73-5	PFBS (perfluorobutanesulfonic acid)	----	----	0.0012	No TF Applied	6.17E+01	----
355-46-4	PFHxS (perfluorohexanesulfonic acid)	----	----	0.000077	No TF Applied	1.12E+02	----
375-95-1	PFNA (perfluorononanoic acid)	----	----	0.000012	No TF Applied	2.46E+02	----
335-67-1	PFOA (perfluorooctanoic acid)	----	----	0.000002	No TF Applied	1.15E+02	----
1763-23-1	PFOS (perfluorooctanesulfonic acid)	----	----	0.0000077	No TF Applied	3.72E+02	1.64E-04

Class II Groundwater Quality Standard (GQS) Updates						Treatment Factor (TF) Determination Parameters	
CASRN	**Constituent**	**Current Class II GQS mg/L**	**Current Class II GQS Basis / TF Applied to Class I GQS**	**Proposed Updated Class II GQS mg/L**	**Updated Class II GQS Basis / TF Applied to Class I GQS**	**Organic Carbon Partition Coefficient (K_{oc}) L/kg**	**Dimensionless Henry's Law Constant at 20 ^{0}C (H') unitless**
129-00-0	Pyrene	1.05	TF = 5	0.6	TF = 5	5.43E+04	2.71E-04
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)'	0.084	No TF Applied	0.062	No TF Applied	8.91E+01	8.22E-10
118-96-7	TNT (2,4,6-trinitrotoluene)	0.014	No TF Applied	0.039	TF = 5	2.81E+03	5.10E-07
79-00-5	1,1,2-Trichloroethane	0.05	TF = 10	0.005	No TF Applied	6.07E+01	2.61E-02
75-69-4	Trichlorofluoromethane	10.5	TF = 5	6	TF = 5	4.39E+01	3.39E+00
99-35-4	1,3,5-Trinitrobenzene	0.84	No TF Applied	2.3	TF = 5	1.68E+03	1.53E-07

' = An enthalpy of vaporization value cannot be derived for the constituent; therefore, a dimensionless Henry's Law Constant value set at 25 degrees Celsius is used for evaluation of its treatment efficiency.
Organic Carbon Partition Coefficient and Dimensionless Henry's Law Constant values derived from U.S. EPA Regional Screening Level (RSL) tables as of May 2021.

Irrigation = The standard is based on beneficial use for irrigation of crops, per "Water Quality Criteria", by National Academy of Sciences.
Livestock = The standard is based on beneficial use for watering livestock, per "Water Quality Criteria", by National Academy of Sciences.
TF = Treatment Factor. A TF of 5 is applied to the Class I groundwater quality standard, based on Illinois EPA's treatment efficiency determination for chemicals whose constituents are not based on an MCL. A constituent's treatment efficiency is based the effectiveness to treat groundwater at an 80% removal efficiency rate for the constituent. A TF of 5 is applied to a constituent having either an organic carbon partition coefficient ("K_{oc}") greater than ethylbenzene's K_{oc} of 446 L/kg for carbon adsorption efficiency, or a constituent having a dimensionless Henry's Law Constant ("H'") greater than dichloromethane's (methylene chloride) H' of 0.11, when set at a groundwater system temperature of 20 degrees Celsius, for air stripping efficiency. If a constituent does not meet the criteria for an 80% removal efficiency, no TF is applied to the Class I groundwater quality standard.

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Comprehensive reviews of literature dealing with trace element effects on plants are provided by McKee and Wolf (1963),[436] Bolland and Butler (1966),[375] and Chapman (1966).[386] Hodgson (1963)[417] presented a review dealing with reactions of trace elements in soils.

In developing a workable program to determine acceptable limits for trace elements in irrigation waters, three considerations should be recognized:

- Many factors affect the uptake of and tolerance to trace elements. The most important of these are the natural variability in tolerances of plants and of animals that consume plants, in reactions within the soil, and in nutrient interactions, particularly in the plant.
- A system of tolerance limits should provide sufficient flexibility to cope with the more serious factors listed above.
- At the same time, restrictions must be defined as precisely as possible using presently available, but limited, research information.

Both the concentration of the element in the soil solution, assuming that steady state may be approached, and the total amount of the element added in relation to quantities that have been shown to produce toxicities were used in arriving at recommended maximum concentrations. A water application rate of 3 acre feet/acre/year was used to calculate the yearly rate of trace elements added in irrigation water.

The suggested maximum trace element concentrations for irrigation waters are shown in Table V-13.

The suggested maximum concentrations for continuous use on all soils are set for those sandy soils that have low capacities to react with the element in question. They are generally set at levels less than the concentrations that produce toxicities when the most sensitive plants are grown in nutrient solutions or sand cultures. This level is set, recognizing that concentration increases in the soil as water is evapotranspired, and that the effective concentration in the soil solution, at near steady state, is higher than in the irrigation water. The criteria for short-term use are suggested for soils that have high capacitites to remove from solution the element or elements being considered.

The work of Hodgson (1963)[417] showed that the general tolerance of the soil-plant system to manganese, cobalt, zinc, copper, and boron increased as the pH increased, primarily because of the positive correlation between the capacity of the soil to inactivate these ions and the pH. This same relationship exists with aluminum and probably exists with other elements such as nickel (Pratt et al. 1964)[449] and boron (Sims and Bingham 1968).[465] However, the ability of the soil to inactivate molybdenum decreases with increase in pH, such that the amount of this element that could be added without producing excesses was higher in acid soils.

TABLE V-13—Recommended Maximum Concentrations of Trace Elements in Irrigation Waters

Element	For waters used continuously on all soil	For use up to 20 years on fine textured soils of pH 6.0 to 8.5
	mg/l	mg/l
Aluminum	5.0	20.0
Arsenic	0.10	2.0
Beryllium	0.10	0.50
Boron	0.75	2.0
Cadmium	0.010	0.050
Chromium	0.10	1.0
Cobalt	0.050	5.0
Copper	0.20	5.0
Fluoride	1.0	15.0
Iron	5.0	20.0
Lead	5.0	10.0
Lithium	2.5[a]	2.5[a]
Manganese	0.20	10.0
Molybdenum	0.010	0.050[d]
Nickel	0.20	2.0
Selenium	0.020	0.020
Tin[c]		
Titanium[c]		
Tungsten[c]		
Vanadium	0.10	1.0
Zinc	2.0	10.0

[a] These levels will normally not adversely affect plants or soils.
[b] Recommended maximum concentration for irrigating citrus is 0.075 mg/l.
[c] See text for a discussion of these elements.
[d] For only acid fine textured soils or acid soils with relatively high iron oxide contents.

In addition to pH control (i.e., liming acid soils), another important management factor that has a large effect on the capacity of soils to adsorb some trace elements without development of plant toxicities is the available phosphorus level. Large applications of phosphate are known to induce deficiencies of such elements as copper and zinc and greatly reduce aluminum toxicity (Chapman 1966).[386]

The concentrations given in Table V-13 are for ionic and soluble forms of the elements. If insoluble forms are present as particulate matter, these should be removed by filtration before the water is analyzed.

Aluminum

The toxicity of this ion is considered to be one of the main causes of nonproductivity in acid soils (Coleman and Thomas 1967,[391] Reeve and Sumner 1970,[453] Hoyt and Nyborg 1971a[419]).

At pH values from about 5.5 to 8.0, soils have great capacities to precipitate soluble aluminum and to eliminate its toxicity. Most irrigated soils are naturally alkaline, and many are highly buffered with calcium carbonate. In these situations aluminum toxicity is effectively prevented.

With only a few exceptions, as soils become more acid ($pH < 5.5$), exchangeable and soluble aluminum develop by dissolution of oxides and hydroxides or by decomposition of clay minerals. Thus, without the introduction of aluminum, a toxicity of this element usually develops as soils are acidified, and limestone must be added to keep the soil productive.

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toxicity in acid sandy soils. A safety margin would be obtained by liming these soils. A concentration of copper at 5.0 mg/l applied in irrigation water at the rate of 3 acre feet of water per year for a 20-year period would add 800 pounds of copper in 20 years.

Recommendations

Based on toxicity levels in nutrient solutions and the limited soils data available, a maximum concentration of 0.20 mg/l copper is recommended for continuous use on all soils. On neutral and alkaline fine textured soils for use over a 20-year period, a maximum concentration of 5.0 mg/l is recommended.

Fluoride

Applications of soluble fluoride salts to acid soils can produce toxicity to plants. Prince et al. (1949)[450] found that 360 pounds fluoride per acre, added as sodium fluoride, reduced the yields of buckwheat at pH 4.5, but at pH values above 5.5 this rate produced no injury.

MacIntire et al. (1942)[435] found that 1,150 pounds of fluoride in superphosphate, 575 pounds of fluoride in slag, or 2,300 pounds of fluoride as calcium fluoride per acre had no detrimental effects on germination or plant growth on well-limed neutral soils, and that vegetation grown on these soils showed only a slight increase in fluoride as compared to those grown in acid soils. However, Shirley et al. (1970)[464] found that bones of cows that had grazed pastures fertilized with raw rock and colloidal phosphate, which contained approximately two to three per cent fluorides, for seven to 16 years averaged approximately 2,900 and 2,300 mg of fluorine per kilogram of bone, respectively. The bones of cows that had grazed on pastures fertilized with relatively fluorine free superphosphate, concentrated superphosphate, and basic slag fertilizer contained only 1400 mg/kg fluorine.

Recommendations

Because of the capacity of neutral and alkaline soils to inactivate fluoride, a relatively high maximum concentration for continuous use on these soils is recommended. Recommended maximum concentrations are 1.0 mg/l for continuous use on all soils and 15 mg/l for use for a 20-year period on neutral and alkaline fine textured soils.

Iron

Iron in irrigation waters is not likely to create a problem of plant toxicities. It is so insoluble in aerated soils at all pH values in which plants grow well, that it is not toxic. In fact, the problems with this element are deficiencies in alkaline soils. In reduced (flooded) soils soluble ferrous ions develop from inherent compounds in soils, so that quantities that might be added in waters would be of no concern. However, Rhoads (1971)[458] found large reductions in the quality of tobacco when plants were sprinkler irrigated with water containing 5 or more mg soluble iron/l, because of precipitation of iron oxides on the leaves. Rhoad's experience would suggest caution when irrigating any crops using sprinkler systems and waters having sufficient reducing conditions to produce reduced and soluble ferrous iron.

The disadvantages of soluble iron salts in waters are that these would contribute to soil acidification, and the precipitated iron would increase the fixation of such essential elements as phosphorous and molybdenum.

Recommendations

A maximum concentration of 5.0 mg/l is recommended for continuous use on all soils, and a maximum concentration of 20 mg/l is recommended on neutral to alkaline soils for a 20-year period. The use of waters with large concentrations of suspended freshly precipitated iron oxides and hydroxides is not recommended, because these materials also increase the fixation of phosphorous and molybdenum.

Lead

The phytotoxicity of lead is relatively low. Berry (1924)[374] found that a concentration of lead nitrate of 25 mg/l was required for toxicity to oats and tomato plants. At a concentration of 50 mg/l, death of plants occurred. Hopper (1937)[418] found that 30 mg/l of lead in nutrient solutions was toxic to bean plants. Wilkins (1957)[479] found that lead at 10 mg/l as lead nitrate reduced root growth. Since soluble lead contents in soils were usually from 0.05 to 5.0 mg/kg (Brewer 1966),[383] little toxicity can be expected. It was shown that the principal entry of lead into plants was from aerial deposits rather than from absorption from soils (Page et al. 1971)[445] indicating that lead that falls onto the soil is not available to plants.

In a summary on the effects of lead on plants, the Committee on the Biological Effects of Atmosphere Pollutants (NRC 1972)[441] concluded that there is not sufficient evidence to indicate that lead, as it occurs in nature, is toxic to vegetation. However, in studies using roots of some plants and very high concentrations of lead, this element was reported to be concentrated in cell walls and nuclei during mitosis and to inhibit cell proliferation.

Recommendations

Recommended maximum concentrations of lead are 5.0 mg/l for continuous use on all soils and 10 mg/l for a 20-year period on neutral and alkaline fine textured soils.

Lithium

Most crops can tolerate lithium in nutrient solutions at concentrations up to 5 mg/l (Oertli 1962,[443] Bingham et al. 1964,[377] Bollard and Butler 1966[378]). But research revealed

that citrus was more sensitive (Aldrich et al. 1951,[309] Bradford 1963b,[381] Hilgeman et al. 1970[415]). Hilgeman et al. (1970)[415] found that grapefruit developed severe symptoms of lithium toxicity when irrigated with waters containing lithium at 0.18 to 0.25 mg/l. Bradford (1963a)[380] reported that experience in California indicated slight toxicity of lithium to citrus at 0.06 to 0.10 mg/l in the water.

Lithium is one of the most mobile of cations in soils. It tends to be replaced by other cations in waters or fertilizers and is removed by leaching. On the other hand, it is not precipitated by any known process.

Recommendations

Recommendations for maximum concentrations of lithium, based on its phytotoxicity, are 2.5 mg/l for continuous use on all soils, except for citrus where the recommended maximum concentration is 0.075 mg/l for all soils. For short-term use on fine textured soils the same maximum concentrations are recommended because of lack of inactivation in soils.

Manganese

Manganese concentrations at a few tenths to a few milligrams per liter in nutrient solutions are toxic to a number of crops as shown by Morris and Pierre (1949),[440] Adams and Wear (1957),[364] Hewitt (1965),[414] and others. However, toxicities of this element are associated with acid soils, and applications of proper quantities of ground limestone successfully eliminated the problem. Increasing the pH to the 5.5 to 6.0 range usually reduced the active manganese to below the toxic level (Adams and Wear 1957).[364] Hoyt and Nyborg (1971b)[420] found that available manganese in the soil and manganese content of plants were negatively correlated with soil pH. However, the definite association of toxicity with soil pH as found with aluminum was not found with manganese, which has a more complex chemistry. Thus, more care must be taken in setting water quality criteria for manganese than for aluminum (i.e., management for control of toxicities is not certain).

Recommendations

Recommended maximum concentrations for manganese in irrigation waters are set at 0.20 mg/l for continued use on all soils and 10 mg/l for use up to 20 years on neutral and alkaline fine textured soils. Concentrations for continued use can be increased with alkaline or calcareous soils, and also with crops that have higher tolerance levels.

Molybdenum

This element presents no problems of toxicity to plants at concentrations usually found in soils and waters. The problem is one of toxicity to animals from molybdenum ingested from forage that has been grown in soils with relatively high amounts of avaiable molybdenum. Dye and O'Hara (1959)[398] reported that the molybdenum concentration in forage that produced toxicity in ruminants was 5 to 30 mg/kg. Lesperance and Bohman (1963)[430] found that toxicity was not simply associated with the molybdenum content of forage but was influenced by the amounts of other elements, particularly copper. Jensen and Lesperance (1971)[423] found that the accumulation of molybdenum in plants was proportional to the amount of the element added to the soil.

Kubota et al. (1963)[426] found that molybdenum concentrations of 0.01 mg/l or greater in soil solutions were associated with animal toxicity levels of this element in alsike clover. Bingham et al. (1970)[376] reported that molybdosis of cattle was associated with soils that had 0.01 to 0.10 mg/l of molybdenum in saturation extracts of soils.

Recommendations

The recommended maximum concentration of molybdenum for continued use of water on all soils, based on animal toxicities from forage, is 0.010 mg/l. For short term use on soils that react with this element, a concentration of 0.050 mg/l is recommended.

Nickel

According to Vanselow (1966b),[474] many experiments with sand and solution cultures have shown that nickel at 0.5 to 1.0 mg/l is toxic to a number of plants. Chang and Sherman (1953)[385] found that tomato seedlings were injured by 0.5 mg/l. Millikan (1949)[437] found that 0.5 to 5.0 mg/l were toxic to flax. Brenchley (1938)[382] reported toxicity to barley and beans from 2 mg/l. Crooke (1954)[395] found that 2.5 mg/l was toxic to oats. Legg and Ormerod (1958)[429] found that 1.0 mg/l produced toxicity in hop plants. Vergnano and Hunter (1953)[473] found that 1.0 mg/l in solutions flushed through sand cultures was toxic to oats. Soane and Saunders (1959)[466] found that tobacco plants showed no toxicity at 30 mg/l, and that corn showed no toxicity at 2 mg/l but showed toxicity at 10 mg/l.

Work by Mizuno (1968)[435] and Halstead et al. (1969)[409] and the review of Vanselow (1966b)[474] showed that increasing the pH of soils reduces the toxicity of added nickel.

Halstead et al. (1969)[409] found the greatest capacity to adsorb nickel without development of toxicity was by a soil with 21 per cent organic matter.

Recommendations

Based on both toxicity in nutrient solutions and on quantities that produce toxicities in soils, the recommended maximum concentration of nickel in irrigation waters is 0.20 mg/l for continued use on all soils. For neutral fine textured soils for a period up to 20 years, the recommended maximum is 2.0 mg/l.

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Recommendations

Recommended maximum concentrations for manganese in irrigation waters are set at 0.20 mg/l for continued use on all soils and 10 mg/l for use up to 20 years on neutral and alkaline fine textured soils. Concentrations for continued use can be increased with alkaline or calcareous soils, and also with crops that have higher tolerance levels.

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Halstead et al. (1969)[409] found the greatest capacity to adsorb nickel without development of toxicity was by a soil with 21 per cent organic matter.

Recommendations

Based on both toxicity in nutrient solutions and on quantities that produce toxicities in soils, the recommended maximum concentration of nickel in irrigation waters is 0.20 mg/l for continued use on all soils. For neutral fine textured soils for a period up to 20 years, the recommended maximum is 2.0 mg/l.

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salinities near this limit may be observed, seri-nomic losses or serious physiological disturbances should rarely, if ever, result from their use.

TOXIC SUBSTANCES IN LIVESTOCK WATERS

There are many substances dissolved or suspended in waters that may be toxic. These include inorganic elements and their salts, certain organic wastes from man's activities, pathogens and parasitic organisms, herbicide and pesticide residues, some biologically produced toxins, and radionuclides.

For any of the above, the concentrations at which they render a water undesirable for use for livestock is subject to a number of variables. These include age, sex, species, and physiological state of the animals; water intake, diet and its composition, the chemical form of any toxic element present, and the temperature of the environment. Naturally, if feeds and waters both contain a toxic substance, this must be taken into account. Both short and long term effects and interactions with other ions or compounds must also be considered.

The development of recommendations for safe concentrations of toxic substances in water for livestock is extremely difficult. Careful attention must be given to the discussion that follows as well as the recommendations and to any additional experimental findings that may develop. Based on available research, an appropriate margin of safety, under almost all conditions, of specific toxic substances harmful to livestock that drink the waters and to man who consumes the livestock or their products, is reviewed below. Although the margin of safety recommended is usually large, the criteria suggested cannot be used as a guide in diagnosing livestock losses, since they are well below toxic levels for domestic animals.

Toxic Elements and Ions

Those ions largely responsible for salinity in water (sodium, calcium, magnesium, chloride, sulfate, and bicarbonate) are in themselves not very toxic. There are, however, a number of others that occur naturally or as the result of man's activities at troublesome concentrations. If feeds and water both contain a toxic ion, both must be considered. Interactions with other ions, if known, must be taken into account. Elements or ions become objectionable in water when they are at levels toxic to animals, where they seriously reduce the palatability of the water, or when they accumulate excessively in tissues or body fluids, rendering the meat, milk, eggs, or other edible product unsafe or unfit for human use.

Aluminum

Soluble aluminum has been found in surface waters of the United States in amounts to 3 mg/l, but its occurrence at such concentrations is rare because it readily precipitates as the hydroxide (Kopp and Kroner 1970).[152]

Most edible grasses contain about 250–500 mg of the element. However, there is no evidence that it is essential for animal growth, and very little is found deposited in animal tissues (Underwood 1971).[254] It is not highly toxic (McKee and Wolf 1963,[103] Underwood 1971),[254] but Deobald and Elvehjem (1935)[188] found that a level of 4,000 mg aluminum per kilogram of diet caused phosphorus deficiency in chicks. Its occurrence in water should not cause problems for livestock, except under unusual conditions and with acid waters.

Recommendation

Livestock should be protected where natural drinking waters contain no more than 5 mg/l aluminum.

Arsenic

Arsenic has long been notorious as a poison. Nevertheless, it is present in all living tissues in the inorganic and in certain organic forms. It has also been used medicinally. It is accepted as a safe feed additive for certain domestic animals. It has not been shown to be a required nutrient for animals, possibly because its ubiquity has precluded the compounding of deficient diets (Frost 1967).[149]

The toxicity of arsenic can depend on its chemical form, its inorganic oxides being considerably more toxic than organic forms occurring in living tissues or used as feed additives. Differences in toxicities of the various forms are clearly related to the rate of their excretion, the least toxic being the most rapidly eliminated (Frost 1967,[149] Underwood 1971).[254] Except in unusual cases, this element should occur in waters largely as inorganic oxides. In waters carrying or in contact with natural colloidal material, the soluble arsenic content may be decreased to a very low level by adsorption.

Wadsworth (1952)[260] gave the acute toxicity of inorganic arsenic for farm animals as follows: poultry, 0.05–0.10 g per animal; swine, 0.5–1.0 g per animal; sheep, goats, and horses, 10.0–15.0 g per animal; and cattle, 15–30 g per animal. Franke and Moxon (1936)[148] concluded that the minimum dose required to kill 75 per cent of rats given intraperitoneal injections of arsenate was 14–18 mg arsenic per kilogram, while for arsenite it was 4.25–4.75 mg/kg of body weight.

When mice were given drinking water containing 5 mg/l of arsenic as arsenite from weaning to natural death, there was some accumulation of the element in the tissues of several organs, a somewhat shortened life span, but no carcinogenic effect (Schroeder and Balassa 1967).[233] In a similar study with rats (Schroeder et al. 1968b),[236] neither toxicity nor carcinogenic effects were observed, but large amounts accumulated in the tissues.

Peoples (1964)[220] fed arsenic acid at levels up to 1.25 mg/kg of body weight per day for eight weeks to lactating cows. This is equivalent to an intake of 60 liters of water

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Table A: Toxicity Metadata for Determining Similar-Acting Noncarcinogen Constituents

CASRN	Constituent	Health Effects	Target Organ
83-32-9	Acenaphthene	Hepatotoxicity	Liver
67-64-1	Acetone	Neuropathy	Nervous System
116-06-3	Aldicarb	AChe inhibition	Cholinesterase Inhibition
7429-90-5	Aluminum	Minimal neurotoxicity in the offspring of mice	Nervous System; Developmental
120-12-7	Anthracene	Cellular necrosis	Whole Body
7440-36-0	Antimony	Longevity, blood glucose; increased serum lipids	Circulatory; Liver; Whole Body
7440-38-2	Arsenic	Hyperpigmentation; Keratosis; Possible Vascular Complications	Circulatory; Dermal
1912-24-9	Atrazine	Cardiac toxicity and dilation of the right atrium; Decreased body weight gain	Circulatory; Decreased Body Weight
7440-39-3	Barium	Nephropathy	Nervous System
65-85-0	Benzoic acid	Cellular necrosis	Whole Body
7440-41-7	Beryllium	Small intestine lesions	Gastrointestinal System
78-93-3	2-Butanone (methyl ethyl ketone)	Decreased pup body weight	Decreased Body Weight; Developmental
7440-43-9	Cadmium	Significant proteinuria	Kidney
1563-66-2	Carbofuran	Red blood cell and plasma cholinesterase inhibition; Testicular and uterine effects	Cholinesterase Inhibition**;** Circulatory**;** Reproductive System
75-15-0	Carbon disulfide	Fetal toxicity/malformations	Developmental; Reproductive System
12798-03-6	Chlordane	Hepatic necrosis	Liver
108-90-7	Chlorobenzene	Histopathologic changes in liver	Liver
67-66-3	Chloroform	Moderate/marked fatty cyst formation in the liver and elevated SGPT	Liver
7440-47-3	Chromium (total)	Developmental and fetotoxic effects (as chromium VI)	Developmental; Reproductive System
7440-48-4	Cobalt	Decreased iodine uptake	Thyroid
143-33-9	Cyanide (sodium cyanide)	Decreased cauda epididymis weight in male F344/N rats	Reproductive System
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)	Hematologic, hepatic and renal toxicity	Circulatory; Kidney; Liver
75-99-0	Dalapon	Increased kidney body weight ratio	Kidney
1918-00-9	Dicamba	Maternal and fetal toxicity	Reproductive System

CASRN	Constituent	Health Effects	Target Organ
75-71-8	Dichlorodifluoromethane	Reduced body weight	Decreased Body weight
75-34-3	1,1-Dichloroethane	Renal injury	Kidney
75-35-4	1,1-Dichloroethylene	Liver toxicity (fatty change)	Liver
156-59-2	*cis*-1,2-Dichloroethylene	Increased relative kidney weight in male rats	Kidney
156-60-5	*trans*-1,2-Dichloroethylene	Decrease in number of antibody forming cells (AFCs) against sheep red blood cells (sRBCs) in male mice	Circulatory; Immune System
84-66-2	Diethyl phthalate	Decreased growth rate, food consumption and altered organ weights	Decreased Body Weight; Developmental
84-74-2	Di-*n*-butyl phthalate	Increased mortality	Whole body
99-65-0	1,3-Dinitrobenzene	Increased splenic weight	Spleen
88-85-7	Dinoseb	Decreased fetal weight	Developmental
72-20-8	Endrin	Mild histological lesions in liver, occasional convulsions	Liver; Nervous System
145-73-3	Endothall	Increased absolute and relative weights of stomach and small intestine	Gastrointestinal System
100-41-4	Ethylbenzene	Liver and kidney toxicity	Kidney; Liver
206-44-0	Fluoranthene	Hematological alterations; Nephropathy; Increased liver weights	Circulatory; Kidney; Liver
86-73-7	Fluorene	Decreased red blood count, packed cell volume and hemoglobin	Circulatory
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	Liver toxicity; Decreased red blood cell count, hemoglobin, and hematocrit; Kidney necrosis and hyperplasia; Reproductive and developmental effects; Immunotoxicity.	Liver; Circulatory; Kidney; Developmental; Immune System
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	Hepatic lesions	Liver
1024-57-3	Heptachlor epoxide	Increased liver-to-body weight ratio in both males and females	Liver
77-47-4	Hexachlorocyclopentadiene	Chronic irritation	Gastrointestinal System
98-82-8	Isopropylbenzene (cumene)	Increased average kidney weights in female rats	Kidney
7439-93-2	Lithium	Multiple Adverse effects	Developmental; Kidney; Nervous System; Reproductive System
93-65-2	MCPP (Mecoprop)	Increased absolute and relative kidney weights	Kidney
1634-04-4	MTBE (methyl tertiary-butyl ether)	Persistent diarrhea, increased serum lipids	Gastrointestinal System/ Liver

CASRN	Constituent	Health Effects	Target Organ
7439-96-5	Manganese	CNS effects	Nervous System
7487-94-7	Mercury (mercuric chloride)	Autoimmune effects (autoimmune glomerulonephritis)	Immune System; Kidney
72-43-5	Methoxychlor	Excessive loss of litters	Reproductive System
90-12-0	1-Methylnaphthalene	Increased incidence of pulmonary alveolar proteinosis	Lungs
91-57-6	2-Methylnaphthalene	Pulmonary alveolar proteinosis	Lungs
95-48-7	2-Methylphenol (*o*-cresol)	Decreased body weights and neurotoxicity	Decreased Body Weight; Nervous System
7439-98-7	Molybdenum	Increased uric acid levels	Kidney
91-20-3	Naphthalene	Decreased mean terminal body weight in males	Decreased Body weight
7440-02-0	Nickel	Decreased body and organ weights	Decreased Body weight
98-95-3	Nitrobenzene	Increased methemoglobin levels	Circulatory System
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)	Ocular exudate, inflamed and prominent meibomian glands, distorted nail growth, decreased vaccine response (as Alachlor 1254)	Immune System; Skin
375-73-5	PFBS (perfluorobutanesulfonic acid)	Decreased serum total T4 in newborn (PND1) mice	Developmental; Kidney; Reproductive System; Thyroid
355-46-4	PFHxS (perfluorohexanesulfonic acid)	Neurodevelopmental; Decreased weight of offspring; Increased triglycerides; Decreased levels of hemoglobin, hemacrit and red blood cells; Thyroid damage; Liver effects	Circulatory System; Developmental; Immune System; Liver; Thyroid
375-95-1	PFNA (perfluorononanoic acid)	Decreased pup body weight, developmental delays, increased serum lipids, liver effects	Decreased Body Weight; Developmental; Liver
1763-23-1	PFOS (perfluorooctanesulfonic acid)	Pregnancy induced hypertension and pre-eclampsia, increased serum lipids, thyroid disease, decreased vaccine response, decreased fertility, decreased pup weight, neurodevelopmental delays, skeletal malformations	Developmental; Immune System; Liver; Nervous System; Reproductive System
335-67-1	PFOA (perfluorooctanoic acid)	Hepatocellular hypertrophy and necrosis; Reduced Antibody Response; Impairment of mammary glands; Neurodevelopmental; Skeletal alterations	Developmental; Immune System; Liver
14797-73-0	Perchlorate	Radioactive iodide uptake inhibition (RAIU) in the thyroid	Thyroid
1918-02-01	Picloram	Increased liver weights	Liver
108-95-2	Phenol	Decreased maternal weight gain	Decreased Body Weight; Reproductive System

CASRN	Constituent	Health Effects	Target Organ
129-00-0	Pyrene	Kidney effects (renal tubular pathology, decreased kidney weights)	Kidney
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	Convulsions in F344 rats	Nervous System
7440-22-4	Silver	Argyria	Skin
122-34-9	Simazine	Hematological changes in females; Reduction in weight gains	Circulatory System; Decreased Body Weight
100-42-5	Styrene	Red blood cell and liver effects	Circulatory System; Liver
118-96-7	TNT (2,4,6-trinitrotoluene)	Liver effects	Liver
93-72-1	2,4,5-TP (silvex)	Histopathological changes in the liver	Liver
7440-28-0	Thallium	Histopathology	Skin
120-82-1	1,2,4-Trichlorobenzene	Increased adrenal weights; vacuolization of zona fasciculata in the cortex	Adrenal gland
71-55-6	1,1,1-Trichloroethane	Reduced body weight	Decreased Body Weight
79-00-5	1,1,2-Trichloroethane	Clinical serum chemistry	Liver
79-01-6	Trichloroethylene	Increased fetal cardiac malformations in Sprague-Dawley rats	Circulatory System; Developmental
75-69-4	Trichlorofluoromethane	Survival and histopathology	Whole Body
99-35-4	1,3,5-Trinitrobenzene	Methemoglobinemia and spleen-erythroid cell hyperplasia	Circulatory System; Spleen
108-88-3	Toluene	Increased kidney weight	Kidney
7440-62-2	Vanadium	Histopathology	Kidney
1330-20-7	Xylenes	Decreased body weight, increased mortality	Decreased Body Weight; Whole Body
7440-66-6	Zinc	Decreased erythrocyte Cu, Zn-superoxide dismutase (ESOD) activity	Circulatory System

Primary Source: U.S. EPA Regional Screening Levels (RSL) Calculator Oral Reference Dose Metadata.
Available at: https://www.epa.gov/risk/regional-screening-levels-rsls

Other Sources
HFPO-DA ((hexafluoropropylene oxide dimer acid, GenX):
PFHxS (perfluorohexanesulfonic acid): Agency for Toxic Substances & Disease Registry (ATSDR). May 2021. Toxicological Profile for Perfluoroalkyls. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237
PFNA (perfluorononanoic acid): Agency for Toxic Substances & Disease Registry (ATSDR). May 2021. Toxicological Profile for Perfluoroalkyls. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237

PFOS (perfluorooctanesulfonic acid): Agency for Toxic Substances & Disease Registry (ATSDR). May 2021. Toxicological Profile for Perfluoroalkyls. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237

Vandium: Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency. Provisional Peer-Reviewed Toxicity Values for Vandium and Its Soluble Inorganic Compounds Other Than Vandium Pentoxide (CASRN 7440-62-2 and Others). September 2009. Available at: https://cfpub.epa.gov/ncea/pprtv/documents/Vanadium.pdf

Table B: Toxicity Metadata for Determining Similar-Acting Carcinogen Constituents

CASRN	**Constituent**	**Health Effects**	**Target Organ**
7440-38-2	Arsenic	Skin cancer	Skin
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)	Hepatic nodules and hepatocellular carcinomas	Liver
71-43-2	Benzene	Leukemia	Circulatory System
56-55-3	Benzo(a)anthracene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
205-99-2	Benzo(b)fluoranthene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
207-08-9	Benzo(k)fluoranthene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
50-32-8	Benzo(a)pyrene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
56-23-5	Carbon tetrachloride	Hepatocellular adenoma or carcinoma	Liver
12798-03-6	Chlordane	Carcinoma	Liver
67-66-3	Chloroform	Renal cell carcinoma	Kidney
218-01-9	Chrysene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
53-70-3	Dibenzo(a,h)anthracene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)	Renal tubular cell adenoma or carcinoma	Kidney
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)	Hepatocarcinogenicity	Liver
107-06-2	1,2-Dichloroethane	Hemangiosarcomas	Circulatory System
75-09-2	Dichloromethane (methylene chloride)	Hepatocellular carcinomas or adenomas	Liver
78-87-5	1,2-Dichloropropane	Hepatocellular adenoma or carcinoma	Liver
117-81-7	Di(2-ethylhexyl)phthalate	Hepatocellular carcinoma and adenoma	Liver
121-14-2	2,4-Dinitrotoluene	Hepatocellular carcinomas, kidney and mammary gland tumors	Kidney; Liver; Mammary Glands
606-20-2	2,6-Dinitrotoluene	Hepatocellular carcinomas, kidney and mammary gland tumors	Kidney; Liver; Mammary Glands
123-91-1	1,4-Dioxane (*p*-dioxane)	Hepatocellular adenoma and carcinoma	Liver
100-41-4	Ethylbenzene	Renal tubule carcinomas or adenomas (combined) in male rats	Kidney

CASRN	Constituent	Health Effects	Target Organ
106-93-4	Ethylene dibromide (1,2-dibromoethane)	Forestomach tumors, hemangiosarcomas, thyroid follicular cell adenomas or carcinomas	Circulatory System; Gastrointestinal System; Thyroid
58-89-9	gamma-HCH (gamma-hexachlorocyclohexane, lindane)	Liver tumors	Liver
76-44-8	Heptachlor	Hepatocellular carcinomas	Liver
1024-57-3	Heptachlor epoxide	Hepatocellular carcinomas	Liver
193-39-5	Indeno(1,2,3-c,d)pyrene	Forestomach, esophagus, tongue, and larynx tumors	Gastrointestinal System
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)	Liver hepatocellular adenomas, carcinomas, cholangiomas, or cholangiocarcinomas	Liver
335-67-1	PFOA (perfluorooctanoic acid)	Leydig cell adenomas, hepatocellular adenomas, pancreatic acinar cell adenomas/carcinomas	Liver; Pancreas; Testes
87-86-5	Pentachlorophenol	Hepatocellular adenomas or carcinomas and adrenal benign or malignant pheochromocytomas	Adrenal Gland; Liver
127-18-4	Tetrachloroethylene	Hepatocellular adenomas or carcinomas	Liver
8001-35-2	Toxaphene	Hepatocellular carcinomas and neoplastic nodules	Liver
79-01-6	Trichloroethylene	Renal cell carcinoma, non-Hodgkin's lymphoma, and liver tumors	Kidney; Liver
75-01-4	Vinyl Chloride	Liver cell polymorphism	Liver

Primary Source
U.S. EPA Regional Screening Levels (RSL) Calculator Oral Slope Factor Metadata.
Available at: https://www.epa.gov/risk/regional-screening-levels-rsls

Other Sources
PFOA (perfluorooctanoic acid): California, Office of Environmental Health Hazard Assessment. 2019. Notification Level Recommendations Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Drinking Water.
Available at: https://oehha.ca.gov/media/downloads/water/chemicals/nl/final-pfoa-pfosnl082119.pdf

ATTACHMENT 2

Pre-filed Testimony of Lynn E. Dunaway, P.G.

My name is Lynn E. Dunaway. I am employed as an Environmental Protection Specialist, have a Bachelor of Science degree in geology and am an Illinois, licensed professional geologist. I work in the Hydrogeology and Compliance Unit, Groundwater Section, Division of Public Water Supplies, Bureau of Water, Illinois Environmental Protection Agency ("Agency"). I have worked in the Groundwater Section since 1988 and have completed multiple courses to enhance my professional knowledge. My curriculum vitae (Attachment 2A), is included for further detail about my training and experience. My duties in the Groundwater Section include: Providing review of hydrogeolgic assessments and reports, and providing technical input on the same as well as special projects requiring geologic expertise; Providing geologic and hydrogeologic expertise to Bureau of Water permit programs (industrial, municipal and mines) and the Public Water Supply, Permit Section; Responding to questions from the regulated community, public and other governmental agencies about the provisions of the Illinois Environmental Protection Act ("Act") and Illinois Pollution Control Board ("Board") rules adopted thereunder; Project management of sites subject to corrective actions under the Act or Board rules; Assist the regulated community with required steps to be compliant with setback zones, technology control regulations (35 Ill. Adm. Code 615 and 35 Ill. Adm Code 616) and minimal hazard certification; Testifying before the Board on proposed rules and setback zone exceptions; assisting in the implementation of source water protection programs and; Mentoring interns and student workers. My pre-filed testimony focuses primarily on changes to the definition of Class I: Potable Resource Groundwater in Section 620.210, additional required submissions with groundwater management zone applications in Section 620.250 and the establishment of site specific numerical groundwater standards within

previously listed Class III: Special Resource Groundwater areas in Section 620.230. I can give further explanation of my pre-filed testimony and answer additional questions as needed.

Section 620.210 Class I: Potable Resource Groundwater

The Agency is proposing to the change methods by which groundwater may be determined to be Class I, in subsection 620.210(a)(4)(B). The Agency is proposing to eliminate the permeameter as method for determining hydraulic conductivity and add the wellhead protection area of a community water supply well or well field.

The permeameter is being proposed for elimination as a method, because samples of earth materials must be collected and transported to the permeameter to run the hydraulic conductivity test. Sample collection may cause a change from the in-situ hydraulic conductivity, because the tool used to collect the sample can cause compaction as it penetrates the geologic formation, thereby reducing the measured hydraulic conductivity. Conversely, removal of the tool from the geologic formation, or subsequent handling can create fractures within the sample, thereby increasing the measured hydraulic conductivity. Further, samples for permeameter tests are usually collected above the saturated zone, and therefore are not representative of material containing groundwater.

The Agency is proposing the addition of the wellhead protection area (Section 620.110) for a community well or well field which is a two dimensional projection on the surface, of a three-dimensional region containing groundwater that will recharge a well or well field within a five-year period. The wellhead protection area is an appropriate region for inclusion as Class I groundwater because these three-dimensional areas have been delineated using site specific hydrogeologic parameters (e.g. transmissivity, specific capacity, gradient, etc.). These site specific

hydrogeologic parameters are measurements that reflect flow conditions within the geologic formations that contain groundwater. See the Illinois "Wellhead Protection Program" incorporated by reference in Section 620.125. The parameters representing groundwater flow conditions can then be used in calculations and models to estimate the area within the geologic formation where groundwater will be flowing towards a well and the region within the geologic formation which is expected to yield groundwater into a well within the next five years. The subsurface regions of groundwater flow towards a community well or well field are then projected onto the overlying ground surface, forming the wellhead protection area, which extends to the depth of the well or wells being delineated. Given the relatively short five-year time-period being predicted, the Agency believes the entire three-dimensional region of the projected two dimensional wellhead protection area should be included as Class I groundwater.

Section 620.250 Groundwater Management Zone

The Agency is proposing the addition of a list of information that must be included with each groundwater management zone (GMZ) application. Though the list of technical documents and submissions the Agency is proposing to add as subsection 620.250(g) have generally been submitted to the Agency as part of the GMZ process, they may only be submitted as the Agency requests the information from the applicant as the Agency's evaluation of the groundwater conditionals and the appropriateness of the proposed corrective action progresses. Adopting a standardized list of technical information, which in the Agency's experience is typically needed to understand the hydrogeologic conditions at a site, will make the GMZ process more effective. The proposed technical submissions are useful for evaluating the adequacy of a proposed correct action as required by subsections 620.250(a) and (b).

The Agency is also proposing to have the GMZ applicant provide a description of the selected corrective action, how the applicant determined the corrective action would effectively achieve the applicable groundwater standards and the monitoring that will be conducted to demonstrate the predicted success of the selected and Agency approved corrective action. The Agency is proposing the inclusion of these additional descriptions and demonstrations in recognition that corrective action is rarely a one step process. For example: removing a storage tank that has caused groundwater contamination is a corrective action, but groundwater contamination will persist, for some period of time, dependent upon site specific conditions. Therefore, monitored natural attenuation or some other additional corrective action will often be necessary to achieve groundwater quality standards. The GMZ application should include explanations of all the steps or phases of corrective action and the amount of time expected to complete them, so that it is clear to anyone reviewing the site when corrective action is complete, and if, or when, the Section 620.450 Alternative Groundwater Quality Standards are applicable.

Therefore, the Agency is proposing new subsection 620.250(g) to make GMZ applications easier to review by requiring submission information typically needed at the beginning of the application process and by requiring a description of the corrective action processes, the amount of time needed to achieve completed corrective action and documentation supporting the efficacy of the selected corrective action.

Section 620.430 Site Specific Standards

Section 620.230 provides the requirements to establish Class III: Special Resource Groundwater within any larger groundwater resource that contributes to a dedicated nature preserve ("DNP"). Since January 1999 when the first, Class III groundwater area was published in the

Environmental Register for final listing, the Agency has completed reviews of the required information and published 30 DNPs for final listing, to create Class III groundwater areas. (Attachment 2B list of nature preserves with Class III areas). Listing a Class III area associated with a DNP establishes a Class III groundwater standard within the Class III groundwater area, equal to the Class I groundwater standard set forth in Section 620.410 and makes the non-degradation provisions of Part 620; Subpart C, applicable to all groundwater within the Class III groundwater area, unless the Board has adopted a different standard pursuant to Section 620.260.

Section 620.230 requires that a general description of the existing groundwater quality at and surrounding a DNP be provided for Agency review prior to listing. While each listed DNP has met this obligation, some DNPs have much more data available than others. The Agency reviewed groundwater monitoring data that was collected by the Illinois State Geological Survey for a number of DNPs. As a first step to evaluate appropriate standards specific to particular DNPs, the Agency statistically evaluated the existing chemical concentrations of available groundwater monitoring data. The power of a statistical analysis increases with the number of valid samples included in the analysis. Therefore, any DNP that did not have at least eight samples in the data set was eliminated from further review for site specific standards, for this proposal.

Of the DNPs for which the Agency was able to find quantitative groundwater quality data, six DNPs met the initial (eight sample minimum) criteria. The DNPs that met the criteria are two wetland DNPs and four cave DNPs. The wetland DNPs are Spring Grove Fen and Cotton Creek Marsh, both in McHenry County, and both listed as Class III groundwater in 2012. The cave DNPs are Fogelpole Cave, Monroe County, listed in 2003; Pautler Cave, Monroe County and Stemler Cave, St. Clair and Monroe Counties, both listed in 2005; and Armin Krueger Speleological, Monroe County, listed in 2010 (Attachments 2C and 2D maps of the DNP and Class III

areas). Statistical methods discussed in the Unified Guidance (March 2009 Unified Guidance) are broad enough to be generally applicable to other groundwater monitoring programs beyond the Resource Conservation and Recovery Act ("RCRA"). The Unified Guidance recommends that false positives for evaluation of groundwater monitoring data not exceed a range of .01 to .05, or inversely, should have a confidence of 99% to 95%, respectively. Since 95% confidence is more environmentally conservative (i.e. more likely to indicate an exceedence of a given concentration when one has not actually occurred) the Agency opted for evaluation methods using 95% confidence. The Agency believes a conservative approach is appropriate since Class III groundwater is intended to protect the groundwater resource contributing to sensitive and unique areas.

The simplest standard is a value to which other values can be directly compared to determine compliance with the standard. Further, the Illinois Groundwater Protection Act ("IGPA") (415 ILCS 55/8) states a preference for numerical standards instead of narrative standards. Therefore, the Agency used statistical methods with existing water quality to calculate a tolerance limit where appropriate, which with 95% confidence, represents the maximum expected groundwater concentration of a given chemical constituent as a single numerical value. Before calculating a tolerance limit, the Agency used the Ryan-Joiner normality test in the Minitab software to determine if the data used was normally distributed. Statistical p-values for data, or log-normal transformed data, equal to or greater than 0.1 were considered to have a normal distribution. Once normality was determined an equation for parametrically (i.e. normally) or an equation for non-parametrically distributed data, as applicable, was used to calculate the tolerance limit for the applicable chemical constituent. In addition to its potential use as a numerical groundwater standard, calculation of tolerance limits allows quantitative and qualitative comparison of expected

maximum results within a Class III area with measured results from relevant research and site specific circumstances. Site specific conditions and scientific research were taken into consideration when proposing standards for the DNPs. The research and site specific circumstances are explained in greater detail in the constituent specific discussions.

The Agency's evaluation of chemical constituents for consideration as site specific Class III groundwater standards took into consideration whether there was an adequate number of samples for statistical analysis, the constituents for which monitoring data is available, the nature of the DNP(s) that the specific groundwater serves, relevant research, site specific conditions and previously adopted Class I groundwater standards. Existence of a Class I standard is significant because it first assures that the criteria for establishment of a groundwater standard as required by Section 8 of the IGPA have been met, and further provides a context within which to assess ambient groundwater conditions within the Class III areas. For example, nitrate monitoring was available for a number of the DNPs. Nitrate can promote the growth of invasive plant species in wetlands and change nutrient cycling within cave systems. Due to the nitrate concentrations and distributions in the available data, the tolerance limits calculated for nitrate at the DNPs, where nitrate data was available, indicated that concentrations of nitrate were likely to exceed the Class I: Potable Resource numeric standard of 10 mg/L with regularity. Therefore, consistently meeting the Class I nitrate standard, which is already applicable within the listed Class III areas, would be a first step towards reducing nutrient levels within the Class III areas assessed. The need for more stringent standards can be assessed and proposed later, after first meeting the Class I, mile stone.

Chloride

The Section 620.410 groundwater standard for chloride is 200 mg/L. Chloride data was available for all of the DNPs that met the Agency's initial data availability assessment. Chloride was the only chemical constituent available for evaluation at Cotton Creek Marsh DNP. The Agency determined a lower site specific standard for chloride would be beneficial to all of the DNPs and the associated Class III areas assessed in this proposal. Fens are wetlands fed primarily by discharging groundwater which is highly mineralized and typically has a neutral to basic pH. According to information from a study by the Illinois State Geological Survey, Native Landscapes and Science and Technology Management, Inc. (Panno et al 1999) (Document 1), the ionic content of groundwater discharging to fens is dominated by calcium and bicarbonate. Both anthropogenic and natural sources of other ions may occur in fens. For example, the Panno et al 1999 study found that sodium and chloride from septic systems or road salt could alter groundwater quality to the point that the groundwater became a sodium and chloride type groundwater instead of a calcium and bicarbonate type. The Panno et al 1999 study correlated the number of chloride tolerant and chloride intolerant plants relative to the ionic charter of the groundwater. The study found that high levels of sodium and chloride favor more salt tolerant, generalist species, such as narrow leaf cattails, to the exclusion of rarer specially adapted species. However, a fen recharged by a calcium and bicarbonate dominated groundwater supported a diverse plant population. The Panno et al 1999 study used a graphical technique to group groundwater into ionic types (e.g. calcium/bicarbonate or sodium/chloride). The concentration of a particular ion that represents an inflection point between groundwater types is called a threshold concentration. The threshold concentration of chloride calculated in the Panno et al 1999 study that would represent a change from a calcium/bicarbonate groundwater to a sodium/chloride groundwater is 45 mg/L chloride. The Agency calculated a tolerance limit (rounded to the nearest whole number) based

on available groundwater monitoring data near Spring Grove Fen and Cotton Creek Marsh of 175 mg/L chloride and 89 mg/L chloride, respectively. Chloride at Spring Grove Fen had a median of 87.4 and a mean of 75.8. This distribution reflects chloride concentrations generally in excess of the 45 mg/L threshold value established by Panno et al 1999, but below the 200 mg/L Class I standard, with some groundwater chloride values still below the threshold. At Cotton Creek Marsh the median chloride was 7.8 mg/L, with a chloride mean of 22.9 mg/L. Chloride distribution at Cotton Creek Marsh represents concentrations generally below the threshold with occasional elevated concentrations of chloride. What the numerical values of the individual samples don't show is the relative volume of groundwater contributed to these two wetlands, from the groundwater flow paths being intercepted by each of the sampling points. The State endangered purple-flowering raspberry (Rubus odoratus), the State threatened bog buckbean (Menyanthes trifoliata) and the State threatened black sandshell mussel (Ligumia recta) occur in both of these wetland DNPs. The presence of these rare wetland species indicates that the averaged flow of groundwater into the DNPs represents groundwater quality which is still generally favorable to a fen environment. Therefore, a lower chloride standard will benefit both Spring Grove Fen and Cotton Creek Marsh, by maintaining and potentially improving groundwater quality.

Accordingly, the Agency is proposing that the Board adopt a site specific groundwater standard of 45 mg/L for both the Spring Grove Fen and Cotton Creek Marsh, Class III groundwater areas to protect the fen environments within the dedicated nature preserves.

Chloride is also being proposed for a lower standard at Stemler, Pautler, Fogelpole and Armin Krueger caves. Data from the Fogelpole Class III area and Armin Krueger Class III groundwater area was considered together because dye tracing tests indicate that under certain flow conditions these cave systems share recharge. Chloride was evaluated for a site specific

standard because there is an existing groundwater quality standard, adequate chloride data exists for each of the cave systems proposed and a more stringent standard in these Class III areas may aid in the protection and potentially the recovery of the federally and State endangered Illinois Cave Amphipod (Gammarus acherondytes). This species exists only in caves in southwestern Illinois. Many other rare species of cave dwelling creatures also inhabit these cave systems. Studies show that the survival of some amphipod species is negatively impacted by increased concentrations of ions such as potassium, sodium and chloride in water (Taylor, Webb and Panno 2000) (Document 2). Changes in these dissolved constituents make it difficult for aquatic creatures to maintain the osmotic balance within their bodies. Even concentrations of these ions that are not immediately lethal to a given species, may cause stress, which when combined with other factors may impact the creatures viability. David Sutcliffe (Sutcliffe 2000) (Document 3) studied Gammarus duebeni a common amphipod in Europe, which occupies environments ranging from hypersaline pools to fresh water streams. Though G. duebeni is recognized as a single species, Sutcliffe found that a number of morphological features exist to regulate osmotic balance within the species depending on the environment inhabited. It therefore stands to reason that over many generations G. acherondytes would adapt to groundwater quality within cave systems. Relatively sudden changes to the chloride content of groundwater caused by urbanization and agriculture would almost certainly cause distress to a creature adapted to live in non-impacted cave groundwater.

Because groundwater within cave systems fluctuates between being dominated by surface infiltration and slower moving longer residence time groundwater, large data sets such as the one available for the Fogelpole/Armin Krueger Class III areas reflect a bimodal distribution. Therefore,

a single tolerance limit doesn't predict cave groundwater quality very well, since it is essentially divided into two different groundwater types.

For example, the Fogelpole/Armin Krueger Class III areas have a large data set, which would, under strictly porous media conditions, yield a useful tolerance limit. However, the data displays different results due to the bimodal groundwater sources. A tolerance limit was calculated for the Fogelpole/Armin Krueger Class III areas, which predicts an upper maximum value, with 95% confidence of 46.5 mg/L. However, the actual measured maximum value was nearly three times that value at 127 mg/L, while the chloride mean and median values were 19.6 and 17.0 mg/L, respectively. The extreme contrast between the maximum detected value, the "average" groundwater quality and a single statistically based calculation suggest that a different metric should be considered to establish Class III groundwater standards for caves. Pautler Cave, which had a population of G. acherondytes when last surveyed, before its entrance was sealed sometime prior to 1993 (Webb 1993) (Document 4), has a mean chloride concentration of 18.3 mg/L, a median concentration of 12.0 mg/L and a maximum concentration of 51 mg/L in its Class III area. In contrast, the Stemler Cave, Class III area has a mean, median and maximum recorded chloride concentration of 51.6 mg/L, 48.0 mg/L and 83.0 mg/L, respectively. These comparative statistics of chloride concentration in the Stemler Class III area result from consistently higher concentrations of chloride than are usually recorded in the Fogelpole/Armin Krueger and Pautler Class III areas. The Agency believes this contrast in chloride concentrations to be important because the Fogelpole/Armin Krueger and Pautler cave systems are reported to have good populations of G. acherondytes, while G. acherondytes has not been found in Stemler Cave since 1965.

Since the Class III areas that contribute to Fogelpole/Armin Krueger and Pautler Caves have generally low chloride concentrations (mean and median concentrations less than 20 mg/L) and chloride has been identified as an ion that may cause stress to the osmotic balance of amphipods, the Agency believes a site specific chloride standard lower than the Class I groundwater standard, that more closely resembles groundwater quality in the cave systems that support G. acherondytes, would be of benefit to the DNPs that contain caves.

With these considerations in mind and to aid in the protection of G. acherondytes in the Fogelpole Cave DNP, the Armin Krueger Cave DNP and the Pautler Cave DNP and potentially assist in the recovery of G. acherondytes in the Stemler Cave DNP, the Agency is proposing that the Board adopt a Class III site specific groundwater standard of 20 mg/L in all four of these listed Class III groundwater areas, since this concentration approximates the mean and median values reported in those cave systems with G. acherondytes populations.

The Agency is aware that private septic systems are common within the Class III areas of Stemler, Fogelpole/Armin Krueger and Pautler Caves, and also exist within the Class III areas of Spring Grove Fen and Cotton Creek Marsh. Septic systems can be a source of contaminants, including chloride, especially in shallow groundwater. To assess the potential economic impact a reduced groundwater standard for chloride might have on the use of private septic systems by residents within Class III areas, the Agency conducted a literature search for documents assessing septic system impacts to groundwater. The Agency found United States Geological Survey, Water Resources Investigations Report 91-4011 (USGS 1991), (Document 5). The USGS 1991 report examines groundwater quality proximate to two different subdivisions, served by private septic systems where highly fractured limestone bedrock is located near the land surface. The subdivisions are located in a suburban area near Nashville Tennessee. Monitoring wells within

the bedrock, domestic wells and springs discharging groundwater down gradient of the subdivisions were sampled to determine if there was a link between groundwater and the septic systems. Since many of the contaminants found in septic effluent occur naturally and may also have other anthropogenic sources the USGS sampled for optical brighteners, which are found in laundry detergents, thus establishing a marker that could be used to evaluate the linkage between septic effluent and groundwater. Optical brighteners were detected in groundwater, thereby establishing a link between septic effluent and groundwater down gradient of the subdivisions. In addition to the optic brighteners, the USGS also monitored for chloride and other water quality parameters. The USGS detected chloride in all samples collected with a range in concentration of 2.1 mg/L to 38 mg/L. The reported groundwater chloride concentrations from USGS 1991 had a mean and median concentration of 13.0mg/L and 12.0mg/L, respectively. These mean and median chloride concentrations are similar to the groundwater in the Fogelpole/Armin Krueger and Pautler Class III areas. The maximum concentration, and the mean and median concentrations reported in USGS 1991, are also below the inflection point used as the proposed site specific chloride standard for Spring Grove Fen and Cotton Creek Marsh. Therefore, groundwater in a karst setting, with more discrete groundwater flow through factures, which is hydraulically connected with septic effluent does not necessarily yield excessive chloride concentrations in groundwater. In comparison to a karst setting, the groundwater flow in sand and gravel is more diffuse. Therefore, average groundwater concentrations of chloride from septic systems in the Spring Grove Fen and Cotton Creek Marsh Class III areas should not be greater than found in a karst setting. Based on this comparison, the Agency does not believe that properly designed and operated private septic systems are implicitly incompatible with Class III groundwater areas. Therefore, adoption of a more stringent chloride standard would not impose an economic burden by precluding the use of

septic systems within the Class III areas. While other anthropogenic sources of chloride may have an impact on groundwater quality, no new groundwater remediation programs are created by these site specific standards, nor are any new groundwater monitoring requirements imposed within the Class III areas by adoption of these site specific standards. Therefore, the Agency believes the economic impact of the site specific chloride standards will be minimal, while the potential benefit to the endangered and threatened species which rely on this groundwater is significant.

pH

The Taylor, Webb and Panno 2000 report identifies pH as a characteristic of groundwater quality that is likely important to the survival of G. acherondytes. The Class I groundwater standard for pH is a range of 6.5 to 9.0 standard units (SU). Groundwater monitoring data was available for assessment of pH within all of the Class III areas that contribute to cave DNPs. As with the chloride analysis, the pH values for the Fogelpole Class III area and the Armin Krueger Class III area were evaluated together since those two cave systems share groundwater under certain flow conditions.

Because groundwater within cave systems fluctuates between being dominated by rapid surface infiltration and slower moving, longer residence time groundwater, large data sets such as the one available for the Fogelpole/Armin Krueger Class III areas reflect this bimodal distribution. A single tolerance limit doesn't predict cave groundwater quality very well, since it is essentially divided into two different groundwater types. Therefore, the Agency has used minimum, maximum and mean pH values to evaluate pH in the cave Class III areas. Comparison of the groundwater pH values for the Class III groundwater areas of the cave systems yield mean pH values of 7.6, 7.1 and 6.7 SU at Fogelpole/Armin Krueger, Pautler and Stemler,

respectively. While the lowest pH recorded within the Fogelpole/Armin Krueger Class III areas (6.1 SU) was below that recorded in the Stemler Class III area (6.4 SU), none of the pH values recorded from the Stemler Class III area were over 7.0 SU (neutral). All pH values recorded for Stemler Cave are on the acidic side of neutral. The maximum pH values were 8.5, 7.4 and 6.9 SU at Fogelpole/Armin Krueger, Pautler and Stemler, respectively. As discussed previously, Fogelpole Cave, Armin Krueger Cave and Pautler Cave have populations of G. acherondytes, while Stemler Cave has no known extant population. As suggested by Taylor, Webb and Panno 2000 the lower pH values in the Stemler Cave Class III groundwater may result from a closer link between surface water and groundwater within the cave. Extended contact with the limestone in which the caves are formed would be expected to raise pH values. Though a modified pH standard can't change the natural conditions, it could insure that surface and subsurface discharges that reach groundwater do not contribute to decreased pH levels. While pH may combine with other factors to determine G. acherondytes survival, it appears, based on available data that pH values consistently below 7.0 SU, at a minimum, may add additional stress to G. acherondytes populations.

Much of the land use contained in the Fogelpole/Armin Krueger, Pautler and Stemler cave Class III groundwater areas is rural, agricultural land and low density residential housing, utilization of septic systems as a means of sanitary waste disposal are common. Therefore, the Agency evaluated available information regarding the pH of septic system effluent. According to the Washington State Department of Health, Wastewater Management Program (2004) (Document 6) the optimum pH for bacterial growth, which septic systems rely on for waste treatment, is between 6.5 and 9.0 SU. The proposed site specific pH standard is 7.0 to 9.0 SU, which is within the operating range for septic systems. None of the reported pH values in groundwater collected by

USGS 1991 were less than 7.0 SU. Taken together, these pieces of information indicate that even though properly operated septic system effluent itself may occasionally be below 7.0 SU, the pH operating range is often higher than 7.0 SU and groundwater would also buffer the pH rather quickly. This suggests that properly installed and operated private septic systems should not adversely impact pH in karst environments. Therefore, use of private septic systems, which are a common waste disposal method in the Fogelpole/Armin Krueger, Pautler and Stemler cave Class III groundwater areas, could continue, and thus not have a negative economic impact on area residents. Further, the site specific pH standard does not create any new remedial action or groundwater monitoring programs. Therefore, the Agency believes the economic impact of the site specific pH standard will be minimal, while the potential benefit to the endangered and threatened species which rely on this groundwater is significant.

Based on the importance of pH attributed by Taylor, Webb and Panno 2000 to the survival of G. acherondytes and the data demonstrating higher pH in the groundwater within the Class III areas that support populations of G. acherondytes, the Agency believes a site specific standard for pH is warranted. Therefore, to aid in the protection of G. acherondytes in the Fogelpole Cave DNP, the Armin Krueger Cave DNP and the Pautler Cave DNP and potentially assist in the recovery of G. acherondytes in the Stemler Cave DNP, the Agency is proposing that the Board adopt a Class III site specific groundwater standard of 7.0 to 9.0 standard units in all four of these listed Class III groundwater areas.

ATTACHMENT 2A

Lynn E. Dunaway

516 South Cherokee
Taylorville, Illinois 62568
Phone (H) 217/827-7960 (W) 217/785-2762

Professional Experience

Illinois Environmental Protection Agency **February 1988 to Present**
Bureau of Water
Division of Public Water Supplies
Groundwater Section
Springfield, Illinois

Environmental Protection Specialist IV **March, 2017 to Present**
Duties include: Currently in this position: provide review and technical input on hydrogeologic assessments and reports to all Bureau of Water Programs and Office of Emergency Response on groundwater issues; regularly respond to questions concerning the Illinois Environmental Protection Act (Act) and associated regulations, from the public, press, other governmental bodies and industry; review and respond to documents submitted pursuant to the regulations; remedial project management at facilities under Bureau of Water permits and unpermitted sites; assist with the development of regulations in support of the Act including testimony before the Illinois Pollution Control Board (Board) and at public hearings; provide technical input for special projects requiring geologic expertise, including pre-trial documents and testimony in court and before the Board; design and routine maintenance of tracking logs and data bases for the support of various groundwater programs; assist in the preparation of routine reports concerning various aspects of the States groundwater protection programs; Participate as mentor in the Graduate Public Service Internship (GPSI) program and the Governor's Environmental Corp (GEC) program.

Environmental Protection Specialist III **April, 1991 to March 2017**
Duties include: Currently in this position: provide review and technical input on hydrogeologic assessments and reports to all Bureau of Water Programs and Office of Emergency Response on groundwater issues; regularly respond to questions concerning the Act and associated regulations, from the public, press, other governmental bodies and industry; review and respond to documents submitted pursuant to the regulations; remedial project management at facilities under Bureau of Water permits and unpermitted sites; assist with the development of regulations in support of the Act including testimony before the Board and at public hearings; provide technical input for special projects requiring geologic expertise, including pre-trial documents and testimony in court and before the Board; design and routine maintenance of tracking logs and data bases for the support of various groundwater programs; assist in the preparation of routine reports concerning various aspects of the States groundwater protection programs; assist in the design of a data base to track and enhance compliance with the regulations under the Act; Participate as mentor in the GPSI program and the GEC program;

Temporary assignment as Unit Supervisor during supervisor's absence.

Environmental Protection Specialist II **June, 1989 to April, 1991**
Duties include: the quality control process used for the Sections ambient groundwater monitoring programs before entry into the SAFE system for periodic transfer to the STORET data base; use the SAFE System; use of the STORET System; assist with the development of regulations in support of the Illinois Groundwater Protection Act (Act); regularly respond to questions concerning the Act; provide technical review of assessments submitted to the Section; provide technical input for special projects requiring geologic expertise; lead worker for the Agency's first Draft submittal for approval of the (WHPP); assist in the preparation of routine combined section 106/319 grant reports; design and routine maintenance of tracking logs and data bases for the support of various groundwater programs; assist in the preparation of routine reports concerning various aspects of the States groundwater protection programs.

Environmental Protection Specialist I **February, 1988 to June, 1989**
Duties include: learn the quality control process used for the Sections ambient groundwater monitoring programs before entry into the SAFE System for periodic transfer to the STORET data base; learn to use the SAFE System; occasionally respond to questions concerning the Act; provide technical input for special projects requiring geologic expertise; routine maintenance of tracking logs for the support of various groundwater programs; assist in the preparation of routine reports concerning various aspects of the States groundwater protection programs.

Analytical Logging Inc. **August, 1982 to January, 1986**
Shreveport Louisiana
South Texas District
Corpus Christi, Texas

Lead Well Site Geologist **March, 1984 to January, 1986**
Duties Included: over site of a two or three man team; provide daily progress reports summarizing drilling activities and important hydrocarbon detections to field office and home office geologists and engineers; correlation and interpretation of geophysical logs; geologic evaluation of lithologic samples to determine geologic formation and hydrocarbon potential; packed column gas chromatography for hydrocarbon analysis; evaluation techniques to predict high pressure zones; routine maintenance of all systems utilized; creating a graphical representation correlating the geology, drilling parameters and hydrocarbon detections for each well.

Well Site Geologist **August, 1982 to February, 1984**
Duties Included: correlation and interpretation of geophysical logs; geologic evaluation of lithologic samples to determine geologic formation and hydrocarbon potential; assist with packed column gas chromatography for hydrocarbon analysis; learn evaluation techniques to predict high pressure zones; learn routine maintenance of all systems utilized; creating a graphical representation correlating the geology, drilling parameters

and hydrocarbon detections for each well.

Other Work Experience

Grain/ Livestock Farm Hand	**February 1986 to July 1986 and October 1987 to December 1987**
Shipping/Receiving Clerk	**August 1986 to November 1986**
Agrichemical Service Company Laborer	**March 1987 to June 1987**
Meat Packing Company Laborer	**July 1987 to September 1987**

Education and Training

Bradley University **August, 1978 to May, 1982**
Peoria, Illinois
Bachelor of Science; Geology

Northern Illinois University **May, 1982 to July, 1982**
Dekalb, Illinois
Post-graduate work; Field Mapping of the Black Hills Region, South Dakota

USEPA Groundwater Monitoring and Restoration **June 1 & 2, 1993**
Short course on behavior of DNAPLs in the subsurface and case studies

USEPA Risk Assessment Guidance for Superfund **October 18-21, 1993**
Environmental response training and case studies

Computer Modeling for Groundwater Systems **August 21, to December 16, 1995**
Basis of groundwater models, Dr. Larry Barrows, Illinois State University

Applied Ground Water Statistics for Landfills Short Course **July 8 & 9, 1997**
Statistical techniques for detection and compliance monitoring

Statistical Methods in Water Resources **August 6-10, 2001**
Application of statistical methods, University of Illinois, Springfield

Ozark Underground Laboratory Karst Short Course **March 12, 2003**
Unique features and case studies in karst geologic settings

Aqueous Geochemistry for Environmental Regulators **March 9 & 10, 2004**
Short Course by Dr. Stephen Van der Hoven, Illinois State University

Overview of Environmental Geophysics **May 6, 2004**
Review of common equipment advantages/disadvantages, by USEPA and Tetra Tech.

Geotechnology for Non-Engineers **April 20, 2005**
Key principles and concepts of Geotechnolgy, by Dr. Timothy Stark, University of Illinois

Fate and Transport Processes and Models **March 29 & 30, 2006**
Key elements of transport, models & assumptions, by Dr. Atul Salhotra, RAM, Inc.

Introduction to ArcGIS I **March 10 & 11, 2008**
Introduction to the features and functions of ArcGIS and use thereof, by Carmen Maso', USEPA

National Groundwater Association Conference **June 26 & 27, 2012**
Day 1: Presentations of general interest for groundwater assessment and protection, Day 2: Presentations related to hydraulic fracturing and groundwater quality

The Environmental Sampling Field Course **August 21-24, 2012**
Classroom and field training collecting soil, surface water and groundwater samples, by David & Gillian Nielson

Practical Geophysics
for Engineering, Archeology and Hydrogeology **September 27 & 28, 2012**
Classroom and hands on field demonstrations of resistivity, seismic, down-hole logging, by Drs. Ismail, Larson and Young, Illinois State Geological Survey

Forty Hour Safety Training with Annual Eight Hour Refresher per 29 CFR 1910.120
Last refresher 3/7/2013

Licenses

Licensed Professional Geologist (Illinois) **March 31, 1998**

License Number: 196.000608
Expiration Date: March 31, 2023

ATTACHMENT 2B

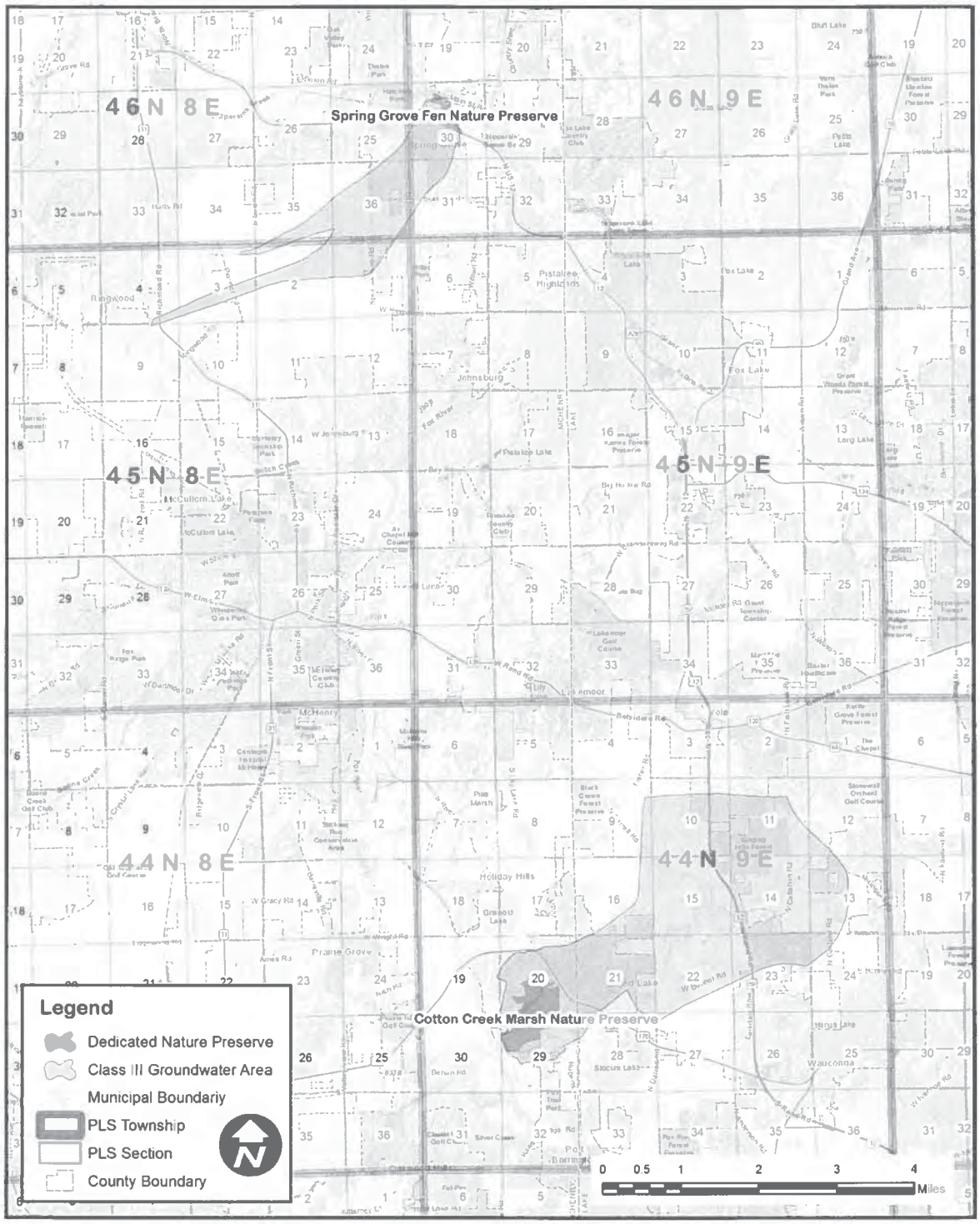
Dedicated Nature Preserves Listed for Class III Groundwater

Sand Ridge Nature Preserve	Cook County
Bluff Spring Fen Nature Preserve	Cook County
Cranberry Slough Nature Preserve	Cook County
McMahon Woods & Fen Nature Preserve	Cook County
Goose Lake Prairie Nature Preserve	Grundy County
Apple River Canyon Nature Preserve	Jo Daviess County
Trout Park Nature Preserve	Kane County
Volo Bog Nature Preserve	Lake County
Illinois Beach Nature Preserve	Lake County
Elizabeth Lake Nature Preserve	McHenry County
Spring Grove Fen Nature Preserve	McHenry County
Boone Creek Fen Nature Preserve	McHenry County
Gladstone Fen Nature Preserve	McHenry County
Julia M. and Royce L. Parker Fen Nature Preserve	McHenry County
Lake in the Hills Fen Nature Preserve	McHenry County
Cotton Creek Marsh Nature Preserve	McHenry County
Yonder Prairie Nature Preserve	McHenry County
Barber Fen Nature Preserve	McHenry County
Pautler Nature Preserve	Monroe County
Armin Krueger Speleological Nature Preserve	Monroe County
Fogelpole Cave Nature Preserve	Monroe County
George B. Fell Nature Preserve	Ogle County

Thomas W. and Elizabeth Moews Dore Seep Nature Preserve	Putnam County
Stemler Cave Woods Nature Preserve	St. Claire County
Bennett's Terraqueous Gardens & Fon du Lac Seep N. P.	Tazewell County
Romeoville Prairie Nature Preserve	Will County
Lockport Prairie Nature Preserve	Will County
Braidwood Dunes and Savanna Nature Preserve	Will County
Long Run Seep Nature Preserve	Will County
Searles Park Prairie Nature Preserve	Winnebago County

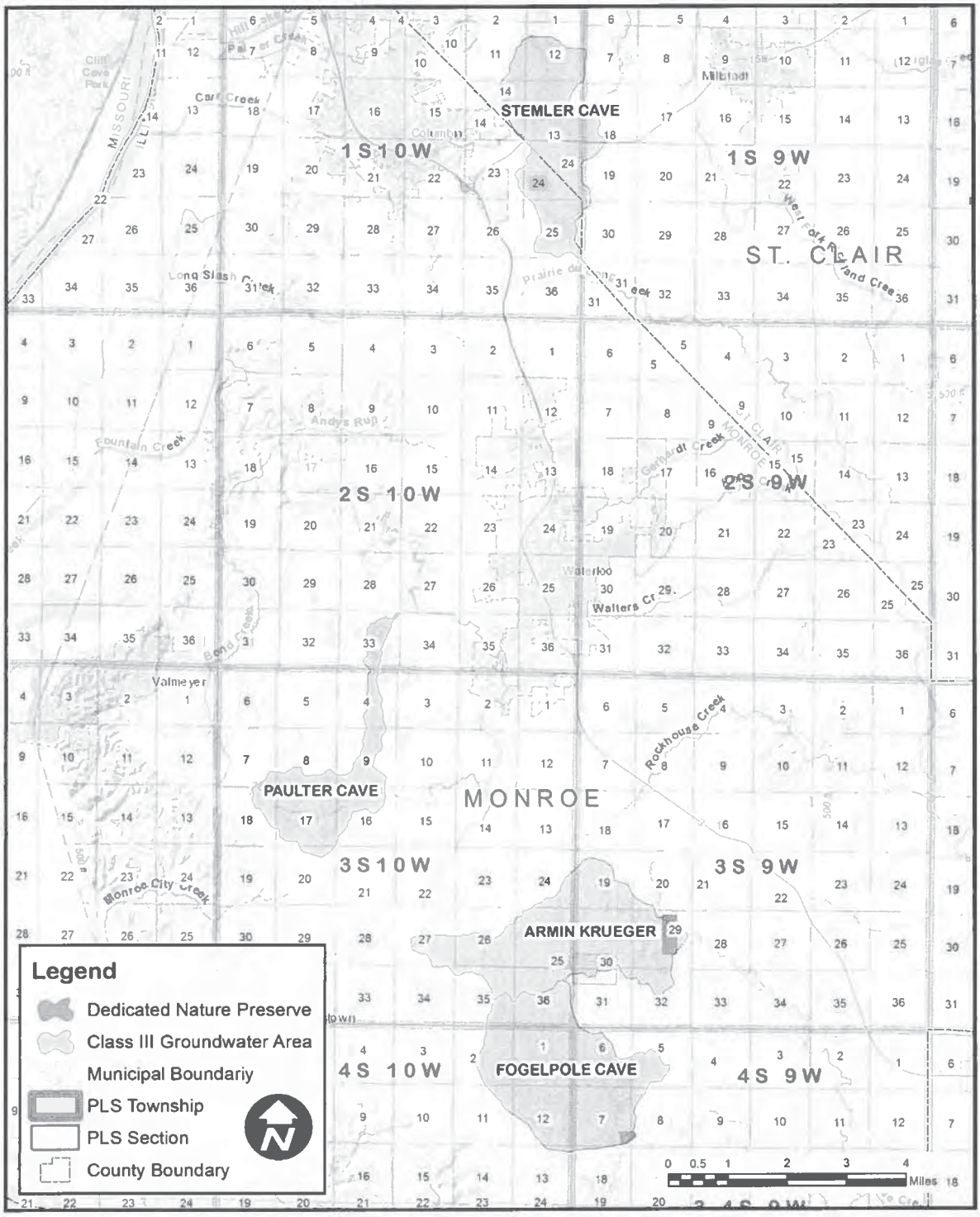
ATTACHMENT 2C

SPRING GROVE FEN AND COTTON CREEK MARSH CLASS III GROUNDWATER AREAS



ATTACHMENT 2D

PAUTLER, STEMLER, ARMIN KRUEGER AND FOGELPOLE CAVES CLASS III GROUNDWATER AREAS



ATTACHMENT 3

Illinois EPA's Development of Proposed PFAS Groundwater Quality Standards for 35 IAC Part 620

Difference between Groundwater Quality Standards and Maximum Contaminant Levels (MCLs)

Groundwater quality standards and MCLs are NOT the same, other than in some cases the absolute value of the MCL may be used as a basis for the numerical groundwater quality standards under the Illinois Pollution Control Board's (Board) Part 620. Groundwater quality standards apply in-situ and are enforceable numerical standards intended to protect and restore the beneficial uses of resource groundwater.

MCLs apply at the entry point to a public water supply distribution system. Finished or treated drinking water must meet MCLs before distribution to consumers to provide assuredly safe drinking water. MCLs consider not only the toxicity of the contaminant, but also the occurrence, best available treatment (BAT), and laboratory analytical methods.

How Illinois EPA's proposed Groundwater Quality Standards are derived

35 Ill. Adm. Code 620, Appendix A, provides procedures for determining a Human Threshold Toxicant Advisory Concentration (HTTAC) for those substances for which US EPA has not adopted a Maximum Contaminant Level Goal (MCLG). Appendix A, subsection (a) provides an equation required for the calculation of the HTTAC:

$$HTTAC = \frac{RSC \bullet ADE}{W}$$

Where:

HTTAC	=	Human Threshold Toxicant Advisory Concentration (mg/L).
RSC	=	Relative contribution of the amount of the exposure to a chemical via drinking water when compared to the total exposure to that chemical from all sources. If valid chemical-specific data are not available, a value of 20% (= 0.20) must be used.
ADE	=	Acceptable daily exposure of substance in milligrams per day (mg/d).
W	=	Per capita daily water consumption equal to 2 liters per day (L/d).

The equation is consistent with the method used by the US EPA for determining drinking water advisories for chemicals for which a maximum contaminant level (MCL) or maximum contaminant level goal (MCLG) is not prescribed by the US EPA. The Illinois EPA uses the equation for calculating a Class I potable resource groundwater quality standard in the absence of an MCL or MCLG.

Appendix A, subsection (b), provides procedures for determining Acceptable Daily Exposures (ADEs) for Class I groundwater. The ADE represents the maximum amount of a threshold toxicant in milligrams per day (mg/d), which if ingested daily for a lifetime results in no adverse effects to humans. Subsection (b)(2), states that for those substances for which the US EPA has derived a verified oral reference dose (RfD) given in milligrams per kilogram per day (mg/kg/d), the reference dose must be used. The ADE equals the product of multiplying the RfD by 70 kilograms (kg), which is the assumed average weight of an adult human.

When determining the appropriate RfD to use in calculating a HTTAC, the Illinois EPA uses the following hierarchy as its source:

1. US EPA Integrated Risk Information System (IRIS)
2. US EPA Provision Peer-Reviewed Toxicity Values Program (PPRTV)
3. US EPA Office of Pesticide Programs (OPP)
4. US Department of Health and Human Services Agency for Toxic Substances and Disease Registry (ATSDR)

In June of 2018, ATSDR issued a draft Toxicological Profile for Perfluoroalkyls for public comment. The comment period closed on August 20, 2018. The toxicological profile focused on four perfluoroalkyls and recommended the following intermediate oral reference doses:

- Perfluorooctanoic Acid (PFOA) = 3E-06 mg/kg/d
- Perfluorooctane Sulfonic Acid (PFOS) = 2E-06 mg/kg/d
- Perfluorohexane Sulfonic Acid (PFHxS) = 2E-05 mg/kg/d
- Perfluorononanoic Acid (PFNA) = 3E-06 mg/kg/d

In 2014, PPRTV issued a chronic reference dose of 2E-02 mg/kg/d for perfluorobutane sulfonic acid (PFBS).

The RfDs are calculated from animal studies and 35 Ill. Adm. Code 620, Appendix A, subsection (b) allows for the use of subchronic (intermediate) RfDs when chronic values are not available. The Illinois EPA expects ATSDR values to be finalized; therefore, chose the ATSDR RfDs for calculating the proposed groundwater quality standards for PFOA, PFOS, PFHxS and PFNA. The Illinois EPA used the PPRTV RfD for calculating the PFBS groundwater quality standard. As updated data becomes available, reference doses will be adjusted and updated Class I standards will be proposed.

Using the equation and values specified in Appendix A, the Class I groundwater quality standards are calculated as follows:

$$HTTAC = \frac{0.20 \bullet ADE\ (\frac{mg}{d})}{2\ (\frac{L}{d})}$$

Where:

PFOA ADE	=	0.000003 mg/kg/d • 70 kg = 0.00021 mg/d
PFOS ADE	=	0.000002 mg/kg/d • 70 kg = 0.00014 mg/d
PFHxS ADE	=	0.00002 mg/kg/d • 70 kg = 0.0014 mg/d
PFNA ADE	=	0.000003 mg/kg/d • 70 kg = 0.00021 mg/d
PFBS ADE	=	0.02 mg/kg/d • 70 kg = 1.4 mg/d

The resulting groundwater quality standards are as follows:

PFOA	=	0.000021 mg/L (21 ng/L)
PFOS	=	0.000014 mg/L (14 ng/L)
PFHxS	=	0.00014 mg/L (140 ng/L)
PFNA	=	0.000021 mg/L (21 ng/L
PFBS	=	0.14 mg/L (140,000 ng/L)

These values are also protective of developmental and infant exposure through umbilical cord blood and breast-milk/formula feeding, based on US EPA exposure factor assumptions of a 7.8 kg child ingesting 1.13 L/d, with an RSC of 100% coming from umbilical cord or ingestion of breast-milk/formula. The proposed groundwater quality standards equal ATSDR's minimal risk levels for a child.

Proposed Updates to 35 Ill. Adm. Code 620.410 and 620.420

(Class I and Class II Groundwater Quality Standards)

By Carol Hawbaker
Environmental Risk Assessor
Illinois EPA Office of Toxicity Assessment

February 13, 2020

Introduction of New Chemicals

<u>The amendments propose the addition of 9 chemicals:</u>

- Aluminum
- Lithium
- 1-Methylnaphthalene
- Molybdenum
- Perfluorobutane Sulfonic Acid (PFBS)
- Perfluorohexane Sulfonic Acid (PFHxS)
- Perfluorononanoic Acid (PFNA)
- Perfluorooctanoic Acid (PFOA)
- Perfluorooctane Sulfonic Acid (PFOS)

Addition of Atrazine Metabolites

The amendments propose the addition of 3 atrazine metabolites to be included in the total atrazine Class I and Class II groundwater quality standards.

<u>Metabolites</u>

- Desethyl-atrazine (DEA)
- Desisopropyl-atrazine (DIA)
- Diaminochlorotriazine (DACT)

Combination of Radium 226 and 228

The amendments propose combined Radium 226 + 228 Class I and Class II groundwater quality standards.

The proposed value for Class I and Class II is the Federal MCL.

Combined PFOA + PFOS Value

The amendments propose both individual and combined values for PFOA and PFOS.

- Individual:

  PFOA = 21 ng/L

  PFOS = 14 ng/L

- Combined Not to Exceed 21 ng/L

Updates to Carcinogenic Designations

<u>Carcinogen designations updated for the following chemicals:</u>

- para-Dichlorobenzene
- Ethylbenzene
- gamma-Hexachlorocyclohexane (lindane)
- 1,2,4-Trichlorobenzene

Updates to Class I: Potable Resource Groundwater Quality Standards (Section 620.410)

In addition to new chemicals, updated Class I Groundwater Quality Standards are proposed for 19 chemicals presently listed in Section 620.410. Proposed new and updated standards are based on the following factors:

- Updates to US EPA or Illinois Maximum Contaminant Levels (MCLs)
- Updates to Toxicity Values
- Improvements in Laboratory Technologies (allowing for lower levels of quantification)
- Irrigation or Livestock Criteria (used when irrigation or livestock values are more stringent than MCLs or toxicity-based values)
- Correction of Typo from Previous Amendments - Vanadium

Updated Toxicity Values

Illinois EPA Hierarchy for Determining Toxicity Values

Hierarchy is derived from US EPA OSWER Directive 9285.7-53, dated December 5, 2003 and discussed in the Illinois Pollution Control Board Rulemaking R08-18: Proposed Amendments to Groundwater Quality Standards, 35 Ill. Adm. Code 620.

Tier 1: Integrated Risk Information System (IRIS)

Tier 2: Provisional Peer Reviewed Toxicity Values (PPRTV)

Tier 3: Other Toxicity Values

> "Priority given to sources of information that are the most current, the basis for which is transparent and publicly available, and which has been peer-reviewed."
>
> OSWER Directive 9285.7-53

Updated Toxicity Values

Tier 3: Other Toxicity Values

Additional sources referenced in OSWER Directive 9285.7-53, include, but are not limited to:

- California Environmental Protection Agency (Cal EPA) Toxicity Values
- Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Levels
- Health Effects Assessment Summary Table (HEAST)

Methods for Calculating Groundwater Quality Standards for Substances for which US EPA Has Not Adopted a MCL

Methodologies are found at Section 620, Subpart F and Appendix A.

- Human Nonthreshold Toxicant Advisory Concentration (HNTAC) for Carcinogens
- Human Threshold Toxicant Advisory Concentration (HTTAC) for Non-Carcinogens

Human Nonthreshold Toxicant Advisory Concentrations (Carcinogen)

$$HNTAC(\frac{mg}{L}) = \frac{TR \bullet BW \bullet AT \bullet 365 \frac{days}{year}}{SF_o \bullet IR \bullet EF \bullet ED}$$

Where:

TR = Target Risk = 1.0E-06

BW = Body Weight = 70 kilograms

AT = Averaging Time = 70 years

SF_o = Oral Slope Factor ((milligrams/kilogram/day)$^{-1}$) = Chemical-Specific

IR = Daily Water Ingestion Rate = 2 liters/day

EF = Exposure Frequency = 350 days/year

ED = Exposure Duration = 30 years

Human Threshold Toxicant Advisory Concentration (Non-Carcinogen)

$$HTTAC\ \left(\frac{mg}{L}\right) = \frac{RSC \bullet ADE}{W}$$

Where:

RSC = Relative contribution of the amount of the exposure to a chemical via drinking water when compared to the total exposure to that chemical from all sources.

ADE= Acceptable Daily Exposure of substance in milligrams per day. The ADE represents the maximum amount of a threshold toxicant, which, if ingested daily for a lifetime, results in no adverse effects to humans. The ADE equals the product of multiplying a US EPA hierarchy oral reference dose given in milligrams per kilogram per day by 70 kilograms, which is the assumed average weight of an adult human.

W = Per capita daily water consumption equal to 2 liters per day

Livestock and Irrigation Values

Livestock or Irrigation Values are Currently Used as Class I Groundwater Quality Standards for the Following Chemicals:

- Boron - Irrigation
- Cobalt - Livestock
- Nickel - Livestock

Based upon support of a use other than potability (livestock watering, irrigation, industrial use) where the different use requires a more stringent standard.

Proposing livestock values for copper and fluoride, and an irrigation value for selenium.

Updates to Class II: General Resource Groundwater Quality Standards (Section 620.420)

In addition to new chemicals, updated Class II Groundwater Quality Standards are proposed for 40 chemicals presently listed in Section 620.420. Proposed new and updated standards are based on the following factors:

- Updated Class I Groundwater Quality Standards
- Updated Treatment Factors
- Irrigation or Livestock Criteria

Updated Treatment Factors

Criteria for Application of Best Available Technology (BAT) Treatment Factors:

- Dimensionless Henry's Constant (H') Value Greater Than Methylene Chloride's (H') Value of 0.133 at a 20 ^{0}C Groundwater System Temperature.

OR

- Organic Carbon Partition Coefficient (K_{oc}) Value Greater Than Ethylbenzene's (K_{oc}) Value of 446 cm^3/gram.

If a chemical's chemical/physical values meet the criteria, a Treatment Factor of 5 is applied to the Class I Groundwater Quality Standard to calculate a Class II Groundwater Quality Standard.

Source of Values: US EPA Regional Screening Levels

Source of Criteria: Illinois Pollution Control Board R08-18

PFAS

Per and Poly-Fluoroalkyl Substances = PFAS

PFAS are a group of man-made chemicals applied to many consumers products to make them waterproof, stain resistant or non-stick.

- Non-Stick Pans
- Stain-Resistant Carpets and Textiles
- Water-Proof Clothing and Footwear
- Pizza Boxes, Food Wrappers, Microwave Popcorn Bags
- Fire-Fighting Foam

PFAS

PFAS are chemicals of emerging concern:

- "Forever Chemicals": PFAS chemicals do not degrade naturally in the environment
- PFAS chemicals have an affinity for water and can migrate long distances

Health Effects of PFAS

Epidemiology Studies Suggest Associations Between PFAS Exposure and Several Health Effects:

- Pregnancy-Induced Hypertension/Pre-Eclampsia
- Liver Damage
- High Cholesterol
- Thyroid Disease
- Decreased Response to Vaccines
- Decreased Fertility
- Decreased Birth Weight

Source: Agency for Toxic Substance and Disease Registry (ATSDR)

Health Effects of PFAS

Carcinogenic Potential:

The Illinois Environmental Protection Act defines a "carcinogen" as:

a contaminant that is classified as a category A1 or A2 carcinogen by the American Conference of Governmental Industrial Hygienists; a category 1 or 2A/2B carcinogen by the World Health Organization's International Agency for Research on Cancer; a "human carcinogen" or "anticipated human carcinogen" by the United States Department of Health and Human Service National Toxicological Program; or a category A or B1/B2 carcinogen or as "carcinogenic to humans" or "likely to be carcinogenic to humans" *by the United States Environmental Protection Agency in the integrated risk information system or a final rule issued in a Federal Register notice by the USEPA.* [415 ILCS 5/58.2]

Health Effects of PFAS

- PFOA: Designated a Group 2B Carcinogen (possibly carcinogenic to humans) by International Agency for Research on Cancer in 2017. PFOA is considered a carcinogen per the Act's definition of a carcinogen.

- PFOS: Designated as "Suggestive of Carcinogenic Potential" by US EPA Integrated Risk Information System in 2016. PFOS is not considered a carcinogen per the Act's definition of a carcinogen.

Suggestive of Increases in Testicular and Kidney Cancer.

Insufficient Data to Develop Cancer Toxicity Values.

PFAS

The Illinois EPA proposes to add 5 PFAS Chemicals to the 620 Regulations:

- Perfluorohexane Sulfonic Acid (PFHxS):

  Class I and Class II Standards = 0.00014 mg/L (140 ng/L)
- Perfluorononanoic Acid (PFNA):

  Class I and II Standards = 0.000021 mg/L (21 ng/L):
- Perfluorooctanoic Acid (PFOA):

  Class I and II Standards = 0.000021 mg/L (21 ng/L)
- Perfluorooctane Sulfonic Acid (PFOS):

  Class I and Class II Standards = 0.000014 mg/L (14 ng/L)
- Perfluorobutane Sulfonic Acid (PFBS):

  Class I and Class II Standards = 0.14 mg/L (140,000 ng/L)

PFAS

The Illinois EPA proposed the addition of 5 PFAS chemicals to the 620 regulations because toxicity values are available from sources within Illinois EPA and US EPA hierarchy for the selection of toxicity values.

- Agency for Toxic Substances and Disease Registry (ATSDR) – PFOA, PFOS, PFNA, PFHxS
- Provisional Peer Reviewed Toxicity Values (PPRTV) - PFBS

PFAS

- PFOA, PFOS, PFNA, PFHxS Source for Toxicity Values:

  Agency for Toxic Substances and Disease Registry (ATSDR)

  Draft

  Draft Profile is in final review by US Office of Management and Budget (OMB). ATSDR did not make revisions following public comment and peer-review.

  The MRLs proposed in the ATSDR Toxicological Profile are used by US Department of Health and Human Services and Illinois Department of Public Health in human health and risk assessments.

PFAS

ATSDR MRLs Used in the Development of Groundwater Quality Standards:

- PFOA: 0.000003 (3E-06) mg/kg/d
- PFOS: 0.000002 (2E-06) mg/kg/d
- PFNA: 0.000003 (3E-06) mg/kg/d
- PFHxS: 0.00002 (2E-05) mg/kg/d

PFAS

- PFBS Source for Toxicity Values:
  Provisional Peer Reviewed Toxicity Values (PPRTV)

In 2014, PPRTV issued a chronic reference dose of 0.02 (2E-02) mg/kg/d for PFBS.

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY
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JB PRITZKER, GOVERNOR JOHN J. KIM, DIRECTOR

Date: February 21, 2020

Re: Illinois EPA Tables Describing the Basis for Developing the Proposed Class I and Class II Groundwater Quality Standards (35 Ill. Adm. Code 620.410 and 620.420)

The attachment document consists of 3 tables:

Table 1: Groundwater Quality Standards (GQS) Proposed for Updates at 620.410: Class I Potable Resource Groundwater

Table 2: Groundwater Quality Standards (GQS) Proposed for Updates at 620.420: Class II General Resource Groundwater

Table 3: Sources for Carcinogenicity Updates

A page is also provided for definitions of acronyms used in the tables.

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Definitions:

ATSDR: Agency for Toxic Substances and Disease Registry

CalEPA: California Environmental Protection Agency

CAS No.: Chemical Abstract Services Number

HNTAC: Human Non-Threshold Toxicant Advisory Concentration (620.605(b)(2))

HTTAC: Human Threshold Toxicant Advisory Concentration (620.Appendix A(a))

IARC: International Agency for Research on Cancer

IRIS: Integrated Risk Information System

MCL: Federal Maximum Contaminant Level

MRL: Minimal Risk Level (milligrams/kilogram/day) – non-carcinogen toxicity value

PPRTV: Provisional Peer Reviewed Toxicity Values

PQL: Practical Quantitation Limit

RfD: Oral Reference Dose (milligrams/kilogram/day) – non-carcinogen toxicity value

RSC: Relative Source Contribution

SF_o: Oral Slope Factor ((milligrams/kilogram/day)$^{-1}$) – carcinogen toxicity value

Treatability Factor: Treatability Factor is determined by best available treatment technologies for removal efficiency. Criteria discussed at Illinois Pollution Control Board (IPCB) R08-18, Rick Cobb prefiled testimony dated May 29, 2008. Criteria for when a treatment factor of 5 is applied is: a dimensionless Henry's constant greater than methylene chloride at 20 ^{0}C (0.11), or an organic carbon partition coefficient greater than ethylbenzene (446 cm^3/g).

WQC: *Water Quality Criteria*, 1972

Table 1: Groundwater Quality Standards (GQS) Proposed for Updates at 620.410: Class I Potable Resource Groundwater

		Groundwater Quality Standards (GQS) Currently in 35 Ill. Adm. Code (IAC) 620					Updated Groundwater Quality Standards Proposed for 35 IAC 620				
CAS No.	Constituent	GQS (mg/L)	Basis of GQS	Toxicity Value Used in HNTAC and HTTAC Methodology in 620	Toxicity Value Source	RSC Applied	GQS (mg/L)	Basis of GQS	Toxicity Value Used for HNTAC and HTTAC Methodology in 620	Toxicity Value Source	RSC Applied
620.410(a)											
7429-90-5	Aluminum	----	----	----	----	----	3.5	HTTAC	RfD = 1E+00	PPRTV 2006	0.1
7440-42-8	Boron	2.0	WQC, 1972 (irrigation)	----	----	----	1.4	HTTAC	RfD = 2E-01	IRIS 2004	0.2
7440-48-4	Cobalt	1.0	WQC, 1972 (livestock)	----	----	----	0.0021	HTTAC	RfD = 3E-04	PPRTV 2008	0.2
7440-50-8	Copper	0.65			----	----	0.5	WQC, 1972 (livestock)	----	----	----
16984-48-8	Fluoride	4.0	MCL	----	----	----	2.0	WQC, 1972 (livestock)	----	----	----
7439-93-2	Lithium	----	----	----	----	----	0.014	HTTAC	RfD = 2E-03	PPRTV 2008	0.2
7439-98-7	Molybdenum	----	----	----	----	----	0.035	HTTAC	RfD = 5E-03	IRIS 1992	0.2
13982-63-3	Radium 226 (pCi/L)	20.0		----	----	----	5.0	Combined 226+228 MCL	----	—	—
15262-20-1	Radium 228 (pCi/L)	20.0		----	----	----					
7782-49-2	Selenium	0.05	MCL	----	----	----	0.02	WQC 1972 (irrigation)	----	----	----
7440-22-4	Silver	0.05	MAC (35 IAC 604)[1]	----	----	----	0.035	HTTAC	RfD = 5E-03	IRIS 1991	0.2
7440-62-2	Vanadium	0.049	(HTTAC) Calculation Error	RfD = 7E-05	----	----	0.00049	HTTAC	RfD = 7E-05	PPRTV 2009	0.2
620.410(b)											
56-55-3	Benzo(a)anthracene	0.00013	PQL	----	----	----	0.00085	HNTAC	SF_o = 1.0E-01	IRIS 2017	0.2
205-99-2	Benzo(b)fluoranthene	0.00018	PQL	----	----	----	0.00085	HNTAC	SF_o = 1.0E-01	IRIS 2017	----
207-08-9	Benzo(k)fluoranthene	0.00017	PQL	----	----	----	0.0085	HNTAC	SF_o = 1.0E-02	IRIS 2017	----
218-01-9	Chrysene	0.012	HNTAC	SF_o = 7.3E-03	IRIS 1994	----	0.085	HNTAC	SF_o = 1.0E-03	IRIS 2017	----
53-70-3	Dibenzo(a,h)anthracene	0.0003	PQL	----	----	----	0.000085	HNTAC	SF_o = 1.0E+00	IRIS 2017	----
121-42-2	2,4-Dinitrotoluene	0.0001	HNTAC	SF_o = 6.8E-01	IRIS 1990[2]	----	0.00027	HNTAC	SF_o = 3.1E-01	CalEPA 2009	----
606-20-0	2,6-Dinitrotoluene	0.00031	PQL	----	----	----	0.000057	HNTAC	SF_o = 1.5E+00	PPRTV 2013	----
123-91-1	1,4-Dioxane (p-dioxane)	0.0077	HNTAC	SF_o = 1.1E-02	IRIS 1990	----	0.00085	HNTAC	SF_o = 1.0E-01	IRIS 2013	----
319-84-6	Hexachlorocyclohexane, alpha-	0.00011	PQL	----	----	----	0.000014	HNTAC	SF_o = 6.3E+00	IRIS 1987	----
193-39-5	Indeno(1,2,3-c,d)pyrene	0.00043	PQL		----	----	0.00085	HNTAC	SF_o = 1.0E-01	IRIS 2017	----
90-12-0	1-Methylnaphthalene	----	----	----	----	----	0.49	HTTAC	MRL = 7E-02	ATSDR 2005	0.2
375-73-5	Perfluorobutane Sulfonic Acid (PFBS)	----	----	----	----	----	0.14	HTTAC	RfD = 2E-02	PPRTV 2014	0.2
355-46-4	Perfluorohexane Sulfonic Acid (PFHxS)	----	----	----	----	----	0.00014	HTTAC	MRL = 2E-05	ATSDR 2016[3]	0.2
375-95-1	Perfluorononanoic Acid (PFNA)	----	----	----	----	----	0.000021	HTTAC	MRL = 3E-06	ATSDR 2016[3]	0.2
121-82-4	RDX (Royal Demolition Explosive, Cyclonite)	0.084	HTTAC	RfD = 3E-03	IRIS 1993	0.8	0.07	HTTAC	RfD = 4E-03	IRIS 2018	0.5
620.410(c)											
1912-24-9	Atrazine	0.003	MCL	----	----	----	0.003 (Total Atrazine + Metabolites)	MCL (metabolites added per *WHO Guidelines for Drinking Water Quality*, 2010)	----	----	----
metabolite	Desethyl-atrazine (DEA)	----	----	----	----	----					
metabolite	Desisopropyl-atrazine (DIA)	----	----	----	----	----					
metabolite	Diaminochlorotriazine (DACT)	----	----	----	----	----					
335-67-1	Perfluorooctanoic Acid (PFOA)	----	----	----	----	----	0.000021	HTTAC	MRL = 3E-06	ATSDR 2016[3]	0.2
1763-23-1	Perfluorooctane Sulfonic Acid (PFOS)	----	----	----	----	----	0.000014	HTTAC	MRL = 2E-06	ATSDR 2016[3]	0.2
	Combined PFOA+PFOS	----	----	----	----	----	0.000021	----	----	----	----

[1] 35 IAC 604 was repealed in 1993, with parts being transferred to 35 IAC 611: Primary Drinking Water Standards. Silver is not listed in Part 611.

[2] IRIS 1990 oral slope factor is for a 2,4-/2,6-ditnitroloune mixture.

[3] ATSDR toxicological profile is draft, pending final review with US Office of Management and Budget (OMB).

Table 2: Groundwater Quality Standards (GQS) Proposed for Updates at 620.420: Class II General Resource Groundwater

CAS No.	Constituent	Groundwater Quality Standards (GQS) Currently in IAC 620			Updated Groundwater Quality Standards Proposed for 35 IAC 620			Chemical/Physical Properties Determining Treatability	
		Class I GQS (mg/L)	Class II GQS (mg/L)	Class II Source	Class I GQS (mg/L)	Class II GQS (mg/L)	Class II Source	Dimensionless Henry's Constant (H') at 20 ^{0}C (unitless)	Organic Carbon Partition Coefficient (K_{oc}) (cm^3/g)
620.420(a)(1)									
16984-48-8	Fluoride	4.0	4.0	Class I	2.0	2.0	Class I		
7439-93-2	Lithium	----	----	----	0.014	2.5	WQC, 1972 (irrigation)		
7439-98-7	Molybdenum	----	----	----	0.035	0.05	WQC, 1972 (irrigation)		
620.420(a)(2)									
7429-90-5	Aluminum	----	----	----	3.5	5.0	WQC, 1972 (livestock)		
7440-50-8	Copper	0.65	0.65	Class I	0.5	0.5	Class I		
13982-63-3	Radium 226 (pCi/L)	20	----	----	5 (Radium 226+228)	5 (Radium 226+228)	Class I		
15262-20-1	Radium 228 (pCi/L)	20	----	----					
7782-49-2	Selenium	0.05	0.05	Class I	0.02	0.02	Class I		
7440-22-4	Silver	0.05	----	----	0.035	0.035	Class I		
620.420(b)(1)				**Treatability Factor Applied to Class I (organics only)**			**Treatability Factor Applied to Class I (organics only)**		
15972-60-8	Alachlor	0.002	0.01	5	0.002	0.002	1	3.40E-07[1]	3.12E+02
116-06-3	Aldicarb	0.003	0.015	5	0.003	0.003	1	5.89E-08[1]	2.46E+01
56-55-3	Benzo(a)anthracene	0.00013	0.00065		0.00085	0.0043			
205-99-2	Benzo(b)fluoranthene	0.00018	0.0009		0.00085	0.0043			
207-08-9	Benzo(k)fluoranthene	0.00017	0.006		0.0085	0.043			
1563-66-2	Carbofuran	0.04	0.2	5	0.04	0.04	1	1.26E-07[1]	9.53E+01
108-90-7	Chlorobenzene	0.1	0.5	5	0.1	0.1	1	9.78E-02	2.34E+02
218-01-9	Chrysene	0.012	0.06		0.085	0.43			
94-75-7	2,4-D	0.07	0.35	5	0.07	0.07	1	1.45E-06	2.96E+01
75-99-0	Dalapon	0.2	2.0	10	0.2	0.2	1	1.68E-06	3.23E+00
53-70-3	Dibenzo(a,h)anthracene	0.0003	0.0015		0.000085	0.00043			
96-12-8	1,2-Dibromo-3-Chloropropane	0.0002	0.002	10	0.0002	0.0002	1	4.29E-03	1.16E+02
95-50-1	ortho-Dichlorobenzene	0.6	1.5	2.5	0.6	0.6	1	5.76E-02	3.83E+02
106-46-7	para-Dichlorobenzene	0.075	0.375	5	0.075	0.075	1	7.23E-02	3.75E+02
107-06-2	1,2-Dichloroethane	0.005	0.025	5	0.005	0.005	1	3.85E-02	3.96E+01
156-59-2	cis-1,2-Dichloroethylene	0.07	0.2	3	0.07	0.35	5	1.36E-01	3.96E+01
75-09-2	Dichloromethane (methylene chloride)	0.005	0.05	10	0.005	0.005	1	1.11E-01	2.17E+01
78-87-5	1,2-Dichloropropane	0.005	0.025	5	0.005	0.005	1	9.18E-02	6.07E+01
121-42-2	2,4-Dinitrotoluene	0.0001	0.0001	1	0.00027	0.0014	5	1.19E-06	5.76E+02
606-20-0	2,6-Dinitrotoluene	0.00031	0.00031	1	0.000057	0.00029	5	1.71E-05	5.87E+02
123-91-1	1,4-Dioxane (p-dioxane)	0.0077	0.0077		0.00085	0.00085			
100-41-4	Ethylbenzene	0.7	1.0	1.5	0.7	3.5	5	2.45E-01	4.46E+02
106-93-4	Ethylene Dibromide	0.00005	0.0005	10	0.00005	0.00005	1	2.06E-02	3.96E+01
319-84-6	Hexachlorocyclohexane, alpha-	0.00011	0.00055		0.000014	0.00007			
2691-41-0	HMX (High Melting Explosive, Octogen)	1.4	1.4	1	1.4	7.0	5	3.54E-08[1]	5.32E+02
193-39-5	Indeno(1,2,3-c,d)pyrene	0.00043	0.0022		0.00085	0.0043			
90-12-0	1-Methylnaphthalene	----	----	----	0.49	2.5	5	1.29E-02	2.53E+03
375-73-5	Perfluorobutane Sulfonic Acid (PFBS)	----	----	----	0.14	0.14	1	----	6.17E+01
355-46-4	Perfluorohexane Sulfonic Acid (PFHxS)	----	----	----	0.00014	0.00014	1	----	----

Table 2: Groundwater Quality Standards (GQS) Proposed for Updates at 620.420: Class II General Resource Groundwater (con't)

CAS No.	Constituent	Groundwater Quality Standards (GQS) Currently in IAC 620			Updated Groundwater Quality Standards Proposed for 35 IAC 620			Chemical/Physical Properties Determining Treatability	
		Class I GQS (mg/L)	Class II GQS (mg/L)	Treatability Factor Applied to Class I (organics only)	Class I GQS (mg/L)	Class II GQS (mg/L)	Treatability Factor Applied to Class I (organics only)	Dimensionless Henry's Constant (H') at 20 °C (unitless)	Organic Carbon Partition Coefficient (K_{oc}) (cm^3/g)
375-95-1	Perfluorononanoic Acid (PFNA)	----	----	----	0.000021	0.000021	1	----	----
1918-02-1	Picloram	0.5	5.0	10	0.5	0.5	1	2.18E-12[1]	3.88E+01
121-82-4	RDX (Royal Demolition Explosive, Cyclonite)	0.084	0.084	1	0.07	0.07			
122-34-9	Simazine	0.004	0.04	10	0.004	0.004	1	3.85E-08[1]	1.47E+02
100-42-5	Styrene	0.1	0.5	5	0.1	0.1	1	8.49E-02	4.46E+02
93-72-1	2,4,5-TP (Silvex)	0.05	0.25	5	0.05	0.05	1	3.70E-07[1]	1.75E+02
108-88-3	Toluene	1.0	2.5	2.5	1.0	5.0	5	2.13E-01	2.34E+02
79-00-5	1,1,2-Trichloroethane	0.005	0.05	10	0.005	0.005	1	2.61E-02	6.07E+01
99-35-4	1,3,5-Trinitrobenzene	0.84	0.84	1	0.84	4.2	5	1.53E-07	1.68E+03
118-96-7	2,4,6-Trinitrotoluene (TNT)	0.014	0.014	1	0.014	0.07	5	5.10E-07	2.81E+03
620.420(c)									
71-43-2	Benzene	0.005	0.025	5	0.005	0.025			
	Total BETX	11.705	13.525	----	11.705	18.525[2]	----		
1912-24-9	Atrazine	0.003	0.015	5	0.003 (Total Atrazine + Metabolites)	0.003 (Total Atrazine + Metabolites)	1	9.65E-08[1]	2.25E+02
metabolite	Desethyl-atrazine (DEA)	----	----	----				----	----
metabolite	Desisopropyl-atrazine (DIA)	----	----	----				----	----
metabolite	Diaminochlorotriazine (DACT)	----	----	----				----	----
335-67-1	Perfluorooctanoic Acid (PFOA)	----	----	----	0.000021	0.000021	1	1.64E-04[1]	1.15E+02
1763-23-1	Perfluorooctane Sulfonic Acid (PFOS)	----	----	----	0.000014	0.000014	1	----	3.72E+02
	Combined PFOA + PFOS	----	----	----	0.000021	0.000021	----	----	----

[1] The value is the dimensionless Henry's Constant at 25 °C. The enthalpy of vaporization is not known; therefore, an adjustment to the Henry's Constant due temperature changes cannot be calculated.

[2] The proposed update to the Class II total BETX standard is due to proposed updates to ethylbenzene and toluene standards.

Table 3: Sources for Carcinogenicity Updates

CAS No.	Constituent	Carcinogen Classification	Source	Year Classified
106-46-7	para-Dichlorobenzene	2B	IARC	1999
100-41-4	Ethylbenzene	2B	IARC	2000
58-89-9	Hexachlorocyclohexane, gamma-(Lindane)	1	IARC	2018
335-67-1	Perfluorooctanoic Acid (PFOA)	2B	IARC	2017

The Illinois Environmental Protection Act (415 ILCS) defines a carcinogen as:

> "A contaminant that is classified as a category A1 or A2 carcinogen by the American Conference of Governmental Industrial Hygienists; a category 1 or 2A/2B carcinogen by the World Health Organization's International Agency for Research on Cancer; a "human carcinogen" or "anticipated human carcinogen" by the United States Department of Health and Human Service National Toxicological Program; or a category A or B1/B2 carcinogen or as "carcinogenic to humans" or "likely to be carcinogenic to humans" by the United States Environmental Protection Agency in the integrated risk information system or a final rule issued in a Federal Register notice by the USEPA." [415 ILCS 5/58.2]

ATTACHMENT 4

Illinois Environmental Protection Agency Proposed Updates to 35 Ill. Adm. Code 620

By: Carol Hawbaker
Environmental Risk Assessor
Illinois Environmental Protection Agency
Office of Toxicity Assessment

May 26, 2021

Proposed Updates to 35 Ill. Adm. Code 620

The presentation will cover the following topics:

- Introduction of nine new constituents.
- Addition of three metabolites to be evaluated with atrazine for compliance with groundwater quality standards (GQS).
- Combination of radium 226 and radium 228 to form a new constituent: radium (combined 226+228).
- Addition of carcinogen designations for four existing constituents.
- Updates to constituents in the tables at Section 620.310(a)(3)(A)(i) and (ii).

Proposed Updates to 35 Ill. Adm. Code 620

The presentation will cover the following topics (continued):

- Updates of Class I GQS for three inorganic constituents from MCLs to irrigation/livestock water quality standards, based on beneficial use of groundwater.
- For constituents which Class I GQS are based on procedures found in Section 620, Subpart F and Appendix A:
  - Updates to toxicity values and relative source contribution (RSC) values;
  - Updates to exposure factors;
  - Addition of a mutagenic method for the development of a carcinogen Class I GQS for constituents with a mutagenic mode of action.

Proposed Updates to 35 Ill. Adm. Code 620

The presentation will cover the following topics (continued):

- Updates to Class II GQS.
- Introduction of tables (Appendix E) listing constituents that have similar-acting health effects or affect the same target organ.

Introduction of New Constituents

- Aluminum
- Lithium
- 1-Methylnaphthalene
- Molybdenum

- Five Per-and Polyfluoroalkyl Substances (PFAS):
  - PFBS (Perfluorobutanesulfonic Acid)
  - PFHxS (Perfluorohexanesulfonic Acid)
  - PFNA (Perfluorononanoic Acid)
  - PFOA (Perfluorooctanoic Acid)
  - PFOS (Perfluorooctanesulfonic Acid)

Introduction of New Constituents

Proposed Class I and Class II GQS:

CASRN	Constituent	Proposed Class I GQS (mg/L)	Class I Source	Proposed Class II GQS (mg/L)	Class II Source
7429-90-5	Aluminum	1.9	Subpart F	5	Livestock
7439-93-2	Lithium	0.01	Subpart F	2.5	Irrigation
90-12-0	1-Methylnaphthalene	0.27	Subpart F	0.27	Subpart F
7439-98-7	Molybdenum	0.019	Subpart F	0.05	Irrigation

Per and Poly-Fluoroalkyl Substances (PFAS)

PFAS are a group of human-made constituents applied to many consumers products to make them waterproof, stain resistant or non-stick.

- Food packaging - fast food containers, lunch meat paper, disposable plates and bowls, and oil-, water- and grease-resistant coatings on food packaging (pizza boxes);
- Commercial household products - non-stick coated cookware (Teflon), cleaning products, waxes, polishes, and adhesives;
- Clothing and fabric textiles - stain- and water-resistant carpeting and upholstery, water repellant clothing, tents, umbrellas, shoes, and leather goods;
- Cosmetics and personal care products - shampoos, conditioners, sunscreens, cosmetics, and dental floss;
- Building and exterior use products - paints and sealants;
- Industrial use - metal plating and finishing, wire coatings, automotive fluids, and the manufacture of artificial turf;
- Firefighting foam - aqueous film-forming foam (AFFF).

Per and Poly-Fluoroalkyl Substances (PFAS)

PFAS are constituents of emerging concern:

- "Forever Chemicals": PFAS not degrade naturally in the environment.
- PFAS constituents have an affinity for water and can migrate long distances.
- PFAS can bioaccumulate in plants, fish and wildlife, and humans.
- PFAS are a group of chemicals consisting of over 5,000 substances.
- Toxicological studies and assessments are being conducted by several agencies.
- Limited toxicological data for most PFAS.

Per and Poly-Fluoroalkyl Substances (PFAS)

Epidemiology and Animal Studies Suggest Associations Between PFAS Exposure and Several Health Effects:

- Pregnancy-Induced Hypertension/Pre-Eclampsia
- Liver Damage
- High Cholesterol
- Thyroid Disease
- Decreased Response to Vaccines
- Decreased Fertility
- Decreased Birth Weight
- Developmental Delays

Per and Poly-Fluoroalkyl Substances (PFAS)

- PFOA meets Illinois EPA's definition of a carcinogen. The International Agency for Research on Cancer (IARC) classified PFOA as a "2B" carcinogen in 2017.
- A "2B" classification means the constituent is possibly carcinogenic to humans.
- U.S. EPA concluded there was "suggestive potential" for PFOS to be carcinogenic to humans; however, PFOS does not meet Illinois EPA's definition of a carcinogen at this time.
- Possible Cancer Links:
  - Kidney
  - Testicular
  - Prostate
  - Liver
  - Pancreas

Per and Poly-Fluoroalkyl Substances (PFAS)

Proposed Class I GQS are based on proposed procedures for 35 Ill. Adm. Code 620, Subpart F and Appendix A.

CASRN	Constituent	Class I and Class II GQS (mg/L or ppm)	Class I and Class II GQS (ng/L or ppt)	Toxicity Value	Toxicity Value Source	Relative Source Contribution Value for Noncarcinogens
375-73-5	PFBS	0.0012	1,200	3E-04	PPRTV	0.2
355-46-4	PFHxS	0.000077	77	2E-05	ATSDR	0.2
375-95-1	PFNA	0.000012	12	3E-06	ATSDR	0.2
335-67-1	PFOA	0.000002	2	1.4E+02	OEHHA	Not Applicable
1763-23-1	PFOS	0.0000077	7.7	2E-06	ATSDR	0.2

PPRTV: Provisional Peer Reviewed Toxicity Values.

ATSDR: Agency for Toxic Substance and Disease Registry.

OEHHA: California EPA Office of Environmental Health Hazard Assessments.

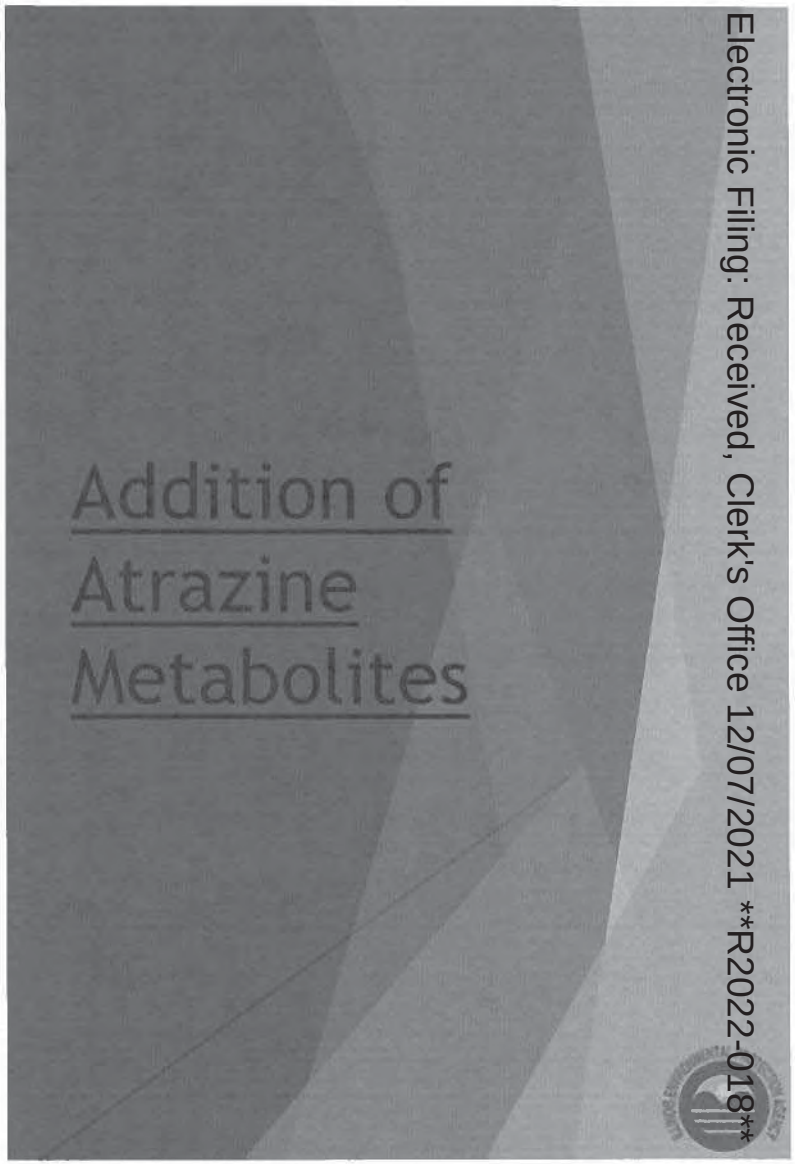
PFBS, PFHxS, PFNA and PFOS toxicity values are oral reference doses (RfDs) for noncarcinogen effects in units of mg/kg-day.

PFOA toxicity value is an oral slope factor (SF_o) for cancer risks in units of $(mg/kg\text{-}day)^{-1}$. The GQS is the minimum reporting level, per Subpart F.

The amendments propose the addition of 3 atrazine metabolites to be included when comparing atrazine concentrations to GQS.

Added Metabolites

- DEA (Desethyl-atrazine)
- DIA (Desisopropyl-atrazine)
- DACT (Diaminochlorotriazine)



Combination of Radium 226 and 228

Presently, radium 226 and radium 228 have individual Class I GQS. They are not listed in the Class II GQS.

The amendments propose radium (combined 226+228) Class I and Class II GQS.

The proposed value for the Class I and Class II GQS is based on the Federal maximum contaminant level (MCL) for radium (combined 226+228) of 5 picocuries per liter (pCi/L).

Proposed Updates to Carcinogen Designations

Carcinogen designations are updated for the following constituents:

- *p*-Dichlorobenzene (1,4-dichlorobenzene)
  - Classified "2B" by International Agency for Research on Cancer (IARC) - 1999
- Ethylbenzene
  - Classified "2B" by IARC - 2000
- *gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)
  - Classified "1" by IARC - 2018
- Isopropylbenzene (cumene)
  - Classified "2B" by IARC - 2013

Proposed Updates to Constituents in Tables at 35 Ill. Adm. Code 620.310(a)(3)(A)(i) and (ii) – Preventive Response Activities

The following constituents are removed from the tables due to carcinogenicity classifications, based on the Board Note at Section 620.310(a)(3)(A).

- *p*-Dichlorobenzene (1,4-dichlorobenzene)
- Ethylbenzene
- Arsenic
- *gamma*-HCH (lindane)
- Isopropylbenzene (cumene)

MCPP (mecoprop) is removed as the constituent's proposed Class I GQS is based on its lowest level of quantitation (LLOQ) or lowest concentration minimum reporting level (LCMRL), formerly termed practical quantitation limit (PQL).

Proposed Updates to Constituents in Tables at 35 Ill. Adm. Code 620.310(a)(3)(A)(i) and (ii) – Preventive Response Activities

Constituents Added to Tables

- Aluminum
- Molybdenum
- 1-Methylnaphthalene
- PFBS
- PFHxS
- PFNA
- PFOS
- Antimony
- HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
- Nitrobenzene
- RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine
- TNT (2,4,6-trinitrotoluene)
- 1,3,5-Trinitrobenzene

Proposed Updates of Class I GQS for Three Inorganic Constituents Based on More Stringent Irrigation or Livestock Values

Class I potable resource groundwater may also be used for irrigation and watering of livestock. The following constituents are proposed to be updated as follows:

CASRN	Constituent	Current Class I GQS (mg/L)	Current Source	Proposed Class I GQS (mg/L)	Proposed Source
7440-50-8	Copper	0.65	Lead/Copper Rule	0.5	Livestock
7681-49-4	Fluoride	4	U.S. EPA MCL	2	Livestock
7782-49-2	Selenium	0.05	U.S. EPA MCL	0.02	Irrigation

Proposed Updates to Subpart F and Appendix A

Out of 115 total constituents presently listed at 35 Ill. Adm. Code 620.410, 40 utilize the procedures in Subpart F and Appendix A to develop Class I GQS (35% of constituents):

- 30 constituents utilize the Human Threshold Toxicant Advisory Concentration (HTTAC) calculation at Appendix A(a) for noncarcinogens.

- 10 constituents utilize the Human Nonthreshold Toxicant Advisory Concentration (HNTAC) calculation at 35 Ill. Adm. Code 620.605(b)(2), for carcinogens. Of these 10, 7 constituents utilize a practical quantitation limit (PQL), because the calculated HNTAC is less than the PQL.

Proposed Updates to Appendix A

Illinois EPA's Hierarchy for Determining Toxicity Values

Basis for hierarchy is derived from U.S. EPA OSWER Directive 9285.7-53, dated December 5, 2003, and discussed in the Illinois Pollution Control Board Rulemaking R08-18: Proposed Amendments to Groundwater Quality Standards, 35 Ill. Adm. Code 620.

- Tier 1 Toxicity Value Source: Integrated Risk Information System (IRIS)
- Tier 2 Toxicity Value Source: Provisional Peer Reviewed Toxicity Values (PPRTV)
- Tier 3 Other Toxicity Values:

  "Priority given to sources of information that are the most current, the basis for which is transparent and publicly available, and which has been peer-reviewed."

  OSWER Directive 9285.7-53

Proposed Updates to Appendix A

Additional Guidance Regarding the Selection of Tier 3 Toxicity Values derived from U.S. EPA OSWER Directive 9285.7-86, dated May 16, 2013. Tier 3 sources are ranked as follows:

1. Agency for Toxic Substances and Disease Registry (ATSDR) minimal risk levels.
2. California EPA, Office of Environmental Health Hazard Assessment (OEHHA), toxicity values.
3. PPRTV Appendix "Screening Toxicity Values."
4. Health Effect Assessment Summary Table (HEAST) toxicity values.

Proposed Updates to Appendix A

Updates Procedures for Determining an Oral Reference Dose (RfD) When an RfD is Not Available from the Listed Toxicity Values Sources.

- Due to outdated methodology, proposes to update the procedures found at 35 Ill. Adm. Code 620, Appendix A(b)(3)-(c) for when there is no "verified" RfD, The proposed updated method is based on the methodology used by IRIS, U.S. EPA's Tier 1 toxicity source.

- Only 1 constituent (MTBE) utilized the methodology at Appendix A(b)(3)-(c) for developing an RfD.

Proposed Updates to Appendix A

Updates to Exposure Factors in the HTTAC calculation

(updates are applied for a more sensitive receptor population - children)

Current Exposure Factors	Proposed Exposure Factors
Body Weight (BW) = 70 kg (equivalent for an average adult)	Body Weight (BW) = 15 kg (equivalent for a child 0 - 6 years) of age
Daily Water Ingestion Rate (W) = 2 L/day (equivalent for an average adult)	Daily Water Ingestion Rate (W) = 0.78 L/day (equivalent for a child 0 - 6 years of age)

Proposed Updates to Appendix A

Updates to HNTAC Calculation

(moved from 35 Ill. Adm. Code 620.605(b) to Appendix A)

HNTAC calculation for carcinogens is based on methodology found in U.S. EPA's Risk Assessment Guidance for Superfund (RAGs), Part B.

Supplemental Guidance from U.S. EPA updates the carcinogen calculation to account for age-adjusted daily water ingestion rates, as opposed to adult only water ingestion rates currently used in the calculation.

Supplemental Guidance also applies adjustment factors to the age-adjusted daily water ingestion rates for to account for toxicokinetic differences between children of various age groups and adults for carcinogens with a mutagenic mode of action for carcinogenesis.

Updated equations used to calculate U.S. EPA Regional Screening Levels (RSLs) for ingestion of tapwater.

Proposed Updates to Appendix A

Updates to HNTAC Calculation

Illinois EPA proposes to update the HNTAC calculation by incorporating updated guidance to adjust for childhood exposures to carcinogens. This includes:

- Updating the HNTAC carcinogen calculation, including updating exposure factors.

- Adding a HNTAC mutagen calculation for carcinogen constituents which operate by a mutagenic mode of action for carcinogenesis. 11 constituents are classified as mutagens; 6 rely on the HNTAC calculation to determine Class I GQS.

Proposed Updates to Appendix A

Current HNTAC Calculation

$$HNTAC(mg/L) = \frac{TR \bullet BW \bullet AT \bullet 365 \frac{days}{year}}{SF_o \bullet IR \bullet EF \bullet ED}$$

Where:

Symbol (units)	Parameter	Existing Value
TR (unitless)	Target Cancer Risk – 1 in 1 Million Risk	1.0E-06
BW (kg)	Body Weight	70
AT (years)	Averaging Time for Carcinogens	70
SF_o ((mg/kg-day)$^{-1}$)	Oral Slope Factor – Toxicological Value	Chemical-Specific
IR (L/day)	Daily Water Ingestion Rate	2
EF (days/year)	Exposure Frequency	350
ED (year)	Exposure Duration	30

Proposed Updates to Appendix A

Proposed Updated HNTAC Calculation

$$HNTAC\ (mg/L) = \frac{TR \bullet \left(AT \bullet 365 \frac{days}{year}\right)}{SF_o \bullet IFW_{adj}}$$

Where:

Symbol (units)	Parameter	Proposed Value
TR (unitless)	Target Cancer Risk - 1 in 1 million	1.0E-06
AT (years)	Averaging Time for Carcinogens	70
SF_o ((mg/kg-day)$^{-1}$)	Oral Slope Factor - Toxicological Value	Chemical-Specific
IFW_{adj} (L/kg)	Age-Adjusted Daily Water Ingestion Rate	327.95

Proposed Updates to Appendix A

IFW_{adj} Calculation

$$IFW_{adj}(327.95\ L/kg) = \left[\left(\frac{EF_{child} \bullet ED_{child} \bullet IRW_{child}}{BW_{child}}\right) + \left(\frac{EF_{adult} \bullet ED_{adult} \bullet IRW_{adult}}{BW_{adult}}\right)\right]$$

Where:

Symbol (units)	Parameter	Value
EF all (days/year)	Exposure Frequency	350
ED_{child} (years)	Exposure Duration - child (0 - 6 years)	6
IRW_{child} (L/day)	Daily Water Ingestion Rate - child (0 - 6 years)	0.78
BW_{child} (kg)	Body Weight - child (0 - 6 years)	15
ED_{adult} (year)	Exposure Duration - adult	20
IRW_{adult} (L/day)	Daily Water Ingestion Rate - adult	2.5
BW_{adult} (kg)	Body Weight - adult	80

Proposed Updates to Appendix A

Proposed Introduction of an HNTAC Calculation for Mutagens

$$HNTAC_{MUT}\ (mg/L) = \frac{TR \bullet \left(AT \bullet 365\,\frac{days}{year}\right)}{SF_o \bullet IFWM_{adj}}$$

Where:

Symbol (units)	Parameter	Value
TR (unitless)	Target Cancer Risk – 1 in 1 million	1.0E-06
AT (years)	Averaging Time for Carcinogens	70
SF_o ($(mg/kg\text{-}day)^{-1}$)	Oral Slope Factor – Toxicological Value	Chemical-Specific
$IFWM_{adj}$ (L/kg)	Age-Adjusted Daily Water Ingestion Rate for Mutagens	1,019.9

Proposed Updates to Appendix A

$IFWM_{adj}$ Calculation

$$IFWM_{adj}\,(1019.9\,L/kg) = \left[\left(\frac{EF_{0-2} \bullet ED_{0-2} \bullet IRW_{0-2} \bullet 10}{BW_{0-2}}\right) + \left(\frac{EF_{2-6} \bullet ED_{2-6} \bullet IRW_{2-6} \bullet 3}{BW_{2-6}}\right) + \left(\frac{EF_{6-16} \bullet ED_{6-16} \bullet IRW_{6-16} \bullet 3}{BW_{6-16}}\right) + \left(\frac{EF_{16-26} \bullet ED_{16-26} \bullet IRW_{16-26} \bullet 1}{BW_{16-26}}\right)\right]$$

Adjustment Factors of 10, 3 and 1 are used to account for different risks from exposure during different life stages.

Proposed Updates to Appendix A

$IFWM_{adj}$ Calculation

$IFWM_{adj}$ Parameter Values:

Symbol	Parameter	Proposed Value
EF - all (days/year)	Exposure Frequency	350
ED_{0-2} (years)	Exposure Duration: 0-2 years of age	2
ED_{2-6} (years)	Exposure Duration: 2-6 years of age	4
ED_{6-16}, ED_{16-26} (years)	Exposure Duration: 6-16 and 16-26 years of age	10
IRW_{0-2}, IRW_{2-6} (L/day)	Daily Water Ingestion Rate: 0-2 and 2-6 years of age	0.78
IRW_{6-16}, IRW_{16-26} (L/day)	Daily Water Ingestion Rate: 6-16 and 16-26 years of age	2.5
BW_{0-2}, BW_{2-6} (kg)	Body Weight: 0-2 and 2-6 years of age	15
BW_{6-16}, BW_{16-26} (kg)	Body Weight: 6-16 and 16-26 years of age	80

Updates to Class II: General Resource Groundwater Quality Standards (Section 620.420)

In addition to the new constituents, updated Class II GQS are proposed for 74 constituents or mixtures currently listed in Section 620.420. Proposed updated standards are based on the following factors:

- Updated Class I Groundwater Quality Standards
- Irrigation or Livestock Criteria
- Updated Treatment Factors

Updated Treatment Factors

Treatment Factors are applied based on the effectiveness to treat the constituent in the groundwater at an 80% removal efficiency rate:

- For removal via air stripping, an 80% removal efficiency rate is assumed for constituents having a Dimensionless Henry's Law Constant (H') value greater than methylene chloride's (H') value of 0.111 at a 20°C groundwater system temperature.

OR

- For removal via carbon adsorption, an 80% removal efficiency rate is assumed for constituents having an Organic Carbon Partition Coefficient (K_{oc}) value greater than ethylbenzene's (K_{oc}) value of 446 L/kg.

If a constituent's chemical/physical values meet either of the criteria, a Treatment Factor of 5 is applied to the Class I Groundwater Quality Standard to calculate a Class II Groundwater Quality Standard.

- Source of Chemical/Physical Values: U.S. EPA Regional Screening Levels
- Source of Treatment Factor Criteria: Illinois Pollution Control Board R08-18

Proposed Addition of Tables at Appendix E for Similar-Acting Chemicals

- 35 Ill. Adm. Code 620, Appendix B and Appendix C provide procedures for mixtures of similar-acting substances within the groundwater.
  - Table A lists similar-acting constituents based on noncarcinogenic health effects or target organs.
  - Table B lists similar-acting constituents based on cancer effects.

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35 Ill. Adm. Code 620; Groundwater Quality
Pre-Filing Public Comment Period
Factsheet and Overview of Proposed Changes

Draft Proposed Rules

The Illinois EPA is proposing draft language to update 35 Ill. Adm. Code 620. The proposed updates include nine new chemicals, three new atrazine metabolites, and procedures for selecting toxicity values consistent with current federal guidance. Definitions are updated and references are consistent with those criteria and practices as incorporated. Site specific groundwater standards for designated Class III Special Resource Groundwater are also added. Exposure factors are updated, and the Human Non-Threshold Toxicant Advisory Concentration model is updated. Tables for similar-acting constituents are added. Finally, this proposal includes groundwater quality standards for five Per- and Polyfluoroalkyl Substances (PFAS): perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorononanoic acid (PFNA), perfluorohexanesulfonic acid (PFHxS), and perfluorobutanesulfonic acid (PFBS).

A summary of the key provisions is below. More information concerning the draft proposed rule may be found at
https://www2.illinois.gov/epa/about-us/rules-regs/water/Pages/620-Groundwater-Quality.aspx

Public Comment

Prior to submitting proposed rules to the Illinois Pollution Control Board for review and final adoption, the Illinois EPA is soliciting public comment on draft proposed rules. The Illinois EPA will accept written public comment until **June 25, 2021**. Comments should be submitted to
EPA.620.rulemaking@illinois.gov

All comments, including proposed alternative language, received by Illinois EPA will be considered prior to the Agency filing the proposed rule with Illinois Pollution Control Board. Questions about the process or rulemaking should be submitted to the e-mail address above.

Public Meeting

The Illinois EPA will host a virtual public meeting to review the proposed changes and answer questions concerning the proposal. **The meeting will be held at 1:00 pm on May 26, 2021.**
The meeting link is:
https://illinois.webex.com/illinois/j.php?MTID=m19e9dc943bb9f835453fc6b6e8823826
Computer and telephone connection instructions are provided at the bottom of this Notice. If you have questions about connecting to the meeting, contact Jeff Guy at (217) 785-8724 or by submitting an e-mail to **EPA.620.rulemaking@illinois.gov.**

Key Provisions

1. Updates the methodology located in Appendix A for developing oral reference doses (RfDs), when a verified RfD is not available. The updated methodology is the method used by U.S. EPA Integrated Risk Information System (IRIS), the Tier 1 source for selecting toxicity criteria.
2. Provides the hierarchy for selecting a verified RfD from various sources. The hierarchy is in Appendix A.
3. Updates the Exposure Factors used in the Human Threshold Toxicant Advisory Concentration (HTTAC) equation and the Human Non-Threshold Toxicant Advisory Concentration (HNTAC) equations for both carcinogens and mutagens to be consistent with the U.S. EPA Exposure Factors Handbook (2011) and U.S. EPA Regional Screening Level calculator. Updates the exposure population from an average adult to a child ages 0-6 years for the HTTAC equation.
4. Updates Class I groundwater quality standards in tables at Part 620.410, based on updates to toxicity values, exposure factors and other methodologies.
5. Updates Class II groundwater quality standards in tables at Part 620.420, based on updates to Class I groundwater quality standards and updates to treatment factors, based on updates to dimensionless Henry's Law Constants when calculated at 20 °C and organic carbon partition coefficients.
6. Establishes groundwater quality standards for nine new chemicals, adds three metabolites as a mixture to atrazine, and moves atrazine and its metabolites tables to Part 620.410(c)(2) and Part 620.420(c)(2) for complex mixtures. Combines Radium 226 and 228 to form CASRN 7440-14-4: Radium (combined 226+228), updates the Class I groundwater quality standard for radium (combined 226+228) to an updated standard of 5 pCi/L, equal to the U.S. EPA Drinking Water MCL, and adds a Class II groundwater quality standard for radium (combined 226+228) at Part 620.420(a)(2). Establishes a Class II groundwater quality standard for silver and adds it to the table at Part 620.420(a)(2).
7. Updates constituent tables to include Chemical Abstract Services Registry Numbers (CASRNs) as additional identifiers for the constituents.
8. Adds footnotes to tables identifying the sources or methods for determining the groundwater quality standards.
9. Removes the explosive constituents at Parts 620.410(c) and 620.420(c); integrates the constituents into Parts 620.410(b) and 620.420(b).
10. Adds Appendix E, providing tables for similar-acting non-carcinogenic constituents by health effect (Table A) and similar-acting carcinogen constituents by cancer effect (Table B).
11. Updates the names of eleven constituents.
12. Adds carcinogen designations for four existing chemicals and one new chemical.
13. Adds mutagen designations for eleven chemicals.
14. Updates toxicity values for the constituents whose groundwater quality standards are based on the Human Threshold Toxicant Advisory Concentration (HTTAC) equation for noncarcinogens or the Human Nonthreshold Toxicant Advisory Concentration (HNTAC) equation for carcinogens.

A detailed list of Key Provisions can be found at
https://www2.illinois.gov/epa/about-us/rules-regs/water/Pages/620-Groundwater-Quality.aspx

Proposed Changes to 620 Sub Part A-C

Sub Part	Section	Proposed Changes
Part A	**620.110**	Adds definition of "Chemical Abstract Service Registry Numbers (CASRN)", "Lowest Concentration Minimum Reporting Level", and "Mutagen". Updates definition of "Carcinogen" to be consistent with updates to terminology used by U.S. EPA Integrated Risk Information System, and definition of "Detection" to language currently used in test methods. Removes the definition of "Practical Quantitation Level".
	620.125	Updates CFR references to most recent iteration of the code. Adds Illinois EPA "Integrated Water Quality Report and Section 303(d) List" and National Academy of Science "Water Quality Criteria" (1973) to incorporated references and updates several test methods. Adds references from the U.S. EPA Office of Research and Development, National Center for Environmental Assessment, and reference from U.S. EPA Office of Resource Conservation and Recovery. Updated for groundwater guidance from USEPA 2017.
Part B	**620.210**	Removes permeameter as an acceptable means to determine hydraulic conductivity. Adds the wellhead protection area of a community water supply well or well field as a specific area to which Class I groundwater quality standards are applicable.
	620.250	Lists a standard set of documentation that must be included with all groundwater management zone applications.
Part C	**620.302**	Adds to the list of examples of persons who do groundwater monitoring.
	620.310	Updates table at Part 620.310(a)(3)(A)(i) to include CASRN for each constituent; and removes para-dichlorobenzene and ethylbenzene from the table due to their updated carcinogen classification and the Board Note for 620.310(a)(3)(A). Adds a table at Part 620.310(a)(3)(A)(ii) depicting the constituents in the subsection; and removes *gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane) and isopropylbenzene (cumene) due to their updated carcinogen classification and the Board Note for 620.310(a)(3)(A). Amends Board Note for 620.310(a)(3)(A) to revised outdated language.

Proposed Changes to 620 Sub Part D-F

Sub Part	Section	Proposed Changes
Part D	**620.410**	Adds Class I groundwater quality standards for nine new chemicals. Updates constituent tables to add CASRN for each constituent. Adds footnotes detailing the sources of the standards. Updates Class I groundwater quality standards as applicable. Removes explosive constituents table at 620.410(c) and integrates the constituents into table at 620.410(b). Moves atrazine from 620.410(b) to the complex chemical mixtures tables at 620.410(c) with the addition of atrazine metabolites.
	620.420	Adds Class II groundwater quality standards for nine new chemicals and two chemicals listed in 620.410 without prior Class II groundwater quality standards. Updates constituent tables to add a CASRN for each constituent, and update Class II groundwater quality standards as applicable. Adds footnotes detailing the sources of the standards. Removes explosive constituents table at 620.420(c) and integrates the constituents into table at 620.420(b). Moves atrazine from 620.420(b) to the complex chemical mixtures tables at 620.420(c) with the addition of atrazine metabolites.
	620.430	Establishes site specific Class III groundwater quality standards for chloride and pH at four dedicated nature preserves, which are caves, pursuant to 620.230(b). Establishes site specific Class III groundwater quality standards for chloride at two dedicated nature preserves, which are wetlands, pursuant to 620.230(b).
	620.440	Updates names of explosive constituents.
	620.450	Updates names of explosive constituents.
Part E	**620.510**	Requires that the 2009 Unified Guidance be used to determine background groundwater quality unless other methods are specified by regulation. Replaces the use of the PQL with the LLOQ, LCMRL or MDL, as appropriate to the nature of the chemical.
Part F	**620.601**	(b)-Updates code reference to 604.200.
	620.605	(b)(1) Designates the more stringent toxicity value of the (Human Threshold Toxicant Advisory Concentration (HTTAC) or Human Nonthreshold Toxicant Advisory Concentration (HNTAC) as the guidance value in the absence of a Maximum Contaminant Level (MCL) or Maximum Contaminant Level Goal (MCLG).
		(b)(2) Removes the Human Nonthreshold Toxicant Advisory Concentration (HNTAC) language and equation and relocates it to Appendix A.

Proposed Changes to 620 Appendices

Appendix	Section	Proposed Changes
A	(a)	Updates exposure factors representative of a child for the HTTAC model, which is consistent with Illinois Administrative Code Part 742 and U.S. EPA Regional Screening Levels (per capita daily water consumption = 0.78 liters per day, assumed average weight of a child 0-6 years of age = 15 kg).
	(b)(2)	Incorporates U.S. EPA's hierarchy of toxicity sources from "*Tier 3 Toxicity Value White Paper*", dated May 16, 2013, by U.S. EPA Office of Solid Waste and Emergency Response Human Health Regional Risk Assessors Forum (OSWER) for determining an appropriate verified oral reference dose.
	(b)(3)	Revises methodology used to calculate guidance values when a verified oral reference dose is not available to make language consistent with U.S. EPA Reference Dose Guidance.
	(b)(4)	Clarifies usage of uncertainty factors.
	(c)(1)	Adds equation for calculating HNTAC guidance level for chemicals designated as mutagens.
	(c)(2)	Updates equation for calculating HNTAC guidance levels for chemicals designated as carcinogens that are not designated as mutagens.
B	(c)	Removes language specific to mixtures of ortho-dichlorobenzene and para-dichlorobenzene, and 1,1-dichloroethane and 1,1,1-trichloroethane, and adds reference to Appendix E.
E		Provides tables of similar acting non-carcinogenic and carcinogenic constituents.

ATTACHMENT 5

GAC Recommendations to Proposed 35 Ill. Adm. Code 620

The Illinois Groundwater Advisory Council (GAC) held meetings on June 16, 2021, July 19, 2021, and August 25, 2021 in part to discuss the Illinois Environmental Protection Agency's (Illinois EPA) current draft Part 620 rulemaking proposal and public comments regarding the same. The Illinois EPA has expressed an urgency to file these proposed rules with the Illinois Pollution Control Board (IPCB), but has provided no specific reason for the urgency other than it being over 12 years since Part 620's last update

After the three GAC meetings referenced above and reviewing the public comments submitted before the May 25th deadline, the GAC believes the Illinois EPA has not yet provided the following necessary information:

1. A description of the basis for the Illinois EPA's reluctance to work with all impacted parties during the drafting of these rules, which could have resulted in discussions answering many of the questions raised during the comment period that ended May 25, 2021. Per the 12/17/20 GAC remote meeting call, the Illinois EPA Agency had already started working on the draft proposal.
2. A description of the basis for the Illinois EPA's urgency to file these proposed rules with the IPCB without prior response to all comments submitted during the comment period that ended May 25, 2021.
3. A description of the basis for not following the federal or surrounding state approaches, methodologies, and standards.
4. A description of the basis for justifying Illinois standards to be more stringent than federal and/or surrounding state approaches, as well as why the Illinois EPA is seeking to revise these standards before the US EPA has updated their federal standards.
5. A description of how testing will be performed in state laboratories at the levels recommended in the proposal, including calculation assumptions and technical research references.
6. A description of the methods regulated entities should use to analyze for per/polyfluoroalkyl (PFA's) substances and other materials in wastewater, biosolids, and other products.
7. A description of state and/or federal resources available for regulated entities to treat to the proposed standards.

Based upon Illinois EPA statements at the August 25th meeting that they would not provide responses to raised nor previously submitted questions prior to proposing the amendments to the IPCB, the GAC will not have the benefit of knowing the Illinois EPA's responses and/or how it might revise the proposal. As a result, the GAC cannot offer a recommendation that the Illinois EPA move forward in this filing. Should the Illinois EPA continue to move forward with the proposal, it is the GAC's position that the Illinois EPA sufficiently address these questions in

the Proposed Rulemaking and/or Statement of Reasons to provide the most robust and transparent proposal to the Illinois Pollution Control Board for a more effective and workable standard.

September 17, 2021

Bob Elvert – Chair

John Liberg

Lauren Lurkins

Monica Rios

Dawn Walker

Pius Weibel

ATTACHMENT 6

ILLINOIS ENVIRONMENTAL PROTECTION AGENCY
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JB PRITZKER, GOVERNOR JOHN J. KIM, DIRECTOR

Memorandum

Date: September 29, 2021

To: Robert Elvert, Chair
Groundwater Advisory Council

From: Michael Summers, P.G.,
Chairman, Intergovernmental Coordinating Committee on Groundwater

Subject: Intergovernmental Coordinating Committee on Groundwater's Response to the Groundwater Advisory Council's Recommendation

---

PURPOSE: The purpose of this Memorandum is to meet the statutory requirements in the Illinois Groundwater Protection Act and to provide a written response to the Groundwater Advisory Council's (GAC or Council) Recommendation. *See 415 ILCS 55/4*

BACKGROUND: The Intergovernmental coordinating Committee on Groundwater (ICCG or Committee) convened a remote meeting (via WebEx) on September 22, 2021 at 1:00pm, with the following members present:

Michael Summers, Chairman - Illinois Environmental Protection Agency (Illinois EPA)
James Bentley, Office of State Fire Marshall
Vickie Broomhead, Department of Natural Resources, Office of Mines and Minerals
Wes Cattoor, Department of Natural Resources, Office of Water Resources
Charles Jones, Department of Public Health
Doug Liniger, Department of Transportation
Randy Locke, Illinois State Geological Survey
Adnan, Khayyat, Office of Emergency Management

Also present at the meeting:
Kari Beckum, Illinois EPA
Gerrin Cheekbutler, Department of Public Health
Alex Davis, Illinois Environmental Regulatory Group
Sara Terranova, Illinois EPA

Representatives from the Department of Agriculture, Department of Nuclear Safety, and Department of Commerce and Economic Opportunity did not attend the meeting.

The purpose of the ICCG meeting was to discuss the proposed changes to the 35 Ill. Adm. Code 620 Groundwater Quality standards, and to discuss the GAC Recommendation concerning these proposed changes. By Statue *"[t]he Committee shall consider findings and recommendations that are provided by the Council and respond in writing regarding such matters. The Chairman of the Committee shall designate a liaison person to serve as a facilitator of communications with the Council."* See 415 ILCS 55/4. The Chairman has designated himself as the liaison with the GAC.

The GAC Recommendation is the result of three GAC remote meetings (June 16, 2021, July 19, 2021, and August 25, 2021), in which GAC members and interested public parties participated. The GAC Recommendations, dated September 19, 2021, was sent directly to ICCG Chairman, Illinois EPA representatives, and GAC members. The ICCG Chairman and Illinois EPA representatives received the GAC Recommendation via email on September 19, 2021 and by hard by copy on September 22, 2021,

The ICCG Chair forwarded a copy of the GAC Recommendations to all Committee members prior to the September 22, 2021 Committee meeting. A copy of the GAC Recommendations is included as an attachment to this Memorandum.

RESPONSE: Discussion of the GAC Recommendation during the Committee meeting resulted in the following statement being unanimously approved by the Committee. The Chair did not voice an opinion:

> "The ICCG as a whole entity does not have the expertise to answer or comment on the GAC questions/comments on the proposed changes to the 35 Ill. Adm. Code 620 Groundwater Quality standards. These changes to the Groundwater Quality standards are being proposed by the Illinois EPA, who has the expertise and knowledge to address this (GAC) Recommendation. Therefore, it is the Committee's stated opinion that the GAC Recommendation should be addressed by the Illinois EPA in the Statement of Reason or before the Illinois Pollution Control Board. Further, this Response by ICCG does not specifically endorse or disapprove of the proposed rule changes and individual ICCG member reserves the right to provide additional comment, questions, or concerns during the rule making process."

CONCLUSION: By providing this written Memorandum and the Response within to the GAC, the ICCG has met its Statutory obligations under the Illinois Groundwater Protection Act.

ATTACHMENT 7

Terranova, Sara

From: Terranova, Sara
Sent: Thursday, November 18, 2021 12:34 PM
To: elvfam@wowway.com
Cc: Diers, Stefanie
Subject: GAC - 35 Ill. Adm. Code 620

Mr. Elvert,

The Illinois Environmental Protection Agency (Agency) has received and reviewed the Groundwater Advisory Council's (GAC) Recommendations to Proposed 35 Ill. Adm. Code 620. The Agency believes each applicable point of concern raised by the GAC has been sufficiently addressed in the Statement of Reasons and the accompanied Testimony that is to be filed before the Illinois Pollution Control Board (Board). However, if any outstanding issues remain, each concern may be raised and further addressed during the 35 Ill. Adm. Code 620 rulemaking proceeding before Board.

Thank you,
Sara Terranova
Assistant Counsel
Division of Legal Counsel

TITLE 35: ENVIRONMENTAL PROTECTION
SUBTITLE F: PUBLIC WATER SUPPLIES
CHAPTER I: POLLUTION CONTROL BOARD

PART 620
GROUNDWATER QUALITY

SUBPART A: GENERAL

SUBPART B: GROUNDWATER CLASSIFICATION

SUBPART C: NONDEGRADATION PROVISIONS
FOR APPROPRIATE GROUNDWATERS

SUBPART D: GROUNDWATER QUALITY STANDARDS

SUBTITLE F

SUBPART E: GROUNDWATER MONITORING AND ANALYTICAL PROCEDURES

SUBPART F: HEALTH ADVISORIES

AUTHORITY: Implementing and authorized by Section 8 of the Illinois Groundwater Protection Act [415 ILCS 55/8] and authorized by Section 27 of the Illinois Environmental Protection Act [415 ILCS 5/27].

SOURCE: Adopted in R89-14(B) at 15 Ill. Reg. 17614, effective November 25, 1991; amended in R89-14(C) at 16 Ill. Reg. 14667, effective September 11, 1992; amended in R93-27 at 18 Ill.

Reg. 14084, effective August 24, 1994; amended in R96-18 at 21 Ill. Reg. 6518, effective May 8, 1997; amended in R97-11 at 21 Ill. Reg. 7869, effective July 1, 1997; amended in R01-14 at 26 Ill. Reg. 2662, effective February 5, 2002; amended in R08-18 at 36 Ill. Reg. 15206, effective October 5, 2012; amended in R08-18(B) at 37 Ill. Reg. 16529, effective October 7, 2013; amended in _____ at ____ Ill. Reg. _____, effective _____

Section 620.110 Definitions

The definitions of the Environmental Protection Act [415 ILCS 5] and the Groundwater Protection Act [415 ILCS 55] apply to this Part. The following definitions also apply to this Part:

"Act" means the Environmental Protection Act [415 ILCS 5].

"Agency" means the Illinois Environmental Protection Agency.

"Aquifer" means saturated (with groundwater) soils and geologic materials which are sufficiently permeable to readily yield economically useful quantities of water to wells, springs, or streams under ordinary hydraulic gradients. [415 ILCS 55/3(b)]

"BETX" means the sum of the concentrations of benzene, ethylbenzene, toluene, and xylenes.

"Board" means the Illinois Pollution Control Board.

"Chemical Abstract Services Registry Number" or "CASRN" means a unique numerical identifier designated for only one substance, assigned by the Chemical Abstracts Service for that substance.

"Carcinogen" means a contaminant that is classified as a Category A1 or A2 Carcinogen by the American Conference of Governmental Industrial Hygienists; or a Category 1 or 2A/2B carcinogen by the World Health Organization's International Agency for Research on Cancer; or a "Human carcinogen" or "Anticipated Human Carcinogen" by the United States Department of Health and Human Service National Toxicological Program; or a Category A or B1/B2 Carcinogen or as "carcinogenic to humans" or "likely to become carcinogenic to humans" *by the United States Environmental Protection Agency in Integrated Risk Information System or a Final Rule issued in a Federal Register notice by the USEPA.* [415 ILCS 5/58.2]

"Community water supply" means a public supply which serves or is intended to serve at least 15 service connections used by residents or regularly serves at least 25 residents. [415 ILCS 5/3.145]

"Contaminant" means any solid, liquid, or gaseous matter, any odor, or any form of energy, from whatever source. [415 ILCS 5/3.165]

"Corrective action process" means those procedures and practices that may be imposed by a regulatory agency when a determination has been made that contamination of groundwater has taken place, and are necessary to address a potential or existing violation of the standards set forth in Subpart D.

"Cumulative impact area" means the area, including the coal mine area permitted under the Surface Coal Mining Land Conservation and Reclamation Act [225 ILCS 720] and 62 Ill. Adm. Code 1700 through 1850, within which impacts resulting from the proposed operation may interact with the impacts of all anticipated mining on surface water and groundwater systems.

"Department" means the Illinois Department of Natural Resources.

"Detection" means the identification of a contaminant in a sample at a value equal to or greater than the:

"Method Detection Limit" or "MDL" means the minimum measured concentration of a substance that can be ~~measured as~~ reported with 99% ~~percent~~ confidence that the ~~true value is greater than zero~~ measured concentration is distinguishable from method blank results, pursuant to 40 CFR 136, appendix B (2017~~2006~~), incorporated by reference at Section 620.125; or

"Lower Limit of Quantitation ~~Method Quantitation Limit~~" or "LLOQ~~MQL~~" means the minimum concentration ~~of a substance~~ that can be measured or ~~and~~ reported pursuant to "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods," ~~,~~ incorporated by reference at Section 620.125.

"Groundwater" means underground water which occurs within the saturated zone and geologic materials where the fluid pressure in the pore space is equal to or greater than atmospheric pressure. [415 ILCS 5/3.210]

"Hydrologic balance" means the relationship between the quality and quantity of water inflow to, water outflow from, and water storage in a hydrologic unit such as a drainage basin, aquifer, soil zone, lake, or reservoir. It encompasses the dynamic relationships among precipitation, runoff, evaporation, and changes in ground and surface water storage.

"Lowest Concentration Minimum Reporting Level" or "LCMRL" means the lowest spiking concentration such that the probability of spike recovery in the

50% to 150% range is at least 99%.

"IGPA" means the Illinois Groundwater Protection Act. ~~[~~415 ILCS 55~~]~~.

"Lowest observable adverse effect level" or "LOAEL" ~~or "Lowest observable adverse effect level"~~ means the lowest tested concentration of a chemical or substance that produces a statistically significant increase in frequency or severity of non-overt adverse effects between the exposed population and its appropriate control. ~~LOAEL may be determined for a human population (LOAEL-H) or an animal population (LOAEL-A).~~

"Licensed Professional Engineer" or "LPE" means a person, corporation, or partnership licensed under the laws of the State of Illinois to practice professional engineering. [415 ILCS 5/57.2]

"Licensed Professional Geologist" or "LPG" means an individual who is licensed under the Professional Geologist Licensing Act *to engage in the practice of professional geology in Illinois.* [225 ILCS 745/15]

"Mutagen" means a carcinogenic constituent that operates by a mutagenic mode of action for carcinogenesis. Carcinogens with a mutagenic mode of action would be expected to cause irreversible changes to DNA and would exhibit greater effects in early life versus later life exposure.

~~"NOAEL" or~~ "No observable adverse effect level" or "NOAEL" means the highest tested concentration of a chemical or substance that does not produce a statistically significant increase in frequency or severity of non-overt adverse effects between the exposed population and its appropriate control. ~~NOAEL may be determined for a human population (NOAEL-H) or an animal population (NOAEL-A).~~

"Non-community water supply" means a public water supply that is not a community water supply. [415 ILCS 5/3.145]

"Off-site" means not on-site.

"On-site" means on the same or geographically contiguous property that may be divided by public or private right-of-way, provided the entrance and exit between properties is at a crossroads intersection and access is by crossing as opposed to going along the right-of-way. Noncontiguous properties owned by the same person but connected by a right-of-way that he controls and that the public does not have access to is also considered on-site property.

"Operator" means the person responsible for the operation of a site, facility or unit.

"Owner" means the person who owns a site, facility, or unit; ~~or~~ part of a site, facility, or unit; ~~,~~ or who owns the land on which the site, facility, or unit is located.

"Potable" means generally fit for human consumption in accordance with accepted water supply principles and practices. [415 ILCS 5/3.340]

"Potential primary source" means any unit at a facility or site not currently subject to a removal or remedial action which:

Is utilized for the treatment, storage, or disposal of any hazardous or special waste not generated at the site; or

Is utilized for the disposal of municipal waste not generated at the site, other than landscape waste and construction and demolition debris; or

Is utilized for the landfilling, land treating, surface impounding or piling of any hazardous or special waste that is generated on the site or at other sites owned, controlled or operated by the same person; or

Stores or accumulates at any time more than 75,000 pounds above ground, or more than 7,500 pounds below ground, of any hazardous substances. [415 ILCS 5/3.345]

"Potential route" means abandoned and improperly plugged wells of all kinds, drainage wells, all injection wells, including closed loop heat pump wells, and any excavation for the discovery, development or production of stone, sand or gravel. This term does not include closed loop heat pump wells using USP (U.S. Pharmacopeia) *food grade propylene glycol.* [415 ILCS 5/3.350]

"Potential secondary source" means any unit at a facility or a site not currently subject to a removal or remedial action, other than a potential primary source, which:

Is utilized for the landfilling, land treating, or surface impounding of waste that is generated on the site or at other sites owned, controlled or operated by the same person, other than livestock and landscape waste, and construction and demolition debris; or

Stores or accumulates at any time more than 25,000 but not more than 75,000 pounds above ground, or more than 2,500 but not more than 7,500 pounds below ground, of any hazardous substance; or

Stores or accumulates at any time more than 25,000 gallons above ground, or more than 500 gallons below ground, of petroleum, including crude oil or any fraction thereof which is not otherwise specifically listed or designated as a hazardous substance; or

Stores or accumulates pesticides, fertilizers, or road oils for purposes of commercial application or for distribution to retail sales outlets; or

Stores or accumulates at any time more than 50,000 pounds of any de-icing agent; or

Is utilized for handling livestock waste or for treating domestic wastewaters other than private sewage disposal systems as defined in the Private Sewage Disposal Licensing Act [225 ILCS 225]. [415 ILCS 5/3.355]

~~"Practical Quantitation Limit" or "PQL" means the lowest concentration or level that can be reliably measured within specified limits of precision and accuracy during routine laboratory operating conditions in accordance with "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846, incorporated by reference at Section 620.125.~~

"Previously mined area" means land disturbed or affected by coal mining operations prior to February 1, 1983.

BOARD NOTE: February 1, 1983, is the effective date of the Illinois permanent program regulations implementing the Surface Coal Mining Land Conservation and Reclamation Act [225 ILCS 720] as codified in 62 Ill. Adm. Code 1700 through 1850.

"Property class" means the class assigned by a tax assessor to real property for purposes of real estate taxes.

BOARD NOTE: The property class (rural property, residential vacant land, residential with dwelling, commercial residence, commercial business, commercial office, or industrial) is identified on the property record card maintained by the tax assessor in accordance with the Illinois Real Property

Appraisal Manual (February 1987), published by the Illinois Department of Revenue, Property Tax Administration Bureau.

"Public water supply" means all mains, pipes and structures through which water is obtained and distributed to the public, including wells and well structures, intakes and cribs, pumping stations, treatment plants, reservoirs, storage tanks and appurtenances, collectively or severally, actually used or intended for use for the purpose of furnishing water for drinking or general domestic use and which serve at least 15 service connections or which regularly serve at least 25 persons at least 60 days per year. A public water supply is either a "community water supply" or a "non-community water supply". [415 ILCS 5/3.365]

"Regulated entity" means a facility or unit regulated for groundwater protection by any State or federal agency.

"Regulatory agency" means the Illinois Environmental Protection Agency, Department of Public Health, Department of Agriculture, the Office of Mines and Minerals in the Department of Natural Resources, and the Office of State Fire Marshal.

"Regulated recharge area" means a compact geographic area, as determined by the Board pursuant to Section 17.4 of the Act, *the geology of which renders a potable resource groundwater particularly susceptible to contamination.* [415 ILCS 5/3.390]

"Resource groundwater" means groundwater that is presently being, or in the future is capable of being, put to beneficial use by reason of being of suitable quality. [415 ILCS 5/3.430]

"Saturated zone" means a subsurface zone in which all the interstices or voids are filled with water under pressure greater than that of the atmosphere.

"Setback zone" means a geographic area, designated pursuant to this Act, containing a potable water supply well or a potential source or potential route having a continuous boundary, and within which certain prohibitions or regulations are applicable in order to protect groundwaters. [415 ILCS 5/3.450]

"Site" means any location, place, tract of land and facilities, including but not limited to, buildings and improvements used for the purposes subject to regulation or control by the Act or regulations thereunder. [415 ILCS 5/3.460]

"Spring" means a natural surface discharge of an aquifer from rock or soil.

"Threshold dose" means the lowest dose of a chemical at which a specified measurable effect is observed and below which it is not observed.

"Treatment" means the technology, treatment techniques, or other procedures for compliance with 35 Ill. Adm. Code,- Subtitle F.

"Unit" means any device, mechanism, equipment, or area (exclusive of land utilized only for agricultural production). [415 ILCS 5/3.515]

"U.S. EPA" means the United States Environmental Protection Agency.

"Wellhead protection area" or "WHPA" means the surface and subsurface recharge area surrounding a community water supply well or well field, delineated outside of any applicable setback zones (pursuant to Section 17.1 of the Act [415 ILCS 5/17.1]), and pursuant to Illinois' Wellhead Protection Program, through which contaminants are reasonably likely to move toward such well or well field.

"Wellhead Protection Program" or "WHPP" means the wellhead protection program for the State of Illinois, approved by U.S. EPA under 42 USC 300h-7. BOARD NOTE: Derived from 40 CFR 141.71(b) (2003). The wellhead protection program includes the "groundwater protection needs assessment" under Section 17.1 of the Act [415 ILCS 5/17.1] and 35 Ill. Adm. Code 615-617.

(Source: Amended at ~~36~~ Ill. Reg. ~~15206~~, effective ~~October 5, 2012~~)

Section 620.125 Incorporations by Reference

a) The Board incorporates the following material by reference:

ASTM International. 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959 (610) 832-9500.

"Standard Practice for Classification of Soils for Engineering Purposes (Unified Classification System)" -ASTM D2487-06.

"Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)" ASTM D7979-20.

CFR (Code of Federal Regulations). Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 (202) 783-3238.

Method Detection Limit Definition, appendix B to Part 136, 40 CFR 136, appendix B – Revision 2 (82 FR 40939, Aug. 28, 2017)~~(2006)~~.

Control of Lead and Copper, general requirements, 40 CFR 141.80 (72 FR 57814, Oct. 10, 2007)~~(2006)~~.

Maximum contaminant levels for organic contaminants, 40 CFR 141.61 (59 FR 34324, July 1, 1994) ~~(2006)~~.

Maximum contaminant levels for inorganic contaminants, 40 CFR 141.62 (69 FR 38855, June 29, 2004) ~~(2006)~~.

Maximum contaminant levels for radionuclides, 40 CFR 141.66 (65 FR 76748, Dec. 7, 2000) ~~(2006)~~.

GPO. Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20401 (202) 783-3238~~)~~.

U.S. EPA Guidelines for Carcinogenic Risk Assessment, 51 Fed. Reg. 33992-34003 (September 24, 1986).

Illinois Environmental Protection Agency, 1020 North Grand Avenue East, P.O. Box 19276, Springfield, IL 62794-9276 (217) 785-4787.

"Guidance Document for Groundwater Protection Needs Assessments," Agency, Illinois State Water Survey, and Illinois State Geologic Survey Joint Report, January 1995.

"Illinois Integrated Water Quality Report and Section 303(d) List, 2018," Agency, February 2021.

"The Illinois Wellhead Protection Program Pursuant to Section 1428 of the Federal Safe Drinking Water Act," Agency, # 22480, October 1992.

NAS. National Academy of Sciences, Engineering, and Medicine, 550 5th St. NW, Washington DC (202) 334-2000.

"Water Quality Criteria 1972", EPA.R3.73-033, 1973. https://nepis.epa.gov

NCRP. National Council on Radiation Protection, 7910 Woodmont Ave., Bethesda, MD (301) 657-2652.

"Maximum Permissible Body Burdens and Maximum Permissible Concentrations of Radionuclides in Air and in Water for Occupational Exposure", NCRP Report Number 22, June 5, 1959.

U. S. Environmental Protection Agency, 1200 Pennsylvania Avenue, N.W., Washington, DC 20460 ~~NTIS. National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 605-6000. NTIS. National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161 (703) 605-6000.~~

"Low Stress (low flow) Purging and Sampling Procedure for the Collection of Groundwater Samples from Monitoring Wells~~Practical Guide for Ground-Water Sampling~~", EPA Publication EQASOP-GW4, Region 1 Low-Stress (low flow) SOP Revision No. 4, July 30, 1996; Revised September 19, 2017.~~No. EPA/600/2-85/104 (September 1985), Doc. No. PB 86-137304~~.

"Methods for Chemical Analysis of Water and Wastes," March 1983, Doc. No. PB84-128677. EPA 600/4-79-020 (available online at http://nepis.epa.gov/).

"Methods for the Determination of Inorganic Substances in Environmental Samples," August 1993, PB94-120821 (referred to as "U.S. EPA Environmental Inorganic Methods"). EPA 600/R-93-100 (available online at http://nepis.epa.gov/).

"Methods for the Determination of Metals in Environmental Samples," June 1991, Doc. No. PB91-231498. EPA 600/4-91-010 (available online at http://nepis.epa.gov/).

"Methods for the Determination of Metals in Environmental Samples – Supplement I," May 1994, Doc. No. PB95-125472. EPA 600/R-94-111 (available online at http://nepis.epa.gov/).

"Methods for the Determination of Organic Compounds in Drinking Water," Doc. No. PB91-231480. EPA/600/4-88/039 (December 1988 (revised July 1991)) (available online at http://nepis.epa.gov/).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement I," Doc. No. PB91-146027. EPA/600/4-90/020 (July 1990) (available online at http://nepis.epa.gov/).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement II," Doc. No. PB92-207703. EPA/600/R-92/129 (August 1992) (available online at http://nepis.epa.gov/).

"Methods for the Determination of Organic Compounds in Drinking Water, Supplement III," Doc. No. PB95-261616. EPA/600/R-95/131 (August 1995) (available online at http://nepis.epa.gov/).

"Methods for the Determination of Organic and Inorganic Compounds in Drinking Water"- Volume I: EPA 815-R-00-014 (August 2000) (available online at http://nepis.epa.gov/).

"Prescribed Procedures for Measurement of Radioactivity in Drinking Water," Doc. No. PB80-224744. EPA 600/4-80-032, (August 1980) (available online at http://nepis.epa.gov/).

"Procedures for Radiochemical Analysis of Nuclear Reactor Aqueous Solutions," H.L. Krieger and S. Gold, Doc. No. PB222-154/7BA. EPA-R4-73-014, May 1973.

"Radiochemical Analytical Procedures for Analysis of Environmental Samples," March 1979, Doc. No. EMSL LV 053917.

"Radiochemistry Procedures Manual," Doc. No. PB-84-215581. EPA-520/5-84-006, December 1987.

"Selected Analytical Methods for Environmental Remediation and Recovery (SAM), 2017. Record last revision date February 10, 2020. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NHSRC&dirEntryId=339252.

"Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," U.S. EPA Publication No. SW-846, Third Edition, Final Updates I (1993), II (1995), IIA (1994), IIB (1995), III (1997), IIIA (1999), IIIB (2005), IV (2008), V (2015), VI Phase 1 (2017), VI Phase 2 (2018), VI Phase 3 (2019), and VII Phase 1 (2020). http://www.epa.gov/hw-sw846/sw-846-compendium.
~~as amended by Updates I, II, IIA, IIB, III, IIIA, and IIIB (Doc. No. 955-001-00000-1) (available on line at http://www.epa.gov/epaoswer/hazwaste/test/main.htm).~~

U.S. Environmental Protection Agency, Office of Ground Water and Drinking Water, Standards and Risk Management Division.

"Method 533: Determination of Per-and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry," November 2019. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf).

U.S. Environmental Protection Agency, Office of Research and

Development, ~~National~~ Center for Environmental Solutions & Emergency Response.~~Assessment~~

Shoemaker, J. and Dan Tettenhorst. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, ~~National~~ Center for Environmental Assessment, Washington, DC. Version 2~~1~~.0, March 2020~~November 2018~~.

United States Environmental Protection Agency, Office of Resource Conservation and Recovery.

"Statistical Analysis of Groundwater Monitoring Data at RCRA Facilities, (March 2009 Unified Guidance)", EPA 530/R-09-007.

United States Environmental Protection Agency, Risk Assessment Forum, Washington, D.C.

"A Review of the Reference Dose and References Concentration Process", EPA/630/P-02/002F, December 2002".

"Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation", EPA/R-14/002F, September 2014.

"Guidelines for Carcinogen Risk Assessment", EPA/630/P-03/001F, March 2005".

"Supplemental Guidance for Assessing Susceptibility for Early-Life Exposure to Carcinogens", EPA/630/R-03/003F, March 2005.

USGS. United States Geological Survey, 1961 Stout St., Denver, CO 80294 (303) 844-4169

"Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected Unstable Constituents", Book I, Chapter D2 (1976).

b) This Section incorporates no later editions or amendments.

(Source: Amended at ~~36~~ Ill. Reg. ~~15206~~, effective ~~October 5, 2012~~)

Section 620.210 Class I: Potable Resource Groundwater

Except as provided in Sections 620.230, 620.240, or 620.250, Potable Resource Groundwater is:

a) Groundwater located 10 feet or more below the land surface and within:

1) The minimum setback zone of a well which serves as a potable water supply and to the bottom of such well;

2) Unconsolidated sand, gravel, or sand and gravel which is 5 feet or more in thickness and that contains 12% ~~percent~~ or less of fines (i.e., fines which pass through a No. 200 sieve tested according to ASTM Standard Practice D2487-06, incorporated by reference at Section 620.125);

3) Sandstone which is 10 feet or more in thickness, or fractured carbonate which is 15 feet or more in thickness; ~~or~~

4) Any geologic material which is capable of a:

A) Sustained groundwater yield, from up to a 12-inch borehole, of 150 gallons per day or more from a thickness of 15 feet or less; or

B) Hydraulic conductivity of 1 x 10^{-4} cm/sec or greater using one of the following test methods or its equivalent:

i) Slug test; or~~Permeameter;~~

ii) Pump test~~Slug test; or~~

~~iii) Pump test.~~

5) The wellhead protection area of a community water supply well or well field, as defined in Section 620.110 and delineated pursuant to the methods incorporated by reference in Section 620.125. For the purposes of this Subpart, when a maximum setback zone has been adopted pursuant to Section 14.3 of the Act, the WHPA includes the delineated area within the maximum setback zone.

b) Any groundwater which is determined by the Board pursuant to petition procedures set forth in Section 620.260, to be capable of potable use.

BOARD NOTE: Any portion of the thickness associated with the geologic

materials as described in subsections 620.210(a)(2), (a)(3), or (a)(4) should be designated as Class I: Potable Resource Groundwater if located 10 feet or more below the land surface.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.250 Groundwater Management Zone

a) Within any class of groundwater, a groundwater management zone may be established as a three-dimensional region containing groundwater being managed to mitigate impairment caused by the release of contaminants from a site:

1) That is subject to a corrective action process approved by the Agency; or

2) For which the owner or operator undertakes an adequate corrective action in a timely and appropriate manner and provides a written confirmation to the Agency. Such confirmation shall ~~must~~ be provided in a form as prescribed by the Agency.

b) A groundwater management zone is established upon concurrence by the Agency that the conditions as specified in subsection (a) are met and groundwater management continues for a period of time consistent with the action described in that subsection.

c) A groundwater management zone expires upon the Agency's receipt of appropriate documentation which confirms the completion of the action taken pursuant to subsection (a) and which confirms the attainment of applicable standards as set forth in Subpart D. The Agency shall review the on-going adequacy of controls and continued management at the site if concentrations of chemical constituents, as specified in Section 620.450(a)(4)(B), remain in groundwater at the site following completion of such action. The review shall ~~must~~ take place no less often than every 5 years and the results shall be presented to the Agency in a written report.

d) Notwithstanding subsections (a) and (b) above, a groundwater management zone as defined in 35 Ill. Adm. Code 740.120 may be established in accordance with the requirements of 35 Ill. Adm. Code 740.530 for sites undergoing remediation pursuant to the Site Remediation Program. Such a groundwater management zone shall remain in effect until the requirements set forth at 35 Ill. Adm. Code 740.530(c) are met.

e) While the groundwater management zone established in accordance with 35 Ill. Adm. Code 740.530 is in effect, the otherwise applicable standards as specified in Subpart D of this Part shall not be applicable to the "contaminants of concern", as defined at 35 Ill. Adm. Code 740.120, for which groundwater remediation objectives have been approved in accordance with the procedures of 35 Ill. Adm. Code 740.

f) Notwithstanding subsection (c) above, the review requirements concerning the ongoing adequacy of controls and continued management at the site shall not apply to groundwater within a three-dimensional region formerly encompassed by a groundwater management zone established in accordance with 35 Ill. Adm. Code 740.530 while a No Further Remediation Letter issued in accordance with the procedures of 35 Ill. Adm. Code 740 is in effect.

g) All groundwater management zone applications submitted pursuant to subsection (a) shall contain the following:

1) Facility information. This includes the name, address, and county where the site is located.

2) Identification of specific units (operating or closed) present at the facility.

3) Maps and engineering drawings showing the facility and units at the facility.

4) Statement of the groundwater classification(s) at the facility.

5) Identification of the chemical constituents released to the groundwater.

6) Description of how groundwater will be monitored to determine the rate and extent of the release, and if it has migrated off site.

7) Schedule for investigation of the extent of the release.

8) Results of available soil testing and groundwater monitoring associated with a release, locations and depths of samples, and monitoring well construction details with well logs.

9) Remedy

A) Description of selected remedy and why it was chosen;

B) Results of groundwater contaminant transport modeling or calculations showing how the selected remedy will achieve compliance with the applicable groundwater standards;

C) Description of the fate and transport of contaminants with selected remedy over time; and

D) A statement of how groundwater at the facility will be monitored following implementation of the remedy to ensure that the groundwater standards have been attained.

10) Information requested by the Agency, necessary for its review of the groundwater management zone application.

(Source: Amended at ___ Ill. Reg. _____, effective _____)

(Source: Amended at ~~21 Ill. Reg. 7869~~, effective ~~July 1, 1997~~)

Section 620.302 Applicability of Preventive Notification and Preventive Response Activities

a) Preventive notification and preventive response as specified in Sections 620.305 through 620.310 applies to:

1) Class I groundwater under Section 620.210(a)(1), (a)(2), or (a)(3) that is monitored by the persons listed in subsection (b); or

2) Class III groundwater that is monitored by the persons listed in subsection (b).

b) For purposes of subsection (a), the persons that conduct groundwater monitoring are:

1) An owner or operator of a regulated entity for which groundwater quality monitoring shall ~~must~~ be performed pursuant to State or Federal law or regulation (e.g., section 106 and 107 of the Comprehensive Environmental Response, Compensation and Liability Act (42 USC 9601, et seq.); sections 3004 and 3008 of the Resource Conservation and Recovery Act (42 USC 6901, et seq.); sections 4(q), 4(v), 12(g), 21(d), 21(f), 22.2(f), 22.2(m) and 22.18 of the Act; 35 Ill. Adm. Code 615, 616, 724, 725, 730, 731, 750, 807, 811, ~~and~~ 814, and 815; and 62 Ill. Adm. Code 1780 and 1784);

2) An owner or operator of a public water supply well who conducts groundwater quality monitoring;

3) A State agency that is authorized to conduct, or is the recipient of, groundwater quality monitoring data (e.g., Illinois Environmental Protection Agency, Department of Public Health, Department of Agriculture, Office of State Fire Marshal, or Department of Natural Resources); or

4) An owner or operator of a facility that conducts groundwater quality monitoring pursuant to State or federal judicial or administrative order.

c) If a contaminant exceeds a standard set forth in Section 620.410 or Section 620.430, the appropriate remedy is corrective action and Sections 620.305 and 620.310 do not apply.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.310 Preventive Response Activities

a) The following preventive assessment shall~~must~~ be undertaken:

1) If a preventive notification under Section 620.305(c) is provided by a community water supply:

A) The Agency shall notify the owner or operator of any identified potential primary source, potential secondary source, potential route, or community water supply well that is located within 2,500 feet of the wellhead.

B) The owner or operator notified under subsection (a)(1)(A) shall, within 30 days after the date of issuance of such notice, sample each water well or monitoring well for the contaminant identified in the notice if the contaminant or material containing such contaminant is or has been stored, disposed of, or otherwise handled at the site. If a contaminant identified under Section 620.305(a) is detected, then the well shall ~~must~~ be resampled within 30 days of the date on which the first sample analyses are received. If a contaminant identified under Section 620.305(a) is detected by the resampling, preventive notification shall ~~must~~ be given as set forth in Section 620.305.

C) If the Agency receives analytical results under subsection (a)(1)(B) that show a contaminant identified under Section 620.305(a) has been detected, the Agency shall:

i) Conduct a well site survey pursuant to 415 ILCS 5/17.1(d), if such a survey has not been previously conducted within the last 5 years; and

ii) Identify those sites or activities that represent a hazard to the continued availability of groundwaters for public use unless a groundwater protection needs assessment has been prepared pursuant to 415 ILCS 5/17.1(d).

2) If a preventive notification is provided under Section 620.305(c) by a non-community water supply or for multiple private water supply wells, the Department of Public Health shall conduct a sanitary survey within 1,000 feet of the wellhead of a non-community water supply or within 500 feet of the wellheads for multiple private water supply wells.

3) If a preventive notification under Section 620.305(b) is provided by the owner or operator of a regulated entity and the applicable standard in Subpart D has not been exceeded:

A) The appropriate regulatory agency shall determine if any of the following occurs for Class I: Potable Resource Groundwater:

i) The levels set forth below are exceeded or are changed for pH:

CASRN	Constituent	Criteria (mg/L)
	~~Para-Dichlorobenzene~~	~~0.005~~
95-50-1	o~~rtho~~-Dichlorobenzene (1,2-dichlorobenzene)	0.01
	~~Ethylbenzene~~	~~0.03~~
1634-04-4	MTBE (m~~M~~ethyl t~~T~~ertiary-b~~B~~utyl e~~E~~ther) ~~(MTBE)~~	0.02
108-95-2	Phenols	0.001
100-42-5	Styrene	0.01
108-88-3	Toluene	0.04
1330-20-7	Xylenes	0.02

ii) A statistically significant increase occurs above background (as determined pursuant to other regulatory procedures (e.g., 35 Ill. Adm. Code 616, 724, 725, or 811)) for the following inorganic constituents (except due to natural causes); or for the following organic constituents: ~~for arsenic, beryllium, cadmium, chromium, cyanide, lead, mercury, thallium, or vanadium (except due to natural causes); or for acenaphthene, acetone, aldicarb, anthracene, atrazine, benzoic acid, carbon disulfide, carbofuran, dalapon, 2-butanone (MEK), dicamba, dichlorodifluoromethane, 1,1-dichloroethane, diethyl phthalate, di-n-butyl phthalate, dinoseb, endrin, endothall, fluoranthene, fluorine, hexachlorocyclopentadiene, isopropylbenzene (cumene), lindane (gamma-hexachloro cyclohexane), 2,4-D,1,1 - dichloroethylene, cis-1,2-dichloroethylene, trans-1,2-dichloroethylene, MCPP (mecoprop), 2-methylnaphthalene, methoxychlor, 2-~~

~~methylphenol, monochlorobenzene, naphthalene, picloram, pyrene, simazine, 2,4,5-TP (silvex), 1,2,4-trichlorobenzene, 1,1,2-trichloroethane, 1,1,1trichloroethane, and trichlorofluoromethane.~~

CASRN	Constituent
Inorganics	
7429-90-5	Aluminum
7440-38-2	Arsenic
7440-41-7	Beryllium
7440-43-9	Cadmium
7440-47-3	Chromium (total)
143-33-9	Cyanide (sodium cyanide)
7439-92-1	Lead
7487-94-7	Mercury (mercuric chloride)
7439-98-7	Molybdenum
7440-28-0	Thallium
7440-62-2	Vanadium
Organics	
83-32-9	Acenaphthene
67-64-1	Acetone
116-06-3	Aldicarb
120-12-7	Anthracene
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)
1912-24-9	Atrazine and metabolites DEA, DIA, DACT
71-43-2	Benzene
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
50-32-8	Benzo(a)pyrene
65-85-0	Benzoic acid
78-93-3	2-Butanone (methyl ethyl ketone)
1563-66-2	Carbofuran
75-15-0	Carbon disulfide
56-23-5	Carbon tetrachloride
12798-03-6	Chlordane
108-90-7	Chlorobenzene
67-66-3	Chloroform

CASRN	Constituent
218-01-9	Chrysene
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)
75-99-0	Dalapon
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)
1918-00-9	Dicamba
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)
75-71-8	Dichlorodifluoromethane
75-34-3	1,1-Dichloroethane
75-35-4	1,1-Dichloroethylene
107-06-2	1,2-Dichloroethane
156-59-2	*cis*-1,2-Dichloroethylene
156-60-5	*trans*-1,2-Dichloroethylene
75-09-2	Dichloromethane (methylene chloride)
78-87-5	1,2-Dichloropropane
117-81-7	Di(2-ethylhexyl)phthalate
84-66-2	Diethyl phthalate
84-74-2	Di-*n*-butyl phthalate
88-85-7	Dinoseb
123-91-1	1,4-Dioxane (*p*-dioxane)
145-73-3	Endothall
72-20-8	Endrin
100-41-4	Ethylbenzene
106-93-4	Ethylene dibromide (1,2-dibromoethane)
206-44-0	Fluoranthene
86-73-7	Fluorene
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
76-44-8	Heptachlor
1024-57-3	Heptachlor epoxide
77-47-4	Hexachlorocyclopentadiene
193-39-5	Indeno(1,2,3-c,d)pyrene
98-82-8	Isopropylbenzene (cumene)
72-43-5	Methoxychlor

CASRN	Constituent
90-12-0	1-Methylnaphthalene
91-57-6	2-Methylnaphthalene
95-48-7	2-Methylphenol (*o*-cresol)
91-20-3	Naphthalene
98-95-3	Nitrobenzene
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)
375-73-5	PFBS (perfluorobutanesulfonic acid)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-95-1	PFNA (perfluorononanoic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
87-86-5	Pentachlorophenol
1918-02-1	Picloram
129-00-0	Pyrene
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)
122-34-9	Simazine
118-96-7	TNT (2,4,6-trinitrotoluene)
93-72-1	2,4,5-TP (silvex)
127-18-4	Tetrachloroethylene
8001-35-2	Toxaphene
120-82-1	1,2,4-Trichlorobenzene
71-55-6	1,1,1-Trichloroethane
79-00-5	1,1,2-Trichloroethane
79-01-6	Trichloroethylene
75-69-4	Trichlorofluoromethane
99-35-4	1,3,5-Trinitrobenzene
75-01-4	Vinyl chloride

iii) For a chemical constituent of gasoline, diesel fuel, or heating fuel, the constituent exceeds the following:

Constituent	Criterion (mg/L)
BETX	0.095

iv) For pH, a statistically significant change occurs from background.

~~BOARD NOTE: Constituents that are carcinogens have not been~~

~~listed in subsection (a)(3)(A) because the standard is set at the PQL and any exceedence thereof is a violation subject to corrective action.~~

B) The appropriate agency shall determine if, for Class III: Special Resource Groundwater, the levels as determined by the Board are exceeded.

C) The appropriate regulatory agency shall consider whether the owner or operator reasonably demonstrates that:

i) The contamination is a result of contaminants remaining in groundwater from a prior release for which appropriate action was taken in accordance with laws and regulations in existence at the time of the release;

ii) The source of contamination is not due to the on-site release of contaminants; or

iii) The detection resulted from error in sampling, analysis, or evaluation.

D) The appropriate regulatory agency shall consider actions necessary to minimize the degree and extent of contamination.

b) The appropriate regulatory agency shall determine whether a preventive response shall ~~must~~ be undertaken based on relevant factors including, but not limited to, the considerations in subsection (a)(3).

c) After completion of preventive response pursuant to authority of an appropriate regulatory agency, the concentration of a contaminant listed in subsection (a)(3)(A) in groundwater may exceed 50% ~~percent~~ of the applicable numerical standard in Subpart D only if the following conditions are met:

1) The exceedence ~~has~~ been minimized to the extent practicable;

2) Beneficial use, as appropriate for the class of groundwater, has been assured; and

3) Any threat to public health or the environment has been minimized.

d) Nothing in this Section shall in any way limit the authority of the State or of the

United States to require or perform any corrective action process.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.410 Groundwater Quality Standards for Class I: Potable Resource Groundwater

a) Inorganic Chemical Constituents
Except due to natural causes or as provided in Section 620.450, concentrations of the following chemical constituents shall ~~must~~ not be exceeded in Class I groundwater:

~~Constituent~~	~~Units~~	~~Standard~~
~~Antimony~~	~~mg/L~~	~~0.006~~
~~Arsenic*~~	~~mg/L~~	~~0.010~~
~~Barium~~	~~mg/L~~	~~2.0~~
~~Beryllium~~	~~mg/L~~	~~0.004~~
~~Boron~~	~~mg/L~~	~~2.0~~
~~Cadmium~~	~~mg/L~~	~~0.005~~
~~Chloride~~	~~mg/L~~	~~200.0~~
~~Chromium~~	~~mg/L~~	~~0.1~~
~~Cobalt~~	~~mg/L~~	~~1.0~~
~~Copper~~	~~mg/L~~	~~0.65~~
~~Cyanide~~	~~mg/L~~	~~0.2~~
~~Fluoride~~	~~mg/L~~	~~4.0~~
~~Iron~~	~~mg/L~~	~~5.0~~
~~Lead~~	~~mg/L~~	~~0.0075~~
~~Manganese~~	~~mg/L~~	~~0.15~~
~~Mercury~~	~~mg/L~~	~~0.002~~
~~Nickel~~	~~mg/L~~	~~0.1~~
~~Nitrate as N~~	~~mg/L~~	~~10.0~~
~~Perchlorate~~	~~mg/L~~	~~0.0049~~
~~Radium-226~~	~~pCi/l~~	~~20.0~~
~~Radium-228~~	~~pCi/l~~	~~20.0~~
~~Selenium~~	~~mg/L~~	~~0.05~~
~~Silver~~	~~mg/L~~	~~0.05~~
~~Sulfate~~	~~mg/L~~	~~400.0~~
~~Thallium~~	~~mg/L~~	~~0.002~~
~~Total Dissolved Solids (TDS)~~	~~mg/L~~	~~1,200~~
~~Vanadium~~	~~mg/L~~	~~0.049~~
~~Zinc~~	~~mg/L~~	~~5.0~~

~~*Denotes a carcinogen.~~

CASRN	Constituent	Standard[a]
7429-90-5	Aluminum	1.9[b]
7440-36-0	Antimony	0.006[c]
7440-38-2	Arsenic[d]	0.01[c]
7440-39-3	Barium	2.0[c]
7440-41-7	Beryllium	0.004[c]
7440-42-8	Boron	2.0[e]
7440-43-9	Cadmium	0.005[c]
16887-00-6	Chloride	200[f]
7440-47-3	Chromium (total)	0.1[c]
7440-48-4	Cobalt	0.0012[b]
7440-50-8	Copper	0.5[g]
143-33-9	Cyanide (sodium cyanide)	0.2[c]
7681-49-4	Fluoride (sodium fluoride)	2[g]
7439-89-6	Iron	5[f]
7439-92-1	Lead	0.0075[h]
7439-93-2	Lithium	0.04[i]
7439-96-5	Manganese	0.15[j]
7487-94-7	Mercury (mercuric chloride)	0.002[c]
7439-98-7	Molybdenum	0.019[b]
7440-02-0	Nickel	0.077[b]
14797-55-8	Nitrate as N	10[c]
14797-73-0	Perchlorate	0.0081[b]
7440-14-4	Radium (combined 226+228)	5[c]
7782-49-2	Selenium	0.02[e]
7440-22-4	Silver	0.058[b]
14808-79-8	Sulfate	400[f]
	TDS (total dissolved solids)	1,200[f]
7440-28-0	Thallium	0.002[c]
7440-62-2	Vanadium	0.00027[b]
7440-66-6	Zinc	1.2[b]

Constituent Name and Groundwater Quality Standard Notations

[a] The standard units are milligrams per liter ("mg/L"), except for the radium (combined 226+228) unit of picocuries per liter ("pCi/L").

[b] The standard is calculated using the Human Threshold Toxicant Advisory Concentration ("HTTAC") procedures at Appendix A.

[c] The standard is based on the Maximum Contaminant Level ("MCL"), promulgated by U.S. EPA, Office of Water, and Illinois EPA Primary Drinking

Water Standards at 35 Ill. Adm. Code 611.

[d] The constituent meets the definition of a "carcinogen" at Section 620.110.

[e] The standard is based on beneficial use for irrigation of crops, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[f] The standard is the 95% confidence concentration stated in Illinois EPA's "*Integrated Water Quality Report and Section 303(d) List*", incorporated by reference at Section 620.125.

[g] The standard is based on beneficial use for watering livestock, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[h] The standard is 50% of the U.S. EPA "action level" of 0.015 mg/L for lead. The U.S. EPA action level applies at the service connection. The standard is reduced by 50% as a safety margin, based on the assumption that 50% of water would be treated.

[i] The standard is the "LLOQ" or "LCMRL" as defined in Section 620.110.

[j] The standard is promulgated at 35 Ill. Adm. Code 611.300.

b) Organic Chemical Constituents

Except due to natural causes or as provided in Section 620.450 or subsection (d), concentrations of the following organic chemical constituents shall not be exceeded in Class I groundwater:

~~Constituent~~	~~Standard (mg/L)~~
~~Acenaphthene~~	~~0.42~~
~~Acetone~~	~~6.3~~
~~Alachlor*~~	~~0.002~~
~~Aldicarb~~	~~0.003~~
~~Anthracene~~	~~2.1~~
~~Atrazine~~	~~0.003~~
~~Benzene*~~	~~0.005~~
~~Benzo(a)anthracene*~~	~~0.00013~~
~~Benzo(b)fluoranthene*~~	~~0.00018~~
~~Benzo(k)fluoranthene*~~	~~0.00017~~
~~Benzo(a)pyrene*~~	~~0.0002~~
~~Benzoic acid~~	~~28.0~~
~~2-Butanone (MEK)~~	~~4.2~~
~~Carbofuran~~	~~0.04~~
~~Carbon Disulfide~~	~~0.7~~
~~Carbon Tetrachloride*~~	~~0.005~~
~~Chlordane*~~	~~0.002~~

~~Chloroform*~~	~~0.07~~
~~Chrysene*~~	~~0.012~~
~~Dalapon~~	~~0.2~~
~~Dibenzo(a,h)anthracene*~~	~~0.0003~~
~~Dicamba~~	~~0.21~~
~~Dichlorodifluoromethane~~	~~1.4~~
~~1,1-Dichloroethane~~	~~1.4~~
~~Dichloromethane*~~	~~0.005~~
~~Di(2-ethylhexyl)phthalate*~~	~~0.006~~
~~Diethyl Phthalate~~	~~5.6~~
~~Di-n-butyl Phthalate~~	~~0.7~~
~~Dinoseb~~	~~0.007~~
~~Endothall~~	~~0.1~~
~~Endrin~~	~~0.002~~
~~Ethylene Dibromide*~~	~~0.00005~~
~~Fluoranthene~~	~~0.28~~
~~Fluorene~~	~~0.28~~
~~Heptachlor*~~	~~0.0004~~
~~Heptachlor Epoxide*~~	~~0.0002~~
~~Hexachlorocyclopentadiene~~	~~0.05~~
~~Indeno(1,2,3-cd)pyrene*~~	~~0.00043~~
~~Isopropylbenzene (Cumene)~~	~~0.7~~
~~Lindane (Gamma-Hexachlorocyclohexane)~~	~~0.0002~~
~~2,4-D~~	~~0.07~~
~~ortho-Dichlorobenzene~~	~~0.6~~
~~para-Dichlorobenzene~~	~~0.075~~
~~1,2-Dibromo-3-Chloropropane*~~	~~0.0002~~
~~1,2-Dichloroethane*~~	~~0.005~~
~~1,1-Dichloroethylene~~	~~0.007~~
~~cis-1,2-Dichloroethylene~~	~~0.07~~
~~trans-1,2-Dichloroethylene~~	~~0.1~~
~~1,2-Dichloropropane*~~	~~0.005~~
~~Ethylbenzene~~	~~0.7~~
~~MCPP (Mecoprop)~~	~~0.007~~
~~Methoxychlor~~	~~0.04~~
~~2-Methylnaphthalene~~	~~0.028~~
~~2-Methylphenol~~	~~0.35~~
~~Methyl Tertiary-Butyl Ether (MTBE)~~	~~0.07~~
~~Monochlorobenzene~~	~~0.1~~
~~Naphthalene~~	~~0.14~~

~~P-Dioxane*~~	~~0.0077~~
~~Pentachlorophenol*~~	~~0.001~~
~~Phenols~~	~~0.1~~
~~Picloram~~	~~0.5~~
~~Pyrene~~	~~0.21~~
~~Polychlorinated Biphenyls (PCBs) (as decachloro-biphenyl)*~~	~~0.0005~~
~~alpha-BHC (alpha-Benzene hexachloride)*~~	~~0.00011~~
~~Simazine~~	~~0.004~~
~~Styrene~~	~~0.1~~
~~2,4,5-TP (Silvex)~~	~~0.05~~
~~Tetrachloroethylene*~~	~~0.005~~
~~Toluene~~	~~1.0~~
~~Toxaphene*~~	~~0.003~~
~~1,1,1-Trichloroethane~~	~~0.2~~
~~1,1,2-Trichloroethane~~	~~0.005~~
~~1,2,4-Trichlorobenzene~~	~~0.07~~
~~Trichloroethylene*~~	~~0.005~~
~~Trichlorofluoromethane~~	~~2.1~~
~~Vinyl Chloride*~~	~~0.002~~
~~Xylenes~~	~~10.0~~

~~*Denotes a carcinogen.~~

CASRN	Constituent	Standard (mg/L)
83-32-9	Acenaphthene	0.23[a]
67-64-1	Acetone	3.5[a]
15972-60-8	Alachlor[b]	0.002[c]
116-06-3	Aldicarb	0.003[c]
120-12-7	Anthracene	1.2[a]
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)[b]	0.000012[d]
71-43-2	Benzene[b]	0.005[c]
56-55-3	Benzo(a)anthracene[e]	0.00025[d]
205-99-2	Benzo(b)fluoranthene[e]	0.00025[d]
207-08-9	Benzo(k)fluoranthene[e]	0.0025[d]
50-32-8	Benzo(a)pyrene[e]	0.0002[c]
65-85-0	Benzoic acid	15[a]

CASRN	Constituent	Standard (mg/L)
78-93-3	2-Butanone (methyl ethyl ketone)	2.3[a]
1563-66-2	Carbofuran	0.04[c]
75-15-0	Carbon disulfide	0.38[a]
56-23-5	Carbon tetrachloride[b]	0.005[c]
12798-03-6	Chlordane[b]	0.002[c]
108-90-7	Chlorobenzene	0.1[c]
67-66-3	Chloroform[b]	0.07[f]
218-01-9	Chrysene[e]	0.025[d]
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)	0.07[c]
75-99-0	Dalapon	0.2[c]
53-70-3	Dibenzo(a,h)anthracene[e]	0.0001[g]
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)[e]	0.0002[c]
1918-00-9	Dicamba	0.12[a]
95-50-1	*o*-Dichlorobenzene (1,2-dichlorobenzene)	0.6[c]
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)[b]	0.075[c]
75-71-8	Dichlorodifluoromethane	0.77[a]
75-34-3	1,1-Dichloroethane	0.77[a]
107-06-2	1,2-Dichloroethane[b]	0.005[c]
75-35-4	1,1-Dichloroethylene	0.007[c]
156-59-2	*cis*-1,2-Dichloroethylene	0.07[c]
156-60-5	*trans*-1,2-Dichloroethylene	0.1[c]
75-09-2	Dichloromethane (methylene chloride)[e]	0.005[c]
78-87-5	1,2-Dichloropropane[b]	0.005[c]
117-81-7	Di(2-ethylhexyl)phthalate[b]	0.006[c]
84-66-2	Diethyl phthalate	3.1[a]
84-74-2	Di-*n*-butyl phthalate	0.38[a]
99-65-0	1,3-Dinitrobenzene	0.001[g]
121-14-2	2,4-Dinitrotoluene[b]	0.001[g]
606-20-2	2,6-Dinitrotoluene[b]	0.001[g]
88-85-7	Dinoseb	0.007[c]
123-91-1	1,4-Dioxane (*p*-dioxane)[b]	0.00078[d]
145-73-3	Endothall	0.1[c]
72-20-8	Endrin	0.002[c]
100-41-4	Ethylbenzene[b]	0.7[c]
106-93-4	Ethylene dibromide (1,2-dibromoethane)[b]	0.00005[c]
206-44-0	Fluoranthene	0.15[a]

CASRN	Constituent	Standard (mg/L)
86-73-7	Fluorene	0.15[a]
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)[b]	0.0002[c]
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	0.000012[a]
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	0.77[a]
76-44-8	Heptachlor[b]	0.0004[c]
1024-57-3	Heptachlor epoxide[b]	0.0002[c]
77-47-4	Hexachlorocyclopentadiene	0.05[c]
193-39-5	Indeno(1,2,3-c,d)pyrene[e]	0.00025[d]
98-82-8	Isopropylbenzene (cumene)[b]	0.38[a]
93-65-2	MCPP (mecoprop)	0.1[g]
1634-04-4	MTBE (methyl tertiary-butyl ether)	0.038[a]
72-43-5	Methoxychlor	0.04[c]
90-12-0	1-Methylnaphthalene	0.27[a]
91-57-6	2-Methylnaphthalene	0.015[a]
95-48-7	2-Methylphenol (*o*-cresol)	0.19[a]
91-20-3	Naphthalene	0.077[a]
98-95-3	Nitrobenzene	0.0077[a]
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)[b]	0.0005[c]
375-73-5	PFBS (perfluorobutanesulfonic acid)	0.0012[a]
355-46-4	PFHxS (perfluorohexanesulfonic acid)	0.000077[a]
375-95-1	PFNA (perfluorononanoic acid)	0.000012[a]
335-67-1	PFOA (perfluorooctanoic acid)[b]	0.000002[g]
1763-23-1	PFOS (perfluorooctanesulfonic acid)	0.0000077[a]
87-86-5	Pentachlorophenol[b]	0.001[c]
108-95-2	Phenol	0.1[h]
1918-02-1	Picloram	0.5[c]
129-00-0	Pyrene	0.12[a]
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	0.062[a]
122-34-9	Simazine	0.004[c]
100-42-5	Styrene	0.1[c]
118-96-7	TNT (2,4,6-trinitrotoluene)	0.0077[a]
93-72-1	2,4,5-TP (silvex)	0.05[c]
127-18-4	Tetrachloroethylene[b]	0.005[c]

CASRN	Constituent	Standard (mg/L)
108-88-3	Toluene	1[c]
8001-35-2	Toxaphene[b]	0.003[c]
120-82-1	1,2,4-Trichlorobenzene	0.07[c]
71-55-6	1,1,1-Trichloroethane	0.2[c]
79-00-5	1,1,2-Trichloroethane	0.005[c]
79-01-6	Trichloroethylene[e]	0.005[c]
75-69-4	Trichlorofluoromethane	1.2[a]
99-35-4	1,3,5-Trinitrobenzene	0.46[a]
75-01-4	Vinyl chloride[e]	0.002[c]
1330-20-7	Xylenes	10[c]

Constituent Name and Groundwater Quality Standard Notations

[a] The standard is the Human Threshold Toxicant Advisory Concentration ("HTTAC"), calculated using procedures at Appendix A.
[b] The constituent meets the definition of a "carcinogen" at Section 620.110.
[c] The standard is based on the Maximum Contaminant Level ("MCL"), promulgated by U.S. EPA, Office of Water, and Illinois EPA Primary Drinking Water Standards at 35 Ill. Adm. Code 611.
[d] The standard is the Human Nonthreshold Toxicant Advisory Concentration ("HNTAC"), calculated using procedures at Appendix A.
[e] The constituent meets the definition of a "mutagen" at Section 620.110.
[f] The standard is based on the Maximum Contaminant Level Goal ("MCLG"), promulgated by U.S. EPA, Office of Water.
[g] The standard is the "LLOQ" or "LCMRL" as defined in Section 620.110.
[h] The standard in based on 35 Ill. Adm. Code 302.208.

c) ~~Explosive Constituents~~

~~Concentrations of the following explosive constituents must not exceed the Class I groundwater standard:~~

~~Constituent~~	~~Standard (mg/L)~~
~~1,3-Dinitrobenzene~~	~~0.0007~~
~~2,4-Dinitrotoluene*~~	~~0.0001~~
~~2,6-Dinitrotoluene*~~	~~0.00031~~
~~HMX (High Melting Explosive, Octogen)~~	~~1.4~~

~~Nitrobenzene~~	~~0.014~~
~~RDX (Royal Demolition Explosive, Cyclonite)~~	~~0.084~~
~~1,3,5-Trinitrobenzene~~	~~0.84~~
~~2,4,6-Trinitrotoluene (TNT)~~	~~0.014~~

~~*Denotes a carcinogen.~~

~~d)~~ Complex Organic Chemical Mixtures

1) Concentrations of the following chemical constituents ~~of gasoline, diesel fuel, or heating fuel~~ shall ~~must~~ not be exceeded in Class I groundwater:

~~Constituent~~	~~Standard (mg/L)~~
~~Benzene*~~	~~0.005~~
~~BETX~~	~~11.705~~
~~*Denotes a carcinogen.~~	

CASRN	**Constituent**	**Standard (mg/L)**
71-43-2	Benzene[a]	0.005[b]
	Total BETX	11.705[c]

Constituent Name and Groundwater Quality Standard Notations

[a] The constituent meets the definition of a "carcinogen" at Section 620.110.

[b] The standard is based on the Maximum Contaminant Level ("MCL"), promulgated by U.S. EPA, Office of Water, and Illinois EPA Primary Drinking Water Standards at 35 Ill. Adm. Code 611.

[c] The standard is the total combined standard of benzene, ethylbenzene, toluene, and xylenes.

2) Atrazine and Metabolites

The total concentration of Atrazine plus Atrazine metabolites shall be compared to the Atrazine Class I groundwater standard of 0.003 mg/L.

CASRN	**Constituent**	**Standard**

		(mg/L)
1912-24-9	Atrazine	0.003[a]
	Total Atrazine and Metabolites	0.003
6190-65-4	DEA (desethyl-atrazine)	
1007-28-9	DIA (desisopropyl-atrazine)	
3397-62-4	DACT (diaminochlorotriazine)	

Groundwater Quality Standard Notation

[a] The standard is based on the Maximum Contaminant Level ("MCL"), promulgated by U.S. EPA, Office of Water, and Illinois EPA Primary Drinking Water Standards at 35 Ill. Adm. Code 611.

d)~~e)~~ pH
Except due to natural causes, a pH range of 6.5 - 9.0 units shall ~~must~~ not be exceeded in Class I groundwater.

e)~~f)~~ Beta Particle and Photon Radioactivity

1) Except due to natural causes, the average annual concentration of beta particle and photon radioactivity from man-made radionuclides shall not exceed a dose equivalent to the total body organ greater than 4 mrem/year in Class I groundwater. If two or more radionuclides are present, the sum of their dose equivalent to the total body, or to any internal organ shall not exceed 4 mrem/year in Class I groundwater except due to natural causes.

2) Except for the radionuclides listed in subsection (f)(3), the concentration of man-made radionuclides causing 4 mrem total body or organ dose equivalent shall ~~must~~ be calculated on the basis of a 2 liter per day drinking water intake using the 168-hour data in accordance with the procedure set forth in NCRP Report Number 22, incorporated by reference at Section 620.125(a).

3) Except due to natural causes, the average annual concentration assumed to produce a total body or organ dose of 4 mrem/year of the following chemical constituents shall not be exceeded in Class I groundwater:

~~Constituent~~	~~Critical Organ~~	~~Standard (pCi/L)~~
~~Tritium~~	~~Total body~~	~~20,000.0~~

~~Strontium-90 Bone marrow 8.0~~

CASRN	Constituent	Critical Organ	Standard (pCi/L)
10028-17-8	Tritium	Total Body	20,000
10098-97-2	Strontium-90	Bone Marrow	8.0

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.420 Groundwater Quality Standards for Class II: General Resource Groundwater

a) Inorganic Chemical Constituents

1) Except due to natural causes or as provided in Section 620.450 or subsection (a)(3) or (e) of this Section, concentrations of the following chemical constituents shall ~~must~~ not be exceeded in Class II groundwater:

~~Constituent~~	~~Standard (mg/L)~~
~~Antimony~~	~~0.024~~
~~Arsenic*~~	~~0.2~~
~~Barium~~	~~2.0~~
~~Beryllium~~	~~0.5~~
~~Cadmium~~	~~0.05~~
~~Chromium~~	~~1.0~~
~~Cobalt~~	~~1.0~~
~~Cyanide~~	~~0.6~~
~~Fluoride~~	~~4.0~~
~~Lead~~	~~0.1~~
~~Mercury~~	~~0.01~~
~~Nitrate as N~~	~~100.0~~
~~Perchlorate~~	~~0.0049~~
~~Thallium~~	~~0.02~~
~~Vanadium~~	~~0.1~~

~~*Denotes a carcinogen.~~

CASRN	Constituent	Standard (mg/L)
7440-36-0	Antimony	0.024[a]
7440-38-2	Arsenic[b]	0.2[c]
7440-39-3	Barium	2.0[d]
7440-41-7	Beryllium	0.5[e]
7440-43-9	Cadmium	0.05[f]
7440-47-3	Chromium (total)	1.0[f]
7440-48-4	Cobalt	1[c]

CASRN	Constituent	Standard (mg/L)
143-33-9	Cyanide (sodium cyanide)	0.6[c]
7681-49-4	Fluoride (sodium fluoride)	2[c]
7439-92-1	Lead	0.1[c]
7439-93-2	Lithium	2.5[e]
7487-94-7	Mercury (mercuric chloride)	0.01[c]
7439-98-7	Molybdenum	0.05[e]
14797-55-8	Nitrate as N	100[c]
14797-73-0	Perchlorate	0.0081[d]
7440-28-0	Thallium	0.002[g]
7440-62-2	Vanadium	0.1[c]

Constituent Name and Groundwater Quality Standard Notations

[a] A treatment factor of 4 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at an 75% removal efficiency rate for the constituent.

[b] The constituent meets the definition of a "carcinogen" at Section 620.110.

[c] The standard is based on beneficial use for watering livestock, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[d] The Class II standard is equal to the Class I groundwater quality standard.

[e] The standard is based on beneficial use for irrigation of crops, per "*Water Quality Criteria,*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[f] The standard is based on beneficial use for watering livestock and irrigation of crops, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[g] A treatment factor of 10 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at an 90% removal efficiency rate for the constituent.

2) Except as provided in Section 620.450 or subsection (a)(3) or (e) of this Section, concentrations of the following chemical constituents shall ~~must~~ not be exceeded in Class II groundwater:

~~Constituent~~	~~Standard (mg/L)~~
~~Boron~~	~~2.0~~
~~Chloride~~	~~200.0~~
~~Copper~~	~~0.65~~
~~Iron~~	~~5.0~~
~~Manganese~~	~~10.0~~
~~Nickel~~	~~2.0~~
~~Selenium~~	~~0.05~~
~~Total Dissolved Solids (TDS)~~	~~1,200.0~~
~~Sulfate~~	~~400.0~~
~~Zinc~~	~~10.0~~

CASRN	Constituent	Standard[a]
7429-90-5	Aluminum	5[b]
7440-42-8	Boron	2[c]
16887-00-6	Chloride	200[d]
7440-50-8	Copper	0.5[b]
7439-89-6	Iron	5[d]
7439-96-5	Manganese	10[c]
7440-02-0	Nickel	2[c]
7440-14-4	Radium (combined 226+228)	5[e]
7782-49-2	Selenium	0.02[c]
7440-22-4	Silver	0.058[e]
14808-79-8	Sulfate	400[d]
	TDS (total dissolved solids)	1,200[d]
7440-66-6	Zinc	10[c]

Constituent Name and Groundwater Quality Standard Notations

[a] The standard units are milligrams per liter ("mg/L"), except for the radium (combined 226+228) unit of picocuries per liter ("pCi/L").

[b] The standard is based on beneficial use for watering livestock, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[c] The standard is based on beneficial use for irrigation of crops, per "*Water Quality Criteria*", by National Academy of Sciences, incorporated by reference at Section 620.125.

[d] The standard is the 95% confidence concentration stated in Illinois

EPA's "*Integrated Water Quality Report and Section 303(d) List*", incorporated by reference at Section 620.125.

[e] The Class II standard is equal to the Class I groundwater quality standard.

3) The standard for any inorganic chemical constituent listed in subsection (a)(2) of this Section, for barium, or for pH does not apply to groundwater within fill material or within the upper 10 feet of parent material under such fill material on a site not within the rural property class for which:

A) Prior to November 25, 1991, surficial characteristics have been altered by the placement of such fill material so as to impact the concentration of the parameters listed in subsection (a)(3) of this Section, and any on-site groundwater monitoring of such parameters is available for review by the Agency.

B) On November 25, 1991, surficial characteristics are in the process of being altered by the placement of such fill material, that proceeds in a reasonably continuous manner to completion, so as to impact the concentration of the parameters listed in subsection (a)(3) of this Section, and any on-site groundwater monitoring of such parameters is available for review by the Agency.

4) For purposes of subsection (a)(3) of this Section, the term "fill material" means clean earthen materials, slag, ash, clean demolition debris, or other similar materials.

b) Organic Chemical Constituents

1) Except due to natural causes or as provided in Section 620.450 or subsection (b)(2) or (e) of this Section, concentrations of the following organic chemical constituents shall ~~must~~ not be exceeded in Class II groundwater:

~~Constituent~~	~~Standard (mg/L)~~
~~Acenaphthene~~	~~2.1~~
~~Acetone~~	~~6.3~~
~~Alachlor*~~	~~0.010~~
~~Aldicarb~~	~~0.015~~
~~Anthracene~~	~~10.5~~

~~Atrazine~~	~~0.015~~
~~Benzene*~~	~~0.025~~
~~Benzo(a)anthracene*~~	~~0.00065~~
~~Benzo(b)fluoranthene*~~	~~0.0009~~
~~Benzo(k)fluoranthene*~~	~~0.006~~
~~Benzo(a)pyrene*~~	~~0.002~~
~~Benzoic acid~~	~~28.0~~
~~2-Butanone (MEK)~~	~~4.2~~
~~Carbon Disulfide~~	~~3.5~~
~~Carbofuran~~	~~0.2~~
~~Carbon Tetrachloride*~~	~~0.025~~
~~Chlordane*~~	~~0.01~~
~~Chloroform*~~	~~0.35~~
~~Chrysene*~~	~~0.06~~
~~Dalapon~~	~~2.0~~
~~Dibenzo(a,h)anthracene*~~	~~0.0015~~
~~Dicamba~~	~~0.21~~
~~Dichlorodifluoromethane~~	~~7.0~~
~~1,1-Dichloroethane~~	~~7.0~~
~~Dichloromethane*~~	~~0.05~~
~~Di(2-ethylhexyl)phthalate*~~	~~0.06~~
~~Diethyl Phthalate~~	~~5.6~~
~~Di-n-butyl Phthalate~~	~~3.5~~
~~Dinoseb~~	~~0.07~~
~~Endothall~~	~~0.1~~
~~Endrin~~	~~0.01~~
~~Ethylene Dibromide*~~	~~0.0005~~
~~Fluoranthene~~	~~1.4~~
~~Fluorene~~	~~1.4~~
~~Heptachlor*~~	~~0.002~~
~~Heptachlor Epoxide*~~	~~0.001~~
~~Hexachlorocyclopentadiene~~	~~0.5~~
~~Indeno(1,2,3-cd)pyrene*~~	~~0.0022~~
~~Isopropylbenzene (Cumene)~~	~~3.5~~
~~Lindane (Gamma-Hexachloro cyclophexane)~~	~~0.001~~
~~2,4-D~~	~~0.35~~
~~Ortho-Dichlorobenze~~	~~1.5~~
~~Para-Dichlorobenzene~~	~~0.375~~
~~1,2-Dibromo-3-Chloropropane*~~	~~0.002~~
~~1,2-Dichloroethane*~~	~~0.025~~
~~1,1-Dichloroethylene~~	~~0.035~~

~~cis-1,2-Dichloroethylene~~	~~0.2~~
~~Trans-1,2-Dichloroethylene~~	~~0.5~~
~~1,2-Dichloropropane*~~	~~0.025~~
~~Ehylbenzene~~	~~1.0~~
~~MCPP (Mecoprop)~~	~~0.007~~
~~Methoxychlor~~	~~0.2~~
~~2-Methylnaphthalene~~	~~0.14~~
~~2-Methylphenol~~	~~0.35~~
~~Methyl Tertiary-Butyl Ether (MTBE)~~	~~0.07~~
~~Monochlorobenzene~~	~~0.5~~
~~Naphthalene~~	~~0.22~~
~~P-Dioxane*~~	~~0.0077~~
~~Pentachlorophenol*~~	~~0.005~~
~~Phenols~~	~~0.1~~
~~Picloram~~	~~5.0~~
~~Pyrene~~	~~1.05~~
~~Polychlorinated Biphenyls (PCBs) (as decachloro-biphenyl)*~~	~~0.0025~~
~~alpha-BHC (alpha-Benzene hexachloride)*~~	~~0.00055~~
~~Simazine~~	~~0.04~~
~~Styrene~~	~~0.5~~
~~2,4,5-TP~~	~~0.25~~
~~Tetrachloroethylene*~~	~~0.025~~
~~Toluene~~	~~2.5~~
~~Toxaphene*~~	~~0.015~~
~~1,1,1-Trichloroethane~~	~~1.0~~
~~1,2,4-Trichlorobenzene~~	~~0.7~~
~~1,1,2-Trichloroethane~~	~~0.05~~
~~Trichloroethylene*~~	~~0.025~~
~~Trichlorofluoromethane~~	~~10.5~~
~~Vinyl Chloride*~~	~~0.01~~
~~Xylenes~~	~~10.0~~

~~* Denotes a carcinogen.~~

CASRN	Constituent	Standard (mg/L)
83-32-9	Acenaphthene	1.2[a]
67-64-1	Acetone	3.5[b]
15972-60-8	Alachlor[c]	0.01[a]

CASRN	Constituent	Standard (mg/L)
116-06-3	Aldicarb	0.015[a]
120-12-7	Anthracene	6[a]
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)[c]	0.00006[a]
71-43-2	Benzene[c]	0.025[a]
56-55-3	Benzo(a)anthracene[d]	0.0012[a]
205-99-2	Benzo(b)fluoranthene[d]	0.0012[a]
207-08-9	Benzo(k)fluoranthene[d]	0.012[a]
50-32-8	Benzo(a)pyrene[d]	0.002[e]
65-85-0	Benzoic acid	15[b]
78-93-3	2-Butanone (methyl ethyl ketone)	2.3[b]
1563-66-2	Carbofuran	0.2[a]
75-15-0	Carbon disulfide	1.9[a]
56-23-5	Carbon tetrachloride[c]	0.025[a]
12798-03-6	Chlordane[c]	0.01[a]
108-90-7	Chlorobenzene	0.5[a]
67-66-3	Chloroform[c]	0.35[a]
218-01-9	Chrysene[d]	0.12[a]
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)	0.35[a]
75-99-0	Dalapon	2.0[e]
53-70-3	Dibenzo(a,h)anthracene[d]	0.0005[a]
96-12-8	1,2-Dibromo-3-chloropropane[d]	0.002[e]
1918-00-9	Dicamba	0.12[b]
95-50-1	*o*-Dichlorobenzene (1,2-dichlorobenzene)	1.5[f]
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)[c]	0.375[a]
75-71-8	Dichlorodifluoromethane	3.9[a]
75-34-3	1,1-Dichloroethane	3.9[a]
107-06-2	1,2-Dichloroethane[c]	0.025[a]
75-35-4	1,1-Dichloroethylene	0.035[a]
156-59-2	*cis*-1,2-Dichloroethylene	0.2[g]
156-60-5	*trans*-1,2-Dichloroethylene	0.5[a]
75-09-2	Dichloromethane (methylene chloride)[d]	0.025[a]
78-87-5	1,2-Dichloropropane[b]	0.025[a]
117-81-7	Di(2-ethylhexyl)phthalate[b]	0.06[e]
84-66-2	Diethyl phthalate	3.1[b]
84-74-2	Di-*n*-butyl phthalate	1.9[a]
99-65-0	1,3-Dinitrobenzene	0.001[b]
121-14-2	2,4-Dinitrotoluene[c]	0.005[a]

CASRN	Constituent	Standard (mg/L)
606-20-2	2,6-Dinitrotoluene[c]	0.005[a]
88-85-7	Dinoseb	0.07[e]
123-91-1	1,4-Dioxane (*p*-dioxane)[c]	0.00078[b]
145-73-3	Endothall	0.1[b]
72-20-8	Endrin	0.01[a]
100-41-4	Ethylbenzene[c]	1.0[h]
106-93-4	Ethylene dibromide (1,2-dibromoethane)[c]	0.0005[e]
206-44-0	Fluoranthene	0.75[a]
86-73-7	Fluorene	0.75[a]
58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)[c]	0.001[a]
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)	0.000012[b]
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)	3.9[a]
76-44-8	Heptachlor[c]	0.002[a]
1024-57-3	Heptachlor epoxide[c]	0.001[a]
77-47-4	Hexachlorocyclopentadiene	0.5[e]
193-39-5	Indeno(1,2,3-c,d)pyrene[d]	0.0012[a]
98-82-8	Isopropylbenzene (cumene)[c]	1.9[a]
93-65-2	MCPP (mecoprop)	0.1[b]
1634-04-4	MTBE (methyl tertiary-butyl ether)	0.5[e]
72-43-5	Methoxychlor	0.2[a]
90-12-0	1-Methylnaphthalene	0.27[b]
91-57-6	2-Methylnaphthalene	0.015[b]
95-48-7	2-Methylphenol (*o*-cresol)	0.19[b]
91-20-3	Naphthalene	0.39[a]
98-95-3	Nitrobenzene	0.0077[b]
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)[c]	0.0025[a]
375-73-5	PFBS (perfluorobutanesulfonic acid)	0.0012[b]
355-46-4	PFHxS (perfluorohexanesulfonic acid)	0.000077[b]
375-95-1	PFNA (perfluorononanoic acid)	0.000012[b]
335-67-1	PFOA (perfluorooctanoic acid)[c]	0.000002[b]
1763-23-1	PFOS (perfluorooctanesulfonic acid)	0.0000077[b]
87-86-5	Pentachlorophenol	0.005[a]
108-95-2	Phenol	0.1[i]
1918-02-1	Picloram	5.0[e]

CASRN	Constituent	Standard (mg/L)
129-00-0	Pyrene	0.6[a]
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	0.062[b]
122-34-9	Simazine	0.04[e]
100-42-5	Styrene	0.5[a]
118-96-7	TNT (2,4,6-trinitrotoluene)	0.039[a]
93-72-1	2,4,5-TP (silvex)	0.25[a]
127-18-4	Tetrachloroethylene[c]	0.025[a]
108-88-3	Toluene	2.5[f]
8001-35-2	Toxaphene[c]	0.015[a]
120-82-1	1,2,4-Trichlorobenzene	0.7[e]
71-55-6	1,1,1-Trichloroethane	1[a]
79-00-5	1,1,2-Trichloroethane	0.05[e]
79-01-6	Trichloroethylene[d]	0.025[a]
75-69-4	Trichlorofluoromethane	6[a]
99-35-4	1,3,5-Trinitrobenzene	2.3[a]
75-01-4	Vinyl chloride[d]	0.01[a]
1330-20-7	Xylenes	10[b]

Constituent Name and Groundwater Quality Standard Notations

[a] A treatment factor of 5 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at an 80% removal efficiency rate for the constituent.

[b] Illinois EPA's treatment efficiency determination demonstrates a treatment factor is not applicable for the constituent. The standard is equal to the Class I groundwater quality standard.

[c] The constituent meets the definition of a "carcinogen" at Section 620.110.

[d] The constituent meets the definition of a "mutagen" at Section 620.110.

[e] A treatment factor of 10 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at a 90% removal efficiency rate for the constituent.

[f] A treatment factor of 2.5 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at a 60% removal efficiency rate for the constituent.

[g] A treatment factor of 3 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at a 65% removal efficiency rate for the constituent.

[h] A treatment factor of 1.5 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at a 30% removal efficiency rate for the constituent.

[i] The standard in based on 35 Ill. Adm. Code 302.208.

2) The standards for pesticide chemical constituents listed in subsection (b)(1) of this Section do not apply to groundwater within 10 feet of the land surface, provided that the concentrations of such constituents result from the application of pesticides in a manner consistent with the requirements of the Federal Insecticide, Fungicide and Rodenticide Act (7 USC 136 et seq.), and the Illinois Pesticide Act [415 ILCS 60].

c) ~~Explosive Constituents~~

~~Concentrations of the following explosive constituents must not exceed the Class II groundwater standard:~~

~~Constituent~~	~~Standard (mg/L)~~
~~1,3-Dinitrobenzene~~	~~0.0007~~
~~2,4-Dinitrotoluene*~~	~~0.0001~~
~~2,6-Dinitrotoluene*~~	~~0.00031~~
~~HMX (High Melting Explosive, Octogen)~~	~~1.4~~
~~Nitrobenzene~~	~~0.014~~
~~RDX (Royal Demolition Explosive, Cyclonite)~~	~~0.084~~
~~1,3,5-Trinitrobenzene~~	~~0.84~~
~~2,4,6-Trinitrotoluene (TNT)~~	~~0.014~~

~~*Denotes a carcinogen.~~

~~d)~~ Complex Organic Chemical Mixtures

1) Concentrations of the following organic chemical constituents ~~of gasoline, diesel fuel, or heating fuel~~ shall ~~must~~ not be exceeded in Class II groundwater:

~~Constituent~~ ~~Standard (mg/L)~~

~~Benzene*~~ ~~0.025~~
~~BETX~~ ~~13.525~~

~~*Denotes a carcinogen~~

CASRN	Constituent	Standard (mg/L)
71-43-2	Benzene[a]	0.025[b]
	Total BETX	13.525[c]

Constituent Name and Groundwater Quality Standard Notations

[a] The constituent meets the definition of a "carcinogen" at Section 620.110.

[b] A treatment factor of 5 is applied to the Class I groundwater quality standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at an 80% removal efficiency rate for the constituent.

[c] The standard is the total combined Class II standard of benzene, ethylbenzene, toluene, and xylenes.

2) Atrazine and Metabolites

The total concentration of Atrazine plus Atrazine metabolites shall be compared to the atrazine Class I groundwater standard of 0.015 mg/L.

CASRN	Constituent	Standard (mg/L)
1912-24-9	Atrazine	0.015[a]
	Total Atrazine and Metabolites	0.015
6190-65-4	DEA (desethyl-atrazine)	
1007-28-9	DIA (desisopropyl-atrazine)	
3397-62-4	DACT (diaminochlorotriazine)	

Constituent Name and Groundwater Quality Standard Notations:

[a] A treatment factor of 5 is applied to the Class I groundwater quality

standard. The constituent's treatment efficiency is based on the effectiveness to treat the constituent in the groundwater at an 80% removal efficiency rate for the constituent.

de) pH

Except due to natural causes, a pH range of 6.5 - 9.0 units shall ~~must~~ not be exceeded in Class II groundwater that is within 5 feet of the land surface.

(Source: Amended at ~~37 Ill. Reg. 16529~~, effective ~~October 7, 2013~~)

Section 620.430 Groundwater Quality Standards for Class III: Special Resource Groundwater

Except due to natural causes, concentrations ~~Concentrations of~~ inorganic and organic chemical constituents shall ~~must~~ not exceed the standards set forth in Section 620.410, except for ~~those~~:

a) The chemical constituents for which the Board has adopted a standard pursuant to Section 620.260; and

b) The following standards set forth below for Class III Special Resource Groundwater established in accordance with Section 620.230(b) and depicted in the Environmental Register as indicated for each nature preserve.

1) The following standards are applicable for Pautler Cave Nature Preserve and Stemler Cave Nature Preserve (Environmental Register, May 2005, Num. 611), Fogelpole Cave Nature Preserve (Environmental Register May 2003, Num. 587), and Armin Krueger Speleological Nature Preserve (Environmental Register, December 2009, Num. 666):

Chloride	20 mg/L
pH	range of 7.0-9.0 Standard Units

2) The following standard is applicable for Cotton Creek Marsh Nature Preserve and Spring Grove Fen Nature Preserve (Environmental Register, July 2012, Num 697):

Chloride	45 mg/L

(Source: Amended at __ Ill. Reg. _____, effective _____)

Section 620.440 Groundwater Quality Standards for Class IV: Other Groundwater

a) Except as provided in subsection (b) or (c), Class IV: Other Groundwater standards are equal to the existing concentrations of constituents in groundwater.

b) For groundwater within a zone of attenuation as provided in 35 Ill. Adm. Code 811 and 814, the standards specified in Section 620.420 shall ~~must~~ not be exceeded, except for concentrations of contaminants within leachate released from a permitted unit.

c) For groundwater within a previously mined area, the standards set forth in Section 620.420 shall ~~must~~ not be exceeded, except for concentrations of TDS, chloride, iron, manganese, sulfates, pH, 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine~~high melting explosive, octogen~~), nitrobenzene, RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine~~royal demolition explosive, cyclonite~~), 1,3,5-trinitrobenzene, or TNT (2,4,6-trinitrotoluene ~~(TNT~~). For concentrations of TDS, chloride, iron, manganese, sulfates, pH, 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, HMX, nitrobenzene, RDX, 1,3,5-trinitrobenzene, or ~~2,4,6-trinitrotoluene (~~TNT~~)~~, the standards are the existing concentrations.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.450 Alternative Groundwater Quality Standards

a) Groundwater Quality Restoration Standards

1) Any chemical constituent in groundwater within a groundwater management zone is subject to this Section.

2) Except as provided in subsections (a)(3) or (a)(4), the standards as specified in Sections 620.410, 620.420, 620.430, and 620.440 apply to any chemical constituent in groundwater within a groundwater management zone.

3) Prior to completion of a corrective action described in Section 620.250(a), the standards as specified in Sections 620.410, 620.420, 620.430, and 620.440 are not applicable to such released chemical constituent, provided that the initiated action proceeds in a timely and appropriate manner.

4) After completion of a corrective action as described in Section 620.250(a), the standard for such released chemical constituent is:

A) The standard as set forth in Section 620.410, 620.420, 620.430, or 620.440, if the concentration as determined by groundwater monitoring of such constituent is less than or equal to the standard for the appropriate class set forth in those Sections; or

B) The concentration as determined by groundwater monitoring, if such concentration exceeds the standard for the appropriate class set forth in Section 620.410, 620.420, 620.430, or 620.440 for such constituent, and:

i) To the extent practicable, the exceedence has been minimized and beneficial use, as appropriate for the class of groundwater, has been returned; and

ii) Any threat to public health or the environment has been minimized.

5) The Agency shall develop and maintain a listing of concentrations derived pursuant to subsection (a)(4)(B). This list shall be made available to the public and be updated periodically, but no less frequently than semi-annually. This listing shall be published in the Environmental Register.

b) Coal Reclamation Groundwater Quality Standards

1) Any inorganic chemical constituent or pH in groundwater, within an underground coal mine, or within the cumulative impact area of groundwater for which the hydrologic balance has been disturbed from a permitted coal mine area pursuant to the Surface Coal Mining Land Conservation and Reclamation Act [225 ILCS 720] and 62 Ill. Adm. Code 1700 through 1850, is subject to this Section.

2) Prior to completion of reclamation at a coal mine, the standards as specified in Sections 620.410(a) and (e), 620.420(a) and (e), 620.430, and 620.440 are not applicable to inorganic constituents and pH.

3) After completion of reclamation at a coal mine, the standards as specified in Sections 620.410(a) and (e), 620.420(a), 620.430, and 620.440 are applicable to inorganic constituents and pH, except:

A) The concentration of total dissolved solids ("TDS") shall ~~must~~ not exceed:

i) The post-reclamation concentration or 3000 mg/L, whichever is less, for groundwater within the permitted area; or

ii) The post-reclamation concentration of TDS shall ~~must~~ not exceed the post-reclamation concentration or 5000 mg/L, whichever is less, for groundwater in underground coal mines and in permitted areas reclaimed after surface coal mining if the Illinois Department of Mines and Minerals and the Agency have determined that no significant resource groundwater existed prior to mining (62 Ill. Adm. Code 1780.21(f) and (g)); and

B) For chloride, iron, manganese, and sulfate, the post-reclamation concentration within the permitted area shall ~~must~~ not be exceeded.

C) For pH, the post-reclamation concentration within the permitted area shall ~~must~~ not be exceeded within Class I: Potable Resource Groundwater as specified in Section 620.210(a)(4).

D) For 1,3-dinitrobenzene, 2,4-dinitrotoluene, 2,6-dinitrotoluene, HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine~~high melting explosive, octogen~~), nitrobenzene, RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine~~royal demolition explosive, cyclonite~~), 1,3,5-trinitrobenzene, and TNT (2,4,6-trinitrotoluene ~~(TNT)~~, the post-reclamation concentration within the permitted area shall ~~must~~ not be exceeded.

4) A refuse disposal area (not contained within the area from which overburden has been removed) is subject to the inorganic chemical constituent and pH requirements of:

A) 35 Ill. Adm. Code 302.Subparts B and C, except due to natural causes, for such area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing;

B) Section 620.440(c) for such area that was placed into operation prior to February 1, 1983, and has remained in continuous operation since that date; or

C) Subpart D of this Part for such area that is placed into operation on or after the effective date of this Part.

5) For a refuse disposal area (not contained within the area from which overburden has been removed) that was placed into operation prior to February 1, 1983, and is modified after that date to include additional area, this Section applies to the area that meets the requirements of subsection (b)(4)(C) and the following applies to the additional area:

A) 35 Ill. Adm. Code 302.Subparts B and C, except due to natural causes, for such additional refuse disposal area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing; and

B) Subpart D for such additional area that was placed into operation on or after the effective date of this Part.

6) A coal preparation plant (not located in an area from which overburden has been removed) which contains slurry material, sludge, or other

precipitated process material, is subject to the inorganic chemical constituent and pH requirements of:

A) 35 Ill. Adm. Code 302.Subparts B and C, except due to natural causes, for such plant that was placed into operation after February 1, 1983 and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing;

B) Section 620.440(c) for such plant that was placed into operation prior to February 1, 1983, and has remained in continuous operation since that date; or

C) Subpart D for such plant that is placed into operation on or after the effective date of this Part.

7) For a coal preparation plant (not located in an area from which overburden has been removed) which contains slurry material, sludge or other precipitated process material, that was placed into operation prior to February 1, 1983, and is modified after that date to include additional area, this Section applies to the area that meets the requirements of subsection (b)(6)(C) and the following applies to the additional area:

A) 35 Ill. Adm. Code 302.Subparts B and C, except due to natural causes, for such additional area that was placed into operation after February 1, 1983, and before the effective date of this Part, provided that the groundwater is a present or a potential source of water for public or food processing; and

B) Subpart D for such additional area that was placed into operation on or after the effective date of this Part.

c) Groundwater Quality Standards for Certain Groundwater Subject to a No Further Remediation Letter under Part 740. While a No Further Remediation Letter is in effect for a region formerly encompassed by a groundwater management zone established under 35 Ill. Adm. Code 740.530, the groundwater quality standards for "contaminants of concern", as defined in 35 Ill. Adm. Code 740.120, within such area shall be the groundwater objectives achieved as documented in the approved Remedial Action Completion Report.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.510 Monitoring and Analytical Requirements

a) Representative Samples
A representative sample shall be taken from locations as specified in Section 620.505.

b) Sampling and Analytical Procedures

1) Samples shall ~~must~~ be collected in accordance with the procedures set forth in the documents pertaining to groundwater monitoring and analysis ~~"Methods for Chemical Analysis of Water and Wastes," "Methods for the Determination of Inorganic Substances in Environmental Samples," "Methods for the Determination of Metals in Environmental Samples," "Methods for the Determination of Organic Compounds in Drinking Water," "Methods for the Determination or Organic Compounds in Drinking Water, Supplement I," "Methods for the Determination of Organic Compounds in Drinking Water, Supplement II," "Methods for the Determination of Organic Compounds in Drinking Water, Supplement III," "Methods for the Determination of Organic and Inorganic Compounds in Drinking Water," "Prescribed Procedures for Measurement of Radioactivity in Drinking Water," "Procedures for Radiochemical Analysis of Nuclear Reactor Aqueous Solutions," "Radiochemical Analytical Procedures for Analysis of Environmental Samples," "Radiochemistry Procedures Manual," "Practical Guide for Ground Water Sampling," "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods" (SW-846), 40 CFR 136, appendix B, 40 CFR 141.80, 40 CFR 141.61, and 40 CFR 141.62, "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground Water Samples for Selected Unstable Constituents," "Practical Guide for Ground-Water Sampling," "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected Unstable Constituents,"~~ incorporated by reference at Section 620.125 or other procedures adopted by the appropriate regulatory agency.

2) Groundwater elevation in a groundwater monitoring well shall ~~must~~ be determined and recorded when necessary to determine the gradient.

3) Unless specified otherwise by regulations, statistical methods used to determine naturally occurring groundwater quality background

concentrations of contaminants shall be conducted in accordance with "Statistical Analysis of Groundwater Monitoring Data at RCRA Facilities, (March 2009 Unified Guidance)," as incorporated by reference in Section 620.125 for use with prediction limits and all other statistical tests including, but not limited to, confidence limits and control charts.

43) The analytical methodology used for the analysis of constituents in Subparts C and D shall ~~must~~ be consistent with both of the following:

A) The methodology shall ~~must~~ have a LLOQ or LCMRL~~PQL~~ at or below the preventive response levels of Subpart C or groundwater standard set forth in Subpart D, whichever is applicable; and

B) "Methods for Chemical Analysis of Water and Wastes," "Methods for the Determination of Inorganic Substances in Environmental Samples," "Methods for the Determination of Metals in Environmental Samples," "Methods for the Determination of Organic Compounds in Drinking Water," "Methods for the Determination of Organic Compounds in Drinking Water, Supplement I," "Methods for the Determination of Organic Compounds in Drinking Water, Supplement II," "Methods for the Determination of Organic Compounds in Drinking Water, Supplement III," "Methods for the Determination of Organic and Inorganic Compounds in Drinking Water," "Prescribed Procedures for Measurement of Radioactivity in Drinking Water," "Procedures for Radiochemical Analysis of Nuclear Reactor Aqueous Solutions," "Radiochemical Analytical Procedures for Analysis of Environmental Samples," "Radiochemistry Procedures Manual," "Practical Guide for Ground Water Sampling," "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods" (SW-846), 40 CFR 136, appendix B, 40 CFR 141.80, 40 CFR 141.61, and 40 CFR 141.62, "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground Water Samples for Selected Unstable Constituents," "Practical Guide for Ground-Water Sampling", "Techniques of Water Resources Investigations of the United States Geological Survey, Guidelines for Collection and Field Analysis of Ground-Water Samples for Selected Unstable Constituents", or other procedures incorporated by reference at Section 620.125.

c) Reporting Requirements

At a minimum, groundwater monitoring analytical results shall ~~must~~ include information, procedures, and techniques for:

1) Sample collection (including but not limited to name of sample collector, time and date of the sample, method of collection, and identification of the monitoring location);

2) Sample preservation and shipment (including but not limited to field quality control);

3) Analytical procedures (including but not limited to the MDL, LLOQ, ~~method detection limits and~~ or the LCMRL~~PQLs~~); and

4) Chain of custody control.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

SUBPART F: HEALTH ADVISORIES

Section 620.601 Purpose of a Health Advisory

This Subpart establishes procedures for the issuance of a Health Advisory that sets forth guidance levels that, in the absence of standards under Section 620.410, shall ~~must~~ be considered by the Agency in:

a) Establishing groundwater cleanup or action levels whenever there is a release or substantial threat of a release of:

1) A hazardous substance or pesticide; or

2) Other contaminant that represents a significant hazard to public health or the environment.

b) Determining whether the community water supply is taking its raw water from a site or source consistent with the siting and source water requirements of 35 Ill. Adm. Code 604.200~~611.114 and 611.115~~.

c) Developing Board rulemaking proposals for new or revised numerical standards.

d) Evaluating mixtures of chemical substances.

(Source: Amended at, effective)

Section 620.605 Issuance of a Health Advisory

a) The Agency shall issue a Health Advisory for a chemical substance if all of the following conditions are met:

1) A community water supply well is sampled and a substance is detected and confirmed by resampling;

2) There is no standard under Section 620.410 for such chemical substance; and

3) The chemical substance is toxic or harmful to human health according to the procedures of Appendix A, B, or C.

b) The Health Advisory shall ~~must~~ contain a general description of the characteristics of the chemical substance, the potential adverse health effects, and a guidance level to be determined as follows:

1) If disease or functional impairment is caused due to a physiological mechanism for where there is a threshold dose below which no damage occurs, the guidance level for any such substance shall be the Maximum Contaminant Level Goal ("MCLG"), adopted by U.S. EPA for such substance, 40 CFR 136, appendix B, 40 CFR 141.80, 40 CFR 141.61, and 40 CFR 141.62, incorporated by reference at Section 620.125. If there is no MCLG for the substance, the guidance level is either the Human Threshold Toxicant Advisory Concentration or the Human Nonthreshold Toxicant Advisory Concentration for such substance as determined in accordance with Appendix A, whichever is less, unless the lower concentration for such substance is less than the lowest appropriate LLOQ ~~PQL~~ specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods", EPA Publication No. SW-846 (SW-846), incorporated by reference at Section 620.125, or the LCMRL specified in the drinking water methods incorporated by reference at Section 620.125 for the substance. If the concentration for such substance is less than the lowest appropriate LLOQ or LCMRL~~PQL~~ for the substance ~~specified in SW-846~~, incorporated by reference at Section 620.125, the guidance level is the lowest appropriate LLOQ or LCMRL~~PQL~~.

~~2) If the chemical substance is a carcinogen, the guidance level for any such chemical substance is the one-in-one-million cancer risk concentration, unless the concentration for such substance is less than the lowest~~

~~appropriate PQL specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods," EPA Publication No. SW-846 (SW-846), incorporated by reference at Section 620.125 for such substance. If the concentration for such substance is less than the lowest appropriate PQL for the substance specified in SW-846, the guidance level is the lowest appropriate PQL. The one-in-one-million cancer risk concentration, the Human Nonthreshold Toxicant Advisory Concentration (HNTAC), shall be determined according to the following equation:~~

$$\frac{HNTAC}{(mg/L)} = \frac{TR \times BW \times AT \times 365\ days/year}{SFo \times IR \times EF \times ED}$$

~~Where:~~

~~TR = Target Risk = 1.0E-06~~
~~BW = Body Weight = 70 kg~~
~~AT = Averaging Time = 70 years~~
~~SFo = Oral Slope Factor = Chemical-specific~~
~~IR = Daily Water Ingestion Rate = 2 liters/day~~
~~EF = Exposure Frequency = 350 days/year~~
~~ED = Exposure Duration = 30 years~~

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.APPENDIX A Procedures for Determining Human ~~Threshold~~ Toxicant Advisory Concentrations for Class I: Potable Resource Groundwater

a) Calculating the Human Threshold Toxicant Advisory Concentration for Noncancer Effects

For those substances for which U.S. EPA has not adopted a Maximum Contaminant Level Goal ("MCLG"), the Human Threshold Toxicant Advisory Concentration is calculated as follows:

$$HTTAC = \frac{RSC \bullet ADE}{W}$$

~~$$HTTAC = \frac{RSCxADE}{W}$$~~

Where:

HTTAC = Human Threshold Toxicant Advisory Concentration in milligrams per liter ("mg/L");

RSC = Relative contribution of the amount of the exposure to a chemical via drinking water when compared to the total exposure to that chemical from all sources. Valid chemical-specific data shall be used if available. If valid chemical-specific data are not available, a value of 20% (= 0.20) shall ~~must~~ be used;

ADE = Acceptable Daily Exposure of substance in milligrams per day ("mg/d") as determined pursuant to subsection (b); and

W = Per capita daily water consumption for a child (0-6 years of age, equal to 0.78~~2~~ liters per day ("L/d").

b) Procedures for Determining Acceptable Daily Exposures for Class I: Potable Resource Groundwater

1) The Acceptable Daily Exposure ("ADE") represents the maximum amount of a threshold toxicant in milligrams per day ("mg/d"), which if ingested daily by a child from 0 to 6 years of age~~for a lifetime~~ results in no adverse

effects ~~to humans~~. Subsections (b)(2) through (b)(6) list, in prescribed order, methods for determining the ADE in Class I: Potable Resource Groundwater.

2) ~~For those substances for which the USEPA has derived a Verified Oral Reference Dose for humans, USEPA's Reference Dose given in milligrams per kilogram per day (mg/kg/d), as determined in accordance with methods provided in National Primary and Secondary Drinking Water Regulations, 40 CFR 136, appendix B, 40 CFR 141.80, 40 CFR 141.61, and 40 CFR 141.62, incorporated by reference at Section 620.125, must be used. The ADE equals the product of multiplying the Reference Dose by 70 kilograms (kg), which is the assumed average weight of an adult human.~~ For those substances for which noncancer toxicity values have been derived and presented in units of milligrams per kilogram per day ("mg/kg/day"), the ADE equals the product of multiplying the toxicity value by 15 kilograms ("kg"), which is the assumed average weight of a child 0 to 6 years of age.

3) ~~For those substances for which a no observed adverse effect level for humans (NOAEL-H) exposed to the substance has been derived, the ADE equals the product of multiplying one-tenth of the NOAEL-H given in milligrams of toxicant per kilogram of body weight per day (mg/kg/d) by the average weight of an adult human of 70 kilograms (kg). If two or more studies are available, the lowest NOAEL-H must be used in the calculation of the ADE.~~ For those substances for which an oral reference dose is not available, the ADE equals the value of the most sensitive Point of Departure ("POD") as determined by Benchmark Dose Modeling or the NOAEL/LOAEL approach consistent with current U.S. EPA RfD guidance, followed by the derivation of a Human Equivalent Dose ("HED") using physiologically based pharmacokinetic ("PBPK") modeling or Dose Adjustment Factor ("DAF"), then divided by the total Uncertainty Factor ("UF") and modifying factor ("MF"), if applicable. The value is then multiplied by 15 kg (the assumed average weight of a child 0 – 6 years of age). The equation is depicted below:

$$ADE = \frac{POD}{Total\ UF} \bullet 15kg$$

4) ~~For those substances for which only a lowest observed adverse effect level for humans (LOAEL-H) exposed to the substance has been derived, one-tenth the LOAEL-H must be substituted for the NOAEL-H in subsection~~

~~(b)(3).~~ Uncertainty Factors shall be applied to the Point of Departure ("POD") in increments of 1, 3, or 10, not to exceed a total UF of 10,000, and shall ~~must~~ be used consistent with U.S. EPA guidance. A composite UF of 3 and 10 shall be expressed as 30 whereas a composite UF of 3 and 3 shall be expressed as 10. UFs may be used to account for the following:

A) Interspecies Variability

B) Intraspecies Variability

C) Lowest Observable Adverse Effects Level ("LOAEL") to No Observed Adverse Effects Level ("NOAEL") Uncertainty

D) Database Deficiencies

E) Subchronic to Chronic Duration

~~5) For those substances for which a no observed adverse effect level has been derived from studies of mammalian test species (NOAEL-A) exposed to the substance, the ADE equals the product of multiplying 1/100 of the NOAEL-A given in milligrams toxicant per kilogram of test species weight per day (mg/kg/d) by the average weight of an adult human of 70 kilograms (kg). Preference will be given to animal studies having High Validity, as defined in subsection (c), in the order listed in that subsection. Studies having a Medium Validity must be considered if no studies having High Validity are available. If studies of Low Validity must be used, the ADE must be calculated using 1/1000 of the NOAEL-A having Low Validity instead of 1/100 of the NOAEL-A of High or Medium Validity, except as described in subsection (b)(6). If two or more studies among different animal species are equally valid, the lowest NOAEL-A among animal species must be used in the calculation of the ADE. Additional considerations in selecting the NOAEL-A include:~~

~~A) If the NOAEL-A is given in milligrams of toxicant per liter of water consumed (mg/L), prior to calculating the ADE the NOAEL-A must be multiplied by the average daily volume of water consumed by the mammalian test species in liters per day (L/d) and divided by the average weight of the mammalian test species in kilograms (kg).~~

~~B) If the NOAEL-A is given in milligrams of toxicant per kilogram of~~

~~food consumed (mg/kg), prior to calculating the ADE, the NOAEL-A must be multiplied by the average amount in kilograms of food consumed daily by the mammalian test species (kg/d) and divided by the average weight of the mammalian test species in kilograms (kg).~~

~~C) If the mammalian test species was not exposed to the toxicant each day of the test period, the NOAEL-A must be multiplied by the ratio of days of exposure to the total days of the test period.~~

~~D) If more than one equally valid NOAEL-A is available for the same mammalian test species, the best available data must be used.~~

~~6) For those substances for which a NOAEL-A is not available but the lowest observed adverse effect level (LOAEL-A) has been derived from studies of mammalian test species exposed to the substance, one-tenth of the LOAEL-A may be substituted for the NOAEL-A in subsection (b)(5). The LOAEL-A must be selected in the same manner as that specified in subsection (b)(5). One-tenth the LOAEL-A from a study determined to have Medium Validity may be substituted for a NOAEL-A in subsection (b)(3) if the NOAEL-A is from a study determined to have Low Validity, or if the toxicity endpoint measured in the study having the LOAEL-A of Medium Validity is determined to be more biologically relevant than the toxicity endpoint measured in the study having the NOAEL-A of Low Validity.~~

c) Procedures for Establishing Validity of Data from Animal Studies

1) High Validity Studies

A) High validity studies use a route of exposure by ingestion or gavage, and are based upon:

i) Data from animal carcinogenicity studies with a minimum of 2 dose levels and a control group, 2 species, both sexes, with 50 animals per dose per sex, and at least 50 percent survival at 15 months in mice and 18 months in rats and at least 25 percent survival at 18 months in mice and 24 months in rats;

ii) Data from animal chronic studies with a minimum of 3 dose levels and a control group, 2 species, both sexes, with

40 animals per dose per sex, and at least 50 percent survival at 15 months in mice and 18 months in rats and at least 25 percent survival at 18 months in mice and 24 months in rats, and a well-defined NOAEL; or

iii) Data from animal subchronic studies with a minimum of 3 dose levels and control, 2 species, both sexes, 4 animals per dose per sex for non-rodent species or 10 animals per dose per sex for rodent species, a duration of at least 5% of the test species' lifespan, and a well-defined NOAEL.

B) Supporting studies which reinforce the conclusions of a study of Medium Validity may be considered to raise such a study to High Validity.

2) Medium Validity Studies
Medium validity studies are based upon:

A) Data from animal carcinogenicity, chronic, or subchronic studies in which minor deviations from the study design elements required for a High Validity Study are found, but which otherwise satisfy the standards for a High Validity Study;

B) Data from animal carcinogenicity and chronic studies in which at least 25 percent survival is reported at 15 months in mice and 18 months in rats (a lesser survival is permitted at the conclusion of a longer duration study, but the number of surviving animals should not fall below 20 percent per dose per sex at 18 months for mice and 24 months for rats), but which otherwise satisfy the standards for a High Validity Study;

C) Data from animal subchronic or chronic studies in which a Lowest Observable Adverse Effect Level (LOAEL) is determined, but which otherwise satisfy the standards for a High Validity Study; or

D) Data from animal subchronic or chronic studies which have an inappropriate route of exposure (for example, intraperitoneal injection or inhalation) but which otherwise satisfy the standards for a High Validity Study, with correction factors for conversion to the oral route.

3) Low Validity Studies

Low validity studies are studies not meeting the standards set forth in subsection (c)(1) or (c)(2).

d) Calculating a Human Nonthreshold Toxicant Advisory Concentration ("HNTAC") for Cancer Risk

The Human Nonthreshold Toxicant Advisory Concentration ("HNTAC") is calculated as follows:

1) For chemicals designated by U.S. EPA as "mutagens," the HNTAC is calculated as follows:

$$HNTAC = \frac{TR \bullet \left(AT \bullet 365 \frac{days}{year}\right)}{SF_o \bullet IFWM_{adj}}$$

Where:

HNTAC	=	Human Nonthreshold Toxicant Advisory Concentration, equal to milligrams per liter (mg/L)
TR	=	Target Cancer Risk, equal to one-in-one million cancer risk (1E-06)
AT	=	Averaging Time, equal to 70 years
SF_o	=	Oral Slope Factor (chemical-specific), equal to $(mg/kg\text{-}day)^{-1}$
$IFWM_{adj}$	=	Age-Adjusted Mutagenic Drinking Water Ingestion Rate, equal to 1,019.9 liters per kilogram (L/kg)

2) For chemicals not designated by U.S. EPA as "mutagens," the HNTAC is calculated as follows:

$$HNTAC = \frac{TR \bullet \left(AT \bullet 365 \frac{days}{year}\right)}{SF_o \bullet IFW_{adj}}$$

Where:

HNTAC	=	Human Nonthreshold Toxicant Advisory Concentration, equal to milligrams per liter (mg/L)
TR	=	Target Cancer Risk, equal to one-in-one million cancer risk (1E-06)
AT	=	Averaging Time, equal to 70 years
SF_o	=	Oral Slope Factor (chemical-specific), equal to $(mg/kg\text{-}day)^{-1}$
IFW_{adj}	=	Age-Adjusted Drinking Water Ingestion Rate, equal to 327.95 liters per kilogram (L/kg)

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.APPENDIX B Procedures for Determining Hazard Indices for Class I: Potable Resource Groundwater for Mixtures of Similar-Acting Substances

a) This appendix describes procedures for evaluating mixtures of similar-acting substances which may be present in Class I: Potable Resource Groundwaters. Except as provided otherwise in subsection (c), subsections (d) through (h) describe the procedure for determining the Hazard Index for mixtures of similar-acting substances.

b) For the purposes of this appendix, a "mixture" means two or more substances which are present in Class I: Potable Resource Groundwater which may or may not be related either chemically or commercially, but which are not complex mixtures of related isomers and congeners which are produced as commercial products (for example, PCBs or technical grade chlordane).

c) The following substances listed in Appendix E~~Section 620.410~~ are ~~mixtures of similar actings~~similar-acting substances:

~~1) Mixtures of ortho-Dichlorobenzene and para-Dichlorobenzene. The Hazard Index (HI) for such mixtures is determined as follows:~~

~~HI = [ortho-Dichlorobenzene]/0.6 + [para-Dichlorobenzene]/0.075~~

~~2) Mixtures of 1,1-Dichloroethylene and 1,1,1-trichloroethane. The Hazard Index (HI) for such mixtures is determined as follows:~~

~~HI = [1,1-Dichloroethylene]/0.007 + [1,1,1-trichloroethane]/0.2~~

d) When two or more substances occur together in a mixture, the additivity of the toxicities of some or all of the substances will be considered when determining health-based standards for Class I: Potable Resource Groundwater. This is done by the use of a dose addition model with the development of a Hazard Index for the mixture of substances with similar-acting toxicities. This method does not address synergism or antagonism. Guidelines for determining when the dose addition of similar-acting substances is appropriate are presented in Appendix C. The Hazard Index is calculated as follows:

$$HI = [A]/ALA + [B]/ALB + \ldots [I]/ALI$$

Where:

HI	=	Hazard Index, unitless.
[A], [B], [I]	=	Concentration of each similar-acting substance in groundwater in milligrams per liter ("mg/L").
ALA, ALB, ALI	=	The acceptable level of each similar-acting substance in the mixture in milligrams per liter ("mg/L").

e) For substances that are considered to have a threshold mechanism of toxicity, the acceptable level is:

1) The standards listed in Section 620.410; or

2) For those substances for which standards have not been established in Section 620.410, the Human Threshold Toxicant Advisory Concentration ("HTTAC") as determined in Appendix A.

f) For substances that are carcinogens, the acceptable level is:

1) The standards listed in Section 620.410; or

2) For those substances for which standards have not been established under Section 620.410, the one-in-one-million cancer risk concentration, unless the concentration for such substance is less than the lowest appropriate LLOQ~~PQL~~ specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods," EPA Publication No. SW-846, incorporated by reference at Section 620.125, or the LCMRL specified in the drinking water methods incorporated by reference at Section 620.125 for the substance, incorporated by reference at Section 620.125, the guidance level is ~~in which case~~ the lowest appropriate LLOQ or LCMRL~~PQL shall be the acceptable level~~.

g) Since the assumption of dose addition is most properly applied to substances that induce the same effect by similar modes of action, a separate Hazard Index shall ~~must~~ be generated for each toxicity endpoint of concern.

h) In addition to meeting the individual substance objectives, a Hazard Index shall ~~must~~ be less than or equal to 1 for a mixture of similar-acting substances.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.APPENDIX C Guidelines for Determining When Dose Addition of Similar-Acting Substances in Class I: Potable Resource Groundwaters is Appropriate

a) Substances shall ~~must~~ be considered similar-acting if:

1) The substances have the same target in an organism (for example, the same organ, organ system, receptor, or enzyme).

2) The substances have the same mode of toxic action. These actions may include, for example, central nervous system depression, liver toxicity, or cholinesterase inhibition.

b) Substances that have fundamentally different mechanisms of toxicity (threshold toxicants vs. carcinogens) shall ~~must~~ not be considered similar-acting. However, carcinogens which also cause a threshold toxic effect should be considered in a mixture with other similar-acting substances having the same threshold toxic effect. In such a case, an Acceptable Level for the carcinogen shall ~~must~~ be derived for its threshold effect, using the procedures described in Appendix A.

c) Substances which are components of a complex mixture of related compounds which are produced as commercial products (for example, PCBs or technical grade chlordane) are not mixtures, as defined in Appendix B. Such complex mixtures are equivalent to a single substance. In such a case, the Human Threshold Toxicant Advisory Concentration may be derived for threshold effects of the complex mixture, using the procedures described in Appendix A, if valid toxicological or epidemiological data are available for the complex mixture. If the complex mixture is a carcinogen, the Health Advisory Concentration is the one-in-one-million cancer risk concentration, unless the lower concentration for such substance is less than the lowest appropriate LLOQ~~PQL~~ specified in "Test Methods for Evaluating Solid Wastes, Physical/Chemical Methods," EPA Publication No. SW-846, incorporated by reference at Section 620.125, or the LCMRL specified in the drinking water methods incorporated by reference at Section 620.125 for the substance.~~,~~ If the concentration for such substance is less than ~~in which case~~ the lowest appropriate LLOQ or LCMRL~~PQL~~ for the substance incorporated by reference at Section 620.125, the guidance level is the lowest appropriate LLOQ or LCMRL~~shall be the Health Advisory Concentration~~.

(Source: Amended at ~~36 Ill. Reg. 15206~~, effective ~~October 5, 2012~~)

Section 620.APPENDIX E: Similar-acting Substances

Table A: -Similar-acting Noncarcinogenic Constituents

Cholinesterase Inhibition	
116-06-3	Aldicarb
1563-66-2	Carbofuran
Circulatory System	
7440-36-0	Antimony
7440-38-2	Arsenic
1912-24-9	Atrazine
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)
206-44-0	Fluoranthene
86-73-7	Fluorene
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
98-95-3	Nitrobenzene
355-46-4	PFHxS (perfluorohexanesulfonic acid)
122-34-9	Simazine
100-42-5	Styrene
156-60-5	*trans*-1,2-Dichloroethylene
79-01-6	Trichloroethylene
99-35-4	1,3,5-Trinitrobenzene
7440-66-6	Zinc
Decreased Body Weight	
1912-24-9	Atrazine
78-93-3	2-Butanone (methyl ethyl ketone)
75-71-8	Dichlorodifluoromethane
84-66-2	Diethyl phthalate
95-48-7	2-Methylphenol (*o*-cresol)
91-20-3	Naphthalene
7440-02-0	Nickel
375-95-1	PFNA (perfluorononanoic acid)
108-95-2	Phenol
122-34-9	Simazine
71-55-6	1,1,1-Trichloroethane
1330-20-7	Xylenes
Developmental	
7429-90-5	Aluminum

78-93-3	2-Butanone (methyl ethyl ketone)
75-15-0	Carbon disulfide
7440-47-3	Chromium (as chromium VI)
84-66-2	Diethyl phthalate
88-85-7	Dinoseb
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
7439-93-2	Lithium
375-73-5	PFBS (perfluorobutanesulfonic acid)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-95-1	PFNA (perfluorononanoic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
335-67-1	PFOA (perfluorooctanoic acid)
79-01-6	Trichloroethylene
Gastrointestinal System	
7440-41-7	Beryllium
145-73-3	Endothall
77-47-4	Hexachlorocyclopentadiene
1634-04-4	MTBE (methyl tertiary-butyl-ether)
Immune System	
156-60-5	*trans*-1,2-Dichloroethylene
7487-94-7	Mercury (mercuric chloride)
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
335-67-1	PFOA (perfluorooctanoic acid)
Kidney	
7440-43-9	Cadmium
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)
75-99-0	Dalapon
75-34-3	1,1-Dichloroethane
156-59-2	*cis*-1,2-Dichloroethylene
100-41-4	Ethylbenzene
206-44-0	Fluoranthene
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
98-82-8	Isopropylbenzene (cumene)
7439-93-2	Lithium

93-65-2	MCPP (mecoprop)
7487-94-7	Mercury (mercuric chloride)
7439-98-7	Molybdenum
375-73-5	PFBS (perfluorobutanesulfonic acid)
129-00-0	Pyrene
108-88-3	Toluene
7440-62-2	Vanadium
Liver	
83-32-9	Acenaphthene
7440-36-0	Antimony
12798-03-6	Chlordane
108-90-7	Chlorobenzene
67-66-3	Chloroform
94-75-7	2,4-D (2,4-dichlorophenoxy acetic acid)
75-35-4	1,1-Dichloroethylene
72-20-8	Endrin
100-41-4	Ethylbenzene
206-44-0	Fluoranthene
13252-13-6	HFPO-DA (hexafluoropropylene oxide dimer acid, GenX)
2691-41-0	HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine)
1024-57-3	Heptachlor Epoxide
1634-04-4	MTBE (methyl tertiary-butyl ether)
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-95-1	PFNA (perfluorononanoic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
335-67-1	PFOA (perfluorooctanoic acid)
1918-02-1	Picloram
100-42-5	Styrene
118-96-7	TNT (2,4,6-trinitrotoluene)
93-72-1	2,4,5-TP (silvex)
79-00-5	1,1,2-Trichloroethane
Lungs	
91-57-6	2-Methylnaphthalene
90-12-0	1-Methylnaphthalene
Nervous System	
67-64-1	Acetone
7429-90-5	Aluminum

7440-39-3	Barium
72-20-8	Endrin
7439-93-2	Lithium
7439-96-5	Manganese
95-48-7	2-Methylphenol (*o*-cresol)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
121-82-4	RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)
Reproductive System	
1563-66-2	Carbofuran
75-15-0	Carbon disulfide
7440-47-3	Chromium (as chromium VI)
143-33-9	Cyanide (sodium cyanide)
1918-00-9	Dicamba
7439-93-2	Lithium
72-43-5	Methoxychlor
375-73-5	PFBS (perfluorobutanesulfonic acid)
1763-23-1	PFOS (perfluorooctanesulfonic acid)
108-95-2	Phenol
Skin	
7440-38-2	Arsenic
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)
7440-22-4	Silver
7440-28-0	Thallium
Spleen	
99-65-0	1,3-Dinitrobenzene
99-35-4	1,3,5-Trinitrobenzene
Thyroid	
7440-48-4	Cobalt
14797-73-0	Perchlorate
355-46-4	PFHxS (perfluorohexanesulfonic acid)
375-73-5	PFBS (perfluorobutanesulfonic acid)
8001-35-2	Toxaphene
Whole Body	
120-12-7	Anthracene
7440-36-0	Antimony

65-85-0	Benzoic Acid
84-74-2	Di-*n*-butyl phthalate
75-69-4	Trichlorofluoromethane
1330-20-7	Xylenes

Table B: -Similar-acting Carcinogenic Constituents

Circulatory System	
71-43-2	Benzene
107-06-2	1,2-Dichloroethane
106-93-4	Ethylene dibromide (1,2-dibromoethane)
Gastrointestinal System	
56-55-3	Benzo(a)anthracene
205-99-2	Benzo(b)fluoranthene
207-08-9	Benzo(k)fluoranthene
50-32-8	Benzo(a)pyrene
218-01-9	Chrysene
53-70-3	Dibenzo(a,h)anthracene
106-93-4	Ethylene dibromide (1,2-dibromoethane)
193-39-5	Indeno(1,2,3-c,d)pyrene
Kidney	
67-66-3	Chloroform
96-12-8	1,2-Dibromo-3-chloropropane (dibromochloropropane)
121-14-2	2,4-Dinitrotoluene
606-20-0	2,6-Dinitrotoluene
100-41-4	Ethylbenzene
79-01-6	Trichloroethylene
Liver	
319-84-6	*alpha*-BHC (*alpha*-benzene hexachloride)
56-23-5	Carbon tetrachloride
12798-03-6	Chlordane
106-46-7	*p*-Dichlorobenzene (1,4-dichlorobenzene)
75-09-2	Dichloromethane (methylene chloride)
78-87-5	1,2-Dichloropropane
117-81-7	Di(2-ethylhexyl)phthalate
121-14-2	2,4-Dinitrotoluene
606-20-0	2,6-Dinitrotoluene
123-91-1	1,4-Dioxane (*p*-dioxane)

58-89-9	*gamma*-HCH (*gamma*-hexachlorocyclohexane, lindane)
76-44-8	Heptachlor
1024-57-3	Heptachlor epoxide
1336-36-3	PCBs (polychlorinated biphenyls as decachloro-biphenyl)
335-67-1	PFOA (perfluorooctanoic acid)
87-86-5	Pentachlorophenol
127-18-4	Tetrachloroethylene
8001-35-2	Toxaphene
79-01-6	Trichloroethylene
75-01-4	Vinyl Chloride
Mammary Gland	
121-14-2	2,4-Dinitrotoluene
606-20-0	2,6-Dinitrotoluene